THE EFFICACY OF LAMIVUDINE ALONE, AND IN COMBINATION WITH INTERFERON IN THE TREATMENT OF RESISTANT CHRONIC HEPATITIS B

G. Montazeri,[*] R. Malekzadeh,[*] N. Nouri,[*] M.R. Sohrabi, M. Sotoudeh,[**] N. Kamalian,[**] M. Farhadi[***]

[*]Digestive Disease Research Center, Shariati Hospital, Tehran University of Medical Sciences, [**]Department of Pathology, Shariati Hospital, Tehran University of Medical Sciences, [***]Department of Hematology, Tarbiat Modarres University, Tehran

ABSTRACT

Background/Objective: To evaluate the efficacy of lamivudine alone and in combination with interferon in patients with chronic hepatitis B who were nonresponsive to previous interferon therapy.

Methods: Thirty-six adult patients, who were previously nonresponders to interferon were randomly divided into two groups. Group I received lamivudine100mg daily for nine months, and Group II received lamivudine 100mg daily for nine months in addition to interferon $5\text{-}10\times 10^6$ u three times weekly for 6 months. Patients were followed up for another 6 months without receiving any medication.

The end points were; normalization of liver enzymes, sustained negative DNA and reduction of at least 2 points in the necroinflammatory scores of the liver biopsies on the basis of the modified Knodell HAI system.

Results: In Group I, 55.6% had sustained negative DNA (P=0.001). The mean AST value of 75.22±64.14 u/l before treatment decreased to 28.28±13.19 u/l after treatment (P=0.01). The mean ALT value of 104.22±92.96 u/l before treatment decreased to 31.22±20.10 u/l after treatment (P=0.005). The histological response was 38.8% (P=0.003). In Group II, 38.9% had sustained negative viral DNA (0.004). The mean AST value of 67.29±53.07 prior to treatment decreased to 26.88±15.85u/l after treatment (P=0.008). The mean ALT value of 98.24±75.92 u/l prior to treatment decreased to 28.06±21.53 u/l after treatment (P=0.002). The histological response rate was 27.7% (P=0.004). The P values were not significant comparing the post treatment AST, ALT, sustained negative HBV DNA and histological responses of the two groups.

Conclusions: It is concluded that both groups had a relatively good response rate with no statistically significant difference between the two groups. The adverse effect was more prevalent in Group II. It is suggested that chronic hepatitis B nonresponders to interferon should be treated with lamivudine therapy alone.

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Key Words: • Chronic hepatitis B • lamivudine • interferon • precore mutants

Introduction

Chronic hepatitis B is one of the most common infectious diseases in the world with approximately 350 million persons chronically infected. Seventy-five percent of those affected are of Asian origin.[1,2] Chronically infected persons with viral replication are at high risk for progressive liver disease including cirrhosis and hepatocellular carcinoma.[3-5]

The prevalence of chronic hepatitis B in Iran is approximately 5% with some 3 million persons affected.[7]

The standard treatment for chronic hepatitis B is interferon. Its efficacy is variable. A recent meta-analysis showed that 33% of

Correspondence: *R. Malekzadeh M.D. Digestive Disease Research Center, Shariati Hospital, Tehran University of Medical Sciences, Kargar Shomali Ave, Tehran, Iran, Tel: +98 21-8012992, Fax: +98218012992, E-mail: guiresh@ams.ac.ir*

patients receiving the drug had loss of HBe Ag compared to 12% of control subjects.[8]

Interferon is not as effective in Asian patients as in Caucasians.[9] The relatively poor response could be due to immune tolerance to HBV infection acquired at birth or in early childhood[10] as well as to the presence of precore mutants. In Iran, two thirds of patients with chronic hepatitis B infection do not respond to interferon. It is conceivable that the use of an antiviral agent may prove to be the therapy of choice in such patients.

Lamivudine (2', 3' dideoxy-3'-thiacytidine) is a potent antiviral agent and has been shown to be effective against hepatitis B viruses.[11] Lamivudine used in a one-year trial was shown to be associated with substantial histological improvement in the liver without any significant side effects.[12]

Combination chemotherapy using an antiviral agent (lamivudine) plus an immunomodulatory agent theoretically could reduce the viral load more rapidly and, therefore, the viral elimination could possibly be achieved easier.

In this study, the efficacy of lamivudine alone and in combination with interferon was examined in chronic hepatitis B patients who had received a full course of interferon (5-10×10^6 U three times per week for 6 months) without any improvement.

Materials and methods

Patients:

Eligible patients included 15 to 65-year-old males and females who were non-responders to interferon. A non-responder was defined as a patient who had received a full course of recombinant interferon α_2A, 5-10×10^6u subcutaneously three times per week for 6 months. All non-responders had positive HBV DNA, elevated liver enzymes ALT > 1.5 times normal, AST > 1.5 times normal on at least three successive occasions within 6 months after the completion of interferon therapy. Approximately, half of these patients had negative HBe Ag. We believe that they were carriers of precore mutants with positive HBV DNA, and elevated ALT and AST levels.

Exclusion criteria included decompensated cirrhosis (portal hypertension, esophageal varices, ascites, bilirubin > 2.5 mg/dl, albumin < 3 gm/dl, and a prothrombin time more than 3 seconds above normal) and other causes of chronic hepatitis (HCV, HDV, HIV, metabolic liver disease and auto-immune hepatitis). Excluded were also patients who had received systemic antiviral therapy, cytotoxic agents and corticosteroids within 6 months and lamivudine within 3 months of the beginning of this study.

Study Design:

All patients underwent liver biopsy prior to starting treatment. The patients were randomly assigned either to receive 100 mg lamivudine daily for nine months, or interferon α_2A, 5-10×10^6 U subcutaneously three times per week for six months in addition to lamivudine 100 mg daily for nine months. Because of its expense as well as the lack of firm scientific evidence for the safety of extended lamivudine therapy at the time our patients were being enrolled, lamivudine therapy was carried out only for 9 months in this study. The patients were followed regularly every two weeks for nine months. AST, ALT, CBC and platelet counts were done at the time of each visit. The patients were examined and were questioned regarding adverse effects. PCR for HBV DNA was performed every three months prior to and after the course of treatment. A second liver biopsy was carried out within 3 months after treatment in those patients who consented to the procedure. The patients were followed for an additional 6 months whilst not receiving any medication.

Laboratory Tests:

All biochemical tests were performed in our clinical pathology laboratory using routine automated techniques. Hepatitis B surface antigen, HBe antigen and their antibodies were assayed using an enzyme immunoassay kit

(Organon, Behringer, Biokits, Radim, corporations).

HBV DNA was qualitatively assayed. The limit of detection was 100 copies per ml. Reagents were supplied by Rosh-Diagnostica Mannheim, Germany. The sera were collected with appropriate precautions for PCR measurement as follows:

150μL of the serum was digested with 150μL of proteinase k (10 mg per ml) at 50°C for 2 hours. Phenol-chloroform extraction was then performed at PH=8. The extract was precipitated with isopropanol. Two primers of the conserved S region were used (primer 1=5'-CTC AAG CTT CAT CCA TAT A-3' and primer II=5'-CTT GGA TCC TAT GGG ATG GG-3'), which amplified a segment of 128 bp. Thirty cycles of amplification were conducted with one minute of denaturation at 90°C, one minute of annealing at 52°C and one minute of extension at 72°C using the automated thermocycler (Genius Bm, Gm, UK). Amplified DNA sequences were detected by agarose gel electrophoresis with 2% ethidium bromide staining and visualized by ultraviolet light with a wavelength of 302 nanometers. Positive and negative control samples were included in each run. Size markers of Bm (manufacturer) were also included. The presence of a particular fragment of amplification (128-150 base pair) indicates the presence of HBV in the samples. The results were recorded either visually or by using a photographic method.

Liver Biopsy:

Baseline liver histology was assessed for all the patients. The second biopsy was performed on 14 patients in Group I and on 13 patients in Group II. The remaining patients did not agree to repeating the procedure. A single, pathologist who was unaware of the patients' treatment assignment or the time at which the specimen had been obtained, reviewed all biopsy specimens. The modified Knodell scoring system, which was originally designed by Ishak was used.[13-15] It seems to be preferred over the original Knodell system because of its continuous grading and separation of necroinflammatory activity from fibrosis.[13] This allocates a numerical score for the histological activity of hepatitis B on a scale of 0 to 24, with higher scores indicating more severe abnormalities. The overall Ishak's modified Knodell scores are the sum of scores for grading and staging. The grading consists of: A- periportal or periseptal interface hepatitis (piecemeal necrosis 0-4), B-confluent necrosis (0-6), C-Focal (spotty) lytic necrosis, apoptosis and focal inflammation (0-4), D-Portal inflammation (0-4). The staging consists of architectural changes, fibrosis and cirrhosis (0-6). Response rates were based on the reduction of necro-inflammatory activity (the grading scores). Histological response was defined as at least a 2-point reduction in the necro-inflammatory scores of the second biopsy, obtained from the same patient within three months after the completion of therapy.

End points:

The end points were sustained normalization of ALT, AST, negative HBV DNA and reduction of at least 2 points in the scores of Ishak's grading and staging system (Modified Knodell HAI) at the post-treatment follow-up.

Statistical Analysis:

For qualitative analysis, chi square (χ^2) test was used for the comparison of one variable between two independent groups. Either before or after treatment, the McNamar test was used for the comparison of one variable between the paired (dependent) groups before and after the treatment.

For quantitative analysis, independent t-test was used for the comparison of one

Table 1: Characteristics of patients at the enrollment

Groups	M/F	Age Yrs. Mean (SD)	ALT U/L mean (SD)	AST U/L mean (SD)	HBs Ag + / -	HBe Ag + / -	HBV DNA + / -	Liver Biopsy scores (SD)
Lamivudine (N=18)	15/3	41.22 (±12.54)	104.22 (±92.96)	75.22 (±64.16)	18 /-	7 / 11	18 / -	8.00 (±3.84)
Lamivudine + Interferon (N=18)	16 / 2	39.22 (±12.72)	98.24 (±75.97)	67.29 (±53.07)	18 /-	8 / 10	18 / -	8.6 (±3.68)
P-value	0.63 (NS)	0.638 (NS)	0.837 (NS)	0.694 (NS)		0.735 (NS)		0.656 (NS)

paired t-test was used for the comparisons of one variable between the paired (dependent) groups before and after the treatment.

Results

Study population:

The two groups were similar with respect to demographic parameters, liver function tests, HBV serology and histologic activity index (modified Knodell scoring) of the liver. The p-value of each variable between the two groups was not statistically significant (Table 1).

HBV DNA:

HBV DNA was measured qualitatively by PCR as positive or negative. All the patients had positive HBV DNA in both groups before the treatment. HBV DNA became negative in 15 out of 18 patients (83.3%) in Group I and in 12 patients out of a total of 18 patients (66.7%) in Group II whilst receiving treatment. The rate of conversion to DNA negativity in both groups was highly significant ($p<0.001$ in Group I and $p<0.001$ in Group II, respectively). The patients were followed up for 6 months after the completion of therapy. Eight out of 18 patients (44.4%) remained HBV DNA negative in Group I during the follow-up ($p<0.001$). Seven out of 18 patients (38.9%) remained HBV negative in Group II during the drug-free follow-up period (P=0004). Therefore, the DNA losses in the paired groups were significant. However, comparing the two groups during (P=0.264) and after the

Table 2: HBV DNA loss (Negative PCR) and status of HBe Ag in treatment groups

		Before Treatment	After Treatment	P value
Negative HBe Ag	Group I (No.)	11	16**	0.063
	Group II(No.)	10	13	0.375
Sustained negative HBV DNA	Group I (No.)	0.00	8*	0.001
	Group II(No.)	0.00	7	0.004

* Independent groups (Group I and Group II) sustained negative HBV DNA after completion of therapy, P*= (0.395)

** Independent groups (Group I and Group II) negative HBeAg after completion of therapy, P**= (0.402)

Table 3: Status of SGOT and SGPT of treatment group

		Before Treatment	After Treatment	P value
AST (SGOT)	Group I (No.)	75.22±64.14	28.28±13.19*	0.010±0.008
	Group II(No.)	67.29±53.07	26.88±15.85	
ALT (SGPT)	Group I (No.)	104.22±92.96	31.22±20.10 * *	0.005±0.002
	Group II(No.)	98.24±75.97	28.06±21.53	

* Independent groups (Group I and Group II) AST level, after treatment, P*= (0.778)
** Independent groups (Group I and Group II) ALT level, after treatment, P=** (0.656)

treatment (P = 0.395), the P values were not significant (Table 2). In other words, adding interferon had no additional advantage.

Seroconversion:

We could not select exclusively HBe Ag positive patients. This was because a substantial proportion of our patients (11 out of 18 patients in Group I and 10 out of 18 patients in Group II) were HBe Ag negative. All had positive HBV DNA, and their liver enzymes were elevated. By definition, these patients were infected with precore mutants. By seroconversion we mean those cases who had positive HBe Ag and negative HBe antibody before treatment and who became HBe Ag negative and anti-HBe antibody positive after the treatment. Five out of seven HBe Ag-positive patients in Group I seroconverted (P=0.063). Three out of eight patients in Group II seroconverted. The p-value was not significant (P = 0.375). With respect to the rate of seroconversion, Group I had much better results compared to Group II. (Table 2)

Table 4: Histological Responses

	Group I (Lamivudine)	Group II (Lamivudine + Interferone)
Responses		
Total number on enrollment :	18	18
Improvement - No. (%)	7(38.8) *	5(27.7)
Worsening or no change - No. (%)	7(38.8) *	8(44.4)
Ishak's system (modified Knodell score)		
Patients Rebiopsied: (Total Number)	14	13
Base line(mean ± SD)	8.29±3.79	8.08±3.48
Post treatment(mean ± SD)	8.36±3.25	7.77±3.37
Improvement:		
Base line(mean ± SD)	10.43±3.55	10.00±3.08
Post treatment(mean ± SD)	8.00±3.79	4.80±2.39
Worsening or no change:		
Base line(mean ± SD)	6.00±2.58	6.87±3.31
Post treatment(mean ± SD)	8.71±2.87	9.75±2.49

Histological response was defined as a reduction of two or more points in Ishak's grading and staging system (modified Knodell HAI).The data are from an intention to treat analysis in which patients that are missing or could not be evaluated were considered to have no response.
(P1= 0.003) paired groups (Group I) before and after treatment
(P2= 0.004) paired groups (Group II) before and after treatment
(P3=0.48) independent groups (groups I&II) before and after treatment

Table 5: Adverse effects reported during therapy

Symptoms	Group I (Lamivudine) N = 18	Group II Lamivudine + Interferon N = 18
Fatigue	2	14
Headache	1	15
Transient fever	0.00	17
Muscle pain	1	18
Hair loss	0.00	5
Nervousness	0.00	7
Low Appetite + Weight loss	0.00	5
Thrombocytopenia	0.00	3
Leukopenia	0.00	4

Two patients lost their HBsAg. One was in Group I and the other in Group II.

AST and ALT Status:

The initial mean AST level before treatment was 75.22±64.14 u/l in Group I. This dropped to 28.28±13.19 u/l after treatment (P = 0.01). The initial mean AST level before treatment in Group II was 67.29±53.07 u/l. This dropped to 26.88±15.85 u/l (P=0.008) after treatment. Although ALT levels dropped significantly in the paired groups, this change in the two independent groups was not significant (P= 0.778). The initial mean ALT value before treatment in Group I was 104.22±92.86 u/l. This dropped to 31.22±20.10 after treatment (P=0.005). The initial mean ALT value before treatment in Group II was 98.24±75.97 u/l. This dropped to 28.06±21.53 u/l after treatment (P=0.002). Although ALT levels dropped significantly after treatment in the paired groups, the change in the two independent groups was not significant (P=0.656). (Table 3)

Histological Findings:

Liver Biopsies were done in all patients before they enrolled in this study. Histological response was defined as a reduction of 2 points of necroinflammatory scores. In Group I, the average initial score was 8.00±3.84 and in Group II, the average initial score was 8.6±3.67. We were unable to rebiopsy all patients at the end of treatment. Fourteen out of eighteen patients in Group I and thirteen out of eighteen patients in Group II agreed to post-treatment liver biopsy. Those patients who did not agree to the post-treatment liver biopsy were considered as nonresponders because the intention-to-treat analysis was used in this study.

In Group I, seven patients (38.8%) had histological response (P=0.003). Their mean pretreatment scores decreased from 10.43±3.55 to 8.00±7.9 after therapy. The other seven patients either had no change or worsening of their histological scores. The mean scores of this subset increased from a mean pretreatment score of 6.00±2.58 to 8.71±2.87 after therapy.

In Group II, five patients (27.7%) had histological response (p=0.004). Their mean pretreatment scores decreased from 10.00±3.05 to 4.80±2.39 after therapy. The other eight patients had either no change or worsening of histology, and their mean pretreatment scores increased from 6.87±3.31 to 9.74±2.49 after treatment. Although paired groups had good response rates, the difference between the two independent groups was not statistically significant (P=0.48). (Table 4)

The histological responders were those who had sustained negative DNA and normal liver enzymes at the post-treatment follow-up. The role of HBeAg mutation on the histological

response was not significant in this study, probably due to the small numbers in each subset.

Flare up:

A total of 4 patients (11.1%), two in each group, who had negative viral DNA on treatment became DNA positive and their AST and ALT, which were normal on treatment, developed a two-fold increase. This phenomenon appeared about 7 months after the initiation of therapy. We believe that this was due to YMDD mutation. Molecular biology studies were not performed

Adverse effects:

The adverse effects included fatigue, headache, fever, muscle pain, hair loss, nervousness, decreased appetite and weight loss. Mild leukopenia and thrombocytopenia was observed in several patients. The adverse effects were mostly confined to Group II (Lamivudine + Interferon group). lamivudine alone was well tolerated (Table 5) .

Discussion

In this study, the efficacy of lamivudine alone for the treatment of patients with chronic hepatitis B, who had not responded to interferon therapy, was compared to lamivudine as well as interferon in combination. This study shows that in our patient population, lamivudine plus interferon was not superior to lamivudine alone, at least with this sample size. However, lamivudine therapy alone was more beneficial than its combination with interferon. Theoritically the use of lamivudine, which is a potent anti-HBV agent, and Interferon α_2A, which is an immunomodulatory agent, in combination seems logical because not only can these agents suppress the viral load, but they can also eradicate the virus more efficiently.[16,17] Previous results have been controversial. In one study, it was shown by Marinos et al. that despite adequate suppression of HBV replication, virus specific T-cell response was not restored.[18] In another study by Schalm Sw., combination therapy was more effective than lamivudine mono-therapy in the treatment of chronic hepatitis B. The combination group had a higher HBe Ag clearance rate.[19] Lamivudine and Interferon have been used in combination therapy in one study and it was shown that it had no advantage over lamivudine monotherapy in interferon non-responders.[20]

About half of our patients were clinically precore mutants carriers (HBe Ag-, HBeAb+, HBVDNA+ and with elevated liver enzymes). Precore mutants are not present as a major species in the HBe Ag positive phase of chronic hepatitis B; they emerge during or sometimes after seroconversion to anti HBe Ab.[21,22] Precore mutants are quite prevalent in anti-HBe+ patients. It was shown that 26 out of 29 patients had either precore mutants or a mixture of wild type and mutants. Patients with pure precore mutants had higher liver enzymes and higher HBV DNA and more severe pathology in comparison to the mixed type and predictably more severe disease.[23] The common form is a single mutation G to A in 1896 (A 1896).[24] Due to the lack of available laboraory facilities, we were not able to clarify our mutant patients on a molecular basis. They could have been either pure mutants or a mixture of precore and wild type.

Lamivudine has been shown to be effective in hepatitis B virus precore mutants before and after liver transplantation. Lamivudine suppression was potent in more than 80% of these cases.[26,27] But the precore mutant response rate was less to interferon in comparison to the wild type.[29] We believe that the high precore mutant rate of our patients could have been a factor in the lack of effect of interferon in their treatment.

Considering all the above, both populations had good response to treatment, in spite of the fact that about half of them were precore mutants. Group I as well as Group II normalized their ALT and AST. In both, there was evidence of suppression of HBV DNA. The paired group had statistically significant

differences. Comparing the pre- and post-treatment, liver biopsy histology which was assessed on the basis of Ishak's grading and staging system (modified Knodell HAI), dropped at least 2 scores in responders of both groups.

The two independent groups were compared for normalization of AST, ALT, suppression of HBV DNA and reduction of necroinflammatory scores pre- and post-therapy. The differences in all these parameters were not statistically significant.

Patients tolerated lamivudine very well and did not have any significant adverse effect. Almost all adverse effects were confined to the Group II patients.

Viral DNA of four patients out of a total of 36 (11.1%) became positive again (DNA break-through), with elevated liver enzymes (two in each group) whilst receiving medications. This was probably due to YMDD mutation, which is a common phenomenon and can happen by using an antiviral agent. In a trial of 52 weeks, the rate of YMDD mutation was 14%(12). This can increase with time to about 60% if lamivudine is continued for 2 years.[28] YMDD mutation (DNA break-through) appears 9-14 months during lamivudine therapy.[29] This could have been the reason why the DNA breakthrough was relatively low in our study.

There are two shortcomings in the present study, which deserve emphasis:

1: The treatment outcome was evaluated only after nine months of therapy because of economic factors and the lack of solid scientific evidence for the safety of extended lamivudine therapy when our patients were enrolled in 1997.

2: PCR for measurement of HBV DNA was qualitative in this study. Quantitative assays of the level of HBV DNA would have been more informative. This goal could not be achieved because of our lack of laboratory facilities in Iran. PCR is a very sensitive assay and having negative HBV DNA by the PCR method would have added to the accuracy of this work.

In conclusion, patients who did not respond to interferon alone had a good response to lamivudine monotherapy. Adding interferon as an immunomodulatory agent did not seem to improve the clinical outcome. Adding interferon increases the expense and seems to produce more adverse effects. Therefore, it is recommended that lamivudine monotherapy be utilized for such patients. We believe further studies with larger sample size are required to answer this question clearly.

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ANTIBODY RESPONSE TO RECOMBINANT HEPATITIS B SURFACE ANTIGEN IN HEALTHY ADULTS FOLLOWING PRIMARY AND SUPPLEMENTARY VACCINATION

M.A. Shokrgozar, F. Shokri***

**National Cell Bank of Iran, the Pasteur Institute of Iran, **Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran*

ABSTRACT

Background: A proportion of healthy adults and neonates do not respond to vaccination with hepatitis B surface antigen (HBsAg). Re vaccination induces a protective antibody response in a minor proportion of non-responders.

Objective: To study the immunogenicity of primary and secondary vaccinations with recombinant hepatitis B vaccine in healthy adults, and to compare the results with those who obtained the vaccine only in the neonatal period.

Methods: Ninety-one healthy adults were immunized with triple doses of recombinant hepatitis B vaccine given at 0, 1 and 6 months intervals. The responder individuals were arbitrarily classified into low and high responders, based on the anti-HBs antibody titer. The non-responder subjects were subsequently re-vaccinated with 2-5 additional doses of the vaccine. Anti-HBs antibody and HBs antigen were detected by a sandwich ELISA, and anti-HBc antibody was detected by a competitive ELISA.

Results: A protective level of anti-HBs antibody (>10 IU/L) was developed in 93.4% (85/91) of the vaccinees following primary vaccination. Only one of the non-responders (16.6%) seroconverted and developed low titer of anti-HBs antibody after administration of additional 3-5 vaccine doses.

Conclusion: Our results suggest that unresponsiveness to hepatitis B virus (HBV) may be differentially regulated in adults as compared to neonates.

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Key Words: • Hepatitis B virus • vaccination • anti-HBs antibody

Introduction

More than a third of the world's population are estimated to have been infected with hepatitis B virus (HBV) of whom, 350 million individuals are chronic carriers. About 25% of carriers eventually develop serious liver diseases such as cirrhosis and hepatocellular carcinoma as a result of the infection.[1]

The incidence and transmission pattern of HBV infection differs in different parts of the world. In areas of intermediate endemicity, such as Iran,[2] the disease transmission patterns are mixed and the disease occurs at all ages, but the predominant period of transmission is at the younger ages.[3,4] Therefore, the effective control of disease transmission may not be possible without mass vaccination of the population, particularly neonates and children.

The development and availability of highly effective vaccines against hepatitis B since 1982 represents a major advance in preventive medicine and public health. Although strategies for immunization against this important infection are still evolving and universal vaccination of infants and adolescents is under examination as a possible strategy to control the transmission of this infection, more than 85 countries now offer HB vaccine to all children.[1] Mass vaccination of neonates and pre-school children has been

Correspondence: *F. Shokri M.D., Dept. of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, P.O.BOX: 6446-14155, I.R.Iran, Fax:- 98-21-6462267*

strongly recommended by the WHO Expanded Programme on Immunization (EPI).[5] This programme has been incorporated in the National Vaccination Scheme in Iran since 1992. Different vaccination schedules have been adapted by the health authorities in different countries. Not all vaccinees, however, respond to vaccination.[6]

In our previous study, immunogenicity of a recombinant HB vaccine was evaluated in a large number of Iranian neonates after primary and supplementary vaccinations.[7,8] In the present study, immunogenicity of the vaccine was investigated in healthy adults, and the results were compared and contrasted with our previous findings in neonates.

Materials and methods

Subjects and vaccination scheme:

A total of 91 healthy adults (68 male and 23 female, aged 17-61 years) were immunized with 20 μg of a yeast-derived recombinant HB vaccine (Heberbiotec, S.A, Cuba). The vaccine was administered intramuscularly at 0,1 and 6-month time intervals. One month after the completion of vaccination, the serum level of anti-HBs antibody was determined. Based on titer of anti-HBs antibody, the vaccinees were arbitrarily classified into 3 subgroups, namely the high-responders (anti-HBs antibody > 500 IU/L , n=68), low-responders (anti-HBs antibody > 10 and < 500 IU/L, n=17) and non-responders (anti-HBs antibody < 10 IU/L, n=6).[6] Non-responder individuals were re-vaccinated with 2-5 extra doses of the same vaccine 3-4 weeks after the completion of the primary vaccination at 4-6 week intervals and anti-HBs antibody was measured in serum 2-3 weeks after each injection.

Detection of HBV markers:

Anti-HBs and anti-HBc antibodies and HBsAg were detected by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Behring, Germany). Anti-HBs antibody was quantitated using appropriate dilutions of a positive sample with a given concentration of the antibody expressed as IU/L provided by the manufacturer.

Statistical analysis:

The differences in variables were analyzed using the Mann-Whitney U test, Chi-square and Fisher tests. *P* values of less than 0.05 were considered significant.

Results

A protective level of anti-HBs antibody (≥10 IU/L) was produced in 93.4% (85/91) of the vaccinated healthy adults following the primary vaccination with triple doses of the vaccine.

No significant differences were observed between male and female vaccinees with regards to the frequency of seroprotection (95% and 89%, respectively). Based on titer of anti-HBs antibody, the responders were arbitrarily classified into 2 subgroups, namely the low (anti-HBs antibody > 10 and < 500 IU/L) and high (anti-HBs antibody > 500 IU/L) responders (Fig. 1). There was no significant difference in the mean titer of anti-HBs antibody between low (195±SD and

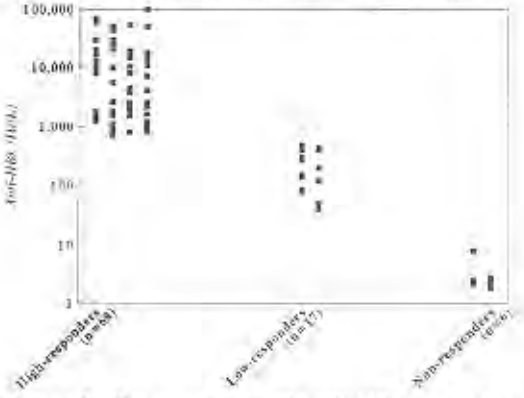

Figure 1: Serum levels of anti-HBs antibody in healthy adult vaccinees following the primary vaccination.

Table 1: Effect of re-vaccination on anti-HBs antibody response in six non-responder adults.

Subjects	Anti-HBs antibody (IU/L)			
	Third dose[1]	Fourth dose	Fifth dose	Sixth dose
NS1	8	8	85	135
NS2*	2.2	2.5	2.5	2.5
NS3*	2.5	2.5	2.5	2.5
NS4	0.5	2	2	NA
NS5	0.8	2.5	2.5	NA
NS6	2.8	2.8	3.2	NA

[1]: Third dose represents the last dose of primary vaccination, *: Anti-HBs titer was less than 10 IU/L even after administration of the 8th vaccine dose, NA: Not administered.

206±SD IU/L) and high (10367±SD and 14459 ±SD IU/L) responders of either sex. If the responder vaccinees are classified, on the basis of age, into 3 groups (17- 31 years, 32-46 years and 47-61 years), no significant differences are observed between the groups with regards to anti-HBs titers. Six vaccinees (3 females and 3 males) failed to develop a protective anti-HBs response following the primary vaccination.

Re-vaccination of the non-responders with the 4th vaccine dose did not induce a protective anti-HBs response in any of the vaccinees (Table 1). Administration of the 5th dose resulted in a low antibody response (85 IU/L) in only 1 subject. No further seroconversion was observed after the administration of the 6th vaccine dose to 3 subjects and also 2 extra doses to 2 of the vaccinees (Table 1). None of the responder and non-responder vaccinees included in this study were tested positive for anti-HBc antibody and HBs antigen (data not presented).

Discussion

We have recently reported on the immunogenicity of a recombinant HB vaccine in a large cohort of Iranian neonates and found a high seroconversion rate following the administration of a single supplementary vaccine dose in neonates non-responding to primary vaccination.[7,8]

In the present study, the immunogenicity of a recombinant HB vaccine and the influence of re-vaccination were investigated in healthy adult individuals. Our results demonstrated a vaccination failure in 6.6% of the subjects, as judged by the production of less than 10 IU/L of anti-HBs antibody.

Despite the usage of two different recombinant vaccines manufactured by different companies, this figure is similar to that obtained in our previous study performed on neonates (7.4%).[7] In a series of studies, it has recently been demonstrated that 2.5-10% of neonates and children,[9-12] and 4.4-14% of healthy adults[13-17] display inadequate antibody response (< 10 IU/L) following a standard vaccination schedule with hepatitis B vaccine, irrespective of the type of the administered vaccine.

The influence of re-vaccination in non-responders to HB vaccine has not been adequately addressed previously and remains a matter of controversy. Different results have been reported in different populations within the last few years.[8-17] Our results, although based on a small number of vaccinees, indicate the inefficiency of re-vaccination in adult healthy HB vaccine non-responders. Only one (16.6%) of the primary non-responder subjects seroconverted following the administration of 2-5 extra vaccine doses. Interestingly, seroconversion in this individual was associated with the production of low levels of anti-HBs antibody (Table 1). These results are significantly different from our previous study conducted on neonates, where 90% (44/49) of primary non-responder neonates responded

Table 2: Outcome of re-vaccination with hepatitis B vaccine in neonates, children and adults.

Age (years)	No.of vaccinees	Type of vaccine	Primary doses	Supplementary Doses	** % Responders	GMT (IU/L)	Ref.
Neonates	49	Rb	3	1	90	7061	8
Neonates	45	PD(Rb)	3	3	100	ND	9
1-5	32	PD(Rb)	4	1	53.1	57.5	10
1-5	32	PD(Rb)	4	2	87.5	104.7	10
1-5	32	PD(Rb)	4	3	100	724.4	10
1	38	Rb	3	2	94.8	ND	11
10	174	PD(Rb)	4	1	95.9	ND	12
31-65	25	PD(Rb)	3	3	36	ND	13
> 30	20	PD	3	1	25	216 RIA units	14
> 30	20	PD	3	2	40	100 RIA units	14
> 30	20	PD	3	3	45	<100 RIA units	14
20-65	11	Rb	3	1	36	41.2	15
39 ± 10*	75	Rb	4	1	32	A	16
22-66	23	Rb	3	1	52	B	17

vigorously to a single supplementary vaccine dose ($P<0.0002$).[8] This difference in the pattern of response to supplementary vaccination between adults and neonates also seems to be reflected in the results reported by other investigators (Table 2).

In neonates and children, re-vaccination was found to induce a protective antibody response in 53-100% of non-responder vaccinees.[8-12] Surprisingly, however, a lower seroconversion rate (16.6-52%) has been reported in adults following re-vaccination[13-17] (Table 2). The difference in the frequency of responsiveness to HB vaccine between neonates/children group[8-12] and adults group,[13-17] as reported by different investigators so far (Table 2), is highly significant ($P<0.001$).

In addition, adults tend to produce lower concentrations of anti-HBs antibody with short-term persistency following re-vaccination.[13-14,17] These findings suggest that unresponsiveness to HB vaccine may be controlled by different mechanisms in adults as compared to neonates and children.

Overall, lack of response to HBV has been attributed to a variety of genetic and environmental factors. Expression of immune suppression genes or lack of immune response genes linked to the HLA system,[18-20] defects in antigen-specific B or T-lymphocyte repertoire [21] and neonatal tolerance [22, 23] are the most widely studied mechanisms. Although some HLA alleles and antigens have been widely reported to be associated with unresponsiveness to HBV,[18-20] as found in our adult vaccines,[24] the high rate of seroconversion subsequent to additional vaccinations may argue against the contribution of HLA in neonatal unresponsiveness. This, however, does not rule out association with some HLA antigens in real non-responder neonates and children who fail to respond to booster vaccinations. This issue is currently being investigated at our laboratory.

Immune tolerance due to exposure to B or T lymphocytes at an early stage of maturation with HBe or HBs antigen, through clonal anergy or deletion, has already been demonstrated in transgenic mice and also in human neonates born to HBV carrier mothers.[25,25] (Although, in our previous study, neither the non-responder neonates nor their mothers were found to be HBs Ag

positive,[8] in the present study too, all the non-responder adults were tested negative for HBs Ag.) However, low-titer HBV DNA infection should not be totally ignored. Indeed, the majority of both neonate and healthy adult non-responders have recently been shown to be infected with low levels of HBV DNA not detectable by the available serological techniques.[25,26,27] In neonates, this occult infection was found to be transitory and largely confined to peripheral blood mononuclear cells (PBMC), acquired through the placenta from infected mothers.[25,26] In adults, however, the source of such trace infection is unknown. In addition to hepatocytes, which are known to be the main target of viral infection and replication, HBV frequently infects PBMC.[28] In the latter situation, replication of the virus is thought to be impaired and non-productive, leading to low-titer viral infection. Such a pattern of infection has been reported in clinically asymptomatic and apparently healthy subjects negative for all serological HBV markers.[27,29] Restricted PBMC infection may result in impaired macrophage/monocyte function and inefficient antigen presentation[28] or destruction of antigen-specific B-cell precursors by CD8+ T-lymphocytes.[30]

One important factor differentiating unresponsiveness in neonates and adults, is the age. It is clearly envisaged in the outcome of natural infection with the wide type virus, where 5-10% of the adults, and 70-90% of the neonates become chronically infected.[22] In neonates, immaturity of the immune system was blamed to be responsible.[31] However, recent observations have shown that neonatal tolerance may reflect an imbalance in the antigen dose inoculated and the ratio of APC/T-lymphocytes.[31,32] Such an imbalance could induce unresponsiveness to a given antigen, either due to impaired co-stimulatory signals[31] or to a shift in Th1 and Th2 responses.[32] The latter mechanism regarding Th1/Th2 imbalances has also been recently reported in healthy adult HB vaccine non-responders.[26,31]

In summary, we have demonstrated that 6.6% of the Iranian healthy adults failed to respond to primary vaccination with triple doses of a recombinant HB vaccine. Re-vaccination induces a protective antibody response in 16.6% of the non-responder individuals.

Our results suggest that unresponsivenss to HBV may be differentially regulated in adults as compared to neonates.

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INDUCTION OF DIFFERENTIATION OF U937 LEUKEMIA CELL LINE BY A NEW NUCLEOSIDE ANALOGUE, 1-PHENOXYMETHYL-6-AZAURACIL

Z. Amirghofran, A. Khalafi-Nezhad,** F. Tasdighi****

Department of Immunology, Medical School, and * Pharmacy School, Shiraz University of Medical Sciences, **Department of Chemistry, Faculty of Science, Shiraz University, Shiraz*

ABSTRACT

Background: Analogues of purines or pyrimidines have been reported to induce differentiation, to inhibit the growth of myeloid cells and to be useful in the treatment of leukemia.

Objective: To investigate the effect of a new nucleoside analogue designated 1-phenoxymethyl-6-azauracil on the growth and differentiation of a monoblast-like human histiocytic lymphoma cell line, U937, capable of terminally differentiating in vitro to functionally mature monocytes.

Methods: The synthesis of 1-phenoxymethyl-6-azauracil is described. Differentiation activity of this compound was measured by the assessment of morphologic maturity and the ability of U937 cell line to induce nitroblue tetrazolium (NBT) reduction. The results were compared with the same effect of retinoic acid (RA), a known inducer of differentiation.

Results: U937 cell line was induced to differentiate into monocyte-like cells by incubation with 1 μM of 1-phenoxymethyl-6-azauracil. At this concentration, NBT reducing activity was observed in 39.3% of compound-treated cells in comparison with 48.3% of RA-treated cells. Treatment of U937 cell line with 1μM of 1-Phenoxymethyl-6-azauracil and the same concentration of RA increased the number of mature cells up to 43.5% and 68.5%, respectively. Increases in the number of mature cells and NBT positivity were correlated with the reduction in the cell number.

Conclusion: The new synthesized compound is able to induce differentiation of U937 cell line.

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Key Words • Nucleoside analogues • U937 cell line • azauracil

Introduction

Nucleoside analogues have gained increasing importance through their biological activity, particularly as anti-cancer and anti-viral compounds.[1] A number of pyrimidine and purine bases have been found to confer activity to nucleoside analogues.[2,3] The study of these analogues and their interaction with proliferative cells may be useful not only for understanding the control of normal cell differentiation but also may imply an alternative approach to the therapy of certain malignancies. 5-flurouracil, 6-thioguanine, 6-mercaptopurine and arabinosyl cytosine are examples of theses analogues. These drugs, in which either the heterocyclic ring or the sugar moiety has been altered, induce toxic effects on cancer cells through the inhibition of specific enzymes or replacement of natural nucleotides during synthesis of DNA or RNA.[1] In addition to the cytotoxic effect, a differentiation induction activity has been reported for some of these drugs.[4,5] Cytosine arabinoside, an important drug in the treatment of acute myeloblastic leukemia, has been shown to induce differentiation of HL-60 and U937 myeloid cell lines.[5] Induction of hemoglobin synthesis by this drug has been

Correspondence: *Z. Amirghofran, Ph.D. Dept. Of Immunology, Medical School, Shiraz University of Medical Sciences. Shiraz, Iran. Fax: +98-711-2334589. E-mail:* amirghof@sums.ac.ir

reported for K562 myelo-erythroid cell line.[5] 6-mercaptopurine and 6-thioagunine have also been reported to induce differentiation of HL-60 myeloid cell line toward mature granulocytes.[6] Several investigators have tried to synthesize other purine and pyrimidine analogues capable of the induction of differentiation of tumor cell lines.[7-9] Makishima et al. investigated the effect of several synthetic analogues on the differentiation of various cell lines.[7] 3-benzyl-5-methyl-3 (ß-D-ribofuranosyl) pyridol [2,3-d] pyrimidine-2, 4 (1H, 3H)-dione has been one of these compounds with activity on HL-60 cell line.[7]

In this study, a new uracil acyclo-aromatic nucleoside derivative was synthesized and its effect on the induction of differentiation of a leukemic cell line, U937, was examined. This cell line is known to differentiate into morphologically mature macrophage-like cells after treatment with retinoic acid (RA), 1,25 dihydroxyvitamin D3, phorbol esters and lymphokines[10-13] and is a useful model for studying the effect of different compounds on the human myeloid cell differentiation.

Materials and Methods

Preparation of 1-phenoxymethyl-6-azauracil:

6-Azauracil (0.01 mole, 1.13 g.) and $(NH_4)_2SO_4$ (0.003 mole, 0.4 gr.) were added to hexamethyldisilazane (50 ml) in a 250 ml round-bottom flask. The mixture was refluxed until a clear solution was obtained (1.5 hours). The solvent was removed at reduced pressure and the residue was dried under vacuum. The residue was dissolved in 1, 2-dichloroethane (75 ml) and then Bu_4NI (0.025 mole) was added. The mixture was refluxed and phenoxymethyl chloride (0.01 mole, 2.19 gr.) was added. After 5 hours, the solution was evaporated to yield a yellow precipitate. The impurity of the residue was extracted with $CHCl_3$(2x50 ml) and then with water (2x50 ml). The solution was dried (Na_2SO_4), filtrated and evaporated. The residue was chromatographed on silica gel using AcOEt to give a yellow solid (65%). m.p. 129 °C, R_f(AcOEt) 0.75, ^{1}H-NMR (DMSO-d^6) δ: 6.22(s, 2H, OCH_2N), 7.15(m, 6H, NH and Ph), 7.7(m, H, H-C5). IR (KBr) ν (cm^{-1}): 3200(NH), 1700(C=O), 1235, 1050(ether). UV λ (A) E(OH(nm): 208.0(2.204), 209.61(2.204), 262.7(1.472).

Media and culture conditions:

U937 cells were grown in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (Sigma USA), 2mM glutamine (Sigma), penicillin (100U/ml) and streptomycin (100 μg/ml). The cells were maintained in culture by serial passages. All cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Induction of differentiation:

Concentrations of 10^{-1} to 10^{-6} M of synthetic compound and RA (gifted by Dr. M. Fazel from Iran-Daru Co. Iran) were dissolved in 95% ethanol and DMSO, respectively. The solutions were diluted 1000-fold into the growth medium, so that the final concentration of ethanol or DMSO was not higher than 0.1%. Suspensions of U937 cells (10^5 cells/ml) with or without varying concentrations of test compound and RA were cultured separately in 24 well plates (Nunc, Denmark). Control cultures included the same concentrations of ethanol or DMSO. All tests were done in triplicate. Cell numbers were determined after the treatment for 7 days. Viability was assessed by trypan blue dye exclusion. Morphologic assessment of the cells was made on cell smears stained with Wright. Differential counts were then performed by at least two independent persons under light microscopy

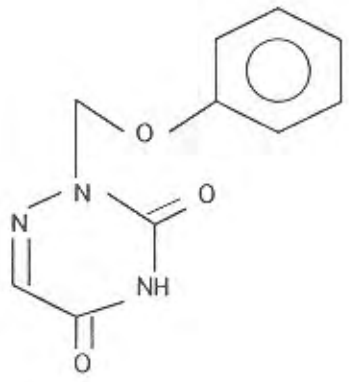

Figure 1: Chemical structure of 1-Phenoxymethyl-6-azauracil

on a minimum of 200 cells.

NBT Reduction:

A latex-stimulated nitroblue tetrazolium (NBT) reduction test was performed as described.[16,17] Cells (10^6/ml) were incubated for 15 minutes at 37°C with an equal volume of 0.2% NBT (Sigma) in phosphate buffered saline. Then 50μl latex particles (0.8μm, Difco Laboratories, USA) were added. After another 15 minutes of incubation in 37°C, the suspension was gently mixed and cell smears were prepared. The percentage of cells that engulfed latex particles and contained intracellular blue-black formazan deposits was then determined on Wright-stained slide preparations. At least 200 cells were assessed by two individuals.

Results

The structure of the tested compound is depicted in Figure 1. The ability of this nucleoside to inhibit growth and induce differentiation of U937 cell line was examined. For this purpose, U937 cells were cultured with various concentrations of the compound (10^{-4} -10^{-9} M) for 7 days. To evaluate the activity of the compound, RA, a known inducer of U937 cell differentiation, was used. The induction of differentiation was measured by NBT reducing activity as a typical marker of myelomonocytic differentiation and by determining the

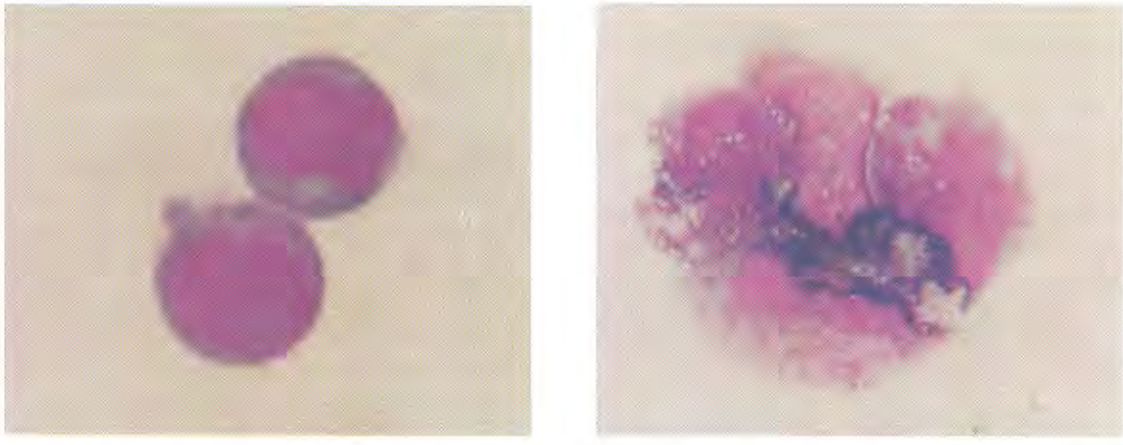

Figure 2: NBT reducing activity before (**left**) and after (**right**) treatment of U937 cells with the compound in a 7 day culture. Dark blue formazan precipitate is observed in the treated cells. Wright staining (X 1000).

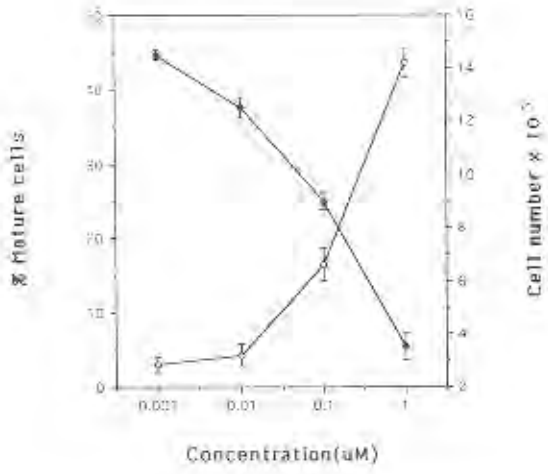

Figure 3: Effect of various concentrations of 1-phenoxymethyl-6-azauracil on growth (•) and maturation (o) of U937 cell line. As the number of mature cells was increased, the growth and proliferation decreased.

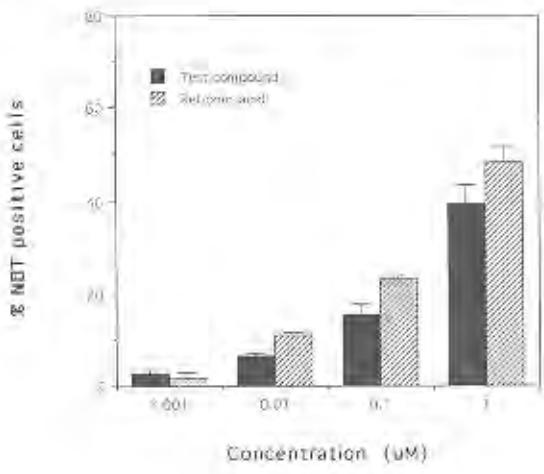

Figure 4: NBT reducing activity of U937 cell line in the presence of test compound and retinoic acid in a 7-day culture. Data represent mean ± SD of triplicate experiments.

percentage of mature cells in treated and untreated cultures (Figure 2). The growth inhibition of the compound was examined by determining the final number of viable cells. Generally, this compound showed a dose-dependent growth inhibitory activity on the U937 cell line. As shown in Figure 3, with increase in the compound concentration from 0.001 to 1 μM, a decrease in the number of cells (14.5 to 3.5×10^5) which corresponded to increase in the number of mature cells (3 to 43.5 %) was observed. In the NBT reduction assay, 1-phenoxymethyl-6-azauracil was able to increase the number of NBT positive cells in a dose-dependent manner (Figure 4). The highest NBT reducing activity was observed at 10^{-6} M, a concentration at which RA caused the most U937 cell differentiation (39.3% *vs* 48.3%). When differential counts on slides were performed, a concentration of 10^{-6} M of the compound produced the highest number of mature cells (43.5%) in comparison to those found after RA treatment (68%). A mature cell with monocyte-like morphology was identified with its pale cytoplasm, irregular or lobulated nuclei and the decreased number of nucleoli.

Discussion

Analogues of purines or pyrimidines have been reported to inhibit the growth of myeloid cells and to be useful in the therapy of leukemias. In this regard, several

myeloid cell lines including HL-60, K562, and U937, have been used as a model.[18-21] These cell lines have the capability to differentiate into mature myeloid cells when induced by certain compounds. Among these cell lines, U937 undergoes morphological and functional maturation when exposed to RA and phorbol esters.

In different attempts, Makishima et al. have investigated the effect of several nucleoside analogues on the U937 cell line.[12,22] Some of these analogues' derivatives of benzyluracil, were effective on the differentiation of HL-60 cells but not the U937 cell line.[7,23] In our study, the differentiation-inducing activity of 1-phenoxymethyl-6-azauracil on the U937 cell line was observed. The most effective concentration of the compound was at 10^{-6} M at which the number of mature cells, as well as NBT positive cells, was increased and the proliferation of cells was inhibited. Comparison of the effect of this compound with the same effect exerted by retinoic acid showed that this compound was a relatively efficient inducer of NBT reduction in the U937 cell line.

The mechanism of action of the compounds effective on cell differentiation is not well known. Compounds such as RA and vitamin D3 act through a sub-family of nuclear hormone receptors which regulate the expression of target genes by binding to specific DNA elements and modulating transcription initiation.[23-26] Phorbol esters act as protein kinase C activators and it has been shown that intracellular signal transudation by the protein kinase C family of enzymes plays a critical role in cellular growth regulation.[27] In the case of nucleoside analogues, the intracellular concentration of guanine nucleosides plays a central role in cellular differentiation.[28] Purine and pyrimidine analogues such as 6-thioguanine and 6-mercaptopurine are known to inhibit several enzymes involved in purine biosynthesis and interfere with guanine nucleoside pool.[1] Nucleoside analogues such as tiazofurin, an anti-cancer drug which inhibits IMP dehydrogenase, decrease cellular GTP concentration, induce differentiation and down-regulate ras and myc oncogenes expression.[19,28] It has been shown that some of the nucleosides, such as adenosine analogues, inhibit RNA and DNA methylation. This effect appears to be responsible for reduced c-myc RNA expression and induction of myeloid differentiation.[29] Further studies are required to determine the mechanisms by which the compound presented here affects.

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POLYMORPHISM OF TUMOR NECROSIS FACTOR- ALPHA PROMOTER REGION IN IRANIAN VACCINEES WITH BCG ADENITIS

E. Kamali-Sarvestani,[*] A.R. Nateghian,[**] B. Gharesi-Fard,[*] A. Behzadbehbahani,[**] A. Alborzi[**]

[*]Department of Immunology, [**]Alborzi Clinical Microbiology Research Center, Nemazi Hospital, Shiraz University of Medical Sciences, Shiraz

ABSTRACT

Background: Tumor necrosis factor-alpha (TNF-α) is a determining factor in macrophage activation and direction of immunologic mechanisms to BCG. By regulating the rate of transcription, allelic polymorphism in the regulatory regions of TNF-α gene can affect the host's ability in BCG containment.

Objective: To study the prevalence of G to A transition polymorphism at position -308 of the TNF-α gene in Iranian BCG vaccinees with lymphadenitis.

Methods: In this study, we determined the polymorphism at position -308 relative to the transcription initiation site of TNF-α gene in 40 patients with BCG adenitis and 42 healthy age-matched infants without reactions by a method based on allele specific PCR. The results of PCR were confirmed by the SSCP method.

Results: The frequency of TNFA2 allele in the patient and the control groups were 0.013 and 0.04, respectively. Statistical analysis showed no significant association of TNF-α promoter polymorphism with susceptibility to BCG adenitis. However, there was a three fold increase in the frequency of TNFA2 allele in the control subjects.

Conclusion: We suggest that the lower frequency of TNFA2 allele in patients might have resulted in weaker immune response that allows bacterial burden and occurrence of lymphadenitis.

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Key Words: • BCG • adenitis • TNF-α • polymorphism

Introduction

BCG vaccination plays an important role in the control of tuberculosis and is used as a part of the national immunization program in Iran. BCG-induced protection is essentially elicited through the generation of appropriate cell mediated immunity or Th1 response.[1] Furthermore, infected macrophages obtain the capability of the production and secretion of pro-inflammatory cytokines, such as TNF-α.[2] In Murine model, TNF-α is required to control mycobacterium infections through the formation and maintenance of granuloma[3] and induction of macrophage activation.[4] The latter leads to an augmentation of bactericidal mechanisms, in particular the induction of inducible nitric oxide synthase.[4]

The use of BCG vaccine may be associated with a significant number of adverse reactions, among which lymphadenitis is the most frequent one.[5] The incidence of this complication depends on many factors, including the type and concentration of vaccine, the age of vaccinees, the use of proper intradermal injection techniques and the characteristics of the recipient population.[5] The role of genetic factors in the susceptibility to BCG-induced adverse reactions have also been taken into consideration.[6]

Cytokines are largely responsible for the regulation of the immune response and may play an important role in the formation of

Correspondence: *E. Kamali-Sarvestani, Ph.D., Dept. of Immunology, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran. P.O.BOX: 71345-1798. Fax: +98-711-2337855. E-mail: immunol2@sums.ac.ir*

adenitis. Recently, several studies have clarified the importance of polymorphism in cytokine genes in different conditions and their influence on cytokine production.[7-9] Several polymorphisms located in the promoter region of cytokine genes affect transcription or translation and not infrequently determine the level of expression of the protein product.[10] Therefore, an interesting and testable hypothesis for the clinical heterogeneity in vaccinees is genetic variation in the cytokine promoter region. As TNF-α is induced in mycobacterium infections[2] and plays an important role in macrophage activation and bacterial containment, we wondered whether di-allelic polymorphism within the TNF-α promoter at -308 bp played a role in lymphadenitis after BCG vaccination. This mutation alters the putative non-consensus binding site for the transcription activator protein AP-2.[11] It has been demonstrated that the TNFA2 allele (corresponding to A at position -308) functions as a more potent transcription activator than the more common TNFA1 allele (G at position -308) in human B-cell line.[12] Furthermore, peripheral blood mononuclear cells (PBMC) from individuals carrying a TNFA2 allele produced more TNF-α under the stimulation of T-lymphocyte activators than did PBMC from homozygous individuals for TNFA1.[13] Reporter gene assays, in which the constructs of the two forms of the TNF-α promoter were inserted upstream of the reporter gene chloramphenicol acetyltransferase (CAT), show a six to seven-fold increase in the level of transcription when the TNFA2 allele is present.[14] Therefore, we investigated the -308 allelic distribution of TNF-α promoter gene in a collective of 40 patients with BCG adenitis. Our results indicate that there is no significant difference between the frequency of two allelic forms of TNF-α promoter in patients with adenitis and controls.

Materials and Methods

Patients and Controls:

Forty infants with BCG adenitis (24 males and 16 females) and 42 normal infants (22 males and 20 females), vaccinated with BCG during the first two days of life, were chosen. Vaccine administration was performed by a group of expert technicians. The type and dose of the vaccine were similar in both study groups. The age of the patient and control groups was between 2 months to 2 years. Both groups were selected from the same population living in Fars Province. The controls were referred for routine check-up, and the patients were recruited from the regional health centers in Shiraz. Both groups had normal immunity in clinical work-up. Written informed consent was obtained from their parents.

Typing of TNF-α polymorphism by allele-specific polymerase chain reaction:

Genomic DNA was extracted from peripheral blood leukocytes by a salting out procedure. PCR-based DNA analysis was carried out with some modifications under the conditions previously described.[15] Based on this method, an allele-specific polymerase chain reaction (ASPCR) was used to detect the G→A transition polymorphism at position -308 of TNF-α gene. Three primers were used for the ASPCR: the 3' primer (C1, position -144/-164: 5'-TCTCGGTTTCTTCTCCATCG-3') was used in combination with either the 5' primer (C2, position -328/-308 G: 5'-ATAGGTTTTGAGGGGCATGG-3'), complementary to the TNFA1 allele, or the 5' primer (C3, position -320/-308 A: 5'-ATAGGTTTTTGAGGGGCATGA-3'),

which is complementary to the TNFA2 allele. Primers C2 and C3 only differ in their 3' terminal nucleotide. When the 3' nucleotide does not match template DNA, amplification does not occur. As an internal control, the β-globin specific primers (5' primer: 5'-ACACAACTGTGTTCACTAG-C-3', and 3' primer: 5'-CAACTTCATCCA-CGTTCACC-3') were included in the reactions. Fifty micro-liters of PCR reaction mixture were comprised of genomic DNA samples (500 ng), 200 μmol/L dNTPs, 2 mM MgCl2, 1X Taq DNA polymerase buffer, 2 units of Taq DNA polymerase (Bohringer, Germany), 10 pmol of each test primer (C1 and C2 or C1 and C3) and 10 pmol of internal control primers. Reaction conditions used with the thermal cycler (master cycler, Ependerof) were as follows: 95°C for 5 minutes; 31 cycles of 95°C for 90 seconds, 61°C for 150 seconds, and 72°C for 60 seconds; and 72°C for 10 minutes.

Reaction products were separated on a 2% agarose gel and were stained with ethidium bromide.

Single strand conformational polymorphism (SSCP) analysis:

Eight μl of PCR product were de-natured with 11 μl of de-ionized formamide containing 0.05% each of bromophenol blue and xylene cyanole FF as markers. This mixture was heated up to 100°C for 5 min; then, it was immediately cooled and kept on ice. Polyacrylamide gel electrophoresis was performed on the whole 20 μl mixture using 10% polyacrylamide (19:1 polyacrylamide/bis) in 0.5X tris borate EDTA (TBE) buffer. Electrophoresis was performed at a constant 16 mA for 3 hours using a 4°C circulating water bath. DNA was visualized using silver staining.

Statistical analysis:

The experimental data for the distribution of TNF-α alleles and clinical data were

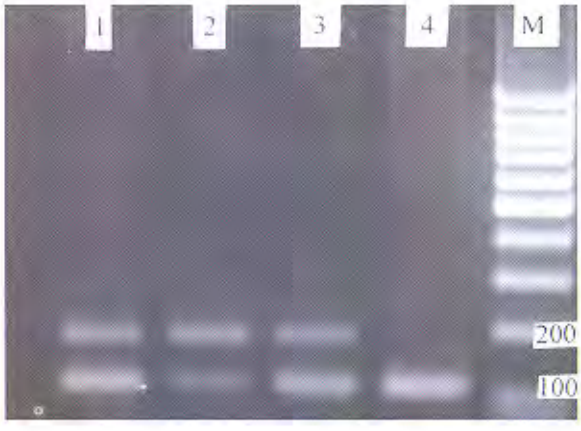

Figure 1: (left) Typical examples of patterns obtained in homozygous and heterozygous individuals for TNF-α -308 polymorphism. Lanes 1 and 3: TNFA1 allele; Lanes 2 and 4: TNFA2 allele. Lanes 1 and 2 correspond to a heterozygous patient (TNFA1/TNFA 2); lanes 3 and 4 correspond to a homozygous patient for TNFA1 (TNFA1/TNFA1). Lane M: molecular weight marker. The bands equal to 184 bp correspond to TNF 1 or 2 alleles and bands equal to 100 bp correspond to internal control.

Figure 2: (right) Single strand conformational polymorphism (SSCP) analysis pattern of TNFA1 allele (line 1) and TNFA2 allele (line 2). The gel was stained with silver nitrate.

Table 1: TNF-α genotype frequencies in control subjects and patients with BCG adenitis.

	Controls (N=42)	BCG adenitis (N=40)
Genotype	F[a]	F[a]
TNFA1/A1	0.93	0.97 NS[b]
TNFA1/A2	0.07	0.03 NS[b]

a: frequencies; b: Not significant. The P value is calculated by Fisher exact test.

compared by $\chi 2$ or Fisher exact tests, using EPI6 statistical software package.

Results

TNF-α genotype was determined in 40 infants with BCG adenitis and 42 normal infant vaccinees. Following ASPCR amplification of the genomic DNA and further verification of the results by SSCP, the patient group and control subjects were categorized for the -308 polymorphism. Grouping into the three genotypes could be accomplished by discriminating between the presence or absence of the two alleles. Figure 1 shows the results of ASPCR amplification and Figure 2 shows the SSCP analysis of the alleles TNFA1 and TNFA2. The frequency of the TNF-α genotypes for the patient and control groups are shown in Table 1.

The frequency of TNFA2 allele in the patient and control groups was 0.013 and 0.04, respectively, which is lower than that found in the European (0.14) and West African (0.11) populations.[16,17] The frequency of TNFA2 allele in our study is approximated to that found in the Japanese population.[18] Although the frequency of TNFA2 allele in the patient group is lower than that in the normal vaccinees, statistical analysis shows no significant difference in genotypes and allele frequencies of the TNF-α gene between the two groups (P=0.62)(Table 1).

Discussion

The knowledge of the genetic basis of susceptibility to disease is an expanding field. The gene for TNF-α lies in the center of the MHC locus on the short arm of human chromosome 6 between HLA-B and HLA-D loci. A polymorphism directly affecting the regulation of TNF-α is located at -308 nucleotides relative to the transcription start site of the gene. TNF-α is a critical mediator of host defense against infectious agents and the association of their allelic forms with susceptibility to severe malaria,[19] leishmaniasis,[20] scarring trachoma,[21] and lepromatous leprosy[22] has been studied. Several *in-vitro* studies have also confirmed the importance of TNF-α production by human monocytes in immunity to BCG.[21] Because of the evidence that different combinations of TNF-α alleles can affect cytokine secretion and the central role the TNF-α plays in immunity to BCG, it was considered important to investigate the relationship between TNF-α polymorphism and BCG adenitis in the present study.

Despite its association with other infectious and inflammatory diseases,[19-22] TNF-α polymorphism was not statistically related to the presence of BCG adenitis. However, the comparison of the TNF 308 A2 allele distribution between the patients and controls revealed that there was an increased frequency (3 times) of this allele in the control subjects. There has been no investigation on the genetic basis of BCG adenitis. We presume that the lower frequency of TNFA2 allele in patients might have resulted in a weaker immune response that allows bacterial burden and the occurrence of lymphadenopathy. In support of this hypothesis, several studies have shown that generalized BCG lymphadenopathy is a

suspected event in immunocompromised patients[24] and in a recent study, the cytomorphologic patterns of lymph nodes in more than 50% of patients were acid-fast bacilli in a necrotic background.[25] Therefore, the phenotype of BCG lymphadenitis may be more relevant to improper immune response. Perhaps a larger study would demonstrate statistically a significant difference of TNFA2 allele distribution between BCG-adenitis individuals and the controls. Another possibility is that TNFA2 may be a minor disease-association allele relative to alleles that lie in the same or other haplotypes. In this respect, the genetic polymorphism in the IL-10 can be mentioned.[26] IL-10 is an anti-inflammatory cytokine that is produced after the infection of human monocyte-derived macrophages by Mycobacterium bovis BCG.[2] This cytokine inhibits IL-12 and TNF-α production by macrophages.[27] IL-10 is also able to inhibit macrophage proliferation. Therefore, it is not surprising if the expression of lymphadenopathy and host ability in anti-bacterial immunity has a relationship to IL-10 gene polymorphism.

In conclusion, the study of polymorphism in the cytokine gene encoding TNF-α did not show a significant deviation in BCG lymphadenitis patients compared to the controls. Further studies are required to elucidate the genetic basis of lymphadenitis.

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Cupola of the Mosque of Shaykh Lutf Allah with delicate decoration of serried arabesques and blue floweres, around 1610 AD, Isfahan

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COMPARISON OF ACCURACY OF THREE ECHOCARDIOGRAPHY METHODS USED FOR DETECTION OF PULMONARY HYPERTENSION

B. Aminian, K. Jamali

Department of Internal Medicine, Cardiology Section, Shiraz University of Medical Sciences, Shiraz

ABSTRACT:

Background: Echocardiography is a valuable tool for detection of pulmonary hypertension (PHT) but little work has been done to compare the accuracy of this non-invasive method with invasive direct trans-catheter pulmonary artery pressure (PAP) measurement. The aim of this study is to compare the accuracy of different echocardiography methods in detection of PHT.

Material and Methods: In a prospective double blind study 49 patients (female: 33 and male: 16) with an age range of 14-68 years (39+12) and various cardiovascular disorders were selected. Tricuspid regurgitation (TR) gradient was measured by continuous wave (CW) doppler, pulmonary artery acceleration time (PAT) by pulse wave doppler, and PA a-dip evaluated by M-mode echocardiography. Data analysis was performed by SPSS software.

Results: Among 46 patients with measurable PAT, 22 had PAT < 100 ms, and 24 had values > 100 ms. In the group with PAT < 100 ms (n=22), 18 had trans-catheter measured mean pulmonary artery pressure (PAP) > 20 mmHg while it was < 20 mmHg in four patients. Among 24 patients with PAT > 100 msec, in 10 patients measured mean pulmonary artery pressure (MPAP) was < 20 mmHg, and in 14 patients, MPAP was > 20 mmHg. (P=0.024, sensitivity: 60%, specificity: 75%). With respect to TR gradient (n=28), in 21 individuals trans-catheter measured MPAP was > 20 mmHg; and in seven patients it was < 20 mmHg (P < 0.0002, sensitivity: 93%, specificity: 98%). In 30 patients with demonstrable PA a-dip, it was present in 20 and absent in 10 patients. In the PA a-dip present group (n=20), 10 patients had MPAP > 20 mmHg, and in the rest of the patients MPAP was < 20 mmHg. In the PA a-dip absent group (n=10), seven patients had MPAP > 20 mmHg, and 3 patients had MPAP < 20 mmHg (P: 0.297, sensitivity: 58%, specificity: 23%). A proposed formula for estimation of PAP through TR gradient was suggested.

Conclusion: This study shows that TR gradient is a non-invasive measurement for detection of PHT, with high grade of catheterization correlation. PAT is moderately specific, while M-mode PA a-dip measurement is neither sensitive nor specific.

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Key Words: • pulmonary hypertension • echocardiography • TR gradient • PA a-dip • pulmonary acceleration time

Introduction

Great technical developments have occurred recently in the field of echocardiography, including trans-esophageal echocardiography, trans-venous ultrasound, tri-dimensional echocardiography, and finally echo-based evaluation of myocardial perfusion.[1] Limited work has been done to compare the power and accuracy of echocardiography as a non-invasive tool for detection of PHT, relative to the gold standard method of invasive angiography.[2,3] In this study, we compared the accuracy of three simple echocardiography methods, used for

Correspondence: *B. Aminian M.D., Dept. of Internal Medicine, Cardiology Section, Shiraz University of Medical Sciences, Shiraz, Iran, Tel: +98711-6260693, E-mail: baminian@sums.ac.ir*

Table 1: Distribution of patients in each of the three echocardiography methods and their relation to trans-catheter pulmonary artery pressure measurement.

	PAT		TR Gradient	PA a-dip	
	>100 ms	<100 ms		Present	Absent
Mean PAP >20 mmHg	10	18	21	10	7
Mean PAP <20 mmHg	14	4	7	10	3
P value	<0.024*		<0.0002*	0.297	

PAT: Pulmonary acceleration time, TR: tricuspid regurgitation, Mean PAP: pulmonary artery pressure determined by catheterization. *: Statistically significant values

detection of PHT, in order to evaluate sensitivity and specificity of each method.

Material and Methods

Patient population:

In this double blind prospective study, 56 patients with various cardiovascular disorders who referred to Nemazee hospital catheterization laboratory for left and right heart catheterization were enrolled. No patient had right ventricular outflow tract (RVOT) obstruction, and all patients in PA a-dip group had normal right ventricular function and were in normal sinus rhythm. Seven patients were excluded because of poor acoustic transthoracic echocardiography windows, and among the remaining 49 patients (male:16, female:33) the age range was 14-68 years, with a mean of 29 years. All these patients underwent echocardiography evaluation the day before cardiac catheterization.

Underlying disorders:

RHD was present in 31 (63%) patients, (male:7, female:24) and ASD in six (12.2%) patients (male:4, female:2). Constrictive pericarditis was detected in 3 (6%) male patients, patent ductus arteiosus (PDA) in 2 (4%) female patients, and ventricular septal defect (VSD) in 3 (6%) patients (male:1, female:2). Four patients (8%), (male:1, female:3) had PHT of undetermined etiology.

Echocardiography evaluation:

All patients underwent transthoracic echocardiography examination by an experienced echocardiographer who was not aware of the results of catheterization data. Echocardiographies were performed on Hewlett-Packard Sonos 1000 echocardiography machine with patients in partial left lateral position. Pulmonary valve a-dip and pulmonary artery acceleration time (PAT) were recorded on the short axis view of the great vessels, by M-mode and pulse wave doppler echocardiography, respectively.[3-5] TR gradient was calculated by modified Bernoulli equation ($4v^2$), obtained by CW or pulse wave doppler guided apical four-chamber view.[1-4,5] Sampling volume in RVOT was obtained in the mid part of pulmonary artery exactly above the pulmonic valve with the angle of 0 (the angle between annulus of pulmonary artery and ultrasound beam) of about 90°.[6-8]

Angiography methods:

All patients underwent left and right heart cardiac catheterization on the day following echocardiography examination by those who were not aware of the results of echocardiography findings. End-expiratory

pressures in all cardiac chambers were recorded by a fluid filled catheter for at least 5-10 cardiac cycles. Mean PA pressure equal or more than 20 mmHg was regarded as pulmonary hypertension.[9]

Statistical analysis:

All data were analyzed through SPSS™ software with values <0.05 as statistically significant.

Results

Among 49 patients who finally enrolled in this study, PAT was measurable in 46. Twenty-two patients (48%) had PAT <100 ms, and in twenty-four individuals (52%) the measured PAT was >100 ms. Among 22 patients with PAT <100 ms, 18 patients (81.8%) had MPAP >20 mmHg by catheterization, whereas four patients in this group (18%) were found during catheterization to have MPAP >20 mmHg. Of the remaining 24 patients with PAT >100 ms, 10 patients (41.7%) had catheterization-based MPAP measurement >20 mmHg, while in 14 patients (58.3%) MPAP was <20 mmHg ($p<0.024$, sensitivity 60%, specificity 75%). Overall, among 46 patients who underwent PAT measurement, trans-catheter MPAP was >20 mmHg in 28 patients, and <20 mmHg in 18 individuals. In the group of patients with MPAP >20 mmHg, the measured PAT was between 50 and 125 ms (85 ± 15 ms), but in the population of patients with MPAP <20 mmHg, PAT was between 87 and 164 ms (135 ± 20 ms). (Table 1).

Pulmonic valve a-dip was detectable in 30 patients. In PA a-dip positive patients ($n=20$), ten had MPAP >20 mmHg, whereas in ten patients MPAP was <20 mmHg. In the remaining 10 patients with absent PA a-dip, seven had MPAP >20 mmHg and three patients had MPAP <20 mmHg ($p=0.297$, sensitivity 58%, specificity 23%) (Table 1). TR flow was obtainable with CW doppler examination in 28 patients. TR gradient was calculated by applying modified Bernoulli equation. In this study, TR gradient differred from MPAP by 7 ± 6 mmHg. Considering the observed mean right atrial pressure in our calculations, the difference was still lower (3 ± 5mmHg) ($p<0.0002$, sensitivity 93%, specificity 97%) (Table 1).

Discussion

Several echocardiography methods have been developed for detection of PHT. The three most frequently used techniques are PA a-dip, TR gradient, and PAT. It is claimed that TR gradient has the highest sensitivity and specificity for detection of PHT.[10-12] Two studies addressing this issue were published.[9,12] PAP was calculated from TR gradient by the following equation: PA systolic pressure (mmHg) = TR gradient + 10 mmHg.[9]

In this study, among the three different echocardiography methods, PAP calculated by TR gradient was the most accurate with the highest sensitivity and specificity, (93%, and 98% respectively). The higher sensitivity and specificity of TR gradient in our study, as compared to the others, are perhaps related to improvements in echo-doppler technology. By applying TR gradient, we suggest the following formula for measurement of PA systolic pressure :

PA systolic pressure (mmHg) $=0.7\times$TR gradient $+20.8$.

This formula accurately detects cases of PHT with mild to moderate severity but underestimates those with severe degrees of pulmonary hypertension.

There is an inverse correlation between PAT and the severity of PA systolic pressure.[4-6,13] Limited work has been done in regard to sensitivity and specificity of this

echo-doppler method for detection of PHT. Although the sensitivity and specificity of PAT for detection of PHT in our study was 60% and 75%, in a substantial number of cases with mean PA systolic pressure >20 mmHg, the mean values of PAT was <100 ms as compared to those with MPAP <20 mmHg (18 *vs.* 4) (Table 1). This study shows that, although compared to TR gradient, PAT has lower sensitivity and specificity profiles, cases with PAT markedly >100 ms have normal PAP, and in those with PAT markedly <100 ms, the probability of having PHT would be high. Older reports suggest the following formula for calculation of PAP:

Mean PAP (mmHg) = 80- PAT/2.[3,5,9] According to our study, this formula is not applicable in all instances, because PATs of 100-120 ms give rise to PAP >20 mmHg that is not acceptable.

PA a-dip is related to diastolic transmission of right atrial pressure to the closed pulmonic valve.[9] In cases of pulmonary hypertension, increased PAP prevents late diastolic notching (a-dip) of closed pulmonic valve. There are a few reports addressing the sensitivity and specificity of absent PA a-dip in cases with pulmonary hypertension.[14] In our study, the sensitivity and specificity of absent PA a-dip in detection of PHT were 23% and 58%, respectively.

Conclusion

Pulmonic valve a-dip is neither sensitive nor specific, for detection of pulmonary hypertension. This study shows that in patients with pulmonary HTN, who have demonstrable tricuspid regurgitation by echo-doppler, TR gradient is a non-invasive method for calculation of systolic PAP and in many instances obviates the need for PAP measurement by cardiac catheterization. Although, as compared to TR gradient, PAT has lower sensitivity and specificity, in cases with acceleration time markedly less or more than 100 ms, PHT could be detected or excluded.

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PRIMARY ATRIAL SEPTAL ANEURYSM AND ITS ASSOCIATED CARDIAC ABNORMALITIES: A TRANSESOPHAGEAL ECHOCARDIOGRAPHIC STUDY

M. Zamirian, G.R. Rezaian, M.B. Sharif Kazemi

Department of Internal Medicine, Cardiology Section, Shiraz University of Medical Sciences, Shiraz

ABSTRACT

Background: Primary atrial septal aneurysm (ASA) is a congenital malformation of the atrial septum which is characterized by its bulging into either atrium. Transesophageal echocardiography (TEE) has recently made it possible to detect ASA more easily and has clarified some of the previously obscure points regarding this infrequent entity.
Objective: Our aim is to report the prevalence of ASA among 270 patients undergoing TEE in this center and to assess their clinical findings and associated cardiac disorders.
Design: Cross sectional study.
Setting: University Hospital
Methods: Over a 2-year period, 270 cases had TEE, of whom, 16 cases were diagnosed as having primary ASA. A protrusion of atrial septum ≥10 mm beyond the plain of atrial septum and a base diameter of ≥ 15 mm were considered diagnostic for ASA.
Results: There were 8 female and 8 male patients with an age range of 19-67 years. The maximal protrusion of ASA ranged from 11-22 mm and the maximal base diameter ranged from 17-25 mm. Oscillating ASAs were present in only 3 cases and in the remaining it was bulging only toward the right atrium. Systolic click was detected in 10 cases, of whom 2 had double clicks. Two patients had documented embolic stroke by history and physical examination and brain CT scan. The most common transesophageal echocardiographic findings included ASD (26%), PFO (26%) and MVP (20%). Of interest was the presence of ostium primum defect in one of our ASD cases. Mild mitral regurgitation and AR were each detected in one case too. Three cases (20%) had no associated cardiac lesions.
Conclusion: Primary ASAs are rare, however once present, they are frequently associated with ASD and PFO. In addition they may not only simulate MVP due to their ausculatatory findings but may actually be associated with it. They should be considered as a potential source for embolic stroke as well.
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Key Words: Atrial septal aneurysm • transesophageal echocardiography

Introduction

Primary atrial septal aneurysm (ASA) is a congenital malformation of the atrial septum which is characterized by its bulging into either atrium.[1] The recent widespread use of transesophageal echocardiography (TEE) has clarified some of the previously obscure points regarding this rare entity.[2-4] The clinical interest in ASA lies in its association with other cardiac pathologies[5] as well as arrhythmias[4,6-8] and embolic complications including the cerebral arteries.[9-11] In this study we report the associated cardiovascular findings in 16 consecutive patients with primary ASA detected by TEE and color flow mapping.

Correspondence: M. Zamirian M.D., Nemazee Hospital, **P.O. Box:** 71935-1334, **Fax:** +98-711- 6279733, Shiraz, Iran

Patients & Methods

Over a two-year period, 270 patients were referred to our echocardiography unit for a combined transthoracic and transesophageal echocardiographic evaluation. Sixteen cases, diagnosed as having primary ASA, underwent a subsequent thorough physical examination,

chest x-ray and a routine 12-lead electrocardiogram.

The echocardiographic studies were performed with a Hewlett-Packard Sonos 1000 ultrasonograph (Hewlett-Packard, Mass), using a 2.5 Mhz transducer for transthoracic and a 5-Mhz monoplane probe for transesophageal studies. All of the echocardiographic examinations were recorded on a VHS videotape for subsequent evaluation by at least 2 independent cardiologists.

Complete echocardiographic and Doppler studies were done to exclude the presence of secondary causes of ASA such as mitral stenosis or cor pulmonale.

In addition, all cases had a concomitant complete color-flow study for the detection of intracardiac shunts (Fig. 1) and valvular leaks. The guidenlines of the American Society of Echocardiography[12] were used for conventional measurements.

A protrusion of atrial septum of ≥10 mm beyond the plane of atrial septum[6] and a base diameter of ≥15mm were considered diagnostic for ASA (Fig. 2). Dynamic protrusion of the interatrial septum into the either atrium was assessed by the re-evaluation of the videotape of each individual case. The diagnosis of patent foramen ovale (PFO) was based on the criteria described by Schneider et al[13].

Results

Of 16 cases, there were 8 females and 8 males with an age range of 19-67 years.

The maximal protrusion of ASA ranged from 11-22 mm and the maximal base diameter ranged from 17 to 25 mm. Oscillating ASA was present in 3 cases and in the remaining 13 cases a fixed aneurysm was noted to protrude toward the right atrium.

Except for two patients who had documented ischemic stroke, proven by history, physical findings and the brain computed tomographic scan, no other embolic events were detected.

The associated cardiac lesions are shown in Table 1. Of interest was the presence of an ostium primum ASD and double clicks in further two patients. One patient with arrhythmia had atrial fibrillation but none of the remaining patients had cardiac rhythm irregularity either on the physical examination and/or routine 12-lead ECGs.

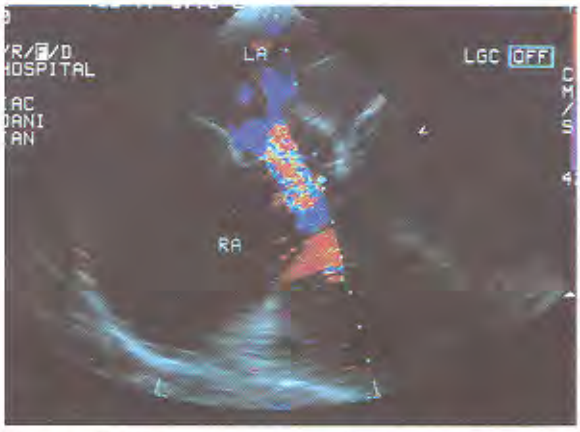
Discussion

The exact prevalence of ASA remains unknown and varies with the population under the study and the diagnostic method used.[2] Of 270 consecutive cases who underwent TEE in our series, 16 (5.9%) had ASA. This figure is similar to 4.9% reported by Burger et al[2] in their 846 consecutive cases who underwent intraoperative TEE. However, all their patients, as most of ours, had an underlying cardiovascular disease. Thus no generalization can be made and the figure obtained cannot be applied to a normal population.

The maximal excursion of the atrial septum in our patients was >11 mm in all cases. However, there is no single-dimensional criterion to make the diagnosis of ASA. Gallet et al,[14] and Longhini et al[15] required a maximal excursion of 6 and 8 mm respectively, while Harely et al[16] proposed a maximal protrusion of 15 mm or greater for the echocardiographic diagnosis of ASA.

Although a higher frequency of ASA has been reported for females, [17] in one study this has not been confirmed by others.[19] In this study equal sex distribution was found.

Embolic complications have been variably described to occur in 20% to 50% of these patients.[16-18] However, in their long term prospective study, Burger et al,[2] failed to find any cerebrovascular or other systemic embolic events. Documented ischemic stroke was noted in only two of our patients. The possible mechanisms for the developments of



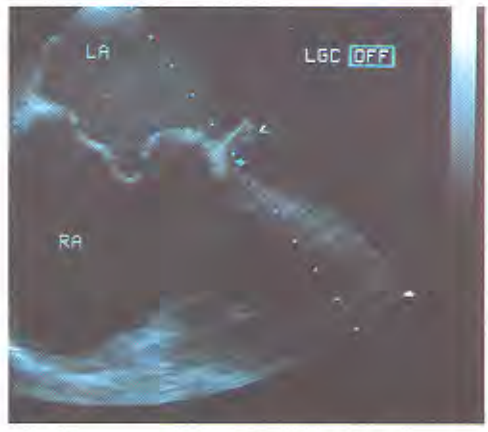


Fig. 1: (left) Transesophageal echocardiogram with color-flow mapping, showing the presence of ASA and its associated ASD.
Fig. 2: (right) Transesophageal echocardiogram of a patient showing protrusion of ASA into the right atrium

CVA may include formation of thrombus in the aneurysm due to stasis, the presence of mitral valve prolapse, supraventricular dysrhythmias and finally paradoxical embolization through a PFO or an ASD.[2] Our patients with ischemic stroke had MVP in addition to ASA but had no arrhythmias, PFO or ASD.

The presence of PFO and ASD have been well documented in patients with ASA.[1,16] Severe tissue attenuation may be the underlying reason for the detection of such anomalies in association with ASA.[19]

The presence of multiple interatrial septal defect, is relatively common, especially in patients with aneurysms of the entrie septum. Only one of our ASD patients had multiple fenestrations, as detected by color-Doppler study. Such lesions are best detected by the use of multiplane probes and contrast echocardiography.

Interestingly, 2 patients had double clicks and the other 5 patients had ASD, of whom one had ostium primum defect. These combinations are, to the best of our knowledge, the first ones reported in the literature.

Atrial septal aneurysms may not only simulate MVP because of the presence of click on cardiac auscultation,[16,20] but they may in fact be associated with it.[1,16,19] Ten out of our 16 cases had systolic click. However, MVP was diagnosed in only 4 of them (Table 1).

Large ASDs may also be associated with

Table 1: The auscultatory and transesophageal echocardiographic findings in 16 patients with primary atrial setpal aneurysm.

	Systolic click	ASD*	PFO**	MVP+	MR§§	AR@
Females n = 8	6	2	2	2	1	-
Males n = 8	4	3	2	2	-	1
Total n = 16	10	5	4	4	1	1

ASD*: Atrial septal defect, PFO**: Patent foramen ovale, MVP+: Mitral valve prolapse, MR§§: Mitral regurgitation, AR@: Aortic regurgitation

MVP. In fact, 2 of our MVP patients had ASD as well.

Barbosa et al[5] have reported a high prevalence of electrocardiographic abnormalities and supraventricular arrhythmias in their 14 cases with ASA. However, this was not the case in our patients. Despite the presence of axis deviation and bundle branch block in our ASD cases, the only observed arrhythmia was atrial fibrilation in only one patient. Holter monitoring is needed to elucidate this issue in these cases.

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CLINICAL TRIAL OF SULFASALAZINE FOR TREATMENT OF UVEITIS IN BEHÇET'S DISEASE

M. Mehryar,[*] S. Samangooee,[**] H. Jahadi Hosseini,[*] N. Kavian,[*] M. Mehdizadeh,[*] S. Safamanesh,[*]

[*]Departments of ophthalmology and [**] Rheumatology, Shiraz University of Medical Sciences, Shiraz

ABSTRACT

Background: Behçet uveitis is a blinding disease with no satandard treatment. New medications with less side effects are being evaluated for this disease.

Objective: This study was done to evaluate the effect of sulfasalazine on uveitis in Behçet's disease.

Method: This study was conducted in Behçet Research Clinic of Shiraz, Nemazi Hospital. A clinical trial was carried out for 15 months to observe the efficacy of two therapeutic regimen. *regimen* I: sulfasalazine + low dose prednisolone (PO) and *regimen II*: cyclophosphamide + regular dose of Prednisolone (PO) in the treatment of uveitis in Behçet's disease. 13 patients received regimen I and 22 patients received regimen II. After at least 3 months, 11 patients in the second group who had no response to regimen II were switched to regimen I and reevaluated for response to therapy. These patients were followed for at least 12 months after starting the trial and were followed every 3 weeks by a vitreoretinal specialist and a rheumatologist.

The eye examination included visual acuity (V/A), slit lamp examination for reaction, indirect ophthalmoscopy, IOP and when needed fleurecin angiography (F/A) for suspected posterior segment lesions. Uveitis was classified according to the Uveitis Scoring System.

Results: Of 13 patients who were initially on regimen I, group A (n=13) 85% (11patient) had good to moderate response during 2-8 weeks. Only 50% of the patients who were on regimen II group B (n=22) had good to moderate response. Therefore, the remaining 50% were selected as group C (n=11) and were put on regimen I. All of this group had favourable response to medication.

Conclusion: Sulfasalazine 1.5-3 g/day with low dose prednisolone (PO) (0.5 mg/Kg) could be a good substitute for the treatmem of uveitis in Behçet's disease

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Key Words: • Behçet's disease • uveitis • sulfasalazine

Introduction

Behçet's disease (BD) is a chronic disease of unknown etiology. Classified under occlusive vasculitides, it affects multiple organs with exacerbations and remissions of unpredictable duration and frequency. This disease is a common cause of uveitis in Mediterranean countries, Middle East and Japan[1] and a leading cause of blindness in these areas; blindness occurring commonly within 3-5 year.[2,3,4]

Behçet uveitis is usually treated using immunosuppressive agent including alkylating agents and agents that inhibit interleukin-2 production, such as cyclosporine and its analogoe, FK506. However, such medication produces severe side effects including CNS symptoms.[5,6]

In the present study, we investigated the efficacy of sulfasalazine in the treatment of uveitis in patients who suffer from persistent or frequent attacks of anterior and/or posterior

Correspondence: *M. Mehryar M.D., Dept. of Ophtalmology, Khalili Hospital, Shiraz University of Medical Sciences, E-mail: mehryarm@sums.ac.ir, Shiraz, Iran.*

Table 1: Distribution of patients in each group

Numbers of Patients in each group	Good response	Moderate response	No. response	Response to therapy
Group A = 13	8(62%)	3(23%)	2(15%)	85%
Group B = 22	11(50%)		11(50%)	50%
Group C = 11	8(73%)	3(27%)	0(0%)	100%

uveitis (pan-uveitis) to control uveitis with low side effects.

Materials and Methods

This study comprises 35 patients with BD and uveitis according to the international study group for Behçet disease and international uveitis study group. All of our patients had examinations of visual acuity and fundoscopy. The anterior chamber and in the vitreous cavity were assessed. Flourecein angiography was performed when lesions in the retina or on vessels were seen. Uveitis was graded according to a scoring system by international uveitis study group.[7,8]

Thirty-five patients with Behçet uveitis reffering to Behçet research clinic at Nemazi Hospital were classified into two groups. 13 patients with persistent or recurrent uveitis for 3 months or more, receiving only PO with topical steroids, were selected as group A. These patients were put on regimen 1, consisting of sulfasalazine 1.5-3g/day plus prednisolon (PO) 0.5mg/kg/day. They were visited at 3 week intervals and followed for 12 months.

The response was recorded as good when uveitis disappeared completely, moderate when the uveitis was diminished and no response when no change or worsening of the uveitis was seen.

Group B comprised 22 cases with panuveitis and retinal involvement who were under treatment with PO prednisolone(1-2 mg/kg) + cyclophosphamide (1-2 mg/kg) (regimen II).

Group C consisted of 11 patients of a subgroup of group B who failed to show good response after at least 3 months of treatment. So, they were put on regimen I.

All of our patients had the same follow up schedule. If patients had good response in group A after 3 weeks, prednisolon (PO) could be reduced to 5-10 mg/day and the starting dose of sulfasalazine was continued (1.5 g/day). If moderate response was seen, then sulfasalazine was increased to 2-3 g/day without changing the initial dose of PO steroid. (0.5mg/kg)

Paraclinical work-ups such as CBC, ESR, LFT, G6PD & U/A were done before & during treatment. HLA B_5, HLA B_{51}, and HLA B_{27} were checked in all the patients. Folic acid (1mg/day) was given to prevent sulfasalazine-induced folic acid deficiency.

Results

The study included 35 patients with an age range of 21 to 65 year. There were 29 males and 6 females; 25 patients had bilateral and 10 patients had unilateral involvement. The success rate of our study is shown in Table 1.

The result of regimen I was good in 62% (n=8) of group A, and 72% (n=8) of group C. Moderate response was seen in 23% (n=3) of group A and 27% (n=3) of group C.

No response was seen in 15% (n=2) of group A and 0% (n=0) of group C.

Recovery rates which were measured as good and moderate response were 85% (n=11) in group A, 50% (n=11) in group B and 100% (n=11) in group C. The average

time to respond to regimen I was 4 weeks (range: 2-8 weeks) in the majority of patients

Discussion

BD is an autoimmune disease both on clinical and experimental grounds. Pathologically, neutrophils in BD is known to have enhanced chemotaxis, phagocytosis, active oxygen production, lysosomal secretion and production of large amount of leukoter in B4, which finally result in tissue injury. In patients with BD, despite its obscure cause, the presence of a migration inhibition factor in the anterior chamber of the eye suggests that cell-mediated immunity might play a significant role. This view is corroborated by previous observation of high levels of interleukin 1 (IL-1) and IL-2 in the aqueous humor and vitreous aspirate of three patients during the active phase of disease.[9,10,11]

The treatment of BD depends on the clinical course of disease and the activity and severity of these manifestations at the time of presentation. Since the etiology of BD is unknown, treatments used are mainly symptomatic and individualized, with no drug or therapeutic regimen addressed capable of relieving all the symptoms. The clinical course of BD is characterized by exacerbation and remission of unpredictable duration and frequency and this natural history makes the evaluation of therapy difficult. Often the treatment of uveitis is unsatisfactory. Although steroids suppress many inflammatory features of the various lesions, recurrence, progression and even appearance of new lesions during systemic steriods therapy are frequent. Corticosteroids not only do not prevent recurrence of ocular disorders, but the long term administration may also lead to decreased vision due to cataract and glaucoma formation. The unsatisfactory results, obtained from steroids alone, led to the use of this medication in combination with immunosuppressive therapy such as azothioprine, cholorambucil, cyclophosphamide, cyclosporin, FK506 and recently interferon-α. Sufficient work has not been done concerning the method and duration of administration and mechanism of the onset of the effect of sulfasalazine.[12-18] In BD, cytotoxic chemotherapy is usually reserved for severe forms of the ocular, central nervous system and vascular involvement. The choice of immunosupressive therapy in BD lies between alkylating agents and agents that inhibit IL-2 production (FK 506).

Cyclosporine A has been reported to be highly effective in the treatment of BD particularly in severe uveitis resistant to cytotoxic therapy.[19] However, reactiviation of ocular attacks after cessation of medication was the main disadvantage and serious side effects such as nephrotoxicity and neurotoxicity limited its long term use. In a study on 12 patients, one patient has developed nephrotoxicity, all the six females developed hirsutism and 8 patients had developed neuropathy.[17]

Colchicine also has been used in the treatment of BD but a double blind study showed that is more effective in oral and genital ulcer and ineffective in the treatment of ocular disease.[20]

Tabara KF has questioned the use of chlorambucil because of the associated effect on spermatogenesis.

In 9 out of 22 patients who had received azathioprine, other medications such as cyclosporine A and cyclophosphomide had to be added. Despite this, three patients developed complete blindness.[4,21]

In this study, sulfasalazine (1.5-3 g/day) with low dose of prednisolone (0.5 mg/kg/day) showed less side effects compared to the above mentioned regimens and revealed 100% effectiveness in controlling uveitis in a group of patients non-responsive to initial cyclophosphamide therapy (group C).

Sulfasalazine and its metabolite (5-ASA) have proved to affect certain aspects of cellular

immune functions including the cell-mediated cytotoxicity. Although the significance of this is not clear, in recent studies, however, it has been shown that 5- ASA abrogates T-cell proliferation by blocking interleukin-2 production in peripheral blood mononuclear cells and inhibition of IL-2 production as an additional mechanism of action for 5-ASA. It is suggested that sulfasalazine may act on gut-associated lymphoid tissues. Sulfasalazine and its metabolites 5-ASA can inhibit the production of leukotriene B4 and other leukotrienes (A, C, D, F) by blocking the lipooxygenase pathway of arachidonic acid metabolism in human leukocytes.[22]

Sulfasalazine has been proved to be effective in the management of mild attacks of ulcerative colitis and in the reduction of the frequency of relapses of ulcerative colitis as well as the Crohn's colitis.[22] Sulfasalazine is also effective on rheumatoid arthritis and in ankylosing spondylitis. It is also used in BD with arthritis and gasterointestinal manifestation.[22] In this study, we noted satisfactory effect of sulfasalazine in the treatment of uveitis in BD.

Conclusion

Our results showed that sulfasalazine (1.5-3 g/day) along with low dose prednisolon (0.5 mg/day) might be a good substitute for treatment in BD patients who suffer from recurrent anterior and posterior uveitis, before considering the patient for chemotherapy. Sulfasalazine may also be a remissions-inducing agent in patients with pan- uveitis who have persistent active uveitis despite other modalities of therapy.

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EFFECTS OF PARENTERAL- ENTERAL VERSUS ENTERAL NUTRITION ON NEUROLOGICAL STATUS AND OUTCOME OF HEAD-INJURED PATIENTS

A. Kamgarpour, E. Sherafat Kazemzadeh,* Sh. Ravanshad***

**Departments of Neurosurgery and **Nutrition, Shiraz University of Medical Sciences, Shiraz*

ABSTRACT

Background: Head injury is a major worldwide health problem and may cause metabolic disruption toward increased energy expenditure and protein catabolism. In head-injured patients, inadequate nutritional support can cause impaired healing, increased tendency to infections, multiple organ failure and poor recovery and outcome.

Objective: To compare the effects of parenteral-enteral nutrition versus enteral support alone on complications, recovery and final outcome.

Methods: A prospective quasi-experimental, randomized, non-blinded study was performed on 66 purely head-injured patients (55 males and 11 females, aged 18-72 years) with 24-hour-admission peak Glasgow Coma Scale (GCS) scores of 4 to 10. Patients were randomly divided into two groups: Group 1 received total parenteral nutrition, gradually changing to enteral nutrition, and Group 2, received enteral nutrition alone. They were studied for 14 days in hospital and followed after 3 and 6 months post-injury by Glasgow Outcome Score (GOS).

Results: GCSs on the 14th day and GOSs three months post-injury were significantly higher in Group 1 than Group 2 ($p=0.023$, $p=0.039$, respectively), but GOSs after 6 months were not significantly different. Coma duration and mortality rate revealed no statistically significant difference between the two groups.

While a statistically significant difference was found in complication rate and the total WBC counts between the two groups, the lymphocyte count was significantly higher in Group 1 ($p<0.001$). Anthropometric indices were not statistically significantly different, except for the percentage of body weight loss and the difference between the weights of the 1st and the 14th days, which were significantly higher ($P<0.001$). Group 1 received a higher calorie intake (36.07± 3.55 cal/kg/day vs. 30.86 ± 5.42 cal/kg/day in Group 2, $p<0.001$), and had a higher cumulative calorie balance (83.38 ± 6.36% vs. 67.49 ± 9.54%, $p<0.001$). Mean protein intake in Group 1 was 1.35 ± 0.15 gm/kg/day, as compared to 0.98 ± 0.18 gm/kg/day in Group 2 ($p<0.001$).

Conclusion: Head-injured patients can receive more calories and proteins via the parenteral-enteral route. Recovery occurs more rapidly in these patients with better early nutritional support, but long-term follow-up reveals no statistically significant difference in the effectiveness of each nutritional regimen on the final outcome.

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Key Words: • Head injury • enteral nutrition • nutritional assessment • outcome • parenteral nutrition.

Introduction

Recent advances in the management of head-injured patients especially nutritional therapy, have improved the outcome of these patients. It has become an important adjunctive medical therapy for patients suffering from head injury. These patients are hypermetabolic and hypercatabolic, similar to patients with burns and major trauma.[1-5] Energy requirements rise to 135%-200% above the normal, and nitrogen excretion is significantly increased for up to 4 weeks.[2,6]

Considering these requirements, the holistic care of a patient with head injury is a challenging subject and may extend for many

Correspondence: *A. Kamgarpour M.D., Department of Neurosurgery, Shiraz University of Medical Sciences, Shiraz, Iran*

weeks, until the patient overcomes central and systemic insults. The strategy of head injury management should be to minimize avoidable mortality and morbidity not only by reducing secondary brain damage, but also by adequate calorie and nutrient replacements in order to secure an optimal environment for neuronal sprouting and regeneration. All of these factors will undoubtedly improve the final outcome.[7]

Three feeding methods may be used in head-injured patients: total parenteral nutrition (TPN), enteral nutrition (EN) and the combined type. Each method has its advantages and disadvantages. For example, TPN may be started early when the patients can not tolerate enteral feeding due to gastric atony or increased intracranial pressure, but EN has less infectious morbidity and can maintain gut mucosal integrity to the better advantage relative to TPN. EN decreases bacterial translocation and is less expensive.[8] Thus, with the combined method, disadvantages of each method may be avoided. This study was undertaken to compare the effects of combined parenteral-enteral nutrition versus enteral support alone on complications, recovery of neurological status as well as final outcome of head-injured patients.

Materials and Methods

A total of 71 purely head-injured patients with 24-hour admission peak Glasgow Coma Scale (GCS) scores[5] of 4 to 10 were randomly divided into two groups to receive parenteral-enteral nutrition (Group 1) or enteral support alone (Group 2). They were studied over an 18-month period from March 1998 to September 1999, following their admission to the Neurosurgery Departments of Shiraz University of Medical Sciences Hospitals (Nemazi and Beheshti Hospitals). Every patient was studied for 14 days whilst hospitalized and reevaluated 3 and 6 months post-injury.

Patients with spinal cord injuries, pre-existing metabolic disorders such as diabetes mellitus, thyroid dysfunction and chronic renal failure, severe orthopedic problems, trauma to the abdomen, were excluded.

Craniotomy was performed to remove intracranial clots, debride the wounds and elevate depressed skull fractures within the first 48 hours after injury in 11 cases of each group.

A urinary catheter was routinely inserted for comatose patients. All patients had nasogastric or orogastric tubes except one who was conscious and orally cooperative. None was treated with corticosteroids, muscle relaxants, or exogenous albumin. Phenytoin was used for prevention of convulsion in all patients. Patients requiring prolonged intubation underwent tracheostomy, performed by the 10th day of hospitalization. Demographic data including age and sex, as well as data on the type of injury (based on CT scan findings), route of nutritional support, GCSs, coma duration and type of operation were recorded. Total WBC and lymphocyte counts were studied. Protein and calorie intake were calculated and analyzed.

The Harris-Benedict equation[9] was used to calculate the basal energy expenditure, and required calories were determined by the Clifton equation.[10]

All discharged patients were subsequently visited 3 and 6 months later and evaluated by the Glasgow Outcome Score (GOS).[5] Parenteral feeding was started 48 hours after trauma in Group 1, and contained sterile aminoacids, dextrose solution (10%), intravenous lipid emulsion, trace elements and vitamins. This feeding formula contained 17% protein, 41% fat, and 42% carbohydrate. After 4 days, the patients were weaned to enteral feeding as soon as bowel sounds were present and gastric residual volumes were less than 100 cc/ 2 hrs.

Each 2000 ml of the standard meal consisted of 100 gm potatoes, 500 gm plain

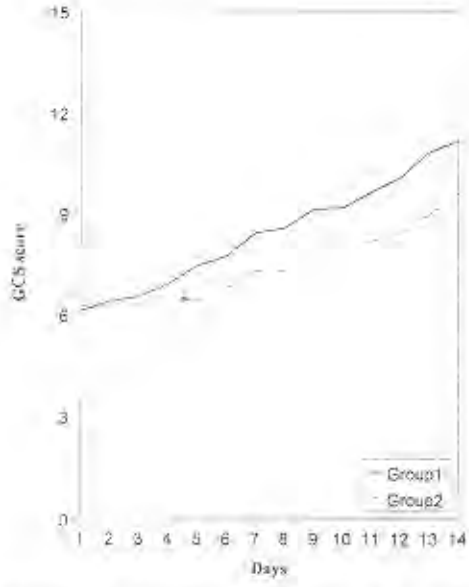

Figure1: Comparison of daily GCS score in two groups during 14 day study.

yogurt, 60 gm low-fat lean meat, 75 gm soya beans, 160 gm sugar, 40 gm sunflower oil, and 60 gm rice flour. It contained 1 Kcal/ml energy, and its osmolality was 443 mOsm/ml.

An enteral nutritional interval was assigned every 4 hours, consisting of an initial volume of 100 ml, and if tolerated by the patient, increased up to a maximal volume of 500 ml/meal every 4 hours. Feedings were held if gastric residues were higher than 150 ml; residues were then rechecked hourly before feeding was resumed.

Parenteral nutrition was tapered as gastric feedings progressed. The mean wean-off period of parenteral nutrition was 7.7 days (7-9 days). In Group 2, 72 hours post- injury, feeding via nasogastric or orogastric tubes was started with the same combination and program as in Group 1 and continued as such. During 5-7 days (mean 5.8 days), the patients received maximum calorie intake delivered by this route. In this regimen, protein, fat and carbohydrate provided 14%, 32% and 54% of daily caloric intake, respectively. In both groups, feeding was discontinued 8 hours before and 4 hours after tracheostomy.

Serum potassium and sodium levels were determined daily by flame photometer (Corning-480, 1995). Total WBC and lymphocyte counts were evaluated weekly by the H1 system (Technicon, 1986) which is based on flow cytometry. All mentioned blood profiles were checked in the morning, between 7:00 a.m. and 9:00 a.m.

The anthropometric indices, including weight, triceps skin fold and midarm circumference, were measured on days 1, 7 and 14 post injury. Height was measured according to Harris-Benedict equation.

Pulmonary as well as urinary complilcations were carefully monitored. All complications were recorded and managed accordingly with the cooperation of other specialists.

A SPSS/PC+ software was used for statistical analysis. Continuous data measured repeatedly over time were calculated by analysis of variance, appropriate for measured data. Demographic responses were evaluated by a two-sample t-test and a chi square test. Categorical responses were tested using the Fisher's exact test and Mann-Whitney U-test.

Results

Five of the original 71 consecutive patients were excluded from the study due to their absence at the 3rd and 6th month post-injury follow-up, leaving 66 patients for statistical analysis: 34 cases in Group 1 and 32 cases in Group 2. There were 55 males and 11 females with an age range of 18 to 71 years.

24 hour admission peak GCSs (Table 1), mean age (34.88 ± 13.63 years in Group 1, 34.94 ± 14.68 years in Group 2), male to female ratio (6.34 (17.65%) in Group 1, 5.32 (15.63%) in Group 2), and height (167.15 ± 6.54 cm in Group 1, 166.84 ± 5.84 cm in Group 2) had no statistically significant differences.

Table1: Neurological status of groups

	Group 1	Group 2	p-Value
24 hour admission peak GCSs	6.09 ± 1.40	6.41 ± 1.39	0.366
GCSs of the 7th day	8.09 ± 2.25	7.30 ± 1.78	0.107
GCSs of the 14th day	10.44 ± 2.80	9.59 ± 1.95	0.023*
GOS in the 3rd month	4.12 ± 1.15	3.53 ± 1.29	0.039*
GOS in the 6th month	4.35 ± 1.18	3.97 ± 1.40	0.303

*$p < 0.05$ statistically significant.

Brain CT scan findings of patients are listed in Table 2. It reveals that 58.82% of Group 1 and 65.62% of Group 2 had no finding or minimal contusion. Craniotomy was performed on 11 patients in Group 1 (32.35%) and 11 patients in Group 2 (34.34%), (p=0.534).

Although the 24-hour admission peak and the 7th day GCSs were not significantly different between the two groups, the changes over time were statistically significant, and the 14th day GCSs were significantly higher in Group 1 (Table 1, Fig. 1). Coma duration had no significant difference between both groups (p=0.126, Fig. 2), and mortality rates were similar (8.32% in Group 1, 9.37% in Group 2, p=1.00).

GOS, as an index of neurological recovery was significantly higher at the 3rd month follow-up in Group 1 than in Group 2 (p=0.039), but after six months, no statistically significant differences were observed between them. (P=0.303). (Table1)

The total WBC count was not statistically significantly different between the two groups (p=0.081). The lymphocyte count was statistically higher in Group 2 (p<0.001) (Fig. 3). These counts were evaluated as immunity indices.

Complications are listed in Table 3. It shows that both groups had no statistically significant difference regarding the infection rate (23.53% in Group 1, 31.25% in Group 2) and total morbidity rate (52.94% in Group 1, 56.25% in Group 2).

The changes over time in triceps skin fold and mid-arm circumference for both groups were not also significantly different (Table 4).

The percentage of weight loss during the 14-day hospitalization and the difference in the

Table2: Brain CT scan findings of groups.

CT scan findings	Group 1		Group 2	
	NO.	%	NO.	%
Subdural hematoma	4	11.76	2	6.25
Epidural hematoma	2	5.88	4	12.5
Intracerebral hematoma	2	5.88	1	3.12
Subdural hematoma with intracerebral hematoma	1	2.94	-	-
Intraventricular hemorrhage	2	5.88	2	6.25
Depressed skull fracture	2	5.88	2	6.25
Basilar skull fracture	1	2.94	-	-
No finding, or minimal contusion	20	58.82	21	65.62
Total	34	100	32	100

Table 3: Complications of groups during study.

Group	Group 1		Group 2		p-value
Complication	No	%	No	%	
Meningitis	3	8.82	2	6.25	
Pneumonia	2	5.88	4	12.5	
Urinary tract infection	–	–	1	3.12	
Wound infection	1	2.94	1	3.12	
Intravascular device infection	2	5.88	–	–	
Septicemia	–	–	1	3.12	
Diarrhea	–	–	1	3.12	
Total no. of infections	8	23.53	10	31.25	0.482
Gastrointestinal bleeding	2	5.88	1	3.12	
Electrolyte imbalance	4	11.76	3	9.37	
Hyponatremia					
Hypernatremia	1	2.94	2	6.25	
Hypokalemia					
	3	8.82	2	6.25	
Total	18	52.94	18	56.25	0.982

weights of the 1st and the 14th days were statistically significantly higher in Group 2 ($p<0.001$) (Table 4).

The mean 14 day caloric and protein intake was statistically significantly higher in Group 1 ($p<0.001$, $p<0.001$, respectively). It was observed that the mean percentage of calorie requirements which were replaced by the patients during this period was statistically significantly higher in Group 1. ($p<0.001$).

Discussion

Head injury can cause hypermetabolism, which may persist for weeks or even up to one year. It appears that provision for adequate energy and nutrients as well as good nutritional supports decreases the morbidity and can improve the outcome of head-injured patients. In this study, GCSs and GOSs, as indices of neurological recovery and final outcome, showed higher levels on the 14th day and in the 3rd month post injury, respectively, but GOSs of the two groups in the 6th month revealed no significant difference. In spite of this finding, Group 1 received more calories and proteins, and more required calories were replaced in this group (83.38% ± 6.86% versus 67.49% ± 9.45% in Group 2). It is therefore concluded that better early nutritional support can accelerate recovery. In 1987, Young et al. obtained similar results;[12] in their study, the percentages of the replacement for required calories were 75.6% ± 5.13% versus 59% ± 4.26% in parenteral-enteral and enteral groups, respectively. The mean intake of protein in our study was 1.35 ± 0.15 gm/kg/day, versus 0.98 ± 0.18 gm/kg/day in Groups 1 and 2, respectively, while in Young's study, these were 1.35 ± 0.12 gm/kg/day versus 0.91 ± 0.9 gm/kg/day in parenteral-enteral and enteral groups, respectively.[12] Their

Table 4:- Anthropometric measurements of two groups.

	Group 1	Group 2	p.value
% weight loss during 14 days	5.83 ± 1.18	7.74 ± 2.49	<0.001*
Difference of weights (kg) of the 1st and the 14th days	3.85 ± 0.82	4.93 ± 1.79	< 0.001*
Mean changes of triceps skin fold (mm)	-1.11 ± 0.42	-1.05 ±0.39	0.561
Mean changes of midarm circumference (cm)	-1.26 ± 0.38	1.29 ±0.47	0.753
Height (cm)	167.15 ±6.54	166.84 ±5.84	0.843

$p < 0.05$ statistically significant

conclusion was similar to ours: better early nutrition represents a more rapid recovery.[12]

Complications were not significantly different between the two groups: 23.53% of Group 1 and 31.25% of Group 2 incurred documented infectious morbidities. Lymphocyte count, as an index of immunity competence, was higher in Group 1 ($p < 0.001$). This finding, with relatively lower incidence of infections in Group 1, showed relatively better immunity with better nutrition. Other factors, however, such as acute phase reactants influencing immunity, should also be considered. Young et al. and Borzotta et al. reported no significant difference in the susceptibility of the groups to infections,[6,12] but in Young's study, no significant difference was found in lymphocyte counts between the two groups. Several anthropometric measurements such as triceps skin fold and mid-arm circumference had no correlation with the route of feeding. This may be the result of sodium and water retention in head-injured patients or slow changes of these somatic measurements during the acute post-traumatic period.[12]

In our study, the percentage of weight loss during hospitalization and the differences in weight on the 1st and the 14th days were significantly lower in the parenteral-enteral group than in the enteral group. This means that the catabolic state of this group was under better control than for the enteral group, which shows the success of the combined method in this field.

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The Bridge of 33 Arches. Built at the begining of the 17th century under Safavid Dynasty, Isfahan

IS AORTA A SUITABLE TISSUE FOR TRACHEAL REPAIR?

H. Hodjati,[*] F. Anoosh,[*] S. Dehghan,[**] N. Tanideh,[**] P.V. Kumar[***]

[*]Departments of Surgery, [**]Animal Lab. and [***]Pathology
Shiraz University of Medical Sciences, Shiraz

ABSTRACT

Background: Tracheal defect repair is a challenging issue in surgery. A variety of prostheses have been used with little success.
Objective: To investigate the suitability of aorta for tracheal repair.
Methods: On the cervical trachea of 11 dogs we made a 5 cm long tracheal defect in the left posterior membranous portion. It was repaired by harvested infrarenal aorta and the aorta was replaced by Dacron graft. One dog died of heart failure, 8 were sacrificed 2 months and two 6 months after the procedure.
Results: Gross and microscopic study showed patent lumen with excellent re-vascularization of the aortic wall and ciliated columnar epithelial growth endoluminally.
Conclusion: Aortic graft is preferable to other substitutes because of its lower antigenicity, lower vascularities, as well as the absence of mucous secretions and peristalsis.
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Key Words: Aorta • trachea • repair • graft

Introduction

Tracheal defects may occur after trauma or prolonged intubation. Resection of tracheal tumors also poses a major challenge for substitution. In an effort to solve this problem, different techniques have been tried with little success.[1-11] We report on a new animal model which showed acceptable results with fewer complications.

Materials and Methods

Eleven healthy adult dogs ranging from 18 kg to 24 kg in weight were studied. General anesthesia was induced with thiopental and maintained with oxygen and 1-3% halothane through an endotracheal tube. Access to the cervical trachea was gained through a longitudinal ventral neck incision by splitting the anterior neck muscles. The trachea was exposed and a 5 cm long window-shaped defect was made.

A laparotomy was simultaneously performed through a midline abdominal incision to expose at least 5 cm of the infrarenal abdominal aorta. Before aortic clamping, 100 unit/kg heparin was given intravenously and 5cm of aorta was excised and replaced by Dacron graft with 3-0 and 4-

Figure 1: Window-shaped healed aortic graft on the trachea.

Correspondence: *H. Hodjati M.D., Dept. Of Department of Surgery, Shiraz University of Medical Sciences, Shiraz, Iran*

Figure 2: Ciliated columnar epithelium over the grafted aorta.

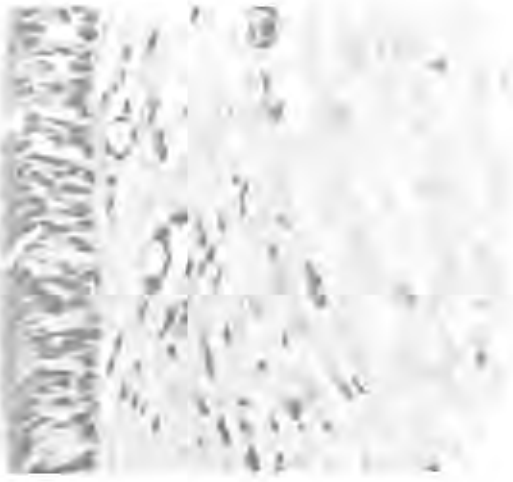

Figure 3: Neovascularization of grafted aortic wall.

0 prolene. The abdomen was then closed in 2 layers by 0-0, 3-0 nylon sutures. The excised aortic segment was cut along the whole length (Fig. 1) and put over the window-shaped defect of cervical trachea by continuous suturing with 4-0 prolene.

The cervical wound was closed in layers with 2-0 chromic and nylon 3-0 (m). The animals were extubated after emerging from anesthesia and put under close observation. Eight animals were sacrificed after 2 months and 2 animals after 6 months and the trachea was evaluated for patency and histological study

Results

Ten animals survived the procedure; one dog died 4 hrs after the operation secondary to heart failure. All wounds healed primarily and there was no respiratory problem clinically. At necropsy, gross examination showed a patent lumen in all specimens. Microscopic examination of the H&E-stained sections of grafted aorta and the anastomotic site revealed well healed anastomosis in all animals together with ciliated columnar epithelium coverage of grafted aorta and neovascularization of the aortic wall (Fig.2,3)

Discussion

Studies on prostheses for repairing the tracheal defects have a long history beginning with Daniel's experiment in 1948. Since then, a variety of prostheses have been used, but results have been disappointing in a significant proportion of cases.[1-9] Although numerous autografts, homografts, and alloplastic materials have also been applied for tracheal defects, none has proved ideal.[1,2,7,10-13] Vascularized autografts such as pectoralis muscle, esophagus, jejunum have shown promising experimental results when used alone,[12] but it is unlikely that soft tissue alone could provide adequate long term structural support to maintain a patent airway.[14,15] Composite implants consisting of a revascularized jejunal autograft in combination with a permanent rigid implantation stent have been utilized in a canine model with promising results. Cryopreserved allografts have been recently performed, first by Tojo et al. on rats and then by Jacops et al. on pediatric allograft. In both instances, it was found that cryopreservation reduced the antigenicity of the transplants.[15,16] In our current study, the aorta as an autogenous tissue was used to replace the trachea. Surgical techniques for aortic segment excision and Dacron replacement with aorto-

tracheal graft are not complicated and feasible to be done in one stage. Secondly, the aorta has no autogenous immunity-related problems and its diameter in human is comparable to the trachea further more it provides a sufficient inner diameter for airway patency.[17]

Due to the disparity between the trachea and the aortic diameter in dogs, the aorta was transplanted to the tracheal defect by anastomosis to the proximal and distal tracheal and posterior membranous parts, but it appears that no such disparity is anticipated in humans. The gross and the microscopic study of transplanted aortic segments showed neovascularization from adjacent tissues providing a good and resistant grafted tissue without vascular pedicle. The growth of endothelial ciliated cells over the grafted aortic segment lumen without mucous secretion and luminal stenosis is another positive point for this method in comparison with other tubular grafts such as esophagus, jejunum and collagen-conjugated mesh.[18]

Conclusion

Aortic graft is preferable to other substitutes because of less antigenicity, less vascularity, and no mucous secretions or peristalsis.

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REDUCTION OF POST-OPERATIVE PERITONEAL ADHESIONS USING METHYLENE BLUE

A. Izadpanah, S.A.A. Payravi

Department of Surgery, Shiraz University of Medical Sciences, Shiraz

ABSTRACT

Background/Objective: Postoperative peritoneal adhesion bands (PABs) are one of the most common complications of laparotomies. Approximately two - third of all intestinal obstructions are caused by adhesion bands.
The use of methylene blue (MB) for prevention of these adhesions has been postulated on account of inhibitory effect of MB on oxygen for the transfer of electrons from flavo-enzymes primarily xantine oxidase.
Methods: In this study 6 groups of guina pigs (n=20 in each group), laparotomy and induction of adhesion was performed in, then MB was administered intraperitoneally, at 0.5, 1, 5, 10 or 20 mg/kg to experimental groups. Control group did not receive MB. After 2 weeks they were sacrificed and their PABs was graded by Nair classification.
Results: MB at 0.5 mg/kg reduced the formation and severity of PABs significantly ($P<0.005$). However, at 1 and 5 mg/kg the PABs were not reduced ($P<0.306$ for G3 and $P<0.219$ for G4). At high doses of 10 and 20 mg/kg MB was lethal to 80% and 100% of the animals, respectively.
Conclusion: In conclusion, MB might be able to prevent PABs at low dose(0.5 mg/kg) in guina pigs. However, at high doses (≥10 mg/kg) it was lethal.
Irn J Med Sci 2001; 26(1&2): 51-54

Key Words: • Peritoneal adhesion bands • methylene blue • postoperative peritoneal adhesions

Introduction

Formation of peritoneal adhesion bands (PABs) is a condition to which every surgeon is familiar with. This condition can cause intestinal obstruction in about 5% of patients who have undergone laparotomy.[1-3] Analyses of large series have demonstrated that approximately two third of all small bowel obstructions are caused by adhesion bands.[1,2]

Many studies have revealed the high frequency of symptomless postoperative adhesions in the general population. Weibel and Majno in their classical work, reviewed autopsy data of 298 subjects who had had previous laparotomies, and found that 67% of these cases showed adhesions. If multiple operations were taken into account, the incidence rose to 93 percent.[2]

It is well established, from clinical observations, that even if large peritoneal lesions were left open, the potent healing capacity of peritoneum would lead to a smooth, glistening, new serosa within a few days.[3-7] However, if the injury is accompanied by vascular damage such as contusion, suturing or ligation, adhesions are more likely to develop. In such cirmustances an active inflammatory-like process will lead to neovascularization.[5-7] Thus, adhesion formation resembles in many ways, an inflammatory process.

Numerous attempts have been made from a number of different aspects, to prevent or minimize the risk of PABs, albeit with limited success.[3-8] These can be classified as follows: 1) Prevention of fibrin deposition in the postoperative peritoneal exudate, using anticoagulants such as sodium citrate.[3] 2) Removal of fibrin exudate by means of intraperitoneal lavage by enzymes such as

Correspondence: *A. Izadpanah M.D., Dept. of General Surgery, Shiraz University of Medical Sciences, P.O.Box :71345-1853, Shiraz., Iran, E-mail :izadpana@sums.ac.ir*

pepsin.[2] 3) Separation of surfaces by methods such as distension of the abdominal cavity with oxygen, stimulation of peristalsis with prostigmine, and the use of substances such as olive oil and liquid paraffin.[3] 4) Inhibition of fibroblastic proliferation has included the use of antihistamines, steroids and cytotoxic drugs.[3] 5) Inhibition of production of oxygen derived free-radicals, by using materials such as MB.[4] Herein, with regard to a previous study[4] showed that MB at 20 mg/kg did prevent the formation of PABs after abdominal surgery in rats. This study was designed to further our understanding of the preventive effect of MB using different doses in guinea pigs.

Materials and Methods

This study was performed on 120 guinea pigs, with an average weight of 750 g (range: 600-1000 g), that were housed in Animal Laboratory Research Center of the Shiraz University of Medical Sciences. The guinea pigs were divided into 6 groups (n=20 for each group). One group served as control group and the other 5 constituded study groups. At the time of operation, the guinea pigs fasting for 12 hours, were anesthetized by intraperitoneal injection of Ketamin 5% and Xylazine 2% (30mg/kg and 8mg/kg, respectively). The abdomen was shaved and scrubbed by povidone-iodine 10% three times. Laparotomy was performed through a 5 cm high midline incision, using sterile surgical technique. The cecum and small bowel were taken out of the wound gently, and about $1cm^2$ of the anterior wall of the cecum was painted with 96° ethylic alcohol by an applicator and about 0.5 g of sterile talcum powder was scattered diffusely, throughout the peritoneal cavity to induce adhesion formation. The abdominal cavity was subsequently closed with nylon 00 continuous suture in 2 layers, and dressing was applied with tie-over technique and tetracycline spray.

No antibiotics were given to the animals in this study. Prior to closure of the abdominal wall, a No. 5 French feeding catheter was passed between the sutures, to allow us for injection of 2ml/kg of MB intraperitoneally in different concentrations (Group 1, no MB: control group; Group 2: 2ml of 0.025% MB= 0.5 mg/kg; Group 3: 2ml of 0.05% MB= 1 mg/kg; Group 4: 2ml of 0.25% MB=5mg/kg; Group 5: 2 ml of 0.5% MB= 10 mg/kg; Group 6: 2 ml of 1%, MB= 20 mg/kg. Guinea pigs in fifth and sixth study groups died in first postoperative days, 80% and 100%, respectively, and were excluded from the study.

Guinea pigs were then housed for 2 weeks and feeding was started from the first postoperative day. At fifteenth postoperative day they were sacrificed by exposure to high doses of ether. Through an ample right paramedian incision, the abdominal cavity was explored and quantitative grading of adhesions was performed by two investigators using the Nair classification for PABs.[9] The data analysis was performed using the Kruskal-Wallis test (Mann-Whitney U, Wilcoxon W).

Results

The total percentage of adhesion band formation was the same in the control group and the three surviving groups, (85% to 90%). However, all groups receiving MB showed reduced severity of PABs ($P<0.016$). Among the surviving groups, adhesion bands of grades 3 and four were less frequent in those receiving the least dose of MB, namely 0.5mg/kg ($P<0.005$). The grades 3 and 4 adhesion bands in group 2 were one-third of that seen in the control group. So we concluding: 1. MB in low doses ≤ 0.5mg/kg

Table 1: Grading of adhesion bands in the study groups

Grading of adhesions (Nair Classification)	G1 Control No MB No.(%)	Group 2, 0.5mg/kg MB No. (%)	Group 3, 1mg/kg MB No. (%)	Group 4, 5 mg/kg MB No. (%)
0	3 (15)	3 (15)	3 (15)	2 (10)
1	0	2 (10)	0	0
2	3 (15)	10 (50)	6 (30)	5 (25)
3	3 (15)	1 (5)	3 (15)	6 (30)
4	11 (55)	4 (20)	8 (40)	7 (35)
All grades	17 (85)	17 (85)	17 (85)	18 (90)

can reduce the PABs, significantly, ($P<0.005$).(Table 1)

2. MB in medium doses, $\geq$1mg/kg, cannot reduce the PABs, Significantly, ($P<0.306$) for group 3, ($P<0.219$) for group 4.

3. MB in doses, $\geq$10mg/kg , was lethal to guinea pigs.

Discussion

Adhesion formation resembles in many ways an inflammatory process. Numerous mediators of inflammation, such as arachidonic acid, cytokines, nitric oxide, and oxygen-derived free radicals might participate in postoperative formation of adhesions.[3-8]

MB is known to inhibit the generation of oxygen radicals such as superoxide by competing with oxygen for the transfer of electrons from flavo-enzymes, primarily xanthine oxidase. This low molecular weight partially liposoluble vital dye, is also a known inhibitor of guanylate cyclase.[4,10,11]

Locally generated free radials such as superoxides, peroxides, and hydroxyl radicals are potential oxidizers of polyunsaturated fatty acids, and are postulated to induce peritoneal adhesions by damaging the cellular membranes.[12] The interference of MB with free-radical generation may be related to the decline in adhesion formation. Kelner et al. suggested that MB might inhibit the production of superoxides by competing with molecular oxygen at the iron-sulfur centers of xanthine oxidase, which allows for the anaerobic oxidation of purine substrates.[13,14]

Salaris and co-workers suggested that MB acts as a parasitic electron acceptor, shunting electron flow from the normal pathway to form leukomethylene blue, and thus effectively bypassing the generation of oxygen free-radicals.[8] Unlike free-radical scavengers such as the enzymes superoxide dismutase and catalase, MB is able to penetrate cells, a property that makes it an effective viable histological stain. In addition, administration of MB to patients has already been proven safe.[4,10] It is relatively nontoxic and can be safely applied over a wide range of concentrations. In humans, MB in doses up to 7 mg/kg has not shown any adverse effects.[4,10]

In this study, intraperitoneal administration of low dose MB (0.5 mg/kg) decreased the formation and severity of PABs, significantly, similar to the study of Galili et al[4]. In contrast to the Galili et al's study, however, we noticed that the protective effect of MB decreased at high concentrations($P<0.016$). In conclusion, it shown that MB at the doses used might be protective against production of adhesion bands in guinea pig. However, the use of MB for such a purpose in human being

needs further trials on other animal species using different doses of MB.

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ERRATUM

In Vol.25 No. 3&4, Page 119, in the Title
Stimulate and Inhibit should read Stimulation an Inhibition.
Page 120, left Column, "oral ulcers and inflammations. A" should be added to the second line.

THE EFFECT OF SODIUM VALPROATE ON MEASLES VIRUS, POLIOVIRUS TYPE 1 AND COXSACKIEVIRUS B3 REPLICATION

M. Kabiri, M. Motamedifar,* S. Akbari,***

**Department of Bacteriology and Virology, Shiraz University of Medical Sciences and **Electron Microscope Unit, Veterinary School, Shiraz University, Shiraz*

ABSTRACT

Background: Sodium valproate, an anticonvulsant drug, has been reported to stimulate the replication of human immunodeficiency virus Type 1 and human cytomegalovirus.

Objective: In view of the fact that epileptic patients receiving this drug may become infected with different viruses, we investigated the effect of sodium valproate on the replication of other viruses especially those affecting neuronal tissues, such as measles virus. The viruses used in our studies include measles virus, poliovirus Type 1 and coxsackievirus B3.

Methods: The replication of measles virus was studied by plaque assay and that for poliovirus Type 1 and coxsackievirus B3 was performed by quantal response method. Electron microscopy was used in evaluation of cell monolayers infected with measles virus.

Results: The results obtained showed that sodium valproate stimulated both measles virus ($p<0.001$) and poliovirus Type 1 replication ($p<0.01$) and had no effect on coxsackievirus B3 infectivity. Electron microscopic studies showed that measles virus nucleocapsids were more abundant in drug treated infected cells as compared with untreated infected control monolayers. The sparsity of intranuclear and intracytoplasmic inclusion bodies was also a prominent feature of cells exposed to the drug and infected with measles virus.

Conclusion: The present preliminary investigation should be assessed by appropriate in vivo studies. Until then caution is to be exercised in prescribing sodium valproate to the patients with neurologic disorders due to infection with measles virus. This is particularly important with respect to the developing countries where measles infection is of higher incidence and severity.

Irn J Med Sci 2001; 26(1&2): 55-61

Key Words • Sodium valproate • Measles virus • Poliovirus • Coxsackievirus B3 • Replication.

Introduction

The measles virus is a member of genus morbillivirus, subfamily paramyxovirinae and family paramyxoviridae. It causes encephalitis (1:1000) and 25% of survivors suffer from permanent neurologic impairments including psychosis or physical disabilities particularly seizure disorders for which SV may be used as a therapeutic agent. Subacute sclerosing panencephalitis (SSPE), occurring in individuals under 20 years of age, also caused by measles virus (1 per 1 to 3 millions) is characterized by progressive neurologic deterioration, failing memory and myoclonus.[3] The poliovirus Type 1, a member of genus enterovirus of family picornaviridae, is the etiologic agent of poliomyelitis and aseptic meningitis. Coxsackievirus B3 belongs to the same genus and is the causative agent of myocarditis and several clinical entities such as aseptic meningitis, Bornholm disease and respiratory infections.[4]

Diverse structural properties and replication strategies of viruses influence

Correspondence: *M. Kabiri, Ph.D., Dept. of Bacteriology & Virology, Shiraz University of Medical Sciences, Shiraz, Iran.* Fax: *+98-7112359847* Tel: *+98-7112305410 - Ext.336,* Email: *hvdept@sums.ac.ir*

viral interaction with physiochemical agents. The anticonvulsant drug, sodium valproate (SV), a branched short chain fatty acid is reported to stimulate human cytomegalovirus (HCMV) and human immunodeficiency virus Type 1 (HIV-1) replication.[1,2] In view of the fact that epileptic patients undergoing SV therapy may suffer from various viral infections, the effect of SV on replication of other viruses is worthy of investigation. This prompted us to extend similar studies to other viruses. The objective of the present work was to study the effect of SV on replication of measles virus, poliovirus type 1 and coxsackievirus B3. The anticonvulsant property of SV and propensity of certain viruses for neuronal tissues render the study of the effect of SV on measles virus replication particularly important. This is especially noteworthy with respect to the developing world and poverty-stricken areas where vaccination programs are inadequate and infection with measles virus is of higher incidence and severity. The ultimate outcome of such preliminary studies await parallel in vivo investigations.

Materials and Methods

Cell cultures:

The HeLa cell culture was grown in Eagle's minimum essential medium (MEM), supplemented with 7% fetal bovine serum (FBS) from GIBCO,U.S. The maintenance medium (MM) was similar but with 3% FBS. Vero cell culture was obtained from the Cell Bank of Pasteur Institute, Tehran, Iran and grown in Dulbecco Minimal Essential Medium (DMEM), containing 5% FBS. Dulbecco maintenance medium (DMM) was similar but with 2.5% FBS. All media contained 100 IU/mL of penicillin & 100 μg/mL of streptomycin sulphate.

Virus stocks:

Coxsackievirus B3 and poliovirus Type 1 (vaccine strain) were propagated in HeLa cells with corresponding $TCID_{50}$ of titers $10^{6.5}$/mL and $10^{6.25}$/mL, respectively. Measles virus (AIK strain) purchased from Razi institute, Tehran, Iran, was grown in Vero cell culture to a titer of $10^{5.25}$/mL $TCID_{50}$.

Sodium valproate (SV):

The drug was purchased from Sigma and was dissolved in MM and sterilized by filtration (0.22mμ, Millipore, US.A); assays for cytotoxicity were performed using trypan blue exclusion dye test.[3] SV concentrations ranging from 4, 2, 1, 0.5, 0.25 and 0.125 mM were used to treat cell monolayers. In view of the low cytotoxic threshold of Vero cells for SV, the experiments on measles virus were not performed with 2 mM and 4 mM of the drug. The protocol for SV treatment of cells exposed to viruses under the study included one hour before and after viral infection, 24 hours before and following inoculation with virus as well as simultaneous exposure of cells to SV and virus. All experiments were performed in 96 microwell plastic plates (Nunc). Following incubation period, the contents of each series of wells were pooled and stored at -70°C along with their corresponding controls for subsequent infectivity titration. Also, mixtures of appropriate concentration of SV and measles virus (10 PFU/mL) were incubated for one hour at room temperature before adding to the cell monlayers.

Infectivity assays:

I) Poliovirus Type 1 and Coxsackievirus B3:

HeLa cells (2×10^5cells/ml) were grown for 48 hours in 96 microwell plastic plates (Nunc). Cell monolayers were then inoculated with ten-fold serial dilution of each virus in MM and incubated under 5% CO_2 for 48 hours at 36°C. The $TCID_{50}$ of viral infectivity was determined using the method of Kärber.[4]

2) Measles virus: A modified liquid overlay method based on the method described by Makino et al.[7] was employed to detect the infectivity of measles virus. Three mLs of each virus dilution prepared in Vero cell suspension (2×10^5 cells/mL) were added to each of four tissue culture plates (60 mm diameter) and incubated as described. Following 48 hours 2 mLs of DMM was added to each plate and incubated for an additional 2 days. The infectivity titer or the mean plaque count of 4 plates were expressed as plaque forming unit (PFU) and determined after staining with 2% crystal violet in 20% ethanol (w/v) for 2 minutes and making correction for dilution factor.

Statistical analysis:

Experiments were performed at least three times in quadruplicates. The statistical analysis was conducted, using Chi-square for detecting the significance of differences in the number of measles virus plaques between control and test series. One way analysis of variance and Dunnett T test were used for comparison of test results with those of controls in the other experiments.

Electron microscopy (EM):

With respect to the importance of neurologic sequelae due to the measles virus, electron microscopic examination was only performed on this virus. Vero cells grown for 48 hours in sterile disposable flasks were treated with 1mM of SV one hour before, one hour after and simultaneous infection with 10

Table 1*: Temporal effect of various SV concentrations on replication of Measles Virus in Vero cells.

No.	Treatment	PFU Mean ± SE	P value
1-1	Control[1] (1)	206.0 ± 8.0	-
1-2	0.25 mM SV 1 hour BVI[2]	282.5 ± 7.5	0.000[3]
1-3	0.50 "	289.5 ± 7.5	0.000
1-4	1.00 "	298.5 ± 8.5	0.000
2-1	Control[1] (2)	112.0 ± 11.0	-
2-2	0.125 mM SV 1 hour AVI[2]	205.5 ± 9.5	0.000
2-3	0.25 "	207.5 ± 10.5	0.000
2-4	0.50 "	218.0 ± 16.0	0.000
2-5	1.00 "	235.5 ± 15.5	0.000
3-1	control[1] (3)	236.0 ± 5.0	-
3-2	0.50 mM SV, SVI[2]	284.0 ± 5.5	0.035
3-3	1.00 "	301.0 ± 4.0	0.000
4-1	control[1] (4)	29.5 ± 8.5	-
4-2	0.125 mM SV 24 hrs BVI[2]	74.0 ± 4.0	0.000
4-3	0.25 "	87.0 ± 2.0	0.000
4-4	0.50 "	145.0 ± 6.0	0.000
4-5	1.00 "	144.5 ± 2.5	0.000

*The table includes only statistically significant data and does not represent drug concentrations without meaningful values.

[1] Each control corresponds to underlying experiments. [2] BVI (Before Virus Inoculation); AVI (After virus inoculation); SVI (Simultaneously with Virus Inoculation); [3] $P<0.001$.

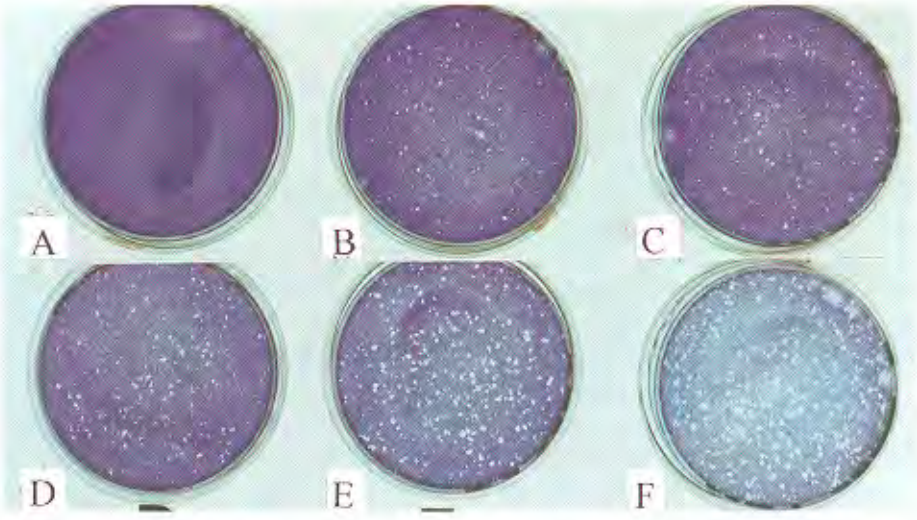

Figure 1: Direct relationship between SV concentrations and stimulation of measles virus replication shown as plaque numbers. A: Uninfected control, B: Virus Control, C, D, E & F, Infected cells treated with 0.125 , 0.25, 0.5 and 1mM of SV respectively.

PFU/mL of measles virus. The monolayers were prepared as described above. Control infected and SV treated infected cells were then scraped by cell scrapers (Nunc) and left in the refrigerator overnight and the resulting cell pellets were fixed in 2.5% glutaraldehyde. Processing for EM was performed according to the method described by Robards and Wilson.[8] The sections were stained with uranyl acetate and lead citrate prior to the examination in a Philips TEM, C. M. 10 electron microscope.

Results

The stimulation of measles virus replication by SV is shown in Table 1. This stimulatory effect is dependent on the sequential exposure of cells to SV and virus. The highest stimulatory effect on virus replication ($P < 0.001$) was observed in cells either treated with SV one hour after viral infection (2-2 to 2-5) or in those treated with the drug 24 hours before viral infection (4-2 to 4-5).

As a rule, a direct relationship was found between increasing SV concentration and stimulation of viral replication (Fig. 1). As for the exposure of cells to SV one hour before virus infection (1-2 to 1-4) all concentrations, excepting 0.125 mM of the drug stimulated viral replication ($p < 0.001$). Simultaneous exposure of cells to the drug and virus (3-2 & 3-3) caused stimulation only with 0.5 mM ($P < 0.05$) and 1.0 mM ($P < 0.001$) of SV. Exposure of cells to the drug 24 hours after virus inoculation and incubation of cells with SV and virus at room temperature prior to exposure of the cells did not stimulate viral replication .

The stimulation of poliovirus Type 1 replication by SV is shown in Table 2. The highest stimulatory effect was found with simultaneous exposure of cells to SV and virus (3-2 to 3-5) or when cells were treated with SV one hour after virus infection (2- 2 to 2-5). However treatment of cells with the drug one hour before virus infection caused stimulation of viral replication only with 4 mM of SV (1-2). Preincubation at room temperature of poliovirus Type 1 for one hour, with various

Table 2*: Effect of various SV concentrations on replication of poliovirus type 1 in HeLa cells.

No.	Treatment	Virus yield (log_{10} of $TCID_{50}$/100 µl Mean ± SE	P value
1-1	control[1] (1)	5.8333 ± 0.0833	1.000
1-2	4.00 mM SV 1 hour BVI[2]	6.3333 ± 0.0833	0.042
2-1	control[1] (2)	5.6667 ± 0.0833	1.000
2-2	0.50 mM SV 1 hour AVI[2]	6.0833 ± 0.0833	0.009
2-3	1.00 "	6.0833 ± 0.0833	0.009
2-4	2.00 "	6.0833 ± 0.0833	0.009
2-5	4.00 "	6.2500 ± 0.0833	0.001
3-1	control[1] (3)	5.5833 ± 0.0833	1.000
3-2	0.50 mM SV, SVI[2]	6.4167 ± 0.0833	0.000[3]
3-3	1.00 "	6.1667 ± 0.0833	0.002
3-4	2.00 "	6.1667 ± 0.0833	0.002
3-5	4.00 "	6.1667 ± 0.0833	0.002

*The table includes only statistically significant data and does not represent drug concentrations without meaningful values.
[1]Controls received no SV, were infected with virus alone and correspond to underlying experiments.
[2] BVI (Before Virus Inoculation); AVI (After virus inoculation); SVI (Simultaneously with Virus Inoculation); [3] $P \ll 0.001$.

SV concentrations had no effect on viral infectivity.

As for coxsackievirus B3, no stimulation of viral replication was observed with any of SV concentrations and time sequences.

Electron microscopy:

Measles virus nucleocapsids (averaging 21 nm in diameter) were more abundant in drug treated infected cells as compared with untreated infected control monolayers (Fig. 2 & Fig. 3). In addition, the sparsity of intranuclear and intracytoplasmic inclusion bodies was a prominent feature of cells exposed to the drug and infected with measles virus (Fig. 4).

Discussion

The mechanism by which SV stimulates viral replication is not fully understood. A study on HCMV[3] suggests that modification of intracellular redox balance, which regulates activation of various transcription factors, is responsible for the stimulation of viral replication by SV. This effect probably reflects induction of higher cellular hydrogen peroxide (H_2O_2) and a decrease in glutathione reductase that produces reduced form of glutathione (GSH), a major antioxidant in cells.[9-11] However the latter mechanism was not involved in the stimulation of HIV-1 replication by SV.[2] Our findings show that SV had no direct effect on measles virus infectivity when it was inoculated with virus for one hour at room temperature before inoculation of Vero cells. It was thus tempting to speculate that preincubation at room temperature of SV and virus induced an irreversible binding of SV to the viral envelope. Upon subsequent fusion of viral envelope with plasma membrane, SV remained attached to the cell membrane and could not be released into the interior of the cell. The simultaneous exposure of cells to SV and measles virus might, to some extent, be influenced by the foregoing mechanism. This is evident by the absence of stimulation of viral

Figure 2: An untreated Vero cell infected with measles virus showing a single viral nuleocapsid tubule measuring 22 nm within the cytoplasm(× 105000)

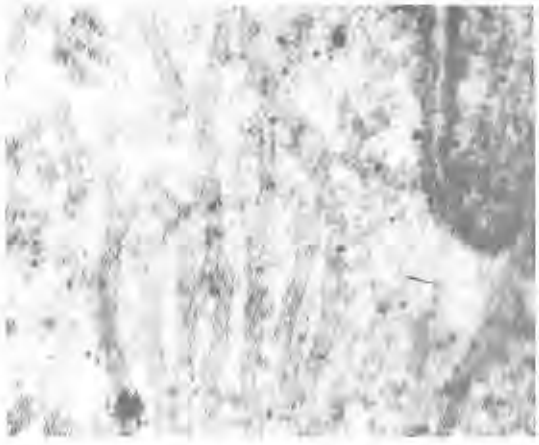

Figure 3: A Vero cell monolayer treated with 1 mM of SV and infected with measles virus. Note several intra-cytoplasmic viral ncleocapsid tubules.approximately 20 nm.

replication with lower concentrations of the drug.

However, the ineffectiveness of SV on virus yield 24 hours after viral infection and its stimulatory activity on viral replication one hour before or after viral infection or when cells were exposed to the drug 24 hours before viral inoculation may suggest that the regulation of the activity of early transcription factors arising during viral replicative cycle are involved in interaction with SV metabolites[12,13] with subsequent stimulation of measles virus replication.

The diversities in structural properties and replication modes of viruses under the study may account for the stimulatory response of drug treated cells. Preincubation at room temperature for one hour of poliovirus Type 1, with various SV concentrations did not affect viral infectivity. This finding may suggest that the binding of SV to the viral capsid would somehow interfere with the drug metabolism.

As for coxsackievirus B3, no stimulatory effect of SV was found in virus infected cells . This would suggest that the replicative cycle of coxsackievirus B3 is devoid of SV specific target molecules involved in the acceleration of viral replication.

An observable consequence of SV treatment of cells infected with measles virus was sparsity of intracytoplasmic and intranuclear inclusion bodies. This might reflect the roles of such inclusions in the modulation of viral

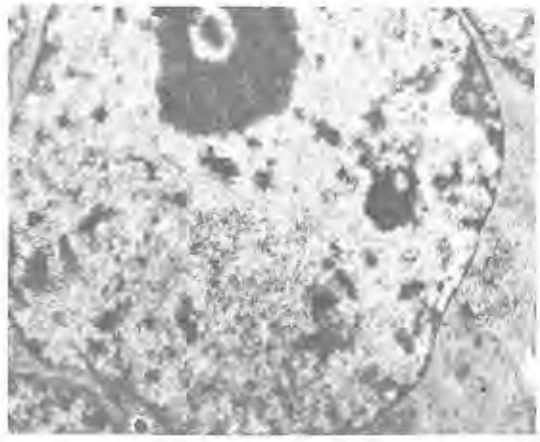

Figure 4: A Vero cell infected with measles virus without exposure to SV. Arrowheads show intranuclear and intracytoplasmic inclusion bodies. (× 8900)

replication. Inclusion bodies from measles virus isolated from clinical specimens usually appear much larger and observable by the light microscope.[10] The considerably smaller size of viral inclusions found in our electron micrographs may be ascribed to an attenuated strain of virus used in our studies (AIK).

Further work is warranted to determine the in vivo effect of SV on aforementioned viruses with particular emphasis on measles virus infectivity. Until then and as suggested by Jennings and Romanelli[15] with respect to the use of valproic acid in HIV-positive patients, caution is justified in prescribing SV to patient with neurologic disorders due to infection with measles virus. Studies are also needed to determine the efficiency of measles virus vaccination in children with epileptic seizures of unknown etiology receiving SV as an anticonvulsant.

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OCULAR TOXICITY OF DIEFFENBACHIA PLANT SAP IN RABBITS

H. Jahadi Hosseini, Sh. Fallah Randjbar, M.H. Rouzitalab

Department of Ophthalmology, School of Medicine, Shiraz University of Medical Sciences, Shiraz

ABSTRACT

Objective: To study the course of events in rabbit eyes exposed to Dieffenbachia plant sap and effect of juice type on the severity of injury.

Methods: Twenty-six rabbits (51 eyes) randomly divided into four groups and exposed to four types of juice (leaf juice, stalk juice, stem free running and stem squeezed extract). The eyes were examined in ambient light and by slit lamp,until the sixth week. For histopathologic examinations; the cornoscleral buttons were stained by hematoxylin and eosin and examined by light microscopy.

Results: The most severe clinical manifestations were seen with the juice of stem. The most common findings in all groups were corneal epithelial erosions and needle shaped stromal crystals, that gradually disappeared spontaneously. Corneal edema was only seen in eight cases of stem juice exposure. It was the best factor related to developing corneal vascularization ($p.v=0.0002$). Permanent corneal vascularization and scarring was seen in four cases, all exposed to stem juice.

Conclusion: Most of ocular injuries caused by dieffenbachia sap are reversible, except for corneal vascularization and scarring which is caused by the sap of mainstem and is highly associated with corneal edema, mandating extensive ophthalmic care upon exposure.

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Key Words: • Dieffenbachia • crystalline keratopathy • toxic keratoconjunctivitis

Introduction

Plant exposures are the fourth most common cause of poisoning, 85% of cases occuring in children.

Dieffenbachia is widely used as an ornamental household plant throughout the world as well as Iran. It has been the second most common cause of plant exposure poisoning.[1]

Although the toxicity after oral ingestion of the plant is well known, unfortunately few studies addressing the ocular toxicity of Dieffenbachia plant sap.[1] This study aims to follow the course of events in the eyes of rabbits exposed to the Diffenbachia plant sap and elucidate the possible effect of different types of juice on the course.

Correspondence: *H. Jahadi Hosseini M.D., Motahari Clinic, P.O. Box: 71935-1338, Shiraz, Iran.*

Materials and Methods

This study was performed on 26 rabbits about 2-6 months old. All of the eyes were examined before exposure. One eye with previous corneal vascularization was excluded.

The Dieffenbachia plants of Sequina species were watered 4-6 hours before the experiment. The plants' leaf, stalk and stem were washed thoroughly before cutting in order to avoid eye contamination with soil.

Four different types of juice were collected as follows: 1)- Leaf juice. 2)- Stalk juice. 3)- Stem free running juice 4)- Stem squeezed juice.

Fifty-one eyes were randomly divided into four groups: Group A, (11 eyes) exposed to leaf juice; Group B, (12 eyes) exposed to the juice of stalk; Group C, (10, eyes) exposed to free running juice of the stem; Group D, (18 eyes) exposed to the stem squeezed juice. Ocular examinations including external eye and slit lamp examination were done daily during the first five days and then weekly until the end of the sixth week. Variables considered in eye examinations included eyelid edema, erythema and discharge, conjunctival congestion and chemosis. Corneal involvement was divided into epithelial and stromal findings. The epithelial involvement consisted of punctate epithelial erosions (P.E.E), linear erosions (L.E.E) and corneal epithelial defects (C.E.D).

The stroma was examined for detection of calcium oxalate crystals with attention to their location (anterior, mid or posterior stroma) and density. The density of crystals were graded from 0 to 4 according to the number of crystals seen: Grade 0, No crystals; Grade 1, <10 crystals; Grade 2, 10-25 crystals; Grade 3, 25-50 crystals; and Grade 4, crystals too numerous to be counted.

Anterior chamber involvement (iritis) included the presence of cell and flare in the aqueous humor. The modified Hogan's grading system was used for the anterior chamber cells and flare (Grade 0 to 4).

For histopathologic examination of the cornea, rabbits were randomly selected and euthanized. Four of them on the third postexposure day (early phase), five at the end of the second week (mid phase), two at the end of the fourth week (late phase) and the rest of rabbits were euthanized at the end of the sixth week (convalescent phase). The corneoscleral buttons were fixed in 10% formalin solution and stained with hematoxylin & eosin. The slides were reviewed by the ophthalmopathologist. The statistical tests used in data analysis included the Chi square, Fisher's exact, Kruskal Wallis and the Withney U test.

Results

A- Clinical Manifestations:

Rabbits in group C and D generally had a more severe clinical course than group A and B.

All of our cases developed blepharokerato-conjunctivitis and crystalline keratopathy.

The most common clinical findings in the first post-exposure day were needle-shape corneal stromal crystals and punctate epithelial erosions (P.E.E.).

Needle shaped polychromatic calcium oxalate crystals were detectable in anterior stroma of 100% of the cases in group A, C, D and in 91.7% of the cases in group B. The number of crystals in anterior stroma decreased gradually, shifting towards the posterior stroma in succeeding days.

The average time needed for crystals to clear from the anterior stroma was eleven days with group C, being the last to resolve.

Punctate epithelial erosion was seen in 81.1%of group A cases, 100% of group B, 90% of group C and 61% of group D cases.

Eyelid edema and erythema was seen in 54.5% of group A cases, 16.7% of group B, 100% of group C and 61.1% of group D cases. The lid findings were significantly more common in group C (p:0.02).

Corneal edema occured rarely and was only seen in groups C and D (30%and 27.7% respectively).

B- Major complications:

Among 51 eyes, four eyes developed permanent corneal vascularization following a severe clinical course, three of them belonged to group D and one to group C.

Variable degrees of corneal edema was seen in all of them. Corneal edema was the factor best related to vascularization ($p=0.0002$, risk$=11.75$, confidence interval 4.60-30.00).

C- Pathologic findings:

Seven eyes belonging to groups A, B and D underwent histopathological examinations in the early phase of study. All of them revealed perilimbal infiltraion of plasma cells, eosinophils and lymphocytes, except for one case in group B that had no evidence of inflammation. Two cases in group D revealed total epithelial necrosis and destruction of Bowmman's area. Degradation and lysis of collagen fibers were also seen in the anterior and mid stroma.

Ten eyes were studied histopatologically in the mid phase. In these samples a moderate inflammation was seen with the same cellular components. No corneal epithelial erosion was seen.

Four eyes were studied in the late phase histopathologically and revealed only a mild inflammatory infiltrate of eosinophils and plasma cells.

Thirty eyes were examined histopathologically at the end of the 6th week (convalescent phase).

The majority of them had no evidence of inflammation or minimal degrees of infiltration. On the other hand, eyes with clinical corneal edema and vascularization had remarkable histopathologic findings. They had heavy eosinophilic, lymphocytic and plasmacytic infiltration in perilimbal and subepithelial region. Russel bodies were also seen. There were subepithelial fibrosis and vascularization in the anterior third to half of corneal stroma. The artificial spaces between collagen fibers were decreased.

No calcium oxalate crystals could be seen in histopathological specimen.

Discussion

Dieffenbachia is a popular household ornamental plant and the second most common cause of all plant poisonings.[1] Little is known about its ocular toxicity. Previous investigations were mainly based on a few case reports of incidental exposure.[3,7] Ellis et al. exposed eight rabbit eyes to Dieffenachia sap and reported severe keratoconjuctivitis with epithelial breakdown and necrosis,[7] but no corneal edema or opacity and scar reported.

In our study the sap extracted from the mainstem produced the most severe clinical course and sequelae. In the first day of the exposure we found the highest concentration of calcium oxalate crystals in the anterior stroma of cornea, decreasing in numbers and migrating towards the posterior stroma in the next days. These findings are in accordance with the Ellis's study.[7]

In the eyes exposed to the stem juice, the crystals persisted for a longer duration, maximally four weeks.

To our konwledge, it is the first time that corneal edema and opacity (scar) due to Dieffenbachia sap toxicity is reported. This observation is important since it shows that toxin can cause endothelial dysfunction and permanent visual loss due to corneal scar.

All of the cases of corneal edema and opacity were exposed to the mainstem sap revealing more toxic nature of this type of the sap.

Ellis et al. reported several cases of stromal vascularization but provided no detailed informations.[7]

In our study four cases in group C and D developed superficial and deep corneal vascularization starting in the second week from the corneal periphery and progressing to total corneal vacularization and opacity at the end of the sixth week. Interestingly all of them suffered from corneal edema and a statistically significant association was found between corneal edema and development of corneal vascularization ($P=0.0002$). So,

whenever corneal edema is detected, a more severe clinical course is possible, calling for more aggressive treatment.

Punctate epithelial erosion (P.E.E.) was a very common finding in our cases that recovered completely within 3-4 weeks. Iritis also was seen in 10% of our cases and all of them were caused by the sap from the mainstem. This complication is previously reported.[8]

The pathogenesis underlying the toxicity of Dieffenbachia has been a matter of controversy. Some believe that the injury is primarily mechanical and the result of penetration of needle-like calcium oxalate crystals present in the sap.[9]

Some evidences also support the possibility of enzymatic and allergic injury.[9]

Presence of eosinophilic, plasma cells and Russel bodies in the histopathologic specimens of our study is in favour of the allergic or immune base of toxicity,[10] however, the possibility of combined mechanism should be considered too.

Conclusion

Fortunately, most of the ocular injuries caused by Dieffenbachia sap are reversible, specially keratoconjunctivitis and crystalline keratopathy. However, exposure to the sap of mainstem and corneal edema should be considered as the risk factors which predispose the eye to permanent corneal scar, vascularization and blindness. Aggressive ophthalmic care is suggested in these situations.

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VALIDITY OF PERSIAN ADULT READING TEST FOR THE ESTIMATION OF PREMORBID IQ

H. Hagh-Shenas, H. Farrashbandi, A. Mani, S. Tahmasbi

Department of Psychiatry, Shiraz University of Medical Sciences, Hafez Hospital, Shiraz

ABSTRACT

Background: The present study was designed to investigate the relationship between the Persian Adult Reading Test (PART) and current intellectual ability in a group of elderly with and without memory problems.

Objective: Reading short and irregular words is the last ability preserved in dementia and brain degenerative diseases. This ability shows the premorbid IQ of patients with memory deficit.

Methods: Seventy five subjects aged above 55 years (23 males and 52 females), visitors of 3 available elderly community houses in Shiraz, Iran, were requested to take part in the study. Four measures of cognitive ability were utilized: 1- The verbal paired association learning task (a sub-test of Wechsler Memory Scale) with delay recall; 2- Computing a series of mental arithmetic; 3- A task of reading 50 Persian words (PART); 4- Finally, they were requested to give response to Raven Standard Progressive Matrices (RSPM) as a measure of general intellectual ability (i.e., IQ).

Results: Positive and significant correlations between PART scores and RSPM ($r=0.36$ for total sample; $r=0.46$ for males; $r=0.35$ for females), and PART and mental arithmetic task ($r=0.36$ for total sample; $r=0.44$ for males; $r=0.33$ for females) were obtained. There were no significant differences between elderly subjects suspected of having dementia and those without memory deficit in PART scores ($p=0.36$) while the two groups were significantly different in RSPM scores ($P<0.0001$).

Conclusions: These findings suggest that PART could be valid for the estimation of premorbid intellectual ability in elderly people, but further study on clinically diagnosed subgroup of demente patients is required.

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Key Words: • Persian Reading Test • PART • dementia • IQ • premorbid IQ

Introduction

The diagnosis of dementia at its early stage is a sensible task for clinical neuropsychologists. Various forms of impairment in original intellectual ability of the individual are typical in patients with this condition. In fact, the hallmark of diagnosis of various forms of dementia is memory impairment together with marked decrease in general intellectual functioning.[1] Hence, it is important for the clinicians to have an estimation of a patient's premorbid intelligence quotient (IQ) and compare it with the current IQ to understand the degree or severity of dementia. Traditionally, the previous education level and socioeconomic status of the patient were granted for the estimation of the premorbid IQ. These indirect measures of premorbid IQ may be subject to distortion and almost lead to under- or overestimations.[2] Vocabulary and related verbal skills have been documented to provide the best estimate of general premorbid ability level in the presence of memory impairment.[2,3] Early attempts to improve the methods of assessment of the cognitive deterioration of patients with a diffusely dementing condition were addressed by Nelson et al.[4] and

Correspondence: *H. Hagh-Shenas Ph.D., Dept. of Psychiatry, Shiraz University of Medical Sciences, Hafez Hospital, Shahid Chamran blvd., Shiraz 71935, Iran.* **E-mail:** *haghshah@sums.ac.ir* Tel: +9871627919

Crawford et al.[5,6] which lead to the introduction and widespread use of the National Adult Reading Test (NART). The NART consists of 50 short, irregular words (e.g., 'ache', and 'gauche') which the subject should read and pronounce. As the words are short, subjects do not need to analyze a series of complex visual stimuli and since the words are irregular, intelligent guesswork will not provide the correct pronunciation. In fact, the subject performance on the test depends more on previous knowledge rather than current cognitive capacity.[7] In an early report, Nelson and McKenna[8] showed that word-reading and general intelligence abilities were highly correlated. This finding was replicated in English and in Dutch-speaking samples.[9,10-12] Furthermore, there is agreement that demented patients show preserved word-reading ability in the absence of normal memory function.[5,7,13,14] Indeed, word-reading ability score consistently did not show any correlation with years of age.[6,7,13,14]

The Persian Adult Reading Test (PART) is a new Persian word-reading test that has been specifically designed for use in Persian speaking adults as a measure for the estimation of premorbid intelligence. It consists of 50 short and irregular Persian words. For each word, intelligent guesswork alone would not result in a correct response. Subjects are requested to read these words; then, the correct pronunciation of each word receives a credit. In a pilot study on 152 healthy subjects, the test was standardized. The result showed high convergent validity between reading scores and years of education ($r = 0.61$), and reading score and RPMS ($r = 0.41$) as a measure of IQ. In the above mentioned study, the test-retest reliability of PART scores within about 2.5 months (mean 73.1 days) was also satisfactory ($r = 90$).

Materials and Methods

Subjects and sampling: Seventy-five subjects aged above 55 years (23 males and 52 females), visitors of 3 available elderly community houses in Shiraz, were requested to take part in the study. The participants were volunteers with the following characteristics (inclusion criteria): age above 55 years, with at least 9 years of formal education, and without a past history of brain damage, and originally Farsi speaker.

The subjects were given a brief introduction about the purpose of the ongoing study, and a few questions regarding the demographic background. The verbal paired association learning task (sub-test of Wechsler Memory Scale Revised - WMS-R)[15] was followed as the second step. The delay-recall task of the learned paired association words was administered after 20 minutes at any point of the following steps. The next step of assessment was followed by asking the subject to compute a series of mental arithmetic tasks (2 times 2, 4 times 2 and so on). The fourth step was a task involving reading of 50 Persian words. Finally, they were requested to give response to the adults form of RPMS.[16] All the subjects were assessed individually, and the assessment took place in 2 or 3 consecutive sessions under the same conditions.

Results

The participants were mostly females and significantly different from men in the years of age and years of formal education. There were no significant differences between the two genders in reading, Raven, and arithmetic tests' scores, but they were significantly different in the total memory and delay recall

Table 1: statistical information for the sample

variables	males (n=23)		Females (n=52)		estimated t	p-values
	mean	SD	mean	SD		
Age (years)	67.9	7.67	62.4	6.10	3.03	0.003
Education (years)	13.8	2.88	11.6	2.01	3.82	<0.0001
Raven raw scores	24.7	10.2	27.3	10.8	-0.95	NS
Reading raw scores	35.0	11.6	36.9	6.81	-0.88	NS
Arithmetic raw scores	9.17	2.76	8.31	2.65	1.29	NS
Recent memory scores	10.8	4.48	15.25	3.65	-4.51	<0.0001
Delay memory scores	5.09	1.86	6.42	1.46	-3.36	0.001

memory scores. The female subjects performed better in the recent memory and delay recall memory tests in contrast to the male subjects. Table 1 presents the relevant statistical findings.

Pearson correlation analyses were carried out to show the relationship between reading test scores and other variables. Table 2 represents the results of these analyses. The data represented in Table 2 show positive and significant correlation between reading scores from one side and Raven test scores, arithmetic scores and years of education from the other side in the total sample. These significant correlations were confirmed for male and female groups in separate analyses. The peak and highest correlation was observed for males' years of education and reading scores. The age of the subjects did not show significant correlation with scores of reading, arithmetic and even Raven test scores.

The last set of repeated paired association of words of WMS-R was compared with the delay recall of the same sets of words for each subject. Those who received 3 scores decrement in delayed recall (about two standard deviations below the mean) were recognized as having memory deficit. Furthermore, subjects who failed to recall less than 4 associations in the initial phase of learning were also added to the memory deficit group. Accordingly, a group with memory

Table 2: Pearson correctional analyses results for Reading Test Scores and other measures

Variables	n	reading test		Arithmetic		Raven		Years of edu.	
		r	p-value	r	p-value	r	p-value	r	p-value
Age -- total	75	-0.07	NS	-0.00	NS	-0.19	NS	0.18	NS
Age - males	22	-0.16	NS	0.14	NS	0.05	NS	-0.24	NS
Age - females	53	0.06	NS	-0.14	NS	-0.24	NS	0.26	NS
Reading - total	75			0.36	0.001	0.36	0.002	0.39	0.001
Reading - males	22			0.46	0.027	0.44	0.048	0.53	0.009
Reading - females	53			0.35	0.012	0.33	0.018	0.42	0.002
Arithmetic - total	75	0.36	0.001			0.32	0.005	0.08	NS
Arithmetic - males	22	0.46	0.02			0.17	NS	0.10	NS
Arithmetic- females	53	0.35	0.012			0.43	0.002	-0.02	NS
Raven - total	75	0.36	0.002	0.32	0.005			-0.04	NS
Raven - males	22	0.43	0.04	0.17	NS			0.10	NS
Raven - females	53	0.33	0.018	0.43	0.002			-0.04	NS
Education - total	75	0.39	0.001	0.08	NS	-0.04	NS		
Education - males	22	0.53	0.009	0.10	NS	0.10	NS		
Education - females	53	0.42	0.002	-0.02	NS	-0.04	NS		

Table 3: Comparison between scores of reading test and the other measures' scores for the group of memory deficit (n=11) and non memory (n=64) deficit subjects.

Variables	memory deficit		non-memory deficit		Estimate	p - values
	mean	SD	mean	SD	d.f. t	
Reading / s	32.5	15.1	37.0	6.83	0.96	0.36
Arithmetic / s	7.45	2.70	8.76	2.66	1.50	0.16
Raven / s	18.80	5.16	27.81	10.75	4.25	<0.0001
Education / y	13.73	3.04	12.00	2.34	1.80	0.097
Age / y	65.9	9.32	63.7	7.29	-0.88	0.38

/ y = years; / s = scores

deficit subjects (n=11) and subjects without detected memory deficit were recognized (n=64). Simply a series of Student t-tests were carried out between the two groups on reading (PART), arithmetic, Raven scores and their years of education. Table 3 presents the results of these analyses.

The results of the analyses showed that the memory deficit group was not different in reading, arithmetic scores and years of education and particularly years of age from the non-memory deficit group. However, they had significantly different scores in Raven test. The memory deficit group performed significantly worse than the non-memory deficit group in this test, although there is a trend in the memory deficit group toward having had more years of education ($p = 0.097$).

Discussion

The present study was designed to investigate the relationships between the Persian Adult Reading Test (PART) scores and two measures of general intelligence ability in a group of elderly people. The data presented shows a positive and significant correlation between the PART scores, Raven Progressive Matrices and mental arithmetic scores as well as years of formal education of the participant subjects. These findings are in agreement with earlier findings in the National Adult Reading Test (NART). The NART, a test designed in English language that was developed in an auempt to provide a valid and reliable method of estimating premorbid intellectual levels.[4,7,8] This measure was devised following the findings that pronounciation of short and irregular words appeared to be preserved in dementing conditions. In their early study, Nelson and O'Connell[7] reported that word reading was resistant to the effects of bilateral atrophy. The original manual indicated that the NART had potentially a wide range of applicability in organic and functional disorders.[4] The Persian version of the test has the same applicability since both NART and PART have the same format and structure, except for the language differences.

The results of the present study also revealed that the scores of PART did not show significant correlation with the age of the subjects. This finding suggests that the reading ability is less likely to deteriorate over time and with aging. The reading test needs less effort by the subjects to carry out and needs less than 5 minutes to be completed. These points are important advantages in using the reading test in contrast to the other time-consuming and complicated forms of intelligence assessment in elderly people, particularly those with depressed mood[17] and dementia.

The findings of the present study showed that elderly people with memory problems were not different in PART scores from the non-deficit memory group, while they had significantly lower scores in general

intelligence ability at the time of assessment (i.e., Ravens test scores). The findings suggest that the PART could be used as a test for estimation of premorbid IQ in elderly people. Further investigations should be carried out to find the accuracy of the test results in patients with different degrees of dementia.

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Tomb of the eminent Iranian poet, Hafez at Shiraz

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Short Communication

PERIURETHRAL AUTOLOGOUS FAT INJECTION IN THE TREATMENT OF FEMALE STRESS URINARY INCONTINENCE

S. Shakeri, M. Yazdani,** D. Irani,* S. Dehbashi***

*Departments of *Urology, **Obstetrics and Gynecology, Shiraz University of Medical Sciences, Shiraz*

ABSTRACT

The need for an easily available and cost-effective treatment for stress urinary incontinence (SUI) of women in the developing communities is a challenging issue for the urologist. We have attempted to determine the efficacy of periurethral autologous fat injection in the treatment of female stress urinary incontinence (SUI).

From October 1995 to December 1998 a total of 22 women, 10 with Type III SUI and 12 with Type I SUI underwent periurethral autologous fat injection. Pre-operative and follow-up evaluations consisted of filling out appropriate questionnaires, vaginal examination, stress test, subjective incontinence scoring, subjective estimation of the quantity of urinary leakage and urodynamic determination of abdominal leak point pressure (ALPP). Fifteen to 35 ml of fat was harvested from the anterior abdominal wall by liposuction and injected periurethrally under direct cystoscopic vision. Injection was done once in 20 patients and 2 patients received two injections. Follow-up period ranged from 13 to 50 months (mean 31 months). Out of 10 patients with Type III SUI, 4 (40%) were cured, 4 (40%) improved and 2 (20%) incurred failure, giving a total success rate of 80%. Out of 12 patients with Type I SUI only 1 (8.3%) was cured, 5 (41.7%) noticed improvement and 6 (50%) failed. The overall success rate in this group was 50%. The total success rate in the 22 women was 63.6%. There were no major complications.

Autologous fat can be a safe and cost-effective material for injection therapy of SUI. It was shown to be an effective alternative method in the treatment of Type III SUI in female, our results in Type I SUI were, however, disappointing.

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Key Words: • Urinary incontinence, stress • sphincter dysfunction • autologous fat

Introduction

Recently, we have witnessed great advances in the treatment of stress urinary incontinence (SUI) including periurethral injection of bulking agents. These materials act by increasing urethral resistance against abdominal pressure. Their role in the therapeutic armamentarium, however, has been a matter of controversy.[1]

Several bulking agents have been utilized in the treatment of SUI.[2] Most of these agents are either not available or highly expensive to be used in developing communities.

Autologous fat due to its availability, low cost and bio-compatibility is the material which fulfill many criteria of an ideal injectable agent in the treatment of female SUI in developing countries.[3]

To date, only a few clinical studies are available with long-term follow-ups.[4] Despite sporadic reports on complications such as pulmonary embolism following fat injection,[5] the main disadvantage of using fat as a bulking substance, remains the variability of

Correspondence: S. Shakeri M.D., Department of Urology, Shiraz University of Medical Sciences, Shiraz, Iran. P.O.Box: 71345-1473
Email: Shakeris@sums.ac.ir

its resorption and connective tissue replacement.[6,7]

Materials and Methods

A total of 22 women, 10 with Type I and 12 with Type III SUI according to Blaivas classification underwent periurethral injection of autologous fat. Pre-operative evaluation comprised total physical examination, stress test, Bonney test, subjective estimation of amount of daily urinary leakage, and measurement of abdominal leak point pressure (ALPP) by urodynamic method.

Autologous fat was obtained from the anterior abdominal wall. Under cystoscopic guidance, 15-35 ml of the harvested fat was injected submucosally, at the bladder neck and proximal urethra, at 9 and 3 o'clock positions. The procedure was performed under general or spinal anesthesia in 19 patients and local anesthesia in the remaining three. The initial follow-up evaluation was conducted at 4 weeks post-treatment. Post-operative ALPP was determined 3 months after the last injection or whenever the procedure failed.

If incontinence did not improve, subsequent injections were performed; two of our patients met the criteria for re-injection.

A cured state was defined as being completely dry for at least 4 months after the last injection. An improved state was defined as a significant decrease in incontinence episodes.[6] The combined total of cure and improved rates were considered as success rate. If no improvement was seen subjectively and/or objectively the procedure was defined as failure.

The follow-up period ranged from 13 to 50 months (mean 31 months). All data were encoded and analyzed by paired t-test, Fisher's exact test and Pearson's correlation using a computer software package.

Results

Four out of ten patients (40%) with Type III SUI were rendered completely dry, 4 (40%) noticed improvement, and the procedure failed in two. The total success rate in this group was 80%. On the other hand, out of 12 patients with Type I SUI, 1 (8.3%) was cured 5 (41.7%) noticed improvement and 6 (50%) incurred failure, resulting in a success rate of 50%. There was a significant difference in cure rates between patients with Type III and Type I SUI (40% versus 8. 3%, $P<0.0001$).

Seventeen out of 22 patients (77%) were dry within the first month after initial or repeated injection. After 1 month, the results gradually deteriorated but there was no significant change in the outcome of each patient beyond 4 months after the last injection and most of the failures occurred during the first 3 months after the injection.

Overall, the improvement after fat injection was highly significant ($P<0.001$). We noticed transient minor complications (pain, dysuria, hematuria) in 18 patients(80%), however 2 patients developed chronic retention that lasted for about 3 months.

Discussion

Stress urinary incontinence secondary to poor or non-functioning proximal urethra[2,8] was our preferred indication for fat injection. However, in order to assess the effects of fat injection on patients with minimal urethral hypermobility and stress urinary incontinence, these patients were also included in the study.[1,8]

Our results of autologous fat injections in Type III SUI showed an acceptable success rate of 80% in a medium-term follow-up. Patients with Type I SUI showed an overall lower success rate of 50. The significant difference in cure rates between the two groups (40% in Type III versus 8.3% in Type I) indicates that even a mild degree of urethral hypermobility

adversely affects the success rate. The overall success rate in 22 patients who received injection was 63.3% when compared to similar studies with success rates of 23% to 63%.[4,5] The higher success rate in our study might be related to: 1- Dividing the injected fat in 2 parts and injecting at 3 and 9 o'clock bladder neck seperately; 2- More fat injection and over-correction of the sphincter defect. Although similar procedures have been performed by different authors, the optimal volume of fat required for perfect results has not yet been determined. While Santarosa and Blaivas used volumes of 5 ml to 15 ml, 15 ml to 35 ml was used in this study.

The main disadvantage of using fat as a bulking substance is the variability and unpredictability of its reabsorption and the extent of eventual connective tissue formation. For this reason, we injected 2-3 times the amount believed to be necessary for urethral coaptation.

Conclusion

Autologous fat injection is an acceptable method for the treatment of type III SUI. Our results show a higher success rate compared to the previous studies and this is due to higher amount of fat injected and also the choice of two injection sites. The material used is available, biocompatible and cost-effective.

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Short Communication

DETECTION OF 63 kDa PROTEIN IN THE CULTURE SUPERNATANT OF *LEISHMANIA TROPICA* AND *LEISHMANIA MAJOR* PROMASTIGOTES

M. Aminlari, S. Ardehali,** H. Taherian**

**Department of Biochemistry, School of Veterinary Medicine, Shiraz Universtiy,*
***Department of Parasitology, School of Medicine, Shiraz University of Medical Sciences, Shiraz*

ABSTRACT

Reference strains of *Leishmania tropica* and *Leishmania major* were recovered from cryopreservation on NNN medium, and adapted to serum-free brain-heart-infusion(BHI) medium. The cultures were centrifuged and supernatants were kept at –20C°. The protein concentrations of the culture supernatants and extracts were determined and subjected to 20% SDS-PAGE with molecular weight markers. Presence of protein with MW of 63kDa was seen in samples of *L. major* and *L. tropica* with no proteins detected in the BHI-only medium. It is not known if the biological functions of the soluble 63 kDa protein is different from intact GP63 as this molecule has been a candidate for vaccine production.
Irn J Med Sci 2001; 26(1&2): 74-76

Key Words: • *Leishmania tropica* • *leishmanian major* • 63 kDa protein • culture supernatant

During the past two decades, the cell surface of *Leishmania* parasites has been a subject of intensive investigation.[1] Two major glycoconjugates have been identified i.e., lipophosphoglycan (LPG) and the surface protease glycoprotein 63 (GP63). LPG, the major cell surface glycoconjugate of the *Leishmania* promastigotes, is termed excreted factor (EF) and is present in the conditioned medium of *Leishmania*.[2] GP63 the major surface glycoprotein of *Leishmania* parasite is a glycoprotein with an apparent MW of 63 kDa.[3] This surface protease is also present in amastigote form at the lower level. The abundance and location of Gp63 suggest that this enzyme may play a significant role in the infection process.[4] There is no report on the presence of GP63 in the cultured media of *Leishmania tropica* promastigotes. This brief communication presents the identificarion of a 63 kDa protein recovered from culture medium of *L. tropica* promastigotes and compared with *L. major* using SDS-PAGE.

Reference strains of *Leishmania tropica* (MHOM/SU/74/K27) and *L. major* (MHOM/SU/73/5ASKH) were recovered from cryopreservation on Novey- McNeal – Nicolle (NNN) medium at 25 °C. Further cultures were made on Brain Heart infusion (BHI) broth (Merck Chemical Co, Germany) plus 10% Fetal Calf Serum. The promastigotes were finally adapted to serum free BHI medium. The cultured promastigotes were harvested at the end of logarithmic phase with the population of promastigotes approximating

Correspondence: S. Ardehali Ph.D., Dept. of Parasitology, School of Medicine, Shiraz University of Medical Sciences, P.O. Box 71345-1741 Shiraz, Iran. Fax: +98 – 711-2359847

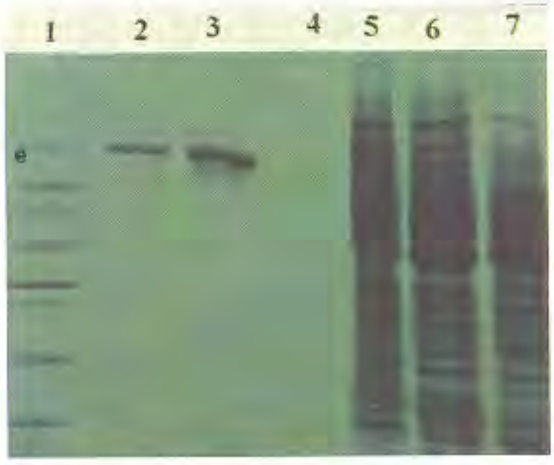

Figure 1: – SDS – Polyacrylamide gel electrophoresis of soluble and intact proteins of *Leishmania* as compared with the MW markers. Column 1 Sigma MW markers. Column 2,3 and 4 present the culture supernatant of *L. major* (5ASKH), *L. tropica* (K27) and the control (BHI only). Column 5,6 and 7 represent the profiles of two *L. major* and one *L. tropica* promastigotes as extracted by Triton X100 respectively.

$6x10^6$/ml. The culture medium containing the parasite was centrifuged at 2000xg in the refrigerated centrifuge at 4 °C for 20 min. The supernatants were satuarated and the protein concentration determined by the method of Lowery et al.[5] BHI medium containing no parasite was used as the control. The culture supernatants were kept at –20 °C until used.

The samples were subjected to 20% SDS–PAGE according to the discontinuous buffer system of Laemmli.[6] Protein samples were prepared by diluting each extract in sample buffer to give final concentration of 1 mg protein/ml in 0.01 M Tris-HCL, pH 6.8, 0.4% SDS, 10% glycerol, 5% 2-mercaptoethanol and 0.04% bromophenol blue. Sigma protein molecular weight markers with molecular weights ranging from 6.5 kDa to 205 kDa were used. Samples from the culture extracts were heated in boiling water for 10 min and 50µl (containing 50 µg protein) was applied to each slot. The protein concentration in culture supernatants was 0.12 mg/ml and no protein was detected in the control BHI medium. 50µl of culture supernatants from *L. tropica* (K27), *L. major* (5ASKH) and control was applied to each slot. The results of SDS-PAGE on extracts of *Leishmania* and culture supernatants are presented in Fig 1. Column 1 represents the protein molecular weight markers ranging from 6.5 kDa to 205 kDa. Columns, 2, 3 and 4 represent the culture supernatants of *L. major, L. tropica* and the control (BHI only). Columns 5,6 and 7 represents two *L. major* and one *L. tropica* promastigotes extracted by Triton x 100. Presence of a protein with the MW of 63kDa is seen in samples 2 and 3. It is not known whether the biological functions of the soluble 63kDa protein is different from the intact GP63 as this molecule has been a candidate for vaccine production. It would be of interest to determine the similarities or differences between these two molecules in regard to their structures and functions. Monoclonal antibodeis against GP63 have been prepared which could help to determine the true identity and biological functions of these molecules.

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Short Communication

A NEW TREATMENT REGIMEN FOR ADVANCED PANCREATIC CANCER: A PRELIMINARY REPORT

M.J. Saalabian

Department of Radiation Oncology, Shiraz University of Medical Sciences, Shiraz

ABSTRACT

The disturbingly rising trend of pancreatic carcinoma (PC) has been an impetus for a growing number of chemotherapy trials in recent years. We report the preliminary results of a novel clinical evaluation of mitomycin + capecitabine in 11 patients with advanced PC. An encouraging 63% overall response rate including two complete remissions and a limited manageable toxicity profile were noted.

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Key Words • Pancreas • cancer • chemotherapy

Introduction

During the past decades we have witnessed a worldwide upsurge in the incidence of pancreatic cancer.[1] Based on the current available data, however, there is no standard effective treatment for the disease and the condition is still associated with a high mortality and morbidity.[2,6]

Materials and Methods

Eleven patients with histologically or cytologically documented adenocarcinoma of the pancreas who attended Shiraz University of Medical Sciences Radiation-Oncology Department were enrolled in this study. Patients with active metastases or medical conditions interfering with the treatment schedule were excluded. All patients were viewed inoperable or had progressive disease following the previous cytodestructive or cytotoxic therapy.[3] Clinical and lab data included adequate hematologic, hepatic and renal function tests. The lesions were assessable to bi-dimensional measurement and evaluated by circulating biomarker (CEA, CA-125)[4,5] relative to the baseline values. Before entering the study, a written consent was taken from each patient.

The treatment consisted of 8 mg/m^2 iv mithomycin-C administered on day 1, and 1500 mg/m^2 capecitabine[7,8] po, administered on days 1 to 14, and days 21 to 35. The regimen cycled every 42 days. Standard antiemetic drugs were administered as needed. If the patients experienced capecitabine-associated dermatitis or hand foot syndrome, 200 mg of pyridoxin bid was added to the regimen.

All included patients had follow-up scans and serum biomarker assays after each two cycles of the treatment. Those with documented response and sustained results at

Correspondence: *M.J. Saalabian, M.D., Dept. of Radiation Oncology, Namazee Hospital, Shiraz, Iran*

four-week intervals were considered as partial or complete responders. The planned regimen aimed at treating patients for a total of 6 cycles of chemotherapy to be interrupted only if there was evidence of progression of the disease or unacceptable toxicity of the drugs.

Results

The preliminary outcome observed in this trial was promising; the overall response rate in the first 11 patients was 63% (2 complete and 5 partial responses). The quality-of-life indicators including pain relief represented a major palliative outcome among responders.

One case showed grade 4 mucositis, 2 developed thrombocytopenia, 4 developed grade 2 anemia, and 3 patients experienced neutropenia. No patient developed nephrotoxicity, pneumotoxicity, febrile neutropenia or mortality due to drug toxicity. Significant hand-foot syndrome was observed in 3 patients. Mild diarrhea and nausea were rare findings.

Discussion

The preliminary results observed in this study represents an alternative to provide alliviation of symptoms in this notoriously dismal condition. Whether chemotherapy with mithomycine-C and capecitabine, combined with radiation therapy, provide better therapeutic results in those with unresectable locoregional pancreatic cancer remains to be solved. Further studies are required to shed light over different aspects of this alternative regimen.

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Case Report

WILSON'S DISEASE PRESENTING WITH THREE UNUSUAL FEATURES

M.H. Imanieh, Z. Karamizadeh

Department of Pediatrics, Shiraz University of Medical Sciences, Shiraz

ABSTRACT

A 9-year-old boy with Wilson's disease and a combination of three uncommon clinical manifestations (cataract, gallstones and bone lesions) without significant CNS and liver involvement is described.

The most common presentations of Wilson's disease are different types of liver involvement, CNS manifestations and hematologic disorders.

Review of literature shows association of Wilson's with different presentations but to the best of our knowledge this patient is the first case of Wilson's disease with a combination of three uncommon features.

Physicians should consider Wilson's disease as a differential diagnosis when encountering these features singly or in combination.

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Key Words: • Wilson's disease • cataract • gallstone • bone lesions

Introduction

When Wilson's disease was first described in 1913, it was considered a degenerative disorder of the central nervous system associated with asymptomatic cirrhosis.[1] Hepatic symptoms were then added by Hall[2] 10 years later, who meanwhile also proposed hepatolenticular degeneration for this entity.

Today, it seems that the disease has a wide variability of expression with involvement of the blood, joints, bone and kidneys, in addition to the liver and brain.[3,4,5]

Here, we describe a boy with Wilson's disease who presented with gallstones, cataract and bone lesions. Although association of these features has been individually described before, we encountered no report with a combination of these manifestations in a single case.

Correspondence: *M.H. Imanieh M.D., Dept. of Pediatrics, Namazee Hospital. Tel: 0711-8222099, Fax: +9871-6264000, E-mail:IMANIEHM@sums.ac.ir*

Case Presentation

This 9 year-old male was jaundiced at the age of 7 which was diagnosed as hepatitis. He then remained asymptomatic until the age of 8, when he developed pain in both knees and thighs with difficulty in ambulation which persisted for a few months.

One year later, he was admitted to the hospital complaining of abdominal pain.

At that time, findings also included Kayser-Fleischer rings, cataract, hepatomegaly and non-palpable spleen. He had tenderness in the right upper quadrant. His serum ceruloplasmin was 0.03 gram/L. (normal 0.233-0.402 gram/L) and urine copper concentration was 600 μg/24hrs (normal > 75 μg/24hrs), respectively.

He also had hyposthenuria and microscopic hematuria. The liver enzyme values and prothrombin time, however, were normal.

Abdominal ultrasonography revealed multiple stones in the gallbladder despite normal reticulocyte count, normal Coombs' test and normal Hgb electrophoresis.

A roentgenographic study showed bone demineralization and lytic lesions in the distal part of both femurs.

The patient underwent cholecystectomy, and D-penicillamine therapy was initiated. The cataracts resolved with medical therapy and did not require surgical management.

After 9 years of therapy, the patient remained asymptomatic with no detectable clinical or laboratory abnormalities. He has graduated from secondary school and is an engineering student now.

Discussion

Hepatolenticular degeneration is a rare inborn disorder of copper metabolism inherited as an autosomal recessive trait.[1] It is characterized by central nervous system degeneration, cirrhosis and renal dysfunction. Other associated features may include Kayser-Fleischer (K.F.) rings, which is the most common non-hepatic, non-neurologic manifestation of Wilson's disease,[6] and sunflower cataract.[7-13]

The diagnosis of this disease is based on a combination of clinical and para-clinical findings (jaundice, hepatomegaly, cataract, positive K.F. rings, low ceruloplasmin, high urinary copper) and good response to D-penicillamine. It is not necessary to perform liver copper measurement or repeated determination of urinary copper for diagnosis.[3]

During the past decades, the less common manifestations of Wilson's disease, including disorders of the blood, joints, and skeletal system, have been recognized more frequently.[16-32] They may occur at any time during the course of the illness but are usually preceded by symptoms of liver or nervous system disease.[6] However, a number of cases have reportedly begun with hemolytic anemia.[19] In our case, gallstones, as a sign of hemolysis and/or liver involvement,[6,33,34] manifested themselves at 9 years of age and only after mild hepatic symptoms.

The gallstones were not accompanied by any sign of hemolysis, abnormal reticulocyte count, positive Coombs' test, or abnormal Hb electrophoresis; unfortunately, no gallstone analysis was performed.

A variety of skeletal changes have been observed in patients with Wilson's disease. These include osteoporosis,[20-23] rickets,[24-26] osteomalacia,[20] spontaneous fractures,[27,28] osteochondritis dissecans and osteoarthritis.[29-32]

Although pediatric patients rarely have significant skeletal changes on radiographs, our patient had bone demineralization and lytic lesions at 9 years of age.

Sunflower cataracts are reported less frequently than K.F. rings[15] and, when present, they are invariably accompanied later by Kayser-Fleischer rings. Most of these cataracts resolve with therapy and do not affect vision. Our patient had sunflower cataract that resolved after 2 years of therapy.

This patient is insofar unique as he incorporated 3 uncommon presentations of the disease (cataract, gallstones, bone disease) in childhood.

To the best of our knowledge, a combination of these manifestations has not been previously reported in a single patient.

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Case Report

COMBINATION OF INSULIN-DEPENDENT DIABETES MELLITUS AND GRAVES' DISEASE IN A PATIENT WITH DOWN'S SYNDROME

H. Karamifar, G.H. Amirhakimi

Division of Pediatric Endocrinology & Metabolism, Shiraz University of Medical Sciences, Shiraz

ABSTRACT

The prevalence of autoimmune disorders is increased in Down's syndrome. The most common autoimmune diseases associated with Down's syndrome, is related to the thyroid gland. Other autoimmune diseases including diabetes mellitus and hypoparathyroidism have also been reported. To our knowledge only two cases with insulin dependent diabetes mellitus and hyperthyroidism associated with Down's syndrome have been reportd previously in the literature. The two reported cases had no ocular complications. We describe a 9 year old girl with Down's syndrome who developed diabetes mellitus six months prior to admission. Physical examination revealed bilateral exophthalmus, mongolian face, a grade 3 goiter and moist skin. Laboratory data revealed blood sugar 731 mg/dl, TSH 0.01 µu/ml, T4 18.1 µg/dl and T3 481 ng/dl. She subsequently developed also cataracts. We suggest that children with Down's syndrome should be evaluated for endocrine diseases and ocular complications.

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Key Words: • Down's syndrome • diabetes mellitus • Graves' disease

Introduction

Down's syndrome is one of the most frequent chromosomal disorders and the most common cause of mental retardation in human.[1] It is well known that patients with Down's syndrome have an increased prevalence of autoimmune disorders.[1-3] Autoimmune disturbances of endocrine system including diabetes mellitus, adrenal insuficiency, chronic active hepatitis and particularly hypothyroidism are common in Down's syndrome. There are also reports of hyperthyroidism in such patients.[4] Hyperthyroidism during childhood with a few exceptions is due to Graves' disease.[5] The prevalence of thyroid diseases in children with Down's syndrome is 3% which is higher than that found in the general population.[3,6]

Herein, we describe a girl with Down's syndrome, hyperthyroidism and diabetes mellitus, who subsequently developed cataract. To the best of our knowledge, only two similar cases with this triad albeit without cataracts have been reported in the literature.[7,8]

Case Report

A 9-year-old girl, a known case of Down's syndrome was admitted at the Pediatric Endocrine Ward of Nemazee Hospital for the control of her blood sugar. She was born to a 34-year-old mother and was the fourth child of

Correspondence: *H. Karamifar M.D. Division of Pediatric Endocrinology & Metabolism, Shiraz University of Medical Sciences, Shiraz, Iran. Fax: +9871-6265024*

the family; her sibblings were healthy and alive. She developed hyperbilirubinemia at 4 days of age for which she underwent an exchange transfusion. The diagnosis of Down's syndrome was made during infancy and chromosome study revealed 47 XX + 21 karyotype. Six months prior to this admission, she developed polyuria, polydypsia and was admitted to a general hospital with a blood sugar level of 790mg/dl. A diagnosis of insulin-dependent diabetes mellitus was made and insulin therapy was begun. She was discharged with daily injections of NPH insulin. At the time of present admission, she had a history of weight loss, sweating, and irritability. On physical examination, she had a pulse rate of 130/minutes, blood pressure of 125/60 mmHg, a temperature of 36.5 °C, a weight of 18.5 kg and a height of 127 centimeters. She had a grade 3 goiter and bilateral exophthalmus with eyelid retraction, tremor of fingers and moist skin.

The thyroid function test was as below: TSH 0.01 μu/ml, (Nl 0.3 - 3.8), T_4 18.1 μg/dl (Nl 4- 12) and T_3 481 ng/dl (Nl 80- 200). The blood sugar was 731 mg/dl, serum sodium 138 mEq/L, potassium 4.2 mEq/L, chloride 104 mEq/L, creatinine 0.7, calcium 9.1 mg/dl, phosphorus 3.7 mg/dl, albumin 3.5 g/dl, with a normal CBC and platelet count. The therapy was started with methimazole 5 mg twice daily. Seventy five days later, the patient became clinically euthyroid and thyroid function tests were as follows: TSH 6.4 μu/ml, T3 173 ng/dl and T4 6.3 μg/dl. The methimazole dose was decreased to 5 mg/day. After 4 years, methimazole was discontinued and she remained euthyroid with a grade 1B goiter. Two years later, she developed bilateral cataracts and surgery was carried out. At this time serum calcium was 9.4 mg/dl, phosphorus 4.6 mg/dl, alkaline phosphatase 457, cholesterol 160 mg/dl, triglyceride 200 mg/dl, BUN 14, sodium 138 mEq/L, potassium 3.8 mEq/L and the blood sugar was 611 mg/dl. Cardiovascular examination revealed no abnormalities. Pain in the left knee joint was the only further complaint during her follow-up. With normal laboratory investigation including negative rheumatoid factor, negative CRP, WBC 9000, polymorphonuclers 76%, lymphocytes 27%, monocytes 6% and ESR of 18, spontaneous improvement was noted after a few weeks.

Discusssion

Auto-antibodies are frequently present in Down's syndrome as markers of the autoimmune disease. The primary defect in this syndrome is in the cell mediated immunity.[5,6] Increased prevalence of autoimmune-induced thyroid disorders in insulin-dependent diabetes mellitus and vice versa is well estabilished.[7] Although thyroid antibodies are common in Down's syndrome, other auto-antibodies are also found and autoimmune diseases including diabetes mellitus and hypoparathyroidism have been reported.[5,6] Karlsson et al.[1] found 2 cases of hyperthyroidism in 85 patients with Down's syndrome. In Down's syndrome, autoimmune thyroid disease is uncommon in pre-school children and usually occurs after the age of 8. The reported prevalence of cataracts in patients with Down's syndrome varies.

DA Cunha et al.[9] reported a prevalence of cataracts, in 13% of patients with Down s syndrome who were mostly above 12 years of age. Cataracts are uncommon in children and young adolescents with diabetes mellitus and are usually associated with prolonged and poor control of the disease.[10] Our patient had a poor metabolic control. Hyperthyroidism could be a contributing factor in the poor metabolic control of the patient.[11] The pathogenesis of diabetic cataracts in human is not well understood. The fact that clinically significant cataracts develop in only a small percentage of diabetic children suggests that the degree of hyperglycemia per se may not be the only factor involved in cataract

formation.[10] When blood sugar is high, the excess glucose within the lens is reduced to sorbitol by the enzyme aldose reductase. Sorbitol accumulates in the cell fibers and draws water into the lens by osmotic pressure and results in cataract formation.[12] It is suggested that children with Down's syndrome should be evaluated for endocrine diseases and eye complications.

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Case Report

NEVOID BASAL CELL CARCINOMA SYNDROME

M. Sardari-Kermani, M. Mehrazin***

**Department of Radiotherapy, the Cancer Institute, and **Department of Neurosurgery, Shariati Hospital, Tehran University of Medical Sciences, Tehran*

ABSTRACT

A 15-year-old man was referred to our department with multiple lesions on the scalp, neck, abdomen, and back which were proved to be basal cell carcinomas. The patient had a history of craniotomy at 3 years of age for exision of a cerebellar medulloblastoma, followed by craniooaxis irradiation post-operatively. On physical examination, hypertelorism, broad nose bridge and a slightly bulged out chest cage were noted. Chest X-ray revealed deformity of the chest wall. A diagnosis of nevoid basal cell carcinoma (NBCC) syndrome was made.

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Key words: • Nevoid BCC syndrome • medulloblastoma • radiotherapy

Introduction

NBCC syndrome is a genetically linked disorder, with an autosomal dominant inheritance.[1] The gene responsible for this syndrome is located on the chromosome 9q. Both sexes are affected equally. Worldwide, the approximate prevalence is reported as between 1/60,000 and 1/200,000. Most patients are Caucasians, but cases have been reported among Africans and Asians.

This syndrome is seen early in life, and is characterized by multiple basal cell carcinomas (BCCs), associated with various skeletal abnormalities and sometimes mental retardation.[2] Skin lesions are usually present in large numbers, and may reach up to several thousands. They are located predominantly on the trunk, face, neck, and in the pre-auricular area.[3] The lesions are nodular, ulcerative, and locally aggressive, causing significant destruction of the underlying tissues.[4] Apart from the involvement of soft tissues, this disorder is often associated with a variety of skeletal, ocular, neurological and endocrine abnormalities. Skeletal abnormalities include cystic bony changes, which may be multiple and unilateral. Defective dentition, bifid ribs, scoliosis, kyphosis,[5] enlarged occipito-frontal circumference, cord defects and broad nasal root are other commonly associated abnormalities.[6] Neurological abnormalities may include congenital agenesis of the corpus callosum and medulloblastoma.[7]

Case report

This was the second referral of a 15-year-old male with multiple lesions on the scalp, neck, abdomen, and back (Fig. 1). Lesions (black spots) initially appeared on the palms and soles and later on extended to the abdomen and back of the neck. Some of the lesions started to grow in size later and a biopsy taken from a couple of lesions revealed BCC.

Correspondence: *M. Sardari-Kermani M.D., Dept. of Radiotherapy, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran.*

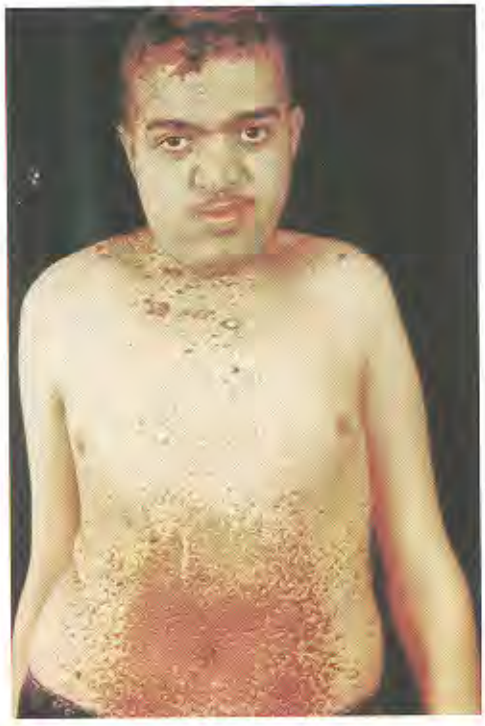

Figure 1: Multiple lesions on the scalp, neck and abdomen in a patient with NBCC.

The patient had a flat nose root with slight pigeon chest deformity. The heart and lungs were normal. No hepatosplenomegaly was found. Chest x-ray showed mild deformity of the chest wall with approximation of the ribs on both sides. OPG showed no evidence of bony cysts.

All laboratory data including CBC, BUN, creatinine and liver function tests were within normal limits.

At his previous referral, twelve years earlier, at the age of three, the patient had been brought to us for radiotherapy following incomplete excision of a medulloblastoma (desmoplastic type) of the cerebellum. A full course of external whole cranioaxis radiation treatment using a cobalt machine, delivered 3000 centigray to the entire cranioaxis plus 2000 centigray to his posterior fossa. He tolerated the treatment well and subsequently had no signs of recurrence.

His mother stated that the skin lesions had developed a month after the initiation of his radiation treatment. The patient refused surgical excision of the large lesions, but flat lesions responded well to efudix (5 -FU) ointment and, as a whole, he responded relatively well to a combination of chemotherapy containing cis-platinum, bleomycin, and high dose methotrexate with calcium folinate (leucovorin).

Discussion

NBCC is an autosomally inherited disease and most commonly manifests itself with either basal cell carcinomas (usually multiple) occurring in the third decade and earlier, or as an odontotic keratocysts presenting in the second or third decade. Diagnosis is based on the presence of major and minor criteria. Despite recent understanding of the underlying genetic basis of NBCC, the diagnosis remains clinical. BCCs in this disorder occur relatively early and tend to develop after exposure to ionizing radiation after a brief latency period.[8] There is a predisposion for BCCs of the skin, medulloblastoma, and ovarian fibromas. Treatment of medulloblastoma with radiotherapy results in a marked increase in the number of BCCs within the field of irradiation. In our case, too multiple BCCs developed shortly after radiotherapy for medulloblastoma. Frequency of medulloblastoma in this syndrome probably does not exceed 5% and may be as low as 1%.[9] The therapeutic choices are similar to those for BCC. The lesions are best treated early by excision, cautery or cryotherapy. Laser treatment has the advantage that lesions can be treated in a short period of time.[10] Bleomycin mediated electrochemotherapy,[11] intralesional application of interferon alpha -2b and photodynamic therapy with 5-amino-levulenic acid may also be valid therapeutic options for flat lesions.[12]

In our case, since some of the lesions were large and ulcerating, we applied low dose x-ray therapy with a combination chemotherapy in order to alleviate the symptoms of the lesions. Apart from one, all of the large lesions healed. Many of the multiple small lesions dropped off, showing more of the underlying skin of the abdomen and back. Superficial flat lesions also responded well to local application of 5-FU 5%.

Acknowledgement

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Letter to the Editor

SERUM FOLATE LEVEL IN MINOR THALASSEMIA

Dear Editor:

The study of folic acid status in beta-thalassemia minor has not been carried out on a large scale, and no data comparing children and adults are available. In two traceable case reports, folate therapy resulted in the elevation of hemoglobin (Hb) and was interpreted as folate deficiency.[1,2] Now, it is routine to prescribe folic acid for minor thalassemic children and pregnant women.[3,4] In this study, the serum level of folic acid was determined in 75 newly diagnosed minor thalassemic (MT) children (aged 3-15 years), their normal siblings as well as normal children and minor thalassemic parents. The history of nutritional status especially in regard to the consumption of fruits, vegetables and salads, was recorded.

Serum folate (SF) was low (<3 ng/ml) in 43 (57.3%) MT children and in 16 (21.3%) MT parents. It was borderline (3.1-20 ng/ml) in 32 (42.7%) children and 59 (78.7%) parents with MT.

In comparison, SF was borderline in 35 (46.7%) healthy siblings and normal in the other 40 (53.3%), while SF was borderline in 23 (30.7%) healthy parents and normal in 52 (69.3%). The difference in SF between MT children and their healthy siblings was statistically significant (P<0.0001). The difference between SF of MT adults and healthy adults was also highly significant (P<0.0001). The history of consumption of salad and vegetables at least three times a week was significantly correlated with the borderline folate level (instead of the low folate level) (p<0.0001). When folic acid was prescribed for minor thalassemic children, it was followed by a rise in Hb

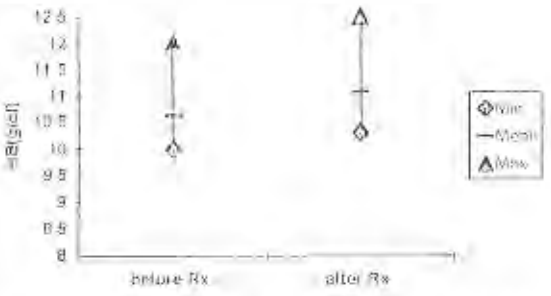

Figure 1: Effect of one miligram of folic acid for 3 months on hemoglobin level of 75 minor thalassemic children

[mean = 0.46 g/dl (range 0.3-1.5 g/dl)] (P<0.0001).(Fig. 1)

We conclude that serum folate is low in both children and adults with minor thalassemia compared to healthy family members. Folate deficiency is also more common in children than adults and it is well correlated with a history of low salad and vegetable intake in children. Moreover, prescription of one mg folic acid QOD is followed by a rise in Hb.

Dr. Shahriari M. M.D.

Department of Pediatrics
Shiraz University of Medical Sciences
Shiraz

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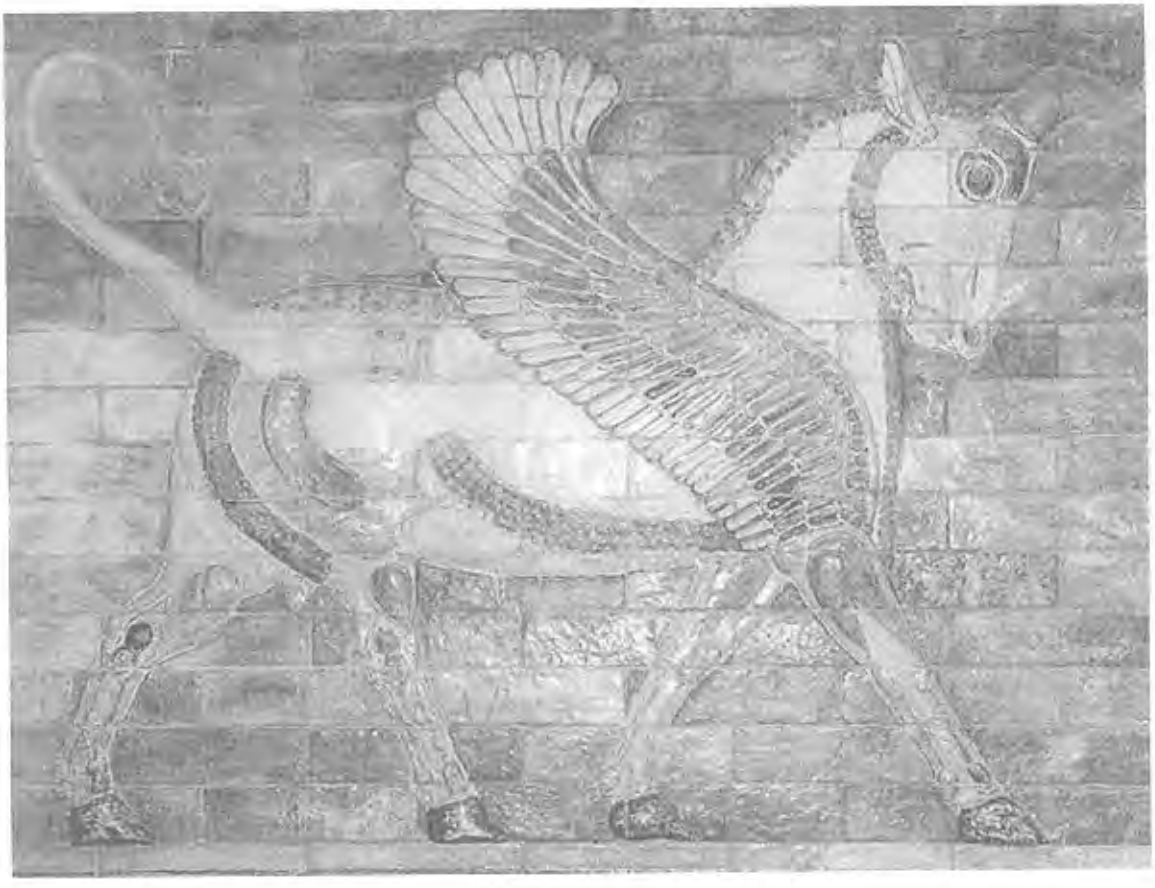

Winged Bull from the Apadana Palace, Achemenid era(558-330 BC), Persepolis, near Shiraz