

Sclerosing Cholangitis Induced by Intra-Biliary Tree Injection of Silver Nitrate or Hypertonic Saline Solutions in Rabbit

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Abstract

Background: One of the most serious late complications, after evacuation of liver hydatid cyst and instillation of scolical agents is sclerosing cholangitis, most probably caused by the type of scolical agents instilled.

Objective: To determine the incidence of sclerosing cholangitis after intra-biliary tract infusion of 5% saline and 0.5% silver nitrate.

Methods: 5% hypertonic saline and 0.5% silver nitrate were injected trans-hepatically into the gallbladder of two equal groups (n=15/group) of rabbits. 4 months later, the livers were biopsied and cholangiography was performed, investigating for signs of sclerosing cholangitis (SC).

Results: 30% of the rabbits in group A (hypertonic saline) and 80% of group B (silver nitrate) developed SC. The prevalence of SC and also the severity of pathological findings were significantly ($p < 0.05$) higher in group B (silver nitrate). 5% hypertonic saline seems to cause a significant amount of sclerosis.

Conclusions: For treatment of hydatid cyst, intra-cystic injection of 5% hypertonic saline or 0.5% silver nitrate is not safe.

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Keywords • Echinococcosis • sclerosing cholangitis • silver nitrate • hypertonic saline • Echinococcus granulosus • Iran

Introduction

Surgery is the treatment of choice for hydatid cyst.¹⁻³ Among currently used methods, the most common surgical procedure is evacuation of the cyst and instillation of a scolical agent into the cyst cavity for sterilization and prevention of spreading.¹⁻⁶ Several scolical agents, like Savlon, hypertonic saline, silver nitrate, chlorhexidine, formaldehyde, ethyl alcohol, hydrogen peroxide, mebendazole, albendazole and povidone iodine have been practiced throughout the world. Nonetheless, many of them have been abandoned due to their early or late complications.⁴⁻¹⁰

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Table 1: Cholangiographic results of groups A and B		
Finding	Group A (n=11)	Group B (n=11)
Normal	7 (64%)	2 (18%)
Non-conclusive	1 (9%)	1 (9%)
Mild narrowing of CBD	1 (9%)	1 (9%)
Mild narrowing of Lt hepatic duct plus moderate narrowing of CHD	0	1 (9%)
Mild beading of CBD and CHD	0	1 (9%)
Moderate narrowing of CBD and CHD	1 (9%)	1 (9%)
Moderate focal stenosis of right hepatic duct	0	2 (18%)
Moderate focal stenosis of left hepatic duct	1 (9%)	0
Severe focal stenosis (beading) in right hepatic duct, mild diffuse stenosis in CHD	0	1 (9%)
Mild diffuse stenosis of CHD	0	1 (9%)
Sclerosing cholangitis	3 (27%)	8 (73%)

CBD: common bile duct, CHD: common hepatic duct

In Iran, where is endemic for echinococcosis,¹¹⁻¹³ evacuation of the cyst and instillation of 0.5% silver nitrate as a scolicedal agent is the most common surgical procedure for treatment of hydatid cyst.⁴⁻⁶

One of the most serious late complications occurred after the evacuation of the cyst and instillation of various scolicedal agents is sclerosing cholangitis (SC), which is a lethal.^{14,15} So far, several investigations on the incidence of this complication after administration of various agents have been performed.¹¹⁻¹⁵ As the result, some of them such as formaldehyde were abandoned.⁴ However, there has been no recent study on silver nitrate and its possible complications.

The aim of this study was to evaluate and compare the incidence of SC following the injection of the two most commonly-used scolicedal agents in Iran, *i.e.*, 0.5% silver nitrate and 5% hypertonic saline solution.

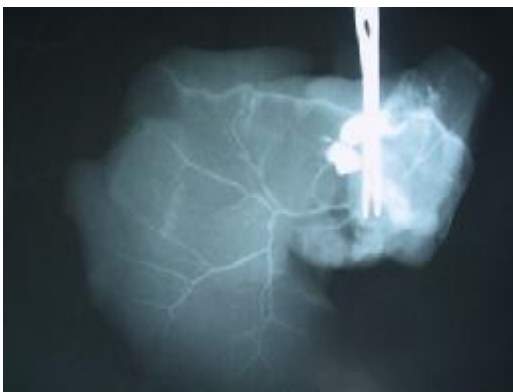


Fig 1: Cholangiogram of a rabbit from group A (silver nitrate) after four months of injection. Findings are in favor of sclerosing cholangitis.

Materials and Methods

Instillation of the agents

Thirty Dutch rabbits weighting 2000 to 2500 gr selected and randomized into two equal groups. After general anesthesia with intramuscular injection of ketamine chlorhydrate (44 mg/kg) plus xylazine (8 mg/kg), after prep and drape, through a right intercostal incision, gallbladder and common bile duct were identified. Then, with a 25G needle the solutions were trans-hepatically injected into the gallbladder, and the common bile duct compressed with the fingers for 5 min. In group A, 0.3 ml of 5% hypertonic saline and in group B, 0.3 ml of 0.5% silver nitrate was injected. Laparotomy incisions were closed in two layers.

Histopathological evaluations

After 4 months, a second operation was performed. Three liver biopsies were taken from each



Fig 2: Cholangiogram of a rabbit from group B (hypertonic saline) after four months of injection. Findings are in favor of sclerosing cholangitis.

Table 2: Pathological findings in biopsies done after 4 months		
Finding	Group A	Group B
Kupffer's cell hyperplasia	4 (36%)	10 (91%)
Hydropic (Ballooning) degeneration	7 (64%)	9 (82%)
Binucleation	4 (36%)	9 (82%)
Portal space lymphocytic infiltration	11 (100%)	11 (100%)
Ductule hyperplasia	2 (18%)	10 (91%)
Sinusoidal dilation	10 (91%)	10 (91%)
Nodule formation	0	2 (18%)
Fibrosis	0	1 (9%)
Flattened epithelial cell	0	1 (9%)
Main duct dilation	2 (18%)	2 (18%)
Piecemeal necrosis	1 (9%)	2 (18%)
Lobular necrosis	0	1 (9%)

rabbit and immediately fixed in 10% formalin solution. Afterwards, the liver, the common bile duct and the duodenum were resected, enblock, inserted into normal saline solution, covered with ice and immediately underwent cholangiographic studies. These specimens were also fixed in 10% formalin for histological examinations. Three pathologists did the histological examination and scorings. The final score reported was the average result reported by the three pathologists. The surgeons, pathologists and radiologists did not know which rabbit belongs to which group and what kind of drug have it received.

Histological examinations were performed to assess the followings:

1. Lesions of the portal and centrilobular veins lining.
2. Kupffer's cell hyperplasia
3. Portal space (for evaluation of its size, biliary epithelial cells height, nuclear orientation and connective tissue) and bile ductile lesions.
4. Sinusoidal lesions (dilatation, fibrosis)

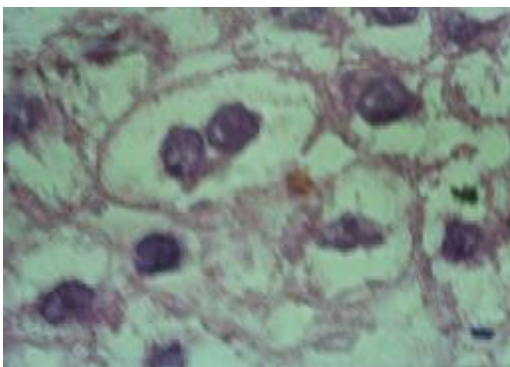


Fig 3: Binucleation of hepatocytes in liver biopsy of a rabbit from group A (silver nitrate) after 4 months of injection.

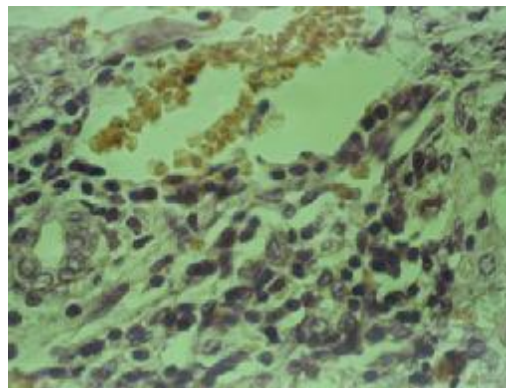


Fig 4: Kupffer's cell hyperplasia, hydropic degeneration of hepatocytes, sinusoidal dilation, and ductule hyperplasia in a rabbit from group A.

5. Cellular infiltration in the lobule or portal space, especially in the periductal areas.
6. Lesions of hepatocytes (Hydropic degeneration, Binucleation)
7. Main bile duct dilatation
8. Nodule formation

Cholangiographic studies

Cholangiograms were performed by injection of 1 ml of 50% contrast agent diluted in normal saline, instilled directly into the gallbladder or common bile duct, while the ampula was clamped. Cholangiograms were reviewed by the team's radiologist, who was blind to the treatment groups. Scoring of mild, moderate and severe changes were recorded.

Statistical analysis

Sample size was estimated based on a two-tailed test of difference between proportions in independent groups at $\alpha = 0.05$. One-sample Kolmogorov-Smirnov test, Student's t-test, generalized estimation equations, and logistic regression model were used to analyze the data. A p value <0.05 was considered significant.

The National Research Council's guide for National law on the care and use of laboratory animals was followed.

Results

Eight rabbits (4 in each group) died during the first and the second weeks post-operation and were excluded from the study.

Radiological results

Thirty percent of the rabbits in group A (hypertonic saline) and 80% of rabbits in group B (silver nitrate) developed radiological findings in favor of

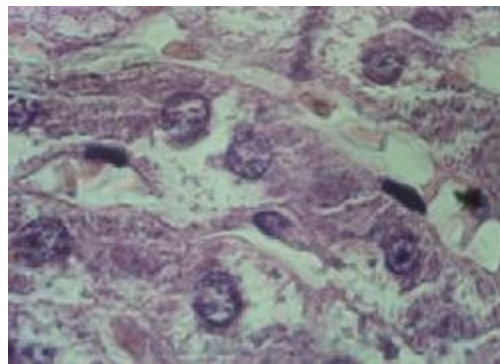
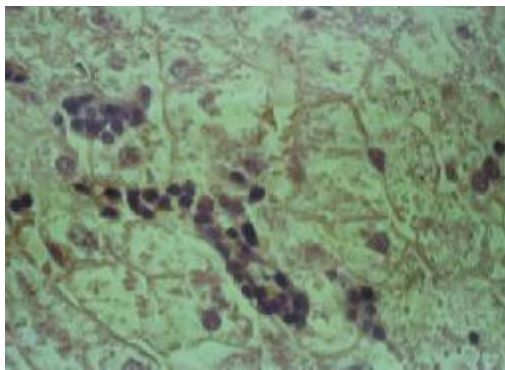


Fig 5a, 5b: Kupffer's cell hyperplasia, hydropic degeneration of hepatocytes, sinusoidal dilation, and portal space lymphocytic infiltration in a rabbit from group B.

SC (Table 1). SC was significantly ($p < 0.05$) more prevalent in group B than group A. Normal cholangiograms and rabbits with SC are shown in Figures 1 and 2.

Pathologic results

According to histological findings, all cases in both groups had some findings in favor of early stages of SC (Table 2). There were no lesions of the central vein in both groups. Portal space lymphocyte infiltration was observed in all of the rabbits. Histological findings and pictures are shown in Figures 3, 4 and 5.

Pathological Scoring

For statistical analysis, we used a quantitative scoring system according to the importance of each pathological finding¹⁵:

Kupffer's cell hyperplasia: Absent (0), mild (1), moderate (2), severe (3).

Hydropic degeneration of hepatocytes: Absent (0), mild (1), moderate (2), severe (3).

Portal space infiltration: Absent (0), mild (1), moderate (2), severe (3).

Sinusoidal dilatation: Absent (0), mild (1), moderate (2), severe (3).

Binucleation of hepatocytes: Rare (1), Frequent (2)

Ductule hyperplasia: Absent (0), present (2)

Nodule formation: Absent (0), present (4)

Piecemeal necrosis: Absent (0), present (4)

Lobular necrosis: Absent (0), present (3)

The mean score for group A was 4 (range: 2–7) and for group B was 9.1 (range: 5–15). Although the reported scores were normal for both groups,¹⁵ the difference between the two groups were statistically significant ($p < 0.001$).

Discussion

The most common surgical procedure used for liver hydatid cyst in Iran and few other countries, is partial cystectomy, omentoplasty, evacuation of the cyst and instillation of the scolicalid agent into the cyst cavity and unroofing the cyst.^{2-6,16} The scolicalid agent is injected to destruct the scoleces and also to prevent spillage and recurrence.

So far, several scolicalid agents have been used. Among them, hypertonic saline solution is the most commonly used scolicalid agent in the world and silver nitrate is the most-commonly used agent in Iran.^{6,11,16}

Silver nitrate solution (0.5%) was suggested to have no toxicity for the host.¹⁶ Additionally, some experimental studies, observed no toxicity or sclerosing cholangitis with injection of 5% hypertonic saline into the biliary tract of rats.⁷ However, several cases of SC have been reported after surgery for liver hydatid cyst.¹⁷ Initially, post-operation sclerosis was hypothesized to be a secondary SC caused by periductal abscess or surgical trauma to the biliary tree, a phenomenon that was eventually ruled out.^{1,14} Therefore, the role of solutions instilled into the cystic cavity has been suspected and several investigations were performed thereafter.^{1-10,17} A cysto-biliary fistula was presumed to allow the passage of these solutions into the biliary tract. Thus, a caustic lesion by the scolicalid solution having passed from the cyst into the biliary tree was regarded as a reasonable mechanism.¹⁴ Nowadays, there is a significant emphasis on not using hypertonic saline or silver nitrate when the fluid color suggests biliary communication.²⁻⁴

In our experiment, the scolicalid solutions were directly injected into the gallbladder and the common bile duct was clamped for 5 min. Histological examination showed that both 0.5% silver nitrate

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and 5% hypertonic saline induced lesions of intra- and extra-hepatic biliary tree. These lesions consisted of Kupffer's cell hyperplasia, proliferation of the bile ductule, hydropic (ballooning) degeneration, binucleation of hepatocytes, piecemeal and lobular necrosis, nodule formation, and sinusoidal and main duct dilation. However, the severity of the pathological changes was significantly ($p < 0.05$) higher in group B than group A.

The pathogenesis of SC secondary to these agents is still unknown. But most likely it has an immunological basis, which can explain their late development and also their development in areas far from areas that are directly exposed to scolical agents.⁷

Although previous experiments in animal models suggested no or minimal effect of hypertonic saline in inducing SC, as supported by an experiment in rats by Houry *et al*,⁷ our experiment documented high incidence of histological and radiological changes in favor of SC in rabbits treated with 5% hypertonic saline. Additionally, there was no experimental study on the real incidence of SC after injection of 0.5% silver nitrate into the biliary tree in the literature. In our experiment, the incidence of this complication was 100%.

In conclusion, we suggest that because of the potential risk for lesions of the biliary tree, the intra-cystic injection of silver nitrate should be avoided. This recommendation might be also valid for 5% hypertonic saline.

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