

Effect of Garlic Extract on Cutaneous Leishmaniasis and the Role of Nitric Oxide

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Abstract

Background: The production of nitric oxide (NO) is important in the final outcome of leishmaniasis in animal models.

Objective: The efficacy of garlic therapy and a combination of garlic and vitamin A were compared with an antimonial drug (glucantime) to assess healing and regulation of NO release in mice infected with *Leishmania major*.

Methods: The mice were subjected to 3×10^5 promastigotes and allowed to induce a lesion. The mice were treated with above compounds twice a day for 45 days. The diameter of the lesion was measured on days 1, 10, 20, 30 and 45. In a separate experiment, NO release by peritoneal macrophages was measured.

Results: The diameter of lesion was reduced by aqueous extract of garlic within 30 days of treatment. However, the maximum reduction was induced when mice were subjected to vitamin A for 10 days before the administration of the aqueous extract for 30 days. A significant correlation between healing and the amount of NO release was also found.

Conclusion: A combination of aqueous extract of garlic and vitamin A can treat leishmaniasis.

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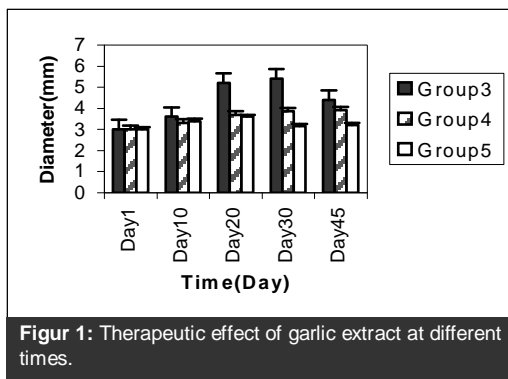
Keywords • Leishmaniasis • *Leishmania major* • garlic • nitric oxide

Introduction

There are a number of reports indicating that natural garlic might have therapeutic effect in some diseases such as *Helicobacter pylori*^{1,2}, cancers³, fungal⁴, bacterial⁵ and parasitic^{6,7} infections. Control of the leishmania parasites is critically dependent on type-1 CD4⁺ T helper (Th₁) cells which evolve in the presence of interleukin-2 and activate the macrophages to kill the intracellular amastigote leishmania stage by the production of γ -IFN which is required for the start of the healing process. On the contrary, Th₂-type response is incapable of mediating parasite clearance and interferes with the action of Th₁-derived cytokines.^{8,9} The killing process involves reactive oxygen and nitrogen intermediates.¹⁰⁻¹³ Recently, Ghazanfari et al.¹⁴ indicated that garlic therapy has been more effective than the antimonial drug, meglumine antimoniate in curing the infection. The aim of this study was to evaluate the thera-

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peutic effect of garlic extract on cutaneous leishmaniasis and the possible role of NO release in this mechanism.

Materials and Methods

Leishmania promastigote

Leishmania promastigotes were grown on NNN medium following several passages on complete RPMI-1640 medium. A suspension of 3×10^5 promastigotes in 1 ml RPMI-1640 was prepared. Forty-two female balb/c mice aged 8-10 weeks were inoculated at the bottom of the tail and experiments were carried out on 7 groups of each six mice harboring a 3-mm lesion.

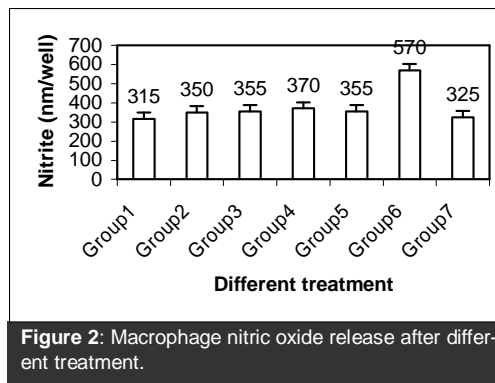
Preparation of garlic extract

Dried garlic powder (500 g) was suspended in 50 ml distilled water and mixed in a blender. The suspension was centrifuged and the supernatant was used as the aqueous extract.

The first group of mice received no treatment and served as control group. The second group was injected with 100 μ l of glucantime close to the lesion as the standard group. The other 3 groups (i.e, 3, 4, and 5) were treated topically with aqueous garlic extract for different periods of time (10, 20, 30 days). The sixth group was subjected to external vitamin A lotion for 10 days before topical administration of aqueous garlic extract for 30 days. The last group was subjected only to external vitamin A lotion, for 40 days. The diameter of lesion was measured between days 10-45. In all cases except glucantime-treated group external lotions were used to cover the lesion.

Macrophage nitric oxide production

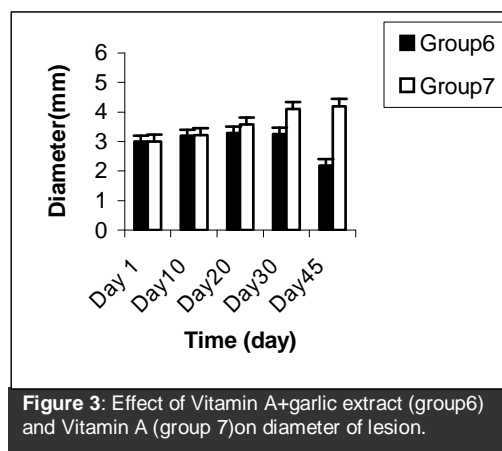
In a separate experiment, 42 female balb/c mice aged 8-10 weeks were inoculated at the bottom of the tail and experiments were carried out on 7 groups, each with six mice, harboring 3 mm le-



sion. The mice were treated as above, and peritoneal cells were collected from all groups as explained previously.¹⁵ Briefly, all mice were sacrificed by decapitation and 10 ml of ice cold PBS was injected into peritoneum. After massaging the location, peritoneal cells were collected and washed by PBS for three times. Cells were counted and plated out at 1×10^6 macrophage/well in 2 ml complete RPMI 1640, and incubated for 2 h at 37°C under air containing 5% CO₂. After removing non-adherent cells, macrophages were treated with 10 μ g/ml LPS. Plates were incubated for 24 h further and NO₂ was measured as an indicator of NO formation by Greiss method.¹⁶

Results

Administration of aqueous garlic extract for 10, 20 or 30 days was unable to inhibit lesion growth until day 30 (Fig 1), but nitric oxide generation in these groups was found to be more than in groups 1 and 2 (Fig 2). Fig 3 shows that removing the pustule



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from the lesion by using vitamin A for ten days before treating with aqueous extract, reduced the lesion size by 0.8 mm while in group 7 which received vitamin A for 40 days, no reduction was seen in lesion diameter. Fig 4 shows that the lesion in group 2, which was treated with glucantime, was reduced to less than 1 mm after 30 days, while in group 1 which received no treatment, the diameter of lesion was increased to more than 5 mm in the same duration, of time. Fig 2 shows that macrophages from group1 had less NO release compared to other groups. Group 2, in which the diameter of lesion had the largest reduction, produced less NO compared to groups 3, 4, 5 and 6. The sixth group produced the greatest amount of NO.

Discussion

It has been previously demonstrated that garlic extract can stimulate nitric oxide (NO) release by peritoneal macrophages in response to leishmania promastogote.¹⁷ There are also reports showing the effect of garlic extract on macrophages and T lymphocyte function.^{18,19} In this study, it was found that administering aqueous garlic extract to mice for 10 or 20 days did not suppress lesion growth. Using the same regimen for 30 days lead to a suppression of growth yet unable to reduce the size of the lesion. Our results showed that removal of the pustule by external use of vitamin A before treatment with aqueous extract can reduce the size of the lesion. Therefore, it seems that one of the reasons the aqueous extract reduces the lesion size might be due to impenetrability of the aqueous extract into the thick pustule. Therefore, it is likely that the external application of vitamin A has induced a suitable environment by reduction of the lesion thickness so that the garlic extract could penetrate into the host tissue. In order to find out if this preventive effect is due to garlic extract or vitamin A, mice in group 7 were subjected to vitamin A without garlic extract for 40 days. The results from this group showed that the external use of vitamin A without garlic extract did not reduce the lesion diameter, suggesting that vitamin A is not responsible for the reduction of the lesion size.

Nitric Oxide Release

While NO release pattern by macrophages of groups 2-5 was similar and no significant difference was seen among these groups, nitric oxide release by macrophages of the sixth group was found to be more than the others, indicating the possible correlation between leishmania healing and NO release. Our results have similarities and differences with the results reported by Ghazanfari et al.¹⁴ They found that garlic-treated mice developed Th₁-type

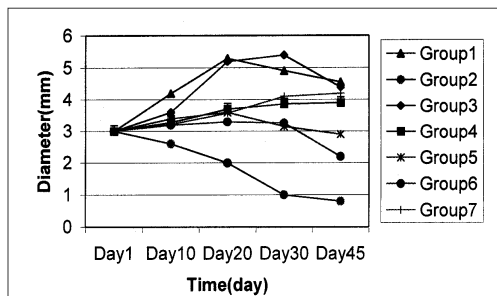


Figure 4: Comparison of all groups at different times.

cytokine response while glucantime therapy led to a Th₂-type response with a lower level of IL-2. Our results showed that glucantime had the best effect on the reduction of lesion diameter, but macrophages of this group (group 2) produced less NO than of group 6. This result indicates that therapeutic effect of glucantime may not be via NO release.

In our study, peritoneal macrophages from garlic-treated mice produced a high level of NO. On the other hand, there is a report showing that γ -IFN, a Th₁ cytokine, is an important stimulator for macrophages in releasing NO.¹ Therefore, it is likely that garlic extract enhances NO release by stimulating γ -IFN release in Th₁ cells by macrophages. Since NO is an important mediator for killing leishmania. It is possible that garlic extract promotes leishmania healing through NO release. Previous studies have also reported that several properties of garlic might be attributed to its ability to physiologically increase nitric oxide release,^{13,21,22} which is in agreement with our results. In our previous work, we also showed that arginine is required for NO production by macrophages. In the present work macrophages induced NO without arginine supplement to the tissue culture which is in agreement with the report of Das et al.²²

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References

- 1 Graham DY, Anderson SY, Lang T: Garlic or Jalapeno peppers for treatment of Helicobacter pylori infection. *Am J Gastroenterol* 1999; **94(5)**:1200-1202.

- 2 You WC, Zhang L, Gail MH, et al: Helicobacter pylori infection, garlic intake and precancerous lesion in a Chinese population at low risk of gastric cancer. *Int J Epidemiol* 1998; **27(6)**: 941-44.
- 3 Challier B, Peraynau JM, Viel JF: Garlic, onion and cereal fibre as protective factors for breast cancer. *Eur J Epidemiol* 1998; **14(8)**:737-47.
- 4 Sata N, Matsunaga S, Fusetania N, et al: New antifungal and cytotoxic steroidal saponins from the bulbs of an elephant garlic mutant. *Biosci Biotech Biochem* 1998; **62(10)**:1904-11.
- 5 Azad-Chowdhury AK: Efficacy of aqueous extract of garlic and allicin in experimental shigellosis in rabbit Indian. *J Med Res (A)* 1991; **393**:33-6.
- 6 Soffar SA, Mokhtar GM: Evaluation of the antiparasitic effect of aqueous garlic extract on hymenolepiasis nana and giardiasis. *J Egypt Soc Parasitol* 1991; **21(2)**:497-502.
- 7 Lun Z.R, Burri C, Menzinger M, et al: Antiparasitic activity of diallyl trisulfide (Dasuansu) on human and animal pathogenic protozoa (Trypanosoma SP, Entamoeba histolitica and Giardia lamblia) in vitro. *Ann Soc Belg Med Trop.* 1994; **74(1)**:51-9.
- 8 Liew FY: Regulation of cell-mediated immunity in Lishmaniasis. *Curr Trop Microbiol* 1990; **155**: 54-64.
- 9 Scott P: The role of Th₁ and Th₂ cells in experimental cutaneous leishmaniasis. *Exp Parasitol* 1989; **68**:369-72.
- 10 Asprey J, Cunha FQ, Epperlein M, et al: Production of nitric oxide and superoxide by activated macrophages and killing of Lishmania major. *Eur J Immunol* 1994; **24**:627-36.
- 11 Bogdan C, Leishmaniasis: Principles of the immune response and function of nitric oxide. *Brel Munch Tiarztl Wochenscher* 1998; **111 (11-12)**:409-14.
- 12 Cillari E, Arcoleo F, Dieli M: The macrophage activating tetrapeptide tuftsins induces nitric oxide synthesis and stimulates murine macrophages to kill Lishmania parasites in vitro. *Infect. Immunol* 1994; **62(6)**:2649-52.
- 13 Kang NS, Moon EY, Cho CG, et al: Immunomodulating effect of garlic component, allicin, on murine peritoneal macrophages. *Nutrition Research* 2001; **21**:617-26.
- 14 Ghazanfari T, Hassan ZM, Ebtakar M, et al: Garlic induces a shift in cytokine pattern in Leishmania major infected balb/c mice. *Scand J Immunol* 2000; **52(5)**:491-5.
- 15 Ahmadi-Renani K, McCrudden AB: Sex differences in macrophage nitric oxide production. *Iran J Med Sci* 1998; **23(1,2)**: 42-7.
- 16 Green LC, Wagner DA, Glogowski J, et al: Analysis of nitrate, nitrite, and ¹⁵N nitrate in biological fluids. *Anal Biochem* 1982; **126**:131-8.
- 17 Ahmadi-Renani K., Pandooneh A, Esfahani AA: Effect of garlic on nitric oxide production by leishmania activated-macrophages, 11th international congress of Immunology. Sweden, 2001. (Abstract book Monday). 87.
- 18 Lau BH, Yamasaki T, Gridley DS: Garlic compounds modulate macrophage and T-lymphocyte functions. *Mol Biother*, 1991; **3(2)**: 103-7.
- 19 Romano EL, Montano RF, Brito B, et al: Effects of ajoene on lymphocyte and macrophage membrane-dependent functions. *Immunopharm Immunotoxicol* 1997; **19(1)**: 15-36.
- 20 Das IKhan N.S, Sooranna SR: Potent activation of nitric oxide synthase by garlic: a basis for its therapeutic applications. *Current Med Res Opinion* 1995; **13**:257-63.
- 21 Morihara N, Sumioka I, Moriguchi T, et al: Aged garlic extract enhances production of nitric oxide. *Life Sci* 2002; **71**:509- 17.
- 22 Das I, Hirani J, Sooranna S: Arginine is not responsible for the activation of nitric oxide synthase by garlic. *J Ethnopharmacol* 1996; **53**: 5-9.