Schistosomicidal Effect of Curcumin

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Abstract: Swiss albino mice infected with 100 cercariae of S. mansoni were orally treated with a single dose of 20 mg curcumin per kg of body weight and second group was treated with a triple dose, day after day, of 250 mg praziquantel (PZQ) per kg of body weight. The curcumin and PZQ-treated groups revealed a significant (p<0.001) reduction (67.3 and 83.97%, respectively) of worm burden compared to controls. Also, total IgG antibodies against Soluble Worm Antigen Preparations (SWAP) were significantly decreased after treatment with curcumin (p<0.05) compared to infected untreated group. Furthermore, the serum levels of gamma interferon (IFN-γ) and interleukine-2 (IL-2) were significantly (p<0.05) decreased in curcumin-treated group. Finally, the serum activity of Glutamic Pyruvic Transaminase (GPT) was significantly (p<0.05) decreased in curcumin-treated group but not in PZQ-treated group. These results indicate that curcumin may represent a new inexpensive and effective schistosomicidal drug.

Key words: Curcumin, S. mansoni, cytokins

Introduction

Schistosomiasis is a condition caused by parasitic helminthes of the genus Schistosoma, with S. mansoni, S. haematobium and S. Japonicum accounting for most of the world’s estimated 200 million cases of human schistosomiasis. The disease is contracted from water born larvae that penetrate the skin and enter the blood stream, where they develop, pair and reach sexual maturity. Adult pairs of S. mansoni reside in the mesenteric veins of the intestine, where each female lays up to 300 eggs per day (Fitzsimmons et al., 2004). Many of eggs exit the body through the gut. However, a substantial number are trapped in host tissues such as the liver and intestine. Chronic egg-induced inflammation in the liver can lead to fibrosis, portal hypertension, bleeding and eventual death (Hesse et al., 2004).

Treatment of schistosomiasis is based on chemotherapy with praziquantel or oxamniquine (Domingues and Coutinho, 1990; Shehkar, 1991). Praziquantel is the drug of choice because it is more effective than oxamniquine, especially for the treatment of mansoniella (Ismail et al., 1996). It has been reported that human strains of S. mansoni have altered their susceptibility (resistance and/or tolerance) to oxamniquine (Katz et al., 1973; Dias et al., 1978). The tolerance to PZQ has been reported in Senegal (Stelma et al., 1995), Egypt (Ismail et al., 1996) and even in Brazil, where it is rarely used (Gomes et al., 1993; Araujo et al., 1996). Thus, the development of an alternative safe, inexpensive and effective schistosomicidal agent is urgently needed.

During the search for the candidate of anti-parasitic agents from natural products, we have found that curcumin isolated from the roots of Curcuma longa plant has been shown to regulate a number of biological processes (Araujo and Leon, 2001). In addition to its anti-tumorigenic, anti-oxidant and anti-inflammatory effects, curcumin has been shown to possess anti-microbial activity (Araujo and

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León, 2001; Rasmussen et al., 2000). Curcumin has been shown to hinder Leishmania (Koide et al., 2002) and Trypanosoma viability (Nose et al., 1998) and has antimalarial activity (Reddy et al., 2005). The aim of the present study was to evaluate the efficacy of curcumin for the treatment of schistosomiasis in mice infected with S. mansoni compared to PZQ as a known schistosomicidal drug.

Materials and Methods

Host animals, infection, treatment and perfusion Female outbred Swiss albino mice (about 20 g in weight) were used for treatment experiments. A Puerto Rican strain of S. mansoni is regularly maintained by laboratory passage through Biomphalaria glabrata snails and golden hamsters. Groups of female mice (ten mice per group) were infected percutaneously with 100 S. mansoni cercariae. After 6 weeks of infection, using stomach tube, a group of mice (n = 10) was orally treated with a triple dose at three days, day after day, of 250 mg praziquantel (Distaclid, EPICO) suspended in distilled water per kilogram of body weight. At the third dose of PZQ, other group of mice (n = 10) was orally treated with a single dose of 20 mg curcumin (Sigma) suspended in distilled water per kg of body weight. One week after the third dose of PZQ, mice were perfused and worms were collected, washed with saline containing heparin and counted. Also, blood samples were collected and stored at -20°C until use. A group of untreated infected mice (n = 10) with S. mansoni was used as a control group.

Measurement of Antischistosomal IgG Antibodies

A polystyrene flat bottom microtitr plate was coated with 50 μL well-1 of 25 μg mL-1 from schistosome antigenic preparation (SWAP) diluted in carbonate/bicarbonate buffer (pH 9.6) at room temperature over night. After blocking, sera from treated and untreated mice diluted 1:250 in PBS-T20 were added (50 μL well) and incubated at 37°C for 2 h. After washing with PBS-T20, 50 μL well of goat anti-mouse IgG alkaline phosphatase conjugate (Sigma) diluted 1:500 in 0.2% (w/v) non-fat milk in PBS-T20 was added and incubated at 37°C for 1 h. After washing, one ng per mL of para-nitrophenyl phosphate (Sigma) was used as a substrate and the absorbance was read at 405 nm using microplate autoreader (Bio-Tek Instruments, Vermont, USA). The antibody levels were expressed as Optical Densities (OD).

Assay of IFN-γ and IL-2

A polystyrene flat bottom microtitr plate was coated with 50 μL well of treated and untreated mice sera diluted 1:100 in carbonate/bicarbonate buffer, (pH 9.6) overnight. After blocking, rat anti-mouse IFN-γ mAb and goat anti-mouse IL-2 mAb (Sigma) diluted 1:1000 in PBS, pH 7.2 were added and incubated at 37°C for 1 h. After washing, 50 μL well of goat anti-rat IgG alkaline phosphatase conjugate (Sigma) (for IFN-γ) and rabbit anti goat IgG alkaline phosphatase conjugate (Sigma) for IL-2 diluted 1:600 in PBS-T20 were added at room temperature for 2 h. One ng per mL of para-nitrophenyl phosphate (Sigma) was used as a substrate and the absorbance was read at 405 nm using microtitr plate autoreader (Bio-Tek Instruments, Vermont, USA). The cytokine levels were expressed as Optical Densities (OD).

Measurement of Serum Activity of GPT

The serum activity of GPT was determined using the routine kit (Alkane Co.) and the method was done according to the manufacture instruction of the kit.

Statistical Analysis

Student t-test was performed using the statistical program package, instate software, version 2.03 (Graphpad, USA) and on IBM PC/AT compatible computer. The degree of significance was categorized as follow: p<0.05 significant, p<0.01 highly significant and p<0.001 extremely significant.
Results

Worm recovered from treated *S. mansoni* infected mice with curcumin and PZQ. The number of worms recovered from treated *S. mansoni* infected mice with curcumin or PZQ was significantly (p<0.001) reduced compared to the infected untreated mice group. The reduction levels of worm burden in curcumin and PZQ-treated groups were 67.3 and 84.61% respectively (Table 1).

Serum levels of total IgG schistosomal antibodies, IFN-γ and IL-2 in treated *S. mansoni* infected mice. Both of curcumin and PZQ-treated mice groups showed significant decrease of schistosomal antibodies (IgG) titer (p<0.05 and p<0.01, respectively) as shown in Fig. 1. The level of IFN-γ was not affected by the treatment with PZQ, while it was significantly (p<0.05) decreased in curcumin-treated group (Fig. 2). On the other hand, the serum levels of IL-2 were significantly (p<0.05) decreased in PZQ and curcumin-treated groups (Fig. 3).

Serum activity of GPT in treated *S. mansoni* infected mice. Curcumin-treated mice group demonstrated a significant (p<0.05) decrease in serum GPT activity, but in PZQ-treated mice group, the serum GPT activity was not changed significantly (p>0.05) as shown in Table 2.

| Table 1: Evaluation of worm burden in mice infected with 100 *S. mansoni* cercariae and treated with 20 mg kg⁻¹ curcumin and 250 mg kg⁻¹ PZQ |
|------------------|-----------------|-----------------|-----------------|
| Groups (n*)      | No. of worm burden Mean±SD | Worm burden reduction (%) | p-value |
| Infected untreated (10) | 31.2±2.6       | -              | -              |
| Infected treated with 20 mg kg⁻¹ curcumin (10) | 10.2±0.88       | 67.3           | <0.001         |
| Infected treated with 250 mg kg⁻¹ PZQ (10) | 5.6±0.57       | 83.97          | <0.001         |
| (n*) = No. of mice per group. |

| Table 2: Serum activity of glutamic pyruvic transaminase (GPT) in mice infected with 100 *S. mansoni* cercariae and treated with 20 mg kg⁻¹ curcumin and 250 mg kg⁻¹ PZQ |
|------------------|------------------|-----------------|
| Groups (n*)      | Serum GPT(U mL⁻¹) Mean±SD | p-value |
| Infected untreated (10) | 101.3±29.9       | -              |
| Infected treated with 20 mg kg⁻¹ curcumin (10) | 71.1±37.2       | <0.05          |
| Infected treated with 250 mg kg⁻¹ PZQ (10) | 104.1±45.2       | >0.05          |
| (n*) = No. of mice per group. p-values were calculated compared to infected untreated mice group. NS: Not Significant |

Fig. 1: Total IgG antibodies against soluble worm antigens preparation (SWAP) of *S. mansoni*. G1) infected untreated mice group. G2) infected and treated mice group with a single dose of curcumin 20 mg kg⁻¹ after six weeks of infection. G3) infected and treated mice group with a triple dose of PZQ 250 mg kg⁻¹ after six weeks of infection. p-values were calculated compared to infected untreated mice group. Bars represent the mean values of optical densities at 405 nm
Fig. 2: Serum levels of gamma Interferon (IFN-γ). G1) infected untreated mice group. G2) infected and treated mice group with a single dose of curcumin 20 mg kg⁻¹ after six weeks of infection. G3) infected and treated mice group with a triple dose of PZQ 250 mg kg⁻¹ after six weeks of infection. p-values were calculated compared to infected untreated mice group. Bars represent the mean values of optical densities at 405 nm.

Fig. 3: Serum levels of interleukin-2 (IL-2). G1) infected untreated mice group. G2) infected and treated mice group with a single dose of curcumin 20 mg kg⁻¹ after six weeks of infection. G3) infected and treated mice group with a triple dose of PZQ 250 mg kg⁻¹ after six weeks of infection. p-values were calculated compared to infected untreated mice group. Bars represent the mean values of optical densities at 405 nm.

Discussion

A major goal in schistosomiasis controlling field is to produce, identify and synthesize a safe, inexpensive and effective drug. Herbal products are not only safe, but can also have anti-helminthic activity (Sheir et al., 2001). Curcumin (diferuloylmethane) [1,7-bis (4-hydroxy-3-methoxy phenol)-1,6-heptadiene-3,5-dione] is the major yellow pigment extracted from turmeric, a commonly used spice, derived from the rhizome of the herb Curcuma longa Linn (Ireson et al., 2002). To the best of our knowledge, the efficacy of curcumin as anti-schistosomal drug has not been previously studied. In the present study, oral administration of a single dose of 20 mg curcumin per kg body weight induced a significant (p<0.001) reduction (67.3%) of worm burden in infected mice with S. mansoni while, PZQ-treated mice group revealed a more reduction (83.97%) level, this may be attributed to
the triple dose of PZQ. Limited number of studies reported the anti-parasitic activity of curcumin. Koide et al. (2002) found that curcumin has leishmanicidal activity in vitro. Also, it was reported that curcumin showed the cytotoxicity against another protozoan, trypanosomes that cause African sleeping sickness (Nose et al., 1998). More recently, curcumin has been shown to possess antimalarial activity, it inhibits chloroquine-resistant Plasmodium falciparum growth in culture. Additionally, oral administration of curcumin to mice infected with malaria parasite (Plasmodium berghei) reduces blood parasitemia by 80-90% and enhances their survival significantly (Reddy et al., 2005). Other herbal antischistosomal therapy derived from Myrrh has been studied. Myrrh, an oleo-gum resin from the stem of the plant Commiphora molmol, induced a cure rate of 91.7% in patients with schistosomiasis and has been shown to be a safe and effective anti-schistosomal drug at a dose of 10 mg kg\(^{-1}\) day for six days (Sheik et al., 2001).

Worm burden reduction, in our study, was accompanied with a significant decrease in total IgG antibodies level against SWAP in both curcumin and PZQ-treated mice groups compared to infected untreated mice, but this level was lower in PZQ-treated mice than was observed in curcumin-treated mice. This may be accounted for a number of reasons, one of these reasons is related to the triple dose of PZQ comparing to the single dose of curcumin. Moreover, schistosomal IgG antibodies level was assayed two weeks after the first dose of PZQ, while these antibodies level was assayed one week after curcumin treatment. In human, treatment of schistosomiasis induced marked changes in antibody response during the months following treatment (Hesse et al., 2004).

The granuloma that surrounds the S. mansoni egg is the cause of pathology in murine schistosomiasis and its formation is driven by egg Ag-stimulated type 1 (Th1) and type 2 (Th2) cytokines. The normal stimulation of type 2 cytokines by eggs during schistosoma infection is essential to regulate the proinflammatory type 1 cytokines that are elicited during early schistosome infection by the other stages of the parasite life-cycle (Fallon and Dunne, 1999). Our results demonstrated a significant (p<0.05) decrease of IFN-\(\gamma\) and IL-10 levels in infected mice after treatment with curcumin, while only IL-2 level was reduced significantly (p<0.05) in PZQ-treated mice group. These findings suggest that curcumin is responsible for the down-regulation of Th1 responses in schistosome infections, a phenomenon that may enable the parasite to escape potentially harmful cell-mediated responses.

The down-modulatory activity of curcumin may be responsible for control of diseases associated with granulomatous inflammation such as schistosome-induced liver disease. To investigate the efficacy of curcumin in controlling schistosome-induced liver damage, serum activity of GPT, as a highly sensitive indicator of liver damage, was measured. Our results showed a significant decrease in serum GPT activity in infected mice after curcumin treatment compared to infected untreated mice, while no change was observed after PZQ treatment. These findings suggest that curcumin has been shown to possess anti-pathological effect by its immunomodulatory activity.

Several investigators reported immunomodulatory activity of curcumin, Lantz et al. (2005) found that, compounds isolated from Curcuma longa rhizomes are effective inhibitors of inflammatory mediator production in vitro. In addition, curcumin inhibited the expression/production of IL-2 and IFN-\(\gamma\) by splenic T lymphocytes and IL-12 and tumor necrosis factor-alpha (TNF-\(\alpha\)) by peritoneal macrophages irreversibly (Gao et al., 2004). In addition, curcumin inhibits the transmigration and infiltration of neutrophils from blood vessels to the underlying liver tissue and, hence, inhibits the damage to the tissue. Moreover, curcumin blocks the induced expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in liver and lungs. So, it may be useful in attenuating multiple organ injury in pathological conditions arising due to excessive infiltration of neutrophils into the tissues (Madan and Ghosh, 2003).

In regard to the safety of curcumin, some investigators carried out a long-term feeding of curcumin to dogs, guinea pigs and monkeys and they found no evidence of toxicity (Shankar et al., 1980; Deshpande et al., 1998). Also, a phase I human trial using up to 8000 g of curcumin per day for 3
months found no toxicity from curcumin (Cheng et al., 2001), so it will be interesting to examine whether prolonged treatment of curcumin can completely eliminate the parasite and prevent recrudescence (Reddy et al., 2005).

In conclusion, this study is the first to investigate the anti-schistosomal effect of curcumin that has been shown to be a new inexpensive and effective drug isolated from natural products at a single dose of 20 mg kg⁻¹ body weight in mice infected with *S. mansoni*. Further investigations with different protocols should be carried out to improve the potential of curcumin as a schistosomicidal drug.

References


