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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 ($p<0.0001$) 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; Shekhar, 1991). Praziquantel is the drug of choice because it is more effective than oxamniquine, especially for the treatment of mansoniasis (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; Araújo *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 the *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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Leon, 2001; Rasmussen *et al*., 2000). Curcumin has been shown to hinder *Leishamnia* (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 *Biomphlaria glabrata* snails and golden hamsters. Groups of female mice (ten mice per group) were infected percutanously 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 (Distocid, 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 microtiter 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 mg 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 IF N-γ and IL-2

A polystyrene flat bottom microtiter 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 mg per mL of para-nitrophenyl phosphate (Sigma) was used as a substrate and the absorbance was read at 405 nm using microtiter 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 PCIAT 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) titers ($p<0.05$ 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
Inf/untreated (10)	31.2±2.6	-	-
Inf/treated with 20 mg kg^{-1} curcumin (10)	10.2±0.88	67.3	<0.001
Inf/treated with 250 mg kg^{-1} PZQ (10)	5.0±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(IU mL^{-1}) Mean±SD	p-value
Inf/untreated (10)	101.3±29.9	-
Inf/treated with 20 mg kg^{-1} curcumin (10)	71.1±37.2	<0.05
Inf/treated with 250 mg kg^{-1} PZQ (10)	104.1±45.2	>0.05 (NS)

(n*) = No. of mice per group, p-values were calculated compared to infected untreated mice group, NS: Not Significant

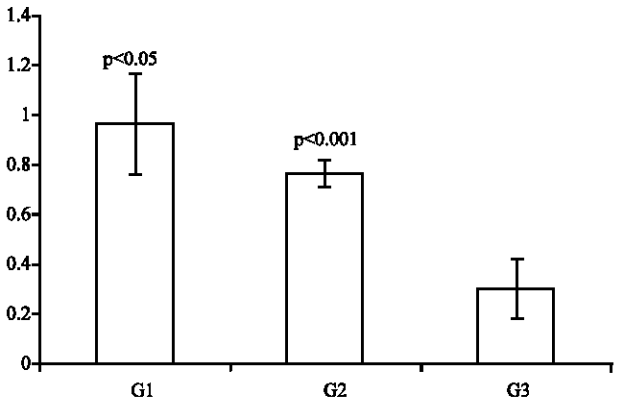

Fig. 1: Total IgG antibodies against soluble worm antigens preparation (SWAP) of *S. mansoni*. G1) infected untreated mice group. G2) infected and treated mice group with a single dose of curcumin 20 mg kg^{-1} after six weeks of infection. G3) infected and treated mice group with a triple dose of PZQ 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 denisities at 405 nm

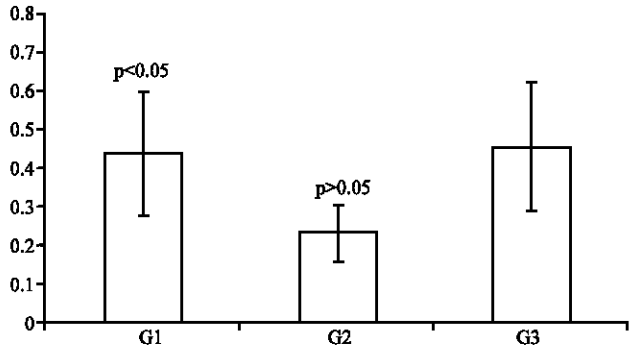

Fig. 2: Serum levels of gamma Interferon (IFN-γ). G1) infected untreated mice group. G2) infected and treated mice group with a single dose of curcumin 20 mg kg^{-1} after six weeks of infection. G3) infected and treated mice group with a triple dose of PZQ 250 mg kg^{-1} after six weeks of infection. p-values were calculated compared to infected untreated mice group. Bars represent the mean values of optical denisities at 405 nm

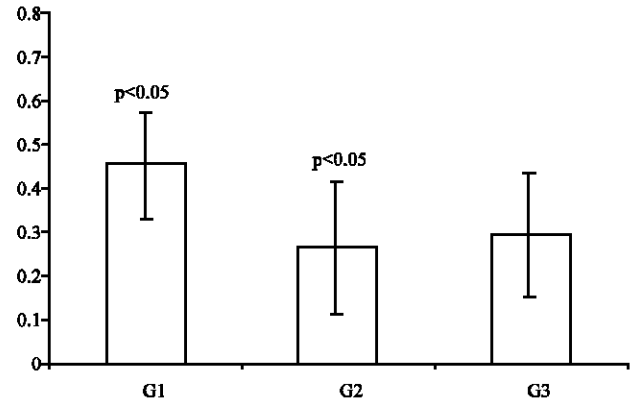

Fig. 3: Serum levels of interleukin-2 (IL-2). G1) infected untreated mice group. G2) infected and treated mice group with a single dose of curcumin 20 mg kg^{-1} after six weeks of infection. G3) infected and treated mice group with a triple dose of PZQ 250 mg kg^{-1} after six weeks of infection. p-values were calculated compared to infected untreated mice group. Bars represent the mean values of optical denisities 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 anti-schistosomal 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 (Sheir *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 type1 (Th1) and type 2 (Th2) cytokines. The normal stimulation of type 2 cytokines by eggs during schistosoma infection is essential to regulate the proinflammatory type1 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-γ and IL-2 levels in infected mice after treatment with curcmin, 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-γ by splenic T lymphocytes and IL-12 and tumor necrosis factor-alpha (TNF-α) by peritoneal macrophages irreversibly (Gao *et al*., 2004). In addition, curcumin inhibits the transmigration and infilteration of neutriphils 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 infilteration 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 1 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

Araujo, C.C. and L.L. Leon, 2001. Biological activities of *Curcuma longa* L. Mem. Inst. Oswaldo. Cruz., 96 :723-728.

Araújo, N., S.P. Souza, L.K.J. Passos and A.J.G. Simpson *et al*., 1996. Suscetibilidade aos agentes quimioterápicos de isolados de *Schistosoma mansoni* oriundos de pacientes tratados com OXA e PZQ e não curados. Rev. Soc. Bras. Med. Trop., 29: 467-476.

Cheng, A.L., C.H. Hsu, J.K. Lin and M.M. Hsu *et al*., 2001. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Res., 21: 2895-2900.

Deshpande, S.S., V.S. Lalitha, A.D. Ingle, A.S. Raste, S.G. Gadre and G.B. Maru, 1998. Subchronic oral toxicity of turmeric and ethanolic turmeric extract in female mice and rats. Toxicol. Lett., 95: 183-193.

Dias, L.C., F.D. Avila-Pires and A.C. Pinto, 1978. Parasitological and ecological aspects of Schistosomiasis *mansoni* in the valley of the Paraiba do Sul River (Sao Paulo State, Brazil) I. Natural infection of small mammals with *Schistosoma mansoni*. Trans. R. Soc. Trop. Med. Hyg., 72: 496-500.

Domingues, A.L. and A.D. Coutinho, 1990. Reduction of morbidity in hepatosplenic schistosomiasis *mansoni* after treatment with praziquantel: A long term study. Rev. Soc. Bras. Med. Trop., 23: 101-107.

Fallon, P.G. and D.W. Dunne, 1999. Tolerization of mice to *Schistosoma mansoni* egg antigens causes elevated type 1 and diminished type 2 cytokine responses and increased mortality in acute infection, J. Immunol., 162: 4122-4132.

Fitzsimmons, C.M., S. Joseph, F.M. Jones, C.M. Reimert and K.F. Hoffmann, 2004. Chemotherapy for schistosomiasis in Ugandan fishermen: Treatment can cause a rapid increase in interleukin-5 levels in plasma but decreased levels of eosinophilia and worm-specific immunoglobulin E. Infect. Immunol., 72: 4023-3040.

Gao, X., J. Kuo, H. Jiang, D. Deeb and Y. Liu *et al*., 2004. Immunomodulatory activity of curcumin: Suppression of lymphocyte proliferation, development of cell-mediated cytotoxicity and cytokine production *in vitro*. Biochem. Pharmacol., 68: 51-61.

Gomes, E., L.C.S. Dias, L. Takaku and G. Stangenhaus, 1993. Biological and morphological characteristics of *Schistosoma mansoni* from the Paranapanema Valley (Ourinhos). São Paulo, Brasil, 4th International Symposium on Schistosomiasis, pp: 51-55.

Hesse, M., C.A. Piccirillo, Y. Belkaid, J. Prufer and M. Mentink-Kane *et al*., 2004. The pathogenesis of schistosomiasis is controlled by cooperating IL-10-producing innate effector and regulatory T cells. J. Immunol., 172: 3157-3166.

Ireson, C.R., D.J.L. Jones, S. Orr and M.W.H. Coughtrie *et al*., 2002. Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine. Cancer Epidemiol. Biomarkers Prev., 11: 105-111.

Ismail, M., A. Metwally, A. Farghaly, J. Bruce, L.F. Tao and J.L. Bennett, 1996. Characterization of isolates of *Schistosoma mansoni* from Egyptian villagers that tolerate high doses of praziquantel. Am. J. Trop. Med. Hyg., 55: 214-218.

Katz, N., J. Pellegrino, E.A. Grinbaum, A. Chaves and F. Zicker, 1973. Preliminary clinical trials with oxamniquine, a new antischistosomal agent. Rev. Inst. Med. Trop. Sao. Paulo., 15: 25-29.

Koide, T., M. Nose, Y. Ogihara, Y . Yabu and N. Ohta, 2002. Leishmanicidal effect of curcumin *in vitro*. Biol. Pharm. Bull., 25: 131-133.

Lantz, R.C., G.J. Ghen, A.M. Solyom, S.D. Jolad and B.N. Timmermann, 2005. The effect of turmeric extracts on inflammatory mediator production. Phytomedicine, 12: 445-452.

Madan, B. and B. Ghosh, 2003. Diferuloylmethane inhibits neutrophil infiltration and improves survival of mice in high-dose endotoxin shock. Shock, 19: 91-96.

Nose, M., T. Koide, Y. Ogihara, Y. Yabu and N. Ohta, 1998. Trypanocidal effects of curcumin *in vitro*. Biol. Pharm. Bull., 21: 643-645.

Rasmussen, H.B., S.B. Christensen, L.P. Kvist and A. Karazmi, 2000. A simple and efficient separation of the curcumins, the antiprotozoal constituents of *Curcuma longa*. Planta Med., 66: 396-398.

Reddy, R.C., P.G. Vatsala V.G. Keshamouni, G. Padmanaban and P.N. Rangarajan, 2005 Curcumin for malaria therapy. Biochem. Biophys. Res. Commun., 326: 472-474.

Shankar, T.N., N.V. Shantha, H.P. Ramesh, I.A. Murthy and V.S. Murthy, 1980. Toxicity studies on turmeric (*Curcuma longa*): Acute toxicity studies in rats, guinea pigs and monkeys. Indian J. Exp. Biol., 18: 73-75.

Sheir, Z., A.A. Nasr, A. Massoud, O. Salama, G.A. Badra, H. El-Shennawy, N. Hassan, S.M. Hammad, 2001. A safe, effective, herbal antischistosomal therapy derived from myrrh. Am. J. Trop. Med. Hyg., 65: 700-704.

Shekhar, K.C., 1991. Schistosomiasis drug therapy and treatment considerations. Drugs., 42: 379-405.

Stelma, F.F., I. Talla, S. Sow, A. Kongs, M. Niang, K. Polman, A.M. Deelder and B. Gryseels, 1995. Efficacy and side effects of praziquantel in an epidemic focus of *Schistosoma mansoni*. Am. J. Trop. Med. Hyg., 53: 167-170.