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Original article

Simvastatin exerts antifibrotic effect and potentiates the antischistosomal effects of praziquantel in a murine model: Role of IL10

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ABSTRACT

Previous studies on simvastatin use in experimental schistosomiasis in mice did not provide a full explanation of its mechanism as antischisome. In this study, we tried to find out the role of IL-10 in the mechanism of action of simvastatin. We used 50 clear mice. Ten were used as normal not treated while 40 were infected with shistosome mansoni then divided into 4 groups; 3 treated groups by praziquantel, simvastatin and combined (praziquantel plus simvastatin) respectively and one group non-treated. The simvastatin treated group showed shortening and loss of the tubercles and disappearance of the spines with swelling and twisted shape of the worms. In addition, it also showed mitigation of ovideposition activity of the worms in the liver and reduction of the fibrous component of the liver granuloma producing a protective effect on the liver. This effect was associated with lowering of IL-10. This may explain the role of IL-10 in the protective effect of simvastatin. Combination of treatment with simvastatin plus praziquantel produced more significant effects in different parameters compared with praziquantel treated group. We recommend using simvastatin as add on therapy to standard antischistosomal therapy, praziquantel. Both drugs affect the worm motility and sucker activity and the ova deposition. Simvastatin has an additional pleiotropic effect halting inflammation and decreasing fibrosis due to increasing IL-10 leading to a hepatoprotective effect. Further clinical studies are needed to further validate these findings.

1. Introduction

Schistosoma mansoni is wide speeded in Africa, Egypt, Brazil, Venezuela, Caribbean, and China. It is transmitted via fresh water snails by Schistosoma mansoni flukes [1]. Praziquantel (PZQ) is the cornerstone for control and treatment of schistosomiasis but unfortunately, the schistosome develops resistance to it with therapeutic failure (10-40%). Its efficacy depends on the presence of effective immune response [2,3]. So, it is necessary to search for newer agents with antischistosomal effect used alone or in combination with PZQ to overcome PZQ resistance or enhance its effect [4,5].

After schistosoma infection, cercaria penetrates the skin and ovideposition starts in the liver after maturation of schistosoma worms resulting in an immunological reaction. This reaction is associated with inflammatory cells and cytokine production resulting in fibrosis to protect the liver from infection [6].

Deposition of ova stimulate Th1 cell immunity which results in secretion of interferon gamma and IL2 in acute stage followed by activation of Th2 cell immunity resulting in release of IL10 and IL4 which has a protective role in the liver and prevent hepatotoxicity [7]

Statins act through inhibition of 3- hydroxyl, 3- methyl glutaryl CoA reductase (HMG-CoA) enzyme. This results in a reduction of mevalonate which acts as a precursor of cholesterol and it is important for egg disposition in schistosomes (Vandewaa et al.,). Statins show several pleiotropic effects including antischistosomal effects [8]. Rojo-Arreola et al. studied the effect of six statins in vitro from which simvastatin showed higher potency compared with other statins used.

Statins show immunomodulatory effects through increasing the secretion of IL-10, IL-5, IL-14, or TGF-β), and decreasing the secretion of IL-2, IL-12, or IFN-δ [10]. IL-10 is produced by both the innate and adaptive immune response after infection. IL-10 mitigates immunopathology in many persistent infections. Effectors and regulatory T cells producing IL-10 co-operate to decrease morbidity and prolong survival in schistosomiasis [7].

In this study, we tried simvastatin alone and in combination with PZQ to detect if it has any antischistosomal effect and tried to figure out

Abbreviations: IL-10, interleukin 10; PZQ, praziquantel; SIM, simvastatin; ALT, alanine amino transferase

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the mechanism of its effects if any. We chose simvastatin because it was the most potent in vitro against schistosoma according to Rojo-Arreola et al. (2014)

2. Material and methods

2.1. Drugs

1. Simvastatin (SIM) (Zocor®): was obtained from MERCK in form of 20 mg tablets.
2. Praziquantel (PZQ) (Distocide ®, Epico Pharma, Cairo, Egypt.

2.2. Infection of animals with schistosoma mansoni

Induction of infection with shistosoma mansoni was done at Theodor Bilharz Research Institute (TBRI) according to Lewis et al. (2003). Adult Biomphalaria alexandrina snails- infected with miracidia- were placed in clean aquaria of dechlorinated water-kept in dark- (25 ± 2 °C) and supplied with lettuce leaves till shedding cercariae. To detect the injection dose, a suspension of cercariae was pulled up into a 1 ml plastic syringe fitted with a 21gauge needle. Suspension stained with iodine was expressed on hemocytometer then count by using a dissecting microscope. Mice were injected subcutaneously with 50 cercaria/0.1 ml dechlorinated water using sterile 1 ml plastic syringe [11].

2.3. Animals grouping

Fifty Swiss male albino mice (CD-1), with weight range (18–20 g) were used throughout this study. Ten of them were not infected and were used as the control group while 40 were infected by schistoaoma mansoni as previously mentioned. After 40 days of parasitic infestation, the infected animals were divided into (non-treated, PZQ, SIM, combined SIM and PZQ treated). SIM was given orally once daily (200 mg/kg/day) suspended in 0.5% methyl-cellulose solution as a vehicle for 2 weeks [12]. While, PZQ was given as 500 mg/kg, p.o.; on 2 successive days [8]. The animals were maintained in our laboratory under a 12 h light/dark cycle in a humidity (75 ± 15%), temperature (20 ± 2 °C), and controlled filtered laminar air flow room with free access to food and water. This protocol is approved by our institutional research board.

2.4. Serum analysis

Animals were killed by decapitation, 2 weeks after initiation of therapy. Sera were used for measurement of

- ALT using commercial kits from abcam (United Kingdom).
- IL10 using commercial kits from abcam (United Kingdom).

2.5. Parasitological study

2.5.1. Adult worms count

Dissection and perfusion of mice were done by inserting a 21-gauge needle into the descending aorta. Pumping perfusion fluid (citrated saline) is done through the venous system. The perfusate included many of the adult worms; can be collected in a pan. Worms were collected on the mesh screen of the sieve. Worms were placed in a petri dish with 0.85% saline [13].

2.5.2. Oogram

Small intestines of mice were cut into segments, each 1 cm in length and compressed between a slide and a cover slip to be examined using a light microscope. Shistosoma *mansoni* eggs (live immature, live mature and dead) were counted in 3 segments per each mouse to calculate the mean number of eggs [14].

2.6. Histopathological assessment and measurement of granuloma size

Liver specimens were fixed in neutral buffered formalin (10%) and embedded in paraffin blocks then cut as 4 μm thick sections stained with hematoxylin and eosin (H & E) for microscopic examination. The granuloma type (cellular, fibro-cellular or fibrous), granuloma number/10 fields/mouse, egg type (intact or degenerated) were assessed. The relative changes of reaction in the granuloma are semi-quantitatively assessed as follows: cellular granuloma if inflammatory cells occupying more than 70% of granuloma size, fibrous granuloma if fibrosis occupying more than 70% of granuloma size and fibro-cellular granuloma if less than 70% of each. Granuloma size was measured and the mean diameter of each granuloma was calculated by taking two perpendicular diameters of the lesion [15].

2.7. Morphometric assessment of fibrosis in the granuloma

Liver sections were stained with Masson trichrome and examined for deposition of collagen fiber. Images of a total of 50 granulomas were taken for each mice randomly with a digital camera connected to (BX41) Olympus optical microscope (Olympus Corporation, Tokyo, Japan). Collagen fibers content in the each granuloma were detected by using Photoshop software to split the picture into blue and green channels and analyzed by the NIH image software (Scion Corp., Frederick, MD). The quantity of fibrosis in each granuloma was expressed as the percentage of the stained area to the total granuloma area. The percentages of collagen content obtained were expressed as the mean ± S.D [16].

2.8. Statistical analysis

Analysis of data was performed using SPSS software Package 20. One way ANOVA was used for analysis of parametric data followed by Tukey post hoc test to detect significance between groups with parametric data. Mann-Whitney test was used to analyze non parametric data. P-values $^{<}0.05$ indicated a significant difference.

Calculation of sample size was done by power analysis method using G*power software. Power of a study was 95%. The initial number of animals used in the study is the corrected sample size after calculation of the expected death of animals.

3. Results

3.1. Effect of simvastatin and PZQ on ALT Iu/L and IL-10

Induction of schistosomiasis resulted in an increase of ALT compared with normal non-infected mice P1 < 0.001 while treatment of different groups with different medication (PZQ or SIM alone or in combination resulted in significant improvement of ALT levels (P2 < 0.00, 0.05, 0.0001 respectively). Combined PZQ & SIM produced a significant reduction of ALT in relation to PZQ only treated group, P3 < 0.01, Table 1.

The IL-10 level has been increased significantly on induction of schistosomiasis compared with the non-infected group, P1 < 0.001. Treatment with either PZQ or SIM alone or in combination resulted in significant increase of IL-10 in relation to infected non treated group (P2 < 0.05, 0.001, 0.001 respectively). Combination of PZQ plus simvastatin produced significant elevation 0f IL-10 in comparison to PZQ only treated one, P3 < 0.001, Table 1.

3.2. Effect of simvastatin and PZQ on oogram, liver & intestinal ova and worms in different groups

Induction of schistosomiasis resulted in the production of ova and worms in the liver and intestine (Fig. 1). Treatment of different infected groups with either PZQ or SIM alone or in combination resulted in