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Full Length Research Paper

Comparative effect of Praziquantel and Mefloquine in Murine Schistosomal Resistance to Reinfection with Regards to Serum IL-4, IL-5 and IL-10 profiles

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This work is a trial to elucidate the effect of Praziquantel and Mefloquine on resistance to reinfection in experimental schistosomiasis *Mansoni*. It is also a mean to clarify the repercussion of giving both drugs on the worm burden, oogram pattern and granuloma measurements in these experimental animals. Again, this study aims to disclose the imprint of giving these drugs upon serum *Interleukins* profile with special reference to IL-4, IL-5 and IL-10 levels. A group of seventy (70) *Swiss Albino* mice was used in the experiment. This group was further subdivided into seven subgroups. Subgroup I: Infected control group: animals were infected with *S. mansoni* cercariae, then sacrificed 9 weeks later. Subgroup II: Infected animals were re-infected 6 weeks post -primary infection then sacrificed 3 weeks later. Subgroup III: Mice were infected, then sacrificed 3 weeks post – infection, where juvenile schistosomes were counted. Subgroup IV: Infected animals were treated with PZQ 6 weeks post infection, then sacrificed 2 weeks post – treatment. Subgroup V: Animals received PZQ 6 weeks post infection. Two weeks later, mice were re-infected, then sacrificed 3 weeks later. Subgroup VI: Mice received MFQ 6 weeks post – infection, then were sacrificed 2 weeks later. Subgroup VII: Infected animals given MFQ 6 weeks post infection. Two weeks post treatment, mice were re-infected ,then sacrificed 3 weeks later. A normal non infected non treated group of mice was used as a control for measuring serum levels of IL-4, IL-5 and IL-10 in response to infection and treatment. In this work, treatment with PZQ 6 weeks post infection, markedly reduced total worm burden with disappearance of female worms. Animals reinfected two weeks post PZQ treatment, revealed 29.2 immature worms. While those given MFQ, then re-infected, yielded only 10.5±6.5 immature worms. Again, Praziquantel (PZQ) resulted in high significant decrease in both mature and immature ova with high percentage of dead ova (95%). Re – infection did not change the oogram pattern. On the other hand, Mefloquine (MFQ) decreased both mature and immature ova with 88.8% dead ova. However, re-infection following MFQ treatment resulted in a higher percentage of dead ova (98.8%). There was a significant drop both in the number and mean hepatic granuloma diameter following treatment with either PZQ or MFQ when compared to the control untreated group. Again, infection markedly increased the IL-10 cytokine level. But treatment with PZQ, did not improve its serum level. On the other hand, MFQ significantly decreased IL-10 serum level, as compared to all infected and infected treated groups. As regards IL-4, it also increased following infection. But treatment with PZQ significantly decreased its level. MFQ also decreased serum IL-4 level. However, this level was still higher than the PZQ treated group. Serum IL-5 level significantly increased post infection. Nevertheless, treatment with MFQ markedly decreased its levels as compared to the other groups.

Keywords: Praziquantel (PZQ), Mefloquine(MFQ), *Schistosoma mansoni* infection, Resistance to Reinfection, *Interleukins* (IL-4, IL-5 and IL-10).

INTRODUCTION

In Africa, both *S. haematobium* and *S. mansoni*, still represent a health problem. It is estimated that 90% of all

cases in the world are concentrated in sub-Saharan Africa (Colley et al 2014, Lai et al 2015). *S. mansoni* is still additionally found in Brazil (Scholte et al 2014), while *S. japonicum* remains endemic in many provinces of China, the Philippines and few small Indonesian foci

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(Utzinger et al 2010).

Although HIV/AIDS, malaria and tuberculosis constitute major health problems worldwide, yet schistosomiasis heads the list of these majors' ailments. Mild symptoms, such as anemia, diarrhea, dysuria and exercise intolerance (King et al 2005, King and Dangerfield 2008), constitute salient features, and even the hallmarks for clinical diagnosis of the disease.

It was found that absence of sanitary facilities in most endemic areas, are challenging factors in fighting schistosomiasis (Rollinson et al 2013, Grimes et al 2014). A variety of approaches, including water, sanitation and hygiene (WASH), information, education and communication (IEC), behavioral change and snail control, have been employed. However, preventive chemotherapy with praziquantel, has been the fundamental approach since the early 1980s (Sokolow et al 2016).

Resistance to reinfection is a product of exposure, curative treatment and rapid re infection, in addition to changes in the immunological status of the host (Karanja et al 2002, Black et al 2010).

S. mansoni-infected patients have been shown to exhibit elevated IL-4, IL-5 and IL-13 (Joseph et al 2004, Brown et al 2005) responses and increased numbers of circulating eosinophils (Kimani et al 1991). Colley and colleagues (1986) observed elevated anti-worm responses in schistosome infected Egyptians, up to two years following PZQ treatment. A similar increase in parasite-specific T cell proliferation, cytokine secretion, and antibody production in infected mice following PZQ treatment, was also found by Mutapi et al (2007).

Praziquantel is currently the only fully effective antischistosomal drug in use. It has been used in preventive chemotherapy programmes in many endemic countries, since more than 15 years (Fenwick et al 2003, Lo et al 2017). Recently, preschool-children, and pregnant women were included in the target population for preventive chemotherapy (Bustinduy et al 2016, Olveda et al 2016). Both these groups are now candidates for praziquantel administration whenever needed (Bustinduy et al 2016).

Xiao et al. (2009) assessed the reversible effect of MFQ and PZQ on the recovery of the motor activity, tegumental changes and parasite survival through an *in vitro* study on juvenile and adult *S. japonicum*. The authors reported that the *in vitro* effect of MFQ against juvenile and adult schistosomes was irreversible, while that of PZQ was reversible. MFQ has been considered T-cell independent (Keiser et al., 2010a) while PZQ requires synergistic interaction with immune response (Hassan et al., 1990).

Immunological studies conducted in schistosome endemic regions, have strongly implicated a negative regulatory role for IL-10 in the development of resistance to re-infection in humans (Van den et al 2002, Leenstra et al 2006). Despite developing enhanced parasite-specific

IL-5 responses following treatment, Van den Bigelaar and colleagues(2002), identified a concurrent increase in parasite-specific IL-10, and proposed that IL-10 was a major risk factor for re-infection. Similarly, Leenstra et al (2006), found that elevated IL-10 predicted a decrease in time to re-infection with several reports describing inverse correlations between IL-10 and *S. mansoni* (Caldas et al 2008) or *S. haematobium* (Mutapi et al 2007) infection intensity.

Praziquantel is the drug of choice for treatment of human schistosomiasis over more than three decades (WHO 2002; Fenwick et al.2003; Chen 2005). Reduced sensitivity to praziquantel has been reported in some *S. mansoni* endemic foci (Ismail et al.1996; Lawn et al. 2003; Melman et al.2009), and there are some cases where there is failure to clear the infections of *S. haematobium* following standard treatment with praziquantel (Alonso et al. 2006; Silva et al. 2008).

The expanded access to praziquantel, including bi-annual treatment schedules (Knopp et al 2016) and use of the drug for treatment of domestic animals in the context of zoonotic schistosomiasis (Hong et al 2011), increases the risk for development of resistance.

Gold and Lengy (1975), stated that failure to confer protection to secondary challenge infection, as previously observed. This was largely due to the inhibitory effects of IL-10, as blockade of IL-10 combined with PZQ treatment raised protective immunity from 0% to more than 50% when compared with PZQ treatment alone.

This study aims at studying the comparative effect of Praziquantel and Mefloquine on resistance to re-infection with murine Schistosomiasis *mansoni*, with evaluation of IL-4, IL-5 and IL-10 profiles.

MATERIAL AND METHODS

Drugs and dosage

Mefloquine (Larium, 250 mg tablet) was provided by F. Hoffmann- La Roche (Basel, Switzerland). Mefloquine was suspended in vehicle (7% (v/v) Tween-80 and 3% (v/v) ethanol) .The drug was administered in a high single dose of 400 mg/kg orally (Keiser et al., 2009).

Praziquantel(PZQ) tablets (Distocide®, EIPICO, El-Asher en Ramadan,Egypt) : PZQ was administered orally as a suspension in 2% cremophore-El (Sigma-Aldrich Chemical Co, St.Louis, MO).The dose was 1,000 mg/kg (Gonnert&Andrews 1977) , divided into two halves, and given on two consecutive days.

Animals

Seventy male Swiss Albino mice (CD-1 strain) weighing 18–20 g were used in the experiment. Animals were obtained from a closed random bred colony at the

Schistosome Biological Supply Center (SBSC) at Theodor Bilharz Research Institute (TBRI), Giza, Egypt. They were housed in polycarbonate boxes with steel-wire tops (not more than six animals per cage) and bedded with wood shavings. Ambient temperature was controlled at 22 ± 3 °C with a relative humidity of $50 \pm 15\%$ and a 12-h light/dark photoperiod. Food and water were provided ad libitum. This study was conducted in accordance with legal ethical guidelines of the Medical Ethical Committee of the Theodor Bilharz Research Institute (TBRI), Giza, Egypt.

Schistosome infection

S. mansoni cercariae (Egyptian strain) were obtained from infected intermediate host snails (*Biomphalaria alexandrina*) maintained at the SBSC. Mice were infected subcutaneously with freshly shed 60 ± 10 cercariae/mouse (Liang et al., 1987). Re-infection was performed 2 weeks post treatment, ie 8 weeks post primary infection, using 120 ± 10 cercariae/mouse. Sacrifice was done 3 weeks later.

Experimental design

A group of seventy (70) Swiss *Albino* mice were used in the experiment. This group was further subdivided into seven subgroups.

Subgroup I: Infected control group: animals were infected then sacrificed 9 weeks later.

Subgroup II: Infected animals were re-infected 6 weeks post -primary infection then sacrificed 3 weeks later.

Subgroup III: Mice were infected, then sacrificed 3 weeks post – infection, where juvenile schistosomes were counted.

Subgroup IV: Infected animals were treated with PZQ 6 weeks post infection, then sacrificed 2 weeks post – treatment.

Subgroup V: Animals received PZQ 6 weeks post infection. Two weeks later, mice were re-infected, then sacrificed 3 weeks later.

Subgroup VI: Mice received MFQ 6 weeks post – infection, then sacrificed 2 weeks later.

Subgroup VII: infected animals given MFQ 6 weeks post infection. Two weeks post treatment; mice were re-infected, and then sacrificed 3 weeks later.

A normal non infected non treated group of mice was used as a control for measuring serum levels of IL-4, IL-5 and IL-10 in response to infection and treatment.

Study of parasitological criteria

Immediately after mice euthanization, blood was collected from the neck blood vessels in centrifuge tubes.

Hepatic and porto-mesenteric vessels were perfused for both mature and immature worm's recovery and subsequent counting (Duvall and De Witt, 1967). The percentage of eggs at various developmental stages was examined in three samples from each mouse and the mean number of eggs at each stage/animal was determined (Pellegrino et al., 1962). Liver specimens were stored at - 80 °C for histopathological study, and were maintained in formalin (10%).

Resistance to re-infection in different groups was calculated according to (Doenhoff, 1978):

$$\% r = 100 - (n/N \times 100)$$

r = Resistance

n = immature (infected challenged) = mean challenged control.

N = immature (infected treated challenged MFQ)
(Infected treated challenged PZQ)

Sample preparation

Serum preparation

Blood samples collected in centrifuge tubes were centrifuged at 3000 rpm for 20 min. Serum was stored at - 20 °C until used for biochemical assays.

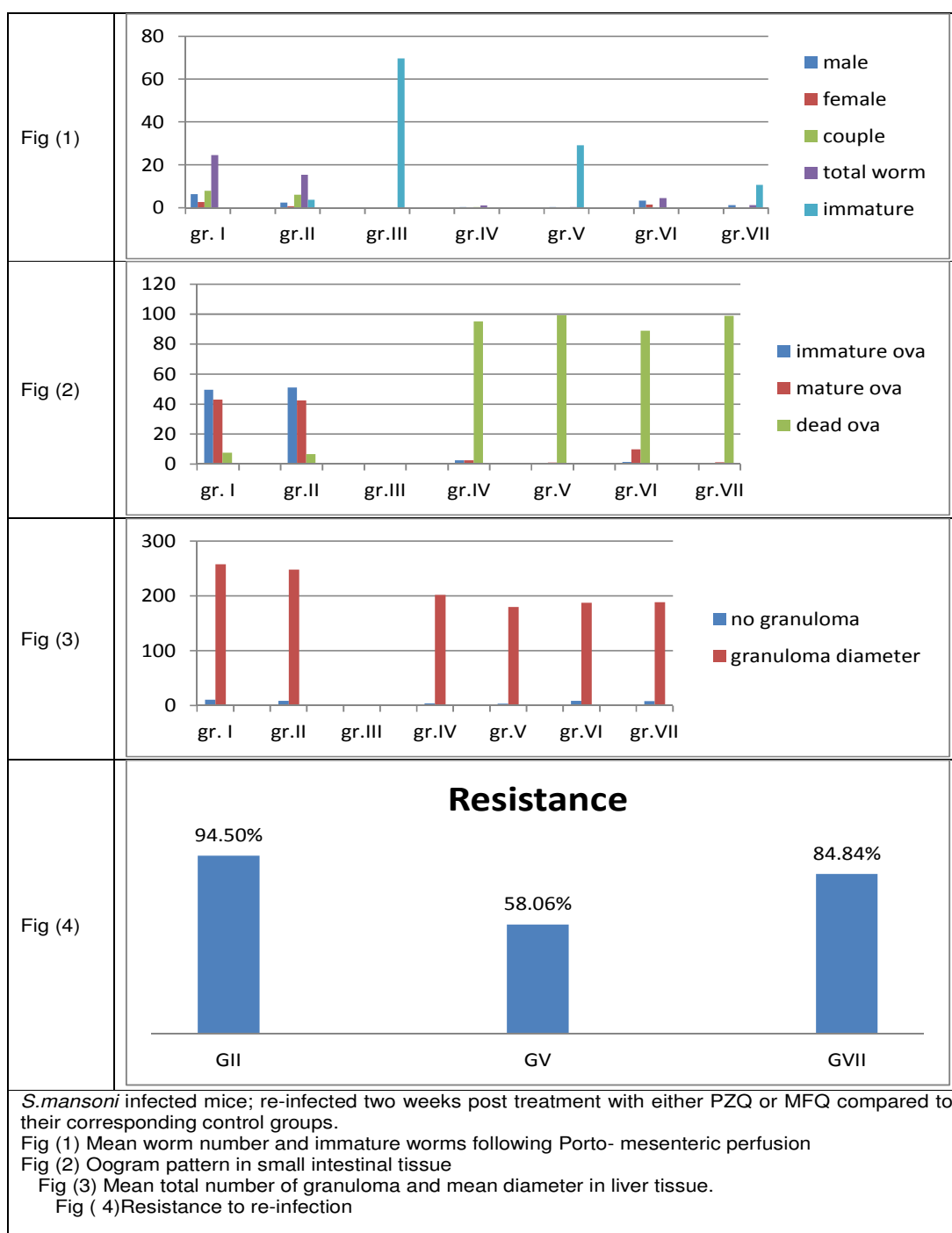
Capture ELISAs with antibody sets from BD Pharmingen following previously published protocols (Joseph et al 2004) were done for IL-4, IL-5 and IL-10 measurements.

RESULTS

Treatment with PZQ 6 weeks post primary infection (PI) markedly reduced total worm burden with disappearance of female worms (group IV). Two weeks following PZQ treatment, reinfection was performed (group V), then 3 weeks later animals were sacrificed. Data revealed 29.2 immature worms in this group. Treatment using MFQ in a dose of 400 mg/kg significantly decreased mean worm burden (group VI). Mice treated with MFQ, then re-infected, yielded only 10.5 ± 6.5 immature worms (group VII).

As regards the oogram pattern, PZQ (group IV) resulted in high significant decrease in both mature and immature ova with high percentage of dead ova (95%). Re –infection did not change the oogram pattern. While animals given Mefloquine (MFQ) in (group VI), showed decrease in both mature and immature ova with 88.8% dead ova. Re-infection following MFQ treatment (group VII), resulted in a higher percentage of dead ova 98.8%.

The number of granulomas decreased significantly following treatment with either PZQ or MFQ. The mean granuloma diameter also showed significant reduction (as compared to control untreated).

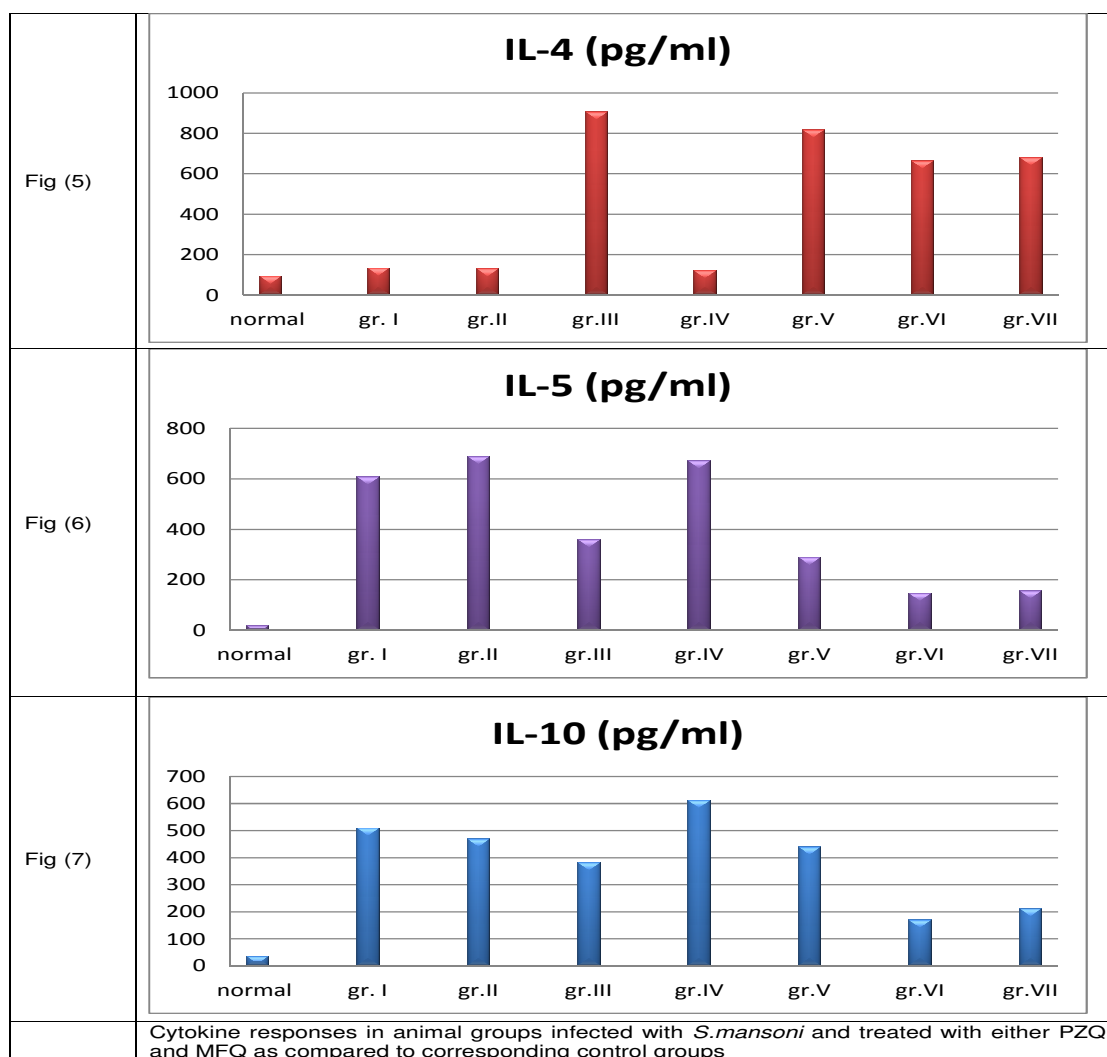


Again, infection markedly increased the IL-10 cytokine level. Treatment with PZQ did not improve its serum level. However, MFQ significantly decreased IL-10 serum level, as compared to all infected untreated and treated groups. As regards IL-4, it also increased following infection. But treatment with PZQ significantly decreased its level. MFQ also decreased serum IL-4 level. However, this level was still higher than the PZQ treated group. Serum IL-5 level significantly increased post infection.

Nevertheless, treatment with MFQ saliently decreased its levels as compared to the other groups.

DISCUSSION

In this work, Mefloquine resulted in reduction in the worm load accompanied by increased percentage of dead ova. This goes with the previous statement of Manneck et al



(2010) and Xiao and Zhang (2010), who noticed that MFQ affects the efficacy of suckers due to changes in their shape and structure. Thus, sluggish fixation of the worms in their place can make them easily swept by the blood stream and diminish their longevity. Again, in this work oral administration of PZQ and MFQ resulted in drop, then absence of hepatic granulomata respectively. This goes with the previous assumption of Abdel- Fattah and Ahmed (2011), who stated that oral administration of single high dose of MFQ (400 mg/kg), 3 weeks post-infection with *S. mansoni* cercariae resulted in absence of hepatic granulomas. While PZQ administrated orally in a dose of 500 mg/kg reduced the granuloma diameter by 20%.

Previously, Gryseels et al. (2001) reported that a major part of resistance to challenge is immunologically based. Botros et al. (2000) reported that *S. mansoni* infected animals, treated and cured with PZQ showed maximal reduction in granuloma diameter, but the state of resistance to re-infection was compromised. The possibility that PZQ may induce anti-fecundity immunity

has important implications for the interpretations of re-infections studies (Polmanet al., 2002). On the other hand, the addition of antigenic extracts from adult worms to PZQ for treatment of infected animal models has provided the complementary goals of improving the state of resistance to re-infection (Botros et al., 2000). Previously, Brindley and Sher (1987), then Doenhoff (1989), stated that PZQ is less effective in T-cell deprived mice, and that its efficacy swas reduced in B cell depleted mice . Later on, Hassan et al (1990) postulated that the reduction in the percentage of resistance to re-infection was accompanied by a reduction in the number of T-helper cells within the hepatic granuloma and also a reduction of granuloma volume. On the other hand, Keiser et al. (2010b) found that MFQ is equally effective against *S. mansoni* in euthymic (T cell-deficient) and immunocompetent mice. Hence, MFQ acts T-cell independently and does not involve synergistic interaction with the immune response for efficacy on *S. mansoni*.

Mefloquine treatment resulted in decreased IL-10 serum levels. This goes with the previous deduction of Wilson et al (2011), who stated that elevated IL-10 after PZQ treatment in combination with IL-10 antagonist resulted in compromising or hindering resistance to reinfection. The authors also postulated that treatment of mice with both IL-10 and PZQ, increased resistance to re-infection.

In this study, a remarkable decrease in the mean number of immature worms was observed in group VII when mefloquine treated mice were challenged with *S.mansoni cercariae* (Figure1).

This goes with the previous assumption of Zhang et al. (2009), then Xiao and Zhang (2010). They stated an additional point regarding interference of MFQ with *S. mansoni* nutrition through an ultrastructural degeneration and dilatation of the gut with focal desquamation of the gut epithelium of *S. mansoni* worms following MFQ treatment. So, these gut changes can reduce the efficacy of blood intake and metabolism by the worms which is important for their development and sexual maturation.

Again, the percentage of resistance increased (Figure 4) significantly when compared to group V treated with PZQ and re-infected two weeks later. This goes with the previous findings of Renganathan and Cioli (1998), then Gryseels et al (2001). The authors found that the immature schistosomes are considered less susceptible to the drug. Hence, the low cure rates may be due to the persistence of immature worms in the patients at the time of treatment. Mefloquine affected schistosomules, and recorded significant reduction in the number of eggs at the early developmental stages following treatment of *S. mansoni* infected mice with single oral dose of 150 mg/kg of MFQ 8 weeks post-infection (Van Nassauw et al. 2008),

Parasitological parameters including oogram pattern, granuloma number and diameter did not reveal significant differences among both treated groups whether challenged or/not. As regards IL-4 levels, *S.mansoni* infection resulted in high responses. It was significantly higher in MFQ treated mice, (group VI and group VII) This could be due to known mastocytosis induced by MFQ. There was a marked drop in IL-4 level, following PZQ treatment. While IL-5 responses increased in all infected groups, treatment with PZQ did not affect its levels considerably. In mefloquine treated groups (VI and VII) the drop in serum levels of IL-10 was significantly comparable to both groups treated with PZQ.

In conclusion, from this study, it was evident that IL-10, derived predominantly from CD4+ lymphocytes, hampers the development of critical effector mechanisms that mediate resistance to schistosome infection following treatment. Therefore, immunomodulators delivered in combination with PZQ treatment are considered the pre-requisite factors to generate the robust and mixed humoral /cell-mediated immune responses required to prevent reinfection with schistosomiasis.

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