Parasitology Research (2019) 118:3399–3408
https://doi.org/10.1007/s00436-019-06465-w

HELMINTHOLOGY - ORIGINAL PAPER

Assessment of the potential therapeutic effects of omeprazole in *Schistosoma mansoni* infected mice

Asmaa R. Ellakany[1] · Dina I. Elgendy[1] · Hanan A. Alshenawy[2] · Amira E. Abdel Ghaffar[1]

Received: 5 January 2019 / Accepted: 22 September 2019 / Published online: 26 October 2019

Abstract

Schistosomiasis is a neglected chronic parasitic disease with a significant lasting morbidity. Currently, praziquantel (PZQ) is the most efficient drug for schistosomiasis worldwide. However, the possibility of the occurrence of resistance to PZQ is increasing. Therefore, there is a vital need to find new antischistosomal drugs or to increase the efficacy of the existing ones. Omeprazole is a proton pump inhibitor which is reported to have antiparasitic properties. Thus, the aim of this study was to assess the potential therapeutic effects of omeprazole in experimental *Schistosoma mansoni* infection either alone or in combination with PZQ. For this aim, 80 laboratory bred mice were divided into 3 groups; uninfected control, infected untreated control, and infected and treated at tenth week P.I. The last group was divided into three subgroups that received either PZQ alone, omeprazole alone, or both drugs. The effectiveness of treatment was assessed by adult worm counts, liver egg count, scanning electron microscopy of adult worms, histopathological, and immunohistochemical (GFAP) examination. There was significant reduction of adult worm counts, liver egg counts, size, diameter of hepatic granulomas, hepatic fibrosis, and GFAP expression in the group that received combined treatment as compared to PZQ group. Moreover, the tegumental changes were more evident in the group that received combined treatment. In conclusion, the administration of omeprazole with PZQ improved the efficacy of PZQ in the treatment of *Schistosomiasis mansoni*.

Keywords *Schistosoma mansoni* · Omeprazole · Scanning electron microscopy (SEM) · Hepatic fibrosis · Glial fibrillary acidic protein (GFAP)

Introduction

Schistosomiasis is a debilitating disease which is prevalent in tropical and subtropical areas worldwide, with the highest incidence of morbidity and mortality in African developing countries (Weerakoon et al. 2015). In Egypt, schistosomiasis is a major public health concern (Othman and Soliman 2015). Moreover, reinfection with schistosomiasis is a persistent dilemma particularly in rural areas due to frequent exposure, despite public health control programs that led to decreased infection rates (Elhenawy et al. 2017).

Adult schistosomes can stay alive in their final hosts for a very long time producing harmful effects on different body systems (Liu et al. 2018). The most characteristic feature in the pathology of schistosomiasis is granulomatous inflammation which is a cell-mediated immune response to antigens secreted from the eggs. Healing of the granulomas results in hepatic fibrosis that leads to portal hypertension which is the major complication of schistosomiasis (Andrade 2009). In spite of the great impact of schistosomiasis on the health and socioeconomic status of the population, it is still a neglected tropical disease (Ajibola et al. 2018).

Currently, praziquantel (PZQ) is the drug of choice for treatment of schistosomiasis (Cioli et al. 2014). The reliance

Section Editor: Ramaswamy Kalyanasundaram

✉ Dina I. Elgendy
drdina83@yahoo.com; dina.elgendy@med.tanta.edu.eg

Asmaa R. Ellakany
arashad20@yahoo.com; asmaa.rashad@med.tanta.edu.eg

Hanan A. Alshenawy
hanan_alshenawy@yahoo.com

Amira E. Abdel Ghaffar
amira29757@gmail.com

[1] Medical Parasitology Department, Faculty of Medicine, Tanta University, Tanta Egypt

[2] Pathology Department, Faculty of Medicine, Tanta University, Tanta Egypt

on it as a single drug and its persistent use in the treatment of this widely spreading disease have brought resistance and tolerance by *Schistosoma mansoni* in various regions (Crellen et al. 2016). Furthermore, after development of schistosomal hepatic and splenic lesions, the benefits of PZQ administration became restricted and the therapeutic dose of PZQ did not notably control these injurious effects (Al-Olayan et al. 2014). Thus, there is an urgent need to investigate new or synergistic compounds against *S. mansoni* parasite that could enhance the activity of PZQ or provide hepatic protection (Gouveia et al. 2018).

Omeprazole is the most commonly prescribed proton pump inhibitor for gastroesophageal hyperacidity (Targownik et al. 2007). It is noteworthy that it is safe, even in high doses, with few side effects (Abuhelwa et al. 2018). It has antibacterial activity and is used in combination with antibiotics for the treatment of *Helicobacter pylori* infection (Yuan et al. 2013). Moreover, the antiparasitic effects of omeprazole were studied against many parasites. It showed effectiveness in killing promastigotes as well as intracellular amastigotes of *Leishmania donovani* (Kochar et al. 2006). In vitro studies showed its great effect against many protozoa, e.g., *Plasmodium falciparum* (Riel et al. 2002), *Trichomonas vaginalis*, *Entamoeba histolytica* (Pérez-Villanueva et al. 2011), and *Giardia lamblia* (Reyes-Vivas et al. 2014). In addition, in vitro use of omeprazole with praziquantel against *S. mansoni* led to increase in worm lethality (Almeida et al. 2015). Therefore, this study aimed to assess the potential therapeutic effects of omeprazole in experimental *S. mansoni* infection either alone or in combination with PZQ.

Material and methods

Animals and *S. mansoni* infection

This study was carried out on 80 laboratory-bred, male Swiss albino mice 6–8 weeks old and 20–25 g weight. Mice were purchased from Theodore Bilharz Research Institute (Giza, Egypt). They were housed in standard cages and were fed on a standard diet and water ad libitum. The housing and infection were performed in *Schistosome* Biological Supply Program (SBSP), Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Laboratory-bred infected *Biomphalaria alexandrina* snails were exposed to light for at least 4 h. All mice in the infected groups were infected subcutaneously with *S. mansoni* cercariae (Egyptian strain) shed from the snails "60 cercariae/animal" as described by Holanda et al. (1974).

Drugs

Praziquantel: (Distocide; 600 mg tablet, EIPICO. Pharmaceuticals, Egypt). It was administered through oral gavages to each mouse in a single dose of 300 mg/kg body weight (Othman et al. 2008). Omeprazole: (Omepral; 20 mg capsules, Memphis, Pharmaceuticals, Egypt). It was administered through oral gavages to each mouse in a dose of 20 μg/day for seven consecutive days (Tian et al. 2014).

Experimental design

Mice were divided into three groups:

Group I (uninfected control): (ten mice) served as the non-infected non-treated control for immunohistochemical staining.
Group II (infected untreated control): (ten mice) *S. mansoni*-infected non-treated control.
Group III: (60 mice) *S. mansoni*-infected mice that received treatment starting 10 weeks P.I. This group was divided into 3 subgroups (20 mice each); group IIIa treated with PZQ only, group IIIb treated with omeprazole only, and group IIIc treated with PZQ and omeprazole.

All mice were sacrificed 12 weeks P.I. For all infected mice, parasitological examination including adult worms count, liver egg count, and SEM of adult worms were done. Parts of the liver of all animals were removed and preserved in formal saline (10%) for further histopathological and immunohistochemical studies.

Parasitological study

Adult worm counts were estimated by using animal perfusion method. Briefly, an incision was done in the portal vein followed by perfusion of citrated saline through the descending aorta. The adult worms were collected into conical sedimentation glasses, transferred by a Pasteur pipette into petri dishes, washed with normal saline, and counted/mouse (Duvall and De Witt 1967).

Liver egg counts were estimated in all infected groups. One gram from each liver was weighed, placed in a test tube containing 2 ml of 5% KOH, and left overnight at room temperature. On the second day, all test tubes were placed in the incubator at 37 °C for 6 h. Each test tube was shaken, then 0.1 ml of the digest was examined microscopically for counting *S. mansoni* eggs. The total egg count in 1 g liver tissue was then calculated (Cheever 1968).

Scanning electron microscopic examination of adult worms

Samples of *S. mansoni* adult worms were washed several times with normal saline, fixed with 2.5% glutaraldehyde, followed by dehydration with serial dilutions of ethanol. Then, the worms were dried using a CO_2 critical point drier. Specimens were coated with a gold sputter coater. Coated

worms were examined and photographed using SEM (Inspect S; FEI, Holland) at the Electron Microscopy Unit of Theodor Bilharz Research Institute (Xavier et al. 2010).

Histopathological and immunohistochemical study

Histopathological examination

Paraffin blocks were made from the livers previously fixed in 10% formal saline. Serial sections (5 μm) were cut from each specimen and stained with hematoxylin and eosin (H&E). Ten sections were examined/mouse. The number of the granulomas was determined in ten high power fields (× 400)/liver section and then the mean number of granulomas/liver section was calculated. The diameter of liver granulomas was measured in two perpendicular lines using ocular micrometer lens fixed on the light microscope, for each slide ten granulomas were measured and their mean diameter was calculated (Bancroft and Steven 1975; Jacobs et al. 1997).

Immunohistochemical examination

It was done for demonstration of activated hepatic stellate cells using antibodies against GFAP that help to assess the degree of hepatic fibrosis. Immunohistochemical staining was performed on 3–5 μm sections, using the UltraVision Detection System (AntiPolyvalent, HRP/DAB "Ready-to-Use," Cat. #TP-015-HD, Lab Vision, USA). The procedure of immunostaining was conducted according to the manufacturer's protocol as follows: sections were deparaffinized in xylene then rehydrated in descending grades of alcohol. The endogenous peroxidase positivity was blocked by incubation in 0.3% hydrogen peroxide in methanol. Microwave antigen retrieval was carried out in citrate buffer. After washing with phosphate buffer, sections were incubated with Ultra V block followed by rinsing with phosphate buffer again. An overnight incubation of the sections with antibody against GFAP was done followed by washing with phosphate buffer and incubation with biotinylated goat anti-polyvalent (secondary antibody) for 10 min then washing again. Sections were then incubated with streptavidin peroxidase solution for 10 min and washed with phosphate buffer. The reaction products were visualized using 3-3-diamino-benzidine-tetrahydrochloride (DAB). The sections were then counterstained with Mayer's hematoxylin, dehydrated in alcohol, and mounted in dibutylphthalate polystyrene xylene (DPX). Positive and negative control slides were included within each session. Sections from the brain of a mouse were used as positive controls, while negative controls were prepared by omission of the primary antibody. A semi-quantitative score of three grades was assigned based on the number of cells showing immunopositivity for GFAP as follows: (grade 1) for weak staining, (grade 2) for moderate staining, and (grade 3) for intense staining (Gibelli et al. 2008)

Ethics statement

The study protocol was approved and conducted according to the guidelines of the Laboratory Animal Centre for Research Ethics Committee, Faculty of Medicine, Tanta University (code number 31446/03/17).

Statistical analysis

Data were presented as means ± standard deviation. Analysis of variance (ANOVA) was used to compare more than two groups and the probability of significant differences among dual means of groups was determined by post hoc test. Chi-square test was used for comparison of qualitative data between two groups. Differences were considered nonsignificant when ($P > 0.05$), significant when ($P < 0.05$), and highly significant when ($P < 0.001$). The statistical analyses were processed according to the conventional procedures using Statistical Program of Social Sciences (SPSS) (SPSS Inc., Chicago, Illinois, USA), software for windows, version (20).

Results

Adult *S. mansoni* worms count

At 12 weeks P.I., when comparing the mean number of adult worms recovered from infected control group (II) with that of other groups, it was found that there was significant decrease in both PZQ (IIIa) group and the group that received combined treatment (IIIc), but the decrease was not significant in omeprazole group (IIIb). Additionally, there was a significant decrease in the group that received combined treatment (IIIc) in comparison with both PZQ (IIIa) and omeprazole (IIIb) groups (Table 1).

Liver egg count

At 12 weeks P.I., both the group that received PZQ alone (IIIa) and the group that received combined treatment (IIIc) showed highly significant decrease ($P < 0.001$) in mean numbers of eggs trapped in the liver compared to the infected control group (II) (6022.00 ± 349.57 and 2452.80 ± 593.87 vs. 8927.20 ± 1233.53) respectively. Moreover, a statistically significant reduction of liver egg count ($P < 0.001$) was found between PZQ group and the group that received combined treatment. There was no significant difference in the mean numbers of liver egg count between the group that received

Table 1 Adult worms count (mean ± SD) and the mean number of *S. mansoni* eggs/g liver in all studied groups at 12 weeks post infection ($n = 10$)

	Group	Mean ± SD	Reduction percentage	*F* test	*P* value	Post hoc test	
Adult worms count	Infected control	25.80 ± 2.42	–	768.157	< 0.001**	P1	< 0.001**
	PZQ	7.80 ± 0.77	69.77%			P2	0.497
						P3	< 0.001**
	Omeprazole	25.00 ± 2.05	3.10%			P4	< 0.001**
						P5	< 0.001**
	Combined treatment	4.47 ± 1.47	82.67%			P6	< 0.001**
S. mansoni eggs count / gm liver	Infected control	8927.20 ± 1233.53	–	226.638	< 0.001**	P1	< 0.001**
	PZQ	6022.00 ± 349.57	32.54%			P2	0.262
						P3	< 0.001**
	Omeprazole	8609.20 ± 1086.31	3.56%			P4	< 0.001**
						P5	< 0.001**
	Combined treatment	2452.80 ± 593.87	72.52%			*P6*	< 0.001**

*P*1: Infected control vs. PZQ—$^{**}P < 0.001$

*P*2: Infected control vs. Omeprazole—$^{*}P < 0.05$

*P*3: Infected control vs. combined treatment

*P*4: PZQ vs Omeprazole

*P*5: PZQ vs combined treatment

*P*6: Omeprazole vs combined treatment

n Number of studied mice in each group

Reduction percentage: percentage of reduction between each group and infected control group

omeprazole and the infected control group ($P = 0.262$) (Table 1).

Scanning electron microscopy

At 12 weeks P.I., SEM images of male worms from the infected control group showed oral and ventral suckers with normal integrity and well-developed tubercles of homogeneous size and distribution. The tegumental surface showed apically directed spines (Fig. 1a, b). Worms from the PZQ group showed tegumental damage in the form of distorted suckers, flattening of tubercles and reduction in the size of the spines (Fig. 1d, e). Additionally, worms from omeprazole group showed swelling and deformation of suckers, swelling of the whole body, smoothing of tubercles, and loss of spines (Fig. 1g, h). Worms from the group that received combined treatment showed marked distortion of the suckers and severe tegumental damage in the form of peeling of tubercles, erosion of the surface with appearance of subtegumental tissues, blebbing of the tegument, and loss of spines (Fig. 1j, k). As regard female worms, SEM images of worms from the infected control group showed intact tegumental surface with normal integrity (Fig. 1c). Worms from the PZQ group showed tegumental contraction, corrugation, and blebbing of the tegument (Fig. 1f). Furthermore, worms of omeprazole group showed body corrugation and blebbing of the tegument (Fig. 1i). Worms from the combined treatment group showed marked erosion of the surface with appearance of subtegumental tissues (Fig. 1l).

Histopathological and immunohistochemical results

Histopathological findings of the liver parenchyma at 12 weeks P.I.

Regarding the mean number of granulomas/liver section of different groups, there was a highly significant reduction in the mean number of granulomas in both PZQ group and the group that received combined treatment in comparison to the infected control group, but the decrease was statistically non-significant in omeprazole group. Additionally, there was a significant decrease in the group that received combined treatment ($P < 0.001$) in comparison with both PZQ and omeprazole groups (Table 2).

As regards the mean diameter of hepatic granulomas, there was a significant decrease in the mean diameter of hepatic granulomas in all groups in comparison with the infected control group. The percentages of reduction in the diameter of granulomas in the group that received combined treatment was highly significant (60.11%) as compared with that of PZQ group (45.05%) (Table 2). Liver section of infected-untreated mice showed multiple portal and parenchymal granulomas, most of them were of fibrous type with intervening inflammatory mononuclear cells (Fig. 2a). As regards PZQ-treated mice, the granulomas were fibrocellular composed mainly of mononuclear cells with mild fibrosis around the ova (Fig. 2b). In omeprazole group, fibrocellular granulomas were prominent with increased cellularity (Fig. 2c). In the group that received combined treatment, very scarce granulomas composed

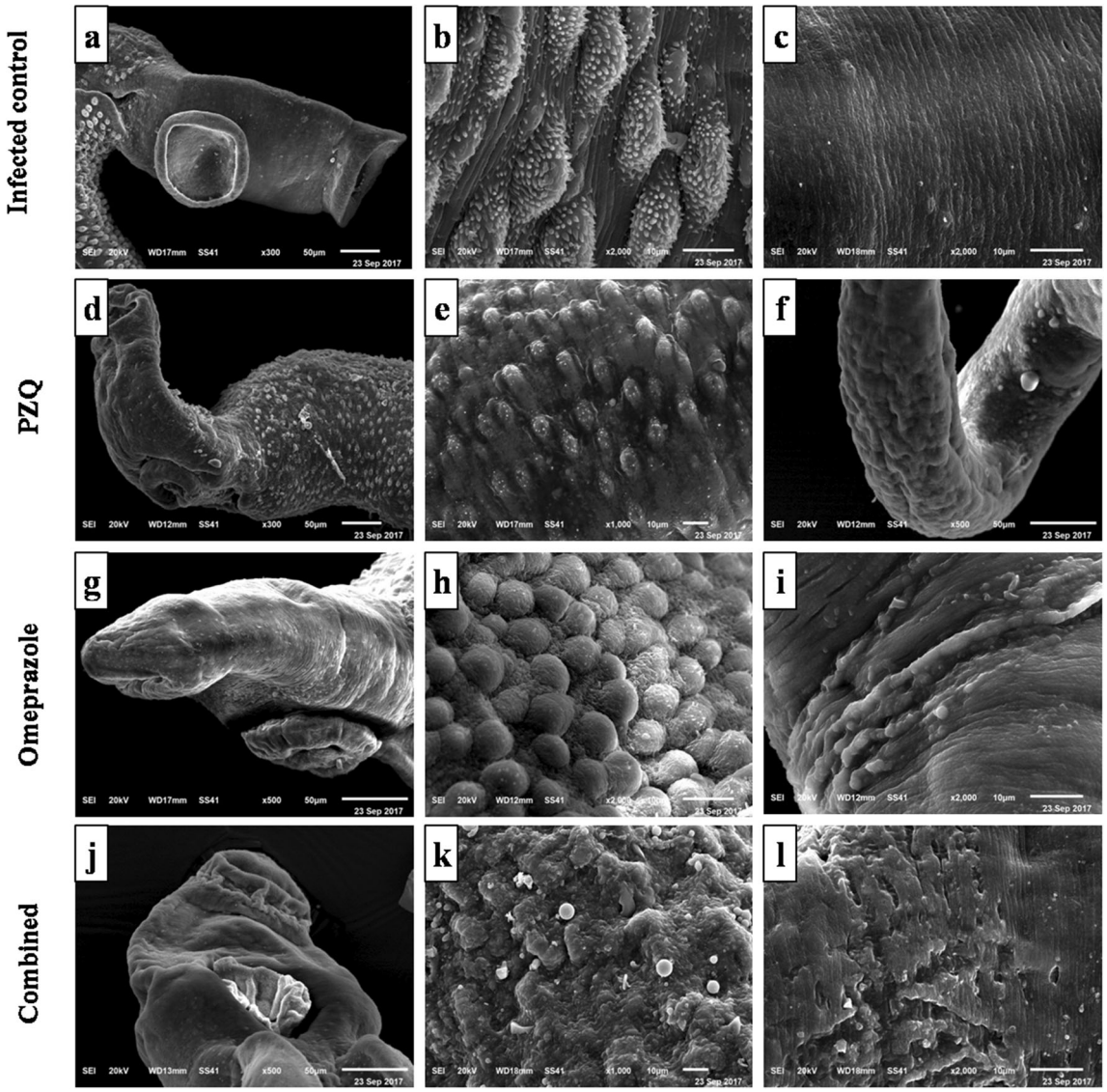

Fig. 1 SEM of *Schistosoma mansoni* adult worm retrived 12 weeks P.I. **a** male worm from infected control group showing oral and ventral suckers with normal integrity (× 300). **b** Male worm from infected control group showing intact tegumental surface with well-developed tubercles of uniform size and distribution and apically directed spines (× 2000). **c** Female worm from infected control group showing intact tegumental surface with normal integrity (× 2000). **d** Male worm from PZQ group showing distortion of the suckers and body contraction (× 300). **e** Male worm from PZQ group showing flattening and destruction of tubercles with partial loss of spines. (× 1000). **f** Female worm from PZQ group showing tegumental contraction, corrugation and blebbing of the tegument (× 500). **g** Male worm from omeprazole group showing swelling and distortion of suckers (× 500). **h** Male worm from omeprazole group showing smoothing and swelling of tubercles and loss of spines (× 2000). **i** Female worm from omeprazole group showing corrugation and blebbing of the tegument (× 2000). **j** male worm from the group received combined treatment showing marked distortion of the suckers (× 500). **k** Male worm from the group received combined treatment showing erosion of the surface, appearance of subtegumental tissues and loss of tubercles and spines (× 1000). **l** Female worm from the group received combined treatment showing extensive erosion of the surface with appearance of subtegumental tissues (× 2000)

mainly of few mononuclear cells and histocytes with very mild fibrosis were detected (Fig. 2d).

Immunohistochemical results (GFAP immunoreactivity) at 12 weeks P.I.

The immunoreactivity of GFAP in hepatic tissues was assessed in all studied groups (Table 3). The highest positivity of the stain was observed in the infected control group, where strong GFAP positivity (grade 3) was detected diffusely in perisinusoidal, periportal areas, and around granulomas (Fig. 3a). Either omeprazole or PZQ administration led to decrease in stellate cell activation, where omeprazole group (Fig. 3b) or PZQ group (Fig. 3c) showed only weak to moderate staining intensity. The weakest staining intensity was detected in the group that received combined treatment. Very weak positivity

Table 2 The number/liver section and diameter (μm) of hepatic granulomas in infected mice in all studied groups at 12 weeks post infection ($n = 10$)

	Group	Mean ± S.D	Reduction percentage	*F* test	*P* value	Post hoc test	
Granuloma number	Infected control	43.10 ± 3.16	±	123.937	< 0.001**	P1	< 0.001**
	PZQ	20.60 ± 1.14	52.20%			P2	0.295
						P3	< 0.001**
	Omeprazole	41.50 ± 8.90	3.71%			P4	< 0.001**
						P5	< 0.001**
	Combined treatment	9.50 ± 1.24	77.96%			P6	< 0.001**
Granuloma diameter	Infected control	321.37 ± 47.26	–	121.962	< 0.001**	P1	< 0.001**
	PZQ	176.60 ± 22.39	45.05%			P2	0.009*
						P3	< 0.001**
	Omeprazole	290.12 ± 39.77	9.72%			P4	< 0.001**
						P5	< 0.001**
	Combined treatment	128.19 ± 34.48	60.11%			P6	< 0.001**

*P*1: Infected control vs. PZQ—$^{**}P < 0.001$

*P*2: Infected control vs. Omeprazole—$^{*}P < 0.05$

*P*3: Infected control vs. combined treatment

*P*4: PZQ vs. Omeprazole

*P*5: PZQ vs. combined treatment

*P*6: Omeprazole vs. combined treatment

n Number of studied mice in each group

Reduction percentage: percentage of reduction between each group and infected control group

was detected in the perisinusoidal area with only some cells within the granulomas (Fig. 3d).

Discussion

Schistosomiasis is a major endemic parasitic disease characterized by chronic course with considerable lasting morbidity (Weerakoon et al. 2015; Kovač et al. 2017). The existing basis of its management is PZQ (Amara et al. 2018). Unfortunately, there have been various information about inadequate or even no response of *S. mansoni* infections to PZQ in some areas. This may be due to excessive use of the drug (McManus et al. 2018). This raises the attention to search for different adjuvants to enhance the efficacy of PZQ (Yang et al. 2015). Omeprazole is a proton pump inhibitor and one of the most potent acid reducers (Lundell 2015). It has been used as an adjuvant in treatment of many disorders for its anti-inflammatory (Kedika et al. 2009), antioxidative stress (Namazi and Jowkar 2008), anti-apoptotic (Biswas et al.

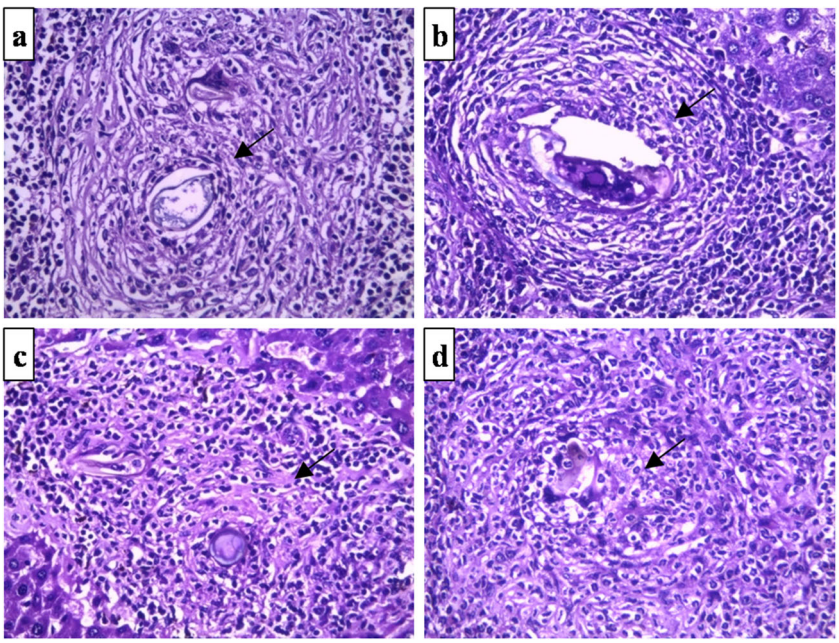

Fig. 2 Photomicrographs of liver sections retrived 12 weeks P.I. (H&E × 400), **a** from infected control group showing fibrous granuloma around the ova with intervening inflammatory mononuclear cells, **b** from PZQ group showing fibrocellular granuloma formed mainly of mononuclear cells with mild fibrosis around the ova, **c** from omeprazole group showing fibrocellular granuloma with increase in its cellular components, and **d** from the group received combined treatment showing fibrocellular granuloma with marked increase in its cellular components and very mild fibrosis

Table 3 Comparison of GFAP expression in all studied groups at 12 weeks post infection (n = 10)

Group	Grade 1	Grade 2	Grade 3	Chi-square	P value
Uninfected control	10 (100%)	0 (0%)	0 (0%)	34.643	MC
Infected control	0 (0%)	2 (20%)	8 (80%)		0.001*
PZQ	5 (50%)	3 (30%)	2 (20%)		
Omeprazole	4 (40%)	4 (40%)	2 (20%)		
Combined treatment	9 (90%)	1 (10%)	0 (0%)		

MC Monte Carlo exact test for chi square

n Number of studied mice in each group

*$P < 0.05$

2003), anti-proliferative, and antifibrotic properties (Ghebre and Raghu 2016). Based on the promising in vitro results of Almeida et al. (2015), we found it good to assess the potential therapeutic effects of omeprazole in experimental *S. mansoni* infection either alone or in combination with PZQ.

In the current study, the percentage of reduction in adult worm counts was significantly increased in *S. mansoni*-infected mice in the group that received combined treatment when compared to mice treated with PZQ or omeprazole alone. However, no statistically significant difference could be detected in omeprazole-treated group in comparison with the infected control group. This reflects the potent effect of combined therapy on the adult worms. These findings are consistent with the in vitro results of Almeida et al. (2015) who reported that omeprazole alone did not kill viable adult worms, but it just affects PZQ-stressed parasites.

Two mechanisms could help in explanation of the synergistic action of omeprazole and PZQ against *S. mansoni* adult worms. First, omeprazole inhibits ATP1A2 Na/K-ATPase in adult worms, which is over expressed under the effect of PZQ, leading to increased mortality of adult worms (Almeida et al. 2015). Second, omeprazole inhibits ATP-dependent efflux transporter P-glycoprotein (a member of the ATP binding cassette multidrug transporter family) in PZQ-stressed parasites leading to increased retention of PZQ in these parasites which could potentiate its antiparasitic effects (Pauli-Magnus et al. 2001; Kasinathan et al. 2014).

As regards the effects on the liver egg loads in the present study, the PZQ group showed a statistically significant reduction in the egg load per gram of liver when compared with the infected control group. Although the reduction of liver egg loads in the omeprazole group versus the infected control group was insignificant, its co-administration with PZQ showed the highest statistically significant reduction (72.52%). These results could be explained by killing of the adult parasites that cut the source of eggs in the tissues (Garba et al. 2001) which is more prominent in the group that received combined treatment than in the PZQ group.

The tegument of an adult schistosome is a defensive covering that plays a role in the defense mechanisms as well as in

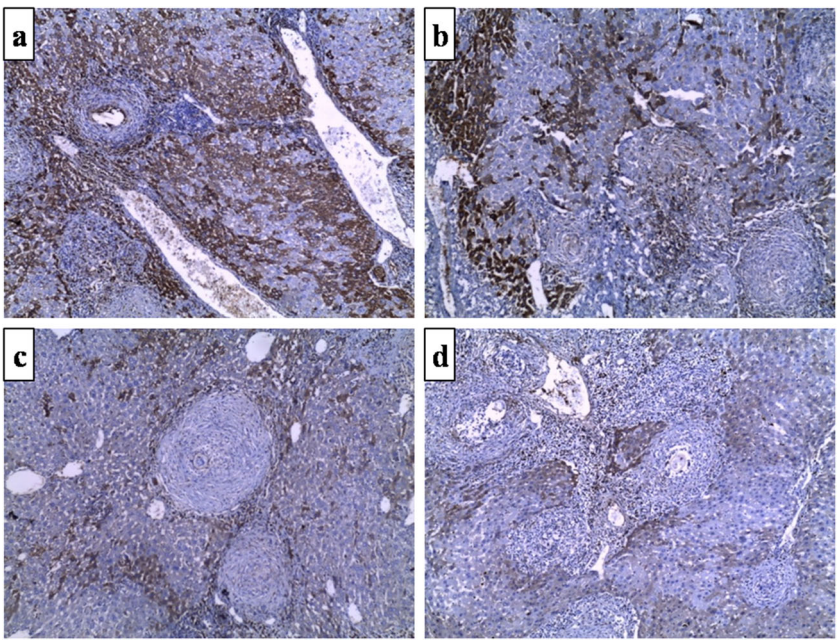

Fig. 3 Photomicrographs of liver sections retrived 12 weeks P.I. showing immunohistochemical expression of GFAP within hepatic stellate cells (Immunoperoxidase × 100) **a** from infected control group showing grade 3 immunoreactivity, **b** from PZQ group showing grade 2 immunoreactivity, **c** from omeprazole group showing grade 2 immunoreactivity, and **d** from the group received combined treatment showing grade 1 immunoreactivity

the nutrition, osmoregulation, and excretion of the parasite. It is a vital border between the parasite and the host (El-Shabasy et al. 2015). It is worth mentioning that sucker distortion lessens the parasite's capability to stay in blood vessels and hinders the process of nourishment. Additionally, tegumental damage weakens its performance (Xiao et al. 2000).

Concerning the ultrastructure and tegumental changes of *S. mansoni* adult worms in response to PZQ in the current study, the tegument showed distorted suckers, coiling of the body, flattening of tubercles, and reduction in the size of the spines. These findings are consistent with the previous several in vitro and in vivo studies on *Schistosoma* worms under the effect of PZQ (Xiao et al. 2000; de Oliveira et al. 2012; Kamel and Bayaumy 2017). Besides, worms from omeprazole group showed tegumental changes in the form of swelling and distortion of suckers, smoothing of tubercles. These results are very important as this study is considered the first in vivo one to investigate the effect of omeprazole on the tegument of *S. mansoni* adult worms. The possibility exists that omeprazole exerts these protective effects by other mechanisms which are yet in need for further investigations.

In addition, the tegumental changes were more evident in the group that received combined treatment with marked loss of spines, erosion of the surface, and appearance of subtegumental tissues. These results are in agreement with the results of Kasinathan et al. (2014) who reported that the co-administration of PZQ with ATP-dependent efflux transporter inhibitors such as omeprazole resulted in complete loss of motility and disruption of the tegument with appearance of multiple blebs on the surface of the parasites.

Regarding the effects on the number and diameter of hepatic granulomas, data from the present study showed that treatment of *S. mansoni*-infected mice with PZQ and omeprazole together significantly reduced granuloma number in the liver by (77.96%) as well as granuloma diameter in the liver by (60.11%). These percentages of reduction are significantly higher than those of PZQ-treated group.

The decreased number of the granulomas was in accordance with diminished egg counts in the liver. It could be explained by the elimination of adult worms with the administered treatment resulting in less egg deposition and decreased eggs induced immunopathology (Pearce 2005). Additionally, PZQ causes a considerable modulatory effect on cell-mediated immune responses which decreases CD4 T cells and enhances CD8 T cells resulting in diminution of the diameter of hepatic schistosomal granuloma (El-Lakkany and Nosseir 2007). Moreover, it has been reported that proton pump inhibitors have potent anti-inflammatory properties (Ghebremariam et al. 2015), which may add to the effect of PZQ and lead to more decrease in granuloma size.

Concerning the histopathological examination of the liver tissues in the present study, the group that received combined treatment showed marked improvement in hepatic pathology with more decrease in the inflammatory cells than other groups. The superiority of combined therapy over the corresponding drug can be attributed to the ability of omeprazole to potentiate the effect of PZQ in control of inflammation by suppressing the expression of pro-inflammatory molecules including vascular cell adhesion molecule-1, inducible nitric oxide synthase, TNF-α, IL-1β, and IL-6 as well as decreased adherence of inflammatory cells to vascular wall (Tanigawa et al. 2009; Ghebremariam et al. 2015).

Liver fibrosis is a major aspect of hepatic pathology which occurs in chronic schistosomal infection. It results from chronic injury of the liver and activation of HSCs which produces excessive amount of extracellular matrix elements (Bartley et al. 2006). Accumulation of extracellular matrix lastly leads to architectural alterations in the liver tissue (Wang et al. 2010). It is noteworthy that one of the most important aims in treatment of schistosomiasis is to stop or to lessen the mechanisms that produce liver fibrosis. Multiple studies have confirmed a positive relationship between the severity of hepatic fibrosis and the number of activated stellate cells present in the liver (Russo et al. 2005, Cassiman et al. 2002). It has been recognized that GFAP could be a valuable indicator of early HSCs activation as its intensity increases significantly during liver fibrosis (Carotti et al. 2008).

In this study, evident reduction of GFAP expression was observed in all treated groups but it was maximum in the group that received combined treatment. We assume that omeprazole potentiates the antifibrotic effect of PZQ. This could be explained by the ability of PPIs to inhibit a number of pro-inflammatory/pro-fibrotic cytokines. They lead to decreased expression of inducible nitric oxide synthase (iNOS) and several other proinflammatory cytokines (Nelson et al. 2017). In addition, cell culture studies revealed that esomeprazole (a member of PPIs) produced potent inhibition of fibroblast proliferation as well as downregulation of profibrotic proteins including receptors for TGF-β, fibronectin, and matrix metalloproteinases (Ghebremariam et al. 2015).

Conclusion

This work has revealed for the first time, as far as we know, a hepatic protective effect of omeprazole against the damage produced by *S. mansoni* infection. Besides, liver fibrosis in the groups that received this drug was greatly reduced. Moreover, omeprazole increased the effectiveness of anti-schistosomal therapy. Therefore, omeprazole could be considered as a valuable adjuvant therapy in hepatic *Schistosomiasis mansoni*, and additional studies and controlled human trials on it are worth consideration in schistosomiasis.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abuhelwa AY, Mudge S, Upton RN, Foster DJR (2018) Mechanistic assessment of the effect of omeprazole on the in vivo pharmacokinetics of itraconazole in healthy volunteers. Eur J Drug Metab Pharmacokinet 44(2):201–215 1–15

Ajibola O, Gulumbe BH, Eze AA, Obishakin E (2018) Tools for detection of schistosomiasis in resource limited settings. Med Sci 6(2): 39–50

Almeida GT, Lage RCG, Anderson L, Venancio TM, Nakaya HI, Miyasato PA, Rofatto HK, Zerlotini A, Nakano E, Oliveira G, Verjovski-Almeida S (2015) Synergy of omeprazole and praziquantel in vitro treatment against *Schistosoma mansoni* adult worms. PLoS Negl Trop Dis 9(9):4086–4108

Al-Olayan EM, El-Khadragy MF, Aref AM, Othman MS, Kassab RB, Abdel Moneim AE (2014) The potential protective effect of Physalis peruviana L. against carbon tetrachloride-induced hepatotoxicity in rats is mediated by suppression of oxidative stress and downregulation of MMP-9 expression. Oxidative Med Cell Longev 2014:381413

Amara RO, Ramadan AA, El-Moslemany RM, Eissa MM, El-Azzouni MZ, El-Khordagui LK (2018) Praziquantel–lipid nanocapsules: an oral nanotherapeutic with potential *Schistosoma mansoni* tegumental targeting. Int J Nanomedicine 13:4493–4505

Andrade ZA (2009) Schistosomiasis and liver fibrosis. Parasite Immunol 31:656–663

Bancroft JD, Steven A (1975) Histopathological stains and their diagnostic uses, 1st edn. Churchill Livingstone, New York, pp 1–20

Bartley PB, Ramm GA, Jones MK, Ruddel RG, Li Y, McManus DP (2006) A contributory role for activated hepatic stellate cells in the dynamics of *Schistosoma japonicum* egg-induced fibrosis. Int J Parasitol 36(9):993–1001

Biswas K, Bandyopadhyay U, Chattopadhyay I, Varadaraj A, Ali E, Banerjee RK (2003) A novel antioxidant and antiapoptotic role of omeprazole to block gastric ulcer through scavenging of hydroxyl radical. J Biol Chem 278(13):10993–11001

Carotti S, Morini S, Corradini SG, Burza MA, Molinaro A, Carpino G, Merli M, De Santis A, Muda AO, Rossi M, Attili AF, Gaudio E (2008) Glial fibrillary acidic protein as an early marker of hepatic stellate cell activation in chronic and posttransplant recurrent hepatitis C. Liver Transpl 14(6):806–814

Cassiman D, Libbrecht L, Desmet V, Denef C, Roskams T (2002) Hepatic stellate cell/myofibroblast subpopulations in fibrotic human and rat livers. J Hepatol 36:200–209

Cheever AW (1968) Conditions affecting the accuracy of potassium hydroxide digestion in techniques for counting *Schistosoma mansoni* eggs in tissues. Bull World Health Organ 39:328–331

Cioli D, Pica-Mattoccia L, Basso A, Guidi A (2014) Schistosomiasis control: Praziquantel forever? Mol Biochem Parasitol 195(1):23–29

Crellen T, Walker M, Lamberton PH, Kabatereine NB, Tukahebwa EM, Cotton JA, Webster JP (2016) Reduced efficacy of praziquantel against *Schistosoma mansoni* is associated with multiple rounds of mass drug administration. Clin Infect Dis 63(9):1151–1159

de Oliveira RN, Rehder VLG, Oliveira ASS, Júnior ÍM, de Carvalho JE, de Ruiz AL, Jeraldo VL, Linhares AX, Allegretti SM (2012) *Schistosoma mansoni*: *in vitro* schistosomicidal activity of essential oil of *Baccharis trimera* (Less) DC. Exp Parasitol 132(2):135–143

Duvall RH, De Witt WB (1967) An improved perfusion technique for recovering adult schistosomes from laboratory animals. Am J Trop Med Hyg 16:483–486

Elhenawy AA, Ashour RH, Nabih N, Shalaby NM, Megahed N (2017) Possible antifibrotic effect of GDC-0449 (Vismodegib), a hedgehog-pathway inhibitor, in mice model of *Schistosoma*–induced liver fibrosis. Parasitol Int 66:545–554

El-Lakkany N, Nosseir M (2007) Pharcodynamics of pentoxifylline and /or praziquantel in murine schistosomiasis mansoni. APMJS. 115: 184–194

El-Shabasy EA, Reda ES, Abdeen SH, Said AE, Ouhtit A (2015) Transmission electron microscopic observations on ultrastructural alterations in *Schistosoma mansoni* adult worms recovered from C57BL/6 mice treated with radiation attenuated vaccine and/or praziquantel in addition to passive immunization with normal and vaccinated rabbit sera against infection. Parasitol Res 114(4):1563–1580

Garba A, Tohon Z, Sidiki A, Chippaux JP, de Chabalier F (2001) Efficacity of praziquantel in school-aged children in a hyperendemic zone for *Schistoma haematobium* (Niger, 1999). Bull Soc Pathol Exot 94(1):42–45

Ghebre YT, Raghu G (2016) Idiopathic pulmonary fibrosis: novel concepts of proton pump inhibitors as antifibrotic drugs. Am J Respir Crit Care Med 193:1345–1352

Ghebremariam YT, Cooke JP, Gerhart W, Griego C, Brower JB, Doyle-Eisele M, Moeller BC, Zhou Q, Ho L, de Andrade J, Raghu G, Peterson L, Rivera A, Rosen GD (2015) Pleiotropic effect of the proton pump inhibitor esomeprazole leading to suppression of lung inflammation and fibrosis. J Transl Med 13:249–268

Gibelli NE, Tannuri U, and Mello ES (2008) Immunohistochemical studies of stellate cells in experimental cholestasis in newborn and adult rats. Clinics (Sao Paulo) 63(5):689–694

Gouveia MJ, Brindley PJ, Gärtner F, da Costa JMC, Vale N (2018) Drug repurposing for schistosomiasis: combinations of drugs or biomolecules. Pharmaceuticals 11(1):15

Holanda JC, Pellegrino J, Gazzinelli G (1974) Infection of mice with cercariae and schistosomula of *Schistosoma mansoni* by intravenous and subcutaneous routes. Rev Inst Med Trop 16:132–134

Jacobs W, Bogers J, Deelder A, Wéry M, Van Marck E (1997) Adult *Schistosoma mansoni* worms positively modulate soluble egg antigen-induced inflammatory hepatic granuloma formation in vivo. Stereological analysis and immunophenotyping of extracellular matrix proteins, adhesion molecules, and chemokines. Am J Pathol 150(6):2033–2045

Kamel ROA, Bayaumy FE-ZA (2017) Ultrastructural alterations in *Schistosoma mansoni* juvenile and adult male worms after *in vitro* incubation with primaquine. Mem Inst Oswaldo Cruz 112(4):247–254

Kasinathan RS, Sharma LK, Cunningham C, Webb TR, Greenberg RM (2014) Inhibition or knockdown of ABC transporters enhances susceptibility of adult and juvenile schistosomes to Praziquantel. PLoS Negl Trop Dis 8:e3265

Kedika RR, Souza RF, Spechler SJ (2009) Potential anti-inflammatory effects of proton pump inhibitors: a review and discussion of the clinical implications. Dig Dis Sci 54(11):2312–2317

Kochar DK, Saini G, Kochar SK, Bumb RA, Mehta RD, Sirohi P, Purohit SK (2006) A double blind randomized placebo controlled trial of rifampicin with omeprazole in the treatment of human cutaneous leishmaniasis. J Vector Borne Dis 43(4):161–167

Kovač J, Vargas M, Keiser J (2017) *In vitro* and in vivo activity of R- and S- praziquantel enantiomers and the main human metabolite trans-4-hydroxy-praziquantel against *Schistosoma haematobium*. Parasit Vectors 10(1):365–369

Liu J, Giri BR, Chen Y, Luo R, Xia T, Grevelding CG, Cheng G (2018) *Schistosoma japonicum* IAP and Teg20 safeguard tegumental

integrity by inhibiting cellular apoptosis. PLoS Negl Trop Dis 12(7): e0006654

Lundell L (2015) The physiological background behind and course of development of the first proton pump inhibitor. Scand J Gastroenterol 50(6):680–684

McManus DP, Dunne DW, Sacko M, Utzinger J, Vennervald BJ, Zhou XN (2018) Schistosomiasis. Nat Rev Dis Primers 4(1):13–31

Namazi MR, Jowkar F (2008) A succinct review of the general and immunological pharmacologic effects of proton pump inhibitors. J Clin Pharm Ther 33:215–217

Nelson C, Lee J, Ko K, Sikora AG, Bonnen MD, Enkhbaatar P, Ghebre YT (2017) Therapeutic efficacy of esomeprazole in cotton smoke-induced lung injury model. Front Immunol 8:16

Othman AA, Soliman RH (2015) Schistosomiasis in Egypt: a never-ending story? Acta Trop 148:179–190

Othman AA, Shoheib ZS, Abdel-Aleem GA, Shareef MM (2008) Experimental schistosomal hepatitis: protective effect of coenzyme-Q10 against the state of oxidative stress. Exp Parasitol 120:147–155

Pauli-Magnus C, Rekersbrink S, Klotz U, Fromm MF (2001) Interaction of omeprazole, lansoprazole and pantoprazole with P-glycoprotein. Naunyn Schmiedeberg's Arch Pharmacol 364:551–557

Pearce EJ (2005) Priming of the immune response by Schistosome eggs. Parasite Immunol 27:265–270

Pérez-Villanueva J, Romo-Mancillas A, Hernández-Campos A, Yépez-Mulia L, Hernández-Luis F, Castillo R (2011) Antiprotozoal activity of proton-pump inhibitors. Bioorg Med Chem Lett 21(24):7351–7354

Reyes-Vivas H, Mora I, Castillo-Villanueva A, Yépez-Mulia L, Hernández-Alcántara G, Figueroa-Salazar R, García-Torres I, Gómez-Manzo S, Méndez ST, Vanoye-Carlo A, Marcial-Quino J, Torres-Arroyo A, Oria-Hernández J, Gutiérrez-Castrellón P, Enríquez-Flores S, López-Velázquez G (2014) Giardial triosephosphate isomerase as possible target of the cytotoxic effect of omeprazole in *Giardia lamblia*. Antimicrob Agents Chemother 58(12):7072–7082

Riel MA, Kyle DE, Bhattacharjee AK, Milhous WK (2002) Efficacy of proton pump inhibitor drugs against *Plasmodium falciparum in vitro* and their probable pharmacophores. Antimicrob Agents Chemother 46:2627–2632

Russo MW, Firpi RJ, Nelson DR, Schoonhoven R, Shrestha R, Fried MW (2005) Early hepatic stellate cell activation is associated with advanced fibrosis after liver transplantation in recipients with hepatitis C. Liver Transpl 11(10):1235–1241

Tanigawa T, Watanabe T, Higuchi K, Machida H, Okazaki H, Yamagami H, Watanabe K, Tominaga K, Fujiwara Y, Oshitani N, Arakawa T (2009) Lansoprazole, a proton pump inhibitor, suppresses production of tumor necrosis factor-alpha and interleukin-1beta induced by lipopolysaccharide and Helicobacter pylori bacterial components in human monocytic cells via inhibition of activation of nuclear factor-kappaB and extracellular signal-regulated kinase. J Clin Biochem Nutr 45(1):86–92

Targownik LE, Metge C, Roos L, Leung S (2007) The prevalence of and the clinical and demographic characteristics associated with high-intensity proton pump inhibitor use. Am J Gastroenterol 102(5): 942–950

Tian A, Xu T, Liu K, Zou Q, Yan X (2014) Anti-*Helicobacter pylori* effect of total alkaloids of sophora alopecuroides in vivo. Chin Med J 127(13):2484–2491

Wang BB, Cheng JY, Gao HH, Zhang Y, Chen ZN, Bian H (2010) Hepatic stellate cells in inflammation-fibrosis-carcinoma axis. Anat Rec 293(9):1492–1496

Weerakoon KG, Gobert GN, Cai P, McManus DP (2015) Advances in the diagnosis of human schistosomiasis. Clin Microbiol Rev 28(4):939–967

Xavier AML, Magalhães JAS, Cunha GDP, Silva AC, Tavares DAG, Sarro-Silva MD (2010) Morphological tegument alterations of adult *Schistosoma mansoni* harbored in non antihelminthic treated, high-immune-tolerogenic and low-inflammatory mice. Acta Trop 116(1): 95–99

Xiao SH, Shen BG, Chollet J, Tanner M (2000) Tegumental changes in 21 day-old *Schistosoma mansoni* harboured in mice treated with artemether. Acta Trop 75(3):341–348

Yang JJ, Boissier J, Chen JL, Yao H, Yang S, Rognon A, Qiao C (2015) Design, synthesis and biological evaluation of praziquantel and endoperoxide conjugates as antischistosomal agents. Future Med Chem 7(6):713–725

Yuan Y, Ford AC, Khan KJ, Gisbert JP, Forman D, Leontiadis GI, Tse F, Calvet X, Fallone C, Fischbach L, Oderda G, Bazzoli F and Moayyedi P (2013): Optimum duration of regimens for *Helicobacter pylori* eradication. Cochrane Database Syst Rev, (12):CD008337.