Experimental schistosomal hepatitis: Protective effect of coenzyme-Q10 against the state of oxidative stress

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ABSTRACT

Schistosoma mansoni (S. mansoni) eggs trapped in the host liver elicit a chain of oxidative processes that may be, at least in part, responsible for the pathology and progression of fibrosis associated with schistosomal hepatitis. This study was designed to assess the protective effect of the antioxidant coenzyme-Q10 (Co-Q10) against experimental S. mansoni-induced oxidative stress in the liver, and its potential role as an adjuvant to praziquantel (PZQ) therapy. The oxidative stress and overall liver function were improved under Co-Q10 therapy as evidenced by significant reduction in oxidative stress markers and preservation of antioxidant factors. Liver fibrosis was also reduced with a positive impact on liver function. Moreover, addition of Co-Q10 to PZQ therapy caused significant reduction of liver egg load, significant improvement of the redox status, and lastly decreased liver fibrosis.

1. Introduction

Schistosomiasis is one of the leading causes of morbidity and mortality associated with parasitic infections in endemic areas, affecting between 200 and 300 million people in 77 countries all over the world (Curtis and Minchella, 2004). Adult worms of Schistosoma mansoni (S. mansoni) reside in the mesenteric venous plexus of the bowel, from where the eggs are frequently swept back to the liver via the portal blood to induce delayed hypersensitivity response (Wynn et al., 2004). The major pathology is granulomatous inflammation which is a cellular response to antigens secreted by the internal membrane and miracidia of schistosome eggs. Subsequently, the granulomas heal, resulting in a unique form of periportal fibrosis followed by portal hypertension as the main complication (Caulfield et al., 1985; Stavitsky, 2004). Although praziquantel (PZQ) is still considered the treatment of choice for human schistosomiasis, there is considerable concern about its decreased efficacy which may be attributed partly to the development of drug resistance (Sangster, 2001).

Oxidative stress is a general term used to describe the disturbed balance between the steady state of reactive oxygen species (ROS) formation in tissues and their efficient consumption by antioxidants. Recently, it was proved that the state of oxidative stress at the sites of inflammation is responsible to a large extent for cellular damage and progression of fibrosis in chronic liver diseases including schistosomal hepatitis (Ames et al., 1993; Gharib et al., 1999). Activated neutrophils, eosinophils, macrophages, and Kupffer cells are major sources of ROS in the vicinity of schistosome eggs during inflammation. Eosinophils, which are abundant in Schistosoma-induced hepatic granulomas, generate reactive oxygen (O2 •−) and hydroxyl (OH •) radicals following stimulation. Also, hydrogen peroxide (H2O2) is released in significant amounts by the macrophages. As a result, the endogenous antioxidants such as glutathione and glutathione peroxidase are depleted, and oxidative stress ensues (Abdallah et al., 1999; Kaplowitz, 2000).

Hepatic stellate cells (HSCs) play a major role in the process of fibrous tissue formation in the liver. It was found that these cells are more vulnerable to the oxidative stress-related molecules owing to their low levels of endogenous antioxidant enzymes (Parola and Robino, 2001). This oxidative stress presents a direct or indirect relevant pro-fibrogenic stimulus for HSCs where signs of oxidative stress and lipid peroxidation were found to be concomitant or precede HSC activation and collagen deposition (Tsukamoto et al., 1995). Therefore, antioxidant treatment in vivo was found to be effective in preventing or reducing liver fibrosis, as demonstrated in several experimental models (Pietrangelo et al., 1995; Lieber, 2000; Loguercio and Federico, 2003).

The normal liver is a well equipped organ in terms of antioxidant mechanisms either enzymatic such as catalase, glutathione...
peroxidase, and glutathione, or non-enzymatic ones such as α-tocopherol, ubiquinol, and ascorbic acid (Parola and Robino, 2001; Zhen et al., 2007). Moreover, paraoxonase and arylesterase which are detoxifying hepatic enzymes were proved to have antioxidative activities. Recently, both enzymes were proved useful as biomarkers of liver function (Gangadharan et al., 2007; Camps et al., 2007). Nitric oxide (NO) is a short-lived gaseous free radical which can act either as a cytoprotective or a cytotoxic agent. Protective effects, for example reducing the proliferative responses of activated HSCs, predominate in the presence of low levels of ROS (Grisham et al., 1998; Carnovale et al., 2000).

Coenzyme-Q10 (Co-Q10), also known as ubiquinone, is a vitamin-like substance present in all human cells in the membranes of endoplasmic reticulum, peroxisomes, lysosomes, and the inner membrane of mitochondria. It is an important component of the electron transport chain in mitochondria responsible for generation of energy. Furthermore, its reduced form serves as an important antioxidant in both mitochondria and lipid membranes where it protects the body against the deleterious effects of ROS. Therefore, the enzyme has been therapeutically employed in many disorders for its cytoprotective and antioxidant properties (Alleva et al., 2001; Ruiz-Jimenez et al., 2007).

The aim of the present study was to investigate the effect of Co-Q10 on the state of oxidative stress during Schistosoma-induced hepatitis in an experimental animal model. Further, the potential role of this coenzyme as an adjuvant to the classic antihepatic treatment was to be assessed.

2. Materials and methods

2.1. Parasite

Laboratory bred Biomphalaria alexandrina snails were purchased from the Schistosome Biological Supply Program, Theodore Bilharz Research Institute (Giza, Egypt). After exposure to light for at least 4 h, S. mansoni cercariae shed from the snails were used to infect the experimental animals of the study.

2.2. Drugs

Co-Q10 (MEPACO, Egypt) was presented as 30 mg tablets. Daily oral dose for each animal was 5 mg/kg-body weight according to Singh et al. (2000). PZQ (Bayer) was available as Biltricide 600 mg tablets. It was given orally in a dose of 300 mg/kg-body weight according to Ismail et al. (1996).

2.3. Experimental design

One hundred and sixty, laboratory bred and parasite free male Swiss albino mice “6 weeks old and 20–25 g weight” were included in this study. Ten mice were left uninfected and served as the non-infected non-treated control for biochemical parameters and immunohistochemical staining (group N). The remaining animals were infected subcutaneously with S. mansoni cercariae “60 ± 10 cercariae/animal” (Peters and Warren, 1969), then divided into four main groups:

Group I included 50 mice that served as infected non-treated control group for comparison in different durations of infection: 8 and 12 weeks post-infection (p.i.).

Group II included 50 mice that received Co-Q10 daily from the first day of infection, and sacrificed at 8 and 12 weeks p.i.

Group III included 25 mice that received single oral dose of PZQ at the 8th week p.i. and sacrificed 4 weeks later.

Group IV included 25 mice that received PZQ therapy at the 8th week p.i., and also received Co-Q10 at the same time for four successive weeks (till the 12th week p.i.).

Mice were purchased from Theodore Bilharz Research Institute (Giza, Egypt) and were housed and infected in accordance with the institutional guidelines. At 8 weeks p.i., half animals of groups (I), (II) and (N) were sacrificed and all the remaining animals were sacrificed at 12 weeks p.i. At both times, the liver of each mouse was immediately removed and divided into three portions for parasitological, biochemical and histopathological studies.

2.4. Parasitological study

Liver egg load was estimated in all groups. One gram from each liver was weighed, and then put in test tube containing 2 ml of 5% KOH and left overnight at room temperature. The second day, all test tubes were put 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).

2.5. Biochemical study

This was carried out on both tissue and serum samples. Malondialdehyde, and nitric oxide were measured as oxidative stress markers, while glutathione, paraoxonase-1, and arylesterase were measured as endogenous antioxidant factors. All chemicals used were of high analytical grade, products of Sigma (USA), Merck and Reidel (Germany), BDH (England), and Fluka (Switzerland) Chemical Co.

2.5.1. Tissue samples

A part of the liver of each mouse was removed, washed with saline, blotted with filter paper and weighed. Liver samples were homogenized in phosphate buffer saline 10 mM, pH 7.4 (20% w/v), using Potter-Elvehjem tissue homogenizer. The crude homogenate was centrifuged at 7700 g for 30 min at 4 °C, and the resultant supernatant, free of insoluble materials, was essayed for protein content (Bradford, 1976). Aliquots were adjusted to contain 1 mg/ml protein to make the protein content of the samples fixed throughout the experiment and aliquots were assayed for the following parameters.

2.5.1.1. Estimation of glutathione. Glutathione was estimated by the method of Moron et al. (1979) using sodium phosphate buffer and 2 mM dithiobisnitrobenzoic acid (DTNB). The developed color was read against blank at 412 nm within 5 min in spectrophotometer. The amount of glutathione was calculated as μmol/mg tissue protein used from a standard curve plotted for serial concentrations of glutathione (5–100 μg).

2.5.1.2. Estimation of paraoxonase-1 activity. Paraoxonase activity was determined by using paraoxon (O,O-diethyl-O-p-nitrophenyl phosphate) (1 mM) as a substrate. The initial rate of substrate hydrolysis to p-nitrophenol during 5 min was recorded as paraoxonase activity using spectrophotometer at 405 nm. The enzyme activity was expressed in U/mg; where 1 unit of enzyme hydrolyzes 1 nmol of paraoxon/min/ml (Ferré et al., 2002).

2.5.1.3. Estimation of arylesterase activity. Arylesterase activity was measured using 5 nmol/l of phenylacetate as a substrate. The rate of formation of phenol during 5 min was recorded as arylesterase activity using spectrophotometer at 270 nm. The enzyme activity was expressed in U/mg; where 1 unit of enzyme hydrolyzes 1 nmol of phenylacetate/min/ml (Lorentz et al., 1979).

2.5.1.4. Estimation of lipid peroxides. Lipid peroxides were estimated by thiobarbituric acid reaction using saturated thiobarbituric acid and trichloroacetic acid (20%) solutions. The developed
color was read against a blank at 530 nm in spectrophotometer and calculated as nmole malondialdehyde (MDA)/mg tissue (Ohkawa et al., 1979).

2.5.1.5. Estimation of nitric oxide (NO). Griess method has been conducted for micro-determination of nitrite and nitrate (NO\textsubscript{2}, NO\textsubscript{3}). This procedure, supplemented with deproteinization and reduction of nitrate to nitrite in the presence of NADPH-sensitive nitrate reductase (EC1.6.6.2), was followed by spectrophotometric assay at wave length 540 nm (Ding et al., 1988).

2.5.2. Serum samples

Used to measure levels of liver enzymes: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as liver function tests (Wilkinson et al., 1972).

2.6. Histopathological and immunohistochemical study

Samples of liver tissue were collected and fixed overnight in neutral buffered formalin [10%] and processed for paraffin embedding. Two paraffin sections (each of 4 μm thick) per slide, separated by 20 intervening sections, each 10 μm thick, were cut and the following stains were used:

- **Haematoxylin and eosin** (H&E) stain for routine examination. Digital photos of representative areas were captured by Olympus digital camera (E 330) installed on Olympus microscope CX31. The maximal diameters of the granulomas were measured by image analysis software [Micrometrics SE/CMOS. Version 2.6]. The granulomas were chosen so that each measured granulomas was discrete (non-confluent) and showing a central Schistosoma egg. Ten granulomas in each section were measured, and then the mean diameter of each group was calculated.

- **Masson trichrome** stain for demonstration of type of granuloma. The composition of the granulomas was assessed and the granulomas were divided subjectively into three types (fibrous, cellular, and fibrocellular) according to the predominant cellular component (Costa-Silva et al., 2002).

- **Immunohistochemical** identification of activated hepatic stellate cells (HSCs) by immunostaining for α-smooth muscle actin [α-SMA]. The immuno-stain was done on randomly selected paraffin blocks from each group [2 blocks from group N and 5 blocks from each infected group]. The immuno-stain was done as follows: sections were deparaffinized in xylene then rehydrated in descending grades of alcohol. The endogenous peroxidase positivity was blocked by incubation in 5% hydrogen peroxide in methanol. Microwave antigen retrieval was carried out in citrate buffer. Sections were flooded by the primary antibody [Mouse Monoclonal Antibody against α-SMA Ab-1, NeoMarkers, Fermont, California (Cat. #MS-113-P)] for 2 h and then washed by phosphate buffer. Sections were then treated by the secondary antibodies followed by the avidin–biotin complex and the DAB chromogen. Sections were then dehydrated and mounted in synthetic resin. Negative control was obtained by omitting the primary antibodies, and internal positive control was the α-SMA positive vascular smooth muscle fibers in the vessels of the portal tracts. The immunostaining assessment was carried out in a blinded subjective method for the overall positivity for each section: (+++) for intense positivity, (+) for moderate positivity, (+) for mild positivity and (−) for negative staining.

2.7. Statistical analysis

Data were presented as means ± standard deviation. The probability of significant differences among dual means of groups was determined by Student’s t-test. Differences were considered non-significant when (P > 0.05), significant (P < 0.05), and highly significant (P < 0.001). The statistical analyses were processed according to the conventional procedures using Statistical Program of Social Sciences (SPSS) software for windows, version10.0.

3. Results

3.1. Hepatic egg load

Results of liver egg load were shown in Fig. 1. There were no significant differences in the mean numbers of liver egg count in the group that received Co-Q10 and the control one. Egg counts 8 weeks p.i. were (4301.6 ± 512.5 vs 3842.8 ± 532; P = 0.061) and were (4861.3 ± 786.9 vs 4350.1 ± 761.2; P = 0.157) at 12 weeks p.i. Both group (III) that received PZQ therapy and group (IV) that received combined therapy showed highly significant decrease (P = 0.000) in mean numbers of eggs trapped in the liver compared to the control group: (1245.9 ± 345.2 and 512.1 ± 191.3 vs 4350.1 ± 761.2), respectively. Also, statistically significant reduction of liver egg count (P = 0.003) was found between groups (IV) and (III).

3.2. Biochemical parameters

Results of biochemical study 8 weeks p.i. were demonstrated in Table 1. Glutathione level and activity of paraoxonase and arylesterase were significantly reduced whereas levels of MDA and NO were significantly increased (P < 0.05) in infected control animals in comparison to the non-infected non-treated mice (group N). Administration of Co-Q10 for 8 weeks had a statistically significant positive impact. Measured antioxidant factors were increased and levels of oxidative stress markers were reduced; however, differences with group (N) were statistically significant (P < 0.05). Also, comparison between group (II) and group (I) as regards the previously mentioned values showed significant differences (P < 0.05). Moreover, serum liver enzymes (ALT, AST) were significantly increased (P < 0.05) in group (I) in comparison to group (N). This increase was reduced significantly in group (II).

Table 2 shows results of biochemical parameters 12 weeks p.i. Significant reduction (P < 0.05) of values of antioxidant parameters with significant increase of oxidative stress markers in group (I) in comparison to group (N) was recorded. Administration of Co-Q10 for 12 weeks had a normalizing effect on both antioxidant factors and oxidative stress markers, where their values in group (II) became non-significantly different (P > 0.05) from those of the
revealed significant differences \((P < 0.05)\) and group (II) as regards all the measured biochemical parameters. Comparison between group (I) and (II) showed also less fibrous granulomas 30% vs 83% in group (II) were of the cellular type 67% with only 10% fibrous ones. The reverse was observed in group (I) where fibrous granulomas represented 53% vs 8% of the cellular type. At the second time p.i. group (II) showed also less fibrous granulomas 30% vs 83% in group (I) and higher percentage of cellular granulomas 49% vs 5% in group (I). As regards to group (IV) there was decreased percentage of fibrous granulomas 45% in comparison to 67% in group (III). Also, the cellular granulomas in group (IV) were increased in comparison to group (III), where the calculated percentages were 29% and 12%, respectively.

### 3.3. Histopathological findings

Histopathological examination studied both size (expressed as mean diameter) and type (cellular, fibrocellular or fibrous) of the granulomas. Mean diameter of granulomas of all groups were demonstrated in Table 3. There was a significant reduction \((P < 0.05)\) in the mean diameter of granulomas of group (II) (Fig. 5) in comparison to group (I) (Figs. 3 and 4) at both durations of infection. Also, there was a highly significant reduction \((P < 0.001)\) in the mean diameter of granulomas of group (III) in comparison to group (I). The combined therapy caused more reduction in mean diameter of granulomas with statistically significant difference \((P < 0.05)\) between groups (IV) and (III).

Differential types of granulomas of the studied groups were illustrated in Fig. 2. At 8 weeks p.i., most of the granulomas in group (II) were of the cellular type 67% with only 10% fibrous ones. The reverse was observed in group (I) where fibrous granulomas represented 53% vs 8% of the cellular type. At the second time p.i. group (II) showed also less fibrous granulomas 30% vs 83% in group (I) and higher percentage of cellular granulomas 49% vs 5% in group (I). As regards to group (IV) there was reduced percentage of fibrous granulomas 45% in comparison to 67% in group (III). Also, the cellular granulomas in group (IV) were increased in comparison to group (III), where the calculated percentages were 29% and 12%, respectively.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver homogenate</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glutathione (µmol/mg)</td>
<td>Paraoxonase (U/mg)</td>
</tr>
<tr>
<td>N</td>
<td>1.71 ± 0.04</td>
<td>22 ± 0.75</td>
</tr>
<tr>
<td>Non-infected non-treated control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1.079 ± 0.15</td>
<td>18 ± 0.85</td>
</tr>
<tr>
<td>II</td>
<td>1.503 ± 0.13</td>
<td>19 ± 1.51</td>
</tr>
<tr>
<td>III</td>
<td>1.78 ± 0.05</td>
<td>21 ± 0.49</td>
</tr>
<tr>
<td>IV</td>
<td>1.74 ± 0.06</td>
<td>23.3 ± 1.52</td>
</tr>
<tr>
<td>P1 (I vs N)</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>P2 (I1 vs N)</td>
<td>0.025</td>
<td>0.003</td>
</tr>
<tr>
<td>P3 (I1 vs II)</td>
<td>0.005</td>
<td>0.001</td>
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</table>

### Table 2

<table>
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<th>Group</th>
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<th>Serum</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Glutathione (µmol/mg)</td>
<td>Paraoxonase (U/mg)</td>
</tr>
<tr>
<td>N</td>
<td>1.71 ± 0.04</td>
<td>22 ± 0.75</td>
</tr>
<tr>
<td>Non-infected non-treated control</td>
<td></td>
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<tr>
<td>I</td>
<td>0.98 ± 0.13</td>
<td>16.33 ± 1.52</td>
</tr>
<tr>
<td>II</td>
<td>1.78 ± 0.05</td>
<td>21 ± 0.49</td>
</tr>
<tr>
<td>III</td>
<td>1.62 ± 0.04</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>IV</td>
<td>1.74 ± 0.06</td>
<td>23.3 ± 1.52</td>
</tr>
<tr>
<td>P1 (I vs N)</td>
<td>0.515</td>
<td>0.148</td>
</tr>
<tr>
<td>P2 (I vs N)</td>
<td>0.110</td>
<td>0.126</td>
</tr>
<tr>
<td>P3 (I vs II)</td>
<td>0.001</td>
<td>0.017</td>
</tr>
</tbody>
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### Table 3

<table>
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<tr>
<th>Group</th>
<th>Diameter of granulomas in µm</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I Infected non-treated 8 wk p.i.</td>
<td>I Infected non-treated 12 wk p.i.</td>
</tr>
<tr>
<td>Diameter of granulomas</td>
<td>465.44 ± 9.4</td>
<td>408.8 ± 8.5</td>
</tr>
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</table>

### Table 4

<table>
<thead>
<tr>
<th>P value</th>
<th>I vs I</th>
<th>I vs I</th>
<th>III vs I</th>
<th>IV vs I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.001</td>
<td>0.002</td>
<td>0.000</td>
<td>0.005</td>
</tr>
</tbody>
</table>

\[ P > 0.05 \rightarrow \text{non-significant}, \ P < 0.05 \rightarrow \text{significant}, \ P < 0.001 \rightarrow \text{highly significant.} \]
3.4. Immunohistochemical localization of \( \alpha \)-SMA

Results of immunohistochemical staining of hepatic stellate cells (HSCs) agreed with those of granuloma type study. The staining in the normal control group (group N) was limited to the vascular walls in the portal areas and to the large and medium sized hepatic venules. The highest positivity of the stain (+++) was observed in the non-treated infected control group, where intense \( \alpha \)-SMA positivity was detected diffusely in the walls of sinusoids, in portal tracts, and areas of fibrosis (Fig. 6). In group (III) the walls served in the non-treated infected control group, where intense \( \alpha \)-SMA positivity was detected diffusely in the walls of sinusoids, in portal tracts, and areas of fibrosis (Fig. 6). In group (III) the walls

![Fig. 2. Percentage of different types of granulomas. Highest percentage of fibrous granulomas was recorded in group (I) 12 weeks p.i. and the lowest percentage was in group (II) 8 weeks p.i. For cellular granulomas, the highest percentage is in group (II) and the lowest in group (I).](image1)

![Fig. 3. Liver section of control group 12 weeks p.i. Many bilharzial granulomas of large size and fibrous type are seen (arrow heads) [H&E 160×, bar: 200 µm].](image2)

![Fig. 4. Liver section of control group 12 weeks p.i., showing mature fibrous granuloma [Masson Trichrome 400×].](image3)

![Fig. 5. Liver section of Co-Q10 treated group 12 weeks p.i. Many small-sized cellular bilharzial granulomas (arrow heads) and few fibrous granulomas (arrows) can be observed [H&E 160×, bar: 200 µm].](image4)

![Fig. 6. Liver section of control infected group 12 weeks p.i. There is intense continuous sinusoidal wall positivity (+++) for \( \alpha \)-SMA [400×, immunoperoxidase \( \alpha \)-SMA].](image5)

![Fig. 7. Liver section of PZQ treated group. Focal intense sinusoidal wall positivity is seen adjacent to the granuloma with some positive cells in the granuloma (asterisk) [400×, immunoperoxidase \( \alpha \)-SMA].](image6)
of the sinusoids showed continuous moderate positivity (++) with more prominent staining adjacent to the granulomas (Fig. 7). Co-Q10 strikingly decreased stellate cell activation, where group (II) showed only mild staining intensity (+) with discontinuous mild sinusoidal wall positivity (Figs. 8 and 9). The least staining intensity (+ or −) was detected in group IV (Fig. 11). Very faint positivity was detected in the wall of the sinusoids close to the granulomas together with few cells in the granulomas themselves (Fig. 10).

4. Discussion

Schistosomiasis is an important public health disease in many developing countries including Egypt. Hepatosplenic schistosomiasis is a serious manifestation of *S. mansoni* infection that may lead to irreversible sequelae (Sayed et al., 2004). Recent research stresses the role of free radicals and oxidative stress in progression of liver injury in various chronic liver diseases such as viral hepatitis, alcoholic hepatitis, and cirrhosis (Parola and Robino, 2001; Loguerchio and Federico, 2003). Schistosomiasis is no exception: oxidative stress occurs in the liver at the site of inflammation in the vicinity of eggs of *S. mansoni*. This state of oxidative stress is attributed to increased generation of ROS and exhaustion of endogenous antioxidant enzymes (Gharib et al., 1999). Therefore, it is tempting to test the efficacy of exogenous antioxidants as an adjuvant therapy in chronic liver diseases including schistosomiasis.

In the present study we aimed at investigating the protective role of Co-Q10 against the oxidative stress induced by experimental *S. mansoni* infection, and its potential role in therapy along with the classical treatment. Co-Q10 is an endogenous antioxidant and an important component of mitochondrial metabolism (Ruiz-Jimenez et al., 2007). It is available commercially in many countries as an over the counter dietary supplement, and is used therapeutically in heart failure, cardiomyopathy, neurodegenerative disorders as well as in diabetes. All these disorders have in common the presence of oxidative stress that damages the tissues. It is noteworthy that the drug is safe, even in high doses, with few reported side effects (Crane, 2001; Miles et al., 2007).

The effect of Co-Q10 on schistosomal hepatitis 8 and 12 weeks p.i. was investigated. As regards the egg count in the liver, no significant effect at both times was recorded compared to control infected animals. Meanwhile we found significant reduction in oxidative stress markers: MDA and NO, and significant increase in endogenous antioxidant factors, namely level of glutathione, and activity of paraoxonase and arylesterase in animals that received Co-Q10 compared with infected control groups at both times p.i.

This study demonstrated that Co-Q10 improved the redox metabolism of the liver as manifested by reduction of oxidative stress markers and preservation of the endogenous antioxidant mechanisms. This could be explained by inactivation of ROS generated during the process of inflammation, by the antioxidant
activity of the drug. The significant decrease of serum level of AST and ALT further confirms the beneficial effect of controlling the oxidative stress on the overall liver function. As Co-Q10 has several cytoprotective functions, the possibility exists that the drug exerts additional protective effect by other mechanisms which are yet in need for further investigation.

Interestingly, at 8 weeks p.i. the mean diameter of granulomas was reduced in the treated group with Co-Q10. The granulomas in the control group were mostly fibrocullular, while in Co-Q10-receiving mice they were mostly cellular. At 12 weeks p.i. these changes were less prominent, probably due to the process of immunomodulation that occurs normally in chronic schistosomiasis where the eggs deposited late induce smaller granulomas than those deposited earlier in the course of infection (Wilson et al., 2007). The situation seems to be beneficial as increased cellularity of early granulomas is presumably detrimental to the eggs, while the host is protected by the reduction of redox metabolism ensured by Co-Q10.

Many studies implemented various antioxidant strategies to protect against oxidative stress in schistosomiasis. One study employed melatonin and found significant reduction of hepatic redox metabolism. Melatonin caused reduction of lipid oxidation products and NO, and increase in vitamin E, catalase, and superoxide dismutase in the liver. The authors attributed these effects to the antioxidant activity of melatonin (El-Sokkary et al., 2001). Similarly, Gharib et al. (2001) have demonstrated that S. mansoni-induced liver inflammation and fibrosis is clearly associated with generation of ROS and depletion of antioxidant enzymes. They further employed molecular hydrogen—a potent antioxidant—which exerted significant protective effects on the liver, namely reduction in fibrosis, lipid peroxide levels, and tumor necrosis factor-α; and increase in antioxidant enzyme activity.

Antioxidants, both natural and synthetic, have been also used as therapeutic agents in liver damage. In vitro and in vivo activation of Kupffer cells, collagen gene expression, hepatitis B virus (HBV) and hepatitis C virus (HCV) replication, were countered by N-acetyl-cysteine, selenium, vitamin C and E (Lieber, 2000; Soltys et al., 2001; Turkodgan et al., 2001).

Fibrosis is the ultimate sequel of chronic schistosomal hepatitis. A characteristic type of severe periportal fibrosis occurs, which leads to presinusoidal portal hypertension and esophageal varices (Stavitsky, 2004). One of the major goals in therapy of S. mansoni is to block the pathways that lead ultimately to liver fibrosis. The hepatic stellate cells (HSCs) play a major role in this process as they are responsible for synthesis of components of extra-cellular matrix and several types of collagen (Parola and Robino, 2001). The different stimuli that initiate and perpetuate HSC activation in chronic liver diseases are poorly understood, but recent studies stress the role of ROS as activators of HSCs and question the potential of various antioxidants at halting the progression of fibrosis (Loguerio and Federico, 2003).

In this study, fibrosis was assessed by Masson trichrome staining, and the activity of HSCs was assessed by α-smooth muscle actin immunohistochemical staining. Evident reduction of activity was observed in groups that received Co-Q10 as compared to control infected groups notably at 12 weeks p.i. We assume that Co-Q10 reduced the oxidative stress at the site of inflammation, thus shut down the stimulatory pathways of HSC activation. Shoheib (2002) has demonstrated reduction in the thickness of the capsules around Trichinella spiralis (T. spiralis) encysted larvae in rats under Co-Q10 therapy, denoting decreased collagen deposition. Further, addition of Co-Q10 to mebendazole during the muscular phase of infection enhanced the efficacy of the latter. Larval counts were reduced and the capsules were damaged compared to animals receiving mebendazole only.

The antifibrotic effect of antioxidants has been explored in several studies whether employing α-tocopherol (vitamin E), or the flavonoids sylimarin (Bedossa et al., 1994; Boigk et al., 1997). Moreover, the antifibrogenic activity displayed by estrogens on di-methyl-nitrosamine-induced hepatic fibrosis is mediated, at least partly, by an antioxidant mechanism (Yasuda et al., 1999). Trials in humans are relatively few, and they have been performed using very different doses or therapeutic combinations for quite limited period of time and on patients with long standing chronic liver disease. However, encouraging results have been obtained with α-tocopherol (Ferro et al., 1999).

Nitric oxide (NO) is an ambivalent agent that acts as a mediator of inflammation and as a regulator of redox metabolism. In absence of oxidative stress, it acts as an endogenous antioxidant (Grisham et al., 1998). Remarkably, nitric oxide can inhibit the initiation or propagation of lipid peroxidation by rapidly reacting with a number of ROS including lipid alkoxyl (LO') and lipid hydroperoxyl (LOO') radicals which are generated as intermediates during lipid peroxidation (Parola and Robino, 2001). In this experiment Co-Q10 caused reduction of oxidative stress, thus favoring the antioxidant activity of NO.

Moreover, relatively high levels of NO• in the tissues were observed in control infected animals, and this may be responsible, in part, for the recruitment of inflammatory cells in the granulomas. Therefore, reduced NO• levels in the tissues under Co-Q10 therapy may be responsible for the reduced size of granulomas. Interestingly, recent data seem to indicate that NO• may block or reduce proliferative response of activated HSCs. Nitric oxide can efficiently inhibit prostaglandin F-dependent proliferation and chemotaxis in activated human HSCs. These effects are more pronounced in lack of oxidative stress (Faiill et al., 2000). So, it seems that Co-Q10 provides a favorable background for the beneficial effects of NO• to appear.

Recently, there has been an increasing interest in paraoxonase and arylesterase as potential biomarkers for chronic liver impairment (Campos et al., 2007; Kilic et al., 2005; Aslan et al., 2007). Decreased activities of both enzymes may contribute to liver dysfunction by effect on lipid metabolism and peroxidation (Aslan et al., 2007). Moreover, paraoxonase plays a role in the regulation of oxidative stress and hepatic cell apoptosis—events that are closely related to liver fibrosis. Paraoxonase activity is thus decreased in patients with chronic hepatitis or liver fibrosis (Campos et al., 2007). In the present study, the infected control group showed reduction in the activity of both enzymes in the liver, which was reversed significantly by treatment with Co-Q10. These findings are consistent with those of Ali (2004) who found that the activities of paraoxonase and arylesterase were partially restored in liver and serum by chronic administration of zinc as compared to control mice in experimental S. mansoni model.

We investigated the role of Co-Q10 as a potential adjuvant in schistosomal therapy where PZQ was given once in a dose of 300 mg/kg and Co-Q10 in a dose of 5 mg/kg-body weight for one month. Significant reduction of hepatic egg count was observed in mice that received combined therapy (PZQ + Co-Q10) in comparison to either, animals that received PZQ alone or control non-treated ones. The reason here is not clear, but a possible explanation is reduction in fibrosis of the intestine, allowing more eggs to pass in the stool instead of being swept away to the liver. We actually observed reduction in fibrosis of the colon in histopathological sections under the effect of Co-Q10 (data not shown). However, further confirmation is needed.

As regards the biochemical parameters, significant reduction in oxidative stress markers in combined therapy as compared with control non-treated and PZQ treated groups was found. Free radical generation was reduced, evidenced by reduction in MDA and NO• levels, while endogenous antioxidant factors, namely glutathione...
level and activities of paraoxonase and arylesterase were increased. Serum levels of ALT and AST were significantly decreased under combined therapy denoting diminished tissue injury and improved liver functions.

Histopathological examination of liver sections during the current study revealed significant reduction in the mean size of granulomas under combined therapy compared to control mice and the best of our knowledge, a protective effect of Co-Q10 on the liver and decreased the intensity of infection. It seems that Co-Q10 exerted protective effect against the deleterious inflammation and fibrosis in experimental schistosomiasis despite lack of effect on the intensity of infection. In contrast, with PZQ the drug exerted protective effect against the state of oxidative stress induced by eosinophil peroxidase activity and concomitant alteration of the antioxidant activity: a new additional test for the improved evaluation of chronic liver fibrosis.

In conclusion, this study has demonstrated for the first time, to the best of our knowledge, a protective effect of Co-Q10 on the liver against the state of oxidative stress induced by S. mansoni infection. Liver fibrosis in the groups that received this drug was also greatly reduced. Moreover, Co-Q10 enhanced the efficacy of anti-schistosomal therapy. Therefore, Co-Q10 could be considered as a useful adjuvant treatment in hepatic schistosomiasis mansoni, and further studies and controlled human trials on it are worth consideration in schistosomiasis and perhaps other chronic liver diseases.

References


Cheever, A.W., 1968. Conditions affecting the accuracy of potassium hydroxide treatment (Antox) in T. spiralis (2000) have found that administration of exogenous antioxidant preparation (Antox) in T. spiralis-infected mice lead to delayed expulsion of adults, increased muscle larval burden, and decreased efficacy of mebendazole therapy in the intestinal phase. In contrast, mebendazole therapy was enhanced by the antioxidant during the muscular phase of infection. The antioxidants seem to exhibit a dual role; on the one hand they reduce the oxidative stress that is detrimental to the host on the other, they may protect the parasite vis-à-vis the host defense mechanisms. The final balance is a quite delicate process.

Fortunately, in the present work, Co-Q10 exerted protective effect against the deleterious inflammation and fibrosis in experimental schistosomiasis despite lack of effect on the intensity of infection. In contrast, with PZQ the drug exerted protective effect on the liver and decreased the intensity of infection. It seems that the drug enhances the anti-parasitic activity of PZQ. These results are highly relevant especially with the multitude of reports of morphometric analysis in comparison to experimentally infected N. cotti/Hc mice. Memorias do Instituto Oswaldo Cruz 97, 129–142.


