Impact of treatment with praziquantel, silymarin and/or β-glucan on pathophysiological markers of liver damage and fibrosis in mice infected with *Mesocestoides vogae* (Cestoda) tetrathyridia

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Abstract

*Mesocestoides vogae* tetrathyridia infection in mice causes hepatocyte injury, hepatic granulomatous inflammation, liver fibrosis and chronic peritonitis manifested with portal hypertension. To reduce the detrimental effect of parasites on the host liver, the effect of the anthelminthic drug praziquantel (PZQ) in combination with natural products silymarin (an antioxidant) and β-glucan (an immunomodulator) was investigated. The therapeutic effect of drugs was assessed by means of aminotransferase (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) activities, content of albumin, total proteins and hyaluronic acid (HA) in sera of ICR mice infected with *M. vogae* larvae. Animals were treated with PZQ suspended in oil emulsion (Group 1), PZQ combined with silymarin incorporated into lipid microspheres (LMS) (Group 2), PZQ combined with β-glucan incorporated into liposomes (LG) (Group 3), PZQ co-administered with LMS and LG (Group 4). Untreated animals (Group 5) served as the control. Treatment of animals started at the early chronic phase of infection (day 14 p.i.) and lasted 10 days; serum samples were collected on days 0, 7, 14, 25, 28, 31, 35 and 45 p.i. ALT and AST activities were significantly (*P* < 0.05) decreased in Groups 2, 3 and 4. HA content was significantly (*P* < 0.05 and 0.01) lower in Groups 2 and 4. Albumin levels were decreased in Groups 2 and 4, total protein concentration decreased in Groups 1 and 3 (*P* < 0.05 and 0.01). These results showed that combined treatment of PZQ with silymarin and/or β-glucan was able to ameliorate or suppress fibrogenesis in the liver, protect liver cells from oxidative damage and, possibly, stimulate regeneration of the parenchyma.

Introduction

The larval stage (tetrathyridium) of the cestode *Mesocestoides vogae* (syn. *M. corti*) multiplies asexually in the liver and peritoneal cavity of mice (Specht & Voge, 1965) and is a suitable experimental model for slowly developing metacestode infection caused by *Echinococcus multilocularis* in pharmacological studies (WHO, 1984; White *et al.*, 1988). Migration and multiplication of tetrathyridia cause severe damage to the liver parenchyma, which results in hepatocyte dysfunction, extensive fibrosis and granulomatous infiltrations (Specht & Widmer, 1972; Riley & Chernin, 1994). Fibrosis is a consequence of chronic liver diseases of any aetiology (e.g. toxic, viral, parasitic) (Pinzani & Rombouts, 2004).
and is a dynamic process resulting from the imbalance between collagen synthesis (i.e. fibrogenesis) and its degradation (i.e. fibrogenolysis). The key issue in the pathogenesis of liver fibrosis is activation of hepatic stellate cells (HSCs, Ito cells), mediated by various cytokines and reactive oxygen species released from the damaged hepatocytes and activated Kupffer cells (Wu & Zern, 2000). Activated HSCs proliferate rapidly and transform into myofibroblasts, which are responsible for increased production of extracellular matrix (ECM) components, mainly collagens and hyaluronic acid. The general approach to the treatment of liver fibrosis involves protection of hepatocytes from injury, suppression of oxidative metabolism of Kupffer cells, and reduction of HSC activation (Wu & Zern, 2000).

In the chemotherapy of many cestode infections, praziquantel (PZQ) is a highly effective drug against adult stages, but it has limited effects towards tissue-dwelling metacestode stages (King & Mahmoud, 1989; Harder, 2002). Immunosuppression is a well-recognized phenomenon occurring in the hosts infected with various species of parasitic helminths, including *M. vogae* tetrathyridia (Kadian et al., 1996). The immunomodulator polysaccharide β-glucan is a potent activator of effector functions of mononuclear phagocytic cells, natural killer (NK) cells and other immune cells (Wakshull et al., 1999; Kogan, 2000), which play a crucial role in the killing of parasites. Moreover, it was demonstrated that water-soluble β-glucans exhibit weak antioxidant activity, but stimulate macrophage free-radical activity, the so-called respiratory burst, a potent cytotoxic mechanism (Tsiapali et al., 2001). Indeed, in our study on mice infected with *M. vogae* larvae, co-administration of immunomodulatory β-glucan with PZQ markedly elevated the efficacy of the drug itself (Hřčková et al., 2006), due to activation of the immune system (Hřčková et al., 2007). We have shown recently that such combined treatment, in comparison with single drug administration, can down-regulate synthesis of total collagen in the liver using the same experimental model (Hřčková et al., 2006).

Administration of hepatoprotective agents offers the possibility of protecting hepatocytes from injury or inducing their regeneration. Silymarin is a polyphenolic flavonoid extracted from the milk thistle, which exhibits cytoprotective, anti-inflammatory and anticarcinogenic effects (Manna et al., 1999; Dvorák et al., 2003; Pascual et al., 2004). It possesses strong antioxidant activity because it acts as a scavenger of the free radicals in damaged tissue (Jia et al., 2001). The consequence of decreased HSC activation by an antioxidant (e.g. silymarin) should be the suppression of liver fibrogenesis (Boigk et al., 1997; Jia et al., 2001). Moreover, during the chronic phase of infection, it would be desirable to use a drug stimulating resorption of ECM components of fibrous tissue. PZQ administration promoted degradation of liver parenchyma collagen in the early phase of murine schistosomiasis (Badawy et al., 1996; Grenard et al., 1997) and resulted in a slow resorption of fibrous tissue within 6 months after treatment (Singh et al., 2004). However, there are a very few data about antifibrotic activity of PZQ in the course of metacestode infections, regarding the ECM metabolism and cross-linking. Development of liver fibrosis also causes chronic peritonitis, seen as an increase in the portal pressure (Lee et al., 1987). Portal hypertension, a severe complication of liver fibrosis, results in a decrease of serum albumins and total protein levels, and was also observed in *M. vogae* infection in mice (White et al., 1982). Therefore these are used, as well as the activities of liver transaminases (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) present in the serum, as markers for monitoring the pathological changes in the liver parenchyma with fibrosis (Ki et al., 2005) before more sensitive and liver-specific biomarkers are developed.

In the present study we investigated the effects of the anthelmintic drug praziquantel in combination with natural compounds – the antioxidant silymarin and the immunomodulator β-glucan – on liver fibrosis in mice experimentally infected with *M. vogae* tetrathyridia, by determination of ALT and AST activities, albumin content, total protein and hyaluronic acid concentrations in the serum. Dynamic changes of these parameters indicated the extent of hepatocyte injury/protection, as well as the process of fibrogenesis/fibrogenolysis after treatment.

**Material and methods**

**Animals, parasites and infection**

Outbred 8-week-old ICR male mice were used in all experiments. Animals were divided into four treated groups, each comprising ten individuals, and one control group of 14 mice. They were kept on a standard diet (commercial pellets) with access to water *ad libitum*.

*M. vogae* tetrathyridia were maintained by intra-ritoneal passage through outbred ICR mice. In the experiment, animals were infected orally with 55–60 tetrathyridia in warm (32°C) Hanks’ balanced salt solution (HBSS) (Sigma Chemical Co., St. Louis, Missouri, USA).

**Chemicals and preparation of drug formulations**

Praziquantel (Sigma) was suspended in 1% cremophor oil (Sigma) in deionized water, giving rise to a suspension with drug concentration of 7 mg ml⁻¹. (1→3)-β-D-Glucan was isolated from the cell walls of the yeast *Saccharomyces cerevisiae* and was carboxymethylated (CM-glucan) according to a procedure described by Machová et al. (1995). CM-glucan was entrapped into liposomes with a negative surface charge (Veľebný et al., 2000) and the liposomal suspension contained CM-glucan and lipids at the same concentration of 1 mg ml⁻¹. Silymarin (Sigma) is only partially water soluble with limited bio-availability. Silymarin used in our study was incorporated into lipid emulsion, referred as lipid microspheres, according to the method of Abrol et al. (2005). Briefly, soyabean lecithin in the form of phospholipon 100H (Phospholipid GmbH, Cologne, Germany) (0.24 g) was dispersed in soya oil (5.5 g) and allowed to dissolve completely at 55°C overnight (oily phase). Tween-80 (4.4 g) was dissolved by stirring in deionized water (40 ml), giving rise to the aqueous phase. This phase was added to the oily phase dropwise with simple agitation, so to obtain a lipid emulsion. Silymarin (0.66 g)
was dissolved in 66 ml 1 m sodium hydroxide and added, under stirring with a magnetic stirrer at a temperature of 50°C, to the prepared emulsion. The final emulsion, with pH value 7.75, containing 1% silymarin, 5% soyabean oil, 1.2% lecithin and 4% Tween-80, was dialysed against distilled water overnight and stored at 4°C.

**Experimental design**

Administration of drug formulations started on day 15 post-infection (p.i.). Praziquantel (PZQ) was given orally (p.o.) at dose of 35 mg kg⁻¹ body weight (b.w.) once a day for ten consecutive days (Group 1). Animals in Group 2 received the same dosage of PZQ and silymarin in lipid microspheres (LMS) at a dose rate 30 mg kg⁻¹ b.w., p.o. (ten doses). In Group 3, PZQ was given at the same dosage as in previous groups and liposomized CM-glucan (LG) was administered at a dose rate of 5 mg kg⁻¹ subcutaneously twice, with the first and second dose of PZQ. Animals in Group 4 received the same dose of PZQ in combination with LMS and LG at the same doses as before. Untreated animals served as the control (Group 5).

**Sample collection and biochemical determinations in serum**

Blood samples were collected from the retro-orbital venous plexus of anaesthetized intact animals and from infected control and treated groups on days 7 and 14, 25, 28, 31, 35 and 45 p.i. Serum ALT and AST activities, serum albumin and total proteins were determined by the commercially available diagnostic kits (Bio-La-Test, PLIVA-Lachema a.s., Brno, Czech Republic). Serum levels of hyaluronic acid (HA) were quantified by the hyaluronic acid quantitative test kit (Corgenix, USA).

**Statistical analysis**

Numerical results were expressed as a mean ± SD. Multiple comparisons of groups were performed by non-parametric Kruskal–Wallis ANOVA and then by the Scheffe post-hoc test (P level is indicated in the tables) using software Statistica 6.0 (Stat Soft Inc., Tulsa, Oklahoma, USA).

**Results**

**Serum activity of aminotransferases**

ALT is confined to the cytoplasm, whereas AST is present both in cytoplasm and mitochondria of parenchymal cells. Therefore serum activities of ALT and AST indicate their leakage due to pathophysiological changes or necrosis of the cells. ALT activity in serum of control animals gradually rose up to day 25 p.i. (1.29 ± 0.08), then declined gradually up to day 45 p.i. (0.68 ± 0.07) (table 1). After termination of treatment (day 25 p.i.) activities of ALT decreased in all treated groups, except that treated with PZQ (Group 1) in comparison with untreated mice. During the follow-up, the lower enzyme values persisted for a week after combined therapies (Groups 2–4), but they were significantly decreased (P < 0.01) only in the animals treated with both PZQ and LMS (Group 2) between days 1 and 11 post-therapy.

In the control group, serum AST activities peaked on day 25 p.i. (1.57 ± 0.07) as did ALT, and then gradually decreased up to day 45 p.i. (1.20 ± 0.06) (table 2). Administration of PZQ alone did not modulate enzyme activities in serum significantly (Group 1); however, a pronounced drop (P < 0.05) was observed on the day following the end of therapy in other treated groups (2, 3 and 4). Within the next period, AST activities levelled off, the values being more or less similar to those in the infected untreated mice (Group 5).

**Serum content of hyaluronic acid**

Hyaluronic acid (HA) plays a prominent role in the pathogenesis of liver fibrosis and is also released into serum. Serum levels (ng l⁻¹) of HA found in our study are summarized in table 3. Concentration of HA in intact mice (73.25 ± 6.35) gradually increased in infected untreated animals (Group 5), reaching the maximal value (379.10 ± 9.34) on the last day of the experiment (day 45 p.i.). Treatment of animals resulted in a

<table>
<thead>
<tr>
<th>Groups of mice/drug formulation administered</th>
<th>Serum ALT activity on following days post-infection (p.i.)/days post-administration (p.a.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25/1</td>
</tr>
<tr>
<td>1. PZQ</td>
<td>1.58 ± 0.10</td>
</tr>
<tr>
<td>2. PZQ + LMS</td>
<td>0.84 ± 0.06*</td>
</tr>
<tr>
<td>3. PZQ + LG</td>
<td>0.82 ± 0.06*</td>
</tr>
<tr>
<td>4. PZQ + LMS + LG</td>
<td>1.07 ± 0.06</td>
</tr>
<tr>
<td>5. Control</td>
<td>1.29 ± 0.08</td>
</tr>
</tbody>
</table>

Each value represents mean ± SD (n = 6).

PZQ, praziquantel; LMS, silymarin incorporated in lipid microspheres; LG, β-glucan entrapped into liposomes.

ALT activity (μkat 1⁻¹) in uninfected mice, 0.41 ± 0.03; on day 7 p.i., 0.65 ± 0.03; on day 14 p.i., 1.11 ± 0.09.

Significant difference in ALT values on corresponding days between control and treated groups of mice: *P < 0.05; **P < 0.01.

Table 1. Activities of alanine aminotransferase (ALT) (μkat 1⁻¹) in sera of mice infected with Mesocestoides vogae tetrathyridia and during the follow-up therapy with drug formulations indicated.
profundely decreased HA concentration in Groups 2 and 4, at different levels of significance, within 7 days after the last dose. However, in the following period, between days 7 and 21 after the last dose of drugs, HA values increased gradually in all treated groups and were not significantly different in comparison with HA content in the control group.

### Serum content of albumin and total proteins

Concentrations of albumin (g l\(^{-1}\)) in serum from intact, control and treated groups of mice are summarized in Table 4. Albumin content found in intact mice (38.90 ± 1.08) dropped after the infection, then increased by day 35 p.i. (39.50 ± 1.59). A second decrease was recorded during the chronic stage of infection, e.g. from day 35 p.i. After treatment with PZQ, albumin levels decreased only moderately. A significant decline (\(P < 0.01\)) was recorded in groups that received PZQ in combination with LMS (Group 2) and LMS plus LG (Group 4) within the following period of 11 days, in comparison with the control. Co-administration of PZQ with LG (Group 3) had a minor and insignificant impact on concentration of albumin in serum.

Concentration of total proteins was proposed as a suitable marker for monitoring liver damage and portal pressure, and values obtained in our study are shown in Table 5. In the control group protein content peaked on day 25 p.i. (81.16 ± 3.05) and declined moderately. A significant decline of serum proteins in comparison with the control was observed only in Group 1 (63.14 ± 3.43) \((P < 0.01)\) and Group 3 (61.93 ± 2.76) \((P < 0.01)\) immediately after the last dose. During further follow-up of either treatment schedule, protein levels oscillated around values similar to those in the control group at corresponding days.

### Discussion

The penetration of *M. vogae* tetrathyridia into the liver, their migration and proliferation, causes the destruction of liver parenchyma, the consequence of which is the severe inflammatory response and development of fibrosis. Following injury of hepatocytes and/or their necrosis, increased levels of aminotransferases (ALT and AST) are released into the circulation and therefore serum activities can be used to monitor pathophysiological processes in the liver (Zilva & Pannall, 1984). In our study, ALT and AST activities gradually increased in the control group and peaked on day 25 p.i., then declined over the next period of examination. Previously, we have shown that administration of PZQ reduced numbers of tetrathyridia significantly, but did not completely eliminate infection (Velebný & Hrčková, 2005; Hrčková et al., 2006). Levels of ALT, which is restricted to the cytoplasm, were increased following administration of PZQ alone, in contrast to the enzyme activities recorded after treatments with PZQ with silymarin and glucan. In our study, AST activities in the PZQ-treated group were lower than in controls. As AST activity is an indicator of whole-cell

### Table 2. Activities of aspartate aminotransferase (AST) (\(\mu\)kat l\(^{-1}\)) in sera of mice infected with *Mesocestoides vogae* tetrathyridia and during the follow-up therapy with drug formulations indicated.

<table>
<thead>
<tr>
<th>Groups of mice/drug formulation administered</th>
<th>Serum AST activity on following days post-infection (p.i.)/days post-administration (p.a.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25/1</td>
</tr>
<tr>
<td>1. PZQ</td>
<td>1.30 ± 0.06</td>
</tr>
<tr>
<td>2. PZQ + LMS</td>
<td>1.15 ± 0.06*</td>
</tr>
<tr>
<td>3. PZQ + LG</td>
<td>1.16 ± 0.05*</td>
</tr>
<tr>
<td>4. PZQ + LMS + LG</td>
<td>1.14 ± 0.04*</td>
</tr>
<tr>
<td>5. Control</td>
<td>1.57 ± 0.07</td>
</tr>
</tbody>
</table>

See footnote to table 1.

AST activity (\(\mu\)kat l\(^{-1}\)) in uninfected mice, 0.48 ± 0.05; on day 7 p.i., 0.76 ± 0.06; on day 14 p.i., 0.98 ± 0.06.

Significant difference in AST values on corresponding days between control and treated groups of mice: \(*P < 0.05\).

### Table 3. Concentration of hyaluronic acid (HA) (ng ml\(^{-1}\)) in sera of mice infected with *Mesocestoides vogae* tetrathyridia and during the follow-up therapy with drug formulations indicated.

<table>
<thead>
<tr>
<th>Groups of mice/drug formulation administered</th>
<th>HA content on following days post-infection (p.i.)/days post-administration (p.a.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25/1</td>
</tr>
<tr>
<td>1. PZQ</td>
<td>297.41 ± 9.84</td>
</tr>
<tr>
<td>2. PZQ + LMS</td>
<td>194.67 ± 12.4**</td>
</tr>
<tr>
<td>3. PZQ + LG</td>
<td>280.67 ± 10.51</td>
</tr>
<tr>
<td>4. PZQ + LMS + LG</td>
<td>251.30 ± 6.04*</td>
</tr>
<tr>
<td>5. Control</td>
<td>312.47 ± 6.89</td>
</tr>
</tbody>
</table>

See footnote to table 1.

HA content (ng ml\(^{-1}\)) in intact mice, 73.25 ± 6.35; on day 7 p.i., 132.50 ± 4.93; on day 14 p.i., 270.34 ± 6.74.

Significant difference in HA values on corresponding days between control and treated groups of mice: \(*P < 0.05; **P < 0.01\).
damage, ALT increase might indicate PZQ-induced changes in the permeability of cell phospholipid membranes, the effect observed by Schepers et al. (1988) and Malheiro et al. (2000), rather than induction of persistent DNA damage in various cells (Herrera et al., 1998). However, this increase may also be a secondary effect of massive worm destruction following PZQ therapy, a hypothesis based on the results obtained by Hutadilok et al. (1983). Our finding that co-administration of PZQ and silymarin resulted in a marked decline of proteins content on corresponding days between control and treated groups of mice: **P < 0.01. Significant difference in HA values on corresponding days between control and treated groups of mice: *P < 0.01.

Table 4. Concentration of total serum albumin (g l⁻¹) in mice infected with *Mesocestoides vogae* tetrathyridia and during the follow-up therapy with drug formulations indicated.

<table>
<thead>
<tr>
<th>Groups of mice/drug formulation administered</th>
<th>Serum albumin content on following days post-infection (p.i.)/days post-administration (p.a.)</th>
<th>25/1</th>
<th>28/4</th>
<th>31/7</th>
<th>35/11</th>
<th>45/21</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PZQ</td>
<td>31.82 ± 1.61</td>
<td>33.90 ± 1.37</td>
<td>35.90 ± 1.76</td>
<td>37.70 ± 1.36</td>
<td>34.70 ± 2.98</td>
<td></td>
</tr>
<tr>
<td>2. PZQ + LMS</td>
<td>26.78 ± 2.23**</td>
<td>25.57 ± 2.27**</td>
<td>25.76 ± 1.80**</td>
<td>27.72 ± 1.48**</td>
<td>31.11 ± 1.59</td>
<td></td>
</tr>
<tr>
<td>3. PZQ + LG</td>
<td>33.61 ± 1.88</td>
<td>34.42 ± 3.11</td>
<td>35.42 ± 2.20</td>
<td>35.26 ± 2.47</td>
<td>33.00 ± 2.57</td>
<td></td>
</tr>
<tr>
<td>4. PZQ + LMS + LG</td>
<td>28.18 ± 1.54**</td>
<td>28.04 ± 2.51**</td>
<td>26.59 ± 1.70**</td>
<td>28.92 ± 1.91**</td>
<td>32.78 ± 1.77</td>
<td></td>
</tr>
<tr>
<td>5. Control</td>
<td>36.23 ± 1.73</td>
<td>38.43 ± 1.79</td>
<td>37.67 ± 1.62</td>
<td>40.50 ± 1.59</td>
<td>34.60 ± 1.95</td>
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</tr>
</tbody>
</table>

See footnote to table 1.

Total albumin content (g l⁻¹) in intact mice, 38.68 ± 1.09; on day 7 p.i, 34.17 ± 1.65; on day 14 p.i, 35.45 ± 1.49.

Table 5. Content of total serum proteins (g l⁻¹) in mice infected with *Mesocestoides vogae* tetrathyridia and during the follow-up therapy with drug formulations indicated.

<table>
<thead>
<tr>
<th>Groups of mice/drug formulation administered</th>
<th>Total protein content on following days post-infection (p.i.)/days post-administration (p.a.)</th>
<th>25/1</th>
<th>28/4</th>
<th>31/7</th>
<th>35/11</th>
<th>45/21</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PZQ</td>
<td>63.14 ± 3.43**</td>
<td>60.04 ± 2.64**</td>
<td>69.75 ± 4.35</td>
<td>68.64 ± 3.70</td>
<td>65.64 ± 4.31</td>
<td></td>
</tr>
<tr>
<td>2. PZQ + LMS</td>
<td>69.11 ± 3.02</td>
<td>76.53 ± 3.29</td>
<td>68.97 ± 3.44</td>
<td>62.79 ± 3.17</td>
<td>60.95 ± 2.65</td>
<td></td>
</tr>
<tr>
<td>3. PZQ + LG</td>
<td>61.93 ± 2.76**</td>
<td>65.06 ± 3.25</td>
<td>63.93 ± 4.19</td>
<td>62.23 ± 4.97</td>
<td>70.27 ± 2.46</td>
<td></td>
</tr>
<tr>
<td>4. PZQ + LMS + LG</td>
<td>76.92 ± 3.45</td>
<td>70.60 ± 2.96</td>
<td>65.64 ± 4.56</td>
<td>72.61 ± 3.32</td>
<td>60.62 ± 4.76</td>
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</tr>
<tr>
<td>5. Control</td>
<td>81.16 ± 3.05</td>
<td>75.74 ± 3.48</td>
<td>65.97 ± 3.02</td>
<td>69.62 ± 5.31</td>
<td>71.05 ± 4.19</td>
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</tr>
</tbody>
</table>

See footnote to table 1.

Total protein content (g l⁻¹) in intact mice, 52.73 ± 3.53; on day 7 p.i, 67.54 ± 3.79; on day 14 p.i, 67.79 ± 5.32.

Significant difference in proteins content on corresponding days between control and treated groups of mice: *P < 0.01.
treatment of PZQ with silymarin was the most potent for hepatocyte protection and regeneration, and did not modulate the larvicidal effect of the drug against liver larvae within a week after therapy (unpublished observation).

Hepatic stellate cells (HSCs) are present in the sinusoidal areas in the liver and under normal conditions quiescent HSCs synthesize physiological levels of ECM proteins, including hyaluronic acid (HA) (Vrochides et al., 1996). Upon activation by Kupffer cells at the site of injury, HSCs rapidly proliferate and undergo transformation to myofibroblasts (Cassiman et al., 2002). The result of induced fibrogenesis is a rapid increase of synthesis of collagens, HA and other ECM proteins (Grzeszczuk & Prokopowicz, 2004). Therefore serum levels of HA are used as a reliable marker in alcoholic liver diseases (Plevris et al., 2000), chronic hepatitis C virus (Guechot et al., 1995) and also hepatic fibrosis during schistosomiasis (Köpke-Aguia et al., 2002). So far this marker has not been used for detection of liver fibrosis induced by metacestode infections. Here we showed that HA levels in sera gradually increased in infected, untreated mice, reaching a threefold concentration on day 45 p.i. In agreement with the study of George et al. (2004) in toxin-induced hepatic fibrosis, we suppose that the early elevation of serum HA was due to its increased synthesis and simultaneous release from the necrotic liver cells. In the later stages of infection, the increase of HA was contributed to by increased synthesis by activated HSCs and reduced clearance by the impaired sinusoidal endothelial cells. We found that PZQ given alone did not change HA levels significantly in comparison with controls. These data suggest that the drug itself has no impact on the balance between synthesis/degradation of HA, indicating its indirect antifibrotic effect. However, treatment with PZQ in combination with silymarin or with silymarin and CM-glucan significantly decreased HA in serum within 7 days after therapy, indicative of the changes of ECM metabolism. Other factors that contribute to the degradation of collagen are matrix metalloproteinases, but the effect of PZQ on these enzymes was not a subject of our study. It was proposed that the antifibrotic effect of silymarin is attributed to its antioxidant properties (Jia et al., 2001). We suppose that diminished levels of HA were the result of elimination of free radical excess in the injured liver, the consequence of which was reduced activation of Kupffer cells and then HSCs. In contrast, co-administration of CM-glucan with PZQ had only a minor impact on HA levels, which seems to be in agreement with the findings from our previous study (Hrčkova et al., 2006), in which total collagen concentrations, determined by means of hydroxyproline in the same experimental model, were nearly the same after treatment with PZQ and PZQ in combination with liposomized CM-glucan.

Development of liver fibrosis of different origins in patients results in an increase in portal pressure, which is manifest by the presence of albumins and other proteins in ascitic fluid accumulated in the peritoneal cavity (Runyon et al., 1992; Oberti et al., 1997). The progressive loss of some serum proteins was also observed in mice infected with M. vogae tetrathyridia (White et al., 1982). Albumin is the body’s predominant serum-binding protein, having several important functions; for example, it maintains colloid osmotic pressure in plasma to counterbalance hydrostatic pressure. It is synthesized by the hepatocytes, from which it is distributed to hepatic interstitial space and then into the intravascular system (Collins et al., 1994). In our study, in comparison with uninfected mice, albumin levels in sera of infected animals became raised within 35 days p.i., which corresponds to the period of massive liver injury and initiation of fibrogenesis by migrating and dividing larvae. Increased albumin levels could be the result of its liberation from damaged liver tissue. Thereafter, a decline was recorded between day 35 and 45 p.i., at which time mature fibrosis occurs in the liver (Specht & Widmer, 1972), which probably diminishes transport of albumin to the intravascular system. A significant decrease of serum albumin was found in groups treated with PZQ in combination with silymarin or with CM-glucan, but not after treatment with PZQ alone. Such controversial findings could be explained indirectly as no similar study on the parasitic hepatic fibrosis model has been conducted with silymarin. It was described previously that silymarin also stimulates liver tissue regeneration (Maglìulo et al., 1973) and one of the possible mechanisms is stimulation of protein synthesis, probably via physiological regulation of RNA polymerase I at specific binding sites, which thus increases the formation of ribosomes (Luper, 1998). We hypothesize that during regeneration of hepatocytes after activation with silymarin administration, albumin synthesis was elevated, but it was first deposited in the interstitial spaces during restoration of normal parenchymal architecture in the liver, at least for 11 days after treatment. During the following period, albumin levels in serum from treated mice increased, indicating the albumin transport from hepatocytes into sinusoids after traversing the space of Disse, due to a significantly decreased amount of ECM fibres. Differences between levels of total proteins in sera were less prominent, and this parameter does not seem to be a sensitive-enough non-invasive marker for monitoring follow-up of therapy with antifibrotic compounds.

In conclusion, the present results show that silymarin exerted potent antifibrotic, cytoprotective and regenerative effects in the liver injured following parasitization with M. vogae larvae, as indicated by markedly decreased ALT, AST, HA and albumin levels. In this respect CM-glucan had a lower capacity, and phospholipid drug carriers used for entrapment of silymarin and CM-glucan probably also contributed to the liver regeneration. Following single praziquantel therapy, none of the serological markers was significantly altered. Therefore, we think that long-term administration of the drug with appropriately dosed antifibrotic and/or immunostimulating compounds could be an alternative treatment approach to asexually developing metacestode infections invading the liver.

Acknowledgements

This work was supported by the Scientific Grant Agency VEGA of the Ministry of Education of Slovak
References


(Accepted 30 January 2008)
First Published Online 8 April 2008
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