

Effect of *Fasciola hepatica* proteins on the functioning of rat hepatocytes

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Abstract Fasciolosis is a hepatic parasitic infection that affects many mammal species and creates a great economic and veterinary problem. Molecular mechanisms of parasite–hepatocyte interactions have not been precisely characterized yet. Therefore, the aim of the study was to investigate alterations in the metabolic activity of rat liver cells exposed to *Fasciola hepatica* somatic proteins. Hepatocytes were incubated with 0–1 mg/ml of fluke's somatic proteins for various periods of time. Afterward, changes in hepatocytes metabolic activity were determined with MTT and enzyme leakage tests. Hepatocytes' capacity to synthesize albumin was also investigated. It was observed that protein concentration, as well as longevity of their action, influenced metabolic activity of rat liver cells. Diminution of hepatocytes survival rate, an increase in enzyme leakage and altered synthetic capacity after treatment with parasite's proteins were reported. It is concluded that somatic proteins of *F. hepatica* may play an important role in liver cell damaging.

Introduction

Fasciolosis, a disease caused by *Fasciola hepatica*, affects many mammal species, mainly ruminants, and occasionally man. It is considered as a great economic and veterinary problem in livestock production, in particular, in sheep and cattle. Economic losses derive from decreased weight gain, fertility, milk yield, wool production and sudden deaths of heavily infected animals. The majority of damage caused in the liver arises from suckers action and spiny tegument abrasions as parasite migrates through the liver parenchyma. Mechanical damage is accompanied by cellular inflammatory reaction generated by the host, which leads to immunopathological condition. Haemorrhage, necrosis, fibrosis and cirrhosis are common findings during the disease. It has been proposed that somatic and/or secreted molecules of the parasite may contribute to hepatic dysfunction. However, molecular mechanisms of parasite–hepatocyte interactions have not been precisely characterized yet. It is known, that some aspects of liver functions such as carbohydrate, protein, lipid, steroid and mitochondrial bioenergetic metabolism have been altered. It has been reported that during fasciolosis, oxidative damage to hepatocytes occurs what was confirmed by indicator enzymes leakage and the presence of lipid peroxidation products in livers and sera of infected animals. In previous work, authors presented that excretory–secretory products of *F. hepatica* play a significant role in rat hepatocytes damaging (Gajewska et al. 2006). The role of somatic proteins in that process has not been characterized yet. Thus, the aim of this study was to examine the effect of *F. hepatica* somatic proteins on enzymatic systems, albumin metabolism and survival rate of hepatocytes.

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Table 1 TBS fraction effect on hepatocytes survival rate

TBS fraction concentration [mg/ml]	Percentage of survival rate					
	2 h	6 h	12 h	24 h	48 h	72 h
0.02	86.70±11.2	88.10±12.0	84.10±6.8 ^a	83.90±9.3 ^a	83.90±9.3 ^a	77.00±15.6 ^a
0.05	86.60±9.7	86.00±10.1	84.90±10.0 ^a	82.40±10.0 ^a	79.80±7.5 ^a	75.50±14.2 ^a
0.10	90.50±10.8	83.30±8.5 ^a	82.80±5.4 ^a	80.50±8.4 ^a	73.60±10.3 ^a	67.10±13.3 ^a
0.20	89.00±12.1	81.80±4.9 ^a	79.00±10.2 ^a	78.5±5.3 ^a	71.20±9.4 ^a	73.30±10.2 ^a
0.40	88.00±7.2 ^a	81.50±7.8 ^a	80.80±8.6 ^a	75.50±10.5 ^a	73.60±10.8 ^a	71.60±8.5 ^a
1.00	89.50±9.1 ^a	75.80±9.2 ^a	76.20±9.5 ^a	75.80±14.3 ^a	72.30±13.1 ^a	74.40±15.2 ^a

^aSignificantly different from control group ($P<0.05$)

Material and methods

***F. hepatica* somatic proteins** Adult specimens of *F. hepatica* were obtained from slaughterhouse in Białystok. Flukes were homogenized in TBS buffer (0.15 M NaCl, 0.02 M TRIS, 0.1 mM PMSF, 0.5 mM EDTA) at 4°C. For 1 mg of parasite, 3 µl of buffer were used. Following centrifugation (15,000×g, 4°C, 30 min), supernatant was collected and stored at -20°C. Afterward, pellet was resuspended in SDS buffer (TBS buffer with 1% SDS) in proportion of 3 µl of buffer for 1 mg of fluke. Probes were then placed for 5 min at 100°C, and subsequently for 20 min at 4°C. After centrifugation (15,000×g, 4°C, 30 min), supernatant was collected and stored at -20°C. TBS fraction contained soluble proteins, whereas SDS fraction contained membrane-bound proteins. Protein concentration was estimated due to Lowry et al. (1951) method.

Experimental animals Twenty male 2-month-old Wistar rats were maintained in plastic cages, fed and watered ad libitum in natural photoperiod condition. Livers were prepared from anaesthetized animals and then hepatocytes were isolated according to INVITTOX No. 20 (1999) protocol recommended by ECVAM. Trypan blue exclusion test was used to determine the number of viable hepatocytes

present in a cell suspension, and suspensions with viability higher than 75% were taken to further analysis.

Survival rate of rat hepatocytes exposed on *F. hepatica* somatic proteins Isolated hepatocytes were cultured in 96-well plates in concentration of 50,000 cells per well in Williams' E medium (Sigma) supplemented with 5% foetal bovine serum (Sigma) and 1× antibiotic antimycotic solution (Sigma). Cells were maintained in a 5% CO₂ atmosphere at 37° and 100% humidity. Plates were placed in an incubator for 12 h to allow cell adhesion; afterward, appropriate concentrations of somatic proteins were added (0.00, 0.02, 0.05, 0.1, 0.2, 0.4, 1 mg/ml). Hepatocytes were cultured for 2, 6, 12, 24, 48 and 72 h. Whenever time of incubation ended, MTT reduction test was assayed. Hepatocytes survival rate in control cells (non-treated with proteins) was assented as 100%, and against that value, percentage of survival rate of treated with proteins cells was calculated.

Enzymatic systems and albumin production in hepatocytes after exposure to *F. hepatica* somatic proteins Hepatocytes were cultured in 6-well plates in concentration of 2×10^6 per well in the same conditions as those presented above. Concentration of proteins from both somatic fractions were

Table 2 SDS fraction effect on hepatocytes survival rate

SDS fraction concentration [mg/ml]	Percentage of survival rate					
	2 h	6 h	12 h	24 h	48 h	72 h
0.02	86.10±10.3	90.20±9.5	86.60±7.4	80.20±6.5 ^a	78.30±10.2 ^a	77.40±11.2 ^a
0.05	85.80±11.3	91.50±6.4	82.00±7.8 ^a	75.60±7.7 ^a	79.60±8.6 ^a	76.20±10.5 ^a
0.10	85.40±12.1	82.70±10.4 ^a	79.00±10.4 ^a	76.70±9.5 ^a	76.70±11.2 ^a	73.30±9.6 ^a
0.20	80.80±8.5 ^a	80.30±13.2 ^a	78.00±13.2 ^a	76.30±8.8 ^a	72.70±10.4 ^a	70.10±14.7 ^a
0.40	70.50±6.3 ^a	67.90±10.30 ^a	55.40±13.2 ^a	56.70±15.3 ^a	60.70±15.3 ^a	57.20±12.7 ^a
1.00	67.60±10.2 ^a	65.10±6.9 ^a	61.30±10.4 ^a	53.80±14.3 ^a	54.50±12.9 ^a	50.30±7.8 ^a

^aSignificantly different from control group ($P<0.05$)

Table 3 TBS fraction effect on percentage of released aspartate transaminase (AST) from hepatocytes

TBS fraction concentration [mg/ml]	Percentage of released AST					
	2 h	6 h	12 h	24 h	48 h	72 h
0.05	8.69±3.2	11.60±4.7	16.20±2.3 ^a	16.63±4.3 ^a	16.96±5.3 ^a	17.72±4.5 ^a
0.10	8.78±4.3	15.80±4.3	17.30±4.2 ^a	17.55±6.3 ^a	17.98±9.2 ^a	20.76±5.3 ^a
0.20	10.55±5.2	14.90±7.2	17.81±4.9 ^a	17.64±5.5 ^a	23.24±7.4 ^a	28.46±6.1 ^a
0.40	17.05±6.8 ^a	19.80±3.4 ^a	18.40±5.1 ^a	18.31±4.9 ^a	23.21±6.8 ^a	29.97±4.1 ^a
1.00	13.42±6.2	18.00±2.5 ^a	17.72±3.7 ^a	18.40±7.3 ^a	27.95±10.1 ^a	28.87±8.4 ^a

^a Significantly different from control group ($P < 0.05$)

0.00, 0.05, 0.1, 0.2, 0.4, 1 mg/ml, and times of incubation were 2, 6, 12, 24, 48 and 72 h. Whenever time of incubation ended, samples of post-incubation medium were collected and activities of aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH-L), gamma-glutamyl-transpeptidase (γ -GT), alkaline phosphatase (ALP) and albumin level were measured (Pointe Scientific tests). Additionally, untreated with proteins, culture cells were treated with ultrasounds to measure total enzyme activity and albumin level in hepatocytes. Results were presented as a percentage of enzyme activity, or albumin level reported in a post-incubation medium in relation to total enzyme activity, or albumin level in cell suspension.

Statistical analysis By means of unpaired Student's *t* test, statistical analysis was done. Results were presented as a mean with standard deviation ($p < 0.05$ in relation to control).

Results

Survival rate of hepatocytes after exposure to *F. hepatica* somatic proteins decreased with the time of incubation and

increasing fluke protein concentration (Tables 1 and 2). The observed declines were higher in cells incubated with SDS fraction (e.g. after 72 h incubation with TBS fraction in concentration of 1.0 mg/ml 74.4% of hepatocytes were viable; in the same configuration with SDS fraction, only 50.3% hepatocytes were viable).

AST, ALT, LDH-L leakage from cells to medium escalated as the time of incubation progressed and protein concentration increased. However, the time of exposition was a critical factor affecting the value of influx (Tables 3, 4, 5, 6, 7 and 8). The influence of the type of a fraction used was also reported, higher enzymatic activities in post-incubation media were observed when hepatocytes were treated with proteins from SDS fraction.

Moreover, ALP and G-GT were released from cells during the experiment (Tables 9, 10, 11 and 12). Increasing protein concentration had no impact on enzymatic leakage. Longevity of exposure to parasite proteins had a decisive role in that process.

As compared to control cells, in hepatocytes exposed to *F. hepatica* proteins, a significant decline in albumin synthesis occurred (Tables 13 and 14). When cells were incubated with SDS fraction, percentage of albumin released to medium was lower than in the case of cells treated with TBS fraction (for e.g. after 2 h exposure to 1 mg/ml SDS fraction, percentage of albumin released to

Table 4 SDS fraction effect on percentage of released aspartate transaminase (AST) from hepatocytes

SDS fraction concentration [mg/ml]	Percentage of released AST					
	2 h	6 h	12 h	24 h	48 h	72 h
0.05	6.41±4.9	12.11±4.9	15.11±3.2 ^a	17.81±3.8 ^a	16.33±4.6 ^a	23.75±9.7 ^a
0.10	7.68±2.6	16.46±4.2 ^a	15.36±6.6 ^a	18.05±3.7 ^a	17.13±5.2 ^a	21.27±5.4 ^a
0.20	6.58±4.3	15.87±6.1 ^a	18.40±3.9 ^a	20.00±5.3 ^a	21.01±4.9 ^a	27.68±6.4 ^a
0.40	11.14±8.3	18.15±3.2 ^a	19.50±7.4 ^a	24.30±8.3 ^a	25.82±6.8 ^a	30.38±8.6 ^a
1.00	12.15±7.2	18.31±2.5 ^a	21.52±6.8 ^a	26.16±10.4 ^a	27.70±11.1 ^a	34.60±12.4 ^a

^a Significantly different from control group ($P < 0.05$)

Table 5 TBS fraction effect on percentage of released alanine transaminase (ALT) from hepatocytes

TBS fraction concentration [mg/ml]	Percentage of released ALT					
	2 h	6 h	12 h	24 h	48 h	72 h
0.05	4.63±1.2	6.68±1.1	7.10±2.5	11.00±2.1 ^a	13.90±2.1 ^a	13.30±6.9 ^a
0.10	7.98±1.5	8.48±1.8	11.10±1.4	11.50±1.8 ^a	14.02±2.7 ^a	15.40±3.5 ^a
0.20	9.64±1.9	10.21±2.3	11.25±1.8 ^a	13.40±1.6 ^a	17.97±2.6 ^a	21.50±2.4 ^a
0.40	10.34±0.9	12.40±0.9 ^a	12.26±1.2 ^a	13.25±1.6 ^a	20.36±2.7 ^a	22.80±4.2 ^a
1.00	12.68±2.0 ^a	14.00±1.5 ^a	14.76±2.9 ^a	14.74±3.0 ^a	21.10±3.7 ^a	26.20±6.9 ^a

^a Significantly different from control group ($P < 0.05$)

medium amounted to 17.9%, whereas percentage of albumin reported in post-incubation medium after 2 h exposure to 1 mg/ml TBS fraction amounted to 39.6%).

Discussion

In somatic extracts from *F. hepatica*, many parasitic proteins are detected, among which molecules of pivotal importance for fluke are present (SDS-PAGE analysis, data not shown). To date, numerous parasite somatic proteins were identified and characterized. Some of them, such as fatty acid binding proteins, glutathione S-transferases, cathepsin L, haemoglobin, Kunitz-type serine inhibitors, phosphoglycerate kinase, were tested as vaccine antigens (Spithill et al. 1999; Hillyer 2005; Jaros et al. 2010). However, molecular interactions between hepatocytes and *F. hepatica* proteins are not characterized very well, and the role of somatic proteins in liver cell damaging have not been revealed. Precise causes of pathology that is observed during the liver fluke infection are still unknown.

Results received in the present experiments suggest that relation between the decline of hepatocytes survival rate and parasite somatic protein concentration, as well as longevity of their action, exist. The higher protein concentration and longer incubation time is, the higher reported

decline of survival rate appears to be. This dependence is more pronounced in cells treated with SDS fraction than in the cells exposed to proteins from TBS fraction. It is believed that SDS has no toxic effect on rat liver cells since its concentration in final dilution is lower than 0.01%. Moreover, significant differences in survival rate values between hepatocytes treated with increasing SDS fraction concentrations were not observed. The decrease in hepatocytes viability may be explained by disturbances in cellular membranes' function and structure. It has been observed that in livers of infected rats, phospholipid and total lipid compounds dramatically decline as the infection progresses (Lenton et al. 1995). Since significant increase in their degradation products or precursors occurs (in particular, non-esterified fatty acids), phospholipase is suggested to have an elevated activity. Moreover, concentration of lipid peroxidation products, such as malondialdehyde, 4-hydroxyketonal and conjugated dienes in liver preparations increases, what is accompanied by lowered antioxidant enzymes activities in serum (Kolodziejczyk et al. 2005, 2006; Kaya et al. 2006). It appears to be the direct evidence for oxidative damage to hepatic lipids in *F. hepatica*-infected animals (Siemieniuk et al. 2008). Alterations in phospholipids content in hepatic membranes lead to many functional changes, among which are respiratory aberrations in mitochondria. It has been reported that mitochon-

Table 6 SDS fraction effect on percentage of released alanine transaminase (ALT) from hepatocytes

SDS fraction concentration [mg/ml]	Percentage of released ALT					
	2 h	6 h	12 h	24 h	48 h	72 h
0.05	9.68±3.4	10.40±3.6	12.05±3.1	15.08±2.9 ^a	16.40±5.5 ^a	15.70±6.3 ^a
0.10	9.82±5.2	10.70±4.2	12.30±2.7	17.40±6.3 ^a	16.40±2.7 ^a	20.80±3.5 ^a
0.20	10.13±2.9	12.00±5.3	15.30±5.3 ^a	16.00±3.2 ^a	22.30±6.3 ^a	23.60±4.7 ^a
0.40	15.12±6.3 ^a	14.70±5.2 ^a	20.40±6.4 ^a	28.90±7.4 ^a	32.00±6.7 ^a	37.20±6.9 ^a
1.00	19.84±7.1 ^a	18.60±4.8 ^a	20.50±4.5 ^a	30.60±5.4 ^a	37.50±8.1 ^a	40.04±10.2 ^a

^a Significantly different from control group ($P < 0.05$)

Table 7 TBS fraction effect on percentage of released lactate dehydrogenase (LDH-L) from hepatocytes

TBS fraction concentration [mg/ml]	Percentage of released LDH-L					
	2 h	6 h	12 h	24 h	48 h	72 h
0.05	6.89±1.4	10.19±3.5	11.94±2.9 ^a	13.68±4.2 ^a	14.12±3.7 ^a	21.69±2.2 ^a
0.10	9.46±2.4	9.32±3.2	12.37±1.7 ^a	18.20±1.9 ^a	17.18±3.4 ^a	23.44±4.1 ^a
0.20	6.55±2.5	9.90±2.4	12.40±1.8 ^a	19.36±1.7 ^a	24.75±4.8 ^a	27.95±3.9 ^a
0.40	9.90±1.8	11.21±4.5	15.53±2.2 ^a	19.51±4.9 ^a	27.75±3.2 ^a	29.88±3.3 ^a
1.00	9.50±1.2	9.75±1.5	14.12±1.6 ^a	19.65±3.3 ^a	28.90±6.7 ^a	29.65±5.1 ^a

^a Significantly different from control group ($P<0.05$)

drial electron transport in rat's mitochondria is not coupled to ATP synthesis, what is essential for all cellular functions (Rule et al. 1989). Consequently, ATP concentration in liver extracts from infected rats decreases as compared with uninfected animals. In addition, changes in mitochondrial membrane permeability are also noticed since mitochondria became permeable to NADH, to which they are normally impermeable (Lenton et al. 1994). Moreover, it was noted that F_1F_0 -ATPase losses its oligomycin sensitivity. Intact rat's hepatocytes demonstrate also abnormal permeability (Hanisch et al. 1992). It was also found that mitochondria isolated from livers of infected animals contain elevated concentration of non-esterified fatty acids, what may be responsible for their uncoupled state (Lenton et al. 1995). Another consequence of diminished phospholipid content is increased permeability of hepatocytes. It was suggested that lysosomes as well as cellular membranes are disrupted, what in consequence can lead to leakage of lysosomal enzymes into the cytosol and next into cellular space (Siemieniuk et al. 2008). Enzymes such as cathepsin B may play carcinogenic role when released into extracellular space (Skrzydłowska et al. 2005). Nevertheless, *F. hepatica* infection is not accompanied by carcinogenic action.

In the present study, alterations in hepatic membranes integrality were confirmed by the leakage of indicator enzymes from liver cells. Changes in levels of hepatic

enzymes such as AST, ALT, LDH-L, are indicators commonly used to monitor fasciolosis progress (Kolodziejczyk et al. 2005; Kaya et al. 2006). In vivo experiments conducted by Jemli et al. (1993) and Ferre et al. (1996) demonstrated that aminotransferases and dehydrogenase activities in sera of animals infected with *F. hepatica* are elevated. Their activities increase significantly during early infection, when parasite migrates through liver parenchyma, and reach a peak value at the end of the parenchymal stage of the disease. During biliary stage, these enzyme activities decrease, but remain higher than in uninfected controls. Results received show that maximal enzyme leakage values observed in this study are related to the highest declines of hepatocytes survival rates. It leads to the conclusion that as the time of incubation with parasite extracts is lengthening, more severe damage to hepatic membranes occurs, which may result in cell death. Influence of parasite extract used on enzyme leakage is also observed. Proteins from SDS fraction display more toxic properties on rat hepatocytes. Additionally, ALP and γ -GT activities in post-incubation medium were also measured. Those enzymes are localized in hepatic membranes. Moreover, γ -GT is an indicator of damage to bile ducts, and its activity peak follows the peaks of the AST, ALT, LDH-L in vivo. Increases of ALP and γ -GT activities in post-incubation medium confirm the severe membrane damage.

Table 8 SDS fraction effect on percentage of released lactate dehydrogenase (LDH-L) from hepatocytes

SDS fraction concentration [mg/ml]	Percentage of released LDH-L					
	2 h	6 h	12 h	24 h	48 h	72 h
0.05	3.09±1.5	7.02±1.4	9.93±4.2	10.07±3.8	20.41±7.5 ^a	26.82±6.8 ^a
0.10	4.25±1.6	6.87±2.3	10.66±1.7	11.09±3.7	20.27±3.6 ^a	24.05±6.6 ^a
0.20	4.50±1.9	7.16±2.4	8.04±2.5	10.37±2.8	21.14±8.4 ^a	25.22±8.4 ^a
0.40	3.23±0.9	7.31±1.4	12.55±5.3	17.09±5.4 ^a	24.04±4.7 ^a	33.58±11.6 ^a
1.00	2.65±1.7	7.60±1.7	12.40±6.4	17.28±5.6 ^a	28.01±7.9 ^a	30.04±12.4 ^a

^a Significantly different from control group ($P<0.05$)

Table 9 TBS fraction effect on percentage of released alkalic phosphatase (ALP) from hepatocytes

TBS fraction concentration [mg/ml]	Percentage of released ALP					
	2 h	6 h	12 h	24 h	48 h	72 h
0.05	0.5±0.4	0.4±0.1	1.3±0.2	1.5±0.3	6.2±1.9	10.5±4.6
0.10	1.2±0.8	0.9±0.5	1.2±0.4	1.3±0.1	5.1±0.2	10.7±1.1
0.20	1.3±0.5	0.8±0.5	1.3±0.3	1.5±0.2	6.4±1.9	10.6±2.3
0.40	1.7±0.8	1.0±0.8	1.5±0.2	1.9±0.3	5.9±1.3	10.0±1.5
1.00	0.8±0.4	1.0±0.4	1.6±0.5	1.8±0.5	4.9±1.6	10.7±2.4

^aSignificantly different from control group ($P<0.05$)

Hepatic damage affects also metabolic activity of cells. Liver is the only site of albumin synthesis, and liver damage compromises functions of that organ, what is reflected in a decline of plasma albumin concentrations. Albumin concentration in post-incubation medium was evaluated in the experiment. When cells were treated with parasite somatic proteins, gradual increase and then decline in albumin concentration was reported. It confirms that hepatocytes are metabolically active, but it must be stressed that at the beginning of the experiment, albumin released might be synthesized earlier, or may leak out through damaged membranes. However, when cells are incubated longer with proteins, the decline in albumin concentration is evident, and it implies that alterations in albumin synthesis occur. The results obtained suggest that proteins from SDS fraction have more toxic influence on rat liver cells than proteins from TBS fraction. It is known from literature data that progressive loss of plasma albumin is common in all infected host species. That loss can be explained by reduced rate of synthesis and by expansion of the plasma volume (Behm and Sangster 1999). Now it is being suggested that somatic proteins of *F. hepatica* may interfere with albumin synthesis and contribute to lower albumin level in plasma of infected animals.

It is concluded that somatic proteins derived from *F. hepatica* may participate in hepatic damage and may contribute to cell death. TBS and SDS fractions cause

lower survival rate of rat liver cells, indicator enzyme leakage and alterations in albumin synthesis. The extent of the damages depends on the protein concentration and longevity of their action. Since hepatocytes are being damaged by parasite molecules, it may be expected that *F. hepatica* somatic proteins play an important role in liver cell damaging, and contribute to liver pathogenesis during fasciolosis. Those proteins may be released from dead parasites which fail to establish in the bile ducts. It is a well-studied phenomenon that the majority of the migrating parasites does not develop to adult stages and become trapped in the liver parenchyma. In naive sheep and cattle infected with *F. hepatica*, metacercariae adult flukes recovery percentages are 16–53% and 11–21%, respectively (Spithill et al. 1999). It is expected that abundance of somatic proteins is released after parasite's death and may exert their effect on hepatocytes. Liver cells may also be exposed to proteins which are located at parasite's surface. Surface proteins may be released during glycocalyx shedding, as well as replacement of damaged tegument. Glycocalyx shedding is a way of evading of immunological attack of the host. In the juvenile stages, continuous glycocalyx turnover occurs approximately every 3 h (Hanna 1980). In turn, tegumental destruction is associated with immune-related damage from the host. Fluke's attempts are to substitute damaged structure with a formation of a new one underneath (Bennet et al. 1980).

Table 10 SDS fraction effect on percentage of released alkalic phosphatase (ALP) from hepatocytes

SDS fraction concentration [mg/ml]	Percentage of released ALP					
	2 h	6 h	12 h	24 h	48 h	72 h
0.05	1.0±0.5	2.0±0.3	4.1±1.6	4.3±1.7	11.0±2.2 ^a	13.2±3.1 ^a
0.10	1.5±0.3	2.1±0.4	5.1±1.2	4.4±1.1	11.3±1.1 ^a	12.9±1.3 ^a
0.20	1.7±0.2	2.4±0.7	4.9±1.3	4.8±1.8	12.90±1.2 ^a	15.1±2.5 ^a
0.40	2.0±0.5	2.5±1.1	6.0±2.2	6.3±2.1	11.9±1.4 ^a	12.5±3.2 ^a
1.00	2.2±1.2	2.4±0.3	6.2±2.3	6.8±1.9	11.6±1.1 ^a	10.9±3.2 ^a

^aSignificantly different from control group ($P<0.05$)

Table 11 TBS fraction effect on percentage of released gamma-glutamyl-transpeptidase (γ -GT) from hepatocytes

TBS fraction concentration [mg/ml]	Percentage of released γ -GT					
	2 h	6 h	12 h	24 h	48 h	72 h
0.05	9.08±2.4	11.67±3.0	11.91±2.6 ^a	12.62±2.1 ^a	21.51±2.7 ^a	22.45±5.6 ^a
0.10	12.15±2.5	10.73±2.8	8.96±2.4	12.74±1.7 ^a	20.75±3.6 ^a	21.89±2.6 ^a
0.20	9.67±3.2	10.85±2.3	12.85±2.2 ^a	12.74±1.6 ^a	22.08±5.2 ^a	24.15±3.9 ^a
0.40	8.73±1.9	10.85±3.1	12.50±1.2 ^a	13.21±1.6 ^a	19.06±5.4 ^a	22.83±5.8 ^a
1.00	9.43±2.1	11.08±2.6	12.15±2.8 ^a	13.44±3.4 ^a	21.51±3.1 ^a	22.64±3.2 ^a

^a Significantly different from control group ($P<0.05$)

Table 12 SDS fraction effect on percentage of released gamma-glutamyl-transpeptidase (γ -GT) from hepatocytes

SDS fraction concentration [mg/ml]	Percentage of released γ -GT					
	2 h	6 h	12 h	24 h	48 h	72 h
0.05	10.24±2.8	10.38±4.7	11.44±2.7	11.08±2.2	16.79±4.7 ^a	17.55±7.1 ^a
0.10	12.97±4.6	10.26±4.8	11.32±2.8	12.26±5.6	17.36±3.6 ^a	19.06±4.7 ^a
0.20	10.50±4.3	10.38±6.2	13.33±3.1 ^a	13.44±1.9 ^a	19.25±3.7 ^a	21.70±9.8 ^a
0.40	10.50±5.2	10.85±6.2	13.09±3.2 ^a	13.33±5.7 ^a	20.99±7.4 ^a	21.93±8.5 ^a
1.00	10.61±6.1	11.08±3.8	13.21±4.1 ^a	13.56±5.4 ^a	19.58±9.1 ^a	20.99±6.7 ^a

^a Significantly different from control group ($P<0.05$)

Table 13 TBS fraction effect on percentage of released albumin from hepatocytes

TBS fraction concentration [mg/ml]	Percentage of released albumin					
	2 h	6 h	12 h	24 h	48 h	72 h
0.05	17.50±5.6 ^a	25.30±2.1 ^a	31.80±4.0 ^a	35.10±3.1 ^a	39.60±4.5 ^a	35.10±2.2 ^a
0.10	36.40±3.1 ^a	39.60±2.9 ^a	44.20±6.7 ^a	42.20±4.7 ^a	44.80±8.1 ^a	32.50±1.3 ^a
0.20	35.70±5.4 ^a	44.20±8.4 ^a	29.20±10.1 ^a	35.70±8.8 ^a	45.50±7.2 ^a	36.40±1.4 ^a
0.40	36.40±3.8 ^a	41.60±4.6 ^a	33.10±9.6 ^a	31.20±3.4 ^a	35.10±3.9 ^a	32.50±2.3 ^a
1.00	39.60±2.7 ^a	48.70±9.5 ^a	37.70±11.6 ^a	32.50±7.2 ^a	29.20±3.1 ^a	31.80±3.0 ^a

^a Significantly different from control group ($P<0.05$)

Table 14 SDS fraction effect on percentage of released albumin from hepatocytes

SDS fraction concentration [mg/ml]	Percentage of released albumin					
	2 h	6 h	12 h	24 h	48 h	72 h
0.05	12.20±6.3	22.80±2.7 ^a	21.70±10.5 ^a	22.30±11.4 ^a	38.00±5.8 ^a	33.70±6.4 ^a
0.10	10.30±2.4	19.00±5.3 ^a	21.70±6.7 ^a	23.90±5.5 ^a	39.10±7.2 ^a	29.30±6.6 ^a
0.20	10.90±3.7	26.10±7.4 ^a	23.90±10.1 ^a	22.30±7.6 ^a	34.80±6.3 ^a	27.70±7.3 ^a
0.40	17.40±2.9 ^a	26.60±4.6 ^a	30.40±9.6 ^a	29.30±3.4 ^a	34.80±11.3 ^a	31.00±2.6 ^a
1.00	17.90±2.6 ^a	27.70±7.3 ^a	29.90±11.60 ^a	31.50±7.2 ^a	31.50±10.4 ^a	26.60±9.5 ^a

^a Significantly different from control group ($P<0.05$)

Eventually, within fractions that were examined in a present study, secretory protein presence is also possible as the molecules are synthesized in cells and are known to have damaging impact on hepatocytes (Gajewska et al. 2006). Parasite proteins, especially those present in SDS fraction, may be a good source for searching new antigen candidates for vaccination against *F. hepatica*. The development of new effective strategies for the control of fasciolosis is currently urgently needed.

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References

- Behm CA, Sangster NC (1999) Pathology, pathophysiology and clinical aspects. In Dalton J.P. (Ed) Fasciolosis, CAB International, Wallingford, UK, pp.185–224.
- Bennet CE, Hughes DL, Harness E (1980) *Fasciola hepatica*: changes in tegument during killing of adult flukes surgically transferred to sensitized rats. *Parasite Immunol* 2:39–55
- Ferre I, López P, Rojo-Vázquez FA, González-Gallego J (1996) Experimental ovine fasciolosis: antipyrine clearance as indicator of liver damage. *Vet Parasitol* 62:93–100
- Gajewska A, Smaga-Kozłowska K, Kotomski G (2006) Effect of excretory-secretory products of *Fasciola hepatica* on the functioning of the rat hepatocytes. *Med Vet* 62:459–462
- Hanisch MJE, Topfer F, Lenton LM, Behm CA, Bygrave FL (1992) Restoration of mitochondrial energy-linked reactions following dexamethasone treatment of rats infected with the liver fluke *Fasciola hepatica*. *Biochim Biophys Acta* 1139:196–202
- Hanna REB (1980) *Fasciola hepatica*: glycocalyx replacement in the juvenile as a possible mechanism for protection against host immunity. *Exp Parasitol* 50:103–114
- Hillyer GV (2005) *Fasciola* antigens as vaccines against fascioliasis and schistosomiasis. *J Helminthol* 79:241–247
- Jaros S, Jaros D, Wesołowska A, Zygner W, Wędrychowicz H (2010) Blocking *Fasciola hepatica* energy metabolism – a pilot study of vaccine potential of a novel gene – phosphoglycerate kinase. *Vet Parasitol* 172:229–237
- Jemli Mh, Braun JP, Dorchies P, Romdhane MN, Kilani M (1993) Exploration biochimique et hématologique chez l'agneau infesté expérimentalement par *Fasciola hepatica*. *Recueil de Médecine Vétérinaire* 169:241–249
- Kaya S, Sutcu R, Cetin ES, Aridogan BC, Delibas N, Demirci M (2006) Lipid peroxidation level and antioxidant enzyme activities in the blood of patients with acute and chronic fascioliasis. *Int J Infect Dis* 11:251–255
- Kolodziejczyk L, Siemieniuk E, Skrzydlewska E (2005) Antioxidant potential of rat liver in experimental infection with *Fasciola hepatica*. *Parasitol Res* 96:367–372
- Kolodziejczyk L, Siemieniuk E, Skrzydlewska E (2006) *Fasciola hepatica*: effects on the antioxidative properties and lipid peroxidation of rat serum. *Exp Parasitol* 113:43–48
- Lenton LM, Behm CA, Bygrave FL (1994) Characterization of the oligomycin-sensitivity properties of the F_1F_0 -ATPase in mitochondria from rats infected with the liver fluke *Fasciola hepatica*. *Biochim Biophys Acta* 1186:237–242
- Lenton LM, Behm CA, Bygrave FL (1995) Abberant mitochondrial respiration in the livers of rats infected with *Fasciola hepatica*: the role of elevated non-esterified fatty acids and altered phospholipid composition. *Methods Enzymol* 186:425–431
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275
- Rule CJ, Behm CA, Bygrave FL (1989) Aberrant energy-linked reactions in mitochondria isolated from the liver of rats infected with the liver fluke *Fasciola hepatica*. *Biochem J* 260:517–523
- Siemieniuk E, Kolodziejczyk L, Skrzydlewska E (2008) Oxidative modifications of rat liver cell components during *Fasciola hepatica* infection. *Toxicol Mech Methods* 18:519–524
- Skrzydlewska E, Sulkowska M, Koda M, Sulkowski S (2005) Proteolytic-antiproteolytic balance and its regulation in carcinogenesis. *World J Gastroenterol* 11:1251–1266
- Spithill TW, Smooker PM, Sexton JL, Bozas E, Morrison CA, Creaney J, Parsons JC (1999) Development of vaccines against *Fasciola hepatica*. In Dalton J.P. (Ed) Fasciolosis, CAB International, Wallingford, UK, pp. 377–410.