Biochemical Profile, Liver and Kidney Selenium (Se) Status during Acanthamoebiasis in a Mouse Model

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The aim of the present study was to determine biochemical parameters and Se concentrations in the main detoxication organs in immunocompetent and immunosuppressed mice infected by Acanthamoeba sp. at the early stage of infection. The mice were divided into 4 groups: immunocompetent non-infected control (C), immunocompetent infected (A), immunosuppressed infected (AS), and immunosuppressed non-infected (CS) mice. Biochemical parameters in serum were determined using an automated clinical chemistry analyzer, ARCHITECT C8000, while selenium concentration in the liver and kidney were determined by spectrofluorimetry. We observed a significant downregulation in chlorine plasma level in the blood of A vs CS groups. We found a significantly higher serum AST level in the A group than in group C. The Acanthamoeba sp. infection did not influence liver Se in immunocompetent mice but significantly increased liver Se levels in mice with compromised immunity. Kidney Se concentration was significantly higher in group AS compared to A. We observed a novel strong relation between Se hepatic concentration and liver enzymes in the AS group (aspartate aminotransferase, AST and alanine aminotransferase, ALT). Moreover, Se liver concentration correlated with plasma AST in group A. Immunological status affected Se levels in the liver and kidney of mice infected by Acanthamoeba sp. This parasite influenced the activity of serum AST regardless of the host’s immunological status. Furthermore, elevated concentrations of hepatic Se were associated with increased levels of plasma ALT and AST.

Key words: Biochemical parameters, selenium, liver, kidney, Acanthamoeba sp., immunological status.

Acanthamoeba sp. are opportunistic protozoan organisms which most often cause lethal brain invasion resulting in granulomatous amebic encephalitis (GAE) (VISVESVARA et al. 2007; TRABELSI et al. 2012). Their biotope may also be the cornea, with contact lens-wearing being a major risk factor of Acanthamoeba keratitis (CARNT & STAPLETON 2016). Moreover, there are also reports of cutaneous acanthamoebiasis, Acanthamoeba rhinosinusitis, osteo-cutaneous and lung invasions.
Selenium’s role in parasitic protozoan invasions has been observed in experimentally induced infections with *Trypanosoma* sp., *Cryptosporidium* sp., *Toxoplasma gondii* and *Plasmodium* sp. (HUANG & YANG 2002; IRIBHOGBE et al. 2013; DA SILVA et al. 2014). Selenium supplementation decreases the parasitemia of *Trypanosoma* sp. infections and reduces important parameters associated with diseases such as anemia and parasite-induced organ damage (DA SILVA et al. 2014). Moreover, Se has potential antimalarial activity and may be of benefit in malaria therapeutics (IRIBHOGBE et al. 2013). Se supplementation or depletion may be beneficial depending on the particular infectious agent and state of the host (JELICKS et al. 2011). Selenium status may vary in an acute phase response to stress or infection (MAEHRA et al. 2002). Parasitic diseases are sometimes accompanied by elevated and/or reduced Se concentrations in the liver and kidney, and may alter the regulation of trace mineral metabolism and homeostasis (PILARCZYK et al. 2008). The liver is the central organ for Se regulation, producing Se forms to regulate whole-body Se (DUNTAS & HUBALEWSKA-DYDEJCZYK 2015). VALIMAKI et al. (1987) observed that Se concentrations were lower in blood and liver tissue in patients with liver disorders (cirrhosis and hepatitis). PILARCZYK et al. (2008) did not report any effect of *Toxocara canis* on the levels of Se in the liver and kidney of infected mice. Importantly, parasite pathological changes in the host may also lead to alterations in the biochemical parameters of serum including liver enzymes, protein, albumin, lipid levels and renal function biomarkers such as urea, creatinine and several trace elements (DAGNACHEW et al. 2015; MOREIRA et al. 2016). The effect of *Acanthamoeba* sp. infection on the biochemical profile may be of paramount importance on the pathophysiological outcome, similar to other protozoan infections (DAGNACHEW et al. 2015). There is no information of how *Acanthamoeba* sp. alters Se concentrations in the liver and kidney, nor serum biochemical parameters in experimentally infected hosts, taking into account host immunological status. Therefore, in the present preliminary study, the main goal was to determine biochemical parameters and Se concentrations in the main detoxication organs in immunocompetent and immunosuppressed mice infected by *Acanthamoeba* sp. at the early stage of infection.

**Material and Methods**

This study was approved by the Local Ethical Committee for Experiments on Animals in Szczecin (No. 29/2015, dated 22 June 2015) and in Poznañ (No. 64/2016 dated 9 September 2016).

**Acanthamoeba sp. strain**

We used *Acanthamoeba* AM 22 strain from a previous study (LANOCHA et al. 2009). Protozoan amoebae were isolated from the bronchoaspirate of a 53-year-old man with low immunity levels. The amoebae were grown on agar plates (NN Agar) covered with a suspension of deactivated (at 70°C for 1 h) *Escherichia coli* and incubated at 37°C for 72 h according to standard parasitological methods.

**Experimental animals**

Experimental acanthamoebiasis in a mouse model were performed as previously described by ŁANOCHA-ARENDARCZYK et al. (2018). Briefly, the study was conducted on 32 male Balb/c mice (6-weeks old) obtained from a licensed breeder – the Centre of Experimental Medicine, Medical University in Białystok, Poland. The infected (A and AS) and control (C and CS) animals were housed in groups of 5 and 6 mice per cage, respectively. The mice were kept in controlled conventional conditions (temperature 22±1°C, relative humidity approximately 50% and 12/12 h light-dark cycle) in the Animal Facility of the Pomeranian Medical University in Szczecin. The animals were fed Labofeed H (Morawski, Kcynia, PL0410004p,
The biochemical profile, liver and kidney selenium (Se) status during Acanthamoebiasis

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Physiological solution (3 and CS) were given the same volume of sterile 10-20 thousand amoebae. Control mice (groups C intra-nasally with 3 and CS) were inoculated with 3 and CS amoebae in 0.1 ml of 0.9% saline intraperitoneally (i.p.) for 5 days (-4, -3, -2, -1, 0 days) before amoeba inoculation. The dose of MPS was based on data from MARKOWITZ et al. (1978). This procedure allowed for the development of an experimental model similar to that of immunosuppressed patients. MPS is administered, among others, to patients treated for acute rejection episodes (LANOCHA-ARENDARCZYK et al. 2018).

The mice (n=32) were divided into 4 groups:
- immunocompetent non-infected mice (C, n=6)
- immunocompetent mice infected with Acanthamoeba sp. (A, n=10)
- immunosuppressed mice infected with Acanthamoeba sp. (AS, n=10)
- immunosuppressed uninfected mice (CS, n=6)

In studies on the influence of parasites on the host, groups of 6-10 individuals form an experimental standard allowing for reliable statistical analysis. The animals (groups A and AS) were inoculated intra-nasally with 3 μl of suspension containing 10-20 thousand amoebae. Control mice (groups C and CS) were given the same volume of sterile physiological solution (3 μl of 0.9% NaCl solution). The mice in all groups were anesthetized by an overdose of pentobarbital sodium (200 mg/kg b.wt. i.p.). The mice were sacrificed at 8 days post Acanthamoeba sp. infection (at the early stage of infection). During necropsy the livers and kidneys were collected for Se concentration analysis.

Blood sample collection for biochemical analysis

Whole blood from the hearts of the mice was sampled by the 1.2 ml blood collection system S Monovette SERUM. We determined the concentrations of sodium (Na), potassium (K), chlorine (Cl), total protein (TP), C-reactive protein (CRP), albumin, creatinine (CR), urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein cholesterol (LDL). A suite of biochemical analyses for the parameters was carried out using an automated clinical chemistry analyzer, ARCHITECT C8000 (Abbott, USA).

Selenium concentrations in liver and kidney

Selenium concentration was determined by spectrofluorometry with a SHIMADZU RF-5001 PC analyzer. Livers and kidneys were wet digested in concentrated HNO3 (230°C/180 min) and HClO4 (310°C/ 20 min). 9% HCl was added to the digested samples to reduce selenate VI to selenate IV. Subsequently, selenate IV was complexed with cyclohexane (Chempur). Fluorescence was measured from the organic layer (cyclohexane) at 518 nm emission wavelength and 378 nm excitation wavelength. The accuracy of the analytical method was based on NCS-ZC 71001 (beef liver) reference material from China NatiAnalysis Center for Iron and Steel (Beijing, China). The determined Se concentration was 90.9% of the standard value. Two replicates were performed for each sample, and statistical analysis used the average of the data, expressed in milligrams per kilogram wet weight (ww).

Statistical analysis

Analyses used Statistica 10.0 software (StatSoft). In order to determine compliance with the expected normal distribution of results, a Kolmogorov-Smirnov test with Lilliefors correction (P < 0.05) was used. The arithmetic means (AM), standard deviations of the AM (SD), and medians (Med) were calculated for each studied group. Nonparametric tests (Kruskal-Wallis and Mann-Whitney U tests) were used as data deviated from a normal distribution. Differences between groups were analyzed with the Mann-Whitney U test for comparison of 2 groups and the Kruskal-Wallis test for comparison of 4 groups. The significance level was P < 0.05. To determine a possible relationship between Se concentration in the liver and kidney in the analyzed infected groups and serum biochemical parameters (ALT and AST levels as important indicators of hepatocyte structural damage), we calculated the Spearman rank correlation coefficient (rS) and determined the significance.

Results

The biochemical findings in the infected and control groups with respect to the host’s immunological status are presented in Table 1. The levels of sodium and potassium were not significantly different among the groups. A significant decrease in chlorine serum level was observed in the blood of the infected immunocompetent mice relative to the uninfected immunosuppressed animals (U = 23.0, P = 0.05). Analysis of serum liver enzyme activities showed that the highest levels of AST and ALT
Table 1

Biochemical parameters of serum results of *Acanthamoeba* sp. infected mice with respect to the host’s immunological status.

<table>
<thead>
<tr>
<th>Parameter/group</th>
<th>C n=6</th>
<th>A n=10</th>
<th>AS n=10</th>
<th>CS n=6</th>
<th>P (K-W test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na mmol/l</td>
<td>AM±SD Median Range</td>
<td>153.0±2.16</td>
<td>152.0</td>
<td>151.0-156.0</td>
<td>152.5±1.0</td>
</tr>
<tr>
<td>K mmol/l</td>
<td>AM±SD Median Range</td>
<td>7.01±0.59</td>
<td>7.10</td>
<td>6.4-7.8</td>
<td>5.93±0.21</td>
</tr>
<tr>
<td>Cl mmol/l</td>
<td>AM±SD Median Range</td>
<td>110.0±3.7</td>
<td>109.5</td>
<td>107.0-114.0</td>
<td>108.5±0.58**</td>
</tr>
<tr>
<td>TP g/l</td>
<td>AM±SD Median Range</td>
<td>48.75±3.1</td>
<td>48.0</td>
<td>46.0-53.0</td>
<td>41.14±4.0</td>
</tr>
<tr>
<td>CRP mg/l</td>
<td>AM±SD Median Range</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Albumin g/l</td>
<td>AM±SD Median Range</td>
<td>25.25±1.26</td>
<td>25.0</td>
<td>23.0-27.0</td>
<td>27.0±0.82</td>
</tr>
<tr>
<td>CR mg/dl</td>
<td>AM±SD Median Range</td>
<td>0.35±0.03</td>
<td>0.35</td>
<td>0.31-0.36</td>
<td>0.36±0.03</td>
</tr>
<tr>
<td>Urea g/l</td>
<td>AM±SD Median Range</td>
<td>48.4±9.18</td>
<td>53.0</td>
<td>37.0-56.0</td>
<td>53.4±3.78</td>
</tr>
<tr>
<td>AST U/l</td>
<td>AM±SD Median Range</td>
<td>272.2±132.1*</td>
<td>273.0</td>
<td>141.0-471.0</td>
<td>546.3±335.9*</td>
</tr>
<tr>
<td>ALT U/l</td>
<td>AM±SD Median Range</td>
<td>113.20±36.6</td>
<td>117.0</td>
<td>58.0-153.0</td>
<td>116.43±68.6</td>
</tr>
<tr>
<td>TC mg/dl</td>
<td>AM±SD Median Range</td>
<td>72.20±2.95</td>
<td>74.0</td>
<td>68.0-75.0</td>
<td>73.14±4.81</td>
</tr>
<tr>
<td>TG mg/l</td>
<td>AM±SD Median Range</td>
<td>122.0±2.65</td>
<td>121.0</td>
<td>120.0-125.0</td>
<td>129.0±26.0</td>
</tr>
<tr>
<td>HDL mg/l</td>
<td>AM±SD Median Range</td>
<td>41.20±3.63</td>
<td>40.0</td>
<td>37.0-45.0</td>
<td>41.20±2.11</td>
</tr>
<tr>
<td>LDL mg/l</td>
<td>AM±SD Median Range</td>
<td>1.20±0.45</td>
<td>1.0</td>
<td>1.0-2.0</td>
<td>1.57±0.53</td>
</tr>
</tbody>
</table>

A – immunocompetent *Acanthamoeba*-infected mice; C – immunocompetent uninfected control group; AS – immunosuppressed *Acanthamoeba*-infected mice; CS – immunosuppressed uninfected mice; Na – sodium, K – potassium, Cl – chlorine, TP – total protein, CRP – C-reactive protein, CR – creatinine, AST – aspartate aminotransferase, ALT – alanine aminotransferase, TC – total cholesterol, TG – triglyceride, HDL – high-density lipoprotein, LDL – low-density lipoprotein cholesterol; AM – arithmetic mean; SD – standard deviation; P – level of significance; P (K-W test) – P-value Kruskal-Wallis test; NS – non-significant difference; Mann-Whitney U-test, P<0.05: *A vs C, ** A vs CS (P-value is not adjusted for multiple comparisons).
were observed in the AS group and mean values did not exceed 680 and 192 U/l, respectively. We found a significantly higher serum AST level in Acanthamoeba-infected immunocompetent mice than in control immunocompetent animals (U=21.0, P=0.02). There was no significant difference in TP, CRP, albumin, CR or urea. Evaluation of lipid levels did not reveal significant differences in TC, HDL and LDL. Significant differences in TG analysis were not found among the groups, but relatively higher mean values were recorded in the immunocompetent animals from the A and C groups, >122 mg/l.

Average Se levels in the livers of the examined groups can be arranged in the following descending order: CS>AS>A>C. The highest Se concentration in livers of Acanthamoeba-infected animals was observed in immunosuppressive mice and ranged from 0.14 mg/kg ww to 1.18 mg/kg ww. We observed a significantly lower Se liver concentration in immunocompetent Acanthamoeba-infected mice than in control immunocompetent animals (U=34.5, P=0.01). To determine the possible association between Se concentration in the liver and kidney in the analyzed groups and serum biochemical parameters (AST and ALT), we calculated the Spearman rank correlation coefficient (rS) and determined its significance. We observed statistically significant correlations between the Se liver concentration and ALT serum level (rS=0.87; P=0.01) as well as AST serum level (rS=0.77, P=0.01) in infected immunosuppressed mice (AS group). Moreover, we noted positive significant correlation for Se concentration in the liver and AST serum level (rS=0.77, P=0.01) in immunocompetent Acanthamoeba sp. infected mice (group A). There were no significant relationships between Se nephric level in the analyzed groups and serum biochemical parameters.

**Discussion**

Limited data from studies on Acanthamoeba sp. infection show that the livers of mice infected by these amoebae are subject to extensive necrosis and a considerable reduction in glycogen levels in hepatocytes. In the kidneys, necrotic changes are reported in tubules and glomeruli (GÓRNİK & KUŽNA-GRYGIEL 2005). We could not find data on biochemical parameters in experimental acanthamoebiasis, with the clinical image of this infection in patients being very diverse and depending on the efficiency of the host’s immune response.
system. In a study by TILAK et al. (2008), a patient with *Acanthamoeba* peritonitis on continuous ambulatory peritoneal dialysis had normal levels of electrolytes Na⁺ and K⁺, and kidney function tests revealed normal ranges of urea, creatinine, serum protein and albumin. Moreover, no changes were observed in liver function. WEBSTER et al. (2012) in an immunocompetent patient with GAE observed that liver enzymes and renal function also remained stable. Similar to our model, KHANNA et al. (2015), in a rare case of meningoencephalitis caused by *Aspergillus* sp. and *Acanthamoeba* sp. coinfection, did not show changes in serum sodium and potassium levels, and urea and creatinine were in normal ranges.

The liver is exposed to many systemic infectious pathogens including, among others, hepatotropic organisms which may directly or indirectly cause liver pathology. Changes in serum ALT and AST are considered important indicators of hepatocyte structural damage. Hepatic enzymes are present in some tissues, including cardiac muscle, liver and brain, in which they take part in energy metabolism involving the transamination of amino acids (LIMDI & HYDE 2003; ADEYEMI & AKANJI 2011). In cases of cellular damage, AST and ALT can leak out into the general circulation leading to elevated activity (EL-SAYED et al. 2016; AL-SALAHY et al. 2016). This study demonstrated that the highest concentrations of AST and ALT were observed in the infected immunosuppressed mice. Moreover, AST level increased in infected immunocompetent compared to non-infected animals. EL-SAYED et al. (2016) showed a significant increase of liver enzymes in the serum of patients infected with the opportunistic parasite *Toxoplasma gondii*. Similarly, AL-SALAHY et al. (2016) reported significantly higher serum ALT and AST in patients with malaria caused by *Plasmodium falciparum*. YUSUF et al. (2012) in experimental trypanosomiasis showed that an increase in the activity of ALT may be due to tissue damage alone, and also as a result of the destruction of trypanosomes by the host defense system. MOREIRA et al. (2016) revealed a renal alteration characterized by an increase in urea and creatinine in *Leishmania infantum* infected hamsters. Our experimental acanthamoebiosis showed no significant difference in total protein, CRP, albumin, creatinine or urea.

Cholesterol may have an important role in cellular immunity, and a low cholesterol level may have destructive effects on lymphocytes and macrophages facilitating development and progression of pneumonia infection (SAHIN & YILDIRIZ 2013). However, the mechanisms involved in lipid changes related to parasite infections remain unclear. Lipid profiling in this study included TC, HDL and LDL, which were all constant, except TG in the immunocompetent animals, with lowest levels noted in non-infected mice with low immunity after corticoid administration. MARTÍNEZ-SUBIELA et al. (2004) suggest that glucocorticoids do not produce an increase in lipid mediators and parameters of lipid profile. There is some evidence of a relationship between acanthamoebiosis and changes in serum triglyceride metabolism in the host. The highest, but nonetheless insignificant, level of triglyceride was observed in *Acanthamoeba*-infected immunocompetent mice and was 1.3 times greater than in infected mice with low immunity. Some researchers suggest that the increase in triglyceride, e.g. in dog babesiosis, may be associated with increased hepatic production or with the inflammatory host response (CARPENTIER & SCRUEL 2002; MRLIJK et al. 2014).

Trace elements including Se are essential for the functioning of immunocompetent cells, and the liver is the main organ in the metabolism and homeostasis of Se (AMINI et al. 2009). This study demonstrated several biochemical disturbances in *Acanthamoeba*-infected mice and a connection between Se concentration in the liver in the analyzed groups and serum biochemical parameters. Selenium levels in the liver and kidney of immunosuppressed *Acanthamoeba*-infected mice were higher than in immunocompetent animals, which may be connected with Se retention. Nephric Se levels in immunocompetent mice were higher in infected group compared to the control. Se levels in serum and tissues of infected hosts are usually lower than in the control. LOGUERCIO et al. (2001) observed that liver cirrhosis induced a decrease in Se level and oxidative stress, as documented by a significant correlation between Se and glutathione. Oxidative stress may be a result of suboptimal Se and Zn concentrations (SCHOMBURG 2014). In contrast, YANG et al. (2016) showed that elevated plasma Se levels were associated not only with nonalcoholic fatty liver disease, but with increased levels of triglycerides, ALT and AST.

This study demonstrated non-significant, but decreasing hepatic Se level only in infected, immuno-suppressed mice compared to control animals with low immunity. PILARCZYK et al. (2008) showed a nephric Se concentration in *T. canis* infected mice at the control level, not exceeding 0.69 mg/kg ww. Moreover, parasite infection did not change Se level in the kidney, which is in line with results in this study in immunocompetent infected hosts.

In patients treated with corticosteroids with anti-inflammatory and immunosuppressive properties, changes in trace elements may be a consequence of the defense response of the organism, mediated by inflammatory-like substances (AL-ZUBAIDI 2001; ONAL et al. 2011). In patients with multiple sclerosis, intravenous administration of methylprednisolone results in decreased serum Se
compared to control, as Se can act as an antioxidant in the extracellular space and in the cell cytosol, in association with the cell membranes, all of which have the potential to influence the immune processes (AL-ZUBAIDI 2001; MILLER et al. 2001). In our animal model of Acanthamoeba-infected and non-infected mice, which were administered methylprednisolone intraperitoneally, Se concentrations in the liver and kidney were greater than without the steroids.

Imbalances in the metabolism of trace metals may lead to metal interactions with potential pathophysiological significance (LANOCHA-AREN-DARCZYK et al. 2015). In the experimentally induced acanthamoebiasis in our study, we observed a novel strong relationship between hepatic Se concentration and liver enzymes (AST and ALT) in infected mice with low immunity. Moreover, Se liver concentration correlated with serum AST in immunocompetent infected mice.

Our results may lead to a better understanding of the pathogenesis of acanthamoebiasis and an improvement in diagnostic efficacy, particularly in patients with low immunity. Immunological status influenced Se level in the liver and kidney of Acanthamoeba-infected mice. The infection did not influence the accumulation of Se in the liver of immunocompetent animals but significantly increased accumulation in mice with low immunity. Infection with this pathogenic amoeba affected the activity of serum AST regardless of the host’s immunological status. Furthermore, the elevated hepatic Se concentrations were associated with increased levels of serum ALT and AST. Hepatic and nephric Se levels and biochemical markers in animals infected by Acanthamoeba sp. might depend on host immunological reactions, but the role of the immune system in the pathogenesis of an Acanthamoeba infection is still unclear.

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**Author Contributions**


**Conflict of Interest**

The authors declare no conflict of interest.

**References**


