Some Variations of Enzyme Parameters in Experimental Hydatidosis of the Sheep

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Abstract. Major parasitic zoonosis, hydatidosis is a public health problem, with the trend of recurrence and mortality at all negligible, causing great suffering to patients. Purpose of the study: to establish how the molecules used to chimio-prevent of hydatidosis influence plasma levels of enzymes. Materials and methods: there have been taken in the study 30 animals (lambs aged 12 months), which were infested with oncospheres of E. granulosus. To determine plasma levels of enzymes is harvested from blood samples on days 0, 30 and 60 of the experiment. Results and discussions: The plasma ASAT was approximately constant at all lots of lambs. In determining the plasma ALAT, the negative group differences between first and last measurement were statistically significant (p = 0.0053). Treatment with albendazole induced statistical variations between the first and last harvest index “p” is 0.0109. Evolutionary dynamics of GGT level in experimental groups of animals was characterized by fluctuations which showed statistical differences for some of the comparison analysis, as in the case group negative value index “p” was 0.0162 (harvested 1 - 3), the positive group index ”p” was p = 0.0400 (1 - 3), the group treated with albendazole, the index ”p” was 0.0122 (1 - 2) and 0.0107 (1 - 3). PAL fluctuation recorded and analyzed according to the comparative experimental time has highlighted the significant differences from a statistical viewpoint. Conclusions: Exploration of liver medicine is to represent a biochemical exploration of the most common because this organ plays a central role in energy metabolism and mechanisms of detoxification of the body.

Key words: Echinococcus granulosus, lambs, ALAT, ASAT, GGT, PAL

INTRODUCTION

Etiological agent, Echinococcus granulosus, is a cosmopolitan parasite, present on all continents, but most of the parasite prevalence was observed in certain areas of Europe, Asia, Africa, Australia and South America. Known and regions where Echinococcosis / hidatidosis was eradicated: Cyprus and New Zealand (Gemmel, 1997).

The process of transmission from host to the final term and vice versa is extremely complex. (Toma, 1996; Iacobiciu, 2005).

Hydatidosis is a disease with a complex character, in addition to direct mechanical action on the body carries on pests, hydatic cyst having an unfavorable influence on the whole body by its action-toxicological allergic.

Echinococcus granulosus can be considered a cosmopolitan parasite, being present in greater or lesser extent on all populated continents. In general, it is recognized as a health issue for all communities who raise sheep, the highest prevalence being recorded in areas where standards of hygiene and development are relatively low.

The main direction of the study was determining how the molecules used to chimio-prevent of hydatidosis influence plasma levels of enzymes, indicators of liver disease,
research being conducted in the Laboratory of Immunology of the Discipline of Parasitology and Parasitic diseases of the Faculty of Veterinary Medicine in Cluj-Napoca. Plasma determinations were made in clinical laboratory of the Faculty of Veterinary Medicine, using UV-VIS SPECTOTOMETRE MASTER +.

Determinations that we performed concerned the assessment of hepatic enzyme indicators, namely: ASAT, ALAT, PAL, and GGT. The methodology of work was the specific clinical lab where they have been measured.

MATERIALS AND METHODS

Experimental batch was composed of 30 lambs aged 12 months from a hatchery in Bistrița Nasaud County. During the experiment the animals were maintained under the same conditions of accommodation and catering. Were collected samples of blood taken from animals in the study by puncture jugular.

The experimental protocol follows up first the distribution of the animals that were divided into 5 groups of 6 lambs each (tab. 1). For the treated lots of lambs, doses were 10 mg / kg / animal, the albendazole group 3, group 4 mebendazol doses of 8.5 mg / kg / animal in group 5 and praziquantel dose of 2.5 mg / kg / animal. Administration was carried out individually p.o. Lot 1 has negative control uninfected and untreated, while Lot 2 has positive control infected and untreated.

Tab. 1:

<table>
<thead>
<tr>
<th>BATCHES</th>
<th>NO. OF THE ANIMALS</th>
<th>TERAPEUTICAL SPECIFICATION</th>
<th>DRUGS DOSES (10 days successive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot I</td>
<td>6</td>
<td>Negative control</td>
<td>-</td>
</tr>
<tr>
<td>Lot II</td>
<td>6</td>
<td>Positive control</td>
<td>-</td>
</tr>
<tr>
<td>Lot III</td>
<td>6</td>
<td>Albendazol</td>
<td>10 mg / kg / animal / day</td>
</tr>
<tr>
<td>Lot IV</td>
<td>6</td>
<td>Mebendazol</td>
<td>10 mg / kg / animal / day</td>
</tr>
<tr>
<td>Lot V</td>
<td>6</td>
<td>Praziquantel</td>
<td>2 mg / kg / animal / day</td>
</tr>
</tbody>
</table>

Regarding the experimental protocol we consider moment "0" of the experiment the day when we made the identification of animals according to the registration number. On day 1 to appeal the collection of blood samples, obtaining blood plasma and enzymatic making determinations. To monitor how the molecules influence the tested biochemical parameters investigated, after the last administration of each therapeutic cure (day 1, 30, 60), were collected blood samples and conducted tests established.

For accurate assessment and interpretation of the results of the analytical epidemiology statistical calculation was performed in the operating system Windows 2000 and the GraphPad In Stat which works with the analyze categorical system ANOVA (Motulsky et al., 1999; Motulsky, 2004).

RESULTS AND DISCUSSIONS

The serum parameters that can be used into the liver diseases to allow the integrity of the liver cells - ALAT and ASAT; billiary system integrity - PA and GGT. In the case of intracellular forms of liver disease enzymes are released into the blood.

Scolicides drugs use can cause serious damage to the system hepatobiliary. In the case study undertaken to examine possible changes of enzyme products and experimental
infections administration of molecules with a role chimio-prevent, the results were based on experimental time and how to compare the levels of the parameters investigated, compared to positive and negative controls.

A. Aspartataminotransferase (ASAT)

In general, ASAT activity is increased when cellular destructions massive muscle, myocardial, nerve, liver. Increased ASAT may encounter in myocardial diseases, muscle dystrophies, nutritional muscle injuries. ASAT activity significance for diagnosing the liver and biliary diseases is lowers other enzymes (I. Coman, 2006).

It was observed that in the case of the negative group plasma was approximately constant, differences were not statistically assured and the same was observed into the case of the positive group of lambs (tab.2).

In group treated with albendazole was increase the relative value of ASAT in the second bleeding and on comparative analysis of these results in terms of time and experimental plots witness, were not found significant differences from a statistical viewpoint (tab. 2).

In group treated with praziquantel is a clear increase in plasma levels of ASAT from the first harvest to the harvesting of a 60 day experiment, but results are not statistically assured, the index "p" is 0.7904 (tab.2).

<table>
<thead>
<tr>
<th>Comparing level</th>
<th>Lot 1 Negative control</th>
<th>Lot 2 Positive control</th>
<th>Lot 3 albendazol</th>
<th>Lot 4 mebendazol</th>
<th>Lot 5 praziquantel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding 1 – 2</td>
<td>0.2591</td>
<td>0.9045</td>
<td>0.5316</td>
<td>0.7497</td>
<td>0.7791</td>
</tr>
<tr>
<td>Bleeding 1 – 3</td>
<td>0.8072</td>
<td>0.7382</td>
<td>0.7645</td>
<td>0.8943</td>
<td>0.7904</td>
</tr>
<tr>
<td>Bleeding 2 – 3</td>
<td>0.4131</td>
<td>0.6201</td>
<td>0.4031</td>
<td>0.7618</td>
<td>0.4836</td>
</tr>
</tbody>
</table>

B. Alaninaminotranspherase (ALAT)

Increased ALAT activity meets in acute hepatitis, chronic hepatitis: cirrhosis, amiloidosis, liver cells carcinoma, Circulatory stasis, anemia, occlusion of liver vessels.

To practice it is important to specify that alaninaminotransferasis (ALAT) is found only in cytoplasm while asparataminotransferasis (ASAT) are both cytoplasm and in mitochondrial (Annoni et al., 1986).

On the negative group has been observed an increase in plasma levels of the first determination to the second and then fall to the 3rd (tab.3). These differences were statistically significant (p = 0.0053) (tab.3).

On the positive group was decreased plasma levels of ALAT from first harvest to the third, but the differences were not statistically assured (tab.3).

Treatment with albendazole induced statistical variations between the first and last harvest, the difference observed was statistically significant index "p" is 0.0109 (tab.3).

In group treated with mebendazol is a relative increase in the level of plasma from the first harvest in the third, but without statistical relevance (tab.3). The same situation can be observed for the lot treated with praziquantel.
Tab 3:

Value index of probability "p" for the ALAT-experimental groups comparing harvest levels 1.2 and 3

<table>
<thead>
<tr>
<th>Comparing level</th>
<th>Lot 1 Negative control</th>
<th>Lot 2 Positive control</th>
<th>Lot 3 albendazol</th>
<th>Lot 4 mebendazol</th>
<th>Lot 5 praziquantel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding 1 – 2</td>
<td>0.0053</td>
<td>0.5014</td>
<td>0.0740</td>
<td>0.8296</td>
<td>0.8118</td>
</tr>
<tr>
<td>Bleeding 1 – 3</td>
<td>0.1026</td>
<td>0.1861</td>
<td>0.0109</td>
<td>0.6333</td>
<td>0.5534</td>
</tr>
<tr>
<td>Bleeding 2 – 3</td>
<td>0.1963</td>
<td>0.2964</td>
<td>0.1747</td>
<td>0.2141</td>
<td>0.4532</td>
</tr>
</tbody>
</table>

C. **Gamma glutamyltransferase (GGT)**

Gamma glutamyltransferase (GGT) in most animal species is present in large amount in renal tissue, pancreatic, liver and intestine thin.

GGT activity measurement is recommended in experimental toxicology, where the level of serum GGT is a marker very early and very sensitive alteration of the cells of the liver (Ghergariu et al., 2000).

Evolutionary dynamics of GGT level in experimental groups was characterized by fluctuations which showed statistical differences for some of the comparison analysis. Please note that in the case of the negative control group, growth figure in the level of GGT collection 1 to collection 3, statistically significant increase in value index "p" is 0.0162. Similar situation was observed in positive group (tab. 4).

Albendazole administration caused a massive increase plasma levels of GGT from bleeding 1 at bleeding 2 and then decreases to a third collection, which provided statistics, the index "p" is 0.0122 (bleeding 1 - 2) respectively 0.0107 (bleeding 1 - 3) (tab.4).

On animal groups treated with praziquantel and mebendazol there are no significant differences in the three harvesting.

Tab. 4:

Value index of probability "p" for the GGT-experimental groups comparing harvest levels 1.2 and 3

<table>
<thead>
<tr>
<th>Comparing level</th>
<th>Lot 1 Negative control</th>
<th>Lot 2 Positive control</th>
<th>Lot 3 albendazol</th>
<th>Lot 4 mebendazol</th>
<th>Lot 5 praziquantel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding 1 – 2</td>
<td>0.8681</td>
<td>0.0503</td>
<td>0.0122</td>
<td>0.3466</td>
<td>0.9236</td>
</tr>
<tr>
<td>Bleeding 1 – 3</td>
<td>0.1382</td>
<td>0.0400</td>
<td>0.0107</td>
<td>0.8529</td>
<td>0.1294</td>
</tr>
<tr>
<td>Bleeding 2 – 3</td>
<td>0.0162</td>
<td>0.5732</td>
<td>0.5322</td>
<td>0.1832</td>
<td>0.1765</td>
</tr>
</tbody>
</table>

D. **Alkaline phosphatase (PAL)**

Alkaline phosphatase is part of a heterogeneous group of enzymes that are widespread in all cells of mammals. Alkaline phosphatase activity is a useful as an indicator of liver lesions, especially those of the biliary channels. Alkaline phosphatase (PAL) is linked to the membrane, almost every tissue. PAL activity increased differentiation is possible by chromatography or electrophoresis (Ghergariu et al., 2000).

If variation in the level of alkaline phosphatase plasma were different depending on the experimental group took the study, namely the substance administered. Fluctuation recorded and analyzed according to the comparative experimental time have highlighted the significant differences from a statistical viewpoint.

In group 1 (negative control) level of PAL increased from the first determination and until the last, but had no statistical value insurance (tab.5).
In the lots treated with albendazole and mebendazol is certain constancy in the three determinations, without an statistical significance (tab.5).

In group treated with praziquantel is observed decreased plasma levels of PAL in the second collection from the first, and then to determine the third is a slight increase, but without statistical value (tab.5).

<table>
<thead>
<tr>
<th>Comparing level</th>
<th>Lot 1 Negative control</th>
<th>Lot 2 Positive control</th>
<th>Lot 3 albendazol</th>
<th>Lot 4 mebendazol</th>
<th>Lot 5 praziquantel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding 1 – 2</td>
<td>0.2664</td>
<td>0.2082</td>
<td>0.1481</td>
<td>0.9195</td>
<td>0.4834</td>
</tr>
<tr>
<td>Bleeding 1 – 3</td>
<td>0.0604</td>
<td>0.0688</td>
<td>0.8403</td>
<td>0.8067</td>
<td>0.4664</td>
</tr>
<tr>
<td>Bleeding 2 – 3</td>
<td>0.3721</td>
<td>0.2717</td>
<td>0.5820</td>
<td>0.8067</td>
<td>0.2496</td>
</tr>
</tbody>
</table>

CONCLUSIONS

The research undertaken is an experimental model for in vivo testing of how the bodies react to certain drug therapies. Lot of animals was composed of 30 lambs in the age of 12 months, and enzymatic determinations were carried out as follows:

✓ In determining the level of plasma ASAT were not statistically supported differences observed in any of the experimental plots and none of the three determinations;
✓ Determination of ALAT revealed highly statistically significant differences in the negative group (uninfected, untreated) in the calculations of the first harvest and the second ("p" = 0.0053);
✓ The group treated with albendazole at the ALAT also observed statistically significant results in the first determining from the third, the index "p" was 0.0109;
✓ Evolutionary dynamics of the level of GGT in experimental groups was characterized by significant fluctuations, in the negative group was observed an important increase in the level of GGT, statistically significant increase, the value of index "p" being 0.0162;
✓ For the positive group was observed also the increasing of plasma levels of GGT, the results being statistically assured by the index "p" from 0.0400;
✓ For the group treated with albendazole it is seen a massive increase in plasma GGT levels from harvesting 1 to 2, then decreases to a third collection, which provided statistics, the index "p" was 0.0122 (bleeding 1 - 2) and 0.0107 (bleeding 1 - 3);
✓ The fluctuation of alkaline phosphatase, recorded and analyzed according to the comparative experimental time have highlighted the significant differences from a statistical viewpoint of all groups of animals.

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