INFLUENCE OF RABBIT INFECTION WITH INTESTINAL COCCIDIA UPON THE ACTIVITY OF ENZYMES PRIMARILY NOT SYNTHESIZED IN THE LIVER

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SUMMARY

Two groups of ten coprologically oocyst free rabbits were infected with 2x10^5 and 4x10^5 coccidia oocysts composed of *Eimeria flavescens* (7%), *E. matsubayashi* (9%), *E. magna* (12%), *E. neoleporis* (19%), *E. perforans* (21%) and *E. media* (32%). A third group of rabbits served as control. Following the artificial infection, a subclinical form of the disease was induced in the rabbits while only 3 animals developed a full-blown disease with diarrhoea. Shortly before and after 4, 7, and 10 days the infection, levels of the following blood enzymes were determined: creatin kinase, gamma glutamil transferase, amylase and alkaline phosphatase. A decrease in the activity of creatin kinase and a rise in the activity of amylase and alkaline phosphatase were registered. The activity of gamma-glutamil transferase was within normal limits.

KEY-WORDS: rabbits, enzymes, intestinal coccidia

INTRODUCTION

A limiting factor in the intensive rabbit meat production is a number of diseases, many of which are anthropozoonoses (HOOP *et al.*, 1993). Of parasitic diseases, the most common is coccidiosis causing a lot of damage while its treatment significantly increases...
production costs. It is not easy to find a rabbit colony free from coccidia oocysts (CATCHPOLE & NORTON, 1979; HOOP et al, 1993; POLOZOWSKI, 1993; TAMBUR et al, 1995). Published studies on coccidiosis have not clarified in detail subtle biochemical mechanisms leading to alterations in enzymes’ activity in the blood of rabbits with coccidiosis (COUDERT et al., 1978; PEETERS et al, 1984; CATCHPOLE & GREGORY, 1985; SHERKOV et al, 1986; COUDERT et al., 1993; FUKATA et al, 1995). Thus we have decided to examine activities of several enzymes in the blood of rabbits infected with intestinal coccidia: creatin kinase (CK), gamma-glutamil transferase (GGT), amylase (AM) and alkaline phosphatase (AP).

**RESULTS AND DISCUSSION**

Only three artificially infected animals developed a complete clinical presentation of coccidiosis with diarrhoea, whereas others displayed milder signs of the disease such as polydipsia, bristling hair and moderate weight loss. On day 10, coprological examination confirmed the presence of intestinal coccidia oocysts in stool of all the infected rabbits.

**Materials and Methods**

Big chinchila male rabbits 52 day-old, weighting 1200-1300 g, were infected with a pool of intestinal coccidia oocysts composed of *Eimeria flavescens* (7%), *E. matsubayashii* (9%), *E. magna* (12%), *E. neoleporis* (19%), *E. perforans* (21%) and *E. media* (32%). Prior to artificial infection, coprologic examination for intestinal coccidia oocysts was consistently negative in all selected rabbits.

Rabbits were divided into three groups of 10 animals each. The first group served as a control (C) - noninfected rabbits. Rabbits of the second (A) and third (B) groups were infected with 2x10⁵ and 4x10⁵ intestinal coccidea oocysts, respectively, by a direct instillation through a tube into the empty stomach. Immediately before the infection, then on days 4, 7, and 10 following the infection, blood samples were drawn and enzymes’ activities were determined. This was performed by the use of a spectrophotometer (Beckmen). GGT and CK were measured by the Warburg optical test, whereas AM and AP were determined spectrophotometrically.

The results were analyzed on a PC IBM compatible computer (software program STATGRAPH ver. 4.2) by bidirectional variance analysis (ANOVA). The significance for intergroup statistical difference (C:A and C:B) was marked as follows: * p<0.05; ** p<0.01; *** p<0.001 and intergroup (A:B): a p<0.05; aa p<0.01; aaa p<0.001.

**The activity of creatin kinase (CK)**

In both groups of rabbits infected with intestinal coccidia oocysts a change in the CK activity was found to be biphasic (Table 01). On day 4 following the infection the CK activity was slightly elevated, somewhat more pronounced in the group which received a lower dose of infectious oocysts. On days 7 and 10, a decrease in the CK activity was observed but it was not significant. The decrease on day 7 was more evident in the group infected with a higher dose of coccidia oocysts, whereas it was more pronounced in the second group on day 10.

We think that changes in the activity of this enzyme suggest energetic, i.e. metabolic exhaustion of a diseased organism. So the activity of CK was highest on day 4 when the animals in an attempt to overcome deranged homeostatic mechanisms use energetic stores because the ATP stores have probably run out. Thereafter in the course of the disease the enzyme activity falls, perhaps, due to a lack of substrate - creatinine levels decrease.

**The activity of gamma-glutamil transferase (GGT)**

Changes in GGT activity in rabbits with coccidiosis were found in both groups of rabbits (Table 02). In the group infected with a lower dose of oocysts, the decrease was most pronounced on day 7, whereas in the second group it was registered on day 10. Intergroup variability was not statistically significant except in group B on day 10.

The activity of GGT varied up or down but always within a physiological range. Our data showed that intestinal coccidiosis did not alter significantly the activity of this enzyme possibly because it originates from the kidney and liver (MAJIKIC-SINGH, 1993).

**The activity of amylase (AM)**

A significant but mild rise in the amylase activity in both groups of infected rabbits was noted over the whole observation period (Table 03). The rise was highest in the group which received a lower dose of intestinal coccidia oocysts. A maximal point was reached on day 7 after infection and was followed by a fall on day 10 (group A). A maximal and statistically significant increase in the second group (B) was found on day 4, followed by a decrease on day 7 and again by an elevation on day 10 (a biphasic effect). Intergroup variability in the AM activity was noted on days 4 and 7 following infection.

The amylase activity was elevated in infected rabbits during the whole observation period. A disorder of the pancreatic function probably forms the basis for the elevation and, in turn, was a consequence of the intestinal lesion caused by intestinal coccidia and absorption of toxic products. The increase in AM activity was significant but rather mild. Our data on the amylase’s activity elevation in
**Table 1** - Activity of creatin kinase (CK) in the blood of rabbits infected with intestinal coccidia (U/L).

<table>
<thead>
<tr>
<th>DAY</th>
<th>CONTROL GROUP</th>
<th>GROUP A</th>
<th>GROUP B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>180.0 ± 169.5</td>
<td>181.4 ± 190.6</td>
<td>176.2 ± 132.5</td>
</tr>
<tr>
<td>4</td>
<td>193.5 ± 201.1</td>
<td>546.9 ± 555.7</td>
<td>345.5 ± 172.7</td>
</tr>
<tr>
<td>7</td>
<td>194.8 ± 197.7</td>
<td>341.0 ± 237.0</td>
<td>199.4 ± 50.4</td>
</tr>
<tr>
<td>10</td>
<td>195.2 ± 161.0</td>
<td>214.2 ± 167.9</td>
<td>250.0 ± 61.0</td>
</tr>
</tbody>
</table>

* * p<0.05; ** p<0.01; *** p<0.001 - significance for intergroup statistical difference (C:A) and (C:B)
a p<0.05; aa p<0.01; aaa p<0.001 - significance for intergroup statistical difference (A:B)

**Table 2** - Activity of gamma-glutamil transferase (GGT) in the blood of rabbits infected with intestinal coccidia (U/L).

<table>
<thead>
<tr>
<th>DAY</th>
<th>CONTROL GROUP</th>
<th>GROUP A</th>
<th>GROUP B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.7 ± 1.8</td>
<td>3.4 ± 2.5</td>
<td>3.1 ± 1.0</td>
</tr>
<tr>
<td>4</td>
<td>3.4 ± 4.1</td>
<td>2.6 ± 1.2</td>
<td>4.0 ± 4.1</td>
</tr>
<tr>
<td>7</td>
<td>3.2 ± 2.6</td>
<td>2.3 ± 0.8</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>10</td>
<td>3.4 ± 2.3</td>
<td>2.9 ± 1.0</td>
<td>1.8 ± 0.9 *; aa</td>
</tr>
</tbody>
</table>

* * p<0.05; ** p<0.01; *** p<0.001 - significance for intergroup statistical difference (C:A) and (C:B)
a p<0.05; aa p<0.01; aaa p<0.001 - significance for intergroup statistical difference (A:B)

**Table 3** - Activity of amylase (AM) in the blood of rabbits infected with intestinal coccidia (U/L).

<table>
<thead>
<tr>
<th>DAY</th>
<th>CONTROL GROUP</th>
<th>GROUP A</th>
<th>GROUP B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>103.5 ± 27.2</td>
<td>99.7 ± 25.9</td>
<td>99.7 ± 25.0</td>
</tr>
<tr>
<td>4</td>
<td>104.8 ± 27.1</td>
<td>117.6 ± 33.5</td>
<td>176.5 ± 35.8 ***; aaa</td>
</tr>
<tr>
<td>7</td>
<td>104.5 ± 26.7</td>
<td>162.1 ± 39.6 ***; aa</td>
<td>117.7 ± 23.7</td>
</tr>
<tr>
<td>10</td>
<td>107.7 ± 24.3</td>
<td>131.2 ± 48.2</td>
<td>140.3 ± 28.9 **</td>
</tr>
</tbody>
</table>

* * p<0.05; ** p<0.01; *** p<0.001 - significance for intergroup statistical difference (C:A) and (C:B)
a p<0.05; aa p<0.01; aaa p<0.001 - significance for intergroup statistical difference (A:B)
rabbits with coccidiosis are in line with previously published results (FUKATA et al., 1995) obtained in the poultry infected with intestinal coccidia.

The activity of alkaline phosphatase (AP)

A significant rise in the activity of alkaline phosphatase in both groups of infected rabbits was observed at all time points (Table 04). It was more evident in the group of rabbits infected with a higher dose of oocysts. In this group, a maximal rise was registered on days 4 and 10 after infection. Intergroup variability was significant on 4 and 10 days after infection.

We propose that the significant increase in the AP activity stems from two independent sources. Alkaline phosphatase is synthesized in the small intestine wall and liver. Although, in our experimental infection, the main pathologic process was confined to the intestines, the liver showed signs of the “enhanced” activity. The central pathologic process occurs in the intestines so we assumed that alkaline phosphatase’s production was elevated and since the intestinal barrier was disrupted, the enzyme entered the circulation where its activity is measured. The enzyme produced in the liver certainly contributed to this activity. According to the literature data (CATCHPOLE & GREGORY, 1985; PEETERS et al., 1984; SHERKOV et al., 1986), the activity of alkaline phosphatase in rabbits and lambs infected with intestinal coccidia oocysts decreases, particularly during the period of diarrhoea. Our own results differed from these data.

Intestinal coccidiosis of rabbits, apart from the local damage of intestinal mucosa, initiates a systemic reaction which manifests itself in changes in certain parameters in the blood, urine and feces. This is illustrated by the results of our experiments. The elevation of activity of amylase and alkaline phosphatase along with monitoring of some other parameters in the blood (erythrocyte numbers, hematocrit, ALT and IgG) may be valuable markers for early diagnosis of this disease. Changes in the activity of creatine kinase and gamma-glutamyl transferase are not specific for intestinal coccidiosis in rabbits.

BIBLIOGRAPHY


