Abstract

Changes in serum tumour necrosis factor-α (TNF-α), nitric oxide (NO), and adenosine deaminase (ADA) levels in cattle babesiosis before and after treatment with diminazene aceturate were determined. Before treatment, TNF-α, NO, and ADA levels were found to be 71.93 pg/ml, 50.03 µmol/L, and 70.18 IU/L, respectively. These levels were significantly higher than those in the control group. After the treatment, the levels of the compounds significantly decreased. In conclusion, besides TNF-α and NO, ADA can also be a predictive and sensitive parameter in the diagnosis and prognosis of babesiosis.

Key words: cattle, babesiosis, tumour necrosis factor-α, nitric oxide, adenosine deaminase.

Babesiosis is a worldwide tick-borne haemolytic disease, caused by intraerythrocytic protozoan parasites of the genus Babesia, order Piroplasmida. It occurs in the tropical and subtropical regions (3). At least six Babesia species appear to be responsible for bovine babesiosis, which is generally characterised with malaria-like syndrome, including fever, anaemia, icterus, haemoglobinuria, and death (10). It is known that cattle babesiosis is widespread in Turkey (17).

Activated macrophages and their soluble products such as TNF-α, IL-12, and IL-1, are effective in stimulating the non-specific immunity (5, 9). Cytokines increase the inducible nitric oxide synthesis (iNOS) secretion and nitric oxide (NO) generation from the macrophages (13). Adenosine deaminase (ADA, adenosine aminohydrolase) is an important enzyme of purine metabolism and is present in all cells. ADA activity is high in many diseases where cellular immunity is stimulated (15).

Chemotherapy is generally effective against bovine babesiosis. Diminazene aceturate, and is widely used in the tropics for the treatment of the disease. However, the mechanisms by which the drug inhibits the development of Babesia sp. proliferation within erythrocytes are largely unknown (19).

In the present study, we aimed to determine the natural process of host-parasite relationship, by determining the changes in the activity of ADA and levels of TNF-α, and NO, in the acute phase of cattle babesiosis, and after treatment with diminazene aceturate. Determining the ADA activity before and after treatment, may provide important information useful for the diagnosis and treatment of the disease.

Material and Methods

In the summer periods of 2001-2002, 40 Holstein cattle, over 1-year-old from the villages of Samsun city Turkey, were used as the material for this study. The animals were divided into 2 groups: 20 healthy cows (control group) and 20 cows with clinical babesiosis (patient group). The animals were selected to proper groups after examination of Giemsa stained thin blood smears, and indirect fluorescent antibody assay (IFAT). The cows showed symptoms suggestive of Babesia bigemina infection, including anorexia, anaemia, fever, icterus, and haemoglobinuria or showed Babesia bigemina-like protozoa in blood smears and Babesia bigemina antibody titers ≥ 80.

The blood samples were collected from the patient group on the day of diagnosis and on days 7, 15 and 30 after treatment with 5 mg/kg (i.m.) of diminazene aceturate (Berenil®). The blood samples were centrifuged for 10 min at 2 000 rpm, and the received
sera were kept frozen (-20°C) in 1.5 ml microtubes until analysis.

Serum ADA activity was determined with Giusti (4) method. It is a colourimetric method based on the principle of measuring absorbance of the coloured indophenole complex at 628 nm. TNF-α level was determined using Immunotech TNF-α enzyme immunoassay kit (1). Serum NO levels were determined using Calbiochem nitric oxide colourimetric assay kit (12).

Dunnet test was used to compare ADA, TNF-α, NO levels between control and patient groups. Dependent t-test was used to compare these parameters within groups after the treatment. Data were presented as mean ± standard deviation (± SD) and P< 0.05 was considered as significant.

Results

Table 1 shows serum TNF-α, NO, and ADA levels in the control and patient groups; and in the patient group on days 7, 15, and 30 after treatment with diminazene aceturate. Serum TNF-α levels in the patient group on days 7 and 15 after the treatment, were found significantly (P<0.05) higher than in the control group.

NO level in the patient group was significantly higher before treatment, as compared to the control group, and decreased significantly (P<0.05) on days 7, 15 and 30 after the treatment.

Serum ADA activity in the patient group was found statistically higher than in the control group. However, this activity decreased to the level of control group one week after the treatment.

Discussion

In the present study, we found out that the serum TNF-α level in the patient group was significantly higher than the control group. It was demonstrated in vitro that increased TNF-α level in babesiosis was related to immunity and inhibition of parasite growth (2, 13). We have suggested that the decrease in the TNF-α levels after the treatment near the values of the control group; and could be related to the inhibition of the parasite growth with the increase in the secretion of NO by TNF-α from the macrophages (14), or to the destruction of the parasite by the drug.

As mentioned in the literature, increase in the NO levels in the patient group may be related to Babesia merozoites that cause an increase in NO secretion by stimulated macrophages (7, 14). It has been demonstrated in studies on B. bovis that NO is effective in the inhibition of the parasite growth, by binding to iron containing fractions of the key enzymes in DNA synthesis of the parasite (6, 13, 14). Goff et al. (6) accepted that NO is a Babesia-killer molecule generated by active macrophages. Goff et al. (7) also reported the necessity of TNF-α presence for NO generation. The after treatment decrease in NO levels, which was high in the patient group, to values of the control group was due to decrease in the severity of parasitic inflammation as the parasites were effectively killed by the drug.

ADA enzyme levels are high in many diseases where cellular immunity is stimulated (15). We could not found in the literature any information on the ADA activity values in cattle with babesiosis. However, Hassan et al. (8) showed in B. divergens extracts that B. divergens is capable to synthesise purine nucleotides.

In the present study, serum ADA activity was significantly higher (P<0.05) in patient group than in the control group. The increase in ADA activity in the patient group expresses phagocytic activity of macrophages, and may have resulted from the erythrocyte damage caused by the parasite in the host. After the treatment, ADA activity decreased near the control group levels very rapidly; with the destruction of the parasite due to cellular immunity by secretion of inflammatory mediators from macrophages, and the parasite killing effect of diminazene aceturate.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Patient</th>
<th>After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
<td>7 d  15 d  30 d</td>
</tr>
<tr>
<td>TNF-α pg/ml</td>
<td>20</td>
<td>22.60±0.92a</td>
<td>71.93±2.56b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>55.82±1.35c</td>
<td>35.35±1.69d</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>27.90±2.05a</td>
<td></td>
</tr>
<tr>
<td>NO µmol/L</td>
<td>20</td>
<td>13.42±0.42a</td>
<td>50.03±3.5b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28.89±1.06c</td>
<td>18.73±0.75ad</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>15.44±0.53a</td>
<td></td>
</tr>
<tr>
<td>ADA IU/L</td>
<td>20</td>
<td>11.07±4.91a</td>
<td>70.18±16.30b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.65±13.55c</td>
<td>14.20±5.71ad</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>11.95±4.05a</td>
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</tr>
</tbody>
</table>

a, b, c, d differences are statistically significant in groups marked with different letters in the same column (P<0.05); ± SD.
ADA is an enzyme that is present in all cells. Measurement of ADA activity is used in the diagnosis and monitoring of autoimmune and inflammatory diseases, especially tuberculosis, due to its easy performance, high sensitivity, and less cost (11).

In the present study we demonstrated the changes in ADA activity and levels of TNF-α and NO in the acute phase of cattle babesiosis and on days 7, 15, and 30, after the treatment with diminazene aceturate. Investigation of acute phase and progression of the disease in vivo, using a natural process to stimulate cytokine production, supported the importance of TNF-α and NO in the immune response to babesiosis. In addition to TNF-α and NO, determining the ADA activity before and after treatment can provide important information for the diagnosis and treatment of bovine babesiosis. Therefore, ADA can be a predictive and sensitive parameter of the disease.

Acknowledgments: This work was supported by TÜBİTAK VHAG -1862/ADP.

References