Plasma nitric oxide concentrations and erythrocyte arginase activities in lambs with contagious ecthyma

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SUMMARY

The aim of the present study was to investigate the plasma nitric oxide concentrations and the erythrocyte arginase activities in lambs with contagious ecthyma, which frequently occurs in lambs and goat kids. The study was conducted on 10 Akkaraman, 1-2 month old lambs with contagious ecthyma (the clinical diagnosis was based on the presence of typical cauliflower-like proliferative lesions in lips, commissura labialis, nostrils and udders) and on 10 apparently healthy lambs (controls) from the same flock. Plasma NO concentrations and erythrocyte arginase activities were determined on all animals using the Griess method and the thiosemicarbazide diacetyl-monoxime urée (TDMU) method, respectively. Compared to the control group, the mean plasma NO concentration was significantly increased in the diseased group while the mean arginase activity was markedly depressed. Abnormally high NO concentrations and low enzyme activities were observed in 9 and 10 diseased lambs, respectively. Additionally, a negative correlation was evidenced between the 2 biochemical parameters. These results show that this viral infection is associated with some perturbations in the arginine metabolism, leading preferentially to the synthesis of NO, which is presumed to exhibit some antiviral properties.

Keywords: Lamb, contagious ecthyma, plasma, nitric oxide, erythrocyte, arginase.

Introduction

Contagious ecthyma is a highly zoonotic viral disease that typically occurs on the nose tips, lips, feet and genital organs in lambs and goat kids, and sometimes crusted papules of udders in adult sheep. The disease is caused by an epitheliotropic virus (parapoxivirus of the family Poxviridae), and is common in spring and summer months [1, 15, 30]. Although the disease itself is not fatal, it is emphasized that secondary infections may occur because the disease hampers the nutrition of the animals and cause loss of weight. It is suggested that multiplication of the necrosis bacteria in ulcerative lesions can cause sepsis [15, 29, 30].

The incubation period of the disease is about 8-10 days. There are three forms of the disease, called labial, pedal and genital forms according to the location of lesions. Diagnosis is based on the appearance of the typical clinical symptoms. However, final diagnosis is made via electron microscopic examination of the pathological materials, PCR and immunohistochemistry [1, 26].

L-arginine plays an important role in congenital and acquired immunity [33]; it is hydrolyzed to nitric oxide by the nitric oxide synthase and to urea and ornithine at the last stage of the urea cycle by the arginase [8]. It is also reported that the nitric oxide, which has antiviral characteristics in both DNA and RNA viruses, plays an important role in preventing RNA synthesis, viral protein accumulation and releasing of the virus [3, 7, 23, 35].

A review of the literature found no previous study on erythrocyte arginase activity and plasma nitric oxide concentrations.
in cases of contagious ecthyma. Therefore, the present study aimed to detect plasma nitric oxide concentrations and erythrocyte arginase activity during contagious ecthyma, which occurs frequently in lambs and goat kids.

**Materials and Methods**

**FLOCK AND ANIMALS**

Firstly, one 1-month old lamb was brought to Firat University Faculty of Veterinary Medicine (Animal Hospital Internal Diseases Clinic) for examination and treatment. Systematic clinical examination revealed lesions typical of contagious ecthyma. The flock was visited and anamnesis revealed lesions of the circumoral parts and gingiva in approximately 30-35% lambs. As they drank milk, some verruca-like lesions were also found in the udders of some sheep. The study material comprised 10 Akkaraman, 1-2 month old lambs diagnosed with contagious ecthyma (Case group) and 10 healthy animals, free of symptoms and clinical signs and diagnosed as healthy after general examination from the same flock (Control group).

Blood samples from all animals were obtained from the V. jugularis puncture and collected into sterile tubes with heparin as anticoagulant. The blood samples were centrifuged at 1500 g for 10 minutes at 4°C. Plasma were carefully separated from the packed cells and stored at -20°C until measurement of nitric oxide concentrations, whereas erythrocytes were washed three times with normal saline (0.9% NaCl) and the isolated erythrocytes were used as the enzyme arginase source.

**BIOCHEMICAL ANALYSES**

The plasma nitric oxide concentrations were determined according to the enzymatic Griess method [24] and the erythrocyte arginase activity was determined using the thioscavogarbazide diacetyl-monoxime urea (TDMU) method [13]. The haemoglobin amount necessary for the determination of the erythrocyte arginase activity was ascertained with the Drabkin method depending on the cyanmethaemoglobin formation [12]. In the present study, 1 unit of enzyme was defined as the amount of enzyme generating 1 µmol urea from L-arginine in 1 hour at 37°C and stated as specific activity urea/hour/g haemoglobin.

**STATISTICAL ANALYSIS**

SPSS (Version 13.0 for Windows) was used in the statistical analysis of the data. The data are shown as arithmetic average and standard error. The independent t-test was used to determine statistically significant variations between groups and differences were considered as significant when \( P \) values were less than 0.05. Correlations were calculated between the 2 parameters using the Pearson test.

**Results**

Systematic clinical examinations of the lambs in the case group showed that the appetite of the animals has remained good. However, there were cauliflower-like proliferative lesions with different sizes in the mucocutaneous parts of the lips, in the skin of the lips, in commissura labialis, and around nostrils and udders in sheep. All lambs from the case group exhibited such lesions.

As shown in the Table I, the mean plasma nitric oxide concentration was markedly increased in the contagious ecthyma affected group (\( P < 0.01 \)) whereas the mean erythrocyte arginase activity was dramatically lowered (\( P < 0.01 \)) compared to the control values. Nine diseased lambs exhibited high nitric oxide concentrations (above the superior threshold value defined as the mean ± standard deviation calculated in controls) and depressed enzyme activities (below the inferior threshold defined as the mean ± standard deviation calculated in controls) were found in 10 diseased animals. In healthy lambs, these abnormal values were observed only in 3 and 5 lambs, respectively (Table II). In addition, it was noted that abnormal values of the plasma nitric oxide concentrations or of the erythrocyte arginase activity were mainly found in lambs which presented the most severe or extended clinical lesions.

Moreover, the 2 biochemical parameters investigated in the study were simultaneously altered in 9 diseased lambs. Plasma nitric oxide concentrations and arginase activities were negatively correlated in all lambs (\( r = -0.329, P < 0.05 \)).

**Discussion**

Contagious ecthyma is common in lambs and goats kids all over the world and is frequently seen in Turkey [11, 14-18, 25, 30]. In cases of complications and secondary infections,

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Control group</th>
<th>Case group</th>
<th>( P )</th>
</tr>
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<tbody>
<tr>
<td>Nitric oxide (µmol/L)</td>
<td>55.93 ± 1.03</td>
<td>61.26 ± 1.20</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Arginase activity (U/g Hb)</td>
<td>80.55 ± 5.35</td>
<td>60.60 ± 4.03</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

*Hb: haemoglobin*

Table I: Plasma nitric oxide concentration and erythrocyte arginase activity in lambs from the Case and Control groups (n = 10 in each group). Results are expressed as mean ± standard deviation.
contagious ecthyma results in very high morbidity and great economic losses of young lambs and goats. In addition, the disease is also considered as a zoonosis [15, 29]. Diagnosis in the present study was made according to reports in the literature [11, 15, 18, 26, 29-31], based on the occurrence of characteristic cauliflower-like proliferative lesions in the mucocutaneous parts of the lips, in the skin of the lip, in the commissura labialis and around the udder and the nostrils. In addition, the detection of similar lesions in the udders of some lambs supports the idea that the disease may be spread from infected youngsters during nursing [26, 30].

It was suggested that arginine, which is a multi-functional amino α-acid in animal cells, is not only a precursor in the protein synthesis but also plays a role in the synthesis of molecules, including the regulation of the nitric oxide, urea, polyamines, pralines, glutamate, creatine and cell homeostasis [34]. It also appears as very important for congenital and acquired immunity [33].

Plasma nitrite and nitrate determinations are increasingly being used in clinical chemistry as markers for the activity of nitric oxide synthase and the production of nitric oxide radicals. Determination of the nitric oxide radical itself is difficult because of its radical nature and its very short half-life. Nitric oxide synthase enzyme activity can be determined only in tissue or cell homogenates [22]. Therefore, the determination of the stable end products of nitric oxide radical, nitrite and nitrate, in plasma is more often used as a measure for the production of nitric oxide radical [24]. Nitric oxide, which has various and very different biological effects, is strongly involved in the cellular responses to the infections caused by a wide range of viruses [5, 6]. In addition to its essential physiological function as neurotransmitter [28, 32] a growing body of evidence indicates that nitric oxide or its derivatives have inhibitory effects on a variety of viral infections. Recently, it has been demonstrated that nitric oxide hinders the productive infection of several animal viruses, including herpes simplex virus type 1, ectromelia virus, vaccine virus [9, 21], vesicular stomatitis virus [6], and murine Friend leukemia retrovirus [2]. In a study of the NO effect on the Vesicular stomatitis viral infection [6], it was observed that the number of viral infected cells was strongly depressed after treatment with NO donors. In agreement with that, the mean plasma NO concentration in the present study was significantly increased in the contagious ecthyma group compared to the control group (P < 0.01). Moreover, the majority (9 cases) of diseased lambs, especially those with severe clinical signs, exhibited abnormally high NO concentrations.

The arginase enzyme is required in the last step of the urea cycle cleaving arginin into ornithine and urea, but it has also many other metabolic features: indeed, it has been previously demonstrated that the enzyme activity is increased in some parasite (e.g. Trypanosome cruzi, Fasciola gigantica) and infectious diseases (e.g. foot-and-mouth disease) [4, 10, 19, 20, 27] and in inflammatory cases [4]. Contrary to these statements, it was found in the present study that the erythrocyte arginase activity compared to the healthy control group was significantly lowered (P < 0.01) in the case group in which all animals exhibited marked low values. It is thought that this situation is related with the clinical course of the disease because the reduction of the enzyme activity was mainly intense in lambs with severe and extended clinical lesions. However, since there are no previous study on the activity of the arginase and the nitric oxide concentration in animals with contagious ecthyma, it was not possible to compare the present findings with similar studies.

It was previously determined that a competition for the metabolic arginin utilisation occurs between two major pathways, in one hand, the hydrolysis into urea and ornithine in the urea cycle catalyzed by the arginase and in the other hand, the conversion into NO and citrulline catalyzed by the NO synthase [8]. In the current study, inverse variations of the mean plasma NO concentration and mean erythrocyte arginase activity were evidenced in the contagious ecthyma group (i.e. increase in the NO concentration and decrease in the enzyme activity) and a significant negative correlation was obtained between the 2 biochemical parameters. These findings comply with the statement that these two factors compete with each other.

**Table II: Comparative distribution of the plasma nitric oxide concentrations and erythrocyte arginase activities in lambs diagnosed with contagious ecthyma (Case group, n = 10) and in healthy lambs (Control group, n = 10). The threshold values were defined as the means ± standard deviations of the 2 respective parameters calculated in healthy controls.**

<table>
<thead>
<tr>
<th>Nitric oxide (μmol/L)</th>
<th>Control group</th>
<th>Case group</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; Superior threshold (56.96 μmol/L)</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Between thresholds</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>&lt; Inferior threshold (54.90 μmol/L)</td>
<td>5</td>
<td>1</td>
</tr>
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<table>
<thead>
<tr>
<th>Arginase activity (U/g Hb)</th>
<th>Control group</th>
<th>Case group</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; Superior threshold (85.90 U/g Hb)</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Between thresholds</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>&lt; Inferior threshold (75.20 U/g Hb)</td>
<td>3</td>
<td>10</td>
</tr>
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*Hb: haemoglobin*
As a conclusion, it was found that, while the arginine activities were reduced, the plasma nitric oxide concentrations markedly increased in cases of contagious ecthyma disease, which is commonly seen in lambs and goat kids. It may therefore be beneficial to conduct further studies on the role played by nitric oxide donors in the immune response and treatments of contagious ecthyma.

References