INFLUENCE OF ZINC ON INNATE IMMUNE SYSTEM IN KIDS

SIMONA MALÁ, FRANTIŠEK KOVÁŘŮ¹, L'UBICA MIŠUROVÁ¹,
LEOŠ PAVLATA¹, RUDOLF DVORÁK¹, AND ANTONÍN LOJEK²

Institute of Physiology, ¹Ruminant Clinic,
University of Veterinary and Pharmaceutical Sciences, 612 42 Brno, Czech Republic
malas@vfu.cz
²Department of Free Radical Pathophysiology, Institute of Biophysics,
Academy of Sciences of the Czech Republic, 612 65 Brno, Czech Republic

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Abstract

The experiment was conducted to determine the effect of adequate zinc level supplementation (inorganic versus organic form) on the innate immune response of kids. The count of white blood cells, leukocyte differential count, phagocytic activity, and phagocytic index (as markers of the immune functions) were determined. Phagocytic activity was not significantly higher in the inorganic-zinc-treated group in comparison to the control (64.7±8.91 vs 61.2 ± 9.15 %). The production of reactive oxygen species (ROS) by goat’s blood neutrophils was detected by luminol-enhanced chemiluminescence (CL). CL was performed to determine integral CL, peak CL, and peak-time after addition of calcium ionophore A23187 (Cal-I), opsonised zymosan (OZP), and phorbol-12-myristate-13-acetate (PMA). A significant ROS increase reflected in peak CL (P≤0.05) was found in the lactate–Zn-treated group when Ca-I was used as activator. In the same group there was a significant integral CL increase (P≤0.05) when we used Ca-I as activator. Other parameters showed no significant changes.

Key words: goat, zinc, neutrophils, reactive oxygen species, innate immunity.

The innate immune system is present in all animals and is the basic and the oldest element of the immune system (2). Activated neutrophils produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) together with microbicidal peptides and proteases (6). Thus the generation and release of ROS contribute to innate immune response against bacterial infection (10). Zinc creates an active site of anti-oxidant enzyme superoxide dismutase (SOD). SOD forms a part of the defence mechanism against oxidative stress (4), which is caused by high amounts of ROS and may damage biological macromolecules of the organism (9). Zinc is an essential trace element associated with over 300 biological functions (7). It is important for all organisms, influencing growth and affecting development and integrity of the immune system (3). The degree of this influence depends on the animal species. In humans and laboratory animals even mild zinc deficiency depresses immune reactions. In contrast to this, marginal zinc deficiency does not appear to impair immune responses in ruminants (12).

The aim of our experiment was to study the possible changes in innate immunity in kids and their mothers by addition of inorganic and organic forms of zinc.

Material and Methods

Twenty-four kids of the white short-haired goat with a strict clinical control of a healthy state were used. The animals were housed at the Ruminant Clinic of University of Veterinary and Pharmaceutical Sciences. The kids were divided into four equal groups. Group 1 served as non-treated control, group 2 was treated with inorganic zinc (zinc oxide), group 3 was treated with zinc lactate (ZINC CHELATE, Agrobac, Czech Republic), and group 4 was treated with zinc proteinate (OPTIMIN ZINC, Trouw Nutrition International, USA).

Content of zinc was 100 mg per 1 kg of concentrate for goats (Biokron, Czech Republic). The animals received the tested substances together with their ration. The mothers (age of 2-4 years) were divided into the same tested groups and received the same doses of zinc as their kids. They started receiving the Zn supplement 3 months before the delivery.

During the after-birth period, the kids from each group were housed in pens with their mothers and they received mother’s milk. The average content of zinc in milk was determined on day 30 of lactation. Milk in the first group contained on average 62.06 mg/L Zn, in the second group 58.88 mg/L, the third group 56.29 mg/L, and in the fourth group 66.37 mg/L. There were
significant differences (P<0.001) observed between the tested groups. The kids were weaned at the age of 69 d and, after weaning, they were fed on concentrate for goats (Biokron, Czech Republic).

The consumption of the concentrate was 300 g per day per animal. Meadow hay and water were available ad libitum. The composition of concentrate in control group was the same but without zinc. The other mineral supplements and vitamins were added in physiologically adequate quantity.

Jugular blood samples were obtained from kids on 120 d of life. Heparin (Leciva, Czech Republic) in concentration of 50 i.u. per 1 ml of blood was used as anticoagulant in all cases. White blood cells were counted in Burker chamber. Differential count of WBC was determined using May-Grünwald-Giemsa stained blood smear. The values of respiratory burst were converted from total count of WBC into the number of neutrophils. Two hundred cells were evaluated on each slide. Luminol-enhanced chemiluminiscence (CL) was used to measure the production of ROS by goat’s blood neutrophils. Calcium ionophore A23187 (Cal-I), opsonised zymosan (OZP), and phorbol-12-myristate-13-acetate (PMA) were used as activators. The concentrations were optimal (unpublished results). Luminol-dependent CL was performed to determine integral CL (RLU*s), peak CL (RLU), and peak-time (min). Luminol-enhanced CL assay was measured using a luminometer Immunotech (Czech Republic). For luminometric measurements the whole peripheral blood was diluted 1:100 in Hanks’ balanced salt solution (HBSS, Sigma-Aldrich, USA) in order to block the effect of haemoglobin. Luminol (Sigma-Aldrich) was diluted in borate buffer (7.628g Na2B4O7, 1.237g H3BO4 (both Fluka), 500 mL of deionised water, pH 9) in order to reach working concentration of 10 mmol/lL; all other chemicals used in this method were diluted in HBSS. The reaction mixture contained diluted blood and 2.5 mmol/lL of luminol. The following agents were used to stimulate the ROS production: OZP at final concentration of 4.8 µmol/L and Ca-I A23187 (all Sigma-Aldrich, USA) at final concentration of 4.8 µmol/L; no activator was added to controls. The height of y-axis and the distance of x-axis from the peak was evaluated as integral, peak and peak-time and the values of the curves were assessed. The obtained values were converted into the constant count of neutrophils using the known number of WBC in the whole blood and the differential count of WBC.

The ability of peripheral blood neutrophils to phagocyte was tested according to Větvicka et al. (15) by using microspheric hydrophilic particles method (Artim, Czech Republic). The results were given as phagocytic activity and phagocytic index. Blood from the kids (10 µl) was collected and mixed in a small plastic test tube with 10 µl of PBS-suspended microspheric hydrophilic particles (4x10⁶ particles). The test tubes were incubated under intermittent shaking for 1 h at 37°C. Two blood smears on slides were prepared from each test tube (in addition to blood smears from original fresh blood) and stained with May-Grünwald-Giemsa. All applied methods were optimized on adult goats. We worked according to recommendation of Lojek et al. (8). The results are presented as average ±SEM. Student’s t-test was used to evaluate the effect of zinc among the tested groups. All calculations were performed with MS-Excel (Microsoft Corp. Inc.) software.

Results

The average body mass of kids on 120 d of their life was in the group 1 - 25.8 kg, in the group 2 - 24.5 kg, in the group 3 - 24.9 kg, and in the group 4 - 30.5 kg. The average body mass was significantly higher in the group 4 (P<0.05).

Mean plasma Zn concentration on day 120 was 10.57 mg/L (control group), 11.0 mg/L (inorganic-Zn-treated group), 10.56 mg/L (lactate-Zn-treated group), and 10.73 mg/L (proteinate-Zn-treated group). There were no significant differences between the tested groups.

All characteristics obtained from tested blood (count of leukocytes, leukocyte differential count) were not significantly different and corresponded to the physiological values of goats’ blood.

In organic–Zn-treated group (group 3), RLU*s increase at the level of significance was observed when PMA was used as the activator. In the same group, there was a significant RLU increase (P<0.05) when Ca-I was used as the activator (Table 1). Assessment of CL activity and thus ROS production were found in the whole blood.

When the data from leukocytes in the whole blood were recounted to neutrophil granulocytes no significant correlation between the tested groups was detected.

Phagocytic activity was insignificantly higher in inorganic-Zn-treated group (64.7±8.91%) compared with the other groups. There were no significant differences between the tested groups in the phagocytic activity and phagocytic index (Table 2).

Discussion

Knowledge related to phagocytic functions of neutrophils in ruminants is very limited. The percentage of phagocytising cells in goat blood reached up to 67.83±5.58% (1). In our study the results amounted to 64.7±8.91% in the inorganic-Zn-treated group and even lower in the control group (61.2±9.15) with a phagocytic index of 22.5 ± 13.45. No significant differences were observed between the tested groups in phagocytic ability but the results suggested that the inorganic form of Zn was beneficial for kids. When inorganic zinc and organic zinc were compared, no enhancement of immune functions in lambs was observed (5).
Table 1
Luminol-enhanced chemiluminescence for all leukocytes

<table>
<thead>
<tr>
<th>Group</th>
<th>Effector</th>
<th>Integral, RLU*s</th>
<th>Peak, RLU</th>
<th>Peak-time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ca-I</td>
<td>62,051.25 ± 3,784.67</td>
<td>31.63 ± 2.16</td>
<td>1,227.13 ± 59.83</td>
</tr>
<tr>
<td></td>
<td>PMA</td>
<td>4,3511.25 ± 4,985.19</td>
<td>18.50 ± 1.78</td>
<td>563.00 ± 209.45</td>
</tr>
<tr>
<td></td>
<td>OZP</td>
<td>193,525.00 ± 10,364.87</td>
<td>76.38 ± 4.95</td>
<td>1,330.25 ± 105.28</td>
</tr>
<tr>
<td>2</td>
<td>Ca-I</td>
<td>75,875.00 ± 5,881.65</td>
<td>52.38 ± 4.09</td>
<td>1,089.75 ± 103.14</td>
</tr>
<tr>
<td></td>
<td>PMA</td>
<td>60,022.50 ± 5,810.74</td>
<td>22.75 ± 2.78</td>
<td>437.00 ± 80.72</td>
</tr>
<tr>
<td></td>
<td>OZP</td>
<td>341,787.50 ± 19,415.38</td>
<td>129.75 ± 10.51</td>
<td>1,387.50 ± 78.29</td>
</tr>
<tr>
<td>3</td>
<td>Ca-I</td>
<td>72,501.25 ± 9,672.44</td>
<td>35.63 ± 5.35</td>
<td>1,387.50 ± 115.42</td>
</tr>
<tr>
<td></td>
<td>PMA</td>
<td>45,875.00 ± 4,729.21</td>
<td>17.63 ± 1.77</td>
<td>1,421.88 ± 387.89</td>
</tr>
<tr>
<td></td>
<td>OZP</td>
<td>186,887.50 ± 38,161.74</td>
<td>69.38 ± 13.63</td>
<td>1,467.63 ± 188.87</td>
</tr>
<tr>
<td>4</td>
<td>Ca-I</td>
<td>60,617.50 ± 6,963.88</td>
<td>47.00 ± 10.77</td>
<td>1,192.63 ± 29.95</td>
</tr>
<tr>
<td></td>
<td>PMA</td>
<td>44,221.25 ± 3,100.35</td>
<td>16.63 ± 1.18</td>
<td>1,891.13 ± 280.19</td>
</tr>
<tr>
<td></td>
<td>OZP</td>
<td>314,837.50 ± 43,776.15</td>
<td>20.85 ± 6.75</td>
<td>1,64.63 ± 63.53</td>
</tr>
</tbody>
</table>

Table 2
Mean plasma Zn concentration and phagocytic function

<table>
<thead>
<tr>
<th>Group</th>
<th>Zn concentration</th>
<th>PA (%)</th>
<th>PI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.57 ±0.49</td>
<td>61.2 ±9.15</td>
<td>22.5 ±13.45</td>
</tr>
<tr>
<td>2</td>
<td>11.0 ±1.03</td>
<td>64.7 ±8.91</td>
<td>23.8 ±10.66</td>
</tr>
<tr>
<td>3</td>
<td>10.5 ±1.04</td>
<td>58.5 ±10.2</td>
<td>20.5 ±14.72</td>
</tr>
<tr>
<td>4</td>
<td>10.73 ±0.74</td>
<td>62.9 ±9.48</td>
<td>22.1 ±9.78</td>
</tr>
</tbody>
</table>

± - SEM; PA – phagocytic activity; PI – phagocytic index.

According to Sunzel et al. (14) no effect on phagocytosis and bacterial killing was found when zinc was used in concentration higher then physiological. Plasma Zn concentration was not significantly affected by the treatment. These findings are in accordance with the data published by Spears et al. (13).

There is little evidence that the form of zinc (organic vs. inorganic) is considerably better absorbed in ruminants (11). Our data on the innate immune response indicate that both forms of Zn can be suitable for enhancement of innate immune system in kids.

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References