Abstract

The aim of this study was to establish the influence of α-ketoglutarate (AKG), administered to pregnant sows from the 91st d of pregnancy to farrowing, and then to piglets from birth to the 30th d of life, on lysozyme and ceruloplasmin activity, serum total protein content, and the WBC counts in blood of piglets, at the age of the 14th and 30th d of their postnatal life. The sows were treated per os with AKG at the dosage of 0.4 g/kg b.w. every day, whereas those of the controls were given saline. Piglets born by sows treated with AKG were divided into two groups: the first group was administered orally saline (AKG/PhS group) and the second group received orally AKG at the dosage of 0.4 g/kg b.w./d (AKG/AKG group), during 30 d of their postnatal life. Administration of AKG to sows during pregnancy increased lysozyme activity in piglets at the age of the 30th d, which reached the value 7.07 mg/L, while that in the controls was 3.90 mg/L. Ceruloplasmin activity decreased during the first 14 d of life in piglets that received AKG as a continuation of the prenatal procedure. At the age of 14 d, ceruloplasmin activity decreased to 90.96 IU/L in comparison with the 117.95 IU/L of the controls, while the level of total protein was higher (71.83 g/L) than that of the controls (64.23 g/L). There is still limited information about the relationship between exposure to AKG during foetal, and/or early postnatal life and altered postnatal immune function in piglets.

Key words: piglets, α-ketoglutarate, lysozyme, ceruloplasmin, total proteins.

α-ketoglutarate (AKG) is called the immunonutrient factor. It modulates the immunity and plays an important role in the general metabolism as well (1, 25, 26). Exogenous AKG is converted to glutamate and glutamine in intestinal cells (9, 15, 16). Glutamine is a conditionally essential amino acid and energy source for all types of cells in the organism constituting more than 60% of the total amino acid pool. It is an important factor in stress and in growth during neonatal life, particularly during weaning and post weaning, when the animals’ diet and intestinal bacterial flora are changed (9, 11, 15, 16, 21). AKG as a precursor of glutamine administered per os, is a main source of energy for intestinal cells and a preferred substrate for both enterocytes and other rapidly dividing cells. It maintains a gut barrier, and reduces bacterial translocation in animals and influences protein synthesis in vivo (3, 5, 14, 18). In clinical studies on septic, traumatic, or surgical patients, AKG has been shown to display beneficial effects by improving the body weight gain, nitrogen balance, all of which are evidence of an AKG-mediated decrease in proteolysis, an increase in protein synthesis, or both. In vitro and in vivo studies showed that AKG decreased protein catabolism in the skeletal muscles and increased protein synthesis in the liver and intestines (3). Other studies showed a different action of AKG on the development of the skeletal system in foetuses and in piglets during their postnatal life (10, 23). Our earlier studies showed that newborn piglets treated with AKG during the first days of neonatal life, presented a decrease in ceruloplasmin activity and γ-globulin level (22). Different factors administered to foetuses by treating the dam or to young animals after birth, may change postnatal immune function more than in the adult and persist for a longer time, leading to different inflammatory
response in the neonate compared with that of the adult (8).

Little is known about the influence of pre-natal and/or early postnatal administration of AKG on non-specific defence mechanism in blood serum of piglets during their development. This motivated us to determine the influence of oral administration of AKG to sows and to piglets on lysozyme and ceruloplasmin activity, total albumin, and white blood cell counts in piglets at the 14th and 30th d of their postnatal life.

Material and Methods

This study was approved by the Local Ethic Committee on Animal Experiments of Agricultural University of Lublin, Poland.

Procedure with pregnant sows. The study was carried out on 12 sows and their 36 newborns, of a Large Polish White breed, housed under standard rearing conditions (temperature and humidity) with free and constant access to fresh water, and fed twice-daily standard commercial diets for pregnant sows. All sows used in the experiment were clinically healthy, and were not prophylactically vaccinated. The experimental procedure was conducted from the 91st d of pregnancy to the parturition (the last 24th d of pregnancy lasting 114 to 116 d). Time of parturition was calculated from the day of the mating. The experimental sows were divided into two groups. The first group (control sows) received orally 300 ml of saline/sow/d. The second group was orally administered with AKG at the dosage of 0.4 g/kg b.w./d (AKG group). Powdered AKG (Gramineer INS. AB, Sweden), with a purity of 99%, was mixed with saline and buffered by the addition of NaOH (POCH, Poland) to a final pH of 7.35.

Procedure with piglets. Piglets chosen randomly from control and experimental sows were divided just after the birth into one control and two experimental groups. Every group consisted of 6 male and 6 female piglets, held in the same boxes with their mothers and naturally fed sows’ milk. Piglets of the control group, born by sows receiving saline during the last 24th d of pregnancy, received saline per os (the same volume as this of prepared AKG solution given to piglets from AKG/AKG group) from their birth through to the 30th d of their postnatal life. Piglets born by sows treated with AKG were divided further into the group orally administered with saline instead of AKG (AKG/PhS group), the group orally administered with AKG at the dosage of 0.4 g/kg b.w./d (AKG/PhS group) and the group orally administered with AKG at the dosage of 0.4 g/kg b.w./d and (AKG/AKG group) during 30 d of their postnatal life. The saline volume given to piglets from AKG/AKG and the control group, was the same as the volume of prepared AKG solution given to piglets from AKG/AKG group and equalled 2 ml /kg b.w./d. Experimental administration of AKG was performed every morning. The total time of the experiment included the last 24 d of pregnancy, when foetuses were under the influence of AKG or saline administered to their mothers, and the 30th d of postnatal life, when the piglets were administered with AKG or saline, depending on their assignment to the experimental group. The piglets at the age of 30 d were euthanised with the lethal doses of pentobarbitalum natrium injected intravenously (Morbital, Biowet Pulawy, Poland).

Procedure with blood samples. The blood samples were collected from 14- and 30-d-old piglets at the time of daily procedure from the subclavian vein. After centrifugation (3 000 x g for 15 min), the obtained serum was rapidly frozen at –25°C and kept at this temperature until the analysis. Serum ceruloplasmin activity was determined with the spectrophotometric method (17) modified by Siwicki and Anderson (20), lysozyme activity with the turbidimetric method, using Micrococcus lysodeikticus (Sigma), modified by Siwicki and Anderson (20), and total protein content with the biuret method (Sigma 690-A). A part of every blood sample was collected to a heparinised tube and analysed immediately. Automatic haematological analyser MS9 (Melet Schloesing Laboratories, France) was used to determine the mean number of white blood cells.

Statistical analysis. All the results are expressed as means ± SEM. Differences between means were tested by the use of two-way ANOVA and post hoc Tukey test as a correction for multiple comparisons. Normal distribution of the data was examined by the use of a Shapiro-Wilk test. Equality of variance was tested by the use of the Levene test. When there was a lack of normal distribution or unequal variance of the data, the differences between the means were tested by the use of the Mann-Whitney U test with a Bonferroni correction for multiple comparisons. P<0.05 was considered as statistically significant. All statistical analyses were carried out by the means of STATISTICA 6.0 software.

Results

An increase in serum lysozyme activity of piglets at the age of the 14th d in the AKG/PhS group when compared with the control was demonstrated. Statistical significance of the lysozyme activity were noted also between piglets, which postnatally received saline after prenatal administration of AKG, and piglets born by AKG sows and treated with AKG during 30 d of their postnatal life. At the age of the 30th d, lysozyme activity was increased in serum of piglets from the AKG/PhS group, when compared with the control, but it was decreased in piglets from the AKG/AKG group. In consequence, lysozyme activity in piglets at the 30th d, significantly differed between the AKG/AKG and AKG/PhS group (Table 1).

Ceruloplasmin activity was significantly lower in the AKG/AKG group of piglets at the 14th d when compared with the control. A tendency to a lower activity of ceruloplasmin was observed in both experimental groups of piglets at the 30th d, when compared with the control (Table 1).
The level of serum total protein in piglets at the 14th d was significantly higher in both experimental groups, when compared with the control. Moreover, when the AKG/AKG group was compared with the AKG/PhS group, the total protein level was significantly lower in the AKG/AKG group of piglets at the 14th d. The level of total protein in piglets at the 30th d did not differ between all the groups (Table 1).

There were no differences between the mean white blood cell count in piglets at the 14th and 30th d from all the investigated groups (Table 1).

**Discussion**

The prenatal period is a time of high sensitivity to chemical agents or their metabolites, which may cross the placenta or influence the foetal immune system by altering the general metabolism of a pregnant mother (8). The immune system is composed of many interdependent cell types that collectively protect the body from bacterial, parasitic, fungal, and viral infections. Many of these cells have specialised functions. Cellular and humoral immune defence in the neonate differs qualitatively and quantitatively from that of the adult. Moreover, piglets are immuno-deficient at birth and dependent on a supply of specific and non-specific immune factors from colostrum or milk (21). The liver is the first place of haematopoiesis during the first days or weeks of gestation, depending on the species. Close to the time of birth, the liver loses its haematopoietic role and starts to play metabolic function. The first leukocytes were observed in the blood of foetuses at the 17th d of gestation in the study of the development of the immune system. The colonisation of the thymus with leukocytes was observed 21 d later (19). Leukocytes in early postnatal immune system are present in lower numbers and decrease their function, such as expression of certain enzymes (8). The changes were described in peripheral lymphocytes of 4-week-old piglets after perinatal influence of polybrominated biphenyl (9). Depending on the character of the used factors, to which the uterus was exposed, the obtained results showed inhibited development of immune organs, like thymus, or altered immune function later in life. (8). Other in vitro studies showed that lymphocyte proliferation in the rat and human was dependent on glutamine administration.

There are also data demonstrating that glutamine is a very important factor for human neutrophil and monocyte growth and bacteria-killing action (26). These observations suggest that glutamine is required for the optimal function of these cells (4). More than 30% of the energy necessary for all the cells in the immune system derives from glutamine. Glutamine is utilised by macrophages, neutrophils, and lymphocytes (25). Other results showed that glutamine supplemented diet increased phagocytic activity of neutrophils in patient with sepsis (25). The present study showed a tendency to a higher mean number of white blood cells in piglets at the 14th and 30th d from AKG/AKG group compared with AKG/PhS. It is known that the gastrointestinal tract of older pigs metabolises AKG when administered per os (12). In our study, AKG was administered to sows during 3 weeks of pregnancy and to piglets before and after closure of the gut for the intestinal immune system formed its integrity required for optimum growth (2). Immune response, such as inflammation or tolerance, depends on maintaining this integrity. Weaning is a very critical time for piglets. It is associated with high mortality engendered by viruses or other pathogens. Ceruloplasmin (Cp) and lysozyme participate in the immune response. Cp is a copper-containing plasma ferroxidase that plays an essential role in mammalian iron homeostasis. This protein is a

**Table 1**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=12)</th>
<th>AKG/PhS (n=12)</th>
<th>AKG/AKG (n=12)</th>
<th>Control (n=12)</th>
<th>AKG/PhS (n=12)</th>
<th>AKG/AKG (n=12)</th>
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<tbody>
<tr>
<td></td>
<td>At 14 d</td>
<td></td>
<td>At 30 d</td>
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<tr>
<td>Lysozyme (mg/L)</td>
<td>0.62±0.17 P&gt;0.05</td>
<td>3.69±0.08 P&gt;0.05</td>
<td>0.79±0.03 P&lt;0.05</td>
<td>3.90±0.43 P&lt;0.05</td>
<td>7.07±0.08 P&lt;0.05</td>
<td>2.85±0.12 P&gt;0.05</td>
</tr>
<tr>
<td>Ceruloplasmin (IU/L)</td>
<td>117.95±9.85 P&lt;0.05</td>
<td>95.68±4.46 P&lt;0.05</td>
<td>90.96±1.36 P&lt;0.05</td>
<td>123.56±3.22 P&lt;0.05</td>
<td>111.16±3.15 P&lt;0.05</td>
<td>119.48±5.61 P&lt;0.05</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>64.23±3.26 P&lt;0.0002</td>
<td>76.35±2.64 P&lt;0.0002</td>
<td>71.83±1.70 P&lt;0.0005</td>
<td>74.84±3.06 P&lt;0.0002</td>
<td>77.57±2.46 P&lt;0.0002</td>
<td>72.76±4.79 P&gt;0.05</td>
</tr>
<tr>
<td>White blood cells (10^9/L)</td>
<td>8.52±1.78 P&gt;0.05</td>
<td>6.02±0.73 P&gt;0.05</td>
<td>7.76±1.53 P&gt;0.05</td>
<td>11.08±1.20 P&gt;0.05</td>
<td>10.63±1.56 P&gt;0.05</td>
<td>12.23±1.46 P&gt;0.05</td>
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P - AKG/AKG or AKG/PhS versus control  P* - AKG/AKG versus AKG/PhS
member of the multicopper oxidase family of enzymes. Its ability to oxidise both organic and inorganic substrates is unique (13). Cp is synthesised in the liver, uterus, placenta, brain, and mammary glands, and secreted into the plasma (6). Recent studies have identified the lung as another major site of Cp synthesis. Bronchial epithelium in baboon foetuses, by 60 d of gestation, was described as place of expression of Cp (7, 24). It functions as an oxidase in the antioxidant system and participates in the acute-phase response to inflammation. Cp participates in angiogenesis as well (6). Although developmental increase in hepatic Cp and serum activity has been described, there are no data included information about the relationship between the Cp activity and AKG administration during prenatal and/or postnatal life. The present study showed the decrease of Cp activity in serum of piglets at the 14th d after both prenatal and postnatal administration of AKG. May be there is a stabilisation in the interactions between bacteria and animal host, which is important for the maintenance of the gut barrier for its normal function and activity.

Lysozymes are a group of enzymes that catalyse the hydrolysis of specific glycosidic bonds in mucopolysaccharides, which constitute some bacterial cell walls. Many mammals, including man, have moderate to high levels of lysozyme in their secretions (tears and saliva), white blood cells, and tissue macrophages. In these settings, lysozyme is thought to be an important antibacterial defence and to have originally evolved for that purpose. Our study showed that lysozyme activity was still increasing in serum of piglets being under only prenatal influence of AKG.

The immune system consists of different elements, of which are structurally and functionally distinct, and in some situations dependent on species, age, diet, and type of placenta. In sows, the epitheliochorial placenta does not permit the transfer of macromolecules from the dam to foetus. This lack of some elements of the perinatal immune system in pigs, gives a model for studies of the influence of nutrient factors on immune development.

References

