EFFECT OF A SINGLE ORAL ADMINISTRATION OF IRON FUMARATE ON HAEMATOLOGICAL INDICES AND ANTIOXIDANT STATUS IN PIGLETS

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Abstract

The aim of this study was to evaluate the efficiency of a single oral iron fumarate administration, and to compare antioxidant status of piglets treated either orally or parenterally. Two days after birth, the piglets in group I (22 piglets) were orally given 200 mg of Fe$$^2+$$ as iron fumarate. At the same time, the piglets in group II (21 piglets) were given intramuscularly 200 mg of Fe$$^3+$$ as iron dextran. The piglets in group III (18 piglets), were given intramuscularly 200 mg of Fe$$^3+$$ at the age of 28 d. At the age of 21 and 28 d Hb, PCV, MCV, MCH and iron concentration in blood plasma of group I were significantly lower compared to group II. No differences in antioxidant status (vitamin E, GSH-Px, total antioxidant capacity) were observed between groups I and II. For group III, on day 28, GSH-Px activity (expressed per 1 T/l RBC) was significantly lower compared to group II. It is concluded that single oral administration of iron fumarate is insufficient for the prevention of iron deficiency and that either parenteral or oral administration do not reduce the antioxidant status of the piglets.

Key words: piglets, iron dextran, vitamin E, glutathion peroxidase, antioxidant capacity.

Iron deficiency anaemia is a well known problem in swine production. The most common method for the prevention of iron deficiency in piglets; is an injection of 200 mg of Fe$$^3+$$ as iron dextran to 2-3-day-old piglets. However, this method is associated with occasional undesirable side effects. Kolb and Hofmann (13) described acute toxicosis after iron dextran injection in antioxidant deficient piglets. Iron toxicosis occurs due to the ability of iron ions to catalyse the formation of free radicals (31). Iron ions facilitate the conversion of hydrogen peroxide to hydroxyl radical. The hydroxyl radical (OH) is believed to be the most important initiator of lipid peroxidation (22). The ability of the organism to counterattack the oxidative stress depends on the availability of antioxidants. The most important antioxidants in piglets are vitamin E (17) and glutathione peroxidase (GSH-Px) (9). The participation of extracellular antioxidants forming total antioxidant capacity (TAS) of blood plasma in defence against iron-induced free radicals was also reported (11). The possible effects of different forms of iron administration on the development of antioxidant status of piglets need to be clarified.

An alternative to iron injection is the oral administration of iron pastes. Iron fumarate (15, 27) and iron lactate (28) are used in pig production. In our previous study, a double oral dose of iron fumarate was used successfully for anaemia prevention (27).

The aim of this study was to evaluate the efficiency of a single oral application of iron fumarate for the prevention of iron deficiency anaemia of piglets; and to compare antioxidant status between parenterally and orally treated piglets.

Material and Methods

Experimental design. Six piglet litters were included in the trial. The piglets were divided into 3 groups using split litters, i.e. each litter was divided into three different groups. The use of split litters minimized the effect of sows. On day 2 after birth, the piglets of group I (22 piglets) were given orally paste containing 200 mg of Fe$$^2+$$ in the form of iron fumarate, and piglets of group II (21 piglets) were injected intramuscularly with 200 mg of Fe$$^3+$$/piglet in the form of iron dextran. Group III (18 piglets) was given intramuscularly 200 mg of Fe$$^3+$$/piglet on day 28 of piglet life. From day 10 all the piglets had access to the starter containing 220 mg/kg of Fe, 0.46 mg/kg of Se and 58 mg/kg of vitamin E (SKS weaning pellets, Slavkovské krmné směsi a.s.).

During pregnancy the sows were fed a feed mixture containing 0.29 mg/kg of Fe, 0.46 mg/kg of Se and 58 mg/kg of vitamin E (Biostan KPB, Biokron s.r.o.). During lactation, a feed mixture containing 0.33 mg/kg of Se and 69.2 mg/kg of vitamin E was fed to the sows.
Sampling and analyses. From day 2 to day 42 of the piglet’s life, blood was collected from the vena cava cranialis. EDTA (ethylenediaminetetraacetic acid) and heparin were used as anticoagulants.

Haematological examination included: haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). These indices were determined by haematological analyser Celtac Alfa (Nihon Kohden).

Iron concentration in blood plasma was determined photometrically, by measuring iron complex with ferrozine (Iron liquid 917, Roche Diagnostic, Germany). For the determination of selenium concentration, samples of whole blood were mineralised in a close system using microwave digestion technology based on HNO₃ and H₂O₂ in MILESTONE MLS – 1200 equipment. After evaporation, the mineralised sample was used to prepare a water solution and 20% HCl was added. Thus prepared sample was tested for Se concentration by AAS hybrid technique using the UNICAM 939 AA spectrometer.

GSH --Px activity in whole heparinized blood was determined by the Paglia and Valentine method (23) using the RANSEL set (Randox) and the COBAS MIRA automatic analyser. The GSH-Px activity in whole blood was calculated to 1 T/l of erythrocytes.

Vitamin E concentration in blood plasma was determined fluorometrically according to Bouda et al. (3) using fluorescence spectrophotometer (204 Perkin – Elmer).

TAS was determined using the RANDOX set and the COBAS MIRA automatic analyser according to the following principle: ABTS ® (2,2’-azino-di-[3-ethylbenzothiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin and H₂O₂) to produce the radical cation ABTS®⁺; it has a relatively stable blue-green colour, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this colour production to a degree, which is proportional to their concentration.

Statistical analyses. The results were evaluated by analyses of variance ANOVA. The results are presented as mean values and standard deviations (SD) of each index. Values in figures 1-8, denoted * or **, express significant difference (P<0.05 or P<0.01, respectively) between groups I and II.

The study was approved by the Ethical Commission of the University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic.

Results

The haematological indices of sows were as follows: Hb: 120.1 ± 4.3, PCV: 0.36 ± 0.007, RBC: 5.49 ± 0.23. The development of red blood cell indices in iron fumarate (group I) and iron dextran group (group II) was similar till the age of 13 d. On days 21 and 28 Hb, PCV, MCV, and MCH in group I were significantly lower compared to group II (all, P<0.01). Two weeks after weaning (day 42), there was a significant increase in Hb, PCV, and Fe in group I (all, P<0.01). No differences in RBC between groups I and II were found during the trial. Fe concentration in blood plasma of group I was found to be significantly lower compared to group II on days 9, 13, 21, and 28 (all, P<0.01).

For group III, Hb, PCV, RBC, MCV, MCH, and Fe were, as expected, significantly lower than in the two other groups (all, P<0.01). Fe application in Group III on day 28 resulted in a significant increase in the element content and red blood cell indices (all, P<0.01) two weeks thereafter. The body weight of piglets in group III was significantly lower compared to the two other groups.

On day 2 after birth, no differences in Se concentration (group I: 83.73±5.97; group II: 82.67±7.26; group III: 80.83±7.01), GSH-Px activity, vitamin E concentration, and TAS were found among the groups I, II, and III.

No differences in vitamin E concentration and TAS of blood plasma were observed among groups I, II, and III in any period of the trial. In group III, on day 28, GSH-Px activity (expressed per 1 T/l of erythrocytes) was significantly lower compared to iron dextran group (P<0.01). No differences in GSH-Px activity between groups I and II were observed during the trial.

Table 1
Antioxidant status of the sows (2 d after farrowing)

<table>
<thead>
<tr>
<th>Index</th>
<th>Se</th>
<th>GSH-Px</th>
<th>Vitamin E</th>
<th>TAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/l in whole blood</td>
<td>µkat/l expressed per 1 T/l RBC</td>
<td>µmol/l in blood plasma</td>
<td>mmol/l of blood plasma</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>139.8 ± 9.94</td>
<td>59.27 ± 4.53</td>
<td>6.45 ± 0.94</td>
<td>0.65 ± 0.08</td>
</tr>
</tbody>
</table>
Fig. 1. Haemoglobin concentration.

Fig. 2. Packed cell volume.

Fig. 3. Red blood cell count.

Fig. 4. MCV.

Fig. 5. MCH.

Fig. 6. MCHC.

Fig. 7. Body weight.

Fig. 8. Iron concentration in blood plasma.
Table 2
Antioxidant status of the piglets (mean ± SD, GSH-Px expressed per 1 T/l RBC, vit. E: µmol/l in blood plasma, TAS: mmol/l of blood plasma)

<table>
<thead>
<tr>
<th>Index</th>
<th>Days of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>GSH</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>46.02</td>
</tr>
<tr>
<td>Px</td>
<td>± 9.66</td>
</tr>
<tr>
<td>Group II</td>
<td>42.87</td>
</tr>
<tr>
<td></td>
<td>± 6.74</td>
</tr>
<tr>
<td>Group III</td>
<td>45.92</td>
</tr>
<tr>
<td></td>
<td>± 7.90</td>
</tr>
<tr>
<td>Vit. E</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>15.80</td>
</tr>
<tr>
<td></td>
<td>± 4.20</td>
</tr>
<tr>
<td>Group II</td>
<td>14.98</td>
</tr>
<tr>
<td></td>
<td>± 4.31</td>
</tr>
<tr>
<td>Group III</td>
<td>15.40</td>
</tr>
<tr>
<td></td>
<td>± 4.12</td>
</tr>
<tr>
<td>TAS</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>0.597</td>
</tr>
<tr>
<td></td>
<td>± 0.19</td>
</tr>
<tr>
<td>Group II</td>
<td>0.580</td>
</tr>
<tr>
<td></td>
<td>± 0.16</td>
</tr>
<tr>
<td>Group III</td>
<td>0.620</td>
</tr>
<tr>
<td></td>
<td>± 0.17</td>
</tr>
</tbody>
</table>

Value with ** express significant difference (P<0.01) between iron dextran group (group II) and the iron deficient group (group III).

Discussion

The efficiency of iron fumarate was tested under experimental conditions by Kotrbáček (15). He found that oral administration of 100 mg of Fe²⁺ as iron fumarate to one-day-old piglets resulted in significant improvement of red blood cell indices. In our previous work, we have found comparable efficiency of repeated (at the age of 6 and 11 d) administration of 200 mg of Fe²⁺-fumarate in anaemia prevention, compared to iron dextran injection (200 mg of Fe³⁺) (27). However, the repeated administration is expensive and additionally stresses the piglets. The first goal of this study was to find out if a single oral dose of iron fumarate could be effective in anaemia prevention.

It is advisable to give iron to piglets after they were left to suckle colostrum. It is known that vitamin E does not cross the placental barrier. This results in its deficiency in newborn piglets. Saturation of piglets with vitamin E increases its content to sufficient levels after colostrum intake (17). Therefore, the common practice is to administer iron to two-three-day-old piglets. During our trial we did not observe any health complications either in orally or parenterally treated piglets.

Simultaneous decreases in red blood cell indices and iron concentration in blood plasma in iron fumarate group, reflects a depletion of iron reserves. The anaemic limit, i.e. the point when anaemia begins to exert a detrimental effect on weight gain and gives clinical symptoms, is set by most authors at an Hb concentration below 80 g/l (7). Even though the average Hb level did not decrease below this limit, it should be avoided. Although no signs of anaemia and growth retardation were present, the low levels of Fe concentration in blood plasma were indicative of latent Fe deficiency. We suggest that the increase in red blood cell indices two weeks after weaning was due to the fact that, the piglets started to feed intensively on the starter diet.

The cases of acute iron toxicosis after iron dextran injection in antioxidant deficient two-day-old piglets were reported (13). The oral administration is believed to be safer. This could be explained by a different mechanism of Fe utilisation. After intramuscular administration, iron dextran is taken by phagocytes of the reticuloendothelial system. Part of Fe ions is released directly to the circulation (14). The elimination of iron dextran from the site of injection takes about 7 d (13). The orally administered Fe is absorbed by mucosa of the small intestine; Fe is then transported from enterocytes to the blood, where it binds to transferrin (10). If an excess of Fe occurs in intestinal mucosal cells, ferritin synthesis is stimulated, and then Fe is deposited as ferritin in order to prevent oxidative damage from ionic iron (26).

Oxidative stress is defined as the imbalance between oxidants and antioxidants in favour of oxidants that potentially leads to tissue damage (24). Under normal conditions, the organism is continuously exposed to oxidants. Endogenous sources of oxidants include mitochondrial respiration, enzymes such as lipoxygenase and xanthine oxidase, NADPH oxidase/myeloperoxidase system of phagocytes and metal ions (8).
Under practical conditions of pig production, repeated administrations of small doses of iron are not feasible. A single administration of relatively high dose of iron is most practical. The question is, if such a procedure reduces the antioxidant status of piglets and thus predisposes them to oxidative damage.

Vitamin E breakslipid peroxidative chain reactions, by scavenging lipid peroxyl and alkoxyl radicals. According to Koyu et al. (16), vitamin E prevents toxic oxidative effect induced by Fe-dependent free radicals in erythrocytes. No differences among the groups were found under conditions of our study. It can be concluded that neither treatment reduced capacity of this antioxidant.

In blood, 98% of GSH-Px activity is bound to erythrocytes. We expressed the GSH-Px activity per 1 T/l of erythrocytes in our study. The literature offers contradictory and limited data on the influence of parenteral Fe treatment on the GSH-Px activity in blood. Lim et al. (20) and Mimic et al. (21) reported that GSH-Px activities were not significantly influenced by Fe overload in human patients. In contrary, Koyu et al. (16) documented decreased GSH-Px activity in iron dextran overloaded rabbits. Isler et al. (12) found that long term treatment of anaemic human patients with repeated parenteral application of iron dextran resulted in significantly decreased GSH-Px activity of erythrocytes. Isler et al. (12) also observed no decrease in GSH-Px activity after oral Fe treatment. This is in agreement with study of Lee et al. (18) conducted on rats, where GSH-Px activity did not respond to increased oxidative stress associated with elevated dietary Fe intake. In our study no differences between parenterally and orally treated piglets were reported in any periods of the trial.

At the age of 28 d, we found in group of Fe deficient piglets lower GSH-Px activity than in the other two groups. The literature offers contradictory data on GSH-Px activity in patients with iron deficiency anaemia. Our finding is in agreement with Cellerino et al. (4) who observed decreased GSH-Px activity in human patients suffering from iron deficiency anaemia. Fe deficiency also caused a significant decrease in GSH-Px activity in red blood cells of rats (19). It could be suggested that Fe is of crucial importance for erythrocyte GSH-Px activity. However, in contrast to these studies, Isler et al. (12) found that GSH-Px activity in Fe deficient human patients was similar to that of healthy controls, and no correlations existed between Fe concentration and GSH-Px activity. It seems that the problem is more complex. Yetgin et al. (32) reported significantly lower Se concentrations in children affected by iron deficiency anaemia. GSH-Px is a Se-dependent enzyme and may thus be affected by Se deficiency. We would like to conduct further studies in the future, trying to clarify this phenomenon in piglets.

TAS is reported to be composed of uric acid, ascorbic acid, vitamin E, protein thiol groups, and other not yet identified antioxidants (1, 30). Research has demonstrated that Fe can induce oxidative stress, such as lipid peroxidation in plasma (2, 6). Chung et al. (11) discovered that Fe can generate reactive oxygen species (ROS) in blood plasma. They also observed that treatment with Fe^{2+} resulted in the reduction of antioxidant capacity of plasma; and was followed by an increase in low-density lipoprotein (LDL) oxidation. Therefore, Fe may indirectly increase the susceptibility of plasma LDL to oxidative stress; by reducing the level of antioxidants in plasma. Negative effects of this phenomenon are well known from human medicine (e.g. a risk factor for coronary heart disease) (5). No differences in TAS among the groups were found during our study.

It can be concluded that single oral administration of iron fumarate is insufficient for the prevention of Fe deficiency and that either parenteral or oral administration do not reduce the antioxidant status of piglets.

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References
12. Isler M., Delibas N., Guela M., Gultekin F., Sutcu R., Babecci M., Kosar A.: Superoxida dismutase and glutathione reductase in erythrocytes of patients with


