Effect of iron supplementation on plasma levels of vitamins A, E and C in piglets

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Abstract

The objective of this study was to investigate the effects of two different commercially available iron–dextran preparations for intramuscular injection of piglets on haematological parameters including haemoglobin, haematocrit and mean corpuscular haemoglobin concentration (MCHC), as well as on plasma levels of vitamins A, E and C. Piglets were either control animals or administered two different iron–dextran preparations (200 mg iron) at day 3 and blood samples were taken by puncture of the cava vein at 3 (before injection), 10, 17 and 24 days after birth. Both iron preparations were efficient to prevent anaemia from day 10 onwards ($P < 0.001$) with no differences between the iron preparations used. While no significant differences were observed for the plasma level of vitamin E, the administration of iron caused a more pronounced decrease in plasma vitamin C than in control animals with significant differences observed already at day 10 ($P < 0.001$). Furthermore, the administration of iron prevented the decrease in the concentration of retinol in plasma as observed in control piglets, resulting in higher plasma retinol levels at day 24 ($P < 0.01$). This would indicate an improvement of the vitamin A status by stabilisation of plasma retinol levels in piglets after birth. Because of the overall role of vitamin A in growth and tissue integrity, this could be of importance for the well-being of the developing piglet.

Keywords: Pig; Iron; Vitamin A; Vitamin E; Vitamin C

1. Introduction

Iron has important functions in the body as a component of haemoglobin and numerous other iron-containing proteins. Increased incidence of infectious diseases associated with iron deficiency may be attributed to an impairment of activities of enzymes containing iron in cells of the immune system (Beard...
et al., 1996). However, apart from being essential to cell metabolism, iron can also promote the damage of lipids and membranes by the generation of free radicals (Herbert et al., 1996). Since iron deficiency and iron overload result in major disturbances, the body iron balance is tightly regulated (Bothwell, 1995). In piglets, iron deficiency is a major problem because body stores at birth are small (Ekman and Jwanska, 1966) and sow milk contains insufficient amounts of iron compared to the need of piglets during the first weeks of life (Kirchgessner et al., 1982). Therefore, it is necessary to supply iron to piglets orally or parenterally shortly after birth in order to maintain proper development (Furugouri, 1972; Gürtler et al., 1979).

Iron is known to interact with numerous other dietary components (Lynch, 1997). In this context, the interaction of iron as a generator of free radicals with the antioxidative vitamins E and C has been studied (Tollerz and Lannek, 1964; Herbert et al., 1996; Berger et al., 1997). Additionally, the interaction of iron and vitamin A has elicited considerable interest because the deficiencies of both micronutrients are two of the most prevalent nutritional problems of humans in developing countries (Bloem, 1995). Numerous studies address the effect of vitamin A on iron metabolism but very little is known with regard to the effect of iron on vitamin A metabolism (Bloem, 1995).

The objective of this field study was to evaluate the effect of two different commercially available, injectable iron–dextran preparations on haematological parameters, retinol and antioxidative vitamins E and C in plasma, because differences in composition might not only affect availability (haematological parameters), but oxidative properties (vitamins) as well.

### 2. Materials and methods

The effects of iron supplementation on haematological parameters and on plasma levels of vitamins A (retinol), E and C were investigated in nursing piglets (German Landrace × German Edelschwein × Piétrain) from day 3 to 24 under field conditions. The animals were assigned to three groups according to their body weight at day 2. Within one litter, the maximal possible number of piglets were equally distributed among the control and treatment groups. During the experiment, piglets remained with their dam. On the third day after birth, the piglets of the two experimental treatments were injected intramuscularly with 1 ml of the iron–dextran preparations containing 200 mg/ml of iron [Ursoferran 200 (iron (III)-hydroxide–dextran–heptonic acid), Serum-Werk Bernburg, Germany – group U (n = 21) or Myofer 200 (iron (III)-hydroxide–dextran), Hoechst Veterinär GmbH, Germany – group M (n = 21)]. Control piglets (group C; n = 21) were injected with 1 ml of physiological saline.

Blood samples were obtained on days 3 (before injection at this day), 10, 17 and 24 after birth from the cava vein. The haematocrit value was measured by microhaematocrit capillaries, and the concentration of haemoglobin was measured with the cyanmethaemoglobin method according to van Kampen and Zijlstra (1961). The mean corpuscular haemoglobin concentration (MCHC) was calculated from haemoglobin and the haematocrit. Vitamin A and E were extracted from plasma after deproteinisation with an equivalent volume of ethanol twice into three volumes of n-hexane (0.05% BHT, butylated hydroxytoluene). The combined organic extracts were dried under nitrogen flow and redissolved in methanol–ethanol (80:20; v/v). Vitamins A (retinol) and E (α-tocopherol) were separated and quantified using a reversed-phase high-performance liquid chromatography (HPLC) system (Waters, Eschborn, Germany) on an RP-18 column (5 μm, 125 x 4 mm; Grom, Germany) with methanol as a solvent at a flow-rate of 1 ml (1–3 min), 1.5 ml (3–7 min) and 2 ml (7–19 min) (Schweigert, 1990). A fluorescence spectrophotometer was used for the quantification of vitamin E (excitation 295 nm; emission 330 nm). Identification and quantification of vitamins were obtained by comparison of retention time and peak areas (at 325 nm for vitamin A) with external standards (Serva, Heidelberg, Germany). Vitamin C was determined in the plasma after deproteinisation with 0.6 M HClO₄ using dinitrophenylhydrazine as reagent (Albanese et al., 1975). All solvents or chemicals used were of high purity commercial grade (Merck, Darmstadt, Germany).

Data are reported as mean ± standard deviation (SD). Because of significant differences between
groups for vitamin C at the beginning of the experiment, changes in response variables in plasma were partitioned using General Linear Model (GLM) procedure of SAS (SAS, 1988) for repeated measurement design. When a significant difference was found a LSD post-hoc test was used to determine the cause of the significant difference. Between-subject factors were transformed into contrast (repeated) for analysis.

3. Results and discussion

3.1. Haematological parameters

Data on haematological parameters (Table 1) showed that from day 10 onwards the piglets of the untreated control group had lower haemoglobin ($P < 0.001$) and haematocrit values ($P < 0.001$) than iron-treated animals. All untreated animals developed a hypochromic anaemia. No differences in haemoglobin, haematocrit and MCHC between the two groups of treated animals were found. The intramuscular administration of iron was an effective way to prevent anaemia in piglets during the first weeks of life. This is in accordance to numerous previous studies in piglets (Furugouri, 1972; Gurtler et al., 1979).

3.2. Vitamins in blood plasma

The effects of iron supplementation on vitamin A, E and C concentrations in plasma are presented in Table 2. No differences between the two groups of piglets treated with different forms of iron were observed. The higher values for vitamin E on day 3 post-partum compared to adult animals may be attributed to the uptake of colostrum and milk rich in this vitamin (Schweigert et al., 1991; Hidiroglou et al., 1993; Mahan, 1994). Plasma values of vitamin C in general and the observed decrease over time correspond to published data (Yen and Pond, 1981, 1983). The decrease of vitamin A level with age in control animals ($P < 0.01$) and the more pronounced decrease in vitamins E and C concentrations in all animals ($P < 0.01$), irrespectively of treatment, may

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of intramuscular iron administration on haematological parameters of piglets (mean±SD and number of samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Day</td>
</tr>
<tr>
<td>Haemoglobin (mmol/l)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3</td>
</tr>
<tr>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>U</td>
<td>19</td>
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</tbody>
</table>

| Haematocrit (l/l) |  |  |  |  |
| C       | 3   | 0.26±0.03 | 0.21±0.03$^{a*}$ | 0.16±0.04$^{a*}$ | 0.15±0.03$^{a}$ |
| 20      | 21  | 0.27±0.05 | 0.34±0.02$^{a*}$ | 0.34±0.02$^{a}$ | 0.34±0.03$^{a}$ |
| U       | 19  | 0.26±0.04 | 0.34±0.02$^{a*}$ | 0.35±0.02$^{a}$ | 0.35±0.02$^{a}$ |

| MCHC (mmol/l) |  |  |  |  |
| C       | 3   | 19.5±1.1 | 19.5±1.1 | 17.9±1.0$^{a*}$ | 17.2±2.4$^{a}$ |
| 20      | 21  | 18.8±1.5 | 19.2±1.7 | 19.8±0.69$^{a}$ | 19.8±1.2$^{a}$ |
| U       | 19  | 19.7±1.21 | 19.2±0.6 | 20.2±1.8$^{a}$ | 20.4±0.9$^{a}$ |

Values in the same column with different superscripts are different (a,b $P < 0.05$; c,d $P < 0.01$; e,f $P < 0.001$). Values in the same row with capital superscripts differ from the previous ones (A $P < 0.05$; B $P < 0.01$; C $P < 0.001$).
Table 2
Effect of intramuscular iron administration on plasma levels of vitamins A, E and C in piglets (mean ± SD and number of samples)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>3</th>
<th>10</th>
<th>17</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retinol (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>225±59</td>
<td>237±53</td>
<td>190±57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>157±77&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>217±56</td>
<td>250±38</td>
<td>222±58</td>
<td>208±43&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>231±66</td>
<td>250±62</td>
<td>227±56</td>
<td>233±34&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin E&lt;sup&gt;f&lt;/sup&gt; (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>4100±1212</td>
<td>2444±715&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2268±687</td>
<td>1936±521</td>
<td></td>
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<tr>
<td>M</td>
<td>3918±1040</td>
<td>2902±1070&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2167±737</td>
<td>1632±461&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>U</td>
<td>3851±1452</td>
<td>2931±821&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2108±812&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1782±639&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td></td>
<td>Vitamin C&lt;sup&gt;f&lt;/sup&gt; (µg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>57.7±8.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>39.4±4.8&lt;sup&gt;ce&lt;/sup&gt;</td>
<td>40.8±7.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.7±6.5&lt;sup&gt;ae&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>58.5±9.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.6±5.8&lt;sup&gt;ce&lt;/sup&gt;</td>
<td>34.0±5.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>29.9±7.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>55.1±11.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.8±5.5&lt;sup&gt;ce&lt;/sup&gt;</td>
<td>32.4±3.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>26.2±5.2&lt;sup&gt;be&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Values in the same column with different superscripts are different (a, b P < 0.05; c, d P < 0.01; e, f P < 0.001). Values in the same row with capital superscripts differ from the previous ones (A P < 0.05; B P < 0.01; C P < 0.001).

Numbers of samples correspond to those given for retinol.

be attributed to a lower supplementation of these micronutrients with advancing lactation, higher demands or a redistribution from plasma into tissues.

Significant differences between treated and untreated animals were observed for plasma concentration of vitamin C already at day 10 (P < 0.001). The slightly higher level of vitamin E in the control group at the end of the experiment compared to treated animals was not significant (P > 0.05). Results for retinol showed a small initial increase at day 10 in all groups. While afterwards in the iron-treated animals plasma concentrations of retinol remained on a high level, plasma values in the control animals decreased continuously, reaching significant differences from iron-treated animals at day 24 (P < 0.01). On day 24 the plasma levels in these animals represented 70% of initial plasma retinol levels on average, while the level in the treated animals remained at 96–101%.

In both humans and animals, vitamin A deficiency results in mild anaemia (Bloem, 1995), a poor immune response and a delayed repair of damaged epithelia. It has thus been observed that under the condition of vitamin A deficiency the severity of some infectious diseases, especially those associated with diarrhea, seems to be increased (Blomhoff and Smeland, 1994). Therefore, our results are of interest in showing that a parenteral supplementation of piglets with iron shortly after birth maintains the plasma level of retinol. This observation could be compared with the results of a recent study on pregnant women in India showing that oral iron supplementation increased plasma retinol and lowered the number of cases with plasma levels of retinol below 300 ng/l (Shatrugna et al., 1997). However, it is not possible to differentiate whether the observed effect of iron on the stabilisation of retinol level in plasma is due to an improvement of the efficiency of vitamin A absorption in the gut or to the mobilisation of additional vitamin A stored in the liver. In this connection, it is furthermore difficult to interpret the lower vitamin A levels in untreated piglets as a sign of vitamin A deficiency, which may contribute to an impaired tissue supply of vitamin A.

Compared to vitamin A, which plays an essential role in differentiation, the maintenance of differentiated tissues and in vision, the function of vitamin C and vitamin E is mainly antioxidative. Since iron can switch back and forth between the ferrous and ferric oxidation states, it is both a strong reductant and...
oxidant. The iron-dependent reduction of $\text{H}_2\text{O}_2$ generates hydroxyl radicals (Herbert et al., 1996). This increase in free radicals promote the damage of lipids and therefore cell membranes, and influences the status of antioxidative vitamins and carotenoids in plasma and body tissues (Whittaker et al., 1996; Parkkila et al., 1996). The lower levels of vitamin C and to a lesser extent of vitamin E in iron-treated piglets might be a consequence of iron-associated mechanism that lead to a disappearance of both antioxidative vitamins from plasma. Similar effects have been observed in pigs, where a parenteral administration of iron results in lower plasma vitamin E concentrations compared to untreated animals (Loudenslager et al., 1986). In addition, vitamin E and synthetic antioxidants can protect vitamin E-deficient piglets from iron toxicity (Tollerz and Lannek, 1964). Recent studies in humans indicate that ascorbic acid acts as an antioxidant toward lipids as well in iron-overloaded plasma (Berger et al., 1997). In studies with plasma lipids, it has been shown that vitamin C is far more effective in inhibiting lipid peroxidation than vitamin E (Frei et al., 1989). The observation that vitamin C can efficiently trap radicals in the aqueous phase before they can initiate lipid peroxidation might explain the result of this study. The differences for vitamin C between treated and control animals were found early after administration with the tendency to appear less obvious later on while a possible effect on the level of vitamin E in plasma may be observed only beyond the period of our investigation.

The known decrease in the plasma level of vitamin E (Loudenslager et al., 1986) and the observed decrease in plasma vitamin C in this study after the parenteral administration of iron to piglets has to be considered because both are important micronutrients in the prevention of infectious diseases. Therefore, it may be advantageous to administer preparations of injectable iron together with appropriate amounts of vitamin C and (or) vitamin E to compensate for the losses of antioxidants caused by iron, and possibly further improve the beneficial effect of iron supplementation to newborn piglets. With regard to vitamin C, however, a possible pro-oxidative action has to be considered. Furthermore, the administration of iron may also be beneficial in stabilizing vitamin A plasma level. Since vitamin A is of importance in supporting normal haematopoiesis as well as the immune system the observed effect of iron on vitamin A levels might be of additional benefit for the growing pig, by possibly reducing infectious diseases as one of the major cause of morbidity and mortality in commercial animal production.

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References


