Effect of an Acute Iron Overdose on PMN Cells Chemiluminescence and Indices of Inner Environment in a Swine Model

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Abstract

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The objective of the study was to evaluate the effect of an acute iron dextran administration on polymorphonuclear cells chemiluminescence and indices of inner environment in piglets. Piglets in the experimental group (n = 16) were given 2000 mg (20 ml) Fe^{3+} in the form of iron dextran i.m. Piglets in the control group (n = 13) were given the same amount of physiological saline. Blood was taken before administration, and 1, 4 and 24 hours after. Iron application resulted in a ten times higher concentration of iron in blood plasma in the experimental group. No differences in spontaneous, PMA- and OZP-stimulated chemiluminescence were recorded between the two groups. The increased activities of CK, AST and LDH are indicative of a muscle injury at the injection site. No changes indicating liver or kidney injury were reported. Our study provides evidence of a good safety of iron dextran after parenteral administration.

Iron dextran, reactive oxygen species, antioxidant

Iron deficiency anaemia is a common problem in pig production. Administration of Fe^{3+} in the form of iron dextran is the most frequently used method for iron administration in piglets. However, this method is not without risks. There is clinical evidence of acute deaths 0.5 - 4 hours after iron dextran injection in antioxidant-deficient 2-day-old piglets (Süveges and Glávits 1976; Kolb and Hoffmann 1989).

Iron dextran is also commonly used for parenteral iron administration in human patients. However, studies evaluating oxidative stress induced by iron dextran in human patients are based mainly on *in vitro* studies (Guo et al. 2002). Therefore experiments conducted on a swine model may provide valuable information. A variety of studies have demonstrated the ability of iron to catalyze the formation of reactive oxygen species (ROS) (Minotti and Aust 1987; Alleman et al. 1985). Evidence indicates that iron acts as a catalyst in the Fenton reaction, facilitating the conversion of hydrogen peroxide to hydroxyl radical (.OH) (Imlay et al. 1988; Halliwell and Gutteridge 1986).

Hydroxyl radicals are the most reactive free radical species known and have the ability to react with a wide range of cellular constituents, including the DNA, as well as to attack membrane lipids to initiate lipid peroxidation (Crichton et al. 2002).

The hydroxyl radical (.OH) is frequently proposed as an initiator of lipid peroxidation (Fridovic and Porter 1981; Girotti and Thomas 1984). Superoxid (O_2^-), hydrogen peroxide (H_2O_2) and iron are proposed to cause lipid peroxidation via Fenton reaction (Miller et al. 1993). Conversely, several studies on iron-catalyzed lipid peroxidation have indicated no correlation with .OH production, leading to the proposal of other initiators of lipid peroxidation, such as Fe^{2+} : Fe^{3+} or iron-oxo complexes (Minotti and Aust 1987; Braughler et al. 1986). Many *in vitro* studies have employed a source of Fe^{3+} and

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a reductant to catalyze lipid peroxidation. However, it is likely that *in vivo* the iron that catalyzes deleterious oxidations exists in a ferrous state (Miller et al. 1993).

It has been recorded that various functions of polymorphonuclear leukocytes (PMN) are depressed by exposure to excess iron. Diminished chemotaxis was observed after incubation of PMN with $FeCl_2$ or $FeCl_3$ (Ward et al. 1975). Inhibition of bactericidal activity of PMN in the presence of $FeSO_4$ has also been demonstrated (Gladstone and Walton 1971). Guo et al. (2002) have reported significant impairment of phagocyte oxidative metabolism of PMN from patients on haemodialysis.

It has been suggested that deleterious effect of iron on phagocytic cells of patients with iron overload could be due to a direct toxic effect of iron on these cells, or due to increased production of toxic oxygen species which may lead to the damage of the cells (Van Asbeck et al. 1982).

Luminol chemiluminescence has recently been used to quantify these active oxygen species (Takahashi et al. 1992).

The aim of the study was to assess the effect of an acute iron overdose on chemiluminescence of PMN and haematological and biochemical indices in a swine model.

Materials and Methods

Experimental design

Six-week-old piglets were used in the study. They were weaned at the age of 28 days. Piglets in the experimental group (n = 16) had a mean body weight of 11.76 ± 1.22 kg, control piglets (n = 13) weighed 12.23 ± 0.70 kg. Piglets in the experimental group were given 20 ml of iron dextran i.m. (Ferridextran 10%, SPOFA a.s.). This is 2 000 mg of Fe^{3+}. Piglets in the control group were given 20 ml of physiological saline solution i. m. Blood was taken before the injection, and 1, 4 and 24 hours after.

Sampling

Blood was collected from vena cava cranialis of the piglets. EDTA (ethylenediaminetetraacetic acid) was used as anticoagulant for the haematological examination. Heparin was used as anticoagulant for the chemiluminescence assay, blood biochemistry, antioxidant status and determination of iron concentration in blood plasma**.**

Chemiluminescence assay of the oxidative burst of neutrophils

The oxidative burst of neutrophils was assessed by luminol-enhanced chemiluminescence using microplate luminometer LM-01T (Immunotech, Czech Republic). The principle of this method was described previously (Lojek et al. 2002). In brief, phagocyte-derived oxidizing species interacted with luminol and resulting light emission was measured at a wavelength of 425 nm. The temperature was maintained at 37 °C. The samples contained 5 µl of whole blood, 25 µl of 10mM luminol (Sigma, USA) in borate buffer and 25 µl of opsonized zymosan (2.5 mg/ml, Sigma, USA) or phorbol myristate acetate (PMA, 1,6 µM/l.). The total volume of 250 µl was attained by adding Hank's balanced salt solution (HBSS, pH = 7.4). Spontaneous chemiluminescence without any activator was also measured in all samples. Light emission, expressed as relative light units (RLU) was recorded continuously for 60 min. The integral value of the CL reaction, which represents the total ROS production by neutrophils were evaluated.

Haematological analysis

Haematological examination included: haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and white blood cell count (WBC). These indices were determined by haematological analyzer Celtac Alfa (Nihon Kohden). Blood smears for determination of differential leukocyte count were prepared and stained according to May-Grünwald and Giemsa- Romanowski.

Iron concentration

Iron concentration in blood plasma (Fe) was determined photometrically measuring iron complex with ferrozin (Iron liquid 917, Roche Diagnostic, Manheim, Germany).

Blood biochemistry

Activities of ALP, ALT, AST, GMT, CK, LDH and concentration of creatinin and urea nitrogen in blood plasma were determined by means of COBAS MIRA automatic analyzer (F. Hoffmann, La-Roche and Co., Switzerland).

Antioxidant status

GSH - Px activity in whole heparinized blood was determined by the Paglia and Valentine method (1967) using the RANSEL set (Randox) and the COBAS MIRA automatic analyzer.

Vitamin E concentration in blood plasma was determined fluorometrically according to Bouda et al. (1980) using fluorescence spectrophotometer 204 Perkin - Elmer.

Total antioxidant capacity was determined using the RANDOX set and the COBAS MIRA automatic analyzer according to the following principle. ABTS® (2,2'-Azino-di-[3- ethylbenzthiazoline suplhonate]) is incubated with a peroxidase (metmyoglobin and H_2O_2) to produce the radical cation ABTS®+. This has a relatively stable blue-green colour, which is measured at 600 nm. Antioxidants in added sample cause suppression of this colour production to a degree which is proportional to their concentration.

Statistical analyses

The results were evaluated statistically by Student's *t*-test.

Results

The results are presented as mean values and standard deviation of each index in Figs. 1 - 4 and in Tables 1 - 3.

Values with * $P < 0.05$, ** $P < 0.01$ express significant difference between the two groups.

Chemiluminescence of PMN (Figs. 1 - 3)

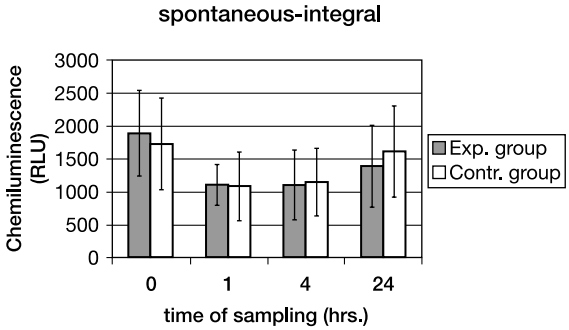

Fig. 1. Spontaneous chemiluminiscence

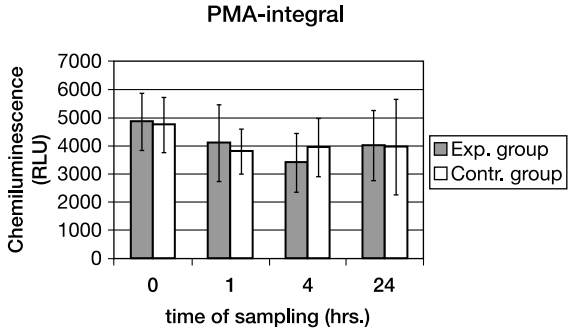

Fig. 2. PMA- stimulated chemiluminiscence

No significant differences in spontaneous, OZP and PMA- activated ROS production of polymorphonuclar granulocytes were found between control and iron-treated piglets during the trial.

Haematological analysis (Table 1)

In all periods of the trial, no significant differences between control and iron-treated

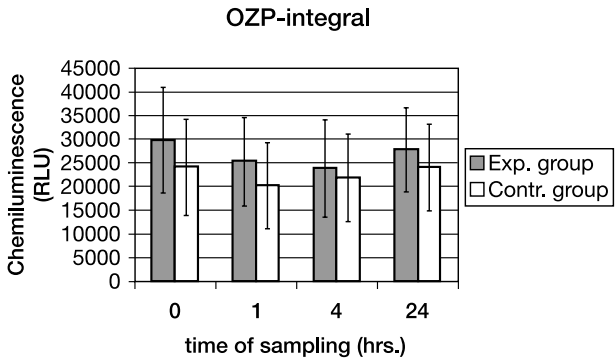

Fig. 3. OZP-stimulated chemiluminiscence

piglets were found. Within both groups, there was an increase of white blood cell count and polymorphonuclear granulocytes numbers between first and subsequent samplings ($P < 0.01$).

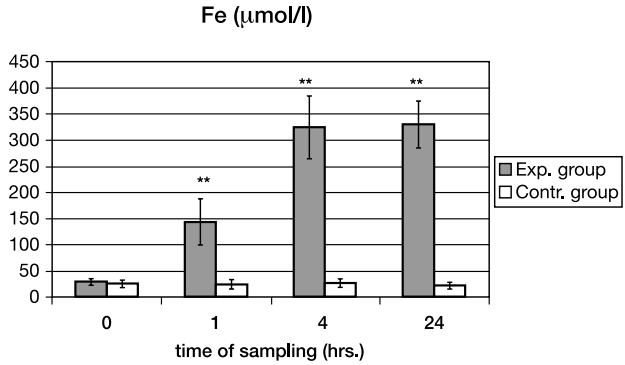

Fig. 4. Iron concentration in blood plasma (Fe) of piglets

Iron concentration (Fig. 4)

The iron concentration in blood plasma increased significantly after application and was significantly higher compared to the control group in all periods of the trial ($P < 0.01$).

Blood biochemistry (Table 2)

The activities of CK, AST and LDH in iron treated group increased and were significantly higher compared to the control group in all periods of the trial ($P < 0.01$). The concentration of creatine in blood plasma was found to be higher in the experimental group 24 hours after iron administration ($P < 0.01$). No other differences in biochemical variables were found between the two groups.

Antioxidant status (Table 3)

No differences in glutathione peroxidase (GSH-Px) activity and total antioxidant status of blood plasma (TAS) were found between the two groups. Vitamin E concentration in blood plasma of the experimental group had decreasing tendency and 24 hours after iron administration it was significantly lower compared to the control group ($P < 0.05$).

Table 1. Haematological indices during the trial

	Unit	Before application Mean ± SD		1 hour after application Mean ± SD		4 hours after application Mean ± SD		24 hours after application Mean ± SD	
		Exp. group	Contr. group	Exp. group	Contr. group	Exp. group	Contr. group	Exp. group	Contr. group
Hb	g/l	105.82 ± 12.6	105.3 ± 10.75	100.72 ± 11.59	95.60 ± 13.71	95.72 ± 11.82	92.3 ±5.93	91.90 ± 9.70	91.33 ± 3.80
PCV	l/l	0.38 ± 0.04	0.38 ± 0.03	0.36 ± 0.037	0.35 ± 0.040	0.36 ± 0.03	0.35 ± 0.03	0.33 ± 0.03	0.32 ± 0.02
RBC	T/L	6.73 ± 0.72	6.84 ± 0.56	6.51 ± 0.53	6.55 ±0.81	6.48 ± 0.45	6.14 ± 0.51	5.95 ± 0.46	5.89 ± 0.43
MCV		57.22 ± 6.66	56.11 ± 5.79	56.68 ± 6.29	53.52 ± 6.28	55.96 ± 6.08	56.58 ± 6.06	55.16 ± 4.06	55.30 ± 3.89
MCH		15.88 ± 2.32	15.47 ± 1.78	15.57 ± 2.15	14.69 ± 2.03	14.91 ± 2.58	15.14 ± 1.71	15.46 ± 1.29	15.56 ± 0.93
MCHC		276.66 ± 11.59	275.53 ± 7.56	274.14 ± 12.21	274.02 ± 10.20	264.91 ± 21.94	268.11 ± 18.5	280.29 ± 9.89	281.86 ±12.0
WBC	G/l	17.88 ± 2.90	18.6 ± 2.93	20.9 ± 5.51	21.07 ± 4.96	21.26 ± 7.62	21.18 ±4.02	22.83 ± 8.88	18.76 ± 4.41
Lymphocytes	%	52.36 ± 13.15	55.2 ± 13.59	35.72 ± 9.09	35.8 ± 13.17	33.27 ± 14.64	41.6 ± 16.82	38.90 ± 15.03	46.66 ± 11.12
	G/l	9.17 ± 1.99	10.21 ± 2.71	7.35 ± 2.38	7.12 ± 2.07	6.34 ± 2.05	8.35 ± 2.54	7.86 ± 3.09	8.76 ± 2.86
Monocytes	%	4.45 ± 2.42	4.5 ± 3.29	2.82 ± 1.79	4.3 ± 3.82	2.36 ± 2.63	1.9 ± 2.21	3.72 ± 2.73	4.77 ± 5.07
	G/l	0.82 ± 0.51	0.81 ± 0.60	0.58 ± 0.35	0.82 ± 0.54	0.52 ± 0.57	0.41 ± 0.48	0.82 ± 0.68	0.86 ± 0.97
Neutrophilic	%	1.54 ± 1.49	1.70 ± 2.32	6.09 ± 3.03	6.20 ± 3.94	4.90 ± 4.64	3.9 ± 2.87	3.27 ± 3.54	2.88 ± 2.51
granulocytes - band	G/l	0.26 ± 0.23	0.30 ± 0.42	1.28 ± 0.75	1.44 ± 1.15	1.33 ± 2.00	0.87 ± 0.72	0.82 ± 1.00	0.59 ± 0.54
Neutrophilic	%	40.45 ± 11.81	37.20 ± 19.95	54.63 ± 9.03	53.60 ± 14.43	59.09 ± 12.10	52.30 ± 16.81	53.36 ± 13.04	43.89 ± 9.98
granulocytes - segment	G/l	7.39 ± 2.51	7.02 ± 2.80	11.52 ± 3.90	11.66 ± 4.51	13.00 ± 6.11	11.49 ± 4.86	13.19 ± 8.66	8.2 ± 2.46
Eosinophilic	%	0.90 ± 0.89	1.1 ± 0.83	0.63 ± 0.97	0.1 ± 0.3	0.18 ± 0.57	0.30 ± 0.64	0.45 ± 0.98	1.77 ± 1.74
granulocytes	G/l	0.17 ± 0.17	0.19 ± 0.13	0.13 ± 0.19	0.02 ± 0.04	0.03 ± 0.10	0.05 ± 0.10	0.08 ± 0.18	0.33 ± 0.34
Basophilic	%	0.27 ± 0.61	0.3 ± 0.64	0.09 ± 0.28	0	0.18 ± 0.38	0	0.27 ± 0.61	0.03 ± 0.07
granulocytes	G/l	0.05 ± 0.11	0.05 ± 0.10	0.02 ± 0.07	0	0.04 ± 0.08	0	0	0

Discussion

The use of weaned piglets was not a random choice. It is known that at this age the immune system of piglets is fully developed and is able of appropriate immune responses (Pastoret et al. 1998). The application of iron dextran resulted in 10 times higher concentrations of iron in blood plasma compared to non-treated control piglets. The rise in iron concentration was evident as early as 1 hour after application. The effects of the iron overdose on the following indices are discussed:

Chemiluminescence of PMN

The respiratory burst is a characteristic response of granulocytes and macrophages when exposed to soluble or particular stimuli (Wang et al. 1993). Due to activation of the membrane-bound NADPH oxidase, the superoxid anion radical (O_2^-) is formed. From this one-electron-reduced form of oxygen, other reactive oxygen species such as hydrogen peroxide, singlet molecular oxygen, hypochlorous acid and the hydroxyl radical are formed by subsequent reactions partly catalyzed by iron ions (Wang et al. 1993).

Table 2. Biochemical indices during the trial

	Index	Unit	Experimental group Mean ± SD	Control group Mean ± SD
Before application	ALP	μkat/l	7.65 ± 3.22	5.82 ± 2.80
	ALT	μkat/l	1.02 ± 0.23	0.94 ± 0.41
	AST	μkat/l	0.77 ± 0.11	0.56 ± 0.17
	GMT	μkat/l	0.59 ± 0.15	0.59 ± 0.12
	CK	μkat/l	5.49 ± 2.13	4.05 ± 1.19
	LDH	μkat/l	13.5 ± 2.18	11.22 ± 2.26
	Creatinine	μkat/l	81.82 ± 11.07	94.32 ± 15.05
	Blood urea nitrogen	mmol/l	1.16 ± 0.22	2.08 ± 0.99
4 hours after application	ALP	μkat/l	8.16 ± 3.22	6.3 ± 3.06
	ALT	μkat/l	0.87 ± 0.25	0.89 ± 0.33
	AST	μkat/l	1.64 ± 0.40**	0.74 ± 0.30
	GMT	μkat/l	0.58 ± 0.15	0.51 ± 0.09
	CK	μkat/l	38.90 ± 17.43**	5.62 ± 1.70
	LDH	μkat/l	16.46 ± 2.23*	11.76 ± 2.52
	Creatinine	μkat/l	96.67 ± 10.48	81.32 ± 19.25
	Blood urea nitrogen	mmol/l	1.52 ± 0.68	2.14 ± 0.62
24 hours after application	ALP	μkat/l	6.80 ± 2.44	5.32 ± 2.02
	ALT	μkat/l	1.12 ± 0.26	0.86 ± 0.22
	AST	μkat/l	1.52 ± 0.38**	0.50 ± 0.11
	GMT	μkat/l	0.50 ± 0.20	0.45 ± 0.09
	CK	μkat/l	20.54 ± 8.43**	3.54 ± 1.15
	LDH	μkat/l	15.01 ± 2.59*	10.77 ± 1.83
	Creatinine	μkat/l	129.77 ± 27.77**	74.92 ± 4.20
	Blood urea nitrogen	mmol/l	2.15 ± 0.66	2.52 ± 0.35

Table 3. Antioxidant status during the trial

	Index	Unit	Experimental group Mean ± SD	Control group Mean ± SD
Before application	Vitamin E	μmol/l	4.40 ± 1.21	3.82 ± 1.71
	GSH-Px	μkat/l	359.32 ± 84.52	355.87 ± 62.83
	TAS	mmo/l	1.11 ± 0.14	1.09 ± 0.14
1 hour after application	Vitamin E	μmol/l	3.59 ± 0.92	3.64 ± 1.60
	GSH-Px	μkat/l	354.96 ± 60.92	343.00 ± 60.72
	TAS	mmo/l	1.12 ± 0.19	1.07 ± 0.15
4 hours after application	Vitamin E	μmol/l	3.65 ± 1.53	3.47 ± 1.50
	GSH-Px	μkat/l	334.13 ± 58.48	335 ± 52.31
	TAS	mmo/l	1.09 ± 0.22	1.00 ± 0.13
24 hours after application	Vitamin E	μmol/l	2.83 ± 0.96*	3.89 ± 1.42
	GSH-Px	μkat/l	335 ± 54.57	327.16 ± 56.71
	TAS	mmo/l	1.05 ± 0.20	0.96 ± 0.14

The generation of reactive oxygen species and metabolites will emit light which can be monitored by luminometers (Van Dyke et al. 1986; Gülüzar and Demiryürek 1998). Light emission can be markedly amplified by luminol which measures a mixture of oxygen-derived species (Van Dyke et al. 1986; Weber 1990; Gülüzar and Demiryürek 1998) or lucigenine which reacts with superoxide, hydrogen peroxide, or singlet oxygen but not hydroxyl radical and hypochlorous acid (Edwards 1987; Weber 1990).

In our study we found no impact of increased iron concentration in blood plasma on PMN chemiluminiscence.

Enhancement of stimulating-dependent response of guinea pig peritoneal neutrophils was observed with Fe^{3+} and ADP- Fe^{3+} *in vitro*. However, the experimental design of that study was different from ours. In their study PMN were cultivated with iron and were subjected to continuous stimulation with respiratory burst stimulant (PMA) (Takahashi et al. 1992). We suggest that enhancement of chemiluminescence in their study was caused by simultaneous action of respiratory burst stimulant and iron.

Impaired respiratory burst activity of human PMN indicated by decreased production of H_2O_2 after exposure to iron dextran *in vitro* was reported by Guo et al. (2002). They evaluated the impact of iron dextran on human polymorphonuclear cells isolated from haemodialysis patients (HD) and healthy subjects. The PMN cells were incubated with iron dextran *in vitro* for 24 hours and were not subjected to any stimulation during the incubation. Iron dextran had no impact on non-stimulated PMN H_2O_2 production in either group. In healthy group only PMA stimulated cells exposed to iron dextran produced less H_2O_2 ($P < 0.05$). In the HD group, iron dextran significantly attenuated H_2O_2 production stimulated by *Staphylococcus aureus*, fMLP, and PMA ($P < 0.01$).

The mechanisms by which iron impairs PMN responsiveness are unclear. It has been shown that peripheral blood leukocytes from HD patients have enhanced vulnerability to lipid peroxidation (Maccarrone et al. 1999). PMN from HD patients, primed by the uremic environment that generates increased basal levels of hydrogen peroxide may be particularly vulnerable to lipid peroxidation-induced injury. This may be related to lower activity of intracellular antioxidant enzymes such as superoxide dismutase and glutathione peroxidase found in HD patients (Shurtz-Swirski et al. 1995). It has been suggested that membrane lipid peroxidation induced by free radicals may also disrupt membrane receptors function such as those for respiratory burst stimulants.

The relative resistance of PMN from healthy subjects to iron dextran may therefore be related to lower levels of spontaneous H_2O_2 production and normal intracellular antioxidant defences.

Furthermore, iron-induced alterations of PMN appear to be ligand-dependent, varying with the nature of the ligand attached to iron ion (van Asbeck et al. 1984). Iron dextran belongs to polynuclear ferric hydroxide complexes. These complexes are relatively inert with a structure relatively similar to that of physiological iron storage protein ferritin. Therefore they have a low reduction potential, less than -324 mV, which would preclude their participation in the Fenton reaction, even when administered at high doses (Legssyer et al. 2003).

Haematological analyses

In both groups there was an increase of white blood cell count and polymorphonuclear granulocytes numbers between first and subsequent samplings. Since no differences between the experimental and control groups were found, these changes could be attributed to stress induced by manipulation and sampling.

Blood biochemistry

Increased activities of CK, AST and LDH, and an increased concentration of creatine were found in the iron dextran-treated group. It has been shown that increased serum CK, AST and LDH activity occur with degenerative or necrotizing muscle injury. CK is a specific indicator of striated muscle damage. Serum AST and LDH are tissue-nonspecific, but muscle and liver are considered a major source of their enzymatic activity (Latimer et al. 2003). Since no differences in activities of other liver enzymes (ALP, ALT, GMT) were found between the control and experimental groups, liver damage can be excluded (Loeb and Quimby 1999).

Since the blood urea nitrogen concentration in the iron-treated group remained on the same

level, the increased creatinine concentration in blood plasma can not be attributed to decreased glomerular filtration rate and kidney damage. It has been documented that increased creatine concentration in blood plasma may result from rhabdomyolysis (Chen et al. 2005).

We conclude that the results of biochemical analysis are indicative of a muscle injury at the application site. This can be explained by accumulation of iron in tissue which leads to an increase in the cellular labile iron pool. Such low molecular weight iron can act as a catalyst in the Fenton reaction and generate free radical species, including hydroxyl radicals .OH (Crichton et al. 2002).

Our results are not indicative of liver and kidney damage. We suppose that this could be contributed to the low reduction potential of ferric hydroxide complexes and to their specific absorption mechanism as explained below. The ferric hydroxide complexes are initially taken up by the reticulo-endothelial system by phagocyting macrophages and are processed to release iron which is either stored in ferritin or released to the circulation and transported by transferrin (Legssyer et al. 2003; Kolb et al. 1992). We suggest that both factors decrease reduction rate of ferric iron to its ferrous state, which is believed to participate in the Fenton reaction.

Antioxidant status

There is clinical evidence of acute deaths 0.5 - 4 hours after iron dextran application in antioxidant-deficient 2-day-old piglets (Süveges and Glávits 1976; Kolb and Hoffmann 1989).

Glutathione peroxidase (GSH-Px) is an intracellular antioxidant and is the major means for removing H_2O_2. In a reaction catalyzed by glutathione peroxidase, the reactive sulfhydryl groups reduce hydrogen peroxide to water (Smith et al. 1996).

No differences between the experimental and control groups were reported in our study. This in agreement with Mimic et al. (2005) and Fletcher et al. (1989) who also reported that GSH-Px activities were not significantly influenced by the iron overload.

Vitamin E can terminate lipid peroxidative chain reactions by scavenging lipid peroxyl and alkoxyl radicals, thus limiting the LP chain reaction (Maiorino et al. 1989). A link between decreasing vitamin E concentration and lipid peroxidation has been implied in the work of Takenaka et al. (1991).

The decline was observed also in our study, where 24 hours after iron application, the vitamin E concentrations in blood plasma were found significantly lower in the iron-treated group.

The total antioxidant status of blood plasma (TAS) was reported to be composed of uric acid, ascorbic acid, vitamin E, protein sulfhydryl groups and others so far unidentified components (Uotila et al. 1994). Chung et al. (2005) discovered that Fe^{2+} iron can generate reactive oxygen species (ROS) with thiols in human plasma. Albumin, the major thiol contributor in plasma also generated ROS with Fe^{2+}. Treatment with Fe^{2+} in their study resulted in significant reduction of oxygen radical absorbance capacity. This suggests that generation of ROS by non-enzymatic reaction of Fe^{2+} with plasma thiols could lead to reduction of total antioxidant capacity in plasma.

In our study TAS was not altered by iron dextran administration. We suggest that this may be due to low reduction potential of iron dextran, which prevents its reduction to ferrous state.

Our study provides evidence of a good safety of iron dextran preparation in a swine model.

Vliv akutního předávkování železem na chemiluminescenci neutrofilních granulocytů a ukazatele vnitřního prostředí u prasat

Cílem práce bylo zhodnotit účinek dextranu železa na chemiluminescenci neutrofilních granulocytů a ukazatele vnitřního prostředí selat. Selatům v experimentální skupině (n = 16)

bylo aplikováno i.m. 2000 mg Fe^{3+} ve formě dextranu železa. Selatům v kontrolní skupině (n = 13) bylo aplikováno stejné množství fyziologického roztoku. Krev byla odebrána před aplikací a 1, 4 a 24 hodin poté. Aplikace železa v experimentální skupině měla za následek desetinásobně větší koncentraci železa v plazmě. Mezi oběma skupinami nebyly zjištěny žádné rozdíly ve spontánní, PMA a OZP stimulované chemiluminiscenci. Zvýšené aktivity CK, AST a LDH indikují poškození svaloviny v místě aplikace. Nebyly zjištěny změny indikující poškození jater a ledvin. Výsledky práce vypovídají o dobré bezpečnosti dextranu železa po parenterální aplikaci.

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References

ALLEMAN MA, KOSTER JF, WILSON JHP, EDIXHOVEN-BOSDIJK A, SLEE RG, KROSS MJ, EIJK HGV 1985: The involvement of iron and lipid peroxidation in the pathogenesis of HCB-induced porphyria. Biochem Pharmacol **34**: 161-166

BOUDA J, JAGOŠ P, DVOŘÁK V 1980: Fluorometric determination of vitamins A and E in blood plasma, colostrum and the liver of cattle (in Czech). Čs Fysiol **29**: 351

BRAUGHLER JM, DUNCAN LA, CHASE RL 1986: The involvement of iron in lipid peroxidation. Importance of ferric to ferrous ratios in initiation. J Biol Chem **261**: 1282-1289

CHEN LL, HSU CW, TIAN YC, FANG JT 2005: Rhabdomyolysis associated with acute renal failure in patients with severe acute respiratory sydrome. Int J Clin Pract **59**: 1162-1166

CHUNG KY, LEE SJ, CHUNG SM, LEE MY, BAE ON, CHUNG JH 2005: Generation of free radical by interaction of iron with thiols in human plasma and its possible significance. Thromb Res **116**: 157-164

CRICHTON RR, WILMET S, LEGSSYER R, WARD RJ 2002: Molecular and cellular mechanisms of iron homeostasis and toxicity in mammalian cells. J Inorg Biochem **91**: 9-18

EDWARDS SW 1987: Luminol- and lucigenin-dependent chemiluminescence of neutrophils: role of degranulation. J Clin Lab Immunol **22**: 35-39

FLETCHER LM, ROBERTS FD, IRVING MG, POWELL LW, HALLIDAY JW 1989: Effect of iron loading on free radical scavenging enzymes and lipid peroxidation in rat liver. Gastroenterology **97**: 1011- 1018

FRIDOROVIC SE, PORTER NA 1981: Oxidation of arachidonic acid in micelles by superoxid and hydrogen peroxide. J Biol Chem **256**: 260-265

GIROTTI AW, THOMAS JP 1984: Damaging effects of oxygen radicals on released erythrocyte ghosts. J Biol Chem **259**: 1744-1752

GLADSTONE GP, WALTON E 1971: The effect of iron and hematin on the killing of staphylococci by rabbit polymorphs. Br J Exp Pathol **52**: 452

GUO D, JABER BL, LEE S, PERIANAYAGAM MC, KING AJ., PEREIRA BJG, BALAKRISHNAN VS 2002: Impact of iron dextran on polymorphonuclear cell function among hemodialysis patients. Clin Nephrol **58**: 134-142

GÜLÜZAR Y, DEMIRYÜREK T 1998: Ferrous induced luminal chemiluminescence: A method for hydroxyl radical study. J Pharmacol Toxicol Method **39**: 179-184

HALLIWELL B, GUTTERIDGE JMC 1986: Iron and free radical reactions. Two aspects of antioxidant protection. Trends Biochem Sci **11**: 372-375

IMLAY JA, CHIN SM, LINN S 1988: Toxic DNA damage by hydrogen peroxide through the Fenton reaction in vivo and in vitro. Science **240**: 640-642

KOLB E, HOFMANN U 1989: Zur frage der zweckmässigen Form der Anwendung von Fe-Dextran, seiner Verwertung sowie des Mechanismus einer möglichen Schädigung. Mh Vet Med **44**: 497-501

KOLB E, HOFMANN U, NESTLER K 1992: Untersuchungen über den Gehalt an Eisen, Kupfer und Zink in verschiedenen Geweben (Magen-Darm-Kanal, Lymphknoten, Muskulatur, Herz, Leber, Milz, Niere, Pankreas, Knochenmark) bei neugeborenen Ferkeln, nach oraler und intramuscularer Verabreichung von Fe-Dextran. Mh Vet Med **47**: 271-278

LATIMER KS, MAHAFFEY EA, PRASSE KW 2003: Muscle. In: Clinical pathology. Duncan &Prasse's, pp. 260-269

LEGSSYER R, GEISSER P, MCARDLE H, CRICHTON RR, WARD RJ 2003: Comparison of injectable iron complexes in their ability to iron load tissues and to induce oxidative stress. Biometals **16**: 425-433

LOEB WF, QUIMBY W 1999: The pig. In: The clinical chemistry of laboratory animals. Edwards Brothers, Ann Arbor, pp. 103-135.

LOJEK A, KUBALA L, ČÍŽOVÁ H, ČÍŽ M 2002: A comparison of whole blood neutrophil chemiluminiscence measured with cuvette and microtitre plate luminometers. Luminiscence **17**: 1-4

MACCARRONE M, TACCONE-GALLUCCI M, MELONI C, COCOCCETTA N, DI VILLHERMOSA SM, CASCIANI CU, FINAYYI-AGRO A 1999: Activation of 5-lipoxygenase and related cell membrane lipoperoxidation in hemodialysis patients. J Am Soc Nephrol **10**: 1991-1996

MAIORINO M, COASSIN M, ROVERI A, URSINI F 1989: Microsomal lipid peroxidation: Effect of vitamin E and its functional interaction with phospholipid hydroperoxide glutathione peroxidase. Lipids **24**: 721-726

MIMIC OKJ, SAVIC RA, PLJESA EM, OPACIC M, SIMIC T, DIMKOVIC N, SIMIC DV 2005: Evaluation of oxidative stress after repeated intravenous iron supplementation. Renal Fail **27**: 345-351

MILLER DM, GROVER TA, NAYINI N, AUST SD 1993: Xanthine oxidase- and iron-dependent lipid peroxidation. Arch Biochem Biophys **301**: 1-7

MINOTTI G, AUST SD 1987: The role of iron in the initiation of lipid peroxidation. Chem Phys Lipids **44**: 191-208

PAGLIA DE, VALENTINE WN 1967: Studies on the quantitative and qualitative characterisation of erythrocyte gluthation peroxidase. J Lab Clin Med **70**: 158-169

PASTORET PP, GRIEBEL P, BAZIN H, GOVAERTS A Immunology of the pig. In: Handbook of vertebrate immunology. Academic Press, pp. 373-407.

SHURTZ-SWIRSKI R, MASHIACH E, KRISTAL B, SHKOLNIK T, SHASHA SM 1995: Antioxidant enzymes activity in polymorphonuclear leukocytes in chronic renal failure. Nephron **71**: 176-179

SMITH C, MARKS A, LIEBERMAN M 1996: Oxygen toxicity and free radical injury. In: Mark's Basic Medical Biochemistry, Lippincott Williams &Wilkins, p. 450.

SÜVEGES T, GLÁVITS R (1976): Piglet losses due to parenteral application of iron-dextran preparations. Acta Vet Hung **26**: 257-262

TAKAHASHI M, IKEDA H, SATO EF, AKIMARU K, EDAMATSU R, INOUE M, UTSUMI K 1992: Stimulus-specific enhancement of luminal chemiluminescence in neutrophils by phosphatidylserine liposomes. Arch Biochem Biophys **298**: 43-48

TAKENAKA Y, MIKI M, YASUDA H, MINO M 1991: The effect of &- tocopherol as an antioxidant on the oxidation of membrane protein thiols induced by free radicals generated in different sites. Arch Biochem Biophys **285**: 344-350

UOTILA JT, KIRKKOLA A-L, RORARIUS M, TUIMALA RJ, METSÄ-KETELÄ T 1994: The total peroxyl radical-trapping ability of plasma and cerospinal fluid in normal and preeclamptic parturients. Free Radical Biol Med **16**: 581-590

VAN ASBECK BS, MARX JJM, STRUYVENBERG VERHOEF J 1982: Defects of phagocytic cells in patients with iron overload. Antonie Van Leeuwenhoek **48**: 192

VAN ASBECK BS, MARX J, STRUYVENBERG A, VAN KATS J, VERHOEF J 1984: Effect of iron (III) in the presence of various ligands on the phagocytic and metabolic activity of human polymorphonuclear leukocytes. J Immunol **132**: 851-856

VAN DYKE K, VAN SCOTT MR, CASTRANOVA V 1986: Measurement of phagocytosis and cell/mediated cytotoxicity by chemiluminescence. Methods Enzymol **132**: 498-507

WARD PA, GOLDSCMIDT P, GREENE MD 1975: Suppressive effects of metal salts on leukocyte and fibroblastic function. J Reticuloendothel Soc **18**: 313

WANG JF, KOMAROV P, DE GROOT H 1993: Luminol chemiluminescence in rat macrophages and granulocytes. The role of NO, O2-/ H2O2 and HOCl. Arch Biochem Biophys **304**: 189-196

WEBER GF 1990: The measurement of oxygen-derived free radicals and related substances in medicine. J Clin Chem Clin Biochem **28** : 569-603