Contents

List of abbreviations

ABE	actual base excess
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
CAT	catalase
CK	creatine kinase
cNOS	Constitutive nitric oxide synthase
DMSO	Dimethyl sulfoxide
EDTA	Ethylendiamine tetraacetic acid
FADH	Flavine adenine dinucleotide
FR	free radical
FRAP	ferric reducing ability of plasma
G6P	Glucose 6 phosphatase
GPX	glutathione peroxidase
GSH	reduced glutathione
GSH-Red	glutathione reductase
GSSG	oxidised glutathione
H2O2	Hydrogen peroxide
Hb	haemoglobin
HCO_3^-	standard bicarbonte concentration
HOCl	hypochlorite
I/R	ischeamia-reperfusion
iNOS	Inducible nitric oxide synthase
IPPV	Intermittent positive pressure ventillation
LDH	lactate dehydrogenase
L-NAME	L-nitroarrginine methyl ester
LP	lipid peroxidation
MDA	malondialdehyde
M4HN	Malondialdehyde+4hydroxi nonenal
$MnCl_2$	Manganese chloride
MPO	myeloperoxidase
NADH	Nicotinamide adenine dinucleotide
NO	nitric oxide
NOS	nitric oxide synthase
O2.-	Superoxide radical
OFR	oxygen free radical
$OH^{\cdot}$	Hydroxil radical
$ONO_2^{\cdot -}$	Peroxynitrit anion radical
$ONO_2^{\cdot -}$	peroxynitrit anion radical
pCO_2	partial carbon dioxyde tension
pO_2	partial oxygen tension
PUFA	polyunsaturated fatty acid
RBC	red blood cell
ROI	reactive oxygen intermediers
SAT	Oxygen saturation of haemoglobine
SOD	superoxide dismutase
SOP	sham operated
SV	Spontaneuos ventillation
TAS	total antioxidant status
TBARS	thiobarbituric acid reactive substances
TCO_2	total carbon dioxyde concentration
TP	total protein
TRAP	Total peroxide radical trapping capacity
UA	Uric acid
vvs	vörösvérsejt
WBC	White blood cell
XDH	Xantine dehydrogenase
XO	Xanitne oxidase

Summary

Free radical induced and mediated processes are present in a great variety of physiological and pathological pathways. The basic source of FR production is the respiratory chain where different oxygen derived free radicals are transformed finally to water and carbon dioxide. On the other hand it was observed that FRs may induce such oxido-reductive cascade mechanisms that can damage protein, nucleic acid and fat molecules of the living cells therefore there are defence mechanisms that involve numerous molecules and enzymes (the antioxidant system) to control their production and elimination. The action of certain basic components of the antioxidant system is investigated in 4 trials in this study.

In **chapter one** age related changes of tissue lipid peroxidation (LP) of liver and brain, as well as plasma antioxidant capacity of broiler chicken cockerels were investigated. Rate of LP was found to be comparatively low in the liver and high in the brain of the 1-day-old broiler chicks. Increased LP was observed in the liver tissue on the 10^{th} and in the brain tissue on the 21^{st} day of life, the former was accompanied by concomitant decrease of plasma antioxidant capacity.

The aim of the study detailed in **chapter 2** was to examine exercise-induced changes of some plasma and red blood cell biochemical and antioxidant parameters in pentathlon horses. Blood samples were taken from horses before, immediately and 24 hours after competing two runs of 1 minute of intense exercise over jumps. The peak intensity periods were preceded by a 20-minute warming up and separated by 20-minute break There were elevated concentrations of plasma TP, lactate, uric acid, CK, LDH and FRAP ($p<0.05$) in the post exercise samples compared to the pre-exercise samples. All parameters returned approximately to the initial values after 24 hours rest. Similar tendencies were observed in the change of plasma TAS and RBC GPX values. Erythrocyte GSH and TBARS concentrations did not show any change immediately after the exercise but decreased TBARS and increased GSH concentrations were observed after 24 hours rest ($p<0.05$). Plasma UA and FRAP values showed good correlation in a linear model. There were opposite changes in FRAP and TAS values calling attention to the fact that assessing the antioxidant capacity by different parameters can give highly different results.

In **chapter 3** LP changes during intestinal ischemia-reperfusion (I/R) in a rat model with and without desferrioxamine and L-arginine treatment are discussed. The only significant change observed in the sham-operated group was the increased intestinal SOD activity after the ischaemic period. In the I/R group significant increase of intestinal M4HN concentration was observed during hypoxia that was followed by similar changes in intestinal and RBC TBARS and plasma FRAP values upon reperfusion. In the deferoxamine treated group the intestinal TBARS and M4HN concentrations were significantly lower while FRAP and NO concentrations were significantly higher compared to the I/R group. At the same time RBC TBARS concentration and GPX activity significantly decreased within group D. In group A the intestinal M4HN concentration was significantly lower than in the I/R group. Plasma FRAP and NO concentration showed similar changes to group D. It is concluded that I/R increased the LPO in the intestinal tissue and altered some parameters of plasma and RBCs, too. Desferrioxamine treatment prevented these effects, while the usefulness of L-arginine remains doubtful.

The study described in **chapter 4** was undertaken to evaluate lipid peroxidation changes in red blood cells and plasma of horses that were operated on for ileus. No significant change over time was observed in any parameter in the control horses. Horses affected with colic were presented with metabolic acidosis as read from significantly lower pH and BE than the control ones. Upon anaesthesia horses having intestinal disorders developed marked mixed type acidosis. In spite of that oxygen tension and saturation of the mixed venous blood showed significant increase during anaesthesia with a peak at two hours after the incision. Plasma uric acid concentrations were some 1,5 times higher in the control group during anaesthesia. Late post-anaesthetic values show opposite picture due to a marked increase seen in the colic horses. Though there are no significant changes over time of FRAP values in either group colic horses exhibited approximately twice higher

concentrations than did the control ones. Plasma NO concentration was significantly higher in the colic horses before the operation and showed a constant decrease thereafter. Erythrocyte TBARS concentration was higher in the colic group at all sampling times during the observed period. The highest TBARS concentration was found 1 hour after incision (ie.: 15-45 minutes after reperfusion). There were no significant alterations observed in the GSH, GSSG, concentrations and GPX and SOD activities of RBCs though the latter one followed a tendency opposite to the TBARS concentration in the colic patients.

It was concluded that the intestinal I/R resulted in marked metabolic acidosis and altered several antioxidant parameters of plasma and erythrocytes. This indicates that maximal effort should be taken to correct metabolic acidosis during the operation of colic horses. Furthermore changes in the blood LP parameters can serve as an indication for antioxidant therapy during the operation of horses with ileus.

Összefoglalás

A szabadgyökök számos élettani és kóros folyamat meghatározó tényezői. Az élő szervezetekben szabadgyökök keletkezésének legfőbb forrása a légzési lánc, amelyben különböző oxigén eredetű szabadgyökök alakulnak sorozatos elektron felvétellel széndioxiddá és vízzé. Ugyanakkor az is bizonyítást nyert, hogy a szabadgyökök a kifejezett reakciókészségükből adadóan károsítani képesek a sejteket felépítő fehérje, nukleinsav és lipid molekulákat. Fentiek okán a káros hatások megelőzésére az élő rendszerekben bonyolult, számos molekulát és enzimet egyesítő ún. antioxidáns rendszer működik. Jelen dolgozat az antioxidáns rendszer egyes elemeinek működésének vizsgálatát célozza, amelyet négy kísérlet keretében végeztem.

Az **első fejezetben** broilercsirkékben vizsgáltam a máj- és agyszövet lipidperoxidációjának, valamint a vérplazma antioxidáns kapacitásának életkorral kapcsolatos változását. Megállapítottam, hogy napos korú broilercsirkékben a lipidperoxidáció magasabb szintű az agyszövetben mint a májban. Fokozott LP-t figyeltem meg a májszövetben tíz napos, míg az agyszövetben 21 napos korú állatokban. A májszövet fokozott LP-jével egyidőben a vérplazma antioxidáns kapacitása jelentősen csökkent értéket mutatott.

A **második fejezetben** bemutatott vizsgálat keretében azt vizsgáltam, hogy öttusa lovakban, versenyterhelés hatására milyen biokémia és antioxidáns-rendszerbeni változások alakulnak ki. A vizsgálatot a lovak torkolati vénájából gyűjtött vérből végeztem, amit kétszer egyperces intenzív, akadálypályán teljesített ugrássor előtt, illetve után közvetlenül, valamint 24 óra elteltével gyűjtöttem. A csúcsintenzitású munkavégzéseket 20 perces bemelegítés előzte meg, illetve kötötte össze. A nyugalmi helyzethez képest magasabb ($p < 0,05$) TP, CK, LDH, húgysav és FRAP értékeket mértem a versenyterhelés után közvetlenül levett vérmintákból. A 24 óra elteltével ismételt vizsgálat alkalmával a jelzett mutatók ismét a kiindulási értékhez közeli tartományban voltak mérhetők. Az fent leírthoz hasonló tendenciát tapasztaltam a vérplazma TAS értékének és a vvs-ek GPX aktivitásának változásában is. A vérplazma TAS és FRAP értékei ellentétes irányú változását figyeltem meg, ami arra figyelmeztet, hogy az össz-antioxidáns kapacitást különböző módszerekkel mérve jelentősen eltérő eredmények adódhatnak.

A **harmadik fejezetben** az intestinalis I/R-nek a LP-s mutatókra kifejtett hatását vizsgáltam deferoxamin és L-arginine kezelés mellett, illetve kezelés nélkül patkány modellben. Az álműtéten átesett állatok (SOP) esetében az egyetlen szignifikánsnak mutatkozó változás a bélszövet SOD aktivitásának az ischaemiás periódus utáni emelkedése volt. A csak intestinalis I/R-n átesestt (I/R) csoportban a bélszövet M4HN koncentrációja szignifikáns emelkedést mutatott a hypoxiás periódus végén, amit bél és vvs TBARS és vérplazma FRAP értékének hasonló irányú változása követett a reperfúzió során. A deferoxaminnal kezelt csoportban a bélszöveti TBARS és M4HN értékek szignifikáns alacsonyabbak, míg a vérplazma FRAP és NO koncentrációk alacsonyabbak voltak az I/R-csoportban mérteknél. Ugyanakkor a vvs-ek TBARS koncentrációja és GPX aktivitása szignifikánsan csökkent a D-csoportban. Az L-argininenal kezelt csoportban az I/R csoporthoz képest szignifikánsan alacsonyabb bélszöveti M4HN értékek voltak mérhetők. A

vérplazma FRAP és NO koncentrációja a D-csoporthoz hasonló változást mutatott. Fentiekből azt a következtetést vontam le, hogy az I/R megemelte a LP-t a bélszövetben és mérhető változásokat okozott a vérplazma és a vvs-ek megfelelő mutatóiban is. Deferoxamin kezeléssel fenti hatások kivédhetők voltak, míg az L-arginine kezelés hatása kérdéses maradt.

A **negyedik fejezetben** a vérplazma és vvs-ek LP-s változásait vizsgáltam kólikás lovak ileus műtétje során gyűjtött mintákból. A kontroll állatokban egyik mért változó sem mutatott szignifikáns idő szerinti változást. Az ileus miatt műtétre került állatok klinikára érkezésükkor metabolikus acidózisra jellemző alacsony pH és BE értékekkel rendelkeztek. Az anesztézia során az ileus műtéten átesett állatoknál kifejezett vegyes típusú acidózis alakult ki. Ennek ellenére a pvO2 és a vénás SAT értékek szignifikánsan emelkedtek az anesztézia során, a hasüreg megnyitásától számított második óránál érve el a legmagasabb szinteket. A vérplazma húgysav szintje mintegy 1,5-szer magasabb volt a kontroll állatokban az anesztézia során. Az altatást követően 12, 24 órával gyűjtött mintákban azonban fordított helyzetet találtam a kólikás lovaknál tapasztalható jelentős koncentrációemelkedésnek köszönhetően. Bár FRAP értékek idő szerinti változása egyik csoportban sem volt szignifikáns, a kólikás lovakban mintegy kétszer magasabb értékeket figyeltem meg mint a kontrollokban. A kólikás csoportban a műtétet megelőzően magas, de későbbi mintavételeknél folyamatos csökkenést mutató vérplazma NO koncentrációt találtam. A vvs-ek TBARS koncentrációja a megfigyelt periódus alatt mindvégig magasabb volt a kólikás állatokban mint a kontrollokban. A legmagasabb TBARS értéket a hasüreg megnyitása után 1 órával (a reperfúziót követő 15-45. percben) vett mintákból mértem. A kólikás betegekben a vvs-ek GSH és GSSG koncentrációjában valamint GPX és SOD aktivitásában nem mutatkozott szignifikáns eltérés, bár ez utóbbi a TBARS koncentráció változásával ellentétes tendenciát mutatott.

Fentiek figyelembevételével elmondható, hogy az intestinalis I/R kifejezett metabolikus/vegyes típusú acidózist idézett elő és megváltoztatta a vérplazma és a vvs-ek bizonyos antioxidáns mutatóit. Ennek alapján fontos, a műtétek során törekedni az acidózis kompenzálására, továbbá a vérplazma és vvs-ek LP-s paramétereinek változása az antioxidáns terápia alkalmazásának szükségességére hívja fel a figyelmet.

Introduction

There is increasing amount of both clinical and basic research done on free radical induced or mediated processes in living systems. It was observed that molecules having unpaired electrons on their outmost molecular orbital may induce such oxido-reductive cascade mechanisms that can damage protein, nucleic acid and fat molecules of the living cells. These molecules are called free radicals (FR) (Del Maestro, 1980, Demopoulos 1973, Pryor 1973, Pacifici and Davies, 1991). On the other hand the generation of free radicals is considered a physiologic process, still they do not cause any damage as there are regulatory mechanisms to control their action (Hornsby and Crivello, 1991, Slater, 1981). The basic source of FR production is the respiratory chain where different oxygen derived free radicals are transformed finally to water and carbon dioxide. These and some other FRs like molecular, sigma and delta singlet oxygen, hydroxil radical anion, superoxide anion radical, perhydroxi radical, hydrogen- and lipid peroxides are called reactive oxygen intermediers (ROI) or oxygen free radicals (OFR) (Fridovich, 1978). Any process that leads to the accumulation of these substances and thus exhausts the capacity of the defence system can cause serious consequences often recognized as special diseases or syndromes. Free radical induced damages most easily occur in the fatty acids of the biological membranes especially in the polyunsaturated fatty acids (PUFA). During the oxidation of these, the structure of the membranes is partially or totally disrupted, cells undergo degeneration and may die while different catabolic enzymes and mediators are released into the interstitial space and to the circulation, which by reaching other organs can worsen the basic processes (Pryor 1973, 1982). Free radical induced damage of membrane lipids is called lipid peroxidation (LP). Many other agents or conditions apart from OFRs can play role in the induction of LP (e.g.: chemicals like paraquat, carbon tetrachloride, halothane etc.), systemic diseases (inflammation, septicaemia, diabetes) diseases of different

organs, operations (reperfusion of hypoxic tissues, organ transplantation), aging, physical exercise (McCay et al., 1983, Parks e al 1983, 19, Shaw et al., 1983, Hunt et al., 1988, Reynolds et al., 1980).

Besides OFRs there is outstanding importance of nitrogen centred free radicals in biological systems. Among these nitric oxide (NO) has the most complex effect. Its basic function is to maintain the resting vasodilator tone of vessels in most organs and also acts as a neurotransmitter in the brain and in the non-cholinergic non-adrenergic enteral nervous system (Salzmann, 1995). The physiological source of NO production is the amino acid L-arginine, the guanidino group of which is utilized by the nitric oxide synthase enzyme (NOS) to produce NO while the original molecule is converted to L-citrulline. There are two basic types of this enzyme. The constitutive one (cNOS) is found in the endothel cells (endothelial NOS) and neurons (neuronal NOS) while the inducible form plays role in certain inflammatory processes and found in the following cells: macrophages, smooth muscle cells, fibroblasts. Besides being a mediator, NO itself can act as a FR, and plays crucial role in many FR reactions. Combining with superoxide it can form the harmful peroxynitrit anion radical ($ONO_2^{\cdot -}$) , which can break down to nitrogen dioxide and $OH^{\cdot}$ (Cristol et al., 1995). The latter one is considered as one of the most potent FRs. Furthermore peroxynitrit can damage proteins forming nitrotyrozine with the tyrosine moieties of protein chains (Beckman et al., 1990).

As it was mentioned before FR production is a basically physiological process. The acceleration of FR production or the accumulation of FRs can lead to the exhaustion of the defence mechanisms. These defence mechanisms involve numerous molecules and enzymes

Generally, molecules that can neutralize FRs by accepting them are called scavengers (Slater et al., 1981). Antioxidants protect the cells against OFRs. There are numerous natural antioxidants that can be further classified on which step of the LP chain reaction they can inhibit. Thus vitamin E inhibits the initiation LP, vitamin E and C hinders the production of lipid hydroperoxides, thiols can break down the latter, deferoxamin, d-penicillamin prevents the Fenton-reaction by metal chelation. Besides vitamin A and E and other carotenoids can directly eliminate FRs by scavenging them. One of the most important antioxidant enzymes is glutathione peroxidase (GPX), a selenium dependent enzyme. The selenium independent form of this molecule is called glutathione S transferase. This enzyme eliminates lipid peroxides or H_2O_2 while oxidizing the reduced form of glutathione (GSH) to glutathione disulfide (GSSG). The latter molecule is converted back to GSH by glutathione reductase (GSH-Red) (Dormandy 1978). Superoxide dismutases (manganese and copper/zinc dependent forms) can neutralize $O_2\cdot^-$ by converting it to the less harmful H_2O_2 that can be further metabolised to water by catalase or GPX (Fridovich, 1975, 1978). Other enzymes can break down the products of LP or repair the molecular damage caused by FRs (eg.: epoxid-hydrolase, aldehyde-reductases, some cytochroms, DNA-repair enzymes).

When trying to understand LP processes in certain pathologic conditions one has to consider that there are many other biological processes going on that can interfere with the LP reactions which are usually very quick making it otherwise difficult to reveal true causes and consequences. As seen from the introduction LP processes are involved in almost if not all physiologic and pathologic process.

Out of this great variety I have studied three phenomenons in four trials.

Chapter/Experiment I.: Age related changes of LP and antioxidant status in developing chicken

Chapter/Experiment II.: - Changes of LP and antioxidant indices in pentathlon horses during exercise

Chapter/Experiment III.: - Role of LP and antioxidants in experimental intestinal ischemia-reperfusion (I/R) in rats

Chapter/Experiment – IV: -. Evaluation of acid base and lipid peroxidation indices in horses with naturally acquired intestinal I/R

Relevant literature to each experiment is summarized in the corresponding chapters.

Rate of lipid peroxidation in brain and liver tissues and the total antioxidant status of blood plasma in developing chicks

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Introduction

Age-related changes of the antioxidant system have been studied in many species previously. Many observations were taken on the embryonic development of the antioxidant system of chicks. It was established that the transport of lipids across the yolk sac membrane occurs only on the third week of incubation (Noble and Cocchi, 1990) and is accompanied by the transport of the antioxidant Vitamin E (Gaál. et al., 1995). The transport of vitamin A precedes this process via an active transport mechanism and reaches its maximum level on the second week of incubation when the development of the retina is going on. Antioxidant defence mechanisms may vary in the organs due to different concentrations of the major antioxidants. Concentration of vitamin A, E, reduced glutathione (GSH) and activities of antioxidant enzymes superoxid-dismutase (SOD) and glutathione-peroxidase (GPX) was found to be significantly higher in the embryonic liver tissue than in the brain, whereas concentration of ascorbic acid was higher in the latter organ (Surai et al., 1996; Gaál et al., 1995). Some of the antioxidant pathways in the developing chick embryo is regulated by the increasing oxygen consumption and causes GSH accumulation in the liver, and higher SOD activity in the brain (Wilson, 1992). In newly hatched chicks the brain tissue is the most capable one to spontaneous and to in vitro iron induced lipid peroxidation (LP), and is characterised by low GPX and catalase (CAT) activity (Surai et al., 1999). The susceptibility of the brain tissue to LP is further exaggerated by the increase in its polyunsaturated fatty acid content during embryonic development (Maldjian et al., 1996). Other investigations revealed many nutritional diseases of poultry that are connected with disturbances of the antioxidant system. The most well known disorder is the nutritional encephalomalacia of poultry that develops on the 7-21st day of life due to absolutely or relatively low concentration of vitamin E in the feed (Salmann et al., 1991; Fuhrmann et al., 1996). Evidence was raised that increasing the n-3 PUFA content in the diet of chicken elevates liver LP and depresses plasma antioxidant capacity. These changes could be weakened by vitamin E supplementation of the diet (Husvéth et al, 2000). Other nutritional diseases like vitamin A, selenium, monensin, and salinomycin-tiamulin toxicosis, tibial dyschondroplasia and pulmonary hypertension (ascites) syndrome are also closely connected with impaired antioxidant mechanisms or increased rate of free radical formation (Mézes et al, 1992, Enkvetchakul et al., 1993, Bottje and Wideman, 1995, Whitehead et al., 1994, Mézes et al., 1997). Ontogenetic changes in the antioxidant mechanisms have been observed in many other species for example rats, cattle and swine (Ledwozyw and Kadziolka, 1989, Carillo et al., 1992, Gunther et al., 1993, Gaál et al., 1996). These findings support the LP theory of ageing (Harman, 1969) and underline the necessity of appropriate antioxidant defence in newborn animals. Recently new methods were developed to characterise the antioxidant status of biological systems with a single parameter instead of measuring the levels of certain antioxidants separately. One of these methods is the total antioxidant status (TAS) assay that was developed by Miller et al (1993) for the evaluation of antioxidant capacity of human plasma. The method was applied to detect changes of antioxidant power of chicken plasma by Gaál and Kopál (1997). In our study we aimed to use this method for the assessment of the plasma antioxidant capacity of growing chickens.

As there are few data available on the antioxidant system changes in newly hatched developing chicks, we carried out this study to evaluate LP in the organs and antioxidant status of plasma of newly hatched chicks from the 1st to the 21st day of life. We hypothesised that the atmospheric oxygen created a significant oxidative stress for the animals causing measurable changes in the rate of LP in brain and liver tissues and in the total antioxidant status of plasma.

Materials and methods:

Animals

Twenty-one one-day-old Ross cockerel chicks were purchased from a commercial hatchery (HE-ROSS Hatcheries Co., Ócsa, Hungary). The animals were kept together during the experimental period in cages in batteries with raised floors and were fed commercial diet („starter diet"-meeting the Hungarian and NRC recommendations for vitamin and nutritional substance content of feed) for broilers. The birds were kept within a controlled environment at 20 to 25 °C. Additional heating was used during the initial 2-week period. Lighting was provided 24 hours a day. The chicks had free access to feed and water. The experiment was approved by the Local Supervising Committee for the Use and Welfare of Experimental Animals (25-9/2000) at the Faculty of Veterinary Science, Szent István University, Budapest.

Sample collection

At each sampling time (1, 10, and 21 days of life) 7 chicks were slaughtered, brain, liver and heparinized blood samples were collected. After blood sampling plasma was separated from the blood cells by centrifugation (3000 rpm for 5 minutes) and approximately 0.5 gram of each tissue sample was homogenised with physiological (160 mmol/l) saline solution. Prepared samples were stored at -20°C to await the analyses for maximum five days.

Chemical analyses

Rate of LP was determined by the modified thiobarbituric acid reaction (TBARS) (Placer et al., 1964). The total antioxidant status (TAS) of plasma was measured by a commercial test kit (TAS kit, Randox Laboratories, Crumlin, UK). All reagents except the Randox TAS kit were purchased from Sigma-Aldrich Ltd. Budapest, Hungary.

Statistical analysis

Data were analysed by means of one-way analysis of variance (ANOVA) and Student's t-test with the help of the STATGRAPHICS statistical software. Significance was accepted at 5% confidence level. Results are expressed as means ± standard error of mean (SEM).

Results

One way ANOVA revealed significant changes according to time in all parameters measured (Table 1).

Table 1: Concentration of TBARS in brain and liver tissues and TAS of plasma in chicks

Parameters / Age in days	TBARS, nmol/g wet tissue		TAS, mmol/l
	brain	liver	plasma
1	$73^{a} \pm 7$	$26^{a} \pm 1$	$1.19^{a} \pm 0.05$
10	$59^{a} \pm 2$	$50^{b} \pm 4$	$0.46^{b} \pm 0.04$
21	$110^{b} \pm 6$	$46^{b} \pm 3$	$1.09^{a} \pm 0.06$

Note: results in the same column with different letters in superscript mean significant ($p<0,05$) differences

The concentration of TBARS was almost three times higher in the brain of the newly hatched chicks than in their liver. At day 10 the TBARS level was almost twice higher in the liver than in the previous samples and there was no significant difference between the two organs. Another ten days later the brain TBARS concentration showed a marked increase while the level of

this substance in the liver remained almost constant throughout the investigated period. The TAS value of plasma of 10 days-old-chicks was less then half of those measured in any other sample, which showed almost the same level.

Discussion

Our data equivocally with those of Husvéth et al., (2000) confirm that the TAS method can be applied to evaluate chick plasma antioxidant status. TAS values of the newborn and developing chick were very similar to those (around 1 mmol/l) measured in other species (Miller et al., 1993, Gaál and Kopál, 1997) except at day 10 when significantly lower TAS value was found. This result suggests that the antioxidant mechanisms of plasma are temporarily exhausted at this period of development. It can be presumed that the antioxidant system is developed well enough to cope with the acute effects of changing from the gas exchange across chorioallantois membrane for the pulmonary ventilation during hatching but later on becomes exhausted by the increased rate of peroxide formation. The above findings are supported by the results of Mézes et al. (1997), who found low activities of CAT and GPX and high concentration of TBARS in plasma of chicks of similar age. The marked post-hatch increase in the liver TBARS concentration could have been caused by the increasing lipid content of the organ due to fat reabsorption from the yolk sac. After this process completed and lipid transport from the liver ceased no further increase in LP could be observed. It seems that the liver may play crucial role in the regulation of the antioxidant mechanisms of plasma, too as the increase of liver TBARS concentration was accompanied by decreased plasma TAS value. The brain has different antioxidant defence system compared to liver as the level of the major antioxidants like vitamin E, GSH CAT, GPX is low in this organ but there is a relatively high vitamin C concentration which is thought to play important role in antioxidant pathways of the embryonic chick brain (Surai et al., 1999). In this study we observed marked increase of TBARS concentration in the brain from the 10^{th} to 21^{st} day of development. Though neither concentration nor activity of individual antioxidants were measured in this experiment one may speculate that these mechanisms are impaired at this developmental stage in the brain tissue. Others found increased LP and decreased GPX-activity in the brain tissue of chicks only at 35 days of age (Mézes, 1988). These results call attention to the fact that the brain has decreased antioxidant capacity around the third week of life in which age the nutritional encephalomalacia, which thought to be the most important disease connected with impaired antioxidant status, most often occurs (Salmann et al.1991, Fuhrmann et al., 1996). Similar tendency was observed in the developing rat brain (i.e.: increasing LP till the third week of life) by Gunther et al. (1993).

In conclusion it is stated that newly hatched chicks have similar plasma antioxidant capacity to other species. Rate of LP is comparatively low in the liver and high in the brain of the 1-day-old broiler chicks. Increased LP was observed in the liver tissue on the 10^{th} and in the brain tissue on the 21^{st} day of life, the former was accompanied by concomitant decrease of plasma antioxidant capacity.

Summary

Age related changes of tissue lipid peroxidation (LP) of liver and brain, as well as plasma antioxidant capacity of broiler chicken cockerels were investigated. Tissue LP was characterised by the spectrophotometric assessment of thiobarbituric acid reactive substances (TBARS). Plasma antioxidant power was evaluated by the measurement of total antioxidant status (TAS). The newly hatched broiler chicks had similar TAS value (1.19 mmol/l) to new-borns of mammalian species. There were significant changes ($p<0.05$) observed in the time course of all parameters. Tissue TBARS concentration was higher in the brain than in the liver at hatching, noting that the latter organ has more effective antioxidant defence during the embryonic life. The concentration of TBARS increased to 10^{th} day in the liver but only to the 21^{st} day in the brain, and the former was accompanied by approximately 50% decrease of plasma antioxidant capacity. This suggests that the liver plays important role in forming the antioxidant defence mechanisms of plasma of broiler chicks.

Changes of some biochemical and antioxidant parameters in plasma and erythrocytes of pentathlon horses before and after exercise

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Introduction

Response to exercise in horses has been studied from several aspects. Many studies were taken to evaluate changes of physiological, biochemical as well as lipid peroxidation parameters in thoroughbred (Snow et al. 1985, Ono et al., 1990, Mills et al., 1996, Harris et al., 1997) and standardbred racehorses (Lindholm and Saltin, 1974, Keenan, 1978, Rose et al., 1983, Gottlie-Vedi et al., 1995, Art et al., 1995, Rasanen et al., 1996 and Avellini et al., 1997) three-day event horses (Williamson et al., 1996) and in endurance horses (Lucke and Hall 1978, Rose et al., 1977, Deldar et al., 1982, Rose et al., 1983). There is only one article on exercise-induced changes in polo horses (Craig et al., 1985) and few papers about show jumping horses (Art et al., 1990a, 1990b). The author is not aware of studies on biochemical and antioxidant changes in pentathlon horses.

During exercise ATP is catabolised to ADP and further to AMP and IMP in the muscle fibres. This process is accompanied by glycogen consumption and increasing glucose-3-phosphate, glycerol and lactate concentration (Snow et al, 1985, Harris et al., 1997, Lindholm and Saltin, 1974,). Exercise induced processes are well reflected in changes of blood constituents. Numerous authors found increased packed cell volume, Hb-concentration, RBC and WBC count as well as increased concentration of plasma total protein (TP), albumin, glucose, urea, creatinine, ketones, bilirubin, iron, phosphate, free fatty acids, cholesterol, lactate, uric acid (UA) cortisol and glucagon. Similarly, higher activities of creatine-kinase, lactate-dehydrogenase alkaline-phosphatase, aspartate-amino-transferase and gamma-glutamil-transferase were documented (Lindholm and Saltin, 1974, Keenan, 1978, Rose et al., 1983a, 1983b, Lucke and Hall 1978,' Deldar et al., 1982). Some other components, for example potassium, calcium and bicarbonate, were found to be decreased by the same authors. There is growing evidence supporting the theory that increased oxygen consumption during exercise creates oxidative stress to the animals. Exercise caused elevation of plasma lipid peroxide (LP) level, and decreased red blood cell (RBC) glutathione peroxidase (GPX) activity while no change in catalase and superoxide dismutase (SOD) activities were observed. In addition, a combination injection of antioxidant vitamin E and Selenium given prior to work decreased resting plasma LP level and could slightly attenuate the effect of exercise on the aforementioned parameters (Ono et al., 1990). Direct proof was produced that during exercise muscle- and RBC-phospholipids are peroxidised and a metastabile end product of lipid peroxidation, malondialdehyde (mostly protein-bound) accumulates in the muscle fibres and in the erythrocytes (Matsuki et al., 1991). In another trial the increase of plasma LP and oxidised glutathione level in RBCs was observed in horses running in a hot and humid environment (Mills et al., 1996). Repeated bouts of exercise in horses were reported to result in increased plasma xantin-oxidase activity, lactate and UA concentration and also in higher plasma total peroxyl radical-trapping (TRAP) ability (Rasanen et al., 1996). While a single bout of exercise was generally found to decrease antioxidant power of different biological systems in horses, regular training increased the erythrocytes' in vitro resistance to LP, lymphocyte GPX activity and decreased resting plasma TBARS concentration. Furthermore, training attenuated plasma vitamin E and TRAP loss in acute exercise (Avellini et al., 1995).

In this study we aimed to evaluate biochemical and antioxidant parameter changes in plasma and RBCs as no previous study described them in horses competing in pentathlon contests. We also wished to introduce the recently developed ferric reducing ability of plasma (FRAP) for the measurement of horse plasma antioxidant capacity.

Materials and methods:

Animals

Altogether fourteen horses: 5 mares, 7 stallions and 2 gelded ones between the age of 5-10 years were used in this trial that took place on a preliminary pentathlon contest preceding the 1999 World Pentathlon Championship in Budapest. At the time of the competition the weather was sunny, temperature was 23-25 °C with a relative humidity of 45%. Each horse had to complete 20 minutes of warming up followed by a 1-minute contest run consisting of 12 jumps of 120 cm height, involving one double and one triple obstacle as well, then after 20 minutes (while the horses were doing the same work as in the warming up) the contest run was repeated by the same pentathlet riding the same horse. Average speed in the contest runs was approximately 7 m/s.

Sample collection

Blood samples were taken from the jugular vein into heparinized collecting tubes 24 hours prior to the competition, immediately after the 2nd contest run and 24 hours after the competition. After sampling, plasma was separated and RBC hemolysates were prepared.

Chemical analysis

The following biochemical parameters of plasma were determined spectrophotometrically: total protein (TP) (biuret method) uric acid (UA) (Uric Acid Diagnostic Reagent Kit, Reanal Fine Chemicals Co. Budapest, Hungary, Cat. No.: 13101-2-99-80) and lactate concentration (Lactate Reagent test kit, Sigma Diagnostics Inc. U:SA. Cat. No.: 735-10), lactate-dehydrogenase (LDH) and creatine-kinase (CK) activities (LDH and CK diagnostic test kits, both from Diagnosztikum Ltd., Budapest, Hungary, Cat. No.: 42101 and 40411, respectively). The total antioxidant status (TAS) of plasma, erythrocyte glutathione-peroxidase (GPX) and superoxid-dismutase (SOD) activities were determined using commercially available kits (TAS, Ransel, and Ransod kits, respectively; all manufactured by Randox, Cork, Ireland),. The ferric reducing ability of plasma (FRAP) was assessed as described by Benzie and Strain (1996). In brief this method is based on the reduction of ferric-tripyridyltriazin reagent to the ferrous form, i.e. by antioxidants in the sample, which has an intense blue colour and can be measured spectrophotometrically at 593nm wavelength. For validation of the method aliquots of horse plasma samples in two different concentration levels were pooled and frozen at -20°C. Within-run precision was calculated from 10 assays done on the same day. Between-run precision was determined from 10 assays done over 20 days. The concentration of thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) in RBCs were measured according to Placer (1964) and Sedlak (1968), respectively. All chemicals for antioxidant parameters except the Randox kits were purchased from Sigma-Aldrich Ltd. Budapest, Hungary.

Statistical analysis

The statistical analysis of the laboratory data was completed by using the Statgraphics software.

Results

Analytical performances of the FRAP assay:

Within-run coefficients of variation (CV) were less than 1,0 % at all values tested (0.284 mmol/l and 0.365 mmol/l). Between run CVs were less than 3.2%.

Plasma and RBC results are shown in Table 2.

Table 2. Biochemical and antioxidant parameters before, immediately and 24 hours after exercise in horse plasma and RBC hemolysates (mean ± standard error)

Values on the same line with different letters are significantly different, $p<0.05$ (ANOVA)

	Parameters	Before exercise		Immediately after exercise		24 hours after exercise	
Plasma	**TP, g/l**	**69**±1	a	**73** ±1	b	**68** ± 1	a
	Lactate, mmol/l	**0.86** ± 0.05	a	**3.73** ± 0.6	b	**1.01**± 0.05	a
	CK, U/l	**106** ± 4	a	**163** ± 21	b	**120** ± 6	a
	LDH, U/l	**364** ± 22	a	**461** ± 34	b	**417** ± 34	ab
	Uric acid, μmol/l	**58** ± 10	a	**72** ± 13	b	**58** ±10	b
	FRAP, mmol/l	**0.31**± 0.08	a	**0.37** ± 0.16	b	**0.33** ± 0.12	a
	TAS, mmol/l	**0.73** ± 0.04		**0.34** ± 0.02		**0.53** ± 0.24	
RBC	**TBARS, nmol/g protein**	**395** ± 23	a	**361** ± 21	a	**290** ± 21	b
	GSH, nmol/g protein	**445** ± 40	a	**479** ± 27 ab		**638** ± 90	b
	GPX, U/g protein	**47** ± 4		**38** ±4		**40** ± 3	
	SOD, U/g protein	**720** ± 80		**768** ± 109		**808** ± 116	

After the exercise horses showed elevated levels of plasma TP, lactate, CK, LDH and FRAP ($p<0.05$) compared to the pre-exercise values and all these returned approximately to the initial values after 24 hours rest. Similar tendencies were observed in the change of plasma TAS and RBC GPX values. GSH and TBARS concentrations did not show any change immediately after the exercise but decreased TBARS and increased GSH concentrations were observed after 24 hours rest (Table 2.). Plasma UA and FRAP values showed good correlation in a linear model. ($p<0.001$, $R^2=0.608$, correlation coefficient: 0.78) (Figure 1)

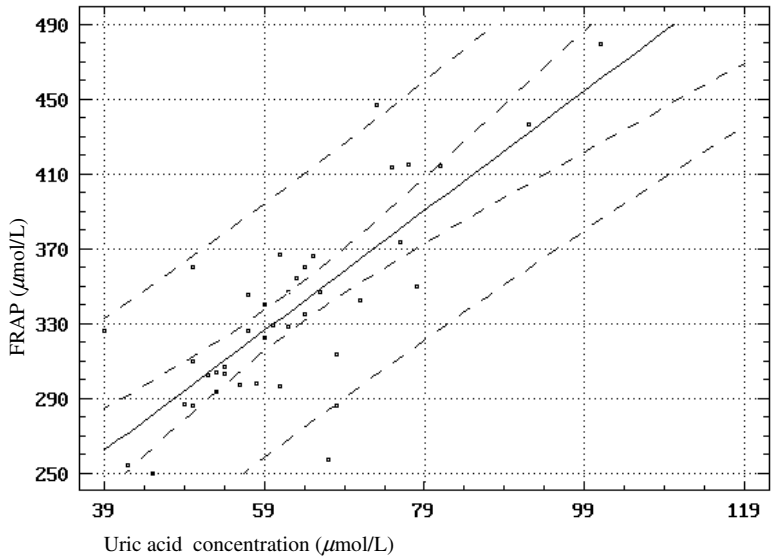

Figure 1. Linear correlation between the ferric reducing ability of plasma (FRAP) and plasma uric acid concentration in 14 horses sampled prior to, immediately after, and 24 hours after exercise (y = 3.02x + 136.9, r =0.78). The solid line indicates the line of equation, the inner pair of dashed lines indicates the 95% probability of the line of equation, the outer pair of dashed lines indicates the 95% probability of the plotted points.

Discussion

All the measured routine plasma biochemical parameters showed significant increase due to the type of exercise used in this study indicating first of all the increased load on muscles leading to enzyme leakage, lactate and uric acid accumulation in plasma. Note that the elevation in TP concentration was though significant but negligible compared to other parameters indicating that not haemoconcentration but metabolic effects played primary role in these changes. FRAP values were also elevated after physical stress. These findings are in good correlation with those of Mcmeniman et al. (1992) and Rasanen et al. (1996). The latter ones also found increased lactate, uric acid concentration and total TRAP levels in trotters. Their suggestion is that the increase of the TRAP value is mainly caused by the accumulation of uric acid in plasma due to purine degradation. According to the original publication of Benzie and Strain (1996) approximately 60 percent of the human plasma FRAP value is given by the uric acid content of the blood. So we can deduct that the rise of the FRAP concentration in this trial was basically caused by the increased UA concentration. On the other hand we have found opposite changes in TAS values, another parameter showing the general plasma antioxidant status. This may seem to contradict the aforementioned results but we have to consider the differences in the two methods we used. In the FRAP method there is direct reduction of the colour forming agent (ferric-tripyridyltriazin) so the antioxidant capacity is proportional to the reducing ability, while in the TAS method a complex free radical cation (2, 2'-azinobis benzothiazoline-6-sulphonic acid) is generated by the means of a pseudoperoxidase (metmyoglobin) and hydrogen peroxide, so the antioxidant capacity will be proportional to the inhibitory ability of the sample on the formation of the coloured complex. In that sense any antioxidant present in the plasma including enzymes as well, can take part in the latter reaction. This deduction is further augmented by the finding of strong correlation (r=0,91) of TAS and catalase activity of liver cells in a toxicological model in rats (O'Brien et al., 1999).

The decreasing tendency of GPX activity due to exercise can denote the effect of oxidative stress on red cells and may explain the late elevation of GSH concentration. The decreased GPX activity in a long term training period reported by Avellini et al (1995), is certainly of other origin (Se-deficiency, prolonged physical stress) than we found in our acute exercise model. Our results are in line with those of Ono et al (1990) who also found decreased GPX and unchanged SOD activity in RBC of exercised horses. It is unclear why the erythrocyte TBARS level did not correlate with other LP indices. We suspect that the method we use was not sensitive enough as LP produces mainly protein bound malondialdehydes in the erythrocytes, the detection of which requires hydrolysis of proteins prior to analyses (Matsuki et al 1991). All parameters except GSH and TBARS returned approximately to the initial values after 24 hours rest that agree the findings of Mills et al (1996). We cannot exclude that the changes we have found in the erythrocytes were parts of longer-term processes due to the continuous training of the horses. It also has to be mentioned that in this study blood was collected from the jugular vein that may not be representative of central circulation for some parameters like pH, pyruvate concentration and partial carbon dioxide and oxygen tension (Miller-Graber et al., 1988). The latter suggests that LP indices also might be affected by the site of sampling. On the other hand the mentioned study proved that other important biochemical parameters like lactate did not differ between the jugular vein and the pulmonary artery so we can accept that our data are representative for the central circulation. Since in our study blood was collected from horses in contest, access to other sampling sites like the pulmonary artery was impossible and we could not perform comparison of parameters measured in blood samples from different vessels.

It is concluded that the type of the applied exercise, which can be considered quite usual for pentathlon horses, caused detectable biochemical and LP changes in the plasma of the horses that were not unequivocally reflected in the erythrocytes. Attention should be paid to the evaluation of results of plasma antioxidant capacity described by different parameters as they can differ significantly.

Summary

The aim of this study was to examine exercise-induced changes of some plasma and red blood cell biochemical and antioxidant parameters in pentathlon horses. Blood samples were taken from fourteen horses before, immediately and 24 hours after competing two runs of 1 minute of intense exercise over jumps. The peak intensity periods were preceded by a 20-minute warming up and separated by 20-minute break The following plasma biochemical parameters were determined: total protein (TP), uric acid (UA) and lactate concentration, lactate-dehydrogenase (LDH) and creatine-kinase (CK) activities. The total antioxidant status (TAS) and the ferric reducing ability of plasma (FRAP) were also measured. Thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), TP levels, glutathione-peroxidase (GSH-Px) and superoxide-dismutase (SOD) activities of red blood cell hemolysates were determined. There were elevated concentrations of plasma TP, lactate, CK, LDH and FRAP ($p<0.05$) in the post exercise samples compared to the pre-exercise samples. All parameters returned approximately to the initial values after 24 hours rest. Similar tendencies were observed in the change of plasma TAS and RBC GSHPx values. Erythrocyte GSH and TBARS concentrations did not show any change immediately after the exercise but decreased TBARS and increased GSH concentrations were observed after 24 hours rest ($p<0.05$). Plasma UA and FRAP values showed good correlation in a linear model. It is concluded that the type of the applied exercise, which can be considered quite usual for pentathlon horses, caused detectable biochemical and lipid peroxidative changes. There were opposite changes in FRAP and TAS values calling attention to the fact that assessing the antioxidant capacity by different parameters can give highly different results.

Effect of deferoxamine and L-arginine treatment on lipid peroxidation in an intestinal ischemia-reperfusion model in rats

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Introduction

Intestinal ischemia-reperfusion (I/R) induces a series of processes that lead to severe gut wall injury finally resulting in tissue necrosis and shock. The role of free radicals in these situations was investigated from several aspects. There are many pathways involved that can generate free radicals in the I/R. In the small intestine the xanthine-oxidase mechanism is one of the most important source of oxygen free radicals while in the colon aldehyde-oxidases are thought to play a similar role (Granger et al., 1981, McCord et al., 1982, Parks et al., 1983). Free radical-caused injury forces endothelial cells to release platelet activating factor and various kinds of leukotriens that trigger neutrophil granulocyte migration towards the damaged mucosa (Granger et al., 1989, Suzuki et al., 1989, Zimmermann et al., 1990, Kubes et al., 1990). Neutrophils via their myeloperoxidase activity contribute to the production of free radicals (Otamiri et al., 1988). Intestinal I/R can also damage the function of distant organs like the lung and heart via the release of different mediators (eg.: tumour necrosis factor, platelet activating factor, leukotriens, prostaglandins) (Caty et al., 1990, Horton et al., 1991).

There were many trials to prevent the deleterious effects of I/R with different drugs like enzyme inhibitors, free radical scavengers, antioxidant enzymes, anti-inflammatory compounds, anti-neutrofil agents and metal-chelators. Deferoxamine is known as the most prominent representative of the latter group and was used successfully to prevent I/R injury (i.e. increased mucosal permeability) in a cat model and improved survival rates after an experimentally induced gastric dilation-volvulus in dogs (Hernandez et al., 1987, Lantz et al., 1992). Furthermore if deferoxamine was added to cardioplegia solution 93% of the left ventricular contractility of the isolated perfused rat hearts was preserved (Ely et al., 1992).

There is growing evidence that supports that nitric oxide (NO) and its derivates play important role in the pathogenesis of intestinal I/R. Nitric oxide is a small lipophylic molecule continuously produced by a constitutive enzyme (constitutive NO synthase, cNOS) expressed in many cells in the intestine and plays important role in the regulation of epithelial permeability (Kubes, 1992). Administration of NO donor (C87-3754) attenuated endothelial dysfunction and improved short-term survival of experimental cats after intestinal I/R (Carey et al., 1992). If rats were given L-arginine (0,5g /kg bw per orally) prior to the establishment of I/R the process of reparation and cell proliferation was more pronounced and levels of polyamines and cGMP also increased. These effects were antagonized by simultaneous administration of NO-synthase inhibitor NG-nitroarginine-methyl-ester (Raul et al., 1995). If cats undergoing intestinal I/R were given NO synthase inhibitor (N- nitro L-arginine methyl ester) the blood to lumen clearance of Cr^{51}-EDTA and I^{125}-albumin was significantly higher than in the control ones. Furthermore L-arginine treatment was able to attenuate these changes (Kubes, 1993). On the other hand NO can take part in different biochemical reactions especially with superoxide to form the harmful peroxynitrite radical (Beckman et al., 1990). There are also a huge number of studies that are focused on the effects of NO, NO-donors, NO-synthesis inhibitors, and peroxynitrite in cardiac, lung and brain I/R, but their results are quite conflicting about the effects of these compounds.

We hypothesized that LP-changes in the intestines undergoing I/R could be reflected in plasma and red blood cell parameters as well. We also aimed to evaluate effects of iron chelation with deferoxamine and NO synthesis enhancement by L-arginine on the aforementioned processes.

Materials and methods

Experimental design

Altogether 56, 3-month-old female White Wistar rats were utilized in this study. Before the experiment the animals were weighed and divided into 4 groups as follows:

The experimental ischemia reperfusion group (Group I/R, n=14): The animals were anaesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg bw). The jugular vein was accessed surgically and blood samples were collected into heparinized collecting tubes. Afterwards, midline laparotomy was performed and the cranial mesenteric artery was ligated using atraumatic surgical clamps. After 30 minutes 1 ml of physiological saline solution (Salsol A infusion, Baxter, Toronto, Canada) was injected into the jugular vein of the animals as the vehicle of the active ingredients used in the treated groups. Another 15 minutes later blood sample was taken from the jugular vein again and full thickness specimens were harvested from the ischaemic jejunum from 7 animals. In the other half of the animals in this group, blood flow was restored in the cranial mesenteric artery and blood sampling was repeated and jejunal sampling was completed after 45 minutes. After collection of the intestinal samples the animals were euthanized by overdosing sodium pentobarbital.

L-arginine (Group A, n=14) and Deferoxamine (Group D, n=14) treated groups: The animals underwent the same procedure as the ones in group I/R, except that L-arginine (300 mg/kg bw, dissolved in physiological saline solution) or deferoxamine (50 mg/kg bw, dissolved in physiological saline solution) was injected to the animals 15 minutes prior to reperfusion. Both chemicals were supplied by Sigma-Aldrich Ltd. Budapest, Hungary.

Sham operated group (Group SOP, n=14). Four rats were anaesthetized and jejunal samples were collected immediately after anaesthesia to serve as negative controls for the operated groups. Samples were collected from the rest of the animals according to the same scheme as in group I/R except no ligature of the cranial mesenteric artery was performed.

The experiment was approved by the Local Supervising Committee for the Use and Welfare of Experimental Animals (25-9/2000 at the Faculty of Veterinary Science, Szent István University, Budapest).

Sample handling

After sampling blood samples were immediately centrifuged at 3000 rpm for five minutes, plasma was removed and red blood cell haemolysates (1:9 v/v with redistilled water) were prepared. Jejunal specimens were flushed with physiological saline solution then approximately half gram was homogenized with 4,5 ml of physiological saline solution. All samples were frozen and stored at -20°C to await the biochemical analysis for maximum 5 days.

Analytical procedures

Erythrocyte and intestinal glutathione-peroxidase (GPX) and superoxide-dismutase (SOD) activities were determined using commercially available kits (Ransel, and Ransod kits, respectively; manufactured by Randox, Cork, Ireland). The concentration of thiobarbituric acid reactive substances (TBARS) in RBCs and intestinal homogenates were measured according to Placer et al. (1966). Overall concentration of lipid peroxidation end products malondialdehyde and 4-hydroxi-nonenal in the jejunal samples was assessed by the LPO-586 kit (Oxis Int. Inc. Bioxytech® LPO-586 Cat. No.: 21012D). The assay is based on the reaction of a chromogenic reagent N-metyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals at 45 °C. Plasma nitric oxide (NO) concentration was measured via the detection of its metabolites nitrite and nitrate (reduced to nitrite with nitrate-reductase) with the Griess-Ilosvay reagent according to Grisham et al. (1996). The ferric reducing ability of plasma (FRAP) was assessed as described by Benzie and Strain (1996). In brief, this method is based on the reduction of ferric-tripyridyltriazin reagent to the ferrous form, i.e. by antioxidants in the sample, which has an intense blue colour that is measurable spectrophotometrically at 593 nm wavelength. Most parameters (except plasma FRAP and NO) are given per gram protein content of the samples. Total protein concentration was determined spectrophotometrically with the biuret reagent (purchased from Diagnosztikum Ltd. Budapest,

Hungary). All other chemicals for antioxidant parameters were purchased from Sigma-Aldrich Ltd. Budapest, Hungary.

Statistical analysis of the laboratory data was completed by the help of Microsoft Excel 5.0 and the Statgraphics 6.0 (Manugistics Inc. 2115, Rockwille, MD, US.) software programs. Data of the intestinal samples and differences of results between each sampling time of plasma and RBC indices (between groups) were compared by two samples Student's t-tests, plasma and RBC results were checked by paired t-tests (within groups). A P value less than 0.05 was considered significant.

Results

There was no significant change of intestinal TBARS concentration in group SOP and D whilst some 2-3 times higher levels ($p<0.05$) were seen in groups I/R and A from the 45th to the 90th minute (i.e. during reperfusion) (414±172 to 941±312 and 378±90 to 1252±415 nmol/g protein, respectively). Furthermore intestinal TBARS concentration was significantly higher after reperfusion in these two groups than in group SOP and D (941±312 and 1252±415 nmol/g protein compared to 433±117 and 270±50 nmol/g protein, respectively (Figure 2).

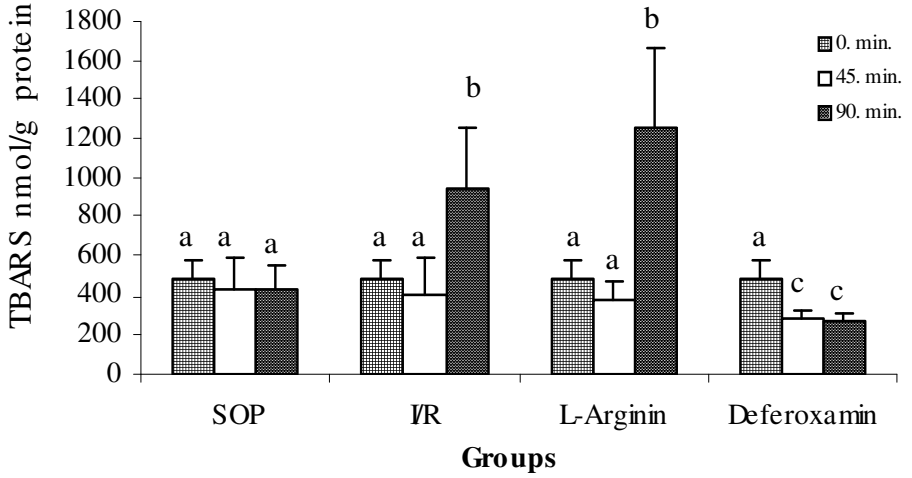

Figure 2. Intestinal TBARS concentration (nmol/g protein). Average ± SD. Columns marked with different letters indicate significant difference of results

Overall concentration of intestinal malondialdehyde and 4-hydroxi-alkenals (M4HN) is shown on Figure 3. There were no change revealed in groups SOP, A and D, however there was 77-87% higher M4HN concentration observed in group I/R after 45 minutes of ischemia staying approximately at the same level after reperfusion as well (98±29 vs.177±43 and 187±64 nmol/g protein, respectively)($p<0.05$). Furthermore post-reperfusion values in group I/R were significantly higher than the corresponding ones in any other group (Figure 3).

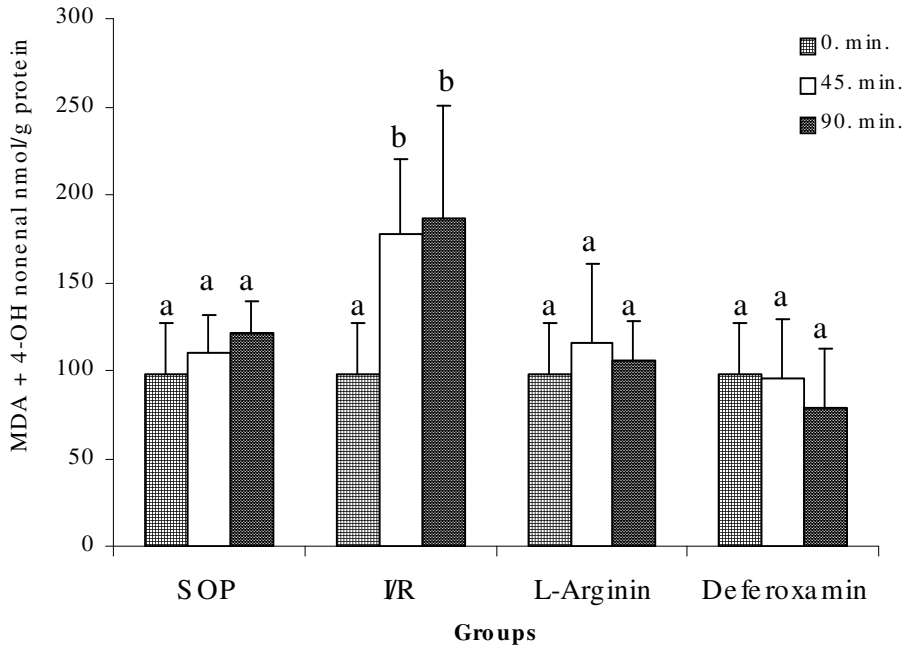

Figure 3. Intestinal MDA and 4-OH nonenal concentration (nmol/g protein). Average ± SD. Columns marked with different letters indicate significant difference of results

The only significant change of the intestinal SOD activity was an increase from 270±54 to 531±174 U/g protein that was found in the SOP group during the first 45 minutes of anaesthesia. Then the values showed about at the same levels for the rest of the experiment (Figure 4). The same tendency was observed in all the other groups.

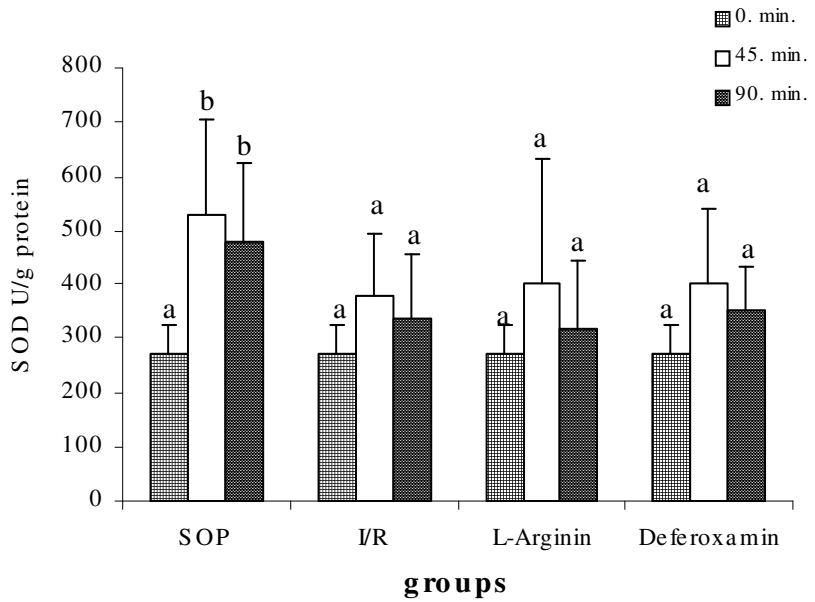

Figure 4. Intestinal SOD activity. Average ± SD. Columns marked with different letters indicate significant difference of results

Intestinal GPX activity showed no significant difference in any group (Figure 5)

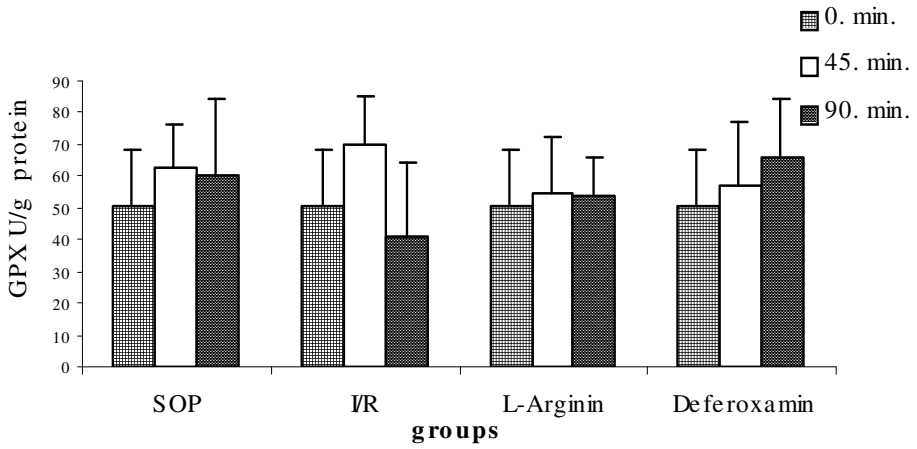

Figure 5. Intestinal GPX activity. Average ± SD

FRAP values did not change in the SOP group. Significantly higher FRAP concentrations measured were in groups I/R, A and D in samples taken at 90 minutes of anaesthesia (1.13±0.27, 2.9±1.64 and 2.25±0.85 mmol/l, respectively) than in those harvested at 45 minutes. In addition FRAP concentration also increased significantly during the first 45 minutes in Group D from 0.70±0.17 to 1.29±0.39 mmol/l (Figure 6).

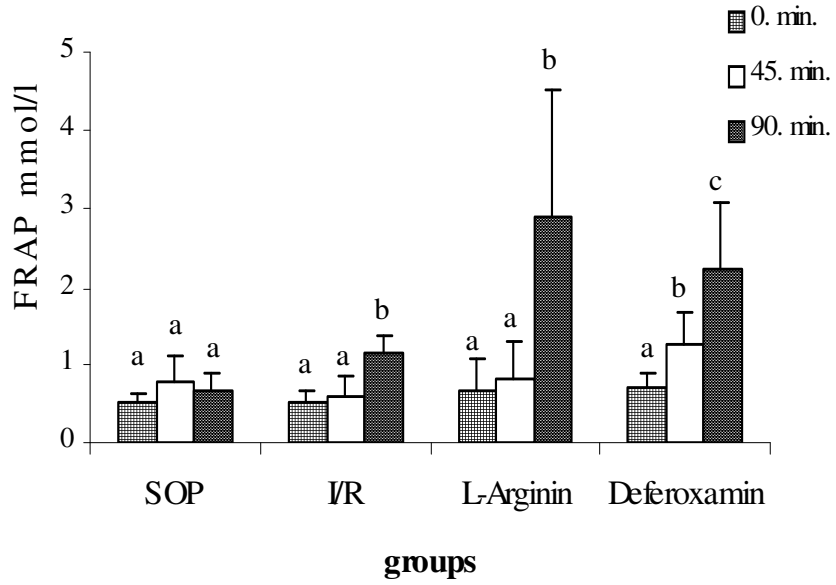

Figure 6. Plasma FRAP values. Average ± SD. Columns marked with different letters indicate significant difference of results within groups

During ischemia, FRAP values showed significantly higher increase in Group D compared to group I/R. By the end of reperfusion the change of FRAP concentration in group I/R (+0.61±0.18 mmol/l) was significantly higher than in the Group SOP (-0.35±0.33 mmol/l) but was significantly lower than in group D (+1.5±0.57 mmol/l). Comparing the changes of FRAP concentration during

the whole experimental period only Groups I/R and D differed significantly (0.69±18 and 1.95±0.95 mmol/l).

Plasma NO concentration did not show any change between sampling times in Groups SOP and I/R (Figure 7). However, there was a significant increase upon reperfusion from 18.0±3.1 to 28.9±7.2 µmol/l in Group D. Similarly, differences of NO concentration were significant only between Group I/R and Group D, considering either 45 or 90 minutes sampling.

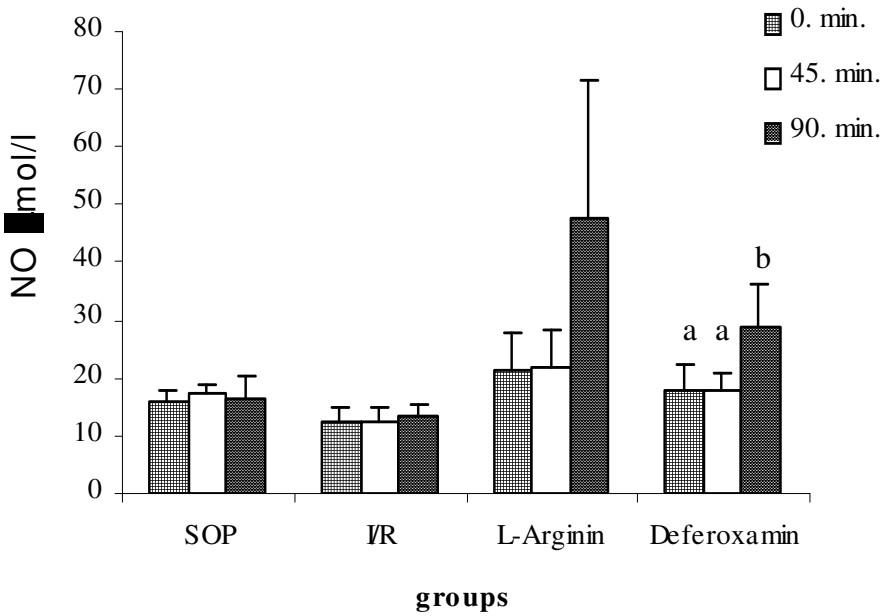

Figure 7. Plasma NO concentrations. Average ± SD. Columns marked with different letters indicate significant difference of results within groups

Erythrocyte TBARS concentration was significantly higher (430±73 nmol/g protein) than the basal value (197±33 nmol/g protein) after 90 minutes in Group I/R; however no significant change was observed at 45 minutes after ischemia compared to the basal values of this group. Similar but not so marked elevation was observed in Group A as well (301±26) vs. (258±30 nmol/g protein) ($p<0.05$; Figure 8).

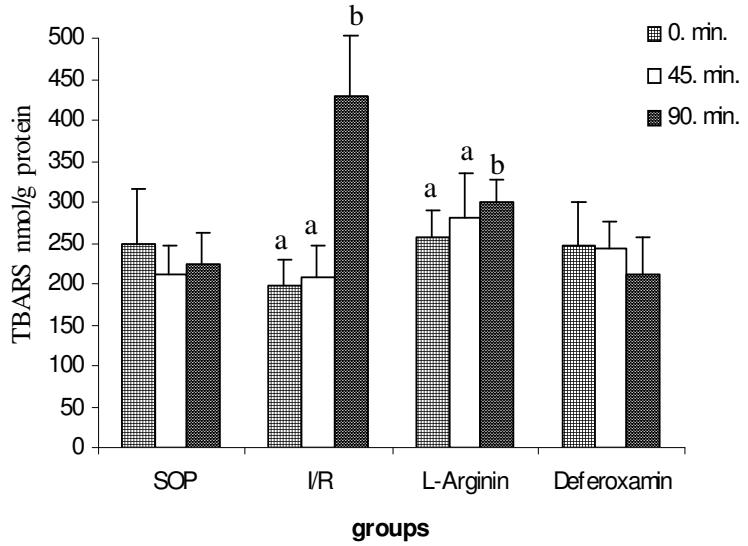

Figure 8. Erythrocyte TBARS concentrations. Average ± SD. Columns marked with different letters indicate significant difference of results within groups

Figure 9 represents that during reperfusion RBC GPX activity decreased from 206±36 to 143±32 U/g protein in Group D. Furthermore the change of RBC GPX activity was significantly different between Group I/R from Group D (+17±35.2 vs. –68.2±64 U/g protein).

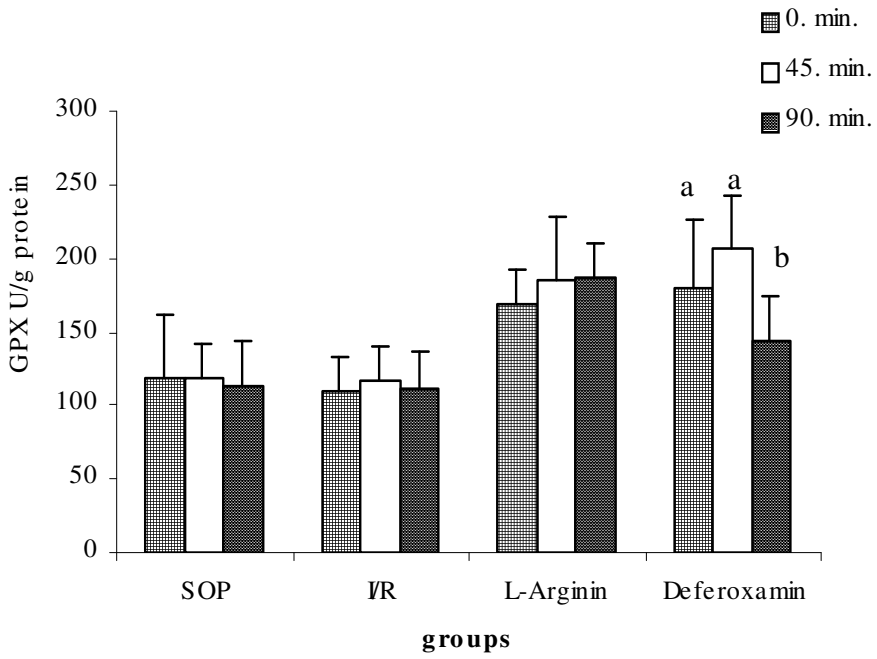

Figure 9. Erythrocyte GPX activities. Average ± SD. Columns marked with different letters indicate significant difference of results within groups

The RBC SOD activity increased significantly during ischemia from 313±61 to 391±31 then returned to the initial values of 307±94 nmol/g protein in Group I/R. Similar tendency charcterised this parameter in Groups A and D as well (Figure 10).

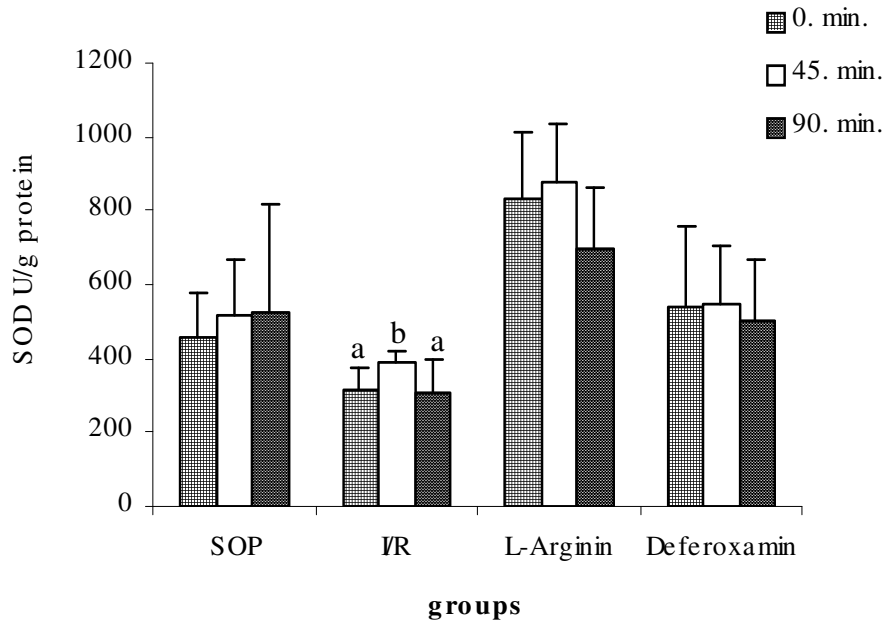

Figure 10. Erythrocyte SOD activities. Average ± SD. Columns marked with different letters indicate significant difference of results within groups.

Discussion

The only significant change observed in the SOP group is the slight increase of SOD activity in the first 45-minute period indicating that the anaesthesia that was reversed by the much greater damage of enzyme activity caused by I/R in all the other groups. Apart from this anaesthesia and laparotomy had no other effect on LP processes.

Increasing intestinal TBARS and M4HN concentration indicates that lipid peroxidation occurred in the jejunal specimens during reperfusion in the I/R group. At the same time the elevation of intestinal TBARS concentration was preceded by an increase in the M4HN concentration during ischemia in this group. (Figures 2 and 3). This incongruence can be explained by the different specificity of the two methods. Many other molecules besides malondialdehyde can react with thiobarbituric acid intefering with the results obtained by the method we applied. It is also possible that a large portion of lipid peroxidation end products is masked by protein-bound complexes reacting with thiobarbituric acid only after hydrolysis that was not performed in this study (Matsuki et al., 1991). We also have to consider that malondialdehyde is a by product of enzymatic thromboxane formation which may occur during intestinal I/R and therefore the malondialdehyde produced in this pathway interfere with LP originated molecules using TBARS or M4HN method.

The situation in case of the L-arginine treated group is even more perplexing as the huge TBARS peak is not reflected in the M4HN results. This may suggest that L-arginine treatment was able to reduce the formation of MDA and 4-OH-nonenal but not other TBA reactive by products of LP. Some data support the latter findings as L-arginine and nitric oxide donors were found to decrease intestinal epithelial permeability especially within the first two hours of reperfusion (Kubes, 1992, Payne and Kubes, 1993, Kanwar et al., 1994, Kawata et al., 2001). Others have not found beneficial effects of increasing the nitric oxide level (ie.: administration of L-arginine or NO donor molecules) and warn that nitric oxide may form peroxynitrite radical with superoxide anion which can even worsen the reperfusion injury in heart and brain I/R models (Beckman et al., 1990; Schulz and Wambolt; 1995, Lopez et al., 1997). Furthermore, evidence was produced for peroxynitrite-formation in intestinal I/R related with constitutive nitric-oxide-synthase (cNOS) activity in a rat experimental model similar to ours (Cuzzocrea et al., 1998).

Erythrocyte TBARS concentration reflects the intestinal events showing that the increased intestinal LP escalated to RBCs as well, though the amplitude of changes is considerably less than in the intestinal samples (Figure 2. and 8.).

The intestinal process did not influence plasma NO level in the I/R group (Figure 7.). This matches the findings of the authors cited above who found no increase in plasma NO level and intestinal inducible nitric oxide synthase (iNOS) expression (Cuzzocrea et al., 1998). In contrast to these, a recent publication reports elevated plasma NO concentration in horses with naturally acquired small intestinal strangulation (Mirza et al., 1999). Other researchers also found increased NO levels during intestinal I/R in wild mice in contrast to iNOS knockout ones, (Suzuki et al., 2000). We found that both L-arginine and deferoxamin administration induced higher plasma NO levels. In case of L-arginine obviously due to increased substrate supply for NO-synthases while in case of deferoxamine probably by preventing iron catalysed superoxide formation that could have reacted with nitric oxide to form peroxynitrite. This molecule is bound by various proteins as nitrotyrozine thereafter. We also have to consider that deferoxamine decreases the non-haem iron pool of cells that are thought to bind NO in the form of different complexes (Keberle 1964; Henry et al., 1991; Reif, 1993). Finally we cannot even rule out with hundred percent certainty that NO is released during the decomposition of deferoxamine in the organism. At the same time in group D a slight decrease was found in the intestinal TBARS and M4HN concentrations showing that the applied deferoxamine therapy was able to protect the tissues from I/R triggered LP (Figure 2 and 3). These findings are well in line with those of Hernandez et al. (1987) who could prevent the development of I/R induced increased intestinal vascular permeability by iron-chelation with deferoxamine and apotransferrin in a cat model. Similarly deferoxamine improved survival rates of dogs with surgically induced gastric dilation volvulus (Lantz et al., 1992). Like in other groups,

erythrocyte TBARS concentration followed similar pattern to the intestinal changes in Group D indicating that deferoxamine administration had beneficial effect of LP processes of RBCs (Figure 8).

Intestinal SOD-activity showed significant increase in Group SOP after the first 45 minutes period possibly due to the anaesthetic procedure and laparotomy and was followed by a slight, not significant decrease. Similar tendencies were observed in all the other groups as well as in case of intestinal GPX activity except that the decrease of GPX activity was reversed by the administration of deferoxamine (Figures 4 and 5).

The SOD activity of erythrocytes followed the changes found in the intestine but no unequivocal tendency was observed in the GPX activity (Figure 9 and 10).

Plasma FRAP concentrations showed marked elevation after reperfusion in all groups where I/R was accomplished (Figure 6). This may serve as a footprint of uric acid release from the reperfused intestinal segments as, according to the original publication of Benzie and Strain some 60% of plasma FRAP is given by the uric acid produced by the xantin oxidase mechanism from hypoxanthine, the intermediery molecule of ATP degradation (Granger et al., 1981, Benzie and Strain, 1996). Unfortunately uric acid was not assayed in this study but assuming that approximately the same amount of this substance got to circulation in all these groups could explain the significantly bigger increase of FRAP values in the treated groups showing that L-arginine and deferoxamin treatment improved the overall plasma antioxidant power (Figure 6). It is interesting that in Group D the elevation of FRAP values were recognised already 15 minutes after drug administration possibly due to the direct hydroxyl-radical scavenging effect of deferoxamine that had previously been evidenced in other studies (Menasche et al., 1987, Morehouse et al., 1987).

It is concluded that intestinal I/R induced changes in LP parameters are reflected in the plasma and red blood cell parameters as well. Deferoxamin treatment was proven to be beneficial in the prevention of I/R induced LP, but the role of L-arginine and nitric oxide remains controversial and necessitates further investigation.

Summary

This study investigated lipid peroxidation (LP) changes during intestinal ischemia-reperfusion (I/R) with and without desferrioxamine and L-arginine treatment. Concentration of thiobarbituric acid reactive substances (TBARS), overall concentration of malondialdehyde and 4-hydroxy-alkenals (M4HN), activities of superoxide-dismutase (SOD) and glutathione-peroxidase (GPX) of the jejunal homogenates were determined. The same analytes except M4HN were assayed in RBC haemolysates. Measurements of ferric reducing ability (FRAP), total antioxidant status (TAS) and nitric oxide (NO) concentrations of plasma samples were also completed. Data were analysed by two samples and paired Student's t-tests, significance level were set at $p<0.05$.

The only significant change observed in the SOP group was increased SOD activity after the ischaemic period. In the I/R group significant increase of intestinal M4HN concentration was observed during hypoxia that was followed by similar changes in intestinal and RBC TBARS and plasma FRAP values upon reperfusion. In group D the intestinal TBARS and M4HN concentrations were significantly lower while FRAP and NO concentrations were significantly higher compared to the I/R group. At the same time RBC TBARS concentration and GPX activity significantly decreased within group D. In group A the intestinal M4HN concentration was significantly lower than in the I/R group. Plasma FRAP and NO concentration showed similar changes to group D.

is concluded that I/R increased the LPO in the intestinal tissue and altered some parameters of plasma and RBCs, too. Desferrioxamine treatment prevented these effects, while the usefulness of L-arginine remains doubtful.

Evaluation of acid-base and antioxidant indices in horses operated on for colic

Introduction

Colic in horses is one of the major causes of death in this species therefore it is of outstanding importance. Though surgical techniques and intensive therapy improved in a huge extent over the last decades there is still high mortality among patients that are finally operated on for colic. This gave rise to enormous research on this topic.

Presently it is established that intestinal ischemia leads to almost complete ATP loss in the affected intestinal segments and that is not completely reversed upon reperfusion. During ischemia ATP is decomposed to hypoxanthine (a substrate for XO/XDH enzyme) that is accumulated in the tissues. Cellular energy deficiency leads to a series of changes. Intra-and extracellular concentration of sodium and potassium increases (McAnulty et al., 1997). In the jejunum the XDH enzyme is converted (by sulphidril oxidation, and proteolytic pathways) to XO form that is known to produce superoxide radicals in the presence of oxygen (i.e.: reperfusion) (Prichard, 1991) In case of intestinal I/R the concentration of lipid peroxidation end products MDA and conjugated diens, was found to be increased (Sullivan et al., 1990; Kooreman et al., 1998). These are accompanied by decreased SOD activity and GSH concentration in the colon, while no change in GPX and G6P activities were observed (Sullivan, 1990; Reeves, 1990). The importance of SOD function was underlined by the finding that in case of in vitro incubation of colonic tissue flaps in SOD containing solution the anoxia induced L-alanine loss and oedema formation could be prevented (Johnston et al., 1991). Cellular and tissue level metabolic changes were mirrored in decreased colonic venous pH, pCO2, pO2, haemoglobin saturation, arteriovenosus oxygen gradient, as well as plasma lactate and glucose concentrations (Kawcak et al., 1995).

Histologically I/R in the horse gut is characterised by decrease in vascular network, epithelial sloughing, serosal and subepithelial oedema formation, shortening of the villi decrease in epithelial surface, neutrophil accumulation in the lamina propria and erythrocyte diapedesis through the damaged endothelium (Arden et al., 1990; Dabareiner et al., 1993; Vatistas et al., 1993; Horne et al., 1994). Epithelial cell degeneration takes place gradually and spreads to almost all cells by the 4^{th} hour of complete ischemia (Snyder et al., 1998). An important component of oedema formation is submucosal albumin accumulation that further augments the oedema itself and the fluid loss from the circulation (Reeves et al., 1990). Neutrophil originated MPO activity was detected in several models and is thought to be one of the major causes of reperfusion injury to the horse intestine as well as a contribution factor to the development of peritoneal adhesions in case of small intestinal resection (Vatistas et al., 1993; Yarbrough et al., 1994; Gerard et al., 1999; Inoue et al., 1998).

Recently NO mediated pathways were found to play important role in intestinal I/R in horses (like in other species) as significantly higher NO levels were found in plasma and intestinal fluid of horses suffering from naturally acquired intestinal ischemia. Besides increased immunohistochemistry staining for iNOS and nitrotyrozine was detected in the affected intestinal segments (Mirza et al., 1999).

Many trials were designed to find chemicals that can protect the intestinal tissue from I/R injury. DMSO was one of the popular drugs the effects of which were investigated but no protective role could be proven (Arden et al., 1990; Moore et al., 1995; Reeves et al., 1990; Horne et al., 1994). MnCl2 having a SOD like action increased only the blood pressure but had no effect on the otherwise increased prostaglandin levels in ischemia (Moore et al., 1994). Allopurinol an inihibitor of XO also gave contradictory results in equine intestinal I/R (Lochner et al., 1989, Moore et al., 1995). Synthetic water-soluble vitamin E derivatives and treatment with complex (macromolecules, antioxidants) infusion solution seem to yield the best results (Dabareiner et al., 1994; Vatistas et al., 1993; Moore et al., 1995)

For better understanding I/R induced changes in horses operated under general anaesthesia one must consider the effects of anaesthesia itself.

It was revealed that increasing the inspired O_2 fraction results in higher expired pCO_2, pO_2, arterial pO_2, pCO_2 as well as arterio-alveolar pO_2 difference but does not alter systemic arterial pressure, central venuos pressure, pulmonary artery pressure, arterial pH and ABE (Cuvelliez et al., 1990). It is also clear that horses under halothane anaesthesia positioned in dorsal recumbency develop lower pO2 than those positioned in lateral recumbency (Steffey et al. 1987). Furthermore, positive end pressure ventilation was proven to be a useful tool in maintaining blood gas values between the reference range (Steffey et al. 1987, Day et al. 1995). Another important aspect is that what kinds of processes take place in the postnarcotic period when horses are disconnected from the ventilatory machine but are still recumbent. In one study authors found significant hypoxaemia in horses with no additional O_2 supply both in dorsal and lateral recumbency or when they were turned to their other side. Administering 10L/min 100% did not, while 50L/min did improve the oxygenation of these animals (Mason et al., 1987)

The present study focuses on alterations of acid base and blood gas parameters in the perioperative period as well as changes in antioxidant indices of plasma and RBCs hypothesizing the intestinal I/R would cause measurable changes in them.

Materials and methods

Animals

The study was completed in a clinical setting. Altogether 14 colic horses of the following breed sex and age were used:

Sex: 6 mares, 5 stalions, 3 geldings.

Breed: 7 Hungarian sport horses, 2 Thoroughbreds, 2 Arabians, 1 Shagya-arab, 1 Lipican, 1 Cold blooded.

Age: 6 months – 10 years.

Intra-operative diagnoses yielded the following results:Left dorsal displacement of the large colon:

-Nephrosplenic intrapment of the large colon (3)
-accompanied by 180° torsion (2)
Retroflexion of the pelvic flexure (2),
Right dorsal displacement (360° torsion of the large colon) (1),
Ileocaecal intussusception (1),
Scrotal herniation of the ileum (1),
Strangulation of the jejunum (4).

Nine horses undergoing orthopedic surgery with the same anaesthetic and recumbency protocol were used as controls for the colic patients.**Sample collection and handling**

Blood samples were harvested from the jugular vein of the animals into heparinized collecting tubes at the following times:

Before the induction of anaesthesia (xylazin-ketamin-diazepam) (1st) right after anaesthesia (dorsal recumbency, halothane+ oxygen) but before the opening of the abdomen (2nd), 30 minutes, 1, 2, 12, 24 hours after the opening of the abdomen (3rd, 4th, 5th, 6th, 7th samples). Blood flow was restored in the displaced intestinal segments 15-45 minutes after the opening of the abdomen.

Immediately after sampling blood gas analysis was performed on a Radiometer ABL 330 analyzer to determine pH ABE, HCO_3^-, TCO_2, pCO_2, pO_2 and oxygen saturation of hemoglobin (SAT). After the completion of the blood gas measurements plasma was separated by centrifugation and red blood cell haemolysates were prepared. All samples were frozen and stored at -20°C to await the biochemical analysis for maximum 5 days.

Analytical procedures

Erythrocyte glutathione-peroxidase (GPX) and superoxide-dismutase (SOD) activities were determined using commercially available kits (Ransel, and Ransod kits, respectively; Randox, Cork, Ireland). Palsma uric acid concentration was determined also by a commercially available kit (Húgysav kit) purchased from Diagnosztikum Ltd. Budapest, Hungary. The concentration of thiobarbituric acid reactive substances (TBARS) in RBCs were measured according to Placer et al.,

(1964). Plasma nitric oxide (NO) concentration was measured via the detection of its metabolites nitrit and nitrate with the Griess-Ilosvay reagent according to Grisham et al. (1996). The ferric reducing ability of plasma (FRAP) was assessed as described by Benzie and Strain (1996). Concentrations of erythrocyte reduced (GSH) and oxidized (GSSG) glutathione were determined according to Sedlak and Lindsay, 1968, and Kosower et al., 1969, respectively). Most parameters are given per gram protein content of the samples that was determined spectrophotometrically with the Biuret reagent.

Statistical analysis

The statistical analysis of the laboratory data was completed by using the Microsoft Excel 5.0 and the Statgraphics 5.1 software programs. Within groups data were analysed by a one way analysis of variance (ANOVA) according to sampling times. Multifactor analysis of variance was used to determine difference between data of the two groups. Two samples t-test was performed to determine difference between groups in samples of the same sampling times. Significance level was set to $p<0.05$.

Results

No significant change over time was observed in any parameter in the control group. Horses operated with colic showed significant changes over time of venous blood pH, ABE, pCO_2, pO_2, HCO_3^-, TCO_2, and oxygen saturation, as well as plasma uric acid and NO, and RBC TBARS concentration. No significant changes over time were observed in other antioxidant indices including plasma FRAP and erythrocyte SOD, GPX activities and GSH and GSSG concentration (One-way ANOVA). Significant interaction occurred between sampling time and the type of groups in case of pH, pCO_2, ABE, uric acid and NO concentration. Results of the two group (regardless of sampling time) were significantly different in case of all measured parameters except SAT, TCO_2, GSH and GSSG concentration.Horses affected with colic were presented with metabolic acidosis as read from significantly lower pH (7,2 ± 0.05) and BE (-2.54±1.58 mmol/L) than the control ones (7.38 ± 0.01 and + 2.62 ± 0.93 mmol/L, respectively) (Figure 11 and 12).

Upon anaesthesia colic horses exhibited marked mixed type acidosis - pH sinking to 7.09 ± 0.04 by the second hour of anaesthesia accompanied by BE –10.32 ± 3.15 mmol/L, pCO_2 66 ± 5 mm Hg, HCO_3^- 20.6 ± 1.4 mmol/L, TCO_2 23.7 ± 1.3 mmol/L (Figures 11-15). In spite of that oxygen tension and saturation of the mixed venous blood showed significant increase during anaesthesia with a peak of 72 ± 15 pO_2 and 83.5 ± 5.2 oxygen saturation two hours after the incision (Figures 16 and 17).

Plasma uric acid concentrations were some 1,5 times higher in the control group during anaesthesia. Late post-anaesthetic values show opposite picture due to a marked increase seen in the colic horses (Figure 18). Though there are no significant changes over time of FRAP values in either group, colic horses exhibited approximately twice higher concentrations than did the control ones (Figure 19). Plasma NO concentration was significantly higher in the colic horses before the operation (33.8 ± 1.6 vs. 26 ± 1 μmol/L and showed a constant decrease to 17.9 ± 2.9 μmol/L thereafter (Figure 20). Erythrocyte TBARS concentration was higher in the colic group at all the sampling times during the observed period. The highest TBARS concentration (458 ± 49 nmol/g protein) was found 1 hour after incision (ie.: 15-45 minutes after reperfusion) (Figure 21.). Though no significant change over time was revealed in the RBC SOD activities of colic horses there were significantly higher values observed in the first samples (926±114 U/g protein) compared to the 2nd, 3rd and 7th ones (670±37, 636±62 and 575±45 U/g protein, respectively) (Figure 22.). There were no significant alterations observed in the GSH, GSSG (Figures 24. and 25), concentrations and GPX activities though the letter one exhibited mildly lower values in colic horses throughout the observed period (significant only at 30 minutes after the incision) (Figure 23.).

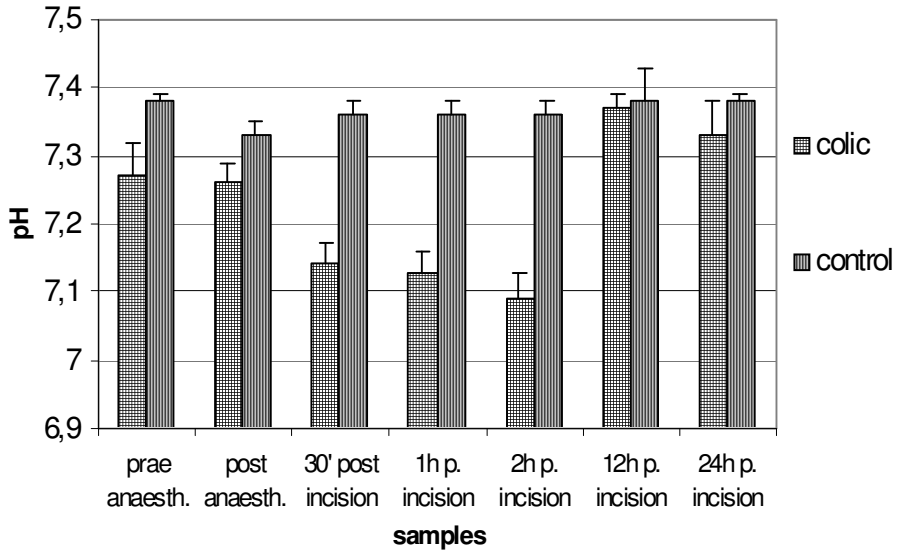

Figure 11. The change of pH in the venous blood of colic (n=14, $p < 0.05$, One–way ANOVA) and control (n=9) horses in the perioperative period. Pattern of change is significantly different in the two groups (Multifactor ANOVA)

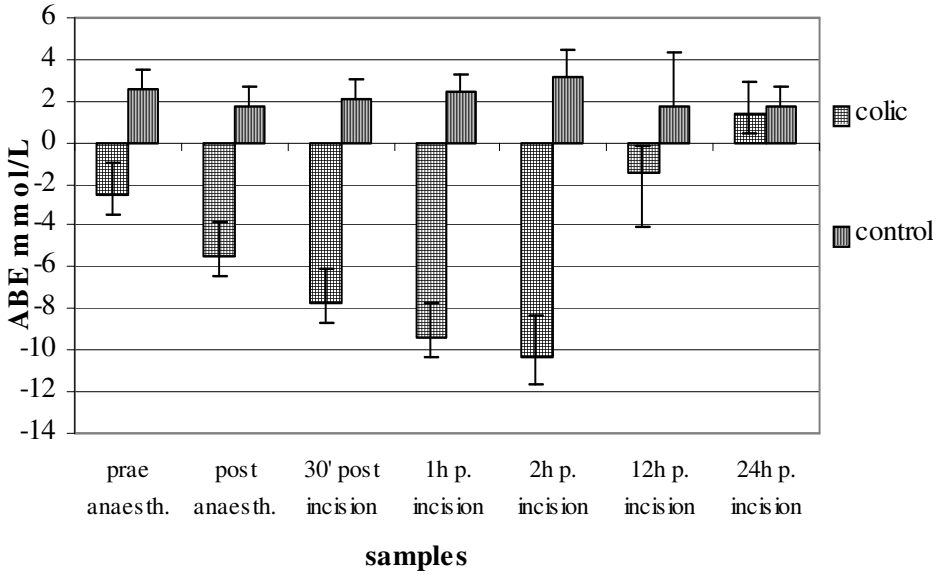

Figure 12. The change of ABE in the venous blood of colic (n=14, $P < 0.05$, ANOVA) and control (n=9) horses in the perioperative period. Pattern of change is significantly different in the two groups (Multifactor ANOVA)

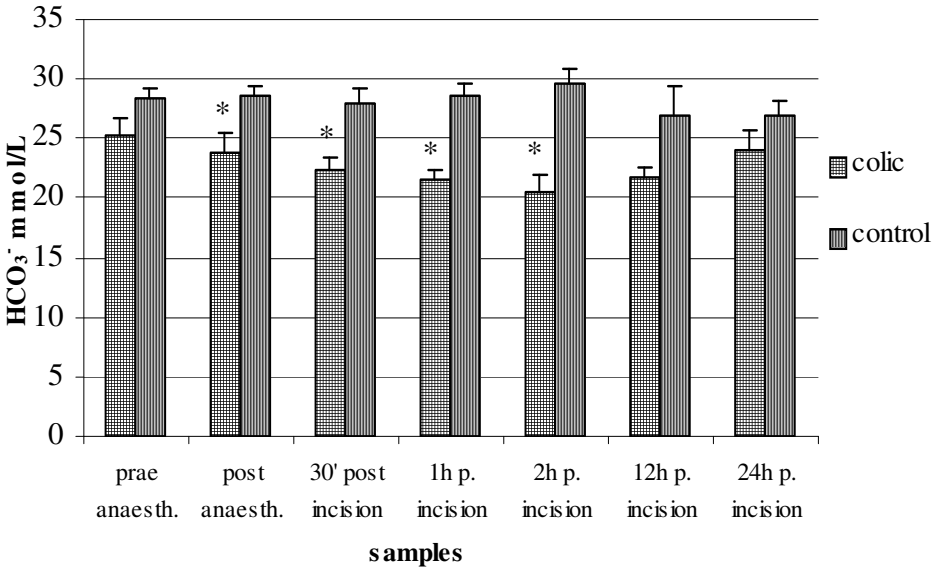

Figure 13. The change of HCO_3^- concentration in the venous blood of colic (n=14, $P < 0.05$, ANOVA) and control (n=9) horses in the perioperative period. An asterisk marks the significant difference between groups at a given sampling time.

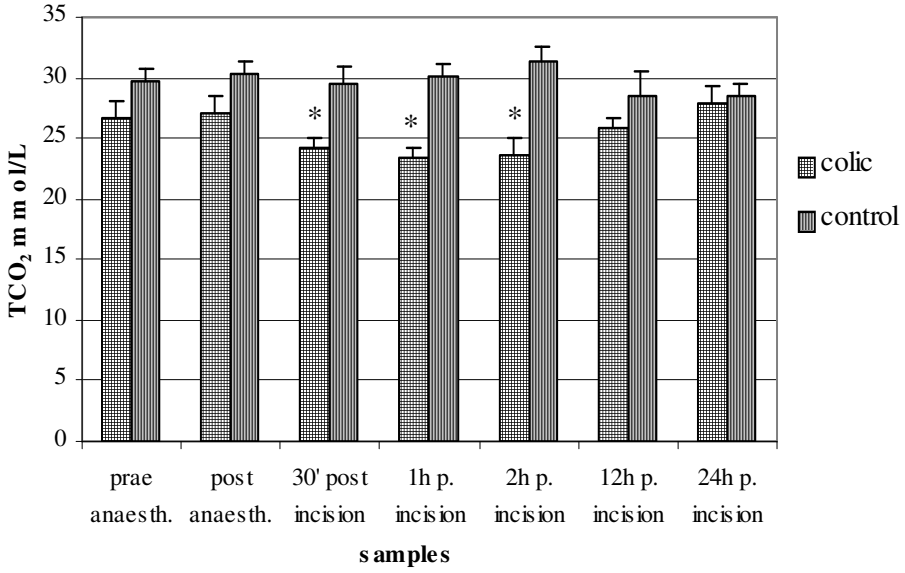

Figure 14. The change of TCO_2 concentration in the venous blood of colic (n=14, $P < 0.05$, ANOVA) and control (n=9) horses in the perioperative period. An asterisk marks the significant difference between groups at a given sampling time.

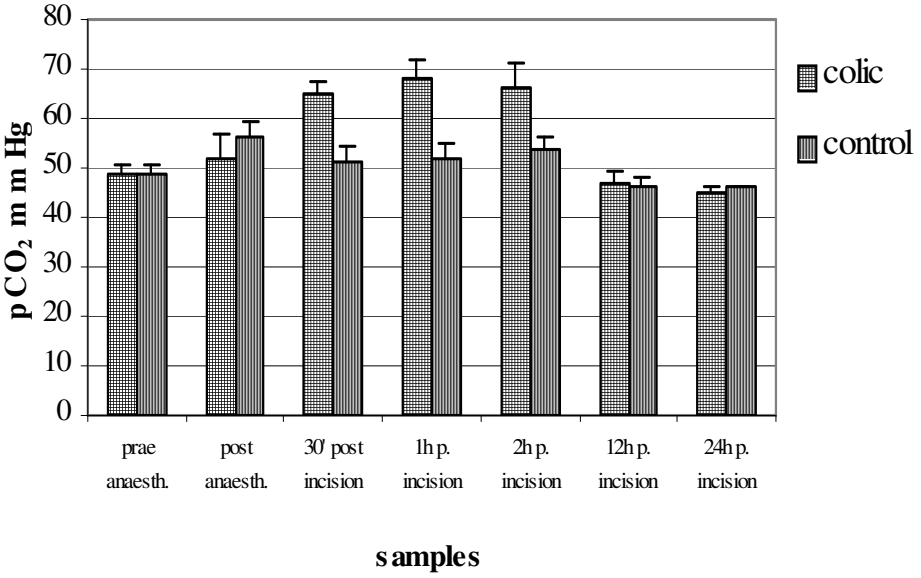

Figure 15. The change of pCO_2 in the venous blood of colic (n=14, $P < 0.05$, ANOVA) and control (n=9) horses in the perioperative period. Pattern of change is significantly different in the two groups (Multifactor ANOVA)

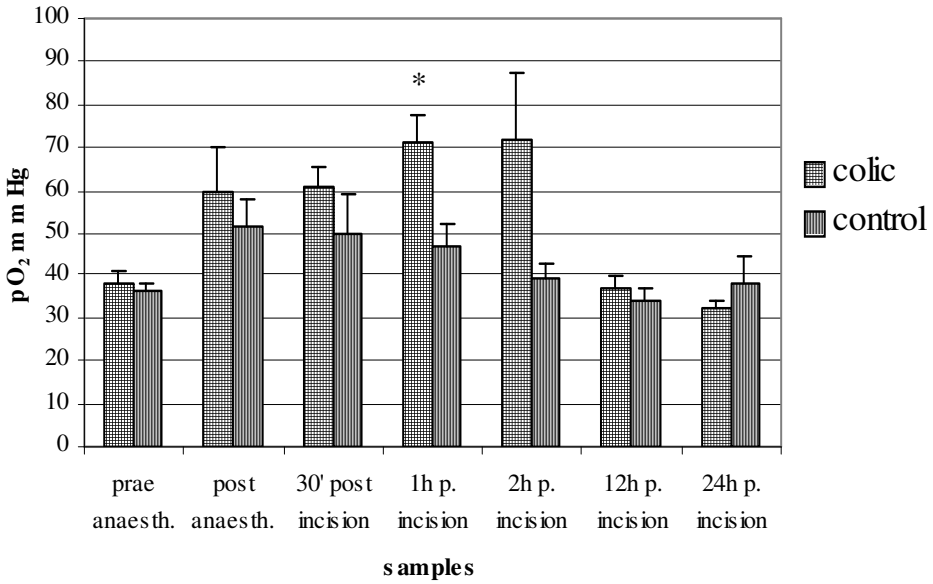

Figure 16. The change of pO_2 in the venous blood of colic (n=14, $P < 0.05$, ANOVA) and control (n=9) horses in the perioperative period. An asterisk marks the significant difference between groups at a given sampling time.

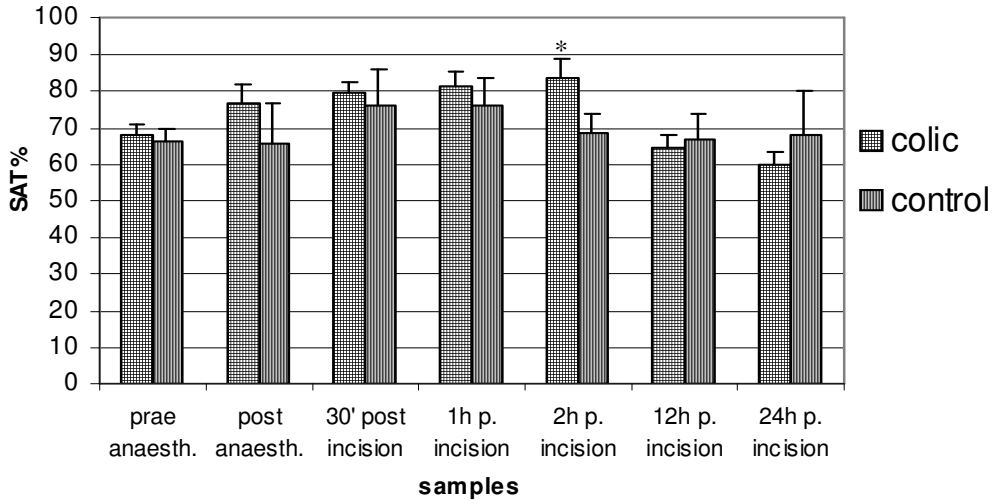

Figure 17. The change of oxygen saturation of haemoglobin in the venous blood of colic (n=14, $P < 0.05$, One-way ANOVA) and control (n=9) horses in the perioperative period. An asterisk marks the significant difference between groups at a given sampling time.

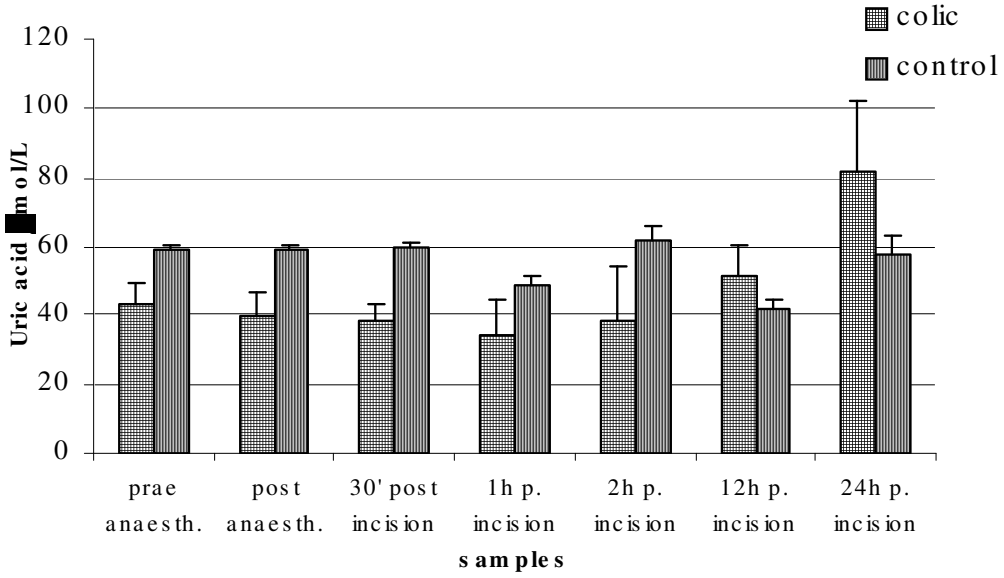

Figure 18. The change of uric acid concentration in the venous blood of colic (n=14, $P < 0.05$, One-way ANOVA) and control (n=9) horses in the perioperative period. Pattern of change is significantly different in the two groups (Multifactor ANOVA)

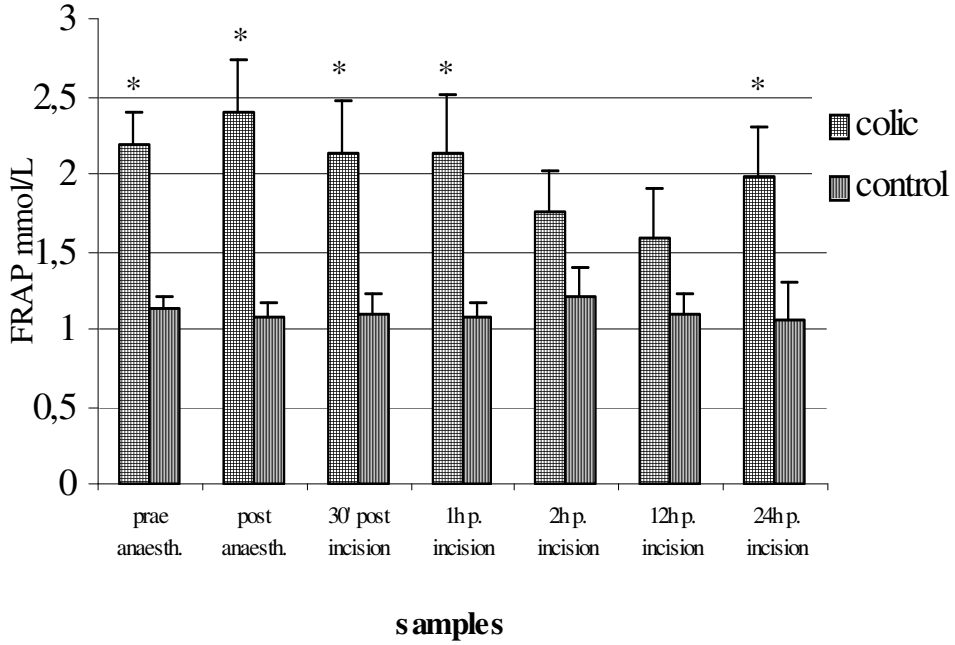

Figure 19. The change of FRAPin the venous blood of colic (n=14) and control (n=9) horses in the perioperative period. An asterisk marks the significant difference between groups at a given sampling time.

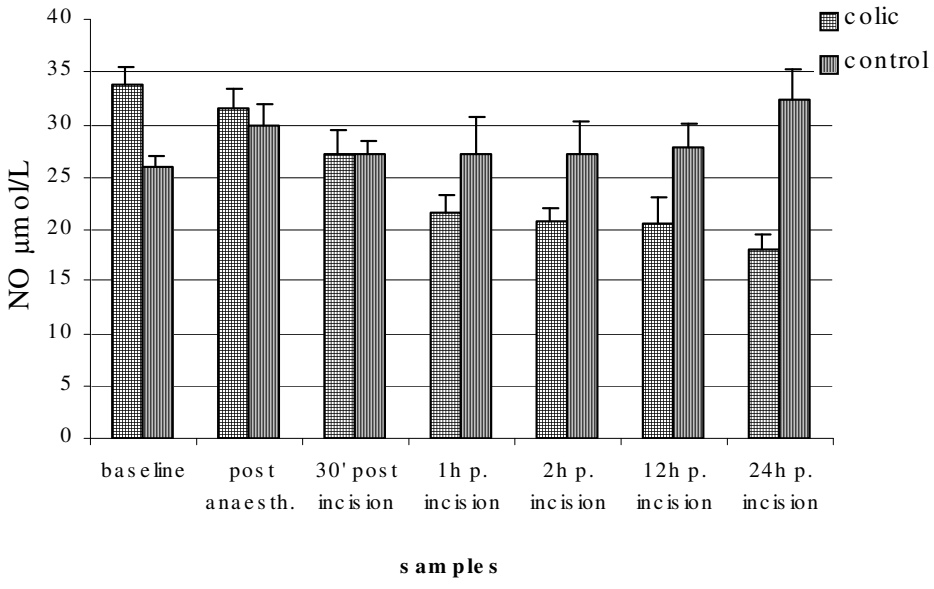

Figure 20. The change of NO concentration in the venous blood of colic (n=14, $P < 0.05$, ANOVA) and control (n=9) horses in the perioperative period. Pattern of change is significantly different in the two groups (Multifactor ANOVA)

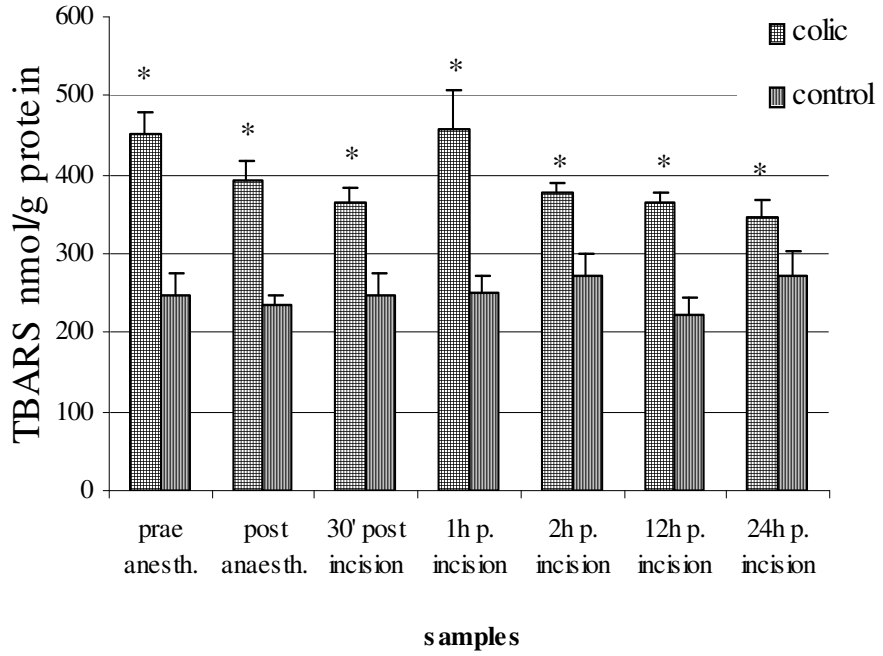

Figure 21. The change of TBARS concentration of erythrocytes in the venous blood of colic (n=14, $P < 0.05$, ANOVA) and control (n=9) horses in the perioperative period. An asterisk marks the significant difference between groups at a given sampling time.

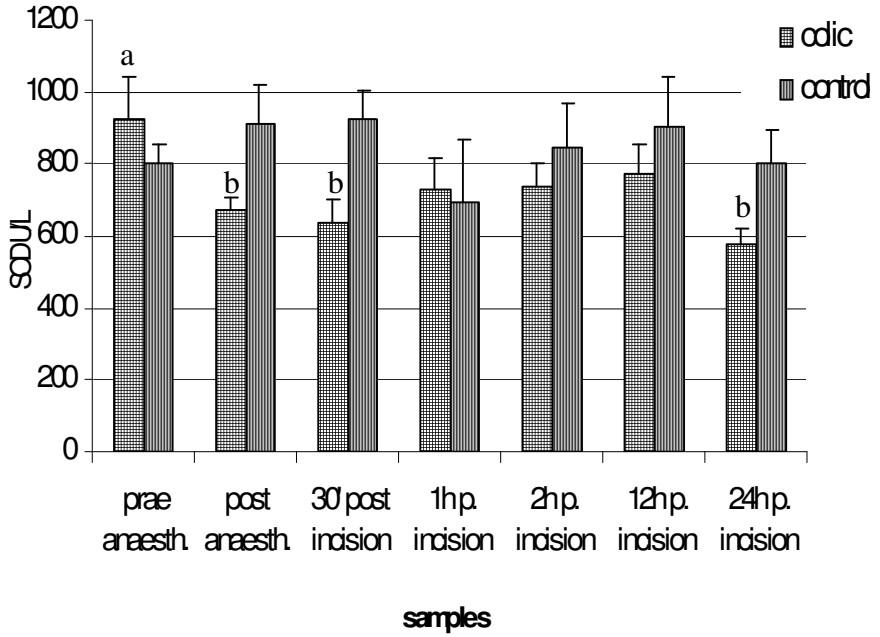

Figure 22. The change of erythrocyte SOD activity of the venous blood of colic (n=14) and control (n=9) horses in the perioperative period. Different letters indicate significant difference betwween the coresponding sampling times within group.

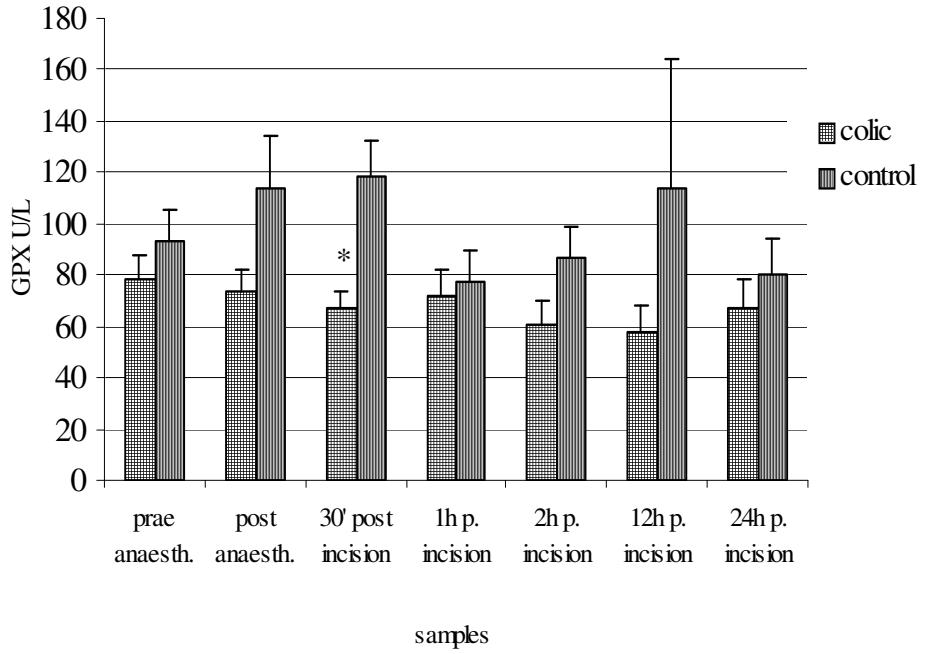

Figure 23. The change of erythrocyte GPX activity the venous blood of colic (n=14) and control (n=9) horses in the perioperative period. An asterisk marks the significant difference between groups at a given sampling time.

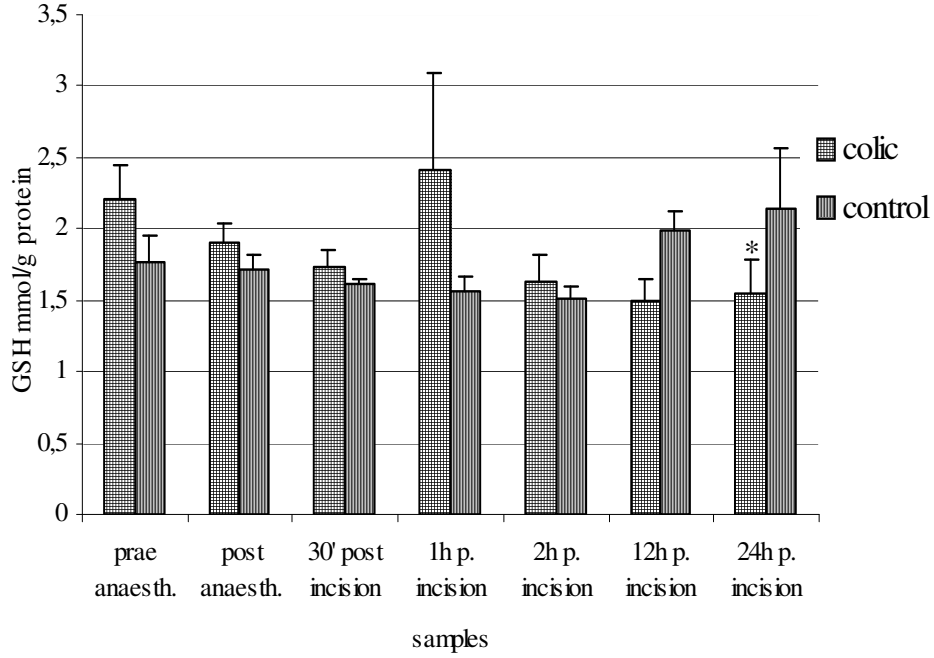

Figure 24. The change of GSH concentration in erythrocytes in the venous blood of colic (n=14) and control (n=9) horses in the perioperative period. An asterisk marks the significant difference between groups at a given sampling time.

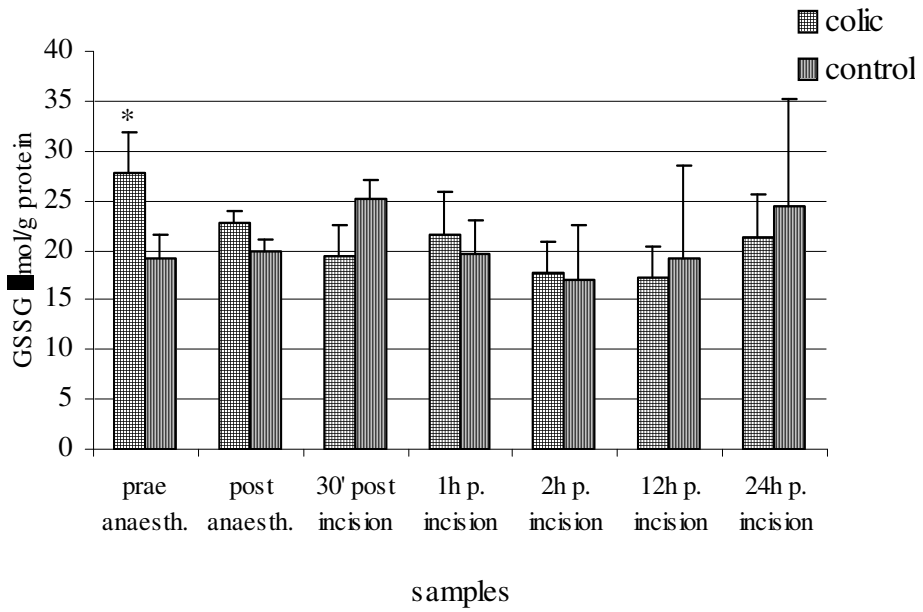

Figure 25. The change of GSSG concetration in erythrocytes from the venous blood of colic (n=14) and control (n=9) horses in the perioperative period. An asterisk marks the significant difference between groups at a given sampling time.

Discussion

Horses operated on for colic involved in this study were presented with moderate uncompensated metabolic acidosis. This is not in line with the findings of Nappert and Johnson (2001) who found lower BE, HCO_3^- and TCO_2 but not pH in horses with similar abdominal disorders. This could have been caused by the different time spent from the beginning of the colic to the arrival at the clinic, which varied between 2-24 hours in this group of horses (unfortunately there is no data in the above mentioned article on this). After the induction of anaesthesia the difference between acid-base and blood gas parameters of the control and colic horses became more dramatic. Though both group of horses were under controlled ventilation and given iv. fluids (Ringer's-infusion, 40% glucose solution) and dobutamin during the anaesthesia these could not prevent further loss of bicarbonate, and accumulation carbon dioxide and obviously the accumulation of other organic acids as seen from the dropping HCO_3, TCO_2, and BE in horses having colic. Efforts to maintain arterial and tissue oxygenation are reflected in the increasing venous oxygen tension and saturation during anaesthesia in the colic group, though similar but not significant tendencies are seen in the control animals as well.

Horses suffering from colic exhibited significantly lower uric acid concentrations than the controls by the time of the first examination at the clinic but showed a significant increase after reperfusion. The reason for the latter phenomenon could be that uric acid is released from the reperfused intestinal segments as a product of the xantin oxidase mechanism that was previously observed to contribute to OFR production in horses and other species (Granger et al., 1981, Prichard et al., 1991). On the other hand another group of authors failed to detect increased XO activity in intestinal I/R though they call attention that the majority of XO is formed in the enterocytes at the tip of the villi, which easily slough after becoming ischaemic. However the uric acid produced by the XO enzyme in these cells could be absorbed afterwards. We also have to consider the possibility that the 24 hours postoperative elevation of uric acid concentration originates from the metabolism of necrotised cellular components (especially nucleic acids) by the liver. At the same time it remains unclear why did colic horses show lower uric acid concentrations at the first 3 sampling times. Plasma NO concentration was significantly higher in the horses with colic than in the control ones and showed constant decrease during and after the surgical procedure finally resulting in significantly lower values than the controls. This agrees the findings of Mirza et al. (1999), who

found increased plasma and abdominal fluid NO concentration in horses with naturally acquired small intestinal obstruction though they did not perform serial sample analysis. The decreasing pattern of NO concentration we found in this group of horses therefore cannot be compared to the results of the aforementioned study. However horses in the cited experiment showed positive iNOS staining in submucosal and mucosal leukocytes and positive nitrotyrosine staining in the latter group of cells. The first finding could explain the higher NO values, the second the decreasing tendency as NO could have been bound by proteins in the form of nitrotyrosine which is formed in the reaction of NO with superoxide (Beckman et al., 1990). Taking into account that superoxide formation is exacerbated upon reperfusion (by the XO mechanism and leukocytes) the decreasing tendency of NO concentration is better understood (Granger et al., 1981). Though the change over time of FRAP values was not significant in the colic patients had significantly higher levels of this analyte than did the control ones. Were the plasma uric acid concentration not determined one could have speculated that this was caused by the accumulation of this compound as uric acid is believed to give 60% of plasma FRAP, at least in human beings (Benzie and Strain, 1996). Though in a previous study we have validated the FRAP assay for equine plasma we did not determine contribution factors for different possible antioxidants co-acting in this parameter (Balogh et al., 2001). Therefore we can only suspect that most likely accumulation of other antioxidants like bilirubin or glucose could have been the major cause of this phenomenon. Higher erythrocyte TBARS concentrations in the colic horses reflect the intestinal lipid peroxidative process with a peak at 1 hour after the incision (15-45 minutes after reperfusion of the ischaemic intestinal segments). To explain these findings we have to consider the following. First, high RBC TBARS prior to anaesthesia indicates oxidative damage of the erythrocytes during the ischaemic period of intestinal I/R possibly due to the developing metabolic acidosis. Second, the increase found during anaesthesia can be of several origin ie.: more pronounced metabolic acidosis, higher oxygen tension, inhalation of halothane, and last but not least OFR radical generation in the affected intestines (XO mechanism, neutrophil infiltration, prostaglandine synthesis) (Granger et al., 1981, Kubes et al., 1990, Otamiri et al., 1988). Our findings are in line with those of Gerard et al (1999) who detected increased intestinal MDA concentration in mid jejunal ischaemic intestinal mucosal scraping samples and Kooreman et al. (1998) who found higher MDA and conjugated dien levels in the jejunum after reperfusion. There is no agreement on the significance of MDA production in the colon of horses during intestinal I/R. One group of authors found no increase after colonic ischemia (Vatistas et al., 1993, Kooreman et al., 1998) while another (Sullivan et al., 1990) did. There were no significant changes seen in the components of the glutathione system of erythrocytes. This is in line with the results of Reeves et al who found no change in plasma glutathione levels during colonic I/R. The same relates to the superoxide dismutase activity. However the change of SOD activity showed opposite pattern to TBARS raising the idea that the decreasing enzyme activity could have been caused by the anaesthesia or the developing acidosis and at least to some extent it could contribute to the elevation of RBC TBARS concentration. At the same time GPX activity was generally lower in the colic horses during the whole observed period, possibly due to the damage or the exhaustion of the enzyme by OFR radicals or metabolic acidosis. However Sullivan et al. (1990) found decreased SOD, but unaltered GPX activity the intestinal tissue indicating that the antioxidant pathways of erythrocytes and the intestine may show significant differences.

Finally we can conclude that the intestinal I/R resulted in marked metabolic acidosis and altered several antioxidant parameters of plasma and erythrocytes. This indicates that maximal effort should be taken to correct metabolic acidosis during the operation of colic horses. Furthermore changes in the blood LP parameters can serve as an indication for antioxidant therapy during the operation of horses with ileus.

Summary

The study was undertaken to evaluate lipid peroxidation changes in red blood cells and plasma of horses that were operated on for ileus. Blood samples were collected from fourteen horses with colic and from 9 control ones operated with orthopaedic problems at the following

times: 1st sample: before inducing anaesthesia; 2nd sample: after performing anaesthesia, but before the reestablishment of intestinal circulation; 3rd sample: 30 minutes after incision; 4th sample: 60 minutes after incision; 5th sample: 180 minutes after incision; 6th, 7th, 8th samples: 12, 24, hours after incision. The red blood cells of the samples were centrifuged and washed in isotonic saline solution and then mixed with distilled water and frozen at -18C to prepare red blood cell haemolysates. The TBRAS concentration, the reduced and oxidized glutathione (GSH and GSSG), and the glutathione-peroxidase (GSH-Px) and superoxide-dismutase (SOD) activities of the haemolysates were determined spectrophotometrically. From the plasma nitrogen monoxide (NO), the FRAP and the uric acid were determined. No significant change over time was observed in any parameter in the control group

Horses affected with colic were presented with metabolic acidosis as read from significantly lower pH (7,2 ± 0.05) and BE (-2.54±1.58 mmol/L) than the control ones (7.38 ± 0.01 and + 2.62 ± 0.93 mmol/L, respectively). Upon anaesthesia horses having intestinal disorders developed marked mixed type acidosis - pH sinking to 7.09 ± 0.04 after the second hour of anaesthesia accompanied by BE –10.32 ± 3.15 mmol/L, pCO_2 66 ± 5 mm Hg, HCO_3^- 20.6 ± 1.4 mmol/L, TCO_2 23.7 ± 1.3 mmol/L In spite of that oxygen tension and saturation of the mixed venous blood showed significant increase during anaesthesia with a peak of 72 ± 15 pO_2 and 83.5 ± 5.2 oxygen saturation two hours after the incision. Plasma uric acid concentrations were some 1,5 times higher in the control group during anaesthesia. Late post-anaesthetic values show opposite picture due to a marked increase seen in the colic horses. Though there are no significant changes over time of FRAP values in either group colic horses exhibited approximately twice higher concentrations than did the control ones. Plasma NO concentration was significantly higher in the colic horses before the operation (33.8 ± 1.6 vs. 26 ± 1 μmol/L and showed a constant decrease to 17.9 ± 2.9 μmol/L thereafter. Erythrocyte TBARS concentration was higher in the colic group at all the sampling times during the observed period. The highest TBARS concentration (458 ± 49 nmol/g protein) was found 1 hour after incision (ie.: 15-45 minutes after reperfusion). There were no significant alterations observed in the GSH, GSSG, concentrations and GPX and SOD activities though the latter one followed a tendency opposite to the TBARS concentration in the colic patients.

It was concluded that the intestinal I/R resulted in marked metabolic acidosis and altered several antioxidant parameters of plasma and erythrocytes. This indicates that maximal effort should be taken to correct metabolic acidosis during the operation of colic horses. Furthermore changes in the blood LPO parameters can serve as an indication for antioxidant therapy during the operation of horses with ileus.

Overview of the new scientific results

1. Newly hatched chicks have similar plasma antioxidant capacity to other species. Rate of LP is comparatively low in the liver and high in the brain of the 1-day-old broiler chicks. Increased LP was occurs in the liver tissue on the 10th and in the brain tissue on the 21st day of life, the former is accompanied by concomitant decrease of plasma antioxidant capacity.
2. The FRAP method can be used as an index of plasma antioxidant capacity in horses. There is strong linear correlation between plasma uric acid and FRAP concentrations in horse plasma. Exercise usual for pentathlon contests causes increased activity of muscle-originated enzymes, CK and LDH in blood plasma together with elevated TP, lactate and uric acid concentration. These biochemical changes are accompanied with significant increase of plasma FRAP concentration and decreased TAS level.
3. Intestinal I/R induced changes in LP parameters are reflected in the plasma and red blood cell parameters in a rat model. Deferoxamine treatment was proven to be beneficial in the

prevention of I/R induced LP, but the role of L-argininee and nitric oxide remains controversial and necessitates further investigation.

4. Horses affected with colic were presented with metabolic acidosis that became more pronounced upon anaesthesia. There were significant changes over time in the blood antioxidant indices of horses having colic as well as compared to the control ones basically due to increased LP. There are high plasma NO levels in colic horses that return to the physiologic levels gradually.

Publications

Original articles

1. **Balogh, N.,** Gaál, T., Husvéth, F., Vajdovich, P. (2001): Rate of lipid peroxidation in brain and liver tissues and the total antioxidant status of blood plasma in developing chicks. Acta Vet Hung. 49 (2):197-202.
2. **Balogh, N**., Gaál, T., Ribiczeyné, P. Sz., Petri, Á. (2001): Biochemical and antioxidant changes in plasma and erythrocytes of pentathlon horses before and after exercise. Vet Clin Path; 30 (4): 214-218.
3. **Balogh, N.,** Krausz, F., Lévai, P., Ribiczeyné, P. S., Vajdovich, P., Gaál, T. (2002): Effect of deferoxamine and L-arginine treatment on lipid peroxidation in an intestinal ischemia reperfusion model in rats Acta Vet Hung; 50 (3): 343-356
4. **Balogh, N**., Gaál, T., Vajdovich, P. Gastrointestinalis ischemia reperfúzió, Kórélettani alapok, terápiás lehetőségek *submitted for publication* Magyar Állatorvosok Lapja

Abstracts

1. **Balogh, N**., Vajdovich, P., Takáts, A., Szlezák, Sz.: The effect of hypoxy-reperfusion on the lipid peroxidation parameters of red blood cells and on the endotoxin levels of plasma during the operation of colic horses - Proceedings, ESVCP Annual Meeting, 1999. 06.04.-06. Verona, Italy Abstract: Comparative Clinical Pathology, 1999, 219-234
2. **Balogh, N.,** Förhécz, A., Gaál, T.: Az antioxidáns védelmi rendszer egyes elemeinek összehasonlítása hús- és tojótípusú csirkékben – MTA Állatorvosi Bizottsága, Akadémia Beszámolók 1999.
3. **Balogh, N.**, Gaál, T., Ribiczeyné, P. S., Petri, Á.: The effect of exercise on some plasma and red blood cell biochemical and lipid peroxidation parameters in pentathlone horses- ESVIM Congress, 1999.10.14-16. Perugia, Italy
4. **Balogh, N.**, Krausz, F., Lévai, P., Ribiczeyné, Sz. P., Vajdovich, P., Gaál, T.: Effect of deferoxamin and L-arginine treatment on the change of lipid peroxidation parameters in an experimental intestinal ischemia-reperfusion model in rats- Proceedings ESVCP Annual meeting 26th June Edinburgh, U.K. Absrtact: J Vet Clin Path 30 (3) 2001 p158
5. **Balogh, N**., Garami, J., Ribiczeyné, Sz. P., Mézes M., Bakos, Z., Bodó, G., Péntek G., Gaál, T., Lukács, Z.: Changes of some plasma and erythrocyte lipid peroxidation indices during the operation of colic horses - preliminary results Proceedings ESVCP Annual meeting 26th June Edinburgh, U.K. Absrtact: J Vet Clin Path 30 (3) 2001 p159
6. **Balogh, N**., Gaál, T., Ribiczeyné, P. S., Kovács, M.: Changes of some antioxidant parameters in foals during the first three weeks of life Proceedings ESVCP Annual Meeting.: 2000. 06. 21. Toulouse France, Abstract: J Vet Clin Path 29 (4) 2000 p140.
7. **Balogh, N**., Garami, J., Ribiczeyné, P. Sz., Mézes, M., Bakos, Z., Bodó, G., Péntek, G., Gaál, T., Lukács, Z., Evaluation of acid base and antioxidant indicies in horses operated on for colic Proceedings ESVCP Annual meeting, München 2002 09.27. Abstract: J Vet Clin Path 31 (4) 2002 p202

References

Arden, W. A., Slocombe, R. F., Stick, J. A. and Parks, A. H. (1990): Morphologic and ultrastructural evaluation of effect of ischemia and dimethyl sulfoxide on equine jejunum Am.J.of Vet.Res. 51(11):1784-1791.

Art, T., Amory, H., Desmecht, D. and Lekeux, P. (1990): Effect of show jumping on heart rate, blood lactate and other plasma biochemical values. Equine Vet J Suppl. Jun;(9):78-82.

Art, T., Desmecht, D., Amory, H., Delogne, O., Buchet, M., Leroy, P., Lekeux, P., (1990): A field study of post-exercise values of blood biochemical constituents in jumping horses: relationship with score, individual and event. Zentralbl Veterinarmed A.; ;37 (3): 231-239.

Art, T., Votion, D. and Lekeux, P. (1995): Physiological measurements in horses after strenuous exercise in hot, humid conditions. Equine Vet J Suppl.; 20: 120-4

Art, T., Votion, D., McEntee, K., Amory, H., Linden, A., Close, R., Lekeux, P. (1994): Cardio-respiratory, haematological and biochemical parameter adjustments to exercise: effect of a probiotic in horses during training.Vet Res.;25(4):361-370

Avellini, L., Silvestrelli, M., Gaiti, A. (1995): Training-induced modifications in some biochemical defences against free radicals in equine erythrocytes. Vet Res Commun.; 19:179-184.

Beckman, J., Beckman, T. W., Chen, J., Marshall, P. A. and Freeman, B. A. (1990): Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide Proc. of Natl. Ac. Sci. USA 87, 1620-24

Benzie, I. F. and Strain, J. J. (1996): The Ferric Reducing Ability of Plasma (FRAP) as a measure of „Antioxidant Power" : The FRAP assay. Analytical Biochemistry.; 239, 70-76,

Bottje, W. and Wideman, R. F. Jr. (1995): Potential role of free radicals in the pathogenesis of pulmonary hypertension syndrome Poultry and Avian Biol. Rev. 6, 211-231

Carey, C., Siegfried, M. R., Ma, X. L., Weyrich, A. S. and Lefer, A. M. (1992): Antishock and endothelial protective actions of a NO donor in mesenteric ischemia and reperfusion. Circulation and Shock, 38: 209-216

Carrillo, M.C., Kanai, S., Sato, Y. and Kitani, K. (1992): Age-related changes in antioxidant enzyme activities are region and organ, as well as sex, selective in the rat. Mech Ageing Dev 65,187-98

Caty, M. G., Guice, K. S.,. Oldham, K. T, Remick, D. G. and Kunkel, S. I. (1990): Evidence for tumor necrosis factor-induced pulmonary microvascular injury after intestinal ischemia-reperfusion injury. Annals of. Surgery; 212, 694-700.

Craig, L., Hintz, H. F., Soderholm, L. V., Shaw K. L. and Schryer, H. F. (1985): Changes in blood constituents accompanying exercise in polo horses. Cornell Vet. Apr;75(2):297-302

Cristol, J. P., Maggi, M. F., Guerin, M. C., Torreilles, J. and Descomps, B. (1995)**: [**Nitric oxide and lipid peroxidation**]** C-R-Seances-Soc-Biol-Fil.; 189(5): 797-809

Cuvelliez, S. G., Eicker, S. W., and McLauchlan C. (1990): Cardiovascular and respiratory effects of inspired oxygen fraction in halothane-anesthetized horses. Am J Vet Res, 51 (8): 1226-1231

Cuzzocrea, S., Zingarelli, B. and Caputi, A. P. (1998): Role of constitutive nitric oxide synthase and peroxynitrite production in a rat model of splanchnic artery occlusion shock Life Sciences, 63, 789-799.

Dabareiner, R. M., Snyder, J. R., Sullins, K. E., White, N. A. and Gardner, I. A. (1993): Evaluation of the microcirculation of the equine jejunum and ascending colon after ischemia and reperfusion. Am. J. of Vet. Res. 54(10): 1683-1692

Dabareiner, R. M., White, N. A. and Lemasters J. J. (1994): Effect of reperfusion solution "Carolina Rinse" on intestinal vascular permeability and blood flow after jejunal ischemia and reperfusion in the horse The 5th Eq. Colic Res. Symp. 28

Day T. K., Gaynor, J. S., Muir, W. W., Bednarski, R. M. and Mason D. E. (1995): Blood Gas Values During Intrmittent Positive Pressure Ventilation in 160 Anesthetized Horses Positioned in Lateral or Dorsal Recumbency, Veterinary Surgery 24:266-276,

Del Maestro, R. F. (1980): An approach to free radicals in medicine and biology. Acta Physiol. Scand. Suppl. 492:153-168,

Deldar, A, Fregin, F. G., Bloom, J. C. and Davanipour, Z. (1982): Changes in selected biochemical constituents of blood collected from horses participating in a 50-mile endurance ride. Am J Vet Res.; Dec; 43: 2239-2243.

Demopoulos, H. B. (1973): The basis of free radical pathology. Fed. Proc. 32:1859-1861,

Dormandy, T. L. (1978): Free radical oxidation and antioxidants. The Lancet, i:, p.: 647-650

Ely, D., Dunphy, G., Dollwet, H., Richter, H., Sellke, F. and Azodi, M. (1992): Maintenance of left ventricular function (90%) after twenty-four-hour heart preservation with deferoxamin. Free Radicals in Biology and Medicine. 12, (6) 479-485.

Enkvetchakul, B., Bottje, W., Anthony, N., Moore, R. and Huff, W. (1993): Compromised antioxidant status associated with ascites in broilers Poultry Sci. 72, 2272-2280

Fridovich, I. (1975): Superoxide Dismutases. Ann. Rev. Biochem. 44: 147-159,

Fridovich, I. (1978): The biology of oxygen radicals. Science 201: 875-880,

Fuhrmann, H., Schultheis, **X.,** Drommer, W., Kaup, F. J. and Sallmann, H. P. (1996): Zur Erfassung der Gewebs-Lipidperoxidation bei der Nutritiven Enzephalomalazie des Broilers. Journal-of-Vet. Med. Sereis-A. 43, 9-21

Gaál, T. and Kopál, D. (1997): Comparative studies on total antioxidant status (TAS) in blood plasma of different animal species Comparative haematology international 7, 179-180

Gaál, T., Mézes, M., Noble, R. C., Dixon, J. and Speake, B. K. (1995): Development of antioxidant capacity in tissues of the chick embryo Comp. Biochem. 112B, 711-716.

Gaál, T., Vajdovich, P., Speake, B. K., Noble, R: C., Surai, P. F. and Mézes, M. (1996): Ageing and lipid peroxidation (in Hungarian with English abstract) Magyar Állatorvosok Lapja 51, 165-169.

Gerard, M. P., Blikslager, A. T., Roberts, M. C., Tate L. P. Jr, and Argenzio, R. A. (1999): The characteristics of intestinal injury peripherial to strangulating obstruction lesions in the equine smaal intestine. Equine vet J. 31(4)331-335

Gottlieb-Vedi, M., Persson, S., Erickson, H. and Korbutiak, E. Cardiovascular, respiratory and metabolic effects of interval training at VLA4. Zentralbl Veterinarmed A. 1995; 42: 165-175

Granger, D. N., Rutili, G. and McCord, J. M. (1981): Superoxide radicals in feline intestinal ischemia. Gastroenterology, 81, 22-29,

Granger, D. N.; Benoit, J. N., Suzuki, M. and Grisham, M. B. (1989): Leukocyte adherence to venular endothelium during ischemia-reperfusion. Am. J. of Phys. 257, G683-688

Grisham, M. B., Johnson, G. G. and. Lancaster, J. R (1996): Quantitation of nitrate and nitrite in extracellular fluids. Meth. in Enzymol. 268, 237-246

Gunther, T., Hollriegl., V. and Vormann, J. (1993): Perinatal development of iron and antioxidant defence systems. J Trace Elem. Electrolytes Health Dis. 7, 47-52

Harman D. (1969): Prolongation of life, role of free radical reactions in ageing., Journal of the Am. Geriatric Soc. 17, 721-731.

Harris, D. B., Harris, R. C., Wilson, A.M. and Goodship, A. (1997): ATP loss with exercise in muscle fibres of the gluteus medius of the thoroughbred horse. Res Vet Sci.; 63: 231-237

Henry, Y., Ducroq, C., Drapier, J. C., Servent, D., Pellat, C., and Giussanai, A. (1991): Nitric oxide a biological effector Electron paramagnetic resonance detection of nytrosil-iron-protein complexes in whole cells. European Biophysics Journal 20, 1-15

Hernandez, L. A., Grisham, M. B. and Granger, D. N. (1987): A role for iron in oxidant-mediated ischaemic injury to intestinal microvasculature. Am. J. of Phys.; 253, G49-53.

Horne M. M., Pascoe, P. J., Duchmare, N. G., Barker, I. K. (1994): Attempts to Modify Reperfusion Injury of Equine jejunal Mucosa Using Dimethylsulfoxid, Allopurinol, and Intraluminar Oxigen. Veterinary Surgery 23: 241-249

Hornsby, P. J. and Crivello, J. F. (1983): The role of lipid peroxidation and biological antioxidants in the function of the adrenal cortex. Part. 1: A background review. Molec. Cell Endocrinol. 30:1-20,

Horton, J. W. and White, D. J. (1991): Cardiac contractile injury after intestinal ischemia-reperfusion. Am. J. of Phys.; 261, H1164-1170.

Hunt, J. V., Dean, R. T. and Wolf, S. P. (1988): Hydroxyl radical production and autoidative glycosylation: glucose autoxidation as the cause of protein damage in the experimental glycation as a model of diabetes and aging. Biochem. J. 256:205-212,

Husvéth, F., Manilla, A. H., Gaál, T., Vajdovich, P., Balogh, N., Wágner, L., Lóth, I. and Németh, K. (2000): Effects of saturated and unsaturated fats with vitamin E supplementation on the antioxidant status of broiler chicken tissues. Acta Vet. Hung. 48, 69-79

Inoue, O. J., Freeman, D. E. and Wallig M. (1998): Effects of hypochlorosus acid and ascorbic acid on conductance, permeábility, and structure of equine colonic mucosa in vitro. Am. J. Vet. Res. 59, 82-7.

Johnston J. K, Freeman D. E., Gilette D. and Soma, L. R. (1991): Effects superoxide dismutase on injury induced by anoxia and reoxygenation in equine small intestine in vitro. , Am. J. Vet. Res. 52, 2050-4.

Kanwar, S., Tepperman, B. L., Payne, D., Sutherland, L. R. and Kubes, P. (1994): Time course of nitric oxide production and epithelial dysfunction during ischemia/reperfusion of the feline small intestine. Circulation and Shock, 42, 135-140.

Kawata, K., Takeyoshi, I., Iwanami, K., Sunose, Y., Aiba, M., Ohwada, S., Matsumoto, K. and Morishita, Y. (2001): A spontaneous nitric oxide donor ameliorates small bowel ischemia-reperfusion injury in dogs. Digestive Diseases and Sciences, 46, 1748-1756

Kawcak, C. E., Baxter, G. M., Getzy, M., Stashak, T. S. and Chapman, P. L. (1995): Abnormalities in oxygenation, coagulation, and fibrinolysis in colonic blood of horses with experimentally induced strangulation obstruction. Am J Vet Res, Vol 56, No. 12, December

Keberle, H. (1964): The biochemistry of Deferoxamine and its relation to iron metabolism Annals New York Academy of Sciences 759-768

Keenan, D. M. (1978) Changes of plasma uric acid levels in horses after galloping Res Vet Sci. 25, 127-128

Kooreman, K., Babb, C. and Fessler J. (1998): Effect of ischémia and reperfusion on oxidative processes in the largre colon and jejunum of horses. Am. J. Vet. Res. 59 (3) 340-346

Kosower, N., Song, K., Kosower, E. and Correa, W. (1969): Glutathion chemical aspects of azoester procedure for oxidation to disulfide. Biochim Biophys Acta 192(1):8.

Kubes, P. (1992): Nitric oxide modulates epithelial permeability in the feline small intestine. Am. J. of Phys. 262, G1139-1142

Kubes, P. (1993): Ischemia-reperfusion in feline small intestine: a role for nitric oxide Am. J. of Phys.; 264, G143-149

Kubes, P., Ibbotson, G., Russell, J.,. Wallace, J.L., and Granger, D. N., (1990): Role of platelet-activating factor in ischemia-reperfusion-induced leukocyte adherence Am. J. of Phys. 259, G300-305

Lantz, G. C., Badylak, S. F., Hiles, M. C. and Arkin, T. E. (1992): Treatment of reperfusion injury in dogs with experimentally induced gastric dilatation-volvulus. Am. J. of Vet.Res.; 53, 1594-1598.

Ledwozyw, A. and Kadziolka, A. (1989): Ontogenesis of antioxidant enzymes in pig (in Polish with English abstract) Polskie-Archiwum-Weterynaryjne., 29, 77-94.

Lindholm, A. and Saltin, B. (1974) The physiological and biochemical response of standardbred horses to exercise of varying speed and duration. Acta Vet Scand.; 15:310-324

Lochner F, Burrowws S. S:, Shawley R., et al. (1989) Effects of allopurinol in experimental endotoxins shock in horses Res. Vet. Sci. 47, 178-184

Lopez, B. L., Gao, L. L., Liu G. L., Christopher, T. A. and Ma, X.L. (1997): Peroxynitrite the product of nitric oxide and superoxide, causes myocardial injury in the isolated perfused rat heart Cor. Art. Dis. 8, 149-153

Lucke, J.N., Hall, G.M. (1980) Long distance exercise in the horse: Golden Horseshoe Ride 1978.Vet Rec.; May 3-17; 106: 405-407

Maldjian, A., Cristofori, C., Noble, R: C., Speake, B. K. (1996): The fatty acid composition of brain phospholipids from chicken and duck embryos Comp. Biochem. Pysiol. 115B, 153-158

Mason, D. E., Muir, W. W., and Wade A. (1987): Arterial blood gas tensions in the horse during recovery from anesthesia. , JAVMA, Vol 190, No. 8, April 15

Matsuki, N., Tamura, S., Ono, K., **et al**., Exercise-induced phospholipid degradation in the equine skeletal muscle and erythrocytes J. Vet. Med. Sci. 1991; 53: 1001-1007

Mcanulty, J.F, Stone, W.C and Darien, B. J. (1997): The Effect of Ischémia and Reperfusion on Mucosal Respiratory Function, Adenosine Tiphophate, Electrolyte and Water Content in the Ascending Colon of Ponies. Veterinary Surgery 26: 172-181, 1997.

McCay, P. B., King, M., Lai, E. K. and Poyer, S. L. (1983): The effect of antioxidants on free radical production during in vivo metabolism of carbon tetrachloride. J. Am. Coll. Toxicol. 2(3): 195,

McCord, J. M. and Roy, R. S. (1982): The pathophysiology of superoxide: roles in inflammation and ischemia. Active oxygen and Medicine. Symposium. Canadian Journal of Physiological Pharmacology, 60, 1346-1352,

McCord, J. M. and Roy, R. S. (1982): The pathophysiology of superoxide: roles in inflammation and ischemia. Active oxygen and Medicine. Symposium. Canadian Journal of Physiological Pharmacology, 60, 1346-1352,

Mcmeniman, N. P. and Hintz, H.F. (1992) Effect of vitamin E status on lipid peroxidation in exercised horses Equine Vet. J.; 24: 482-484

Menasche, P., Grousset, C., Gauduel, Y., Mouas, C. and Piwnica, A. (1987): Prevention of hydroxyl radical formation: A critical concept for improving cardioplegia Protective effects of deferoxamine. Circulation, 76, (Suppl V): V-180-185

Mézes, M. (1988): Changes in lipid peroxidation and activities of some antioxidant enzymes of chicken brain homogenates during early postnatal development In: Matkovics,B., Boda, D. and Kalász H. (eds.): Oxygen Free Radicals and Tissue Injury Akadémiai Kiadó Budapest, 211-215.

Mézes, M., Sályi, G., Bánhidi, G. and Szeberényi, S. (1992): Effect of acute salinomycin-tiamulin toxicity on the lipid peroxide and antioxidant status of broiler chicken. Acta Vet. Hung. 40, 251-257.

Mézes, M., Surai, P., Sályi, G., Speake, B., Gaál, T. and Maldjian, A. (1997): Nutritional metabolic disease of poultry and disorders of the biological antioxidant defence system. Acta Vet. Hung. 45, 349-360

Miller, N. J., Rice-Evans, C., Davies, M. J., Gopinathan, V. and Milner, A. (1993): A novel method for measuring antioxidant capacity and its application to monitoring theantioxidant status in premature neonates Clin. Sci. 84, 407-412.

Miller-Graber P., Lawrence L., Foreman J, **et al 8**1988): Lactate. Pyruvate and blood gases in the carotid artery, jugular vein and pulmonary artery during submaximal exercise Equine Vet. Sci., 8 (4) pp 322-325

Mills, P. C., Smith, N. C., Casas, I., Harris, P., Harris, R.C. and Marlin, D. J. (1996): Effects of exercise intensity and environmental stress on indices of oxidative stress and iron homeostasis during exercise in the horse. Eur J Appl Physiol.; 74: 60-66.

Mirza, M. H., Oliver, J. L., Seahorn, T. L., Hosgood, G. and Moore, R. M. (1999): Detection and comparison of nitric oxide in clinically normal horses and those with naturally acquired small intestinal strangulation obstruction. Can. J. Vet. Res., 63, 230-240

Moore. R. M, Muir, W. W., Bertone, A. L., Bear, W. L., and Stromberg, C. (1995): Effects of dimethyl sulfoxid, allopurinol, 21- aminosteroid U-74389G, and manganese cholride, on

low flow ischemia and reperfusion o the large colon in horses, Am. J. Vet. Res., 56 671-87.

Morehouse, K. M., Flitter, W. D. and Mason, R. P. (1987): The enzymatic oxidation of Desferal to a nitroxide free radical FEBS Letters 22, 246-250

Nappert, G. and Johnson, P. J. (2001) Determination of he acid- base status in 50 horses admitted with colic between December 1998 and May 1999. Can Vet J Volume 42, September

Noble R.C. and Cocchi M. (1990): Lipid metabolism and the neonatal chicken. Prog. Lipid Res. 29, 107-140.

O'Brien, P., Slaughter, M. R., Swain, A., (1999) **et al** Adaptive e response of hepatic antioxidant system to repeated dosing with acetaminophen in rats Abstract Proc. 9th Annual Congress of the European Society of European Internal Medicine 14-16 October, Perugia, Italy

Ono, K., Inui K., Hasegawa, T., (1990) The changes of antioxidative enzyme activities in equine erythrocytes following exercise. Nippon Juigaku Zasshi; 52(4):759-765

Otamiri, T., Lindahl, M. and Tagesson, C. (1988): Phospholipase A2 inhibition prevents mucosal damage associated with small intestinal ischemia in rats. Gut, 29, 489-494.

Pacifici, R. E.; Davies, K.J.: Protein, lipid and DNA repair systems in oxidative stress: the free-radical theory of aging revisited. Gerontology. 37(1-2):166-180, 1991

Parks, D. A., Bulkley, G. B. and Granger, D. N. (1983): Role of oxygen-derived free radicals in digestive tract disease. Surgery, 94, 415-422,

Parks, D. A., Bulkley, G. B. and Granger, D. N. (1983): Role of oxygen free radicals in shock, ishemia, and organ preservation. Surgery 94(3):428-432,

Payne, D. and Kubes, P. (1993): Nitric oxide donors reduce the rise in reperfusion induced intestinal mucosal permeability. Am. J. of Phys. 265, G189-195

Placer, Z., Cushman, L. and Johnson, B. (1966) Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal Biochem; (16): 359-364.

Prichard, M., Ducharme, N. G., Wilkins, P. A., Erb H. N. and Butt M. (1991): Xanthine Oxidase Formation during Experimental Ischémia of the Equine Small Intestine. Can Jvet Res ; 55:310-314.

Pryor, W. A. (1982): Free radical biology: xenobiotics, cancer, aging. Ann. New York Acad. Sci. 293:1-22,

Pryor, W. A. (1973): Free radical reactions and their importance in biochemical systems. Fed. Proc. 32:1862-1869,

Rasanen, L.A., Wiitanen, P.A., Lilius, E. M., Hyyppa, S., Poso, A. R., (1996): Accumulation of uric acid in plasma after repeated bouts of exercise in the horse Comp Biochem Physiol B Biochem Mol Biol; Jun;114:139-144.

Raul, F., Galluser, M., Schleiffer, R., Gosse, F., Hasselmann, M. and Seiler, N. (1995): Beneficial effects of L-argininee on intestinal epithelial restitution after ischaemic damage in rats. Digestion, 56, 400-405.

Reeves, M.J., Vansteenhouse, J., Stashak, T. S., Yovich, J.V. and Cockerell, G. (1990): Failure to demonstrate reperfusion injury following ischemia of the large colon using dimethyl sulfoxide.Equine Vet.J. 22 (2): 126-132,

Reif, D. W. (1993): Ferritin as source of iron for oxidative damage. Free Rad. Biol. Med. 12, 417-427

Reynolds, E. S., Treinen R. J., Moslen, M. T. (1980): Free radical damage in liver in: Free radicals in biology. Vol. IV. Ed.: Pryor, W. A., Academic Press, New York 49-94,

Rose, R. J., Hodgson, D. R., Sampson, D., Stewart, J. and Chan, W. (1983): Responses to submaximal treadmill exercise and training in the horse: changes in haematology, arterial blood gas and acid base measurements, plasma biochemical values and heart rate. Vet Rec.; 24/31; 113: 612-618

Rose, R. J., Purdue, R. A. and Hensley, W. (1983): Changes in plasma biochemistry in horses competing in a 160 km endurance ride. Aust Vet J.; Apr;60:101-105

Rose, R. J., Purdue, R. A. and Hensley, W. (1977): Plasma biochemistry alterations in horses during an endurance ride. Equine Vet J.; Jul; 9(3): 122-126.

Sallmann, H. P., Fuhrmann, H., Molnár, S. and Stegmanns T. (1991): Endogenous lipid peroxidation in broiler chickens under dietary loads. Fat. Sci. Technol. 93, 457-462

Salzman, A. L. (1995): Nitric oxide in the gut. New-Horiz. May; 3(2): 352-64

Schulz, R. and Wambolt, R. (1995): Inhibition of nitric oxide synthesis protects the isolated working rabbit heart from ischemia reperfusion Cardiovasc. Res., 30, 432-439

Sedlak, J, and Lindsay, R. (1968): Estimation of total protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 25: 191-205,.

Shaw, S., Rubin, K. P. and Lieber, C. S. (1983): Depressed hepatic glutathione and increased diene conjugates in alkoholic liver disease. Evidence of lipid peroxidation. Digest. Dis. Sci. 28(7):585-589,

Slater, T. F. (1981): Free radical scavengers. Royal Society of Medicine International Congress and Symposium Series No 47. International Workshop on (+)-cyanidol-3 in disease of the liver. Ed.: Conn, H. O., 11-15,

Snow, D. H., Harris, R. C. and Gash, S. P. (1985): Metabolic response of equine muscle to intermittent maximal exercise. J Am Vet Physiol.; 58: 1689-1697

Snyder, J. R., Harvey, J., Olander, J., Pascoe, R., Holland, M., and Kurpershoek C. J. (1998): Morpgologic alterations observed during experimental ischemia of the equine large colon. Am. J .Vet. Res. Vol 49 No. 6. June

Steffey E. P., Wheat, J. D., Norrie, R. D., McKee, J., Brown, M. and Arnold J. (1987): Body Position and Mode of Ventilation Influences Arterial PH, Oxygen, and Carbon Dioxide tensions in Halothan- Anesthetized Horses. Am J. Vet Res, 38 (3), 379-382

Sullivan, K. E., Snyder ,J. R., Schiedt, M. J., Harman, F. J. and Giri, S. N. (1990): Lipidperoxidation and antioxidative defences during ischemia and reperfusion of the equine ascending colon.Equine Vet.J. Supplement(13):99-101, 31.ref.,

Surai, P. F., Speake, B. K., Noble, R: C., Sparks, N. H: (1999): Tissue-specific antioxidant profiles and susceptibility to lipid peroxidation of the newly hatched chick Biol. Trace Elem. Res. 68, 63-78

Surai, P.F., Noble, R.C. and Speake, B. K. (1996): Tissue-specific differences in antioxidant distribution and susceptibility to lipid peroxidation during development of the chick embryo. Biochim. Biophys. Acta 1304, 1-10

Suzuki, M, Inaunen, W., Kvietys, P. R., Grisham, M. B., Meininge, R. C., Schelling, M. E., Granger, H. J. and. Granger, D. N. (1989): Superoxide mediates reperfusion induced leukocyte-endothelial cell interactions. Am. J. of Phys.: 257, H1740-1745.

Suzuki, Y., Deitch, E. A., Mishima. S., Lu. Q. and Xu, D. (2000): Inducible nitric oxide synthase gene knockout mice have increased resistance to gut injury and bacterial translocation after an intestinal ischemia-reperfusion injury. Crit. Care Med. 28, 3692-3696

Van Hoogmoed L. M., Synder, J. R., Nieto, J. E. and Harmon, F. A. (2000): Use of an extracorporeal circuit to evaluate effecets of ischemia and reperfusion of the equine large colon. AJVR, vol 61, No 9.szeptember

Vatistas, N. J., Snyder, J. R., Hildebrand, S. V., Harmon,F. A., Woliner, M. J., Henry, P., Enos, L .R., Magliano ,D., Brown, S .A. and Drake, C. (1993):Effects of the21-aminosteroid U-74389G on ischemia and reperfusion injury of the ascending colon in horses. Am.J.ofVet.Res.54(12) :2155-2160,49. ref.,

Whitehead, C., Farquharson, C., Rennie, J. S. and McCormack, H. (1994): Nutritional and cellular factors affecting tibial dyschondroplasia in broilers. Proc. Austr. Poultry Sci. Symp. Sydney. P 13-19.

Williamson, L. H., Andrews, F. M., Maykuth, P.L., White, S. L. and Green, E. M. (1996): Biochemical changes in three-day-event horses at the beginning, middle and end of Phase C and after Phase D. Equine Vet J Suppl. 22; Jul; 92-98

Wilson, J. X., Lui, E. M. K. and Del-Maestro, R. F. (1992): Development profiles of antioxidant enzymes and trace metals in chick embryo. Mechanisms-of-Ageing-and-Development. 65, 1, 51-64;
Yarbrough, I. B., Snyder, J. R., Harmon, F. A., O'Conell, K. A. (1994): Evaluations of myeloperxidase concetrtions in experimentally induced equine colonic ischemia and reperfusion. Equine Vet.J.26(1): 67-69, 22 ref.,
Zimmermann, B. J., Grisham, M. B., Granger, D. N. (1990): Role of oxidants in ischemia/reperfusion-induced granulocyte infiltration. Am. J. of Phys.: 258, G185-190.

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