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Original Article

Antioxidant Parameters and Ageing in Some Animal Species

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Abstract. Connection between ageing and some tissue antioxidant parameters have been studied in four experiments on different animal species. Prenatal studies on the developing chick embryos showed discrepancies between the lipid-rich liver and brain antioxidant defence. In the liver, high levels of reduced glutathione (GSH), vitamins A and E and high activities of the antioxidant enzymes glutathione peroxidase (GPX) and superoxide dismutase (SOD) were found whereas brain expressed a high vitamin C concentration. In newborn healthy calves during the first two days of life, atmospheric oxygen tension did not cause either increased lipid peroxidation as reflected in a high malondialdehyde (MDA) level or any changes in GSH, GPX, SOD and catalase (CAT) activities in red blood cells (RBC). Plasma vitamin E and carotene concentrations also did not change. In growing healthy calves during two months after birth increasing MDA, decreasing GSH, GPX and CAT are leading features, whereas plasma vitamin E and carotene concentrations significantly increased. In young (1-year-old) and old (9-year-old) dogs RBC results showed significant differences with the highest MDA and lowest GSH levels in the old males. Activity of GPX and SOD was higher in old dogs than in the young ones, especially in the females.

Keywords: Ageing; Antioxidants; Calf; Chick; Dog; Lipidperoxidation

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Introduction

During phylogenesis several defence mechanisms, such as phagocytosis, haemostasis, regeneration, reparation, antioxidant defence, adaptation to altered environmental temperature, specific immune response etc., have been developed in living organisms to prevent disturbances to homeostasis caused by environmental effects. These defences have their own development during the ontogenesis of a given organism. Their improper capacity predispose the body to severe diseases as frequently occurs in young animals with lower and unstable defence levels compared to adults.

One of the widely studied defences is the antioxidant capacity of animal tissues under physiological and pathological conditions (Igarashi et al. 1983; Nohl 1984; Gritz and Rahko 1990). Some physiological processes and several diseases in both humans and animals are caused by free radical mediated peroxidative processes within protein, lipid, carbohydrate and purin metabolism (van Vleet 1982; Maas et al. 1984; Nohl 1984; Sárközy and Gaál 1989). Nutritive myopathies (white muscle diseases), encephalomalacia in chicken, hepatosis dietetica in swine are examples of widely studied conditions. Frequent consequences of free radical attack can be seen in alterations to lipids during the course of their peroxidation (LP). This predominantly occurs in tissues and cell components rich in polyunsaturated fatty acids (Nohl 1984; Noble and Cocchi 1990). LP processes play a substantial role in organisms during ageing as postulated by Harman (1982) in his free radical theory of ageing. The biological basis of this hypothesis is the fact that free radical-mediated processes included a regular increase in LP with ageing which can be easily detected in tissues (Tappel 1973; Boross et al. 1991).

Unfortunately, age-related gerontological pro-

cedures in domesticated farm and pet animals have not been as frequently studied as in laboratory animals but some information is available (Mosier 1988; Lowseth et al. 1990; Vallois et al. 1990; Quadri and Palazzolo 1991; Rothuizen et al. 1991). An explanation that is frequently used is that in human experimental gerontology, laboratory species (rats, mice) have adequately served as model animals.

For this reason the main goal of the present study was to present data on the antioxidant defence of tissues in a range of species at different age levels. Thus the studies have embraced the prenatal and post hatching period of chick embryos, postnatal/pubertal changes in suckling and growing calves and observation between young and old dogs. Some of the data reported has already been published (Vajdovich et al. 1993; Gaál et al. 1993, 1995; Speake et al. 1996).

Materials and Methods

Animals and Sampling

In total four experiments on different species of different ages were undertaken. In Experiment 1 the ontogenesis of the antioxidant capacity in the prenatal and early postnatal period were studied in a series of experiments on developing chick embryos and 1-day-old chicks. Fertilised eggs were obtained from Ross 1 broiler-breeder parent stock supplied by Ross Poultry Ltd, Inverurie, Scotland and were incubated in a portable bench top incubator. On hatching, the chicks were maintained in the incubator for 1 day with free access to hourly replenished drinking water but with no food provision. Measurement on the initial yolks were performed on unincubated eggs. Yolk and tissue samples, mainly liver and brain, were obtained from incubated eggs around days 14 and 19 of development and from chicks one day after hatching. At each sampling day, between four and eight replicate samples of each tissue were collected. Each replicate sample consisted of the pooled tissues from 3–5 embryos or chicks in order to obtain sufficient material for analyses.

In Experiments 2 and 3 antioxidant parameters of red blood cell homogenates (RBC) from newborn and growing calves were studied. Seven newborn and 10 young Holstein-fries calves were investigated in two separate experiments from the same dairy herd. The calves were housed and fed according to the local practice, i.e. cow's colostrum and fresh milk until 10 days of age, milk replacer and gradually increasing amount of hay and concentrate up to 30 days of age leading to hay and concentrate only. Heparinised blood samples from the jugular vein were taken in both trials and RBC isolated (Gaál et al. 1993). In Experiment 2 involving newborn calves, blood was taken at birth (time zero) and consequently at 3, 6, 9, 12, 24 and 48 h after birth. In Experiment 3 involving young calves blood samples were taken on days 1, 3, 10, 30 and 60 after birth.

In Experiment 4 involving young and old dogs RBC of 28 Beagles in four groups were studied. Groups 1–4 were represented by 1-year-old males (n = 7), 1-year-old females (n = 7), 9-year-old males (n = 7) and 9-year-old females (n = 7), respectively. Dogs were housed according to their sex, in seperate kennels and fed the same ration. Heparinised blood samples were taken from the brachial vein and prepared as for the calves.

Analyses

Malondialdehyde (MDA) as a representative of thiobarbituric acid reactive substances (TBARs), an end-product of LP in tissues, was measured immediately after sample collection and after homogenisation of the samples (Gaál et al. 1993, 1995) according to Placer et al. (1966). For calibration standard curve using suitable solutions of tetramethoxypropane was used. Reduced glutathione (GSH) was determined spectrophotometrically using Ellman's reagent (Sedlak and Lindsay 1968). The homogenates were stored at −20 °C for 2–5 days before the assay of glutathione peroxide (GPX) and superoxide dismutase (SOD). GPX activity in the chick experiment was assayed by the method of Paglia and Valentine (1967) using a standard kit system (Randox Ltd, Crumlin, Northern Ireland). In the other experiments GPX was detected with Ellman's reagent (Lawrence and Burk 1976). SOD activity in chick experiment was also assayed using a standard kit system (Randox Ltd, Crumlin, Northern Ireland), involving xanthine and xanthine oxidase to generate superoxide radicals followed by detection by a formazan dye reaction. The SOD activity was calculated from the degree of inhibition of this reaction. In all other experiments SOD was analysed by the adrenochrom method (Misra and Fridorich 1972). Catalase (CAT) activity was measured using a direct ultraviolet detection system for the qualification of the hydrogen peroxide concentration (Beers and Sizer 1952). In the experiment on chicks intact tissue samples were stored at −20 °C under oxygen-free N_2 for 10–14 days prior to vitamin analyses. Vitamins A and E were determined by HPLC according to the method of McMurray et al. (1980) and vitamin C by the spectrophotometric method of Omaye et al. (1979). In both experiments on calves' plasma total carotene was measured according to Bardos (1988) and vitamin E according to Bieri (1964).

Statistical Analysis

Statistical analysis of the data was performed by one-way ANOVA or Student's t-test and correlation coefficients were calculated using the Genstat 5 (Release 3.1) statistical package. In all cases results are expressed as means with average standard errors of differences (SED) or standard deviations (SD).

Table 1. Levels of antioxidant parameters in liver and brain during chick embryo development

	Tissue	Days of development[a]			SED	*P*
		14	19	22		
Vitamin E	Liver	43.5±2.3	152±16	320±32	24.4	<0.001
(μg/g)	Brain	4.9±0.4	5.2±0.2	5.5±0.7	0.34	NS
Vitamin A	Liver	14.7±0.6	10.4±0.9	12.2±1.4	1.35	<0.001
(μg/g)	Brain	0.19±0.02	0.11±0.01	0.07±0.01	0.02	<0.001
GSH	Liver	3.8±0.2	3.4±0.3	4.1±0.2	0.31	<0.001
(μmol/g)	Brain	2.7±0.04	4.0±0.4	3.4±0.3	0.36	<0.05
GPX	Liver	13.9±1.2	15±0.9	23.8±1.5	1.78	<0.001
(mU/g)	Brain	4.0±0.9	3.0±0.4	6.0±1.1	1.05	<0.05
SOD	Liver	78±1.9	69±2.8	110±5.7	5.27	<0.001
(U/g)	Brain	24.4±1.0	26.2±1.5	23.6±2.1	2.87	NS
MDA	Liver	ND	9.6±0.3	30.7±1.6	2.38	<0.001
(nmol/g)	Brain	ND	20.6±2.2	91.8±3.6	6.26	<0.001

Values represent the means with average standard error of difference (SED) of 4–8 replicate samples and are expressed in terms of tissue fresh weight.
[a]14 and 19 represent days of incubation, 22 represents 1-day-post-hatch.
NS = not significant.
ND = not determined.

Results

Experiment 1: Chick Embryos and 1-day-old Chicks

A major finding of the study was the existence of a consistent disparity between the concentrations of the major antioxidant systems in the brain compared to those of the liver (Table 1). The concentration of vitamin E in the brain at day 14 was only 11% of the concentration in the liver. Moreover, the concentration of this vitamin in the brain did not increase further during the final week of development, so that by hatching (day 22), the level in this tissue was only 1.7% of that in the liver. Similarly, only low levels of vitamin A were detectable in the brain at all stages, such that, after hatching, the concentration was only 0.6% of the liver value. High activities of GPX enzyme were expressed in the liver at day 14, and this activity increased as the development progressed. In contrast, the activity of GPX in the brain did not increase during the final week of development, and by day 22, represented only 25% of the activity measured in the liver. Only the concentrations of GSH were similar in the extracts of brain and liver, and remained relatively constant during the final week of development. The highest activities of SOD was expressed in the liver, and the level of this enzyme in the brain at day 22 was only 21% of the liver activity. The concentrations of MDA, detected in the brain extracts, were two- and three-fold higher than in the liver at days 19 and 22, respectively. Although such antioxidant components were very low in the brain a significant level of ascorbic acid (vitamin C) was detected. Brain and liver tissues of 20-day-old embryos expressed 636±71 and 179±9 μg/g vitamin C, respectively. It means that the concentration of this antioxidant in the liver was only 28% of the brain value.

Experiment 2: Newborn Calves

The development of the RBC and plasma antioxidant components in the first two days of life in the Holstein fries calves are summarised in Table 2. Neither increased LP as reflected in higher levels of MDA nor any changes of the antioxidant components could be detected over the 2-day period. Similarly, plasma total carotene and vitamin E levels also remained unchanged. However, in the cows the concentration of carotenoids was 2–3 times higher and vitamin E levels were some 30% higher than in the calves (results not shown).

Experiment 3: Suckling and Growing Calves

Studies on 10 calves for 60 days after calving provided a good opportunity to compare the results for the suckling period (1–10 days, when the calves are practically monogastric animals) with those of the growing period (30–60 days), when the calves displayed increasing levels of rumination and appropriate dietary changes. As shown in Table 3 and in contrast to the results of Experiment 2 for newborn calves, significant changes occurred over the 2-month period. Increased LP by day 60 was reflected in a 2.5-fold increase of MDA. Simultaneously GSH levels decreased by 40%; GPX and CAT activity decreased by 30% and 40%, respectively, whereas SOD activity remained unchanged. In suckling

Table 2. Changes of antioxidant parameters of RBC and blood plasma of newborn calves (n=7)

	Time of sampling after birth (h)						
	0	3	6	9	12	24	48
MDA (μmol/l)	225 ±36	230 ±22	221 ±34	232 ±25	232 ±38	256 ±60	223 ±63
GSH (mmol/l)	2.6 ±0.45	2.6 ±0.4	2.6 ±0.8	2.1 ±0.9	2.4 ±0.9	2.3 ±0.9	2.3 ±0.6
GPX (U/g protein)	39.6 ±9.5	43.7 ±12.6	42.0 ±9.8	36.1 ±4.2	37.3 ±5.6	37.3 ±6.7	35.3 ±2.3
SOD (U/mg protein)	20.9 ±1.9	24.6 ±8.7	22.2 ±2.5	21.1 ±4.9	22.7 ±6.7	21.7 ±2.6	21.2 ±1.0
CAT (U/g protein)	8.3 ±2.0	9.5 ±1.7	9.2 ±2.1	8.7 ±1.3	8.8 ±1.8	9.8 ±2.2	8.3 ±1.6
Vitamin E (mg/l)	1.15 ±0.54	0.9 ±0.3	0.9 ±0.5	1.1 ±0.4	0.9 ±0.2	1.0 ±0.2	0.9 ±0.02
Total carotene (mg/l)	0.35 ±0.23	0.2 ±0.1	0.1 ±0.07	0.3 ±0.3	0.2 ±0.1	0.2 ±0.2	0.1 ±0.06

No significant differences were found.

Table 3. Changes of antioxidant parameters of RBC and blood plasma of calves (n=10) during the first two months of life

	Days of sampling after birth				
	0	3	10	30	60
MDA (μmol/l)	146^a ±32	161^a ±20	194^a ±28	252^b ±26	367^c ±13
GSH (mmol/l)	1.9^a ±0.5	1.8^a ±0.4	1.6^b ±0.4	1.1^c ±0.1	1.3^c ±0.2
GPX (U/g protein)	44.6^a ±8.9	45.0^a ±8.5	43.8^a ±8.0	29.2^c ±6.3	35.5^b ±1.8
SOD (U/mg protein)	27.5^a ±2.5	28.0^a ±3.4	24.8^a ±5.0	30.4^a ±5.3	27.9^a ±3.6
CAT (U/g protein)	10.5^a ±2.45	11.5^a ±4.4	10.0^a ±3.0	7.0^b ±2.2	3.5^c ±1.5
Vitamin E (mg/l)	1.5^a ±0.12	1.4^a ±0.1	1.4^a ±0.1	1.8^c ±0.2	1.9^c ±0.1
Total carotene (mg/l)	0.43^a ±0.05	0.49^a ±0.04	0.39^a ±0.04	0.93^c ±0.09	0.8^b ±0.3

Significant differences (min. $p<0.05$) are marked with different letters (a, b, c)

Table 4. Antioxidant parameters in RBC of 1-year-old and 9-year-old Beagle dogs

	1-year-old		9-year-old	
	Males (7)	Females (7)	Males (7)	Females (7)
MDA (±μmol/l)	224^a ±22	220^a ±22	380^b ±91	257^a ±38
GSH (mmol/l)	2.5^a ±0.3	2.6^a ±0.4	1.1^b ±0.1	2.3^a ±0.2
GPX (U/g protein)	18.8^a ±0.5	18.9^a ±1.2	29.5^b ±0.3	57.8^c ±1.9
SOD (U/mg protein)	6.7^a ±0.7	8.1^a ±1.2	10.8^b ±2.2	14.1^c ±2.0

Significant differences (min. $p<0.05$) are marked with different letters (a, b, c).

calves, plasma vitamin E and total carotene concentrations were only 50% and 80% of subsequent levels.

Experiment 4: Young and Old Dogs

The total number of dogs (n = 28) made it possible for the study to embrace both ageing and sexual differences. Table 4 summarises the results. Similar MDA and GSH levels for RBC were observed for 1-year-old males and females and 9-year-old females. Only the 9-year-old males showed any extreme results with their MDA some 1.5 times higher and their GSH levels half the values in the other three groups. GPX and SOD activity was similar in the young of both sexes. Higher activities of both antioxidant enzymes were found in older males and females, but particularly in the females.

Discussion

The results of the chick experiment indicate that vitamin A is preferentially transported from the yolk prior to day 14, whereas the major period of vitamin E transfer occurs after this time in parallel with the bulk transfer of lipids. The functional significance of this observation may be the embryo's early requirement for the pro-

vision of retinal to the developing eye. In contrast, vitamin E is transported from the yolk, in accordance with its function as a major lipid-soluble antioxidant. This period being characterised by a major increase in the content and degree of unsaturation of tissue lipid (Griffin 1992). The activities of GPX and SOD were higher in the liver than in the brain. Similar differences between these two tissues of the chick embryo have also been reported by Wilson et al. (1992). In contrast to other antioxidant components, GSH level was similar in the liver and the brain and no correlation was found between GSH and vitamin E results in any tissues. Somashekaraiah et al. (1992) also found liver and brain GSH levels to be similar in 14-day-old chick embryos but their results were some three-fold higher than those presently observed. The paradoxical situation that so far the developing brain displays no effective antioxidant defence can probably be explained by the very high level of vitamin C. These findings accord with the results of Wilson (1990) who showed a high affinity uptake system for ascorbic acid in chick embryo brain.

Results on newborn calves show that the exposure to atmospheric oxygen did not cause increased LP in RBC of healthy calves. In spite of the fact that a hyperoxic enviroment causes peroxidative damages in newborn infants (Cruz et al. 1983; Muller 1987) and rabbits (Wispe et al. 1986), antioxidant defences of newborn calf RBC seems to be effective against oxidative damages. Observation of higher plasma carotenoid and vitamin E levels in cows can be explained rather by nutritional differences than purely by ageing.

In suckling and growing calves generally significant differences were found between the results of the suckling period (1–10 days) and the rumination–adaptation period (30–60 days). An age-related rise of MDA with a decrease in GSH was associated with an increase of LP in RBC. The results, especially the increasing plasma carotenoid and vitamin E levels emphasise again the importance of nutritional conditions with respect to suckling calves as consumption of carotenoids and vitamin E is far less than that within a diet of hay and concentrate received during rumination.

Our results on young and old dogs especially in the old males proved that an elevation of LP in the RBC was reflected in higher MDA and lower GSH levels. This observation correlates well with the free radical theory of ageing (Harman 1982). The situation in the male during ageing underlines an important sex difference arising from hormonal patterns. Permanent movement, sniffing and excited behaviour of the males compared to the uninterested females could also be a contributing factor to the detected results seen especially in the low GSH levels similar to that found earlier during exercise in rats (Pyke et al. 1986). As all dogs were on the same diet, nutritional differences could be excluded from playing any role.

In summary, the conclusion can be made that age-related differences in LP processes and antioxidants can be detected in several species at sites of several tissues. However, the importance of other effects such as nutritional and hormonal factors should also be taken into consideration.

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