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Selenium Induced Lipid Peroxidation in Heart Tissues of Chick Embryos

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During the past three decades research has been carried out to elucidate the role of free radicals and reactive oxygen species play in various pathophysiological processes (Fridovich 1986; Slater et al 1987). Membranes of subcellular organelles contain relatively high concentrations of polyunsaturated lipids as well as hemoproteins which are strong catalysts of lipid peroxidation. Lipid peroxides (LPO) destroy membrane integrity and decrease membrane fluidity and elasticity (Burch and Thayer 1983). Selenium is known both as an important essential trace element and an environmental pollutant (Jacobs and Forst 1981). Selenium has many uses in the industries (Hasan and Guler 1989). The main source of selenium for the mammalian organism is food (from the soil into the vegetables and grain) and to a lesser extent, drinking water. A number of syndromes of selenium toxicity in animals have been described (Yang 1987). Selenium is regarded as the most important biological antioxidant. The antioxidant function of selenium is linked to the activity of seleno enzyme glutathione peroxidase (GPx), which catalyses the reduction of hydroperoxides (Simmons and Jammall 1988). The antioxidant enzymes like superoxide dismutase (SOD) reduce superoxide radicals to H_2O_2 which inturn is converted into water by catalase (CAT) and peroxidases. The preferential oxidation of glutathione by peroxides is catalysed by GPx. The oxidized glutathione is then reduced by glutathione reductase (GR) and maintains the reduced glutathione levels in the system in a cyclic manner. Further, glutathione transferases (GST) catalyses the transformation of a wide variety of electrophilic compounds to less toxic compounds by conjugating them to GSH.

The present study evaluated the biochemical basis of selenium induced lipid peroxidative damage to heart tissues in chick embryos and the role of antioxidant enzymes like GPx, GST, GR, SOD and CAT.

MATERIALS AND METHODS

Freshly laid Bobcock strain zero day old eggs were procured from a poultry farm (Balaji Hatcheries, Tirupati). They were placed in an egg incubator, the temperature of which was already maintained

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at 37°C with 65% relative humidity. The eggs were rotated manually once a day during incubation. Injections of selenium (as selenium dioxide) dissolved in sterile distilled water of different concentrations (12.5 and 37.5 µmoles/kg egg wt.) in 10 µl were given to 14 d old chick embryos. Control embryos were injected with the same amount of distilled water. Injections were given through a small hole directly into the yolk sac and the hole was covered with surgical tape to prevent infection. A minimum of 25 embryos were used in each group. Embryos were sacrificed after different time intervals of selenium exposure to collect heart tissues for the determination of lipid peroxidation and antioxidant enzyme activities. Chick embryos were dissected and heart tissues were removed quickly, weighed and chilled in ice cold 0.9% NaCl. Level of lipid peroxidation was measured using thiobarbituric acid reaction as described by Ohkawa et al (1979). Tissues were collected and homogenized in 3 volumes of 70 mM phosphate buffer pH 7.4 containing 0.25 M sucrose, 10 mM EDTA and 1% Triton X-100. Post mitochondrial supernatants were prepared by centrifuging the homogenates at 12,000 rpm for 10 min and immediately antioxidant enzyme activities were estimated. Superoxide dismutase activity was measured by the method of Misra and Fridovich (1972). Catalase activity was determined by the method of Beer and Sizer (1952). Glutathione peroxidase activity was determined by continuous monitoring of NADPH oxidation in a recycling assay as described by Wendel (1981). Glutathione-S-transferase activity was measured using 1-chloro-2,4-dinitrobenzene as a substrate as previously described by Habig et al (1974). Glutathione reductase activity was assayed as described by Carlberg and Mannervik (1981). The protein content was determined by the method of Lowry et al (1951) using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

A very significant induction of LPO levels was observed with selenium treatment at initial hours of exposure like 3 and 6 hr and returned to normal levels by 48 hr (Fig. 1). We observed a dose dependent increase of LPO levels with selenium treatment and generally the LPO levels in controls decreased with age. There was 120 and 140 per cent induction of LPO levels observed with 37.5 µmoles of Se at 3 and 6 hr respectively. Our results are in agreement with Dougherty and Hoeckstra (1982) who showed acute selenite toxicity induced lipid peroxidation in rat heart. Selenium compounds are known to affect many cellular processes and functions (Ganther 1986). The ability of antioxidative protective systems to metabolize lipid peroxide or inhibit their formation is important in determining the level of LPO in tissues.

Results in Table 1 shows the effect of selenium on SOD and CAT activities at different time intervals. At early hours of selenium exposure both SOD and CAT activities were reduced. Maximum reduction was observed in SOD (73%) and CAT (53%) activities at 6 hr with 37.5 µmoles of Se treatment. Superoxide dismutase (EC 1.15.1.1) and catalase (EC 1.11.1.6) together forms an integral

Table 1. Effect of Selenium on Heart Superoxide dismutase and Catalase Activities in Chick embryos

Treatment with Selenium (μmoles/ kg egg wt.)	Superoxide dismutase			Catalase		
	Units/mg protein			Units/mg protein		
	3 hr	6 hr	48 hr	3 hr	6 hr	48 hr
Control	21.5 ±1.9	18.5 ±1.42	8.8 ±7.6	4.40 ±0.45	4.50 ±0.41	5.78 ±0.51
12.5	20.4 ±1.82	10.83** ±0.92	8.43 ±7.9	4.0 ±0.39	3.05** ±0.29	5.34 ±0.49
37.5	19.6 ±1.00	5.20*** ±0.41	9.03 ±8.2	3.4** ±0.30	2.10** ±0.19	5.52 ±0.50

**P < 0.01 *** P < 0.001 N = 25

Values are average of four separate experiments. Mean ± S.D.

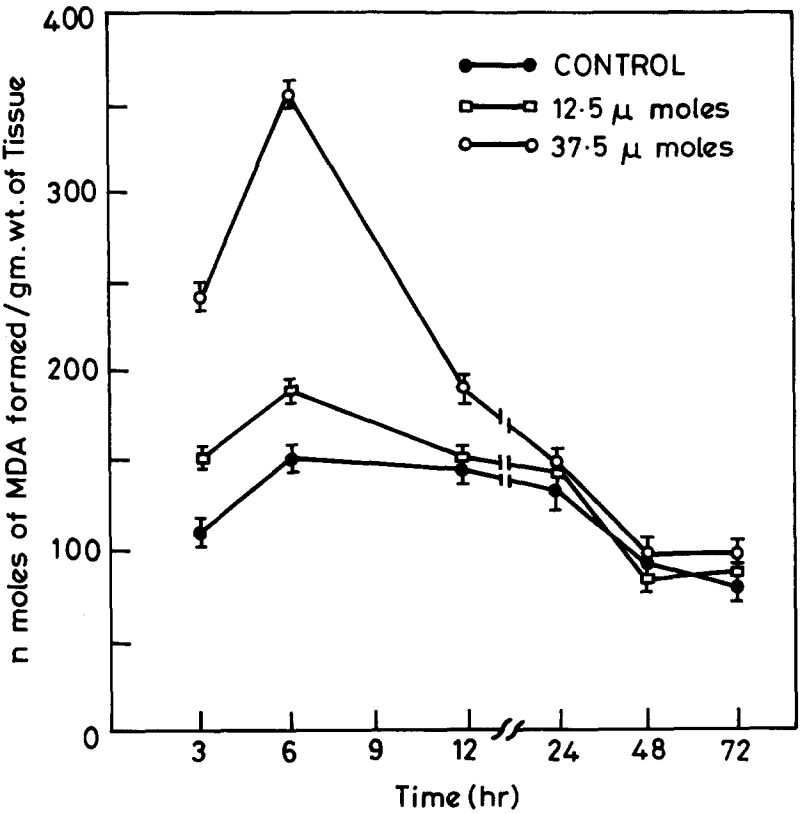

Figure.1.Effect of Selenium on heart lipid peroxide levels of chick embryos. Mean ± S·D·(N = 25)

part of the enzymatic defense against a free radical attack on biological membranes. Hence, the decreased activity of both SOD and CAT may be responsible for increased production of LPO. This is further confirmed by the normal levels of SOD and CAT together with normal LPO levels at 48 hr.

The action of selenium on GSH dependent enzyme system like GPx is shown in Fig. 2. Even excess amounts of Se failed to enhance the GPx activity which is a known selenoenzyme (Simmons and Jammall 1988) that catalyzes the reduction of H_2O_2 and a variety of organic hydroperoxides (ROOH) to water or the corresponding hydroxyl compound. Though the tissues of a majority of species studied contain appreciable levels of GPx activity (Wendel 1980), it is subject to numerous influences including dietary selenium (Scott et al 1977), age (Viarengo et al 1991), environmental factors (Srivastava et al 1988) and ingestion of peroxidized lipids (Reddy and Tappel 1974). Hence, the knowledge of biological and non-biological factors that influence the activity of this enzyme is therefore important in the study of detoxification, aging and other degenerative process. No change in the GPx activity was observed at 3 and 6 hr after exposure, while a

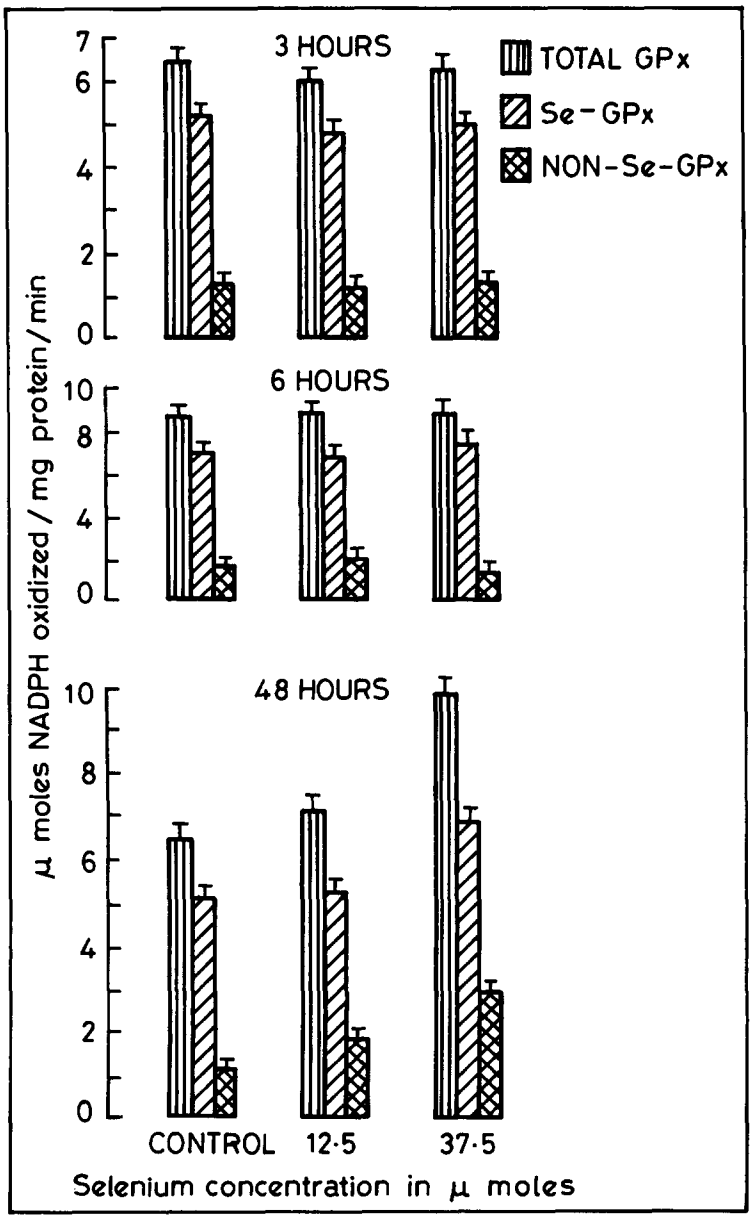

Figure.2.Effect of Selenium on heart GPx activity in chick embryos. Mean ± S·D (N=25)

significant 52 per cent increase was observed after 48 hr. The induction of the antioxidative protective systems are known to be a compensatory reaction against lipid peroxide induced damage (Sagai et al 1982). It is evident from our results that there was considerable reduction of LPO levels with concomitant increase of GPx activity at 48 hr after selenium exposure.

Table 2. Effect of Selenium on Heart Glutathione transferase and Glutathione reductase Activities in Chick embryos

Treatment with Selenium (μmoles/ kg egg wt.)	Glutathione transferase			Glutathione reductase		
	μmoles of GSH-CDNB conjugate formed/mg protein/min.			μmoles of NADPH oxidized/mg protein/min.		
	3 hr	6 hr	48 hr	3 hr	6 hr	48 hr
Control	12.6 ±1.02	12.68 ±1.25	12.0 ±1.19	6.07 ±0.59	6.20 ±0.55	9.87 ±0.87
12.5	12.07 ±1.09	12.02** ±1.22	12.89 ±1.82	6.2 ±0.60	6.40 ±0.54	12.23 ±1.02
37.5	12.32 ±1.21	12.54 ±1.23	15.0** ±1.34	6.2 ±0.61	6.39 ±0.52	18.42*** ±1.32

**P < 0.01 ***P < 0.001 N = 25

Values are average of four separate experiments. Mean ± S.D.

Table 2 shows the effect of selenium on GST and GR activities. The response of GST and GR activities are similar to that of GPx. No change in activities of these two enzymes were observed at 3 and 6 hr, while 87 and 25 per cent increase in GST and GR activities were observed at 48 hr. Glutathione transferases function as a scavenger protein for many reactive intermediates (Younes et al 1980). Few isoenzymes of GST are known to possess Non-Se-GPx activity (Prohaska and Ganther 1977). Hence, it can be suggested that the enhanced activities of GSH dependent enzymes scavenge free radicals and LPO and detoxify them.

It can be concluded from our results that selenium administration caused the accumulation of LPO in heart tissues of chick embryos. The elevated levels of LPO at 6 hr after Se treatment may be due to reduced antioxidant enzymes like SOD and catalase. However, the induction of LPO was considerably reduced at 48 hr with concomitant raise in activities of GPx and GST.

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References

Beer RF, Sizer IW (1952) A spectrophotometric method for measuring the break down of hydrogen peroxide by catalase. J Biol Chem 195: 133-140

Burch RC, Thayer WS (1983) Differential effect of lipid peroxidation on membrane fluidity as determined by electron spin resonance probes. Biochim Biophys Acta 733: 216-222

Carlberg I, Mannervik B (1981) Purification and characterization of glutathione reductase from calf liver. An improved procedure for affinity chromatography on 2′ 5′ ADP Sepharose 4B. Anal Biochem 116: 531-536.

Dougherty JJ, Hoeckstra WG (1982) Stimulation of lipid peroxidation invivo by injected selenite and lack of stimulation by selenate. Proc Soc Exp Biol Med 169: 209-215

Fridovich I (1986) Biological effects of the superoxide radical. Arch Bio Chem 247: 1-11

Ganther HE (1986) Chemistry and metabolism of selenium. In: GF Jr Combs, JE Spallholz, OA Levander, JE Oldfield (eds) Proceedings of the third International Symposium on selenium in biology and medicine. Avi Publ Co, Westport, Conn.

Habig WH, Pabst MJ, Jacoby WB (1974) Glutathione-S-transferase. First enzymatic step in mercapturic acid formation. J Biol Chem 249: 7130-7139

Hasan A, Guler S (1989) Titrimetric determination of selenium in Anodic slimes. Talanta 36: 723-726

Jacobs M, Forst C (1981) Toxicological effects of sodium selenite in Sprangue Dawley rats. J Toxicol Environ Health 8: 575-585

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin-phenol reagent. J Biol Chem 193: 265-275

Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247: 3170-3175

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Bio Chem 95: 351-358

Prohaska JR, Ganther HE (1977) Glutathione peroxidase activity of glutathione-S-transferases purified from rat liver. Biochem Biophys Res Commun 76: 437-445

Reddy K, Tappel AL (1974) Effect of dietary selenium and autoxidized lipids on the glutathione peroxidase system of the gastrointestinal tract and other tissues of the rat. J Nutr 104: 1069-1078.

Sagai M, Ichinose T, Oda H, Kubota K (1982) Studies on biochemical effect of nitrogen dioxide II. Changes of the protective systems in rat lungs and of lipid peroxidation by acute exposure. J Toxicol Environ Hlth 9: 153-164

Scott D, Kelleher J, Losowsky MS (1977) The influence of dietary selenium and Vitamin B on glutathione peroxidase and glutathione in the rat. Biochem Biophys Acta 497: 218-224

Simmons TW, Jammall IS (1988) Significance of alterations in hepatic antioxidant enzymes. Primacy of glutathione peroxidase. Biochem J 25: 913-917

Slater, TF, Cheeseman KH, Davies MJ, Proudfoot K, Xin W (1987) Free radical mechanisms in relation to tissue injury. Proc Nutr Soc 46: 1-12

Srivastava PK, Singh Y, Misra UK (1988) Pulmonary and hepatic glutathione levels, Glutathione shuttle enzymes and lipid peroxidation in rats exposed intratrachelly to coal fly ash. Biochem Int 17: 509-516

Viarengo A, Canesi L, Pertica M, Liningstone DR, Orunesu M (1991) Age related lipid peroxidation in the digestive gland of mussels. The role of the antioxidant defense systems. Experientia 47: 454-457

Wendel A (1980) Glutathione Peroxidase. Enzymatic basis of detoxification. In: WB Jacoby (ed) Vol 1. Academic Press, New York PP 333-353

Wendel A (1981) Glutathione Peroxidase. In: WB Jacoby (ed) Methods in enzymology Vol 77. Academic Press New York PP 325-333

Yang GQ (1987) Research on selenium related problems in human health in China. In: GF Jr Combs, JE Spallholz, OA Levander, JE Oldfield (eds) Selenium in Biology and Medicine. Part A. Avi Publ Co, New York, PP 9-32

Younes M, Schilichting R, Siegers CP (1980) Glutathione-S-transferase activity in rat liver. Effect of some factors influencing the metabolism of xenobiotics. Pharm Res Commun 12: 115-129

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