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Nickel Interactions with Glutathione and Related Enzyme in 11-Day Embryo and Yolk Sac In Rat

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The embryotoxicity and teratogenicity of nickel compounds has been observed in rats (Sunderman 1983). Little is known about the biochemical mechanism(s) of Ni-teratogenesis. Furthermore, nickel induced a rapid, but transient rise in the serum glucose and changes in hormone balance in pregnant rats (Mas et al. 1986) as well as metabolic changes in females (Clary 1975; Sunderman et al. 1978;). On the other hand, Sunderman support the theory that lipid peroxidation constitutes a molecular mechanism of acute nickel toxicity (Sunderman 1987). In this sense, the hepatic lipid peroxidation in $NiCl_2$-treated rats may reflect the transient depletion of hepatic glutathione that occurs in these animals (Sunderman 1984). Glutathione metabolism has been adapted to perform cellular protective functions and is an important component of the system that uses reduced pyridine nucleotide to provide the cell with its reducing properties (Meister 1988). Glutathione reductase (EC 1.6.4.2) (GSSG-R), glutathione peroxidase (EC.1.11.1.9) (GSHpx), and glutathione transferase (EC 2.5.1.18) (GST) enzymatic activities are involved in the defense function of the tripeptide (γ-Glu-CysH-Gly) (GSH). The purpose of this study was to characteizer the effect of $NiCl_2$ on glutathione and related enzymes in 11-day embryo and visceral yolk sac in rat.

MATERIALS AND METHODS

Virgin Wistar albino rats weighing initially 180-200 g were housed together overnight; the presence of a vaginal plug the following morning indicated that copulation had ocurred, and that day was designated pregnancy day 0. Pregnant dams were held in a room with 12-hour light/dark cycle (8 PM to 8 AM dark) and an ambient temperature of 20°. Animals were allowed free access to water and chow.

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Groups of 8-10 pregnant rats were randomly selected, $NiCl_2$ a dosage of 4 mg/kg was i.p. administred. Control rats were included and were given an i.p. injection of the vehicle alone (sterile NaCl solution). Both Ni-treated and control-rats were killed on day 11 gestation at 1 or 24 hours after treatment. Rats were sacrified by an overdose of ether, and conceptuses were removed from the uterus. The whole embryos were placed in petri dishes containing physiological saline solution and examined under a dissecting microscope. Embryos, with 29-27 somites, and visceral yolk sacs were homogenized for GSH and enzymatic analysis. Protein content was determined by the method of Lowry et al. (1951). Total glutathione levels were measured by the DTNB-GSSG reductase recycling assay (Griffith 1980). Embryonal tissue extracts were obtained with 5% sulphosalicilic acid. The reaction system contained 0.1 M Na-phosphate buffer (5mM Na-EDTA), pH 7.5; 0.6 mM DTNB; 0.06 mM NADPH; 2 units of GSSG-R; and suitable amount of neutralized tissular extract and/or water to give a final volume of 1.0 ml. GSH standars were up to 0.5 nmol in assay mixture. Change in color of DTNB was recorded spectrophotometrically at 412 nm with a Hitachi U-2000. On the other hand, embryo and yolk sac extracts were obtained with KCL 150 mM for enzymatic analysis. GSSG-R reaction system contained a suitable amount of sample, 200 mM of NADPH; 2 mM of GSSG, 5 mM FAD; 0.1 M Na-phosphate buffer pH 7.2 in 1 ml of assay mixture (Goldberg DM and Spooner RJ 1983). GSHpx activity using cumene hydroperoxide (CHP) as a substrate was assayed as follows: both sample and reference cuvettes contained 0.1 M of Tris-HCl, pH 8.0; 0.5 mM of EDTA; 0.2 mM of NADPH; 1 U of GSSG-R; 2 mM of GSH; and the appropiate amount of enzyme in 1 ml. GSHpx activity using H_2O_2 as a substrate was assayed as follows: both sample and reference cuvettes contained 0.1 M Na-phosphate buffer, pH 7.0; 0.5 mM of EDTA; 0.2 mM of NADPH, 1 U of GSSG-R; 1 mM of NaN_3; 2 mM of GSH and the appropiate amount of enzyme in 1 ml (Takahashi and Cohen 1986). The oxidation of NADPH regarding both GSSG-R and GSHpx enzymatic activity, was followed at 340 nm. GST reaction system contained a suitable amount of sample, 0.1 M Na-phosphate buffer pH 6.5; 1 mM of GSH; 1 mM of 1-chloro-2-4-dinitrobenzene (CDNB). GST activity was determined by monitoring changes in absorbance at 340 nm (Habig et al. 1974). All results are presented as the mean$\pm$SD. Statistical comparison between groups were done by Student's t test.

RESULTS AND DISCUSSION

The period when a teratogenic agents are most likely to cause malformations is during organogenesis (New 1978). The results found in the present study show a significant effect on GSH metabolism either in rat

Table 1. In vivo total GSH content in 11-day conceptus of Ni-and control-pregnant rats.

Tissue	Treatment	Total GSH
Embryo	Control	23.9±1.25
	1 hour	30.7±1.90*
	24 hours	18.5±0.90*
Yolk Sac	Control	28.4±1.54
	1 hour	41.3±1.98*
	24 hours	29.9±2.64

Values are expressed as nmol GSH/mg protein and mean±S.D. number of 50-60 embryos and visceral yolk sacs. In comparison with controls, *: $P<0.05$. Embryo and visceral yolk sac protein content was 0.20±0.002 and 0.091±0.006 mg respectively.

Table 2. In vivo GSSGR, GSHpx and GST enzymatic activities in 11-day concentus of Ni- and control pregnant rats.

Treatment	GSSGR	GSHpx(CHP)	GSHpx(H_2O_2)	GST(CDNB)
Embryo				
Control	5.80±0.19	54.3±4.14	54.4±2.10	7.26±0.34
1 hour	6.14±0.76	49.1±2.50	51.5±3.30	6.65±0.46
24 hours	4.06±0.33*	58.6±2.50	58.9±2.75	6.54±1.52
Yolk sac				
Control	30.7±3.23	104±12	109±8	10.1±1.09
1 hour	31.9±1.86	95.2±7.10	92.6±7.12	7.71±0.86
24 hours	11.1±4.99*	88.3±6.49	104±8	5.75±0.66*

Values of enzymatic activity are expressed as mU/mg protein and mean±S.D. number of 50-60 embryos and visceral yolk sacs. In comparison with controls, *: $P<0.05$.

embryo and visceral yolk sac of dams that were injected with Ni(II) (4 mg/kg body wt.). This dose was about 60-65 % that of LD_{50} of Ni(II), administred i.p. as $NiCl_2$, in the 11-day pregnant rat (LD_{50}: 6.30 mg Ni/kg body wt) (Mas et al. 1986). Table 1 shows GSH concentrations in these embyonal tissues of control and Ni(II)-treated rats. The effects of Ni(II) on GSH concentration in embryo were different according the time post-treatment considered. At 1 hour after Ni-administration the increase of

embryo GSH levels was 128% that of control, however, at 24 hour post-treatment the GSH concentration was the 77% that of control. In visceral yolk sac at 1 hour after a dose of 4 mg Ni/kg body wt. the increase was about 146% but, after a further 24 hours, the normal level was re-established. Table 2 shows the effects of Ni(II) on GSSGR, GSHpx and GST enzymatic activities in both embryo and visceral yolk sac. Not significant changes were seen at 1 hour post-treatment. However, at 24 hours after Ni-administration the GSSGR enzymatic activity decresed significantly in both embryo (70% that of control) and visceral yolk sac (36% that of control). GST enzymatic activities in visceral yolk sac from dams 24 hours after treatment decreased significantly respect controls dams (57% that of control).

Because conceptus nickel concentrations diminished rapidly following a single administration during organogenesis (Mas et al. 1985), it is not likely that changes on GSH metabolism found in conceptus of Ni-treated pregnant rats were induced by the direct action of residual nickel around the time at which the effects were observable. Therefore, depletion of the tripeptide in embryo may enhance the teratogenic effects of nickel, that appear later in gestation (Sunderman et al. 1978; Mas et al. 1985).

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