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Cobalt chloride induces hepatotoxicity in adult rats and their suckling pups

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ABSTRACT

To assess liver damages in pregnant and lactating rats and in their suckling pups, wistar female rats were given through drinking water 350 ppm of $CoCl_2$ (157 ppm Co^{2+}) from the 14th day of pregnancy until day 14 after delivery. The effects of cobalt chloride on lipid peroxidation levels, antioxidant enzyme activities, lipid profile and histopathology aspects of liver were evaluated. Biochemical results showed that lipid peroxidation increased significantly in Co-treated rats, as evidenced by high liver thiobarbituric acid-reactive substance (TBARS) levels. Alteration of the antioxidant system in treated group was confirmed by the significant decline of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities and reduced glutathione (GSH) content in liver of suckling pups and their mothers. Moreover, $CoCl_2$ exposure induced an increase in the activities of the aspartate transaminase (AST), alanine transaminase (ALT), lactate deshydrogenase (LDH) and bilirubin levels in pups and their mothers while liver LDH activity and plasma albumin level were significantly decreased. On the other hand, cobalt chloride induced a marked hypoglycemia, a significant decline in triglycerides and total cholesterol levels. Histological studies showed an infiltration of mononuclear cells and vascular congestion in liver of pups and their mothers.

Based on the present findings, exposure of rats to $CoCl_2$ during late pregnancy and early postnatal period affects antioxidant enzyme activities and lipid peroxidation indicating liver damage in mothers and their offspring.

Introduction

Heavy metals are too well known as environmental pollutants with dangerous effects on human health due to their wide usage in many industrial branches. They are present everywhere in the air, water and soils (Boguszewska and Pasternak, 2004). Trace metals in soils may originate also from parent rock or from anthropogenic sources, primarily fertilizers, pesticides and herbicides (Smith and Carson, 1981). Among them, cobalt emission to the atmospheric, aquatic and terrestrial environment increased dramatically during the twentieth century. Cobalt is an oligoelement present in almost all the animal and vegetal organisms; its biological importance is due to its essential role in the formation of vitamin B_{12} and others cobalamines. Vitamin B_{12} is necessary for the organism, because it is involved in the formation of some proteins and in the normal functionality of the nervous system. It can cause pernicious anaemia and peripheral nervous system diseases (Karovic et al., 2006). This metal is combined with the auxin herbicide triclopyr to enhance its activity (Morré et al., 2004). Moreover, ^{60}Co γ-radiation is used in order to degrade the herbicide 2, 4-dichlorophenoxyacetic acid (2, 4-D) dimethylamine salt in water in the presence of humic acid (Campos et al., 2003). Data in the literature indicate that cobalt is cytotoxic to many cell types, including neural cells.

Exposure to an excessive amount of Co can have deleterious effects on the human body (ATSDR, 2001). As a class of toxic agents, metals are a concern of the highest priority for human exposure. They are also unique environmental pollutants, which are neither created nor destroyed by humans. They are non-biodegradable and persist in the environment. They are transported and transformed into various products, which in turn affect the growth and longevity of aquatic and terrestrial animals (Pourahmad et al., 2003). They have a vast array of remarkable adverse effects, including those of cardiomyopathy (Kesteloot et al., 1968), adverse pulmonary effects (Wehner et al., 1977), carcinogenicity and hepatotoxicity (Pourahmad et al., 2003).

Human body contains about 1–2 mg of cobalt, incorporated particularly in soft tissues such as, heart, spleen, kidney and liver (Elinder and Friberg, 1986). The last one is a major organ involved in metabolic homeostasis as well as drug and chemical detoxification (Millward-Sadler, 1987). Due to its role in the transformation of environmental xenobiotics, the liver is at great risk of injury, where high intracellular concentrations of such compounds can be reached (Davies and Portmann, 1987).

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Cobalt is suspected to be toxic to many cell types, including neural cells (Yang et al., 2004) and can induce cell death by apoptosis and necrosis (Huk et al., 2004). Cobalt can cause DNA fragmentation (Zou et al., 2001), activation of caspases (Zou et al., 2002) and increases production of reactive oxygen species (ROS) leading to oxidative stress (Olivieri et al., 2001). These free radicals may lead to cellular damage when the rate of their generation overcomes the rate of their decomposition by antioxidant defense systems, such as superoxide dismutase (SOD), catalase (CAT), or reduced GSH (Di Mascio et al., 1991; Mates et al., 1999; Datta et al., 2000).

Although, the toxicity of cobalt in the organism has been studied in humans and adult rats, its role in producing oxidative stress in liver of pregnant and lactating rats and their progeny has not been largely examined.

The purpose of this study was to determine whether administration of cobalt chloride to female rats during late pregnancy and early postnatal periods would have effects on the systemic oxidative stress parameters of their progeny.

Materials and methods

Animals and experimental design

Experiments were performed on Wistar strain female rats weighing about 180 g and purchased from Central Pharmacy (SIPHAT, Tunisia). Animals were kept in an air-conditioned room (temperature 22 ± 3 °C and relative humidity of 40%) with a 12 h light/dark cycle. All animals received *ad libitum* pure water and standard rat chow (SICO, Sfax, Tunisia). After 1 week of acclimatization to the laboratory conditions, male and female rats were housed by pairs in each cage. Pregnant female rats were inspected daily by the presence of the vaginal plug, which indicated day zero of pregnancy. Twelve pregnant female rats were randomly divided into two groups of 6 each: (1) the control rats given drinking pure water; (2) the treated rats given drinking water supplemented with 350 ppm $CoCl_2$ (157 ppm Co^{2+}) from the 14th day of pregnancy until day 14 after delivery. This dose represents 1/9 of $LD_{50.}$ Pregnant female rats were allowed to deliver spontaneously 3 weeks after coitus. The day of birth was considered as postnatal day zero. Within 24 h after delivery, the number of pups born (8–12 pups by litter), their sex and weight were recorded. Excess pups were removed, so the litters were reduced to 8 pups each (4 males and 4 females if possible) in order to maintain a similar lactation performance and to ensure maternal care (Fishbeck and Rasmussen, 1987). Cobalt chloride intake ingested daily by lactating rats was calculated after measuring drinking water as reported in Table 1. So each lactating rat treated with cobalt chloride ingested 3.75 mg of cobalt per day. Moreover, the food intake was monitored daily (Table 1). This study was approved by the Ethical and Research Committee of Tunisian Public Health Ministry. All animal procedures were conducted in strict conformation with the "Institute ethical committee guidelines" for the care and use of laboratory animals (Maryland, National research Council Public Health Service, 1996).

At the end of the experimental period, 96 pups and 12 lactating mothers were sacrificed on postnatal day 14 after anesthesia by intra-abdominal injection with chloral hydrate. Blood samples were collected with heparin from brachial artery of pups and by aortic puncture of dams. They were centrifuged at 2200*g* and plasma samples were drawn and kept at −20 °C until biochemical determinations. Livers were rapidly excised, blotted, weighed and processed for histopathology and biochemical assays. Some portions of livers were rinced in cold saline buffer, weighed, finely minced and homogenized in the same solution (10%, w/v). The resulting supernatants were used for immediate lipid peroxidation assays and homogenate aliquots were stored at −80 °C for further biochemical assays.

Biochemical assays

Thiobarbituric acid-reactive substances (TBARS)

Oxidative damage was determined in fresh liver homogenates using the degree of lipid peroxidation. Malondialdehyde (MDA) was quantified by measuring the formation of thiobarbituric acid-reactive substances (TBARS), which were expressed in terms of MDA content according to Draper and Hadley method (1990). Aliquots of liver homogenates were incubated with 10% trichloroacetic acid and 0.67% thiobarbituric acid. The mixture was heated on a boiling water bath for 30 min. The absorbance of samples was determined at 532 nm. Results were expressed as nmol MDA/g organ.

Antioxidant enzyme activities

Superoxide dismutase (SOD) activity was estimated according to Beauchamp and Fridovich (1971). The reaction mixture contained 50 mM of tissue homogenates in potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM L-methionine, 2 μM riboflavin and 75 μM Nitro Blue Tetrazolium (NBT). The developed blue colour in the reaction was measured at 560 nm. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50% and the activity was expressed as units per mg of protein.

Catalase (CAT) activity was assayed by the method of Aebi (1984). Enzymatic reaction was initiated by adding an aliquot of 20 μl of the homogenized tissue and the substrate (H_2O_2) to a concentration of 0.5 M in a medium containing 100 mM phosphate buffer, pH 7.4. Changes in absorbance were recorded at

Table 1
Body and liver weights of suckling and adult rats, controls and treated with 350 ppm $CoCl_2$ through their drinking water from the 14th day of pregnancy until day 14 after delivery.

Parameters and treatments	Body weight (g)	Absolute liver weight (g)	Food intake (g/day/mother)	Drinking water (ml/day/mother)	Co^{2+} ingested (mg/day/mother)
Controls					
Mothers (n=6)	207.60 ± 9.31	10.57 ± 1.22	28.32 ± 3.08	36.41 ± 1.74	–
Pups (n=48)	24.93 ± 1.72	0.97 ± 0.09	–	–	–
$CoCl_2$					
Mothers (n=6)	195.87 ± 8.18 ns	$9.53 \pm 0.81^{*}$	$19.99 \pm 0.71^{***}$	$24.67 \pm 1.29^{***}$	3.75 ± 0.14
Pups (n=48)	$15.08 \pm 0.69^{***}$	$0.44 \pm 0.05^{***}$	–	–	–

Significant differences: $CoCl_2$ vs. controls, $p^{***}p \le 0.001$ and $^{*}p \le 0.05$.
Number of determinations is indicated between parenthesis.