Bull. Environ. Contam. Toxicol. (1996) 57:327-334

Bulletin of Environmental Contamination and Toxicology

Embryo- and Fetotoxicity of Chromium in Pregestationally Exposed Mice

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Received: 1 June 1995/Accepted: 9 March 1996

Chromium, an essential element in the human body required for proper carbohydrate, protein, and fat metabolism, is reported to impair gestational development of offspring of workers chronically exposed to this metal in the work place. Workers in chromium based industries can be exposed to concentrations two orders of magnitude higher than the general population (Hemminki and Vainio 1984). Among the general population, residents living near chromate production sites may be exposed to high levels of chromium (VI) in air or to elevated levels (40 - 50,000 ppm) of chromium in effluents (Rumar 1987). Shmitova (1978,1980) reported afterbirth and puerpera1 hemorrhages in women industrially exposed to this metal and observed high chromium levels in blood and urine of pregnant women and in fetal and cord blood. Chromium readily passes the placental barrier and reaches the growing fetus (Tipton 1960; Pribluda 1963). Exposure of mice to chromium during various gestational periods resulted in embryo and fetotoxic effects (Junaid et al. 1995, 1996).

Pribluda (1963) reported that the chromium content of bones of pregnant rats decrease with the advancement of gestation. Such released chromium may reach the circulatory system and enter feto-placental tissue through the placental barrier. Therefore, it was thought worthwhile to ascertain the role of body chromium accumulated pregestationally on embryo and fetal development and its subsequent transfer to feto-placental sites.

MATERIALS AND METHODS

Sixty, 4-month old, Swiss albino, female mice (body weight 30 ± 5 gms) of proven fertility from the Industrial Toxicology Research Centre colony were divided into four groups of fifteen mice each. Group I was given drinking water and served as the control, while groups II, III, and IV were treated with 250, 500 and 750 ppm chromium (VI, as potassium dichromate), respectively, in drinking water for 20 days [time required for complete development of an ovarian follicle (Pederson 1970]. The selection of doses was based on our earlier study (Trivedi et al. 1989) and the fact that the average chromium intake of humans is approximately 200 ug/day in drinking water (NRC 1989). The animals were individually housed

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under standard animal house conditions (room temprature 20-22°C, relative humidity 50±5%) where a regular cycle of 12 hrs light: 12 hrs darkness was maintained and were provided with feed pellets (Lipton India Ltd.) and water ad libitum. The dams were observed daily for water intake and clinical signs of toxicity. After 20 days, females were mated with normal healthy, adult males and females were checked for pregnancy the next morning. The day that the vaginal plug was found was designated as '0'day of gestation. Mothers were weighed and kept individually in plastic cages. Ten pregnant females were randomly selected from each group, weighed, and sacrificed on the 19th day of gestation under ether anesthesia and caesarian sections performed. Blood from five animals from each group was withdrawn from the heart in heparinized vials and kept at -20°C for chromium estimation. One fetus plus placenta/litter was also kept at -20°C for chromium estimation. Both ovaries were removed and the number of corpora lutea was determined. Total implantations, the number of fetuses/litter, the number of live/dead fetuses, crown-rump length, the number of resorptions, and the weight of the fetuses and their respective placenta were recorded. Pre and post-implantation loss (%) was calculated as described by Palmer et al. (1978). Remaining fetuses were examined for gross external abnormalities and 1/3 of these fetuses were fixed in Bouin's fluid for examination of visceral abnormalities (Wilson 1965), while the others were fixed in 95% ethanol, eviscerated, and stained by the Alizarin red S method (Staples and Schnell 1964) for examination of skeletal deformities (Kelsey 1974).

Known amounts of maternal blood, placentae and the fetuses were digested in Nitric acid:Perchloric acid (6:l) mixture till a white residue remained at the bottom of the flask. The residue was dissolved in 5.0 ml of 0.1 N Nitric acid and read on DC Plasma Emission Spectrophotometer (Beckman Spectrospan V). Blank and spiked samples were also run and analyzed simultaneously (Trivedi et al. 1989). The embryo- and feto-toxicity data in Table 1 and chromium estimation data in Table 3 were analysed by one-way ANOVA followed by Student's 't' test while gross and skeletal abnormalities data in Table 2 were analysed by Fischer's Exact Test (Brunning and Kintz 1977).

RESULTS AND DISCUSSION

The treated females did not show any notable change in behaviour or external features. Mortality (3 females; 20%) was observed in group IV. Autopsy of these animals could not establish the cause of death. Daily chromium (VI) intake as calculated by water consumed: 1.9 ± 0.02, 3.56 ± 0.03, and 5.23 ± 0.07 mg Cr for groups II, III, and IV, respectively. Water consumption in the control group was 8.52 ± 0.21 ml/mouse/day. No significant change in the weight of the mothers during the treatment was observed. Gestational weight gain of mothers in groups II and III was not significantly different when compared to controls; group IV registered no weight gain during gestation.

We observed an absence of implantation in the uterine horns of

group IV mothers. While corpora lutea were present, their numbers were significantly reduced compared to the rest of the treatment groups.

Group III had a significant ($P<0.05$) increase in the number of resorptions (37%) when compared with the control group. Decrease in fetal weight (39%) and crown rump length (28%) and increase in placental weight (63%) as well as pre-(25%) and post-implantation (37%) loss was evident in group III compared to the control group. No significant difference in the number of corpora lutea was observed in group III compared to group II.

There was a significant ($P<0.05$) decrease in fetal weight (30%), placental weight (7%) and crown-rump length (17%) and an increase in post-implantation loss (18%) in group II compared to the control (Table 1). No dead fetuses were observed in any of the treated groups.

The fetuses of group III had higher ($P<0.05$) number of sub-dermal haemorrhagic patches and kinky and short tails. The number was markedly higher than for the control and group II animals (Table 2)

No major skeletal abnormalites was observed in any of the treated groups. Significantly reduced ossification in caudal, parietal and interparietal bones of the fetuses of group III was observed in treated mothers (Table 2). Soft tissue examination did not reveal any significant deformities in any of the treated groups.

Blood chromium was signifcantly higher in group IV compared to all other groups whereas that of groups II and III was elevated compared to controls. Placental chromium concentration increased in a dose-dependent manner in groups II and III compared to controls. Fetuses of mothers in group III had significantly higher chromium concentrations compared to fetuses of control and group II mothers (Table 3).

Chromium (VI) is reported to pass the placental barrier and accumulate in fetal tissues (Shmitova 1980). The presence of chromium (VI) in fetuses and infants has been reported in women working or living near the dichromate industries (Shmitova 1978). It was also noticed that women working in chromium-based industries for many years experienced abnormal menses, which was attributed to ovarian-hormonal impairment (Ross 1978). Tipton (1960) reported the transfer of chromium from the mother to the bones of the developing fetus in humans. In rats, the pregestationally retained chromium is reported to pass to the developing fetuses if exposure is stopped during gestation (Pribluda 1963).

Chromium speciation, concentration, and duration of exposure are important variables influencing tissue distribution. Gastrointestinal uptake of chromium is 2 - 10 % of the dose in both humans and laboratory animals. Shiraishi and Ichikawa (1972) reported that the bones and kidneys of rats contained the highest chromium concentration in comparison to other tissues monitored following oral administration of chromium (VI).

Table 1. Chromium-induced embryo- and feto-toxicity in mice treated during the pregestational period.

Parameters	Group I (Control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Weight gain in mothers (g)	14.40 ± 1.01	13.43 ± 0.50	12.38 ± 0.49	1.7 ± 0.93
Number of corpora lutea/mice	7.9 ± 1.01	7.4 ± 0.50	7.3 ± 0.37	4.4 ± 0.50 abc*
Number of implantations/mice	7.7 ± 0.74	6.8 ± 0.41	5.4 ± 0.27 a*	0
Number of live fetuses/mice	7.7 ± 0.74	5.6 ± 0.50	3.4 ± 0.24 ab*	0
Number of resorptions/mice	0	1.20 ± 0.44	2.0 ± 0.31 a*	0
Pre-implantation loss (%)	2.77 ± 1.21	8.38 ± 3.53	24.79 ± 2.17 ab*	100 %
Post-implantation loss (%)	0	17.51 ± 2.22 a*	36.66 ± 4.94 ab*	0
Fetal weight (g)	1.59 ± 0.04	1.11 ± 0.04 a*	0.97 ± 0.03 ab*	0
Placental weight (g)	0.137 ± 0.003	0.128 ± 0.005a*	0.223 ± 0.005ab*	0
Crown-rump length (cm)	2.92 ± 0.07	2.41 ± 0.08 a*	2.09 ± 0.08 ab*	0

Value represents mean ± S.E. of 10 female mice in each group.

The significance of the difference among various groups was evaluated by applying one-way ANOVA followed by Student's 't' test (Brunning and Kintz 1977).

* Significance $p < 0.05$. Comparison between two groups: a -vs control; b -vs 250 ppm; c -vs 500 ppm

Table 2. Incidences of gross and skeletal abnormalities in the pups of dams treated with chromium during the pregestational period.

Parameters	Group I (Control)	Group II (250 ppm)	Group III (500 ppm)
Gross abnormalities			
Number of pups/litters observed	72/10	51/10	19/10
Drooping rist	0/10	0/10	6/4 (32)
Sub-dermal hemorrhagic patches	0	8/6 (16)	8/4 (42) a*
Kinky tail	0	0	8/6 (42) a*
Short tail	0	4/4 (9)	10/4 (53) a*
Skeletal abnormalities			
Number of pups/litter observed	48/10	34/10	19/10
Reduced parietal ossification	0	0	12/10 (63) a*
Reduced inter-parietal ossification	0	0	10/10 (53) a*
Reduced caudal ossification	6/4 (12)	18/8 (53) a*	18/10 (95) a*

Gross and skeletal abnormalites are represented as number of abnormal pups/litters observed. The statistical significance was evaluated by Fisher's Exact test (Drunning and Kintz 1977). Percentage in parentheses calculated by the total number of pups observed.

* Significance $p < 0.05$. Comparison between two groups: a-vs control.

Table 3. Chromium concentrations in different tissues of mice treated during the pregestational period

Tissue	GroupI (Control)	Group II (250 ppm)	Group III (500 ppm)	Group IV (750 ppm)
Blood (µg/mL)	0.03 ± 0.007	0.05 ± 0.006 a*	0.06 ± 0.008 a*	0.13 ± 0.007 abc*
Placenta (µg/g:f.w.)	0.09 ± 0.001	0.14 ± 0.008 a*	0.17 ± 0.002 ab*	No implantation
Fetus (µg/g:f.w.)	0.04 ± 0.008	0.07 ± 0.007	0.16 ± 0.013 ab*	No implantation

Values represent mean ± S.E of 5 mice in each group.

The significance of the difference among various groups was evaluated by applying one-way ANOVA followed by Student's 't' test (Brunning and Kintz 1977). * Significance $p < 0.05$.

Comparison between two groups: a -vs control; b -vs 250 ppm; c -vs 500 ppm. f.w. q fresh weight.

In the present study, the treated animals showed an increase in blood chromium concentration compared to controls, with the highest dose group (750 ppm) having the highest chromium concentrations. However, blood chromium concentrations of the 250 and 500 ppm dose group were not significantly different from one another. This may be attributed to the fact that chromium (VI) enters the red blood cells where reduction to chromium (III) and subsequent binding to hemoglobin takes place. Assimilation of chromium (VI) in excess of the amount that can be reduced and sequestered results in longer residence time of chromium (VI) in blood and, hence, greater exposure of body tissues (Saner 1980). Although we have not assessed the extent of chromium transfer from maternal tissues to fetal tissue in the present study, the results from previous studies suggest transfer of prestored chromium from maternal soft tissue and/or bones to the developing fetus (Fitzgerald et al. 1985).

We observed a dose-dependent rise in placental chromium concentration as compared to the fetus. This may be due to the placenta acting as a barrier to retard passage of chromium from the mother to the fetus to safeguard fetal development and growth. The highest close group in this study (750 ppm) did not show any implantation. However, the release of ovum. as evidenced by the presence of corpora lutea, was apparent, although highly reduced in number compared to the rest of the treated and control groups. This reduction in number of' corpora lutea may possibly be due to direct accumulation of chromium in ovarian tissue (Langard 1982) or reduced hormone levels (Mattison et al. 1983).

Pre-implantation loss (100%) in the highest dose group may also be attributed to reduced hormone levels (Mattison et al. 1983) or impaired embryos, as reported earlier (Jacquet and Draye 1982). Chromium passed to the fetus could have resulted in reduced fetal ossification, influencing fetal development either through a direct effect on fetal tissue (Matsumoto et al. 1976) or impairment of placental physiology (Faulk 1981).

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