LOW IRON DIET AND CADMIUM EXPOSURE DISRUPT STEROIDOGENESIS IN THE RAT

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1. INTRODUCTION

The existing evidence in humans is not sufficient to consider female reproductive effects as critical effects of cadmium (Cd) exposure (Järup *et al*., 1998). Further facts argue for Cd as an element of concern as a potential reproductive toxicant in women. It accumulates in internal organs during a lifetime, including tissues of a reproductive axis; more in smokers (Varga *et al*., 1993) and women habitually eating a diet rich in Cd (shell-fish and high fiber diet) (Moberg Wing *et al*., 1992). Cadmium interferes with trace element homeostasis. Iron (Fe) status has been shown to influence the gastrointestinal absorption of Cd both in experimental animals and humans (Berglund *et al*., 1994). Women are high at risk group for Cd effects; 1) they usually show greater tissue Cd concentrations than men; 2) they are prone to Fe deficiency during childbearing age; 3) as evidenced by animal studies, gastrointestinal absorption of Cd is increased during gestation and lactation (Järup *et al*., 1998; Kostial *et al*., 1991a,b).

To date only limited information is available on the effects of Cd exposure on ovarian steroid production (Paksy *et al*., 1989; Piasek and Laskey, 1994). No data on Cd effect on placental steroidogenesis are available. We found that acute Cd exposure in rats appeared to cause endocrine disruption in the ovary with target organ Cd accumulation and concomitant decrease in tissue Fe, which were related to the reproductive stage.

The objective of this work was to evaluate the effect(s) of low Fe diet and concurrent subchronic Cd exposure during gestation on both ovarian and placental steroidogenesis in rats.

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2. MATERIAL AND METHODS

The study was conducted on timed-pregnant 60-days-old Sprague-Dawley rats fed semisynthetic pelleted laboratory diets (Teklad, Madison, WI, USA) with either high Fe (240mg/kg) or low Fe (10mg/kg) content. From gestation day one, the dams were continuously exposed to Cd (chloride) at a total dose of 0 (control), 3, or 5 mg/kg body weight during 19 days (minimum six animals per group) by subcutaneously implanted osmotic pumps (MP 2ML4 Alzet, Alza Co., Palo Alto, CA, USA). On gestation day 19, the dams were exsanguinated by cardiac puncture in CO_2 anesthesia and blood was taken for serum. Right ovaries and two placentas (one from each extreme lateral position in the uterine horn) were removed. Placentas were separated into maternal and fetal portions. Data of the maternal portions of the placentas are presented here.

Progesterone and estradiol serum concentrations and progesterone, testosterone and estradiol productions in the minced whole ovary cultures were analysed as described earlier (Piasek and Laskey, 1994). Placental progesterone and testosterone productions were assessed in the cultures of minced maternal placentas. Specific radioimmunoassay (RIA kits Coat-A-Count®, Los Angeles, CA) was used for steroid hormone analyses. Cadmium was analysed in the placental tissue (composite samples) by electrothemal atomic absorption spectrometry (Varian SpectrAA 300A, Australia). The data were statistically evaluated using both one-way and two-way analysis of variance (ANOVA) and test for linearity in the general linear models procedure (PROC GLM) available in 1985 edition of the Statistical Analysis System (SAS) with dietary Fe and Cd exposure as two independent variables.

3. RESULTS AND DISCUSSION

No Cd effects were found on maternal general health and fetal viability. Low Fe diet itself was associated with lower maternal body weights, decreased maternal and fetal hematocrits, reduced number of viable fetuses and increased late resorptions (data not presented).

Summary effects of diet with low Fe and concomitant parenteral Cd exposure during 19 days of pregnancy are presented in the Table 1. The data show that all dams fed low Fe diet had reduced serum progesterone. With subchronic Cd exposure, serum estradiol concentrations decreased and the effect was linear. The effects of low Fe diet and concurrent Cd exposure on placental steroidogenesis were additive and linear with significant reduction in maternal placental progesterone production at 5 mg/kg Cd dose. No effect was observed on either testosterone production in the maternal portions of the placentas or steroid production in whole ovary cultures (data not shown). With both high and low Fe diets, increase in Cd concentrations in the maternal placentas of exposed dams was dose-related. Average placental Fe concentrations were greater in the rats fed high Fe feed comparing to the low Fe diet group, no matter the Cd exposure (85 ± 3 and 52 ± 2. g/g wet tissue wt., respectively).

The results of our previous studies have suggested that, with acute exposure, Cd appears to interfere with normal rat streoidogenesis at several sites in the biosynthetic pathway, with serum estradiol concentration and ovarian estradiol production most affected in proestrus and in the first third of pregnancy (Piasek and Laskey, 1994). Findings of this study imply that low Fe diet with concomitant subchronic Cd exposure during pregnancy disrupt placental steroid production and concentrations of circulating steroid

Table 1. Effects of low iron (10 mg/kg) diet and concurrent exposure to cadmium (3 and 5 mg/kg body wt. by subcutaneously implanted osmotic pumps from gestation day 1–19) on steroidogenesis and placental cadmium in the rats

EXPOSURE\DIET	Fe 240 mg/kg	Fe 10 mg/kg	CADMIUM EFFECT
	Serum Progesterone [ng/ml]		
Control	(14) 63.9 ± 2.37	(13) 52.5 ± 1.89	(27) 58.4 ± 1.87
Cd 3 mg/kg	(7) 62.1 ± 3.00	(7) 48.8 ± 2.30	(14) 55.4 ± 2.59
Cd 5 mg/kg	(7) 60.9 ± 3.42	(8) 58.4 ± 3.12	(15) 59.6 ± 2.25
IRON EFFECT	(28) 62.7 ± 1.60	(28) 53.3 ± 1.49**[a]	
	Serum Estradiol [pg/ml]		
Control	(12) 19.1 ± 3.07	(13) 15.0 ± 2.32	(25) 17.0 ± 1.91
Cd 3 mg/kg	(6) 13.1 ± 2.82	(7) 13.7 ± 5.52	(13) 13.4 ± 3.12
Cd 5 mg/kg	(7) 11.3 ± 3.61	(8) 7.32 ± 1.96	(17) 9.19 ± 1.98**[b,c]
IRON EFFECT	(25) 15.5 ± 1.98	(28) 12.5 ± 1.87	
	Placental Progesterone Production [ng/g/hr]		
Control	(14) 13.1 ± 1.39	(13) 12.5 ± 1.45	(27) 12.8 ± 0.984
Cd 3 mg/kg	(7) 12.8 ± 1.73	(7) 10.0 ± 1.08	(14) 11.4 ± 1.05
Cd 5 mg/kg	(7) 10.8 ± 1.21	(8) 8.33 ± 1.23*	(15) 9.48 ± 0.896*[b,c]
IRON EFFECT	(28) 12.4 ± 0.862	(28) 10.7 ± 0.854	
	Placental Cadmium [. g/g wet wt.]		
[§]Cd 3 mg/kg	(8) 0.321 ± 0.043	(7) 0.381 ± 0.055	(15) 0.349 ± 0.034
Cd 5 mg/kg	(7) 1.19 ± 0.145**	(8) 1.18 ± 0.169**	(15) 1.18 ± 0.109*[b]
IRON EFFECT	(15) 0.166 ± 0.031	(15) 0.148 ± 0.032	

The results are presented as arithmetic means ± SEM (numbers of rats in parentheses).
[§]Control values were close to the detection limit by electrothermal atomic absorption spectrophotometry (0.5–0.9 ng/g tissue wet wt.).
Significant differences from control: *P < 0.05, **P < 0.01; [a]Iron main effect; [b]Cadmium main effect; [c]Linear cadmium effect (at P < 0.01). No Cd × Fe interaction.

hormones near the full term. This, together with decreased maternal and fetal Fe body stores and increased placental Cd, may pose a risk for growth and development of the young.

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