Effect of Progesterone Pretreatment on Cadmium Toxicity in Male Fischer (F344/NCr) and Wistar (WF/NCr) Rats

Noriyuki Shiraishi, Robert A. Barter, Hiroshi Uno, and Michael P. Waalkes

Laboratory of Comparative Carcinogenesis, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, Maryland

A previous report indicated that progesterone pretreatment can markedly reduce cadmium (Cd) toxicity in male NAW mice. Therefore we examined the effects of progesterone pretreatment on Cd toxicity in male Fischer (F344) and Wistar (WF) rats. A single subcutaneous injection of 10 or 30 nmole (CdCl₂/kg) proved nonlethal over 24 hr but caused the typical spectrum of testicular lesions in these rats. Moreover, when F344 rats were pretreated with progesterone (100 mg/kg, sc, at -48, -24, and 0 hr) and then given cadmium (20 nmole CdCl₂/kg, 0 hr), this dose of cadmium proved very toxic, unexpectedly causing 53% mortality. Progesterone pretreatment had no effect on cadmium-induced lethality in WF rats or on testicular lesions in either strain. Significant elevations in serum lactate dehydrogenase (LDH) activity, indicative of hepatotoxicity, were also observed in progesterone-pretreated F344 rats given cadmium as compared to rats given Cd alone. Progesterone did not induce increases in hepatic or renal metallothionein (MT) and hepatic or testicular MT-I mRNA levels in F344 rats. In contrast, levels of the testicular cadmium-binding protein (TCBP) in progesterone-pretreated F344 rats were doubled. This increase in TCBP provided no protection against cadmium toxicity in the testes. These results indicate that, in contrast to previously reported data for mice, progesterone pretreatment increased the lethality of cadmium in male F344 rats and had no effect on cadmium-induced testicular toxicity in F344 and WF rats. — Environ Health Perspect 102(Suppl 3):277–280 (1994).

Key words: cadmium toxicity, progesterone, hepatotoxicity, metallothionein, testicular cadmium-binding protein

Introduction

Although steroid hormones can modify cadmium toxicity in rodents, very little mechanistic information about the effect of these compounds on cadmium-induced lesions is available. Gunn et al. (1) reported that estrogen and stilbestrol protected CD-1 mouse testes against injury from cadmium, although estrogens did not modify cadmium accumulation in the testes (2). Wolkowski-Tyl and Preston (3) reported that progesterone pretreatment prevented the necrotizing effects of cadmium in NAW mouse testes, but corticosterone and dexamethasone were without effect. That study suggested that progesterone induced a dimer of cadmium-thionein (Cd-bp-D, 19,000 mw) in liver that may have an important role in resistance to cadmium-induced testicular necrosis (3). However, these authors did not show whether progesterone induced cadmium-binding proteins in the testes, and cadmium content in testes was actually increased by prior progesterone treatment. Therefore, the basis of the cadmium tolerance induced by sex hormones is unclear and has not been demonstrated in species other than the mouse. Thus, the current study examined the effects of progesterone pretreatment on cadmium toxicity in male Fischer (F344) and Wistar (WF) rats, and investigated the induction of cadmium-binding proteins by progesterone.

Materials and Methods

Animals and Treatment

Adult male Fischer (F344) and Wistar (WF) rats from the Frederic Cancer Research and Development Center Animal Production Area (Frederick, MD), weighing 300 to 400 g, were used throughout this study. Progesterone (100 mg/kg), dissolved in sesame seed oil, or oil (4 ml/kg) were injected, sc, for three consecutive days. Cadmium chloride (CdCl₂; 10–30 nmole/kg), dissolved in saline, or saline (4 ml/kg) was injected concomitantly with the last injection of progesterone or oil. Rats were sacrificed by CO₂ asphyxia 24 hr after the last injection and liver, kidney, and testes were excised.

Histological Analysis of Tissue Damage

Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. One representative section of liver and both testes and kidneys were examined.

Biochemical Analysis

Serum lactate dehydrogenase (LDH) and blood urea nitrogen (BUN) were assayed with commercially available kits (Sigma Chemical Co., St. Louis, MO). Metallothionein (MT) levels were determined in liver and kidney using the cadmium-hemoglobin method of Onosaka et al. (4) as modified by Eaton and Toal (5). The assay was also used to measure testicular cadmium-binding protein (TCBP) levels (6). MT-I mRNA levels were determined by Northern and slot-blot analysis, which were carried out as described by Coogan et al. (7). Relative MT-I mRNA expression was defined as MT-I mRNA content in relation to β-actin mRNA content. Tissue metal concentrations were measured by atomic absorption spectrometry after acid digestion.
Table 1. Cadmium-induced mortality in male F344 and Wistar rats: effect of progesterone.

| Treatment | Dead/treated | (%) |
|-----------|--------------|-----|
| Control   | 0/10         | 0   |
| Progesterone | 0/10     | 0   |
| Cd (10 μmole/kg) | 0/4   | 0   |
| Cd (20 μmole/kg) | 0/6   | 0   |
| Progesterone + Cd (10 μmole/kg) | 0/4   | 0   |
| Progesterone + Cd (20 μmole/kg) | †0/10† | 53  |

Wistar

| Treatment | Dead/treated | (%) |
|-----------|--------------|-----|
| Control   | 0/4          | 0   |
| Progesterone | 0/4       | 0   |
| Cd (20 μmole/kg) | 0/4   | 0   |
| Cd (30 μmole/kg) | 0/4   | 0   |
| Progesterone + Cd (20 μmole/kg) | 0/10  | 0   |
| Progesterone + Cd (30 μmole/kg) | 1/10  | 10  |

Rats were pretreated with progesterone (100 mg/kg, sc, -48, -24 and 0 hrs) and then given Cd (10 to 30 μmole/kg, 0 hr).

Assessed 24 hrs after Cd treatment. The asterisk indicates a significant difference (p<0.05) from control.

Table 2. Effect of progesterone pretreatment on cadmium distribution to testes, liver and kidney in male F344 rats.

| Treatment | Testes, ng/organ | Liver, μg/g | Kidney, μg/g |
|-----------|------------------|-------------|--------------|
| Control   | ND               | 2.1 ± 1.4a  | 0.6 ± 0.6b  |
| Progesterone | ND        | 0.2 ± 0.5a  | 1.1 ± 0.4a  |
| Cd        | 269 ± 90a       | 29.9 ± 4.7b | 19.7 ± 8.5b |
| Progesterone + Cd | 353 ± 118a | 23.6 ± 5.8b | 20.7 ± 3.2b |

ND, not detected. Rats were pretreated with progesterone (100 mg/kg, sc, -48, -24 and 0 hrs) and then given Cd (20 μmole/kg, 0 hr). Values with differing superscripts (a,b) indicate significant differences (p<0.05) between groups.

Table 3. Effect of progesterone pretreatment on serum LDH and BUN levels in male F344 and WF rats.

| Treatment | LDH,2 | BUN,2 |
|-----------|-------|-------|
| Control   | 82.7 ± 39.9a | 23.5 ± 3.5a |
| Progesterone | 68.4 ± 16.9b | 23.8 ± 3.7a |
| Cd        | 255.9 ± 26.6b | 14.8 ± 0.6b |
| Progesterone + Cd | 540.1 ± 110.7c | 15.7 ± 1.5b |

F344

| Treatment | LDH,2 | BUN,2 |
|-----------|-------|-------|
| Control   | 42.2 ± 10.5a | 18.7 ± 1.2a |
| Progesterone | 46.4 ± 8.9b | 19.2 ± 0.9b |
| Cd        | 96.3 ± 6.5b  | 11.3 ± 2.5b |
| Progesterone + Cd | 175.0 ± 76.5b | 12.7 ± 2.5b |

Rats were pretreated with progesterone (100 mg/kg, sc, -48, -24 and 0 hr), then given Cd (20 μmole/kg, 0 hr). Differing superscripts (a, b, c) indicate significant differences (p<0.05) between groups.

Statistics

Unless otherwise stated, the data represent the mean ± SD of four to eight determinations. Differences between treatment groups were evaluated statistically using one-way analysis of variance (ANOVA) or Duncan’s test. Probability values <0.05 were considered to indicate significant differences.

Results

A single sc injection of 10 to 30 μmole Cd/kg 24 hr earlier proved nonlethal but caused the typical spectrum of testicular lesions in F344 and WF rats. Cadmium-induced degeneration of seminiferous tubules, edematous vasculitis, and hemorrhage in stroma were observed in treated rats. However, in the case of F344 rats pretreated with progesterone (100 mg/kg, sc, -48, -24, and 0 hr) and then treated with cadmium (20 μmole/kg, 0 hr), this dose of cadmium proved extremely toxic, unexpectedly causing a 53% mortality rate. On the other hand, in the case of WF rats, progesterone did not increase cadmium-induced lethality (Table 1). Progesterone pretreatment had little or no effect on cadmium-induced testicular lesions in either F344 or WF rats.

Since progesterone clearly increased the lethality of cadmium in F344 rats, experiments were performed to determine if the increase was due to altered toxicokinetics of cadmium. Progesterone pretreatment had no significant effect on the accumulation of testicular, hepatic, or renal cadmium (Table 2).

Decreased BUN and increases in serum LDH activity were observed in F344 and WF rats treated with cadmium (20 μmole Cd/kg) as compared with untreated rats. The decreases in BUN seen with cadmium treatment likely reflect hepatic insufficiency in the absence of renal damage. The increases in cadmium-induced serum LDH in F344 rats was enhanced 2-fold by progesterone pretreatment, indicating an enhancement of cadmium-induced hepatotoxicity (Table 3). In these rats, progesterone pretreatment caused extensive hepatocellular necrosis (not shown). Progesterone or cadmium alone did not cause any such hepatic lesions. In the case of WF rats, pretreatment with progesterone partially enhanced the increases in serum LDH induced by cadmium (20 μmole Cd/kg) (Table 3).

In F344 rats treated with cadmium, marked increases in hepatic or renal MT were observed as compared to control levels. With progesterone pretreatment, similar cadmium-induced increases were observed. However, increases in hepatic MT in F344 rats treated with 20 μmole Cd/kg following progesterone pretreatment were less than those in rats treated with cadmium alone. An increase in hepatic MT-I mRNA was observed in rats treated with cadmium, but not in progesterone-pretreated rats, as compared with controls. In contrast, a decrease in testicular MT-I mRNA was observed in rats treated with cadmium and progesterone as compared with controls (Table 4).

In contrast to liver and kidney MT, increases in testicular cadmium-binding protein (TCBP) in F344 rats treated with cadmium (20 μmole/kg) were not observed. On the contrary, levels of TCBP in rats treated with cadmium were
Table 4. Effect of progesterone pretreatment on hepatic and renal MT, testicular cadmium-binding protein, and MT mRNA in male F344 rats.

| Treatment | MT, μg/g | or TCBP, μg/organ | MT-1 mRNA | Normalized |
|-----------|---------|------------------|------------|------------|
| Liver     |         |                  |            |            |
| Control   | 11.9 ± 3.8a | 11.9 ± 3.8a | ND | 16 |
| Progesterone | 8.6 ± 1.6  | 8.6 ± 1.6  | ND | 10 |
| Cd       | 223.2 ± 49.6  | 223.2 ± 49.6  | ND | 10 |
| Kidney   |         |                  |            |            |
| Control   | 37.1 ± 8.1  | 37.1 ± 8.1  | ND | 75 |
| Progesterone | 52.3 ± 4.4  | 52.3 ± 4.4  | ND | 22 |
| Cd       | 1277.2 ± 287.9 | 1277.2 ± 287.9 | ND | 36 |
| Progesterone + Cd | 159.9 ± 22.9 | 159.9 ± 22.9 | ND | 75 |

ND: not determined. Rats were pretreated with progesterone (100 μg/kg, sc., 48–24, and 0 h), then given Cd (2 μg/mouse, 0 h). MT levels were determined in liver and kidney, and TCBP levels were determined in testes. Values with differing superscripts (a,b,c) indicate significant differences (p<0.05) between groups.

markedly decreased as compared with untreated rats. Although progesterone pretreatment did not increase hepatic and renal MT, it doubled levels of TCBP. However, progesterone pretreatment did not prevent the reduction of levels of TCBP seen with 20 μmol/kg cadmium treatment (Table 4).

Discussion

The results in this study indicate that, in contrast to previously reported data in the NAW mouse (3), progesterone pretreatment increased the toxicity of cadmium in the F344 rat, as assessed by lethality, and had no effect on cadmium-induced testicular toxicity in either F344 or WF rats. It is suspected that the acute lethal effects of cadmium are due to necrotic lesions in the liver (8) and the increased lethality of cadmium in F344 rats appeared to be due to progesterone potentiation of cadmium hepatotoxicity. The potentialization of cadmium hepatotoxicity has not been reported in mice pretreated with estrogen (1) or progesterone (3). On the other hand, during pregnancy, a time of high circulating progesterone levels, mammals are extremely susceptible to cadmium toxicity (9–12). The rat, for example, Parizek (9,10) showed that a single sc injection of 40 μg CdCl₂/kg on days 17 to 21 of gestation resulted in marked degenerative changes of the placenta and in fetal death in a high proportion of pregnant rats. Pronounced necrotic lesions also occur in the maternal kidney (10–12) and liver (12) of pregnant rats exposed to cadmium. Likewise in humans, multiple pregnancies appear to predispose women to chronic cadmium intoxication, such as in itai-itai disease (13) although any specific role of progesterone in this disorder is as yet obscure. In the present study, progesterone appeared to sensitize the liver to cadmium toxicity, as a single sc injection at a dose normally tolerated (20 μg Cd/kg) given following progesterone pretreatment caused extensive necrotic damage in the liver, a lesion normally observed only with much higher doses of cadmium (8). These results indicate that, similar to previously reported data for pregnant rats (12), progesterone sensitizes at least one strain of rats to cadmium-induced hepatotoxicity.

In contrast to previous results in the mouse, progesterone pretreatment had no effect on testicular toxicity in either strain of rats. Gunn and Gould (2) had also indicated that attempts to protect the testes of Wistar rats from cadmium damage by estrogen pretreatment were not successful. They speculated that this might be related to the fact that the testis of the rat was much more sensitive to antiandrogenic effects of estrogen than that of the mouse. Similarly, there may be a difference in sensitivity to progesterone between rat and mouse.

Wolkowoski-Tyl and Preston (3) suggested that a dimer of cadmium-thionine (Cd-bp-D’, 19,000 mw), induced in liver by progesterone pretreatment, may have an important role in resistance to cadmium-induced testicular necrosis. However, in this study, progesterone pretreatment had no effect on hepatic MT and MT-mRNA levels. Previously, Bracken and Klassen (14) reported that sex steroids (β-estradiol, progesterone, and testosterone) were ineffective as inducers of MT in rat hepatocytes. From these results, we conclude that progesterone alone is not even a weak inducer of hepatic MT in rats. In fact, progesterone pretreatment actually weakened the induction response of MT to cadmium in the liver. Whether or not this diminished response to cadmium plays a role in sensitizing the liver to cadmium intoxication remains to be established.

Progesterone pretreatment doubled levels of the TCBP as assessed by cadmium binding, while MT-1 mRNA expression was reduced by almost 70%. Clearly from these data the levels of TCBP are not correlated with the levels of MT-1 mRNA. This is further evidence of the marked difference between TCBP and authentic MT (6,15,16). Beyond this, it is evident that the increased levels of TCBP had no major effect on the testicular toxicity of cadmium.

In conclusion, the results of this study indicate that, in contrast to previously reported data in mice (3), progesterone pretreatment markedly increased the overall toxicity of cadmium in F344 rats, and had no effect on cadmium-induced testicular toxicity in either F344 or WF rats. The increased lethality in F344 rats appeared to be due to progesterone potentiation of cadmium hepatotoxicity, but further research will be required to fully define this effect.

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