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Androgen catabolism and excretion in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated rats

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Treatment of adult male rats with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)* causes a pronounced androgenic deficiency (ED_{50} 15 μg TCDD/kg). Dose-dependent decreases in plasma testosterone concentrations, with consequent decreases in plasma dihydrotestosterone concentrations and sex organ weights, were observed [1]. Such effects could be due to a decrease in testosterone secretion secondary to testicular, pituitary, and/or hypothalamic dysfunction. Alternatively, the catabolism and excretion of testosterone and/or its metabolites could be accelerated to such an extent that animals can no longer maintain physiological concentrations of androgens. Experiments were conducted to examine the latter possibility.

The induction of hydroxylases is a well known effect of TCDD [2], and effects of TCDD treatment on a variety of androgen metabolism rates *in vitro* have been characterized [3–9]. None of these reports, however, addresses the question of whether androgen disposition is altered *in vivo*. Our primary objective, therefore, was to determine the extent to which enhanced catabolism and/or excretion could account, via mechanisms known or unknown, for the androgenic deficiency in TCDD-treated rats.

Methods

Animals and treatments. Male Sprague–Dawley rats (Harlan Sprague Dawley, Inc., Madison, WI) were obtained 3–7 days before the start of the experiments and housed and cared for essentially as described [1]. On day 0, rats were dosed orally with TCDD (>99% pure, Dow Chemical, Midland, MI) or vehicle (corn oil–acetone, 19:1, v/v, 2 ml/kg). In order to estimate the extent to which effects of TCDD treatment could be attributed to undernutrition and/or body weight loss, each TCDD-treated rat had a weight-matched pair-fed control (PFC) partner. PFC rats were dosed and killed 1 day after their partners. *Ad lib.*-fed control (ALC) rats were dosed and killed with both groups.

Plasma disappearance and biliary excretion of testosterone. Rats (271 ± 2 g, eight per treatment group) were dosed with TCDD (100 μg/kg) or vehicle. Seven days later they were anesthetized with urethane (100 mg/ml in 0.9% NaCl, 10 ml/kg, i.p.) and castrated, and then prepared for blood and bile collection as previously described [10]. One hour after castration, 0.25 ml blood was obtained for radioimmunoassay ([11], without chromatography) of residual endogenous plasma testosterone. [1,2,6,7-^{3}H]Testosterone (313 μCi/μg, 98% pure, Amersham, Arlington Heights, IL) was then injected i.v. (20 μCi/ml in 5% ethanol/95% 0.9% NaCl, 1 ml/kg). Bile was collected at 5-min intervals for 15 min and then at 15-min intervals for 45 min. Heparinized blood (0.25 ml) was collected 1, 2, 4, 8, 16, 30, and 60 min after [^{3}H]testosterone administration.

Bile volume was determined gravimetrically, whereas radioactivity in bile was determined by liquid scintillation counting. Plasma [^{3}H]testosterone concentrations were determined by spotting 20 μl of plasma and 5 μg of carrier testosterone (Sigma, St. Louis, MO) onto silica gel TLC plates (LK5D, Whatman, Clifton, NJ). After developing the preadsorbent region three times with diethyl ether, plates were developed with cyclohexane/ethyl acetate (60:40, v/v). Testosterone-containing spots were visualized under UV light, scraped into vials, and assayed for radioactivity by liquid scintillation counting. Recovery of [^{3}H]testosterone added exogenously to plasma was 90 ± 3%.

Androgenic status of castrated, testosterone-implanted rats. On day 0, rats (282 ± 1 g) were anesthetized with diethyl ether, castrated, implanted s.c. with testosterone-containing capsules, and dosed with TCDD (15 or 100 μg/kg) or vehicle. There were eight rats per treatment group except for the ALC group (N = 12). Capsules were prepared as described [12], except that the tubing (Silastic, 1.57 mm i.d., 3.18 mm o.d., Dow Corning) was slurry packed before being dried and cut into 40 mm lengths. The testosterone (>99.9% pure by HPLC, Sigma) was compressed to 30 mm as the ends were plugged.

On day 7, trunk blood was collected by decapitation during the last 2 hr of the light cycle. Organ weights were determined immediately; seminal fluid was expressed before weighing. Stress control procedures, plasma preparation, and androgen radioimmunoassays were as described [1].

Statistical analysis. Results were analyzed by one-way analysis of variance followed by a least significance difference test [13]. Differences were considered significant at $P < 0.05$.

Results and discussion

Plasma disappearance and biliary excretion of testosterone. Effects of treatment on feed intake, body weight, and ventral prostate weights were similar (data not shown) to those previously reported [1]; TCDD-treated and PFC rats evidenced severe and mild androgenic deficiencies respectively.

To ensure that plasma testosterone concentrations would be similar in all treatment groups when [^{3}H]testosterone was injected, rats were castrated 1 hr beforehand. Endogenous testosterone concentrations decreased to negligible levels (ALC and PFC, 0.13 ± 0.01 ng/ml; TCDD, 0.11 ± 0.01 ng/ml) as a result.

Following i.v. injection of sufficient [^{3}H]testosterone to return plasma concentrations to the low physiological range, testosterone was rapidly cleared from plasma. Neither TCDD treament nor paired feed restriction had any detectable effect on the plasma disappearance rate of [^{3}H]testosterone (Fig. 1, top). ALC and PFC rats excreted 57 and 52%, respectively, of the [^{3}H]testosterone-derived radioactivity in bile within the first hour, while TCDD-treated rats excreted 47% (Fig. 1, bottom). Bile flow rate was slightly decreased in TCDD-treated rats, but rates remained constant within each treatment group throughout the time of collection (data not shown).

The fact that the plasma disappearance rate of testosterone was unaffected by TCDD treatment strongly suggests that no net increase in androgen catabolism and/or excretion occurs even when a severe androgenic deficiency is present. Any such net increases should have been detectable by an increase in plasma testosterone disappearance. The slight decrease in the rate of appearance

* Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PFC, pair-fed control; and ALC, *ad lib.*-fed control.

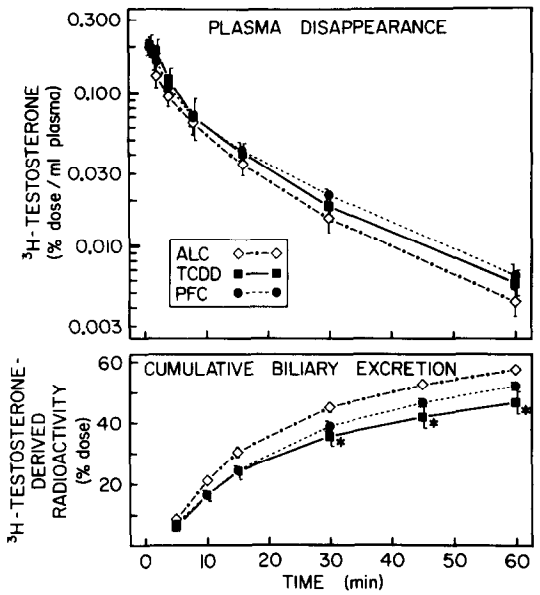

Fig. 1. Effects of TCDD treatment (100 μg/kg) or paired feed restriction on the plasma disappearance rate of testosterone (top) and cumulative biliary excretion of [^{3}H]testosterone-derived radioactivity (bottom). Measurements were taken 7 days after dosing with TCDD or vehicle. Plasma [^{3}H]testosterone concentrations were determined following chromatography. Each point represents the mean ± SE, N = 4–8. Significant differences ($P < 0.05$) between TCDD and ALC are as shown (*).

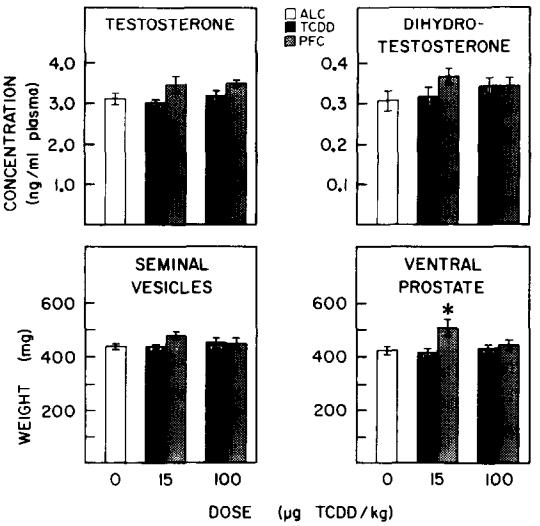

Fig. 2. Effects of TCDD treatment or paired feed restriction on plasma androgen concentrations (top) and accessory sex organ weights (bottom) in castrated, testosterone-implanted rats. Plasma was obtained and organ weights determined 7 days after surgery and dosing with TCDD or vehicle. Each value represents the mean ± SE, N = 7–11. The only significant difference ($P < 0.05$) was in ventral prostate weights, between one group of PFC rats and all other groups, as shown (*).

of testosterone metabolites in bile is consistent with this finding.

Androgenic status of castrated, testosterone-implanted rats. If TCDD treatment causes the androgenic deficiency by enhancing androgen catabolism and/or excretion, it should continue to do so even when exposure of rats to testosterone is held constant within the physiological range. This can be done by implanting testosterone-filled Silastic capsules into castrated rats [12]. Seven days after castration, implantation, and dosing, substantial dose-related decreases in feed intake and body weight were seen in TCDD-treated rats and in their PFC partners (data not shown). Plasma testosterone and dihydrotestosterone concentrations in ALC rats were similar (though about 50% higher on the average) to those we have observed in intact unstressed control male rats [1]. Yet doses of TCDD capable of causing half-maximal and maximal androgenic deficiencies in intact rats a week after dosing (15 and 100 μg/kg respectively [1]) had no detectable effect on plasma testosterone and dihydrotestosterone concentrations or seminal vesicle and ventral prostate weights (Fig. 2). With the exception of a slight increase in ventral prostate weights in the more moderately feed-restricted group, PFC rats were similarly unaffected. These results clearly demonstrate that TCDD (even at overtly toxic doses) cannot cause an androgenic deficiency when rats are exposed to a constant, physiological dose of testosterone.

In summary, despite the fact that *in vitro* androgen catabolism rates are affected by TCDD treatment [3–8], the results of two independent experiments each demonstrated that there were no alterations in either catabolism or excretion rates capable of depleting androgen pools *in vivo*. The proximate cause of the androgenic deficiency, therefore, must be a decrease in testicular testosterone secretion.

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REFERENCES

1. R. W. Moore, C. L. Potter, H. M. Theobald, J. A. Robinson and R. E. Peterson, *Toxic. appl. Pharmac.* **79**, 99 (1985).
2. A. Poland and J. C. Knutson, *A. Rev. Pharmac. Toxic.* **22**, 517 (1982).
3. G. E. R. Hook, T. C. Orton, J. A. Moore and G. W. Lucier, *Biochem. Pharmac.* **24**, 335 (1975).
4. J.-Å. Gustafsson and M. Ingelman-Sundberg, *Biochem. Pharmac.* **28**, 497 (1979).
5. W. Nienstedt, M. Parkki, P. Uotila and A. Aitio, *Toxicology* **13**, 233 (1979).
6. B. Keys, M. Hlavinka, G. Mason and S. Safe, *Can. J. Physiol. Pharmac.* **63**, 1537 (1985).
7. K. T. Shiverick and T. F. Muther, *Biochem. Pharmac.* **32**, 991 (1983).
8. J. C. Mittler, N. H. Ertel, R. X. Peng, C. S. Yang and T. Kiernan, *Ann. N.Y. Acad. Sci.* **438**, 645 (1984).
9. G. W. Lucier, O. S. McDaniel and G. E. R. Hook, *Biochem. Pharmac.* **24**, 325 (1975).
10. K. H. Yang, W. A. Croft and R. E. Peterson, *Toxic. appl. Pharmac.* **40**, 485 (1977).

11. J. A. Robinson, G. Scheffler, S. G. Eisele and R. W. Goy, *Biology Reprod.* **13**, 203 (1975).
12. E. R. Smith, D. A. Damassa and J. M. Davidson, in *Methods in Psychobiology. Advanced Laboratory Techniques in Neuropsychology and Neurobiology* (Ed. R. D. Myers), Vol. 3, p. 259. Academic Press, New York (1977).
13. R. G. D. Steel and J. H. Torrie, *Principles and Procedures of Statistics*, 2nd Edn. McGraw-Hill, New York (1980).

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Depletion of glutathione by the radioprotective agent *S*-2-(3-aminopropylamino)ethyl phosphorothioic acid (WR2721)

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S-2-(3-Aminopropylamino)ethyl phosphorothioic acid (WR2721*) is a free radical scavenger currently in limited clinical use as an adjunct in cancer radio- and chemotherapy [1, 2]. We have previously determined the pharmacokinetics and protein binding characteristics of WR2721 in rabbits and in humans [3, 4]. We have also shown that WR2721 is an effective mucolytic agent in patients with cystic fibrosis. *In vivo*, WR2721 is converted to its free thiol analogue, *N*-2-mercaptoethyl-1,3-diaminopropane (MDP), and, as such, reacts with disulfide bonds in the mucin molecule, altering its rheology [3, 5].

In the course of experiments designed to examine the spectrum of its activity as a free radical scavenger, we have observed that WR2721 in fact exacerbates the toxicity of those free radical-generating agents, such as acetaminophen and 6-hydroxydopamine, which depend upon glutathione for their detoxication. For this reason, we examined the glutathione content of the livers of mice treated with WR2721.

Materials and methods

Chemicals. WR2721 was supplied by Dr. K. Borah of Organon, Inc. (West Orange, NJ). Dithionitrobenzoic acid, NADPH, and glutathione reductase were obtained from the Sigma Chemical Co. (St. Louis, MO).

Animal studies. Determinations of hepatic glutathione (GSH + GSSG) levels were performed on 5- to 7-week-old female CD-1 mice obtained from Charles River Laboratories (Cambridge, MA) and on 7-week-old male A/J mice obtained from Jackson Laboratories (Bar Harbor, ME). There were five mice in each treatment group. Mice were given access to food and water *ad lib.*

WR2721 was administered by orogastric or intraperitoneal instillation as a solution in distilled water. The concentration of the solution was such that a 20 g mouse received 0.2 ml/dose. Control mice (0 mg/kg) received an equal volume of distilled water alone.

Assay for hepatic glutathione (GSH + GSSG). Hepatic glutathione content was determined upon protein-free supernatant fractions of tissue homogenates prepared by mechanical homogenization of weighed samples of mouse liver in 1 ml of 5% trichloroacetic acid, 0.01 M hydrochloric acid in a Tenbroeke glass tissue grinder at 4°. Residual trichloroacetic acid was removed from the resulting supernatant fraction by three ether extractions, and the samples were assayed for GSH + GSSG by the method of Tietze [6]. Glutathione levels for the mice in each group were averaged and compared using a one-tailed *t*-test. Control and treated paradigms were conducted simultaneously to obviate the problem of diurnal variation in glutathione levels.

Results

CD-1 female mice receiving doses of WR2721 up to 400 mg/kg via orogastric tube were clinically indistinguishable from normal animals. The relationship between the dose of WR2721 and the GSH + GSSG content of their livers 6 hr after dosing is shown in Fig. 1. Six hours was chosen because this was the point at which animals given WR2721 in conjunction with acetaminophen or 6-hydroxydopamine were demonstrably clinically impaired, but had not yet succumbed to the treatment (Schor, unpublished observations). Treatment with 200 mg/kg WR2721 resulted in a 30–40% decline in total hepatic glutathione content ($P < 0.05$). Treatment with higher doses did not result in further reduction of the glutathione content. That this is not a strain- or sex-specific effect is also shown in Fig. 1. Male A/J mice showed virtually identical decreases in glutathione content in response to WR2721. The difference between these two strains was significantly only at 100 mg/kg, with a $P < 0.05$. Intraperitoneal injection of WR2721 into A/J male mice also resulted in a similar dose–response curve of glutathione depletion (data not shown), including the inability to drive glutathione below 60% of control values.

Discussion

WR2721 is a thiophosphate which has been developed as a radioprotective agent by the Walter Reed Army Insti-

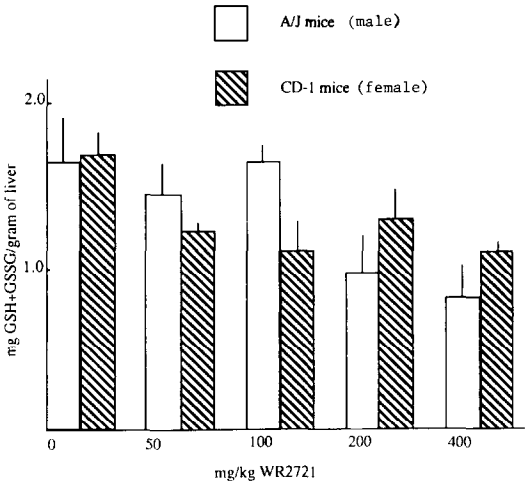

Fig. 1. Total glutathione levels in liver of control mice and mice treated with 50, 100, 200, and 400 mg/kg WR2721 via orogastric tube. Assays were performed 6 hr after dosing. There were five mice in each treatment group.

* Abbreviations: WR2721, *S*-2-(3-aminopropylamino)-ethyl phosphorothioic acid; (GSH + GSSG), total (reduced plus oxidized) glutathione; and MDP, *N*-2-mercaptoethyl-1,3-diaminopropane.