Modulation of TCDD-Induced Wasting Syndrome by Diabetes

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1. Introduction

The salient clinical sign of the acute toxicity of TCDD in laboratory animals is a wasting syndrome, comprising substantial body weight loss before death. The decline of body weight has been shown to predominantly result from reduced feed intake[1]. There is an accumulating body of evidence that the wasting syndrome is not merely a secondary change to general malaise but arises as a consequence of a specific derailment of regulatory systems[2,3]. Whether the primary target of TCDD is the regulation of feed intake or that of body weight, is not known.

TCDD alters drastically the plasma concentrations of a variety of hormones and, conversely, endocrine manipulations modulate the toxicity of TCDD[3]. For example, in the rat a lethal dose of TCDD typically lowers plasma thyroxine but elevates corticosterone levels. Thyroidectomy attenuates the acute lethality of TCDD by prolonging the survival time of the exposed animals[4], whereas adrenalectomy exacerbates its acute toxicity by curtailing the survival time[5].

One of the endocrine factors affected by TCDD is insulin. In humans, TCDD exposure has been associated with an increased risk of diabetes[6]. In the rat, plasma insulin levels decrease within the first 4 days after exposure and persist lowered for about 2 weeks[7]. TCDD treatment also sensitizes rats to the glucose-decreasing effect of insulin thereby rendering them exceptionally susceptible to a fatal insulin shock[7]. This hypersensitivity seems to be at least partially due to a severely suppressed feeding response to insulin-induced hypoglycemia[8].

To date, no published studies exist on the possible interference of diabetes with TCDD toxicity. However, diabetes would provide a potentially useful means to assess the effects of TCDD on body weight and feed intake separately, since it is characterized by hyperphagia in conjunction with subnormal body weight. If TCDD primarily affects body weight, one would anticipate a diminished effect in diabetic animals. On the other hand, if the impact is directed at feed intake, a normal or even exaggerated response should follow. These alternatives were tested in the present study. To enable a long (6-week) follow-up after a high dose of TCDD, we employed Han/Wistar (Kuopio; H/W) rats, which are extremely resistant to the acute lethality of TCDD but exhibit most of its biochemical and morphological effects at the same doses as do more susceptible strains[3].

2. Material and Methods

A. Design. Young adult (age 10-11 weeks) male H/W rats were rendered diabetic by administering them i.p. a single dose of 60 mg/kg streptozotocin (STZ), dissolved in saline acidified to pH 4.5 with citrate. This dose of STZ degranulates pancreatic beta cells without causing necrosis[9]. The control rats were injected with the same volume (1.2 ml/kg) of the vehicle. Three days later, blood glucose was determined from tail-prick samples with a refractometer (Accutrend®). All STZ-treated rats had

glucose values >18 mmol/l, while those for the controls ranged from 5.7 to 8.6 mmol/l. Nine days after this (=day 0), half the STZ-treated rats and half the controls were exposed i.p. to 1000 µg/kg TCDD, dissolved in corn oil. The remainder rats reveived pure corn oil. On day 21, the STZ groups were again split into two. One subgroup of both TCDD-exposed and control rats were put on a daily s.c. insulin treatment. The dose of insulin was initially 5 U/kg (long-acting insulin) s.i.d., but was gradually increased based on blood glucose analyses (see Fig. 2). The final dose was 24-27 U/kg, depending on individual responses.

B. Animal Care. The rats were maintained in a animal room with controlled temperature (21.5±1.0°C), humidity (55±10%) and lighting (12/12h). The rats were housed singly in either plastic metabolic cages (Tecniplast1700®) or suspending wire-mesh cages equipped with feeding tunnels equal to those in the metabolic cages. Both types of cages enabled strict control of feed spillage. Full access to feed (R36, Ewos, Södertälje, Sweden) and tap water was provided for the rats all the time.

3. Results

A. Lethality. A total of 5 rats (out of 30) succumbed during the 42-day follow-up period after TCDD exposure. Two of these rats had been treated with STZ and corn oil (deaths on days 7 and 23), two with STZ and TCDD (31 and 33) and one with saline and TCDD (31). Thus, diabetes did not appreciably aggravate the acute lethality of TCDD.

B. Wasting syndrome. As expected, STZ treatment led to an initial drop of body weight followed by completely

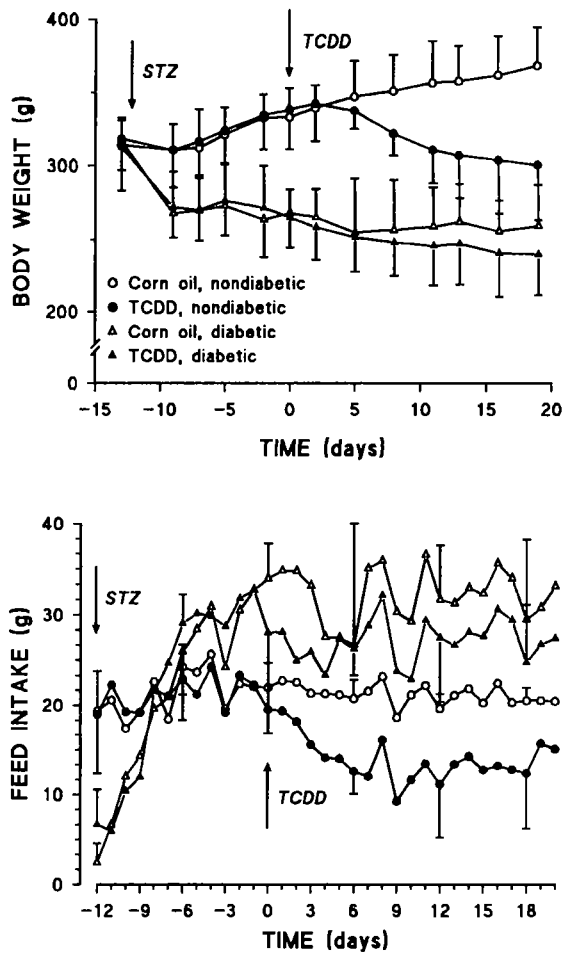

Fig. 1. Changes in body weight (top) and feed intake (bottom) after STZ and/or TCDD treatment. Mean ± SD (for feed intake, only some error bars are shown to retain clarity).

blocked growth (Fig. 1, upper panel). The phase of body weight decline was accompanied by suppressed feed intake, but thereafter STZ-treated rats ate more than the nondiabetic rats (Fig. 1, lower panel). In nondiabetic rats, TCDD reduced body weight by about 10% during the first 14 days. This stage was followed by a stable body weight level up to the end of the follow-up period (Figs. 1 and 2). A greater reduction (about 50%) occurred in feed intake. In diabetic rats, by contrast, both feed intake and (especially so) body weight were only mildly altered by TCDD. For

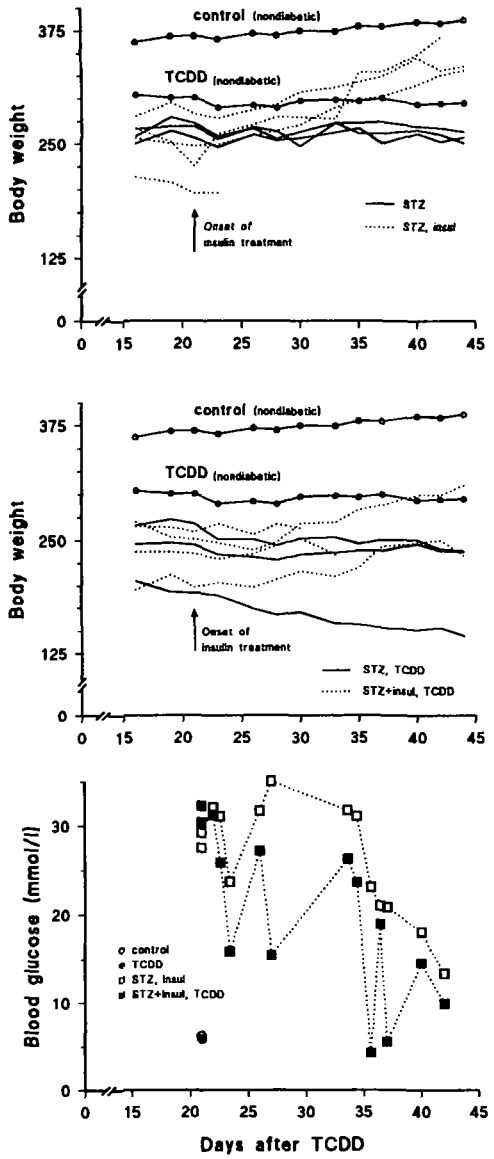

Fig. 2. Effect of the daily insulin treatment on body weight in corn oil- (top panel) or TCDD-exposed (middle panel) diabetic H/W rats. Individual data are shown (the group averages for nondiabetic rats are included for comparison). Bottom panel: influence of insulin on blood glucose in the diabetic H/W rats.

example, the amount of feed consumed daily remained at a higher level in TCDD-dosed diabetic rats than in their nondiabetic corn oil-treated partners (Fig. 1).

Those diabetic control rats which were put on the daily insulin regimen started to grow efficiently. At the end of the observation period, they were approaching the body weight level of the nondiabetic controls (Fig. 2). The response of the TCDD-exposed rats to the growth-stimulating effect of insulin was notably weaker; they approached the body weight of the nondiabetic TCDD-treated rats. This poorer responsiveness occurred despite the fact that blood glucose returned closer to normal values by insulin in TCDD-treated rats than in the controls (Fig. 2).

4. Discussion

One of the major findings in the present study was that depletion of insulin did not exacerbate the acute toxicity of TCDD, at least in this particular strain of rat. In fact, the wasting syndrome was attenuated in STZ-diabetic rats. Since the diabetic animals continued to display hyperphagia relative to nondiabetic control animals even after TCDD treatment, it is unlikely that TCDD affects feed intake directly, as a primary impact. Rather, these results are in keeping with the proposed reduction of body weight set point caused by TCDD[10]. According to that hypothesis, the STZ-induced loss of body weight before TCDD treatment should mitigate the weight-declining effect of TCDD. Hypophagia, although essential for the wasting syndrome, would merely represent a tool for achieving the new set point and should therefore not be depressed in diabetic rats to the extent that occurs in euglycemic animals. This was indeed the case here.

In support of this line of reasoning, a distinctly different outcome was recently reported in ventromedial hypothalamus-lesioned H/W rats after treatment with the

same dose of TCDD[11]. Similar to STZ-diabetic H/W rats, the lesioned rats also exhibited hyperphagia before TCDD administration. However, they were concurrently obese. In these lesioned rats, the wasting syndrome was strikingly aggravated and the rats stopped eating completely for extended periods of time.

The outcome with insulin reinstatement further favored the view of a reset aim level for body weight in TCDD-exposed rats. Despite the fact that their blood glucose values deviated less from normal than those of corn oil-treated diabetic rats, TCDD-exposed H/W rats grew at a slower rate, gradually approaching the body weight level of the nondiabetic TCDD-treated rats.

Hence, TCDD appears to adjust the regulated level of body weight downwards. The biochemical mechanism of this action remains to be determined.

5. References

1) Kelling C.K., B.J. Christian, S.L. Inhorn and R.E. Peterson (1985): Hypophagia-induced weight loss in mice, rats, and guinea pigs treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Fundam. Appl. Toxicol. 5, 700-712
2) Pohjanvirta R., M. Unkila and J. Tuomisto (1994): TCDD-induced hypophagia is not explained by nausea. Pharmacol. Biochem. Behav. 47, 273-282
3) Pohjanvirta R. and J. Tuomisto (1994): Short-term toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in laboratory animals: effects, mechanisms, and animal models. Pharmacol. Rev. (submitted)
4) Rozman K., T. Rozman, E. Scheufler, T. Pazdernik and H. Greim (1985): Thyroid hormones modulate the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). J. Toxicol. Environ. Health 16, 481-491
5) Gorski J.R., T. Rozman, H. Greim and K. Rozman (1988): Corticosterone modulates acute toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in male Sprague-Dawley rats. Fundam. Appl. Toxicol. 11, 494-502
6) Wolfe W., J. Michalek, J. Miner, L. Needham and D. Patterson Jr. (1992): Diabetes versus dioxin body burden in veterans of operation ranc hand. *In* Abstracts of the 12th International Symposium on Dioxins and Related Compounds, 24-28 August 1992, Tampere, Finland (Organohalogen Compounds, vol. 10), pp. 279-280.
7) Gorski J.R. and K. Rozman (1987): Dose-response and time course of hypothyroxinemia and hypoinsulinemia and characterization of insulin hypersensitivity in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-treated rats. Toxicology 44, 297-307
8) Pohjanvirta R., M. Unkila and J. Tuomisto (1990): The loss of glucoprivic feeding is an early-stage alteration in TCDD-treated Han/Wistar rats. Pharmacol. Toxicol. 67, 441-443
9) Arison R.N., E.I. Ciaccio, M.S. Glitzer, J.A. Cassaro and M.P. Pruss (1967): Light and electron microscopy of lesions in rats rendered diabetic with streptozotocin. Diabetes 16, 51-56
10) Seefeld M.D., S.W. Corbett, R.E. Keesey and R.E. Peterson (1984): Characterization of the wasting syndrome in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 73, 311-322, 1984
11) Tuomisto J.T., R. Pohjanvirta, M. Unkila and J. Tuomisto (1993): TCDD reduces feed intake despite hyperphagia by VMH lesion. Pharmacol. Toxicol. (suppl. II) 73, 1152