Effect of Geldanamycin on the Acute Toxicity of TCDD

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Introduction

Most of the biological effects of TCDD are mediated by the AH receptor, a cytosolic bHLH/PAS protein that functions as a ligand-activated transcription factor. The unliganded AH receptor is associated with the hsp90 and the transformation to a DNA-binding form of the receptor involves dissociation of this complex. The activated AH receptor translocates into the nucleus, combines with an other bHLH/PAS protein, ARNT, and then binds to DNA at specific enhancer sites (1). Recently, an alternative cell signaling pathway was suggested for TCDD. It was shown that TCDD induces protein phosphorylation in cell-free conditions (2), that c-Src protein kinase is directly associated with the hsp90-AH receptor complex and activated upon ligand binding (3), and that c-Src-deficient knockout mice are resistant to several facets of TCDD toxicity including the wasting syndrome and acute lethality, but retain their responsiveness to CYP1A1 induction (4). Based on these findings, it was hypothesized that many of the toxic effects of TCDD would not arise via activation of the classic pathway but rather through a protein phosphorylation cascade with c-Src playing a pivotal role in the process (4). An additional interesting finding supporting the presented hypothesis was that geldanamycin (GA), a benzoquinone ansamycin antibiotic that inhibits protein tyrosine kinases (especially v-Src) (5) and disrupts the hsp90-AH receptor complex (6), also protected from TCDD toxicity (4). Targeted gene disruption is currently only possible in mice, but GA can potentially provide a pharmacological means to probe the importance of protein tyrosine phosphorylation for TCDD toxicity in other species such as the rat. Here, we studied the ability of GA to interfere with toxic and biochemical effects of TCDD in rats and mice.

Material and Methods

The experiments were conducted in adult male animals of the most TCDD-susceptible (LD50 10-20 μg/kg) and the most TCDD-resistant (LD50 >9600 μg/kg) rat strains (Long-Evans [*Turku AB*] and Han/Wistar [*Kuopio*], respectively) (7,8). Of mice, adult female NIH/S (LD50 about 500 μg/kg [unpublished]) and male C57BL/6 (LD50 180 μg/kg [9]) animals were used. TCDD was dissolved in corn oil (4-5 ml/kg) and given by gavage on day 0. GA was purchased from Calbiochem (La Jolla, CA, USA) and dissolved in DMSO to a stock solution of 1 mg/ml which was stored in -20°C. The stock solution was always aliquoted on the day of first GA treatment and dissolved to an appropriate concentration for final volumes of 2 or 5 ml/kg in 0.9% saline. GA was injected ip. every 2-3 days

commencing the regimen 3 days prior to the TCDD exposure. The control compound, administered in an equal volume, was corn oil for TCDD and 0.9% saline for GA.

GA was tested in two experimental settings. First, L-E rats and NIH/S mice were exposed to an approximately LD50 dose of TCDD (20 and 500 μg/kg, respectively) and monitored for body weight, feed intake and mortality until the possible interference of GA could be verified. In addition, H/W rats were exposed to a high but nonlethal dose of TCDD (1000 μg/kg) and treated identically to L-E rats. Three different doses of GA (0.2, 2 and 20 μg/kg) were used in mice, but because of toxicity that emerged in L-E rats, only the lowest of these doses was tried in rats. Second, the influence of GA on TCDD-induced changes in organ weights and some selected biochemical variables was studied in a 10-day experiment. To this end, L-E rats were dosed with 20 μg/kg and both NIH/S and C57BL/6 mice with 115 μg/kg TCDD, the latter dose being adopted from a previous study (4). GA was given at 0.2 μg/kg to minimize its own toxicity. On day 10, the animals were killed by decapitation (rats) or cervical dislocation (mice) and the liver, thymus and caudoabdominal fat pad were weighed. The liver was further sampled for EROD activity and glycogen concentration assays.

Results and Discussion

In L-E rats, TCDD (20 μg/kg) dropped feed consumption precipitously over the first 5 days (Fig. 1). GA exerted also a suppressive effect on feed intake as evidenced by a decline of the mean intake from >15 to ca. 5 g/day during days -3 to 0. These impacts were reflected in an additive loss of body weight by the two compounds in L-E rats. In H/W rats dosed with 1000 μg/kg TCDD, there was only a moderate and transient reduction of feed intake along with a concomitant ca. 10% decrease in body weight unrelated to the GA treatment (Fig. 1). By day 19, 3 TCDD + GA and 2 TCDD + NaCl-treated L-E rats had succumbed and the experiment was terminated.

No body weight loss occurred in NIH/S mice after TCDD exposure. On the contrary, the body weight of several individual mice almost doubled before death (data not shown). This difference in response between rats and mice is well known and is due to general edema formation in severely TCDD-intoxicated mice (10). GA turned out to aggravate TCDD toxicity, since all the 5 mice of the TCDD + GA (20 μg/kg) group and 2 out of 5 from the TCDD + GA (2 μg/kg) had died or been euthanized in agony by day 19, whereas there were no deaths in other groups until day 21, when one of the five mice treated with TCDD and NaCl died. The rest of the mice survived until the end of the experiment (day 30).

The dose of TCDD administered in the 10-day experiment (20 μg/kg for L-E rats and 115 μg/kg for NIH/S and C57BL/6 mice) retarded body weight gain in L-E rats (≈ -10% for exposed vs.≈ +1% for controls) and C57BL/6 mice (≈ -1.5% vs.≈ +4%) but not in NIH/S mice (≈ +3% overall). GA had no statistically significant influence on this variable. GA neither interfered appreciably with the effects of TCDD on relative liver or fat pad weights (Table 1). GA did, however, attenuate the thymic atrophy caused by TCDD, but this alleviation was only recorded in NIH/S mice, for which the TCDD dose was lowest relative to the corresponding LD50 value. GA decreased liver glycogen content in both strains of mice but not in L-E rats, TCDD reduced it in all cases.

Fig. 1. Daily feed intake and body weight changes in L-E and H/W rats (means of 4-5 rats)

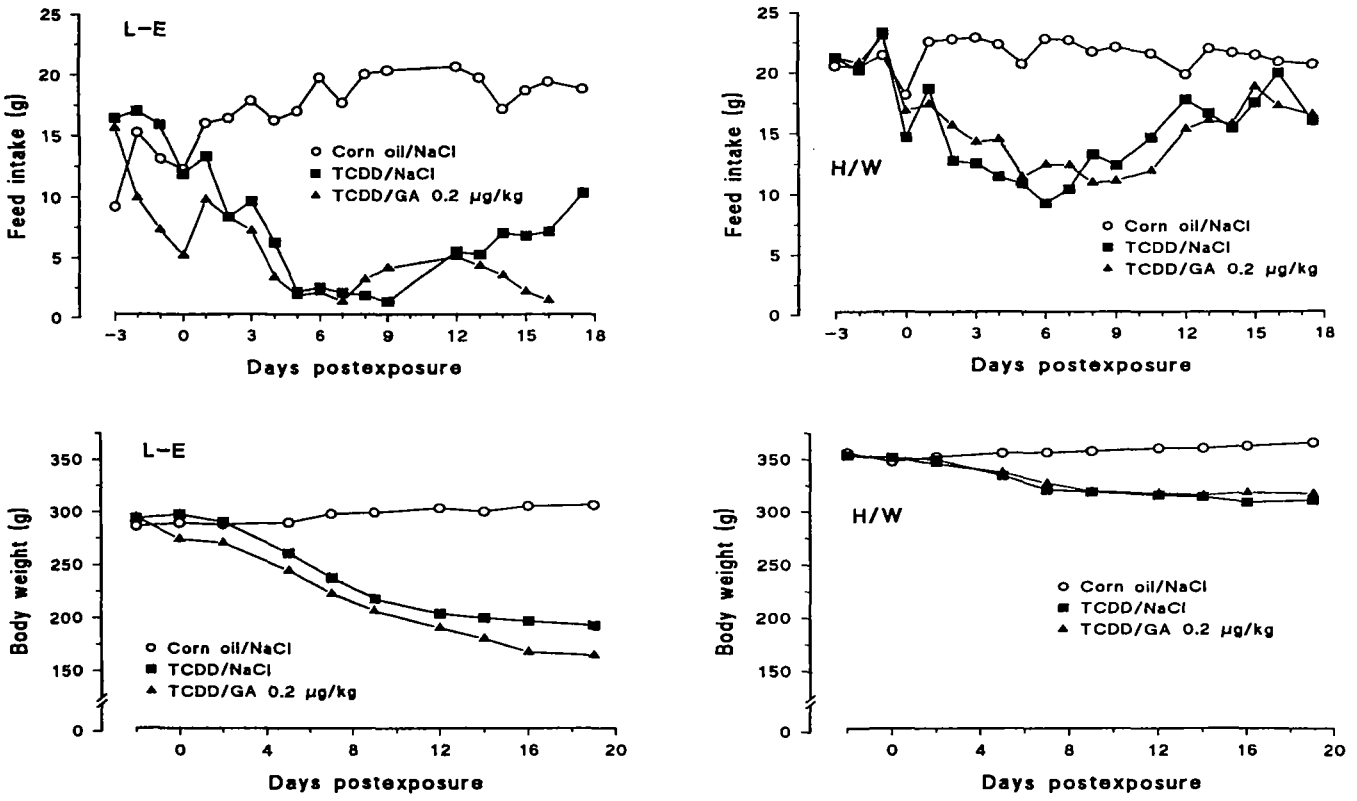

Table 1. Effects of a cotreatment with TCDD and GA on selected variables in the 10-day experiment.

	NIH/S mice				C57BL/6 mice				L-E rats			
	Corn oil + NaCl	Corn oil + GA	TCDD + NaCl	TCDD + GA	Corn oil + NaCl	Corn oil + GA	TCDD + NaCl	TCDD + GA	Corn oil + NaCl	Corn oil + GA	TCDD + NaCl	TCDD + GA
Rel. liver (%BW)	4.51± .08	4.30± .19	6.27± .59 **a,b**	6.20± .72 **a,b**	5.11± .62	4.72± .15	6.11± 1.14	5.93± .69 **b**	3.26± .10	3.26± .16	3.82± .27	3.60± .43
Rel. thymus (%BW)	.23± ..03	.23± .05	.05± .01 **a,b**	.12± .03**a,b,c**	.20± .02	.18± .03	.05± .01 **a,b**	.04± .01 **a,b**	.05± .01	.05± .01	.02± .00 **a,b**	.02± .00 **a,b**
Rel. fat pad (%BW)	1.45± .44	1.16± .33	1.59± .46	1.75± .33	1.33± .15	1.17± .20	1.20± .10	1.14± .17	1.05± .17	1.14± .37	.65± .13 **b**	.67± .32 **b**
EROD (nmol/min/ mg prot.)	94± 19	77± 33	2137± 610 **a,b**	1952± 381 **a,b**	85± 33	80± 38	2002± 536 **a,b**	1607± 464 **a,b**	117± 107	104± 30	591± 159 **a,b**	424± 284 **a,b**
Glycogen content (mg/liver)	53± 5	39± 14 **a**	27± 7 **a**	37± 12 **a**	102± 23	60± 16 **a**	65± 13 **a**	45± 14 **a**	251± 51	290± 63	164± 96 **a,b**	123± 60 **a,b**

The data are given as mean ± SD (n=6 except for L-E, corn oil + GA, where n=5). Statistical comparisons were made by one-way analysis of variance followed by Duncan's range test. In case of nonhomogeneous variances, the data were assessed by the nonparametric Kruskal-Wallis and Mann-Whithey U tests. Statistically significant ($p<0.05$) changes are depicted as follows in bold letters: a, vs. corn oil + NaCl; b, vs. corn oil + GA; and c, vs. TCDD + NaCl.

In conclusion, high doses of GA intensified TCDD toxicity. Attenuation was only seen for thymic atrophy in NIH/S mice. Based on these results, the applicability of GA as a modulator of TCDD toxicity appears to be rather limited.

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