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Report

Therapeutic Effect of Dexamethasone in T-2 Toxicosis

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T-2 Toxin is a mycotoxin that induces toxemia characterized by numerous hematological and biochemical changes. We have previously shown that prostaglandin (PG) production in brain tissue is increased following T-2 toxin. The present study was designed in order to test the effect of dexamethasone on brain prostaglandins and survival of rats subjected to T-2 toxin. Furthermore, the effect of BW 755c, a dual inhibitor of the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism, on the survival of rats exposed to T-2 toxin was also examined. The present study demonstrated that dexamethasone increases the survival of rats exposed to a highly lethal T-2 toxicosis. This effect was demonstrated at low as well as high doses and at different times after T-2 administration. Dexamethasone depressed PGE_2 levels in the brain cortex 6 hr after T-2 toxin but abolished the reduction of PGE_2 in brain cortex seen 24 hr after T-2. BW 755c had no consistent effect on the survival of rats in T-2 toxicosis. It is suggested that dexamethasone might be a useful therapeutic agent in T-2 toxicosis in animals and humans, but its mechanism of action remains obscure.

KEY WORDS: T-2 toxin; trichothecene; prostaglandins; dexamethasone; BW 755c; central nervous system.

INTRODUCTION

T-2 Toxin [4β, 15-diacetoxy-8α-(3-methylbutyryloxy)-12,13-epoxytricho-tec-9-en-3α-ol] is a naturally occurring mycotoxin. As a food contaminant it may cause severe illness when ingested in relatively small amounts (1,2) in experimental animals as well as humans.

T-2 toxicosis is characterized by profound cardiovascular (3,4), metabolic (5,6), hematologic (7), and diffuse organ pathology (8). In this regard T-2 toxicosis has some resemblance to endotoxemia produced by bacterial lipopolysaccharides (LPS). In the latter condition, high levels of circulating prostaglandins (PGs) (9) and increased production of PGs in the brain (10) have been reported, along with a beneficial effect of cyclooxygenase inhibitors of arachidonic acid metabolism (11–13). Increased production of PGs (in particular, PGE_2) in the cerebral cortex and hypothalamus of rats exposed to acute T-2 toxicosis (6) was also reported. Furthermore, Tremel *et al.* (14) have reported a marked reduction in lethality of rats exposed to T-2 toxin when treated with dexamethasone.

Glucocorticoids exert their antiinflammatory effect through several mechanisms including the induction of a protein that inhibits phospholipase A_2 (15), thus inhibiting the release of arachidonic acid from membrane-bound phospholipids and their further metabolism to PGs. In addition, a recent report demonstrated that therapeutic doses of dexamethasone inhibit PG synthesis in certain brain areas of the rat (16).

The present study was designed in order to test the effect of dexamethasone (a potent glucocorticosteroid) on the long-term survival of rats exposed to a highly lethal dose of T-2 toxin and to examine the relationship of this effect of dexamethasone to PGE_2 levels in the brain. Furthermore the effect of BW 755c, a dual inhibitor of both the cyclooxygenase and the lipoxygenase pathways of arachidonate metabolism, on the survival of rats exposed to T-2 toxin was compared to that of dexamethasone. In addition, the effect of T-2 toxin on brain edema and the blood–brain barrier (BBB) of the rat was also studied.

MATERIALS AND METHODS

Effect of Dexamethasone and BW 775c on Survival of Rats Exposed to T-2 Toxin

Male Sprague Dawley rats, weighing 220–280 g, were used in this study. Rats were anesthetized with 2% halothane, their femoral vein was cannulated with PE-50 tubing, and the rats were then allowed to recover overnight. On the following day, 0.55 to 0.75 mg/kg T-2 toxin (dissolved in 10% ethanol in saline) was administered in 200 μl to the rat through the venous line over a 5-min period. Control rats received the vehicles. Survival of the rats was followed up to 7 days after T-2 administration.

The effect of dexamethasone on survival was studied in various protocols as follows: protocol 1—dexamethasone,

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2mg/kg i.v., was given 1 hr after T-2 toxin; protocol 2—dexamethasone, 10 mg/kg i.v., was given 1 and 24 hr after T-2 toxin; protocol 3—dexamethasone 1, mg/kg i.v., was given 1, 24, and 48 hr after T-2 toxin; and protocol 4—dexamethasone, 10 mg/kg was given 3 hr after T-2 toxin. Dexamethasone was dissolved in the same vehicle used for T-2.

BW 755c (dissolved in saline) was given at a dose of 10 mg/kg, i.v., 1, 9, 17, and 25 hr after T-2 toxin and the survival of animals was followed for 72 hr.

Effect of Dexamethasone on PG Production

Six groups of rats were used for these experiments. Control rats received two vehicle injections (200 μl of 10% ethanol in saline) at a 1-hr interval. The remaining rats were treated with T-2 toxin and, 1 hr later, received 1 or 10 mg/kg of dexamethasone. Six and twenty-four hours following T-2 (or vehicle) administration rats were sacrificed by rapid decapitation, and the brains removed within 40 sec, frozen on dry ice, and saved at −70°C. We have previously shown that this method results in the same levels of PGE_2 in the brain as rapid decapitation where the head is dropped directly into liquid nitrogen (17).

Brain PGs were assayed by methods previously described in detail (10). In brief, brain tissue (20 mg) from the frontal cortex and hypothalamus was homogenized in 1 ml of buffer (Tris–EDTA, pH 7.0), the homogenate centrifuged (5000*g* for 10 min), and the supernatant washed three times with ether. Aliquots of 100 μl were taken from the aqueous phase for PGE_2 determination by radioimmunoassay (RIA).

Radioimmunoassay (RIA) was performed by using a specific antibody (purchased from Dr. L. Levine, Brandeis University, Waltham, Mass.) and tritiated PGE_2 (100–200 ci/mmol, New England Nuclear, Boston) as described elsewhere (10). Protein was assayed on an aliquot of the homogenate by the method of Lowry *et al.* (18).

Effect of T-2 Toxin on Brain Edema and the BBB

For the determination of brain water content, brains were weighed fresh and after 48 hr of drying at 87°C. Water content is expressed as a percentage of fresh weight. Six additional animals received 2 ml/kg of 2% Evans blue-albumin solution 30 min before sacrifice. These animals were perfusion-fixed with 10% phosphate-buffered formalin. The brains were then frozen and cut on a cryostat into 10-μm sections. The sections were evaluated for Evans blue fluorescence using a Zeiss microscope equipped with a xenon lamp, filters for red fluorescence, and a photo multiplier tube (19).

Drugs

T-2 toxin and dexamethasone were purchased from Sigma (St. Louis, Mo.). BW755c was kindly provided by Burroughs-Welcome (England).

Data Analysis

The data in text and tables are mean values ± SE for the indicated number of animals. The Fisher exact probability test was used to evaluate differences in survival rate. The levels of PGE_2 in the various experimental groups were compared by analysis of variance (ANOVA) followed by Student–Newman–Keuls' multiple range test.

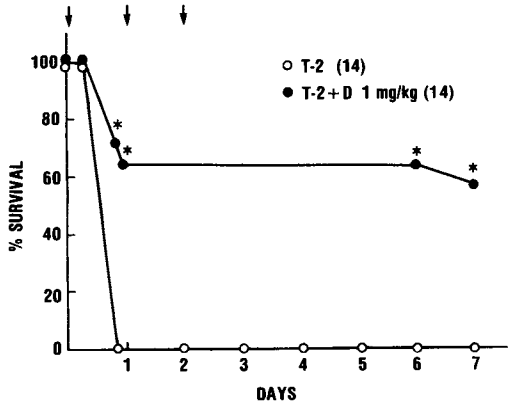

Fig. 1. Effect of dexamethasone on chronic survival of rats exposed to T-2 toxin. Symbols for the experimental groups are given in the figure and numbers of animals in each group are given in parentheses. Arrows denote time of dexamethasone (D) administration. Asterisks denote statistical significance at $P < 0.01$.

RESULTS

Effect of Dexamethasone and BW 775c on Survival of Rats Exposed to T-2 Toxin

The effect of dexamethasone on the survival of rats subjected to T-2 toxin is depicted in Figs. 1 and 2. Figure 1 demonstrates a protective effect of 1 mg/kg dexamethasone given 1, 24, and 48 hr post-T-2. In this study 100% of the vehicle-treated rats died within 21 hr of intoxication; however, only 44% of the dexamethasone-treated rats died by the end of the week.

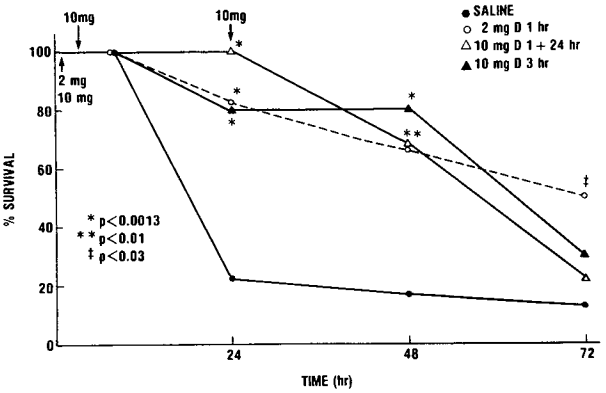

Fig. 2. Effect of various doses of dexamethasone on acute-term survival of rats exposed to T-2 toxin. Symbols for the various experimental groups are given in the figure. The 2-mg/kg doses of dexamethasone (D) was given once, after T-2 toxin; 12 rats were used in this group. The 10-mg dose of dexamethasone was given either once, 3 hr after T-2 toxin, or twice, 1 and 24 hr after T-2 toxin. In these experiments 10 and 10 rats per group were used, respectively. The control group of 18 rats received 10% ethanol in saline (200 ml) 1 and 24 hr after T-2.

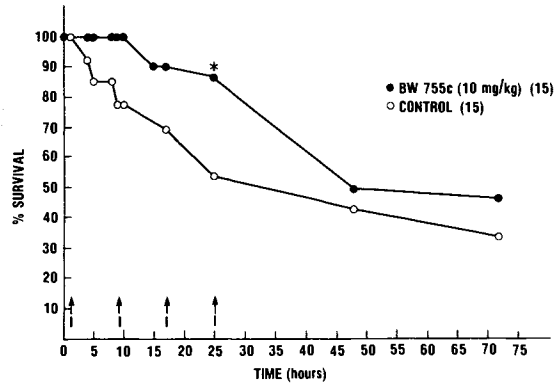

Fig. 3. Effect of BW 755c on survival of rats exposed to T-2 toxicosis. Symbols for each experimental group are given in the figure, and numbers of rats in each group are in parentheses. Arrows denote time of BW 755c administration, 10 mg/kg i.v., every 8 hr. The asterisk denotes statistical significance at $P < 0.05$.

In Fig. 2, the results of different regimes of dexamethasone treatments are shown. It appears that 2 mg/kg given at 1 hr and 10 mg/kg given at 1 and 24 hr were effective at 24 and 48 hr, while at 72 hr only the effect of the 2-mg/kg dose was significant. A single dose of dexamethasone given as late as 3 hr after T-2 administration also prolonged survival up to 48 hr.

Figure 3 summarizes the survival of rats treated with BW 775c following T-2 toxin. In this study an increase in survival rate was found only at 24 hr, whereas at 48 and 72 hr no protective effect of the drug was found.

Effect of Dexamethasone on PG Levels in the Brain

The levels of PGE_2 in the cortex and hypothalamus of normal rats were 202 ± 25 and 94 ± 14 pg/mg protein, respectively. These levels are in accord with previous studies of this species (20). T-2 caused a slight but significant increase in the brain cortex PGE_2 level 6 hr after T-2 administration but a profound decrease at 24 hr after T-2 toxin administration. Dexamethasone, 1 or 10 mg/kg, significantly decreased the PGE_2 concentration in the cortex at 6 hr after T-2 toxin; however, the high dose of dexamethasone prevented the decrease in PGE_2 levels 24 hr after T-2 administration (Table I).

Table II. Effect of T-2 Toxin on Brain Edema[a]

Time (hr)	Control	T-2
6	79.02 ± 0.14 (N = 4)	78.70 ± 0.30 (N = 4)
24	79.25 ± 0.10 (N = 7)	79.27 ± 0.12 (N = 6)

[a] Brain water content (g H_2O/100 g fresh weight × 100) at 6 and 24 hr after T-2 toxin administration to rats. Control animals received vehicle only (0.5 ml 10% ethanol in 0.9% NaCl). No significant differences were found between the groups.

In the hypothalamus, the only significant change in PGE_2 concentrations was seen in the T-2 toxin + 10 mg/kg dexamethasone group at 6 hr after the toxin administration.

Brain Water Content After T-2 Toxin

T-2 toxin did not cause any increase in brain water content either 6 or 24 hr after T-2 toxin (Table II). In addition, there was no evidence for Evans blue extravasation even in discrete areas of the brain, as revealed by the complete absence of fluorescence in animals which had received Evans blue 30 min prior to sacrifice (data not shown).

DISCUSSION

T-2 toxicosis is a highly lethal syndrome where multiple organs and systems are involved. However, in spite of its extreme toxicity, no adequate therapeutic strategies have been developed. We have previously reported prophylactic and therapeutic measures for T-2 toxicosis based on the administration of large doses of monoclonal antibodies directed against T-2 and HT-2 mycotoxins (21). However, such treatment is not available for humans. Dexamethasone was also shown to provide protective and therapeutic effects in moderately lethal T-2 toxicosis (14). In the latter study no protective effect was demonstrated in highly lethal T-2 toxicosis. In the present study, we confirmed and extended these observations to show that doses as low as 1 mg/kg of dexamethasone given after the exposure to T-2 provided substantial protection (over 50% survival) in a highly lethal T-2 intoxication which resulted in 100% mortality in less than 24 hr. Furthermore, in our model, significant prolongation of survival in a highly lethal (LD_{90}) T-2 toxicosis was

Table I. Effect of Dexamethasone on Brain PGE_2 Levels of T-2 Toxin-Treated Rats[a]

	T-2-toxin		T-2 + D-10		T-2 + D-1,
	6 hr	24 hr	6 hr	24 hr	6 hr
Cortex	130 ± 11*	41 ± 7**	65 ± 14***	132 ± 18	74 ± 7*,***
Hypothalamus	131 ± 19	96 ± 23	84 ± 5*	129 ± 28	99 ± 18
N	8	4	4	5	5

[a] PGE_2 levels (presented as percentage of control) extracted from cortex and hypothalamus of rats subjected to T-2 toxin and treated 1 hr later with dexamethasone (1 or 10 mg/kg, D-1 or D-10). Rats were sacrificed 6 or 24 hr post-T-2 administration. N = number of rats in each group.

* $P \leq 0.05$ vs control.

** $P \leq 0.01$ vs control (sham/saline).

*** $P \leq 0.01$ vs T-2 + saline at 6 hr.

demonstrated by dexamethasone administration as late as 3 hr after T-2 toxin. However, a much higher dose of dexamethasone, 10 mg/kg, was needed to provide this desired effect. The difference in the dose of dexamethasone between our study and the previous report might explain the variance in the outcome, i.e., failure to protect a highly lethal toxicosis in the previous study (14).

The mechanism of the protective effect of dexamethasone in T-2 toxicosis is still unknown. Dexamethasone, like other glucocorticosteroids, has multiple actions in many systems and organs. A known action of glucocorticosteroids is inhibition of arachidonic acid release and therefore prostaglandin synthesis. It was previously shown that T-2 toxicosis is associated with elevated plasma levels of some prostanoids (4,5) and specific increments in PGE_2 levels in different brain regions of rats exposed to T-2 toxin (6). In the present study we have extended these observations to show that T-2 causes a late (24-hr) depression of PGE_2 synthesis in the brain which follows the early increases. Dexamethasone treatment caused a decrease in PGE_2 levels in the brain cortex a few hours after its administration but, surprisingly, prevented the decrease in PGE_2 levels later.

The effects of dexamethasone on brain eicosanoids were not related to correction of brain edema since no changes could be identified in brain water content, nor were there any changes in the integrity of the blood–brain barrier during severe T-2 toxicosis.

The failure of BW 755c to protect rats from the lethal effects of T-2 toxin at doses sufficient to block arachidonate metabolism *in vivo* (22,23) might cast some doubt on whether blockade of arachidonate metabolism through the 5-lipoxygenase and cyclooxygenase pathways is indeed essential for the protective action of dexamethasone in T-2 toxicosis. It is, however, possible that dexamethasone-induced phospholipase A_2 inhibition reduces the formation of the plasmalogen derivative platelet-activating factor (PAF-acether) which was suggested to play a role in T-2 toxicosis (24). Thus, the changes in brain PGE_2 might reflect only the effective inhibition of arachidonate turnover and may not necessarily implicate brain prostanoids in the pathophysiology of T-2 toxicosis.

An alternate mechanism for dexamethasone actions in T-2 toxicosis could involve its suppression of the proopiomelanocortin system. Such actions of glucocorticoids are expected to reduce the release of endorphins, which have been argued to play a role in a variety of shock states (25). However, further studies would be necessary to substantiate this possibility.

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