Fatal Placental Hemorrhage in Pregnant CD-1 Mice Following One Oral Dose of T-2 Toxin

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ABSTRACT

Forty-eight hours after oral administration of a single dose (3.0 mg/kg BW) of T-2 toxin to mice on days 7, 8, 10, 11 and 12 of pregnancy, 17% maternal mortality following vaginal hemorrhage was encountered. Necropsy examination of the dead females revealed that massive hemorrhages originating from the placental regions had occurred into the reproductive tract. This observation supports the studies in which hemorrhagic disease has been described as characteristic for intoxications with T-2 toxin. The results suggest that fatal hemorrhage during pregnancy can occur in hemochorial and hemoendotheliochorial placental mammals as a result of T-2 toxin administration.

Key words: Mycotoxins, hemorrhagic diathesis, pregnancy and T-2 toxin.

RÉSUMÉ

Quarante-huit heures après l'administration orale d'une seule dose de 3 mg/kg de toxine T-2 à des souris, gravides depuis sept, huit, dix, 11 ou 12 jours, les auteurs constatèrent un taux de mortalité qui atteignait 17%, à la suite d'une hémorragie vaginale. La nécropsie de ces souris révéla, dans leurs voies génitales, la présence d'une hémorragie massive qui originait des points d'attache des enveloppes foetales. Cette constatation s'accorde avec celle d'études antérieures qui décrivent une maladie hémorragique comme caractéristique des intoxications dues à la toxine T-2. Les résultats de cette expérience permettent de penser qu'une hémorragie fatale peut survenir, au cours de la gestation, à la suite de l'administration de toxine T-2, chez les mammifères dont le placenta est hémochorial ou hémoendothélial.

Mots clés: mycotoxines, diathèse hémorragique, gestation, toxine T-2.

INTRODUCTION

T-2 toxin (3α-hydroxy-4β-,15-diacetoxy-8α-(3-methylbutyryloxy) -12,13-epoxy-trichothec-9-ene) a trichothecene mycotoxin produced mainly by *Fusarium* spp. fungi (1), is one of the more toxic trichothecene mycotoxins. It has significant effects on health of humans and livestock (2,3,4,5,6,7). T-2 toxin and other trichothecenes have a world wide distribution (8) and have been associated with diseases characterized by immunosuppression and cytotoxicity, namely, "alimentary toxic aleukia" in man (1,2,9,10), "moldy corn poisoning" in cattle and swine (4,11) and "fusariotoxicosis" in poultry (6). Some of these diseases are characterized by a hemorrhagic diathesis. Widespread petechiation and ecchymoses of the visceral serosal surfaces, hemorrhage into the peritoneal cavity, intestinal lumen, lung, meninges and nasal passages have been reported in a variety of species (2,4,12,13,14). T-2 toxin, identified by thin layer chromatography (TLC) has been suspected as being the hemorrhagic agent involved in cattle affected with "moldy corn toxicosis" (15). However, severe or generalized hemorrhage is not a typical feature of experimental T-2 toxicosis (16,17,18) and some debate still exists as to whether or not T-2 toxin is capable of producing these hemorrhagic syndromes (16).

This report describes the occurrence of placental hemorrhage and subsequent death of pregnant CD-1 mice from a teratology experiment in which mice were treated once orally with purified T-2 toxin in a propylene glycol vehicle.

MATERIALS AND METHODS

Six week old female and proven breeder male CD-1 mice (Charles River Laboratories, Canada) were bred from 08:00 h to 10:00 h for four consecutive days. The males were housed separately when not breeding, and each day were randomly allocated to a new cage of females. A ratio of five females to one male was used and breeding followed three days of teasing, in which females had five minutes access to the male but were not allowed to mate. When vaginal plugs were detected, the 24 h period following was considered day 0 of pregnancy (19). The mice were kept under a 12 h day-night cycle with darkness from 19:00 h to 07:00 h, housed individually after being diagnosed pregnant and kept in shoebox cages with inert corn hull bedding (Sanicell, Paxton Processing Co. Inc., Illinois).

The diet was semisynthetic in nature to eliminate the possibility of exposure to naturally occurring mycotoxins (20). It was prepared as compacted cubes and supplied *ad libitum* to the females throughout the experiment. One hundred and twenty-six pregnant mice were treated with T-2 toxin and

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52 pregnant mice were used as controls.

T-2 toxin (Myco-Lab Co., Chesterfield, Missouri) was administered as one single dose of 3 mg/kg body weight. This was done on either day 7 or days 8, 10, 11, or 12 of pregnancy via gavage tube in a propylene glycol vehicle (BDH Chemicals, Toronto). The body weight at day 7 of pregnancy was used to determine the dose. Mice were treated between 08:00 and 10:00 h and observed every 24 h. Controls were given similar volumes of propylene glycol and starved for 48 h to simulate the anorexia produced by T-2 toxin. All animals were sacrificed by cervical dislocation on day 18 of pregnancy. Animals that died before day 18 of pregnancy were examined postmortem. Formalinized tissue sections were processed in the usual manner, stained with hematoxylin and eosin (H & E) and phosphotungstic acid and hematoxylin (PTAH) and examined microscopically.

RESULTS

The teratological and reproductive findings are in the process of being reported elsewhere. However, 17% maternal mortality was seen after the administration of T-2 toxin. This mortality peaked at 48 h and was preceded in some instances by vaginal hemorrhage (Table I). No hemorrhage was seen in any of the controls. Necropsy of the females that died revealed the reproductive tract to be full of unclotted dark blood (Fig. 1). Histologically, blood filled the reproductive tract in the periamniotic region (Fig. 2). Phosphotungstic acid and hematoxylin stains did not reveal microthrombi in the placenta or any other organ. No other gross changes were seen in the dams; however, lymphoid necrosis in the spleen and necrosis of crypt epithelium of the small intestine were seen in five examined. The remainder were too autolysed for examination. Some treated females that died did not display hemorrhage. These animals were found to have died from gavaging accidents and hence were not included in the results reported here. No histological lesions were seen in any of the fetuses from the animals that died of placental hemorrhage. A detailed

TABLE I. Fatal Placental Hemorrhage in Pregnant CD-1 Dams Dosed with 3.0 mg/kg T-2 Toxin in Response to Day of Pregnancy of Dosing

Day of Pregnancy Dosed	Hours after Administration of T-2 Toxin			Total (%)
	24	48	72	
7	—	2	—	2/23 (9%)
8	—	2	1	3/21 (10%)
10	—	1	—	1/18 (5%)
11	—	4	—	4/26 (15%)
12	2	8	1	11/38 (30%)

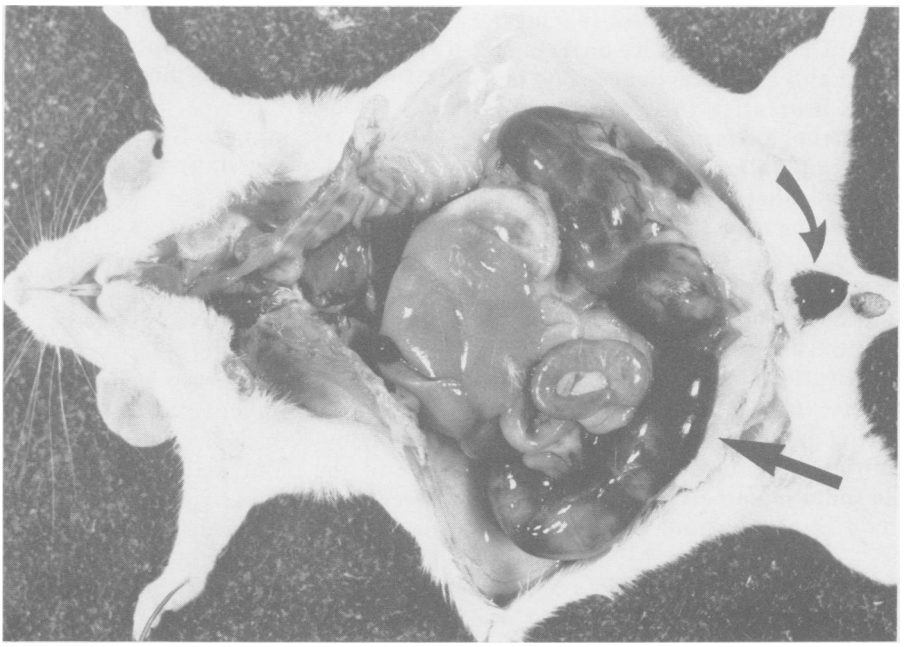

Fig. 1. CD-1 mouse at time of necropsy, 48 h after oral administration of 3.0 mg/kg T-2 toxin in propylene glycol. Note presence of unclotted blood in the vaginal region and the blood filled uterus (arrows).

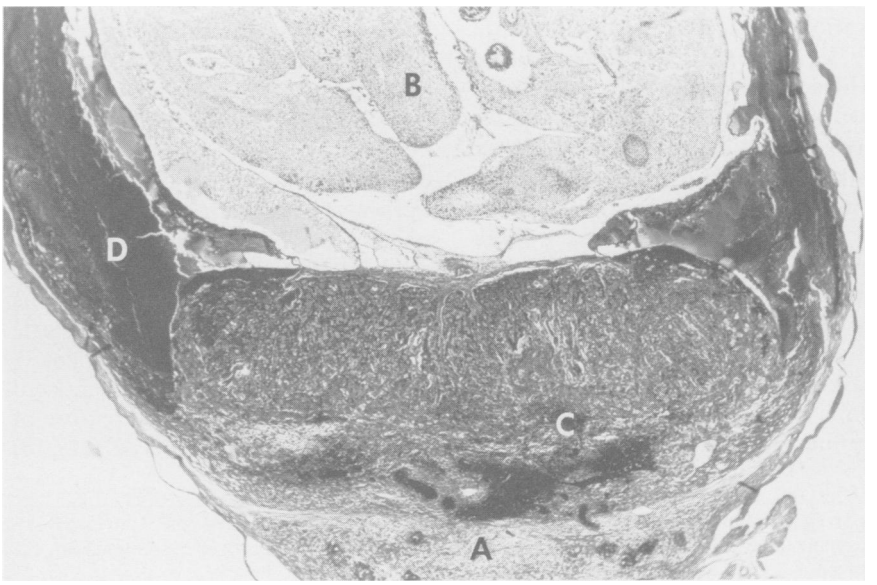

Fig 2. Cross-section of a gravid uterus from the mouse in Figure 1, showing the uterus (a), fetus (b), placenta (c) and blood in the periamniotic region (d). H & E. X15.

examination of the fetuses and uteri was made at day 18 of pregnancy and the findings are reported elsewhere, but no evidence of hemorrhage was seen.

DISCUSSION

Hemorrhage is a common finding in animals exposed to excessive radiation or radiomimetic compounds and certain plant toxins (21). As T-2 toxin is cytotoxic, immunosuppressive and a potent protein and DNA synthesis inhibitor (1,22,23,24), it is considered a radiomimetic agent.

The findings of hemorrhage with blood that failed to clot after death support the suggestion that T-2 toxin is capable of producing hemorrhagic disease in various species (2,7,9,12,13, 15). The fact that T-2 toxin failed to reproduce hemorrhagic diseases in some studies (16,18) does not necessarily mean that T-2 toxin does not have an effect on the coagulation system of most mammals. As a matter of fact, the contrary is true.

Examination of hematological parameters in animals given T-2 toxin revealed decreased numbers of circulating red and white blood cells and altered cell morphology in cats, mice, guinea pigs, chickens and sheep (25,26,27,28,29) and exhaustion of bone marrow in mice (20). Prothrombin times were increased, and hemorrhage was seen in calves given purified T-2 toxin (30) and in a cow given a crude T-2 toxin preparation intramuscularly (13). In contrast, oral administration of both crude and purified T-2 toxin to calves did not result in a hemorrhagic diathesis, yet an increase in plasma clotting times was seen (18). Rats (31,35), chickens (32), rabbits (33) and monkeys (34) all have shown elevated blood clotting times following the administration of T-2 toxin. Other, more detailed, work revealed an increase in the prothrombin time and a decrease in the plasma activity of clotting factors VIII, X, V, prothrombin and fibrinogen when purified T-2 toxin was added to the diet of broiler chickens (36). Similarly, a single intravenous dose of pure T-2 toxin in a rabbit decreased the activities of factors VII, VIII, IX, X and XI by about 40% six hours after administration (37). Spontaneous bleeding problems do not usually occur unless the blood coagulation factor's plasma activity falls below 20% (38). In most studies, application of T-2 toxin results in some reduced coagulation activity (13,18,30,31,32,33,36), but this decrease may not have been sufficient to result in uncontrollable hemorrhage.

The effect on the coagulation profiles is not a result of protein synthesis inhibition as reduction of the clotting factors far exceeds their natural half life (37). Although a consumptive coagulopathy has not yet been reported or evidenced in the form of microthrombi throughout tissues, the decline in fibrinogen concentration in chickens (36) and platelet numbers in cats treated with T-2 toxin indicate that it may be possible (25). However, recent work suggests that T-2 toxin may inhibit platelet adhesion and aggregation and the release of dense bodies (38). This would result in a coagulopathy without the loss of circulating platelets.

Pregnancy indirectly increases the procoagulant activities in species with hemochorial or zonary placentation (39,40), which is related to the delicate nature of this type of placentation (41) and its reliance on the clotting system for its integrity (42). T-2 toxin, because of its cytotoxicity, may have damaged the delicate epithelium initiating hemorrhage which could not be controlled because of defective hemostasis.

At present, one can only speculate as to the mechanisms involved in the hemorrhagic diathesis observed in pregnant mice treated orally with T-2 toxin. This phenomenon is being further investigated.

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