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As observed by others, T-2 mycotoxin can cause a spectrum of injury from erythema through necrosis when exposed to the skin of various species. Of the species tested the skin of rabbit was most sensitive to the toxin, while that of the cynomolgus monkey was the least. Skin absorption of this toxin appeared to be relatively slow with most liquid solvents in the three species studied, as compared to IM dosage. As observed with aflatoxin, a screen barrier was required to prevent oral ingestion and facial contamination of T-2 mycotoxin when applied to the skin. When a screen-barrier was utilized,

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very high doses of toxin were required in the guinea pig and cynomolgus monkey. DMSO appears to increase percutaneous absorption of T-2 in rat, however, it was not as effective in the guinea pig or monkey. The relationship to percutaneous absorption of T-2 mycotoxin through human skin will require additional studies which utlize in vitro model or human skin implants in the nude mouse. Soap and water wash appears to be an effective treatment for removal of toxin from the skin in most solvents. Additional data are required to determine what will be the maximum effective time of the toxin is applied in DMSO.

Mycotoxin Symposium

University of Sydney, Australia

Dermal toxicity of T-2 toxin in guinea pigs, rats, and cynomolgus monkeys.

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

INTRODUCTION

A number of Fusarium fungi produce a chemically related group of trichothecene metabolites which are highly toxic (Bamburg and Strong, 1971; Ueno, 1980). One of the more toxic trichothecenes is T-2 mycotoxin. In addition to its parenteral toxicity, small amounts of T-2 mycotoxin when applied to the skin have strong inflammatory and irritative characteristics, which have been utilized to develop a sensitive bioassay for detection of these toxins (Wei et al., 1972; Chung et al., 1974; Eppley et al., 1974; Hayes and Schiefer, 1979). Preliminary results indicated that larger amounts of this trichothecene when applied to the skin result in death within 48 hours without any noticeable skin reaction (Bamburg and Strong, 1971).

Circumstantial evidence has suggested that trichothecene toxins have been used in the yellow rain attacks in east Asia and Afghanistan (Marshall, 1982; Rosen and Rosen, 1982). Since skin necrosis and absorption of trichothecenes may have accounted for some of the severe toxicity observed in these attacks, various experimental models were investigated to evaluate the pathophysiology associated with topical applications of the fungal toxins.

Another fungal toxin, aflatoxin, when applied topically to the skin of rabbits, causes severe lesions in the epidermis and liver. These rabbits wore collars preventing oral ingestion of toxin during the experiment (Joffe and Ungar, 1969; Ungar and Joffee, 1969). Studies on conscious and unconscious rats, showed that ingestion of the toxin present on the hind foot pads, as a result of grooming, was responsible for the liver necrosis, which occurred after dermal exposure to aflatoxin (Purchase and Steyn, 1973).

When aflatoxin was applied topically to the backs of gerbils for 35 days, no skin or liver changes were observed, even though no precautions were taken

to prevent inadvertent ingestion (Llewellyn, 1978). These studies emphasize the complications of interpreting data on topically applied toxin and the possible species differences in their absorption through the skin or by oral ingestion of the toxin applied to the skin.

Materials and Methods

Determination of Minimum Effective Dose (MED): Twenty-four hours before use in an experimental study, male Fisher 344 rats (50-75 grams); Hartley outbred guinea pigs (350-400 grams), New Zealand white rabbits (2.5-2.9 kg), or cynomolgus monkeys (5-6 kg) had their backs shaved with an electric clipper and remaining hair was removed with a chemical depilatory SURGEX hair-removing cream . Two microliter samples of varying concentrations of T-2 mycotoxin in methanol were applied to the shaved backs of each species. The reactions were scored at 24, 48, 72 hrs by the method of Draiz et al. (1944), with both erythema and edema being scored on a 0-5 scale of increasing intensity. A linear regression was determined between the reaction score and dose of mycotoxin for each species. The MED was defined as that dose of toxin that produced a score of 1.

LD_{50} and mean time to death determinations: Rats, guinea pigs, and cynomolgus monkeys were injected intramuscularly (IM) with 0.1 ml of varying doses of T-2 mycotoxin in ethanol:glycerol:water (2:3:5). Each animal was observed to determine time of death. Selected animals from each species who succumbed to the T-2 mycotoxin were necropsied and tissues saved for histopathological examination.

For topical studies in rats, the back was shaved and hair removed with a depilatory, 24 hrs before applying toxin. Varying concentrations of T-2 mycotoxin in either methanol or dimethylsulfoxide (DMSO) was applied as 0.1 ml sample in a 4 x 4 cm area on the back of the rat. They were observed for the

next 7 days and time of death recorded for each rat. Selected rats that died were necropsied and tissues saved for histopathology.

Hair was shaved from the backs of guinea pigs and varying concentrations of T-2 mycotoxin were applied at 0.1 ml sample in either methanol or DMSO. Guinea pigs were observed for clinical signs and time of death. In another group of guinea pigs a barrier was applied to the skin at time of application of the toxin. The barrier consisted of a vinyl screen attached to a plastic sponge. A 4 x 4 cm hole was made in the plastic sponge. A brass screen was placed on top of the sponge and it and the vinyl screen were attached to the sponge with elastic tape. This barrier was placed with the vinyl screen against the back of the guinea pig in the area where the T-2 toxin had been applied. The barrier was held in place with the elastic tape. Guinea pigs with the barrier attached were observed for 14 days after application of the T-2 mycotoxin and time of death recorded for individual animals.

A right atrial catheter was implanted in four cynomolgus monkeys, after immobilization with Ketamine (Ayerst Labs. Incorp., New York, N. Y.). While still anesthetized, monkeys were fitted with a leather jacket and the catheter was passed through a stainless steel conduit. The flexible conduit was attached to a swivel located on the upper portion of the metabolic cage. A temperature probe was also implanted in the muscles of the back and passed along with the catheter to the swivel. The atrial catheter was attached to a second small swivel, which was located on the top of a big one. A plastic carrier was attached to the lower swivel, in which a temperature probe was maintained. The atrial catheter was kept patent by a slow infusion of 100 ml per day of a solution that contained 4 units per ml of heparin in half-normal saline. Six days after surgery, monkeys were again sedated, jackets opened, chest hair shaved, and either 1, 2, 4, or 8 mg per kg of T-2 mycotoxin in DMSO

was applied to the chest. After the DMSO dried, the area was covered with a plastic screen attached to a quarter inch plastic sponge. This barrier was held in place with elastic tape and the monkey was re-jacketed. Blood samples were obtained at 0, 3, 12, 24, 72 hrs and 7 days after exposure to the toxin. Various clinical parameters were monitored including body temperature, vomiting, bleeding, diarrhea, excitability, listlessness, ability to stand, prone in cage, changes in breathing rate, coma, or death. In addition, daily food intake was recorded for each monkey. Seven days after applying the toxin, the monkeys were sedated, and their jacket and catheter were removed. Lesions on the chest were photographed and the monkeys observed for another 7 days before returning to the colony.

Metabolism of labeled T-2 mycotoxin: A group of 12 guinea pigs were injected IM with a solution that contained 1.0 mg of T-2 mycotoxin and 100 microcuries of ^{3}H-T-2 mycotoxin per kg of body weight, in a mixture of ethanol:glycerol:water (2:3:5). Individual guinea pigs were placed in metabolic cages and daily urine and fecal collections were made for the next 28 days. Daily urine volumes were recorded and a sample was analyzed for radioactive content. Another sample of urine was analyzed by thin layer chromatography to determine radioactivity associated with the T-2 mycotoxin and metabolites (Pace et al., 1983).

Another group of 24 guinea pigs had their backs shaved and 3.8 mg of T-2 toxin and 200 microcuries of ^{3}H-T-2 mycotoxin kg by weight in methanol applied to the skin. Twelve of these guinea pigs had a screen barrier applied over the area of the skin on which the toxin had been painted.

Ability to remove T-2 mycotoxin from skin of the rat: Male, Fisher-344 rats weighing 75-100 grams had their backs shaved and hair removed with a chemical depilatory. Twenty-four (24) hours after removing the hair, 50

microliters of a solution containing 5 mg of T-2 mycotoxin per ml of either methanol, ethanol:glycerol:water (2:3:5) or DMSO was applied to a 1 x 1 square inch of skin. Five rats were each treated 0, 5, 60, or 360 minutes after exposure to the toxin. Treatment consisted of wiping of the back with a soap-saturated 2 x 2 surgical sponge, followed by rinsing for 1 minute with a stream of water. Another group of 5 rats which did not have their backs washed after exposure to toxin served as controls. The skin of each rat was observed on day 1, 2, and 3 after exposure and scored according to procedure of Draize et al. (1944). These scores were utilized to calculate micrograms of toxin from a previously determined standard curve. Concentration of toxin in the treated rats was compared to the control nontreated group in order to determine percentage removal of toxin from the skin.

Results and Discussion

The minimal effective dose (MED) of T-2 toxin to produce a skin response in different species is presented in Table 1. Rabbit skin was the most sensitive to T-2 mycotoxin of the species tested. Almost 2 1/2 times as much T-2 mycotoxin was required to produce a similar response in rats and guinea pigs. These observations are in agreement with previous studies of Hayes and Schiefer (1979). In contrast, the skin of cynomolgus monkey was 10 times less sensitive to the T-2 mycotoxin than that of rabbit. A MED of 200 nanograms in the monkey caused only very slight erythema, while a similar dose in the rabbit, rat, or guinea pig caused a severe erythema and edema which was associated with definite skin necrosis. Variation in dermal response to the T-2 mycotoxin in these species may be related to its ability to penetrate the stratum corneum layer of skin. Regardless of the species, a very strong inflammatory and cytotoxic response was elicited when the toxin was applied to skin at higher doses.

The comparative systemic toxicity and lethality of T-2 mycotoxin of different species was assessed by the intramuscular (IM) route or topical application (Table 2). By IM injection the lethal dose$_{50}$ (LD_{50}) was between 0.5 and 1 milligram kg of body weight for rat, guinea pig, and cynomolgus monkey, with a mean time of death (MTD) of 12-24 hours. In the rat a similar LD_{50} was observed when the T-2 mycotoxin was applied to the skin in DMSO. In contrast, topical application of this mycotoxin in methanol on rats resulted in an LD_{50} of 12.5 mg per kg and a mean time of death of 5.5 days. When the toxin in DMSO was applied to the skin of rats, systemic lesions of necrosis of lymphoid tissues and intestinal epithelieum were observed, with only mild dermal necrosis, but when T-2 toxin in methanol was applied to the skin, the major lesion was skin necrosis with few detectable systemic effects. In contrast to the rat, topical application of T-2 mycotoxin to the skin of a guinea pig in either methanol or DMSO resulted in a similar LD_{50}, which was 4 times that observed by the IM route. Mean time of death for topical exposure to T-2 mycotoxin in either methanol or DMSO was 7 to 8 days for the guinea pig. With both solvents, toxin exposure resulted in severe skin necrosis and marked inflammation characterized by edema, redness and purulent discharge from the eyes, nose, and mouth. This inflammation started to develop about day 3 after applying the toxin to the skin. Histological examination, revealed that in some cases the inflammatory discharge completely blocked the nasal passage. As the mucous membrane irritation developed the guinea pigs stopped eating or drinking water. No other gross histological lesions were observed by microscopic examination. Thus, it would appear, that the guinea pigs, during normal grooming procedures, were able to remove some of the toxin from their backs and managed to contaminate their eyes, noses, and mouths. The result - conjunctivitis and rhinitis ultimately significantly contributed

to death due to either dehydration, anorexia, and/or suffocation. When a screen barrier was applied to the area of the back where the toxin was painted, at a dose of 33 mg per kg of T-2 mycotoxin (8 X LD_{50} without barrier), none of the guinea pigs died. At a dose of approximately 80 mg per kg of toxin painted on the skin in either methanol or DMSO and a barrier applied, 4 of 6 guinea pigs died in each group. The guinea pigs given toxin in DMSO died in approximately 4.2 days, while those that had it applied in methanol died in 6.3 days. With the barrier applied none of the guinea pigs developed the mucous membrane irritation that was observed without the barrier. These data indicate that without the use of a barrier guinea pigs were able to remove some of the T-2 mycotoxin from the back, thus contributing to their deaths. Further, in the studies with the barrier, it can be concluded that this toxin is only very slowly absorbed through the skin of guinea pig.

When up to 8 mg per kg of T-2 toxin in DMSO was applied to the skin of cynomolgus monkey and the area covered with a screen and leather jacket, no deaths were observed. The monkeys developed a mild illness as indicated by slight elevations in serum CPK and mild reduction in food intake. The monkeys did develop severe skin lesions at the site of application of the toxin which were still evident 14 days after exposure. By 28 days post-exposure, the lesions had almost completely resolved. Since a dose that was 10 times the IM LD_{50} for T-2 mycotoxin did not result in death of the monkey, it could be concluded that less than 10% of the toxin, when applied to the skin was absorbed per day.

When labeled T-2 mycotoxin was injected IM in guinea pigs, radioactive compounds were rapidly excreted in the urine (Fig 1). Very little of the labeled T-2 mycotoxin was excreted in the urine and most of the radioactivity

was associated with more polar or conjugated trichothecenes. In contrast, less than 10% of the radioactivity from labeled T-2 that was painted on the skin of guinea pig appeared in the urine over 28 days. If a screen barrier was placed over the site of application labeled T-2 mycotoxin even less radioactivity was excreted in the urine (Fig 1). While 50% of the IM-injected radioactivity in T-2 mycotoxin was excreted in the urine over 7 days and 52% over 14 days (Table 3), significantly less radioactivity was found in the urine when the toxin was applied to the skin of guinea pigs. These data again support the conclusion that T-2 mycotoxin is only slowly absorbed through the skin of guinea pig.

Six hours after applying T-2 mycotoxin to the skin of the rat in either methanol or ethanol:glycerol:water solvent, the majority of toxin could be removed from the skin by washing with soap and water (Table 4). When the toxin was applied to the skin in DMSO, immediate washing, or washing 5 minutes later with soap and water, resulted in significant removal and prevented death. If washing was delayed 60 minutes, all the rats died which suggests that more than 50% of the dose had been absorbed. These data again indicate that T-2 mycotoxin was slowly absorbed through the skin when applied in methanol, but in the rat DMSO significantly increased its rate of absorption. Since the rats were sedated for 60 minutes after application of the toxin in DMSO, the death of the rats who had their skins washed after 60 minutes could not be explained by oral ingestion of toxin.

Conclusion

As observed by others (Bamburg and Strong, 1971), T-2 mycotoxin can cause a spectrum of injury from erythema through necrosis when exposed to the skin of various species. Of the species tested the skin of rabbit was most sensitive to the toxin, while that of the cynomolgus monkey was the least.

Skin absorption of this toxin appeared to be relatively slow with most liquid solvents in the three species studied, as compared to IM dosage. As observed with aflatoxin (Purchase and Steyn, 1973; Llewellyn, 1978), a screen barrier was required to prevent oral ingestion and facial contamination of T-2 mycotoxin when applied to the skin. When a screen-barrier was utilized, very high doses of toxin were required in the guinea pig and cynomolgus monkey. DMSO appears to increase percutaneous absorption of T-2 in rat, however, it was not as effective in the guinea pig or monkey. The relationship to percutaneous absorption of T-2 mycotoxin through human skin will require additional studies which utilize in vitro model (Franz, 1975; Franz, 1978; Bronaugh et al., 1982) or human skin implants in the nude mouse. Soap and water wash appears to be an effective treatment for removal of toxin from the skin in most solvents. Additional data are required to determine what will be the maximum effective time if the toxin is applied in DMSO.

LIST OF CAPTIONS

Fig 1. Cumulative radioactivity in guinea pig urine following a single exposure to labeled T-2 mycotoxin [□] = IM injection; [Δ] = topical application; [●] topical application plus a screen-barrier. The data are calculated as a percent of radioactivity injected or applied to the skin.

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TABLE 1

MINIMAL EFFECTIVE DOSE (MED) OF T-2 MYCOTOXIN TO PRODUCE A SKIN RESPONSE IN DIFFERENT SPECIES

SPECIES	N	MED (ng/2ul) mean +/- SE	SIGNIFICANCE
RABBIT	(8)	20.5 +/- 8.8	P < 0.01, RAT P < 0.05, GUINEA PIG & MONKEY
RAT	(10)	51.3 +/- 3.6	P < 0.01, RABBIT P < 0.05, MONKEY
GUINEA PIG	(10)	53.2 +/- 3.3	P < 0.05, RABBIT
MONKEY	(4)	209 +/- 112	P < 0.05, RAT & RABBIT

MED = Dose to produce score 1

TABLE 2

COMPARATIVE LD-50 AND MEAN TIME TO DEATH OF T-2 MYCOTOXIN BY TOPICAL OR i.m. ROUTES IN DIFFERENT SPECIES

SPECIES	i.m. INJECTION		TOPICAL				TOPICAL + BARRIER			
			METHANOL		DMSO		METHANOL		DMSO	
	LD-50 mg/kg	MTD hr	LD-50 mg/kg	MTD hr	LD-50 mg/kg	MTD hr	LD-50 mg/kg	MTD hr	LD-50 mg/kg	MTD hr
RAT	0.47	15.1	12.48	132	1.50	19.6				
GUINEA PIG	1.03	14.1	4.20	190	4.56	180	79.8 (4 of 6 died)	153	78.8 (4 of 6 died)	100
CYNOMOLOGOUS MONKEY	0.79	18.6							> 8.0	

TABLE 3

CUMULATIVE RATE OF URINARY EXCRETION OF RADIOACTIVITY
FROM A DOSE OF [3-H] T-2 MYCOTOXIN IN GUINEA PIGS

ROUTE OF ADMINISTRATION	% OF EXPOSED DOSE	
	DAY 7	DAY 14
	mean +/- SE (N)	mean +/- SE (N)
IM	50.2 +/- 2.9 (6)	52.3 +/- 2.8 (6)
TOPICAL	4.6 +/- 0.52 (9)[a]	10.21 +/- 0.65 (3)[c]
TOPICAL + BARRIER	1.47 +/- 0.42 (6)[b]	4.81 +/- 0.81 (6)[d]

[a] $P < 0.001$, I.M. or TOPICAL + BARRIER

[b] $P < 0.001$, I.M. or TOPICAL

[c] $P < 0.001$, I.M.; $P < 0.01$, TOPICAL + BARRIER

[d] $P < 0.001$, I.M.; $P < 0.01$, TOPICAL

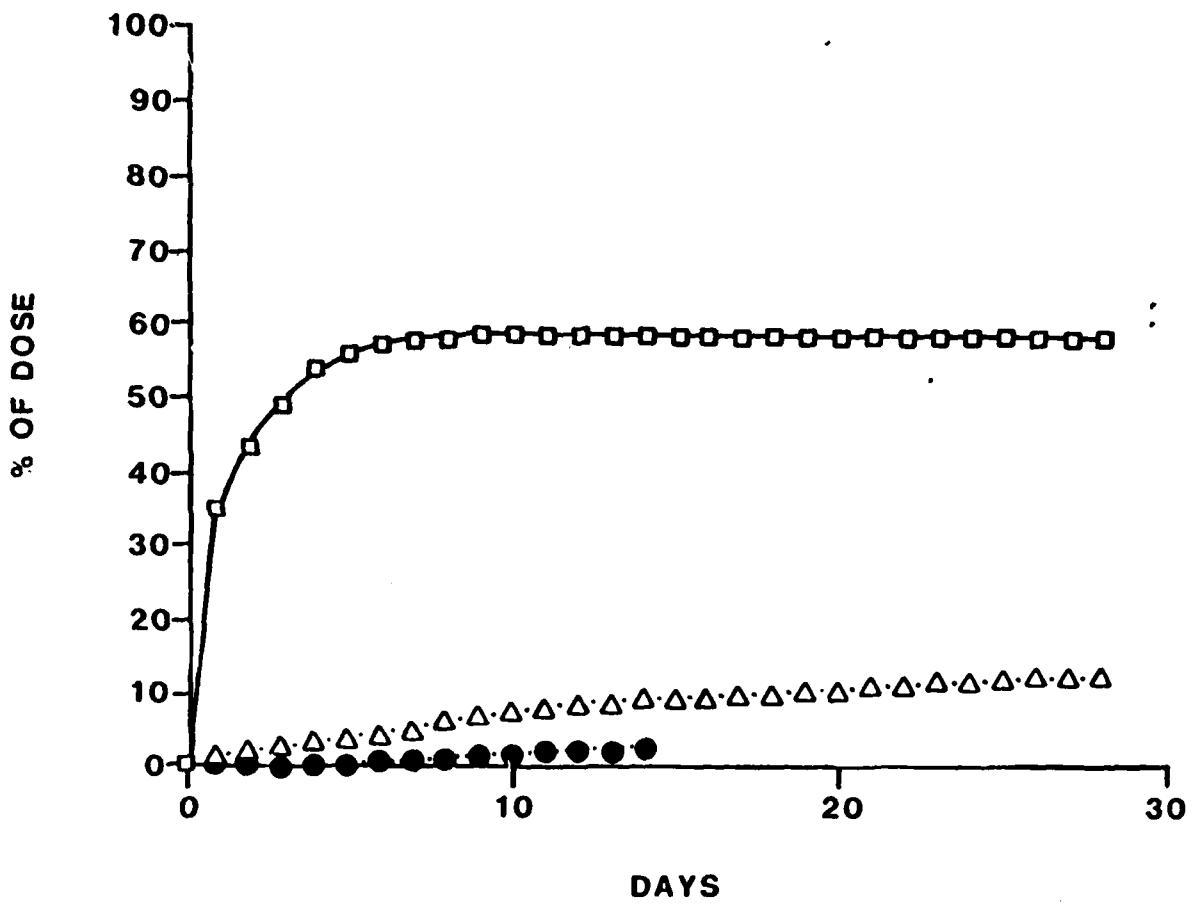
TABLE 4

ABILITY OF SOAP AND WATER TO DETOXIFY T-2 MYCOTOXIN FROM SKIN OF RATS AT VARIOUS TIMES AFTER APPLICATION IN DIFFERENT SOLVENTS

TIME AFTER APPLICATION (MIN)	SOLVENT		
	METHANOL	ETHANOL:GLYCEROL:WATER	DMSO
	% DETOXIFIED	% DETOXIFIED	% DETOXIFIED
0	100 +/- 0[a]	97.1 +/- 1.3	98.2 +/- 2.4
5	93.9 +/- 2.7	93.9 +/- 2.6	75.8 +/- 6.5
60	95.6 +/- 1.8	92.0 +/- 5.5	b
360	58.2 +/- 22.3	70.0 +/- 15.1	b

[a] = Mean +/- standard error of six rats.

[b] = Lethal (all rats dead in 24 hours. Less than 50% of dose detoxified.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