Percutaneous Toxicity of Pyridinethiones in a Dimethylsulfoxide Vehicle

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The percutaneous toxicity of three salts of pyridinethione in water and dimethylsulfoxide is described. Sodium and zinc pyridinethione penetrated the skin of rabbits when applied in a dimethylsulfoxide vehicle and caused hind quarter paralysis. Cadmium pyridinethione did not penetrate the skin in amounts comparable to the other two salts regardless of the vehicle.

THE SALTS of 1-hydroxypyridine-2-thiol have been shown to be effective antifungal and antibacterial agents. The zinc salt (ZnPT) is in current use as an antidandruff agent. Snyder *et al.* (1) have reported on the toxicity of ZnPT in a shampoo base and found no percutaneous effects in rabbits. The sodium salt (NaPT) was shown to penetrate the skin of rabbits causing hind quarter paralysis. Winek and Buehler (2) reported on the intravenous toxicity of the pyridinethiones. Following the recent usage of dimethylsulfoxide (DMSO) by Jacob *et al.* (3, 4) and others as a topical drug for arthritic conditions, considerable work on the enhancement of percutaneous absorption of various compounds by DMSO has been reported. Kligman (5) studied the percutaneous absorption of a large number of different types of compounds in different concentrations of DMSO and other solvents. With most, he found absorption greatly enhanced over other solvents.

The present study concerns the effects of the sodium, zinc, and cadmium salts of pyridinethione in a DMSO vehicle after application to the skin of rabbits.

MATERIALS AND METHODS

Albino rabbits of both sexes with a weight range of 2 to 3 Kg. were used in the study.

Nuded areas on the backs of rabbits, each approximately 100 sq. cm. in size, were dosed for 14 consecutive days. The material was applied using a dermal applicator. Some concentration occurred at the hairline edging the nuded area. The rabbits were fitted with the Newmann (6) restraining harnesses. A commercial depilatory was used to denude the skin. The dose levels were 300 mg./Kg. with sodium-1-hydroxypyridine-2-thiol (NaPT) as a 10% solution in either water or DMSO, 400 mg./Kg. of cadmium-1-hydroxypyridine-2-thiol (CdPT) as a 20% suspension in a 2% solution of methylcellulose[1] in either water or DMSO, and 400 mg./Kg. of zinc-1-hydroxypyridine-2-thiol (ZnPT) as a 10% suspension in a 2% solution of methylcellulose in either water or DMSO. The difference in dose is based on preliminary data concerning toxicity. The number of animals treated is indicated in the tables. A water control without PT salt and two DMSO control animals without PT salt were used for each group.

The pyridinethione salts used were technical, new drug grade obtained from the R. T. Vanderbilt Co. The dimethylsulfoxide was a technical grade obtained from Fisher Scientific. The animals were individually housed with food and water *ad libitum*. Both abraded and nonabraded skin were studied. The head of a small animal clipper was used to abrade the skin.

Animals were observed during the dosing period and for 1 week after treatment. Incoordination, paralysis, and death were the major points of observation. Minor points of observation were diarrhea, weight, and effects on the eyes of the animals.

RESULTS

Results are summarized in Table I; observations on individual animals are presented in Tables II–V. All animals treated with NaPT in DMSO showed signs of incoordination[2] by day 3, while those treated with aqueous NaPT showed these signs on day 5. The NaPT–DMSO treated animals were unable to rise[2] by the sixth day while the two NaPT–water treated animals were unable to rise on the sixth and eighth days, respectively. Seventy percent of the animals dosed with NaPT–DMSO showed sluggish pupillary response by the fourth day and 80% developed diarrhea by the third day. One animal treated with NaPT–water developed a sluggish pupillary response on day 11 and had cloudy drainage of the eye on day 12; it also developed diarrhea.

The animals treated with NaPT–DMSO all died by day 7 with a mean value of 4.3 days, while the two animals treated with NaPT–water died on day 8 and day 13, respectively.

One animal treated with CdPT–water and two treated with CdPT–DMSO showed changes. The remainder of the animals were grossly comparable with the water control. One treated with CdPT–water was unable to rise and showed weight loss. One treated with CdPT–DMSO developed incoordination and weight loss, while the other on the same treatment showed only weight loss.

The animals which were treated with Zn PT–DMSO all developed incoordination from day 2 to day 7, and were unable to rise from times beginning between day 8 and day 13. Twelve animals developed eye drainage, three developed sluggish pupillary responses, and two developed corneal opacities. Thirteen animals died between days 9 and 17. This represents 72% of the animals on this treatment. The other three were sacrificed after 21 days, when they appeared to be recovering. Of the animals dosed with ZnPT–water two died, one on day 2 and one on day 9. These two also developed eye diffi-

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[1] Trademarked as Methocel by the Dow Chemical Co., Midland, Mich.

[2] Struggle to right itself when placed on side: no paralysis. Hind limb paralysis.

TABLE I—SUMMARY OF RESULTS

Material Dosed	Onset of Incoordination: No. Affected / No. Dosed	Onset of Incoordination: Days Av.	Onset of Incoordination: Days Range	Onset of Paralysis: No. Affected / No. Dosed	Onset of Paralysis: Days Av.	Onset of Paralysis: Days Range	Death: No. Affected / No. Dosed	Death: Days Av.	Death: Days Range
DMSO–NaPT	10/10	1.7	1–3	10/10	3.7	2–6	10/10	4.3	2–7
H_2O–NaPT	2/2	5	5–5	2/2	7	6–8	2/2	10.5	8–13
DMSO–CdPT	1/10	2	2	0/10	...	...	0/10	...	...
H_2O–CdPT	1/2	5	5	1/2	5	5	0/2	...	...
DMSO–ZnPT	16/16	5.1	2–7	16/16	9.4	7–13	13/16	10.4	9–17
H_2O–ZnPT	1/7	6	6	1/7	7	7	2/7	5.5	2–9
DMSO	0/6	...		0/6	...		0/6	...	...

TABLE II—RABBITS DOSED WITH 10% NaPT AT A LEVEL OF 300 mg./Kg.[a]

Vehicle	Dose Vol., ml.	Onset of Incoordination, Days	Onset of Paralysis, Days	Death, Days	Wt. Loss, Gm.
DMSO[b]	7.0 N[c]	3	6	7	320
DMSO	6.6 N	2	4	5	510
DMSO	7.2 N	3	3	4	320
DMSO	6.5 N	2	5	6	585
DMSO	6.5 N	2	3	3	335
Water	6.1 N	5	6	8	320
DMSO	7.1 A[d]	2	4	4	500
DMSO	7.1 A	2	4	5	440
DMSO	6.6 A	2	3	4	285
DMSO	6.5 A	2	3	3	335
DMSO	6.6 A	1	2	2	435
Water	6.9 A	5	8	13	410

[a] Daily topical application. [b] Three grams per kilogram. [c] Nonabraded skin. [d] Abraded skin.

TABLE III—RABBITS DOSED WITH 20% CdPT AT A LEVEL OF 400 mg./Kg.[a] (NO DEATHS OCCURRED)

Vehicle[b]	Dose Vol., ml.	Onset of Incoordination, Days	Onset of Paralysis, Days	Wt. Loss, Gm.
DMSO[c]	5.4 N[d]	...	...	+95
DMSO	5.9 N	...	...	+40
DMSO	5.2 N	...	...	+45
DMSO	4.8 N	...	...	−410
DMSO	4.8 N	...	...	+60
Water	5.1 N	5	5	−730
DMSO	4.5 A[e]	...	...	+110
DMSO	4.6 A	2	...	−970
DMSO	5.5 A	...	...	+215
DMSO	5.7 A	...	...	+270
DMSO	3.7 A	...	...	+280
Water	4.8 A	...	...	+120

[a] Daily topical application. [b] With 2% methylcellulose. [c] Two grams per kilogram. [d] Nonabraded skin. [e] Abraded skin.

culties; both had drainage and one developed corneal opacity. Weight change data varied, ranging from the loss of 1035 Gm. to a gain of 360 Gm. The two deaths represent 29% of the animals treated. Figure 1 shows the hind limb paralysis produced by treatment and Fig. 2 shows a control animal.

Lung necrosis was present in seven of the 12 animals which died after dosing with NaPT, and in 14 of the 17 which died after ZnPT treatment.

The control animals which were dosed with DMSO–methylcellulose did not develop any of the outward signs of intoxication, and gained from 30 Gm. to 445 Gm. during the course of the dosing. None of the animals developed corneal opacities or irritations of the eye.

TABLE IV—RABBITS DOSED WITH 10% ZnPT IN DMSO AT A LEVEL OF 400 mg./Kg.[a]

Vehicle[b]	Dose Vol., ml.	Onset of Incoordination, Days	Onset of Paralysis, Days	Death, Days	Wt. Loss, Gm.
DMSO[c]	11.2 N[d]	6	8	15	−760
DMSO	10.2 N	4	12	...	+130
DMSO	10.8 N	6	9	...	−740
DMSO	9.5 N	7	11	15	−610
DMSO	10.4 N	5	12	16	−765
DMSO	12.4 N	5	8	9	−635
DMSO	13.2 N	2	9	...	−530
DMSO	11.2 N	5	8	10	−295
Water	9.6 N	6	7	9[f]	−310
Water	12.8 N	...	...	...	−160
Water	14.4 N	...	...	...	−290
Water	15.6 N	...	...	...	−510
DMSO	10.2 A[e]	6	10	13	−270
DMSO	10.3 A	7	13	15	−480
DMSO	9.8 A	6	7	10	−310
DMSO	11.2 A	6	8	12	−1035
DMSO	10.2 A	5	9	17	−995
DMSO	12.8 A	3	9	10	−360
DMSO	12.4 A	3	9	14	−655
DMSO	13.2 A	6	9	10	−525
Water	10.0 A	...	...	...	+360
Water	10.4 A	...	...	2[f]	−200
Water	12.4 A	...	...	...	+10

[a] Daily topical application. [b] With 2% methylcellulose. [c] Four grams per kilogram. [d] Nonabraded skin. [e] Abraded skin. [f] Animals were observed to lick material from sides of cages.

TABLE V—ANIMALS DOSED WITH DMSO CONTAINING 2% METHYLCELLULOSE[a]

Dose Vol., [b] ml.	Onset of Incoordination, Days	Onset of Paralysis, Days	Death, Days	Wt. Gain, Gm.
4.8 N[c]	...	...	...	30
9.8 N	...	...	...	250
6.6 N	...	...	...	330
4.5 A[d]	...	...	...	140
11.6 A	...	...	...	445
6.9 A	...	...	...	360

[a] The amount used is based on the volume of DMSO used with NaPt, CdPT, and ZnPT. [b] Daily topical application. [c] Nonabraded skin. [d] Abraded skin.

Fig. 1—Hind limb paralysis produced by treatment with ZnPT in DMSO. Animal is in a Newmann harness.

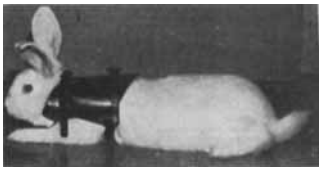

Fig. 2—Control animal in Newmann harness.

All of the animals treated with a pyridinethione salt, either in DMSO or water, showed skin irritation at the site of application which cleared when the treatment was terminated.

DISCUSSION

Dimethylsulfoxide apparently aided in the penetration of both NaPT and ZnPT in comparison with water. The sodium salt readily penetrated the skin when applied in an aqueous solution, but when applied in DMSO, the onset of toxic signs was earlier and paralysis and death resulted earlier in the treatment regimen. The animals all lost weight which is attributed to their inability to eat because of the hind quarter paralysis.

In the ZnPT treated group, DMSO apparently facilitated percutaneous absorption with the production of toxic signs and, in most cases, death. ZnPT in water did not produce paralysis or death except in two cases where the animals were observed to lick applied material from the sides of their cages. A precaution that must be taken in percutaneous studies is to avoid the transfer of applied material from the backs of animals to the sides of the cages. If this occurs, the study becomes an oral ingestion study and not a percutaneous study. In all other cases the material remained on the backs of the animals and was not observed on the sides of the cages. Adequate-size cages can prevent the transfer in most cases.

DMSO apparently did not aid the penetration of CdPT. The present study indicates an apparent lack of penetration regardless of the vehicle. As indicated in Table III, two animals developed toxic signs and lost weight, but recovered completely when treatment ended. All animals were comparable with the water control grossly, except for the animal treated with CdPT in an aqueous vehicle. This animal had lost considerable weight, but returned to normal weight within 1 week after treatment ended. Whether or not the skin was abraded apparently had no effect on penetration. Further study of CdPT in DMSO and aqueous vehicles is planned to gain evidence for the lack of penetration of this salt.

The concurrent use by the public of DMSO and other drugs, cosmetics, and soap products can be a potential hazard, as this study and others indicate. Substances that do not penetrate the skin and have been shown to be safe may become hazardous if applied concurrently with DMSO. This may be the strongest indication for restrictions on the use of DMSO regardless of the safety of DMSO itself.

As a continuation of this study and in an attempt to explain the difference in penetration between the two insoluble salts, ZnPT and CdPT, when applied in DMSO, an analytical method for the analysis of pyridinethione in tissues is in progress. A possible explanation for the difference in penetration is that Cd^{2+} is known to have a higher affinity for sulfur in the lower oxidation states, *i.e.*, S^{2-}, while Zn^{2+} does not show so great an affinity. This might cause a stronger complex with hydroxypyridinethione (PT) as compared with DMSO for Cd^{2+}, and the reverse of this for Zn^{2+}, thereby freeing the pyridinethione for penetration.

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Biosynthesis of Ergot Alkaloids. Origin of the Oxygens of Chanoclavine-I and Elymoclavine

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The oxygen of the hydroxyl groups of chanoclavine-I and elymoclavine has been shown not to be derived from water. This indicates that these hydroxyl groups do not originate from the reaction of an allylic carbonium ion with water, but may be introduced by direct hydroxylation.

ERGOT ALKALOIDS are formed from tryptophan and mevalonic acid (1, 2). A hypothetical biogenetic scheme which accounts for the experimental results so far available has been proposed recently (Scheme I) (3). The formation of the tetracyclic ergoline skeleton, as well as many of the known alkaloid interconversions, involves a number of oxidative cyclizations and hydroxylations. As pointed out by Agurell *et al.* (4), most of these reactions can be accounted for by oxidations at positions allylic to the exocyclic double bond. Very little experimental evidence about the mechanism of these oxidations is, however, available. Ramstad, Taylor, and their co-workers (5) have shown that the conversion of agroclavine to setoclavines and of elymoclavine to penniclavines can be brought about by horseradish peroxidase in the presence of hydrogen peroxide. This oxidation was

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