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THE INHIBITORY EFFECT OF ZINC PYRITHIONE ON THE EPIDERMAL PROLIFERATION OF ANIMAL SKINS

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Abstract. The hypothesis that zinc pyrithione, a highly active anti-dandruff agent, exerts an anti-biosynthetic effect and reduces the epidermal turnover has been tested using guinea pig and hairless mouse skins. It has been found that enhanced mitosis caused by both stripping off the horny layers and sodium dodecyl sulphate, could be suppressed by approximately 50% by the topical application of zinc pyrithione. Furthermore, sodium dodecyl sulphate-induced epidermal hyperproliferation has been found suppressible by the simultaneous application of zinc pyrithione. Thymidine incorporation studies using hairless mice revealed that a single application of zinc pyrithione results in reduction of DNA synthesis. It is suggested that the anti-dandruff effect of zinc pyrithione results primarily from its action as an anti-biosynthetic agent rather than from its anti-yeast action.

Key words: Zinc pyrithione; Dandruff; Anti-biosynthetic effect

The action mechanisms by which anti-dandruff agents clinically suppress dandruff have long been disputed (1–7). Recent microbial studies using highly active anti-dandruff agents such as selenium sulphide (7) and zinc pyrithione (8) have shown that scalp microorganisms do not play any primary role in the pathogenesis of dandruff. It seems likely that anti-dandruff agents influence the skin in a manner differing from the way in which they suppress microorganisms. Kligman et al. (9) have suggested that the mode of their anti-dandruff action is cytostatic and that they reduce epidermal proliferation by inhibiting the multiplication of germinative cells. However, except for selenium sulphide (4) and omadine MDS (13) there has been little direct evidence for the anti-metabolic property of many widely available anti-dandruff agents. In the present study, the effect of zinc pyrithione, a highly active anti-dandruff agent, on cellular mitosis and DNA synthesis which are directly responsible for cellular reproduction has been investigated to test the hypothesis that anti-dandruff agents also suppress the multiplication of mammalian epithelia.

MATERIAL AND METHODS

Materials

Zinc pyrithione (ZPT) was obtained from Olin Japan Inc. Irgasan DP-300 (DP-300) was purchased from Ciba Geigy Company. Colcemid and all other chemicals were purchased from Sigma Chemical Co. (Saint Louis, Miss.). [^{3}H]thymidine (methyl-[^{3}H]thymidine, 2 Ci/mmol) was obtained from New England Nuclear (Boston, Mass.).

Mitosis assay

The anti-mitotic effect of various drugs was assayed utilizing the Colcemid technique to count the number of cells entering metaphase during 4 hours with or without application of these drugs *ad modum* Christopher (10). Guinea pigs (Hartley strain, weighing 250–300 g) were used; their ears were stripped with adhesive tape until discrete glistening of the skin appeared (15 strippings). Zinc pyrithione (ZPT) or Irgasan DP-300 dispersed in saline containing 1% dimethyl sulphoxide (DMSO) was applied topically on the left ear either once on the 4th day or daily for 4 days. The right ear served as control.

In hydroxyurea experiments, aqueous solution of 1.0% concentration was used. In the experiments using sodium dodecyl sulphate (SDS) to enhance mitotis, a mixture consisting of 1% SDS and 1% ZPT in distilled water was applied twice a day on 5 successive days on the left ear of the guinea pigs. The conditions for this application consisted of 1 min washing and 10 sec rinsing with water. No inflammatory reaction was observed on the treated ear during this experiment. The right ear, treated in a corresponding manner with 1% SDS or saline only, served as control. To assess mitotic activity, each animal was injected intraperitoneally with Colcemid in saline at a dose rate of 2 mg/kg body weight 4 hours prior to sacrifice. The animals were sacrificed by cervical dislocation and skin biopsies were fixed in 10% formaldehyde solution and embedded in paraffin wax. Sections were cut perpendicular to the skin surface at 6 μm and stained with hematoxylin and eosin for light microscopy. Twelve sections that were cut from each specimen, separated by at least 30 μm were scanned in the interfollicular epidermis for mitoses. The number of cells arrested in metaphase per 2-mm length of the dermal–epidermal interface were counted. In addition, the thickness of the malpighian layer of the epidermis, i.e. from the dermal–epidermal interface to the junction between the granular and keratin layers, on 30–60 sites in these sections was measured microscopical-

Table I. *Sequential changes in arrested metaphase after 15 times stripping of horny layers of guinea pig ear*

n = number of areas observed

Period after stripping	Number of arrested metaphases per 2 mm length	n
Unstripped animal	1.36±1.32[a]	22
Stripped animal		
4 hours	0.40±0.70**	10
2nd day	0.30±0.48**	13
3rd day	24.96±8.76***	45
4th day	13.71±5.52***	34
7th day	5.63±3.25***	30

[a] Means and standard deviation.
* Value significantly different from unstripped control at $p<0.05$, **, at $p<0.01$, and ***, at $p<0.001$.

ly using a calibrated eyepiece oriented perpendicular to the dermal–epidermal interface.

DNA synthesis assay

Epidermal DNA synthesis was measured on adult hairless mouse skins according to the method of ●tani (11). 1.0% zinc pyrithione dissolved or suspended in dimethysulphoxide or ethanol and a 1% aqueous solution of sodium dodecyl sulphate was applied on the dorsal skin. Mice treated with vehicle only were used as controls. Four hours after these treatments, the animals were injected intraperitoneally with 25 μCi tritiated thymidine and sacrificed 1½ hours later. The dorsal skin was cleansed with ethanol, freed from the subcutaneous tissue and placed on paper towels. 17-mm transparent semirigid plastic discs were fixed to the skin with cyanoacrylate glue. After drying, the skin specimens were heated for 10 min on a hot-plate at 60°C. The skin surrounding the discs was trimmed and placed in glass scintillation vials containing 5 ml of 2 M potassium bromide solution and incubated at 60°C to split the dermis. The epidermis was rinsed and incubated with 0.25% acetic acid for 1 hour at room temperature with gentle agitation to release the non-incorporated thymidine. The discs then were washed with 10 ml distilled water and then with 95% ethanol. After drying, 5 ml of Insta-Gel (Packard) was added to the vial to determine the ^{3}H-content incorporated into the epidermal specimens using an Aloka liquid scintillation spectrometer. Incorporated [^{3}H]thymidine is expressed as dpm/disc.

Statistics

The level of significance of the difference was calculated by Student's t-test.

RESULTS

Effect on stimulated mitoses

Table I demonstrates the sequential changes in the number of metaphases per 2-mm length of the dermal–epidermal interface following stripping of the horny layers and indicates that a peak in mitotic activity occurs on the 3rd day. Table II shows the number of metaphases on the 4th day after a single application of various drugs. It was found that enhanced mitosis after stripping on the horny layers could be suppressed by up to approximately 50% with topically applied ZPT, but not with Irgasan DP-300 (DP-300), and equally active antimicrobial agent which has no anti-dandruff effect in vivo (8). The effects of daily application of these drugs after stripping are shown in Table III. Under these conditions a more marked anti-mitotic effect of ZPT occurs, in contrast to the apparent lack of effect by DP-300. Hydroxyurea (HU) employed as positive control also exhibits an anti-mitotic effect due to its DNA synthesis inhibition.

Fig. 1 shows the histological appearance of the ear skin following daily applications of ZPT for 4 days. As can be seen from the figure, ZPT causes no gross injurious changes to the epidermis.

Table IV shows the effect of a mixture of 1% SDS and 1% ZPT on mitoses, in comparison with 1% SDS or water, in twice-daily 1-minute treatments followed by rinsing, on 5 successive days. While treatments with 1% SDS alone induce a marked increase in the number of metaphases in the basal cells as compared with the control, the addition of ZPT at the 1% concentration to this system reduces the enhanced mitosis by an average of 50%. Furthermore, examination of epidermal thickness (Table V) reveals that, consistent with the

Table II. *Effect of a 4-hour single application of ZPT on the number of arrested metaphases 3 days after 15 strippings of horny layers of guinea pig ear*

NS = not significant, ZPT = zinc pyrithione, DP-300 = Irgasan DP-300, n = number of areas observed

	Number of arrested metaphase per 2 mm length	
	Drug application (left ear)	Control (right ear)
ZPT 1.0%		
Expt. 1	25.1±8.2[a] NS $n=15$	31.6±6.8 $n=10$
Expt. 2	17.9±5.2** $n=15$	38.8±9.4 $n=6$
Expt. 3	7.0±3.6*** $n=18$	14.5±6.7 $n=43$
DP-300 1.0%		
Expt. 1	15.9±4.0 NS $n=17$	15.7±4.3 $n=19$

[a] Means and standard deviations.
* Value significantly different from control at $p<0.05$, ** at $p<0.01$ and *** at $p<0.001$.

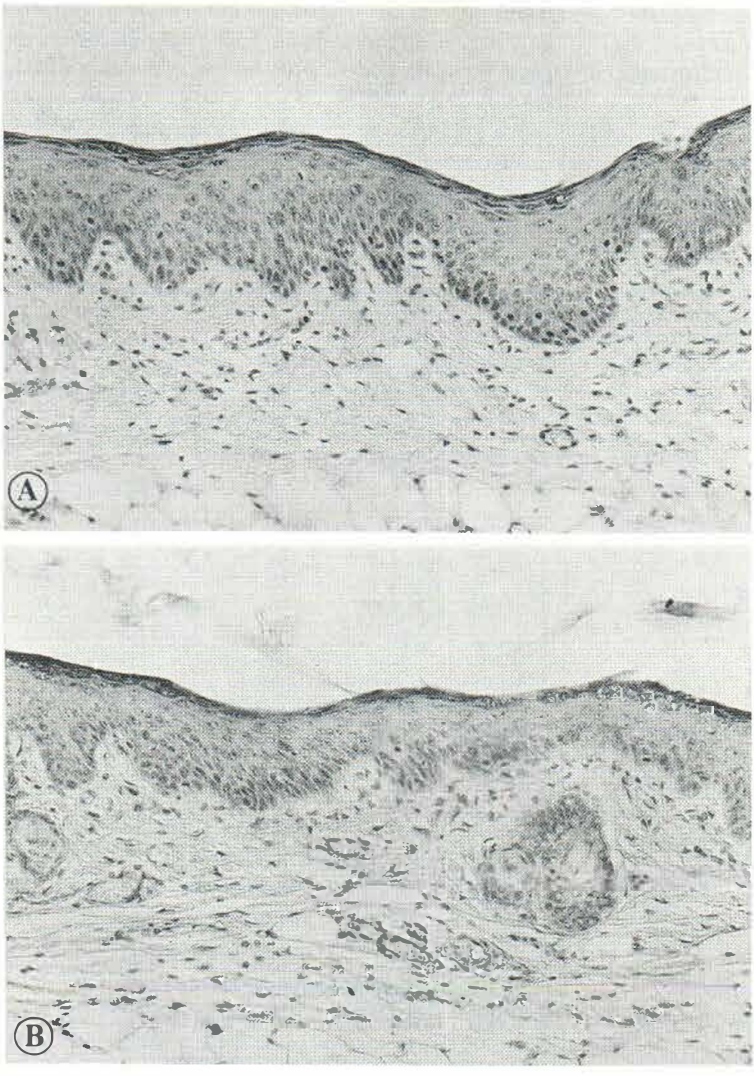

Fig. 1. (A) H & E sections of guinea pig ear skin treated daily for 4 successive days with vehicle only, following 15 times stripping of the horny layers. (B) H & E sections of guinea pig ear skin treated daily for 4 successive days with 1% ZPT solution, following 15 strippings of the horny layers. (×400.)

results indicated by mitotic index, treatments with a mixture of SDS and ZPT induce a reduction in the thickness of epidermis.

Effect on DNA synthesis

Thymidine incorporation into the epidermis has been estimated as a measure of epidermal DNA synthesis after a single application of ZPT at 1.0% concentration in three different vehicles. Table VI shows the effect of ZPT application on epidermal DNA synthesis, as compared with the application of vehicle only. In all cases, topical ZPT caused a significant decrease in epidermal DNA synthesis as compared with the control.

DISCUSSION

Our animal experiments have revealed that enhanced mitosis after stripping of the horny layers could be suppressed by approximately 50% by topical application of ZPT. Since this antimitotic effect of ZPT is seen within 4 hours, which corresponds approximately to the cell cycle period of the G2 phase, it is unlikely that this anti-proliferative effect is similar to hydroxyurea-induced anti-DNA synthesis action. The anti-DNA synthesizing effect of ZPT observed here is not related to its anti-mitotic effect occurring within 4 hours. It is therefore suggested that ZPT has both of these cellular ef-

Table III. *Effect of daily application of ZPT on successive 4 days on number of arrested metaphase after 15 times stripping of horny layers of guinea pig ear*

NS = not significant, ZPT = zinc pyrithione, DP-300 = Irgasan DP-300, n = number of areas observed

	Drug applications (left ear)	Control (right ear)
ZPT 1.0%	28.5±12.1*** $n=15$	78.2±20.9[a] $n=37$
Hydroxyurea 1.0%		
	1) 30.0±13.8*** $n=20$	59.9±20.5 $n=8$
	2) 70.6±19.6*** $n=8$	104.0±20.0 $n=10$
DP-300 1.0%	89.8±12.5 NS $n=15$	89.0±13.9 $n=22$

[a] Means and standard deviations.
* Value significantly different from control at $p<0.05$, ** at $p<0.01$, and *** $p<0.001$.

fects, independently of each other. Comparative experiments revealed that ZPT exerts an anti-mitotic effect whereas DP-300, a similarly active antimicrobial agent which has been previously shown to have no anti-dandruff effect (8), has no anti-mitotic effect.

Priestley et al. (12) demonstrated the acute toxicity of ZPT at concentrations above 0.1 μg/ml for cultured human skin cells and suggested that this non-specific toxicity of ZPT may play a role in suppressing dandruff. If ZPT actually has a marked toxicity for epidermis at lower concentrations, one may reasonably expect to find severe injurious changes in epidermal cells following cumulative treatments of ZPT to 15-times stripped skin. However, our histological observation of ZPT-treated skin recovering from strippings reveals that even in such highly permeable conditions no substantial epidermal damage occurs despite its anti-mitotic effect. In general, toxic reactions are accompanied by the stimulation of epidermal proliferation such as is observed after SDS application. Such epidermal proliferation is necessary for the regeneration of the tissue. However, ZPT by contrast, is found to reduce the capacity of SDS to stimulate the epidermal mitosis or DNA synthesis. Gloor et al. (13) have reported a similar anti-mitotic effect of omadine MDS (chemically similar to zinc pyrithione) on surfactant-stimulated skin of guinea pigs. It is very likely that the anti-biosynthetic effects of ZPT observed here are responsible for its cellular action rather than their general toxic reaction to epidermis.

Table IV. *Effect of two daily application of ZPT on 5 successive days on SDS-induced hyperproliferation*

Data are expressed as number of arrested metaphases within 4 hours. ZPT = zinc pyrithione, SDS = sodium dodecyl sulphate, n = number of areas observed

	Number of arrested metaphase per 2 mm length	
Group no.	ZPT (1%) + SDS (1%) (left ear)	SDS (1%) (right ear)
1	1.9±1.7** $n=15$	5.6±2.4[a] $n=7$
2	2.3±2.1*** $n=11$	9.2±2.6 $n=9$
3	1.8±1.3** $n=11$	4.6±3.1 $n=13$
4	3.5±2.7*** $n=21$	8.3±2.3 $n=8$
5	19.4±4.0* $n=8$	26.0±3.4 $n=6$
6	32.6±3.6*** $n=8$	48.3±2.2 $n=6$
7	3.3±1.4** $n=8$	29.4±8.2 $n=7$
8	48.8±9.6* $n=8$	63.8±10.1

Control (water): 3.2±2.4, n = 39.
[a] Means and standard deviations.
* Value significantly different from SDS-treated skin at $p<0.05$, ** at $p<0.01$, and *** at $p<0.001$.

Table V. *Effect of ZPT on hyperproliferation induced by two daily application of SDS on 5 days as shown by thickness (μm) of the epidermis (Malpighian layer)*

NS = not significant, ZPT = zinc pyrithione, SDS = sodium dodecyl sulphate, n = number of areas observed

Group no.	Left ear	Right ear
	ZPT (1%) + SDS (1%)	SDS (1%)
1	22.6±8.8 NS $n=60$	25.3±6.7[a] $n=60$
2	16.5±5.0*** $n=62$	25.7±6.9 $n=60$
3	13.8±2.9*** $n=59$	26.6±8.0 $n=60$
4	24.2±6.5*** $n=60$	32.2±1.0 $n=60$
	ZPT (1%) + SDS (1%)	Control
1	20.5±5.7 NS $n=30$	23.0±5.7 $n=30$
2	15.3±4.3 NS $n=59$	14.0±4.0 $n=70$
	SDS (1%)	Control
1	16.6±5.4*** $n=50$	9.8±2.6 $n=60$
2	15.1±4.3 NS $n=50$	15.8±6.2 $n=60$
3	34.4±7.6*** $n=70$	16.8±4.5 $n=60$
4	23.9±7.0*** $n=60$	14.5±2.8 $n=60$

[a] Means and standard deviations.
* Value significantly different from control or SDS-treated skin at $p<0.05$, ** at $p<0.01$, and *** at $p<0.001$.

Table VI. *Effect of ZPT on epidermal DNA synthesis of hairless mouse skin after a single 5½-hour application*

DMSO = dimethyl sulphoxide, EtOH = ethanol, SDS = sodium dodecyl sulphate, *n* = number of experiments

Vehicle	Control (dpm/disc)	1% ZPT (dpm/disc)	% inhibition	
1. DMSO	4 339±2 421[a] *n*=5	1 514±1 150 *n*=5	65.1	$p<0.001$
2. EtOH	4 479±1 976 *n*=5	2 651±1 784 *n*=5	40.8	$p<0.001$
3. 1% SDS in water	7 215±1 204 *n*=5	4 122±2 716 *n*=5	42.8	$p<0.001$

[a] Means and standard deviations.

Concerning the observed anti-DNA synthesizing effect of ZPT, it is interesting to note that, apart from the presence of its annular structure, pyrithione is very similar to hydroxy-2-thiourea which also exhibits an anti-DNA synthetic effect. All of the hydroxyurea and related compounds which form complexes with metal ions have been shown to inhibit DNA synthesis in the HeLa cell system (14).

Although microbial analysis of ZPT has been extensively studied to assess its anti-dandruff mechanisms (15), little is known about its other possible biological functions associated with its highly active anti-dandruff effect. Our present evidence indicates that ZPT has an anti-biosynthesis effect on epidermis. These findings suggest the possibility that the reproductive activity of mammalian epithelia might also be suppressed by ZPT, leading to the reduction in dandruff, which is after all as revealed by scalp epidermal kinetics (9), a disorder of hyperproliferation. Cellular mechanisms by which ZPT exerts its anti-biosynthetic action on the epidermis are currently under investigation.

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