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Effect of Zinc Pyrithione on Mitotic Activity in Normal Human Skin

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Summary. Two concentrations (0.02% and 0.002%) of zinc pyrithione (ZPT) in water and a water control were applied to normal forearms – both on normal skin (six subjects) and also where the skin had been stripped to the 'glistening layer' (six subjects) with adhesive tape. Measurements of labelling index (LI), mean epidermal thickness (MET), mean stratum-corneum thickness (MSCT), total epidermal thickness (TET) and basal/granular cell ratio (B/G) showed no significant differences between the three treatments on normal skin or the parameters studied in stripped skin. It is concluded that ZPT has no effect on epidermal renewal in normal skin in vivo.

Introduction

Shampoos containing zinc pyrithione (ZPT) are efficient treatments for dandruff [1, 3, 6]. Although ZPT has an anti-fungal action and has been thought to be effective against dandruff for this reason, it has also been suggested that it exerts its therapeutic effect by an anti-mitotic action [2, 5]. Because of the importance of knowing which of these effects is operative, we conducted two separate studies. The first of these, which dealt with patients with dandruff, has been reported elsewhere (Marks, Pearse and Walker, in press). The present study was designed to establish whether ZPT has an anti-mitotic effect on either stimulated or non-stimulated normal human epidermis in vivo.

Materials and Methods

Twelve human volunteers (five male, seven female) with an average age of 40.2 years (age range, 19–59 years) were randomly divided into two groups of six subjects. Three areas (3 cm^2) were delineated on the extensor aspect of one forearm of each volunteer. In one group, the forearm sites were stripped to the 'glistening layer' with clear adhesive tape (Sellotape). In the second group, the sites were not stripped.

Under polythene-film occlusion, 0.1 ml water, 0,002% ZPT in water or 0.02% ZPT in water were applied three times per day for 2 days to the 3-cm^2 sites (allocated blind) on the left forearm of each subject. The lower ZPT concentration was selected in order to deposit the same level of ZPT per square centimetre as is deposited on the scalp during the normal use of a shampoo containing ZPT (unpublished data); the higher concentration gave a tenfold exaggeration of the deposition level.

Fourty-eight hours after stripping and application, 4-mm punch-biopsy samples were taken from each application site after local infiltration of the site with 1% lignocaine. Each biopsy sample was divided in two. One-half was incubated in the presence of tritiated thymidine (2.5 μCi tritiated methyl thymidine; specific activity, 18 Ci/mmol/ml of unsupplemented Eagle's MEM) for 4 h and subsequently prepared for autoradiography using standard techniques of histological autoradiographic preparation with dipping film. The labelling indices (LI) were obtained by counting the number of autoradiographically labelled basal and suprabasal cells, and expressing these as a percentage of the number of basal cells. The other half was subjected to histometric evaluation using the Quantimet-720 image-analysing system. Mean epidermal thickness (MET; mean thickness of the viable epidermis in microns), total epidermal thickness (TET; mean thickness of the epidermis and stratum corneum in microns) and the basal/granular cell ratio (B/G; ratio of the length of the basal layer to that of the top of the granular cell layer) were the parameters obtained using this technique.

Results

Labelling Index (Table 1)

The mean LI value for the unstripped skin in the water-treated control sites was 8.1%, which is in agreement with the values of 5%–8% which may be expected for normal human skin using the system employed in our laboratory. The values following treatment were 6.8% (0.02% ZPT) and 7.1% (0.002%

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Table 1. Mean labelling indices (%) for the forearm

	Water	0.002% ZPT	0.02% ZPT
Unstripped skin	8.1	7.1	6.8
SD	2.5	2.4	6.8
Stripped skin	26.8	37.3	32.2[a]
SD	8.9	15.2	–

[a] The severe inflammatory response to 0.02% ZPT in stripped skin prevented the counting of a labelling index in basal cells in five out of six subjects

Table 2. Mean epidermal thickness (μm) of the forearm

	Water	0.002% ZPT	0.02% ZPT
Unstripped skin	59.4	52.9	51.5
SD	8.9	13.7	9.7
Stripped skin	77.6	76.0	31.6[a]
SD	9.8	12.0	23.0

[a] This low value was partially due to the erosion of the epidermis by the higher ZPT concentration

Table 3. Mean stratum-corneum thickness (μm) of the forearm

	Water	0.002% ZPT	0.02% ZPT
Unstripped skin	29.3	22.9	24.1
SD	6.4	4.0	5.7

Table 4. Total epidermal thickness (μm) of the forearm

	Water	0.002% ZPT	0.02% ZPT
Unstripped skin	88.7	75.8	75.6
SD	13.3	14.8	14.5

Table 5. Basal/granular cell ratio for the forearm

	Water	0.002% ZPT	0.02% ZPT
Unstripped skin	1.10	1.07	1.09
SD	0.05	0.10	0.05

ZPT). The LI values for the stripped skin were 26.8% in the water-treated sites and 37.3% in the sites treated with 0.002% ZPT. Due to the erosion of the epidermis caused by 0.02% ZPT, it was only possible to determine the LI in one subject from the groups with stripped skin.

Two-way analysis of variance showed no significant differences in the LI in unstripped normal forearm skin following exposure to water, 0.002% ZPT or 0.02% ZPT, and stripped forearm skin following exposure to water or 0.002% ZPT.

Mean Epidermal Thickness (Table 2)

The MET value for unstripped skin in the water-treated control subjects was 59.4 μm; this was slightly higher than the range of 30–50 μm which may be expected for untreated skin using the system employed in our laboratory (possibly reflecting the effect of hydration from occlusion). The values following treatment were 52.9 μm (0.02% ZPT) and 51.5 μm (0.002% ZPT). The treatments did not produce significant differences in MET.

In the stripped-skin group, MET showed a reduction following the application of 0.02% ZPT. In some cases, this was due to the erosion of the epidermis, but there was no significant difference in the MET of sites treated with water or 0.002% ZPT.

Mean Stratum-Corneum Thickness (Table 3) and Total Epidermal Thickness (Table 4)

Unstripped skin in the water-treated control subjects gave values of 29.3 μm for mean stratum-corneum thickness (MSCT) and 88.7 μm for TET. Following treatment, were the values 22.9 μm (0.002% ZPT) and 24.1 μm (0.02% for MSCT, and 75.8 μm (0.002% ZPT) and 75.6 μm (0.02% ZPT) for TET. The treatments did not produce significant differences in MSCT or TET (two-way analysis of variance).

In the unstripped-skin samples, MET, MSCT and TET were consistently (but not significantly) lower following the two ZPT treatments than those after water treatment. There were no significant or consistent differences between the results obtained with the two different concentrations of ZPT.

Basal Granular Cell Ratio (Table 5)

There were no significant, treatment-related differences (two-way analysis of variance) in this ratio (unstripped skin only). The values for the ratio were in the range 1.07–1.10 for both the control and ZPT-treatment groups.

Discussion

The tritiated-thymidine autoradiographic LI is a much-used parameter of the rate of epidermal cell

proliferation. It is not ideal, but when exposure to the tritiated precursor occurs in vitro (as in this study), it is probably the most reliable measure currently available. Epidermal thickness is also a measure of epidermal proliferative activity, assuming that there is little spongiosis or change in epidermal cell size. These measurements did not indicate any alteration in the proliferative status of the epidermis due to ZPT. Separate measurements of MET, MSCT, TET and B/G ratio were made to determine whether any epidermal compartment was preferentially altered.

None of the skin parameters measured here provided any evidence, either on normal human forearm skin or on skin stripped of stratum corneum to stimulate mitotic activity, of anti-mitotic activity by ZPT at the concentration at which it is normally used or at a tenfold greater concentration. These results are consistent with those of a previous investigation (Marks, Pearse and Walker, in press) on the effects of shampoo with and without ZPT on skin parameters in dandruff-affected scalps. They are at variance with the studies and conclusions of Imokawa et al. [2] and Leyden and Kligman [4]. The former used an in vitro culture system, and their study, without further information, cannot be regarded as being indicative of the situation in vivo. The results of the latter study is not surprising, and the apparent anti-mitotic effect may have been due to a general improvement in the dandruff as a result of the use of ZPT. It is concluded that ZPT has no effect on epidermal renewal in normal skin in vivo.

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