HISTOMETRICAL STUDY OF STEROID INDUCED CHANGES IN THE SKIN

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Betamethasone-17-valerate (0.1%) ointment and clobetasol-17-propionate (0.05%) ointment were applied morning and evening to the dorsal shaved skin of guinea pigs for four weeks. Skin samples were excised every week from the treated area. After staining, histometrical study was carried out with the ocular micrometer. Mean reduction in epidermal thickness was moderately significant after 3rd week and highly significant after 4th week with clobetasol-17-propionate (0.05%) ointment topical application. However, with betamethasone-17-valerate (0.1%) ointment topical application, it was only moderately significant even after 4th week.

INTRODUCTION

Potent topical corticosteroids are said to induce skin thinning (Epstein *et al.*, 1963), however, experimental evidence in this regard is lacking. This experiment was designed to establish the shortest time to cause skin thinning with different (betamethasone-17-valerate) (0.1%) and (clobetasol-17-valerate) (0.05%) topical corticosteroids.

MATERIALS AND METHODS

Albino guinea pigs of male gender weighing 500 ± 25 g were selected for the experiment. They were shaved on the back with electric clippers and kept in separate cages. Water and green fodder were provided *ad libitum*. The animals were divided into three groups. The animals in group A were applied betamethasone-17-valerate (0.1%) ointment while those in group B received clobetasol-17-propionate (0.05%) ointment. Group C acted as a control and received the base of the ointment. For four weeks, 0.4 ml of the ointments and the base respectively were applied morning (8.00 a.m.) and evening (8.00 p.m.) on an area of 5 cm^2 on the back of the animals with a glass rod. Four animals from each group were killed weekly. Full thickness skin was excised from the treated area. It was processed, embedded in paraffin, sectioned at 0.6 μ vertically and stained with H & E.

For histometrical analysis, cutaneous thickness was measured in μm by means of a micrometer eyepiece (x 10) and x 20 objective. Measurements were made at points where dermoepidermal boundary was plane and parallel to the surface of the epidermis. The statistical significance of the difference of various quantitative changes between experimental and control groups was evaluated by Z-test. The difference was statistically significant if the calculated Z-value was equal or less than -2.33 which is the table value of Z at $P \leq 0.01$.

RESULTS

The weekly results of the quantitative assessment of epidermal and dermal thickness are shown in Tables 1 and 2.

Table 1. Mean epidermal thickness (μm)

Duration (weeks)	Group C	Group A	Z α-value	Group B	Z α-value
1	80.1 ± 7.67	80.1 ± 8.28	0	79.3 ± 6.83	-0.33
2	80.1 ± 2.06	80.1 ± 8.42	-0.04	79.5 ± 3.81	-0.25
3	80.1 ± 5.56	80.9 ± 3.86	-0.09	63.0 ± 3.22	-8.86*
4	81.8 ± 6.66	59.2 ± 4.05	-10.37*	37.7 ± 2.38	-21.25**

Z α-table value (-2.33) at P≤0.01; * Significant; ** Highly significant.

Table 2. Mean dermal thickness (μm)

Duration (weeks)	Group C	Group A	Z α-value	Group B	Z α-value
1	1052.8 ± 92.18	1050.8 ± 96.96	-1.68	1050.6 ± 89.91	-0.07
2	1052.4 ± 115.02	1050.9 ± 116.76	-0.09	1051.5 ± 110.27	-0.08
3	1052.6 ± 113.29	1050.6 ± 104.82	-0.12	1050.0 ± 100.63	-0.08
4	1052.1 ± 90.32	1049.3 ± 87.04	-0.22	1048.0 ± 91.71	-0.25

Z α-table value (-2.33) at P≤0.01; * Significant; ** Highly significant.

The results obtained during first two weeks of the study do not show any appreciable change in the thickness of skin. During 3rd week, the mean epidermal thickness reduces moderately with clobetasol-17-propionate (0.05%) ointment, while with betamethasone-17-valerate (0.1%) ointment the change is insignificant (Fig. 1). During 4th week, mean epidermal thickness reduces moderately with betamethasone-17-valerate (0.1%) ointment while with clobetasol-17-propionate ointment it reduces markedly. Mean dermal thickness of experimental skin remains the same as that of the control skin even after 4 weeks topical application of both the ointments (Fig. 2).

DISCUSSION

Histometric assessment has been used to demonstrate epidermal and dermal thinning caused by topical steroids both in humans and animals (Jones, 1976). Guinea pig skin is used in this experiment as it is a reasonable rodent model for human for absorption from skin (Andersen *et al.*, 1980). These are easy to handle and large quantities of skin can be used for the experiment. Human skin, if used, may cause artificial changes in skin while taking biopsy. The first two weeks application of both the steroids did not cause any change in the experimental skin. It is the long term application of topical steroids which causes skin thinning (Jablonska *et al.*, 1979). After 3 weeks

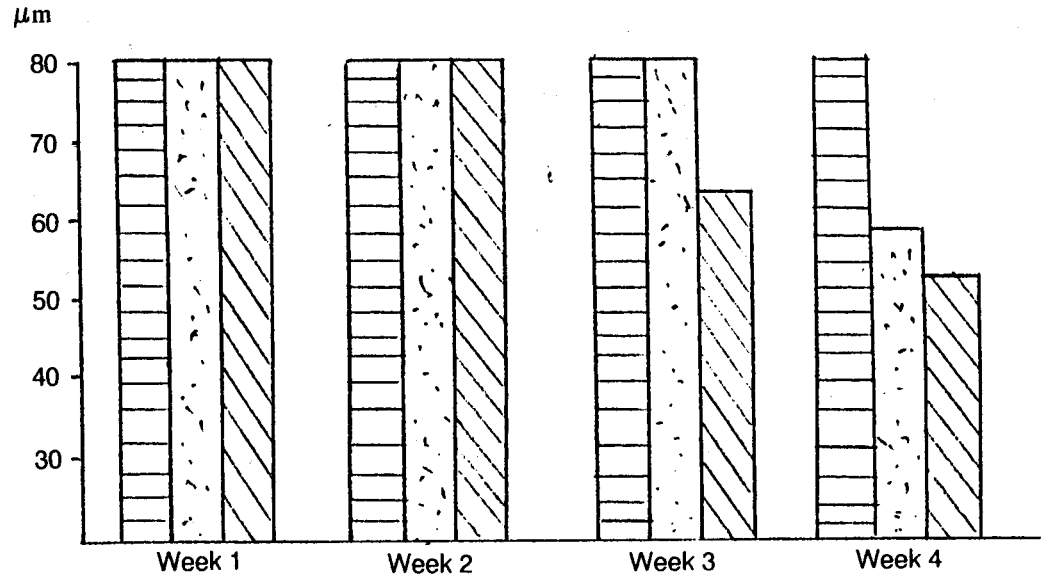

Fig. 1. Weekly mean epidermal thickness change

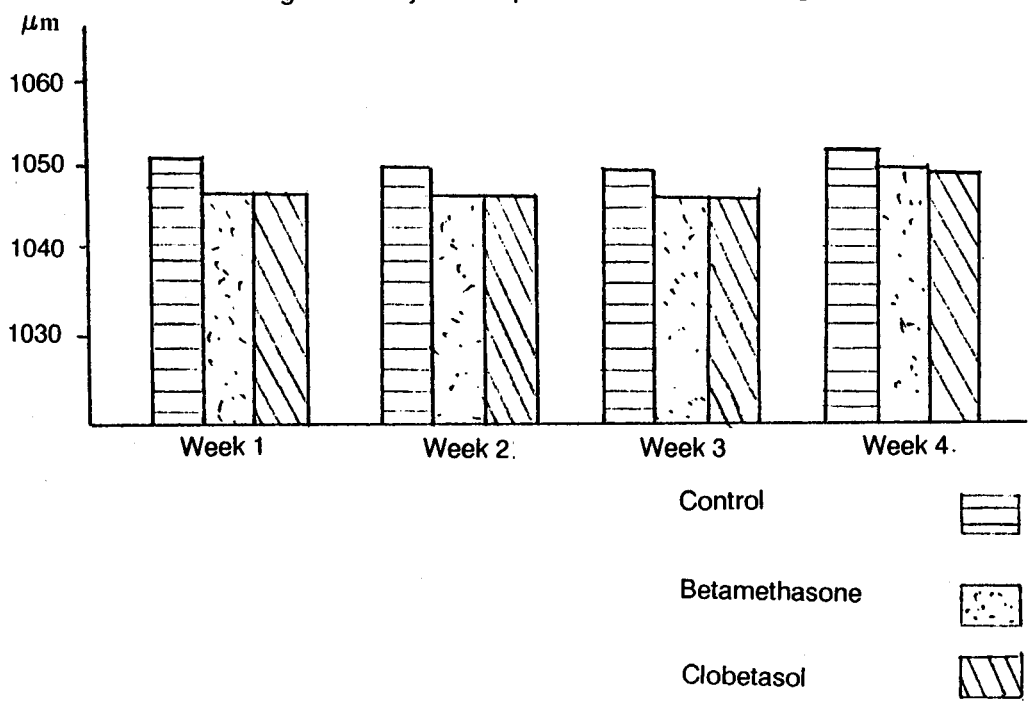

Fig. 2. Weekly mean dermal thickness change

topical application of clobetasol-17-propionate (0.05%) ointment, skin exhibits moderate thinning (63.0 ± 3.22 μm), while the skin of guinea pig treated with betamethasone-17-valerate (0.1%) ointment still does not show visible thinning (80.9 ± 3.86 μm). Clobetasol thus has significantly greater therapeutic and atrophogenic effect than betamethasone (Du Vivier *et al.*, 1978).

The analysis of 4th week results show that the skin treated with clobetasol-17-propionate (0.05%) ointment is highly significantly thin (37.7 ± 2.32 μm) than that treated with betamethasone-17-valerate (0.1%) ointment. With the latter the skin undergoes only moderate thinning (59.2 ± 4.05 μm). The thinning is confined mainly to the epidermis. The dermis, however, shows little or no change as reported by Jones (1976). This is not to say that there are no changes in the epidermis but conventional methods do not reveal them.

The possible processes causing skin thinning are antimitotic and antisynthetic effects on various components of epidermis and dermis. The epidermopoesis, collagen synthesis and fibrobalst proliferation action are inhibited by these steroids (Marks, 1976). It is likely that epidermal thinning is the first step in a sequence of changes that result in dermal atrophy and that the thinned epidermis subsequently allows a far greater penetration of steroids into the dermis to cause atrophy.

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