Alteration of rat skin acid and alkaline phosphatase and β-glucuronidase activity after topical application of fluocinolone acetonide cream

The evaluation of topical anti-inflammatory agents presents some perplexing problems because there is a lack of an appropriate assay procedure; an inability to avoid ingestion without creating stressful conditions; physiological differences between rat and human skin, and difficulty in separating local and systemic effects. The present investigation is an attempt to evaluate and resolve some of these problems.

Male Sprague-Dawley rats (150–160 g) were individually housed and had free access to tap water and rat chow. A pouch was formed caudal to the interscapular region by a subcutaneous injection of air (25 ml). The air pouch served as the site for drug application and as a physical barrier to avoid drug ingestion. The rats were divided into four groups of 20 each and pouch skin samples were obtained from: untreated control rats; shaved skin controls; shaved skin treated with a control cream; shaved skin on which one half of the area received a control cream (D-1) and the other half (D-2) received a cream* containing fluocinolone acetonide, 0·025%. The active preparation (100 mg) or control cream (or both) was applied daily to an area approximately 1 inch square. Ten animals from each group were killed at 12 h and 10 at 4 days. A section of skin over the air pouch was weighed on an analytical balance and placed between two blocks of dry ice for 1 min. The frozen skin was placed in a rubber finger cot and shattered by hitting it with a hammer. The tissue was transferred to a flask containing 10 ml of cold distilled water and was homogenized with a VirTis No. 45 homogenizer until a uniform suspension was obtained (approximately 5 min). The homogenate was diluted to a suitable concentration and aliquots were used for the assay of β-glucuronidase (Talalay, Fishman & Huggins, 1946) and acid (Manning, Babson & others, 1966) and alkaline phosphatase activity (Manning, Butler & others, 1967). Dry weights were obtained by drying pieces of skin under vacuum at 60° for 24 h. The mean and standard error was calculated for all groups and the significance of the differences between control and treated groups was determined by the Student's *t*-test.

The separately determined enzyme levels of the adjacent areas of shaved rat skin treated with the control and active creams (Group D-1 and D-2) were significantly decreased at 12 h and at 4 days when compared with the untreated control Groups A and B, and, except for the acid phosphatase at 4 days, with Group C (Table 1). The acid phosphatase activity on day 4 for Group C (shaved + control cream) was lower than its 12 h value and equal to the shaved rat skins receiving the control and active creams at both time periods All groups had significantly lower alkaline phosphatase levels on day 4 than at 12 h. The shaved-skin controls (Group B) had a reduced β-glucuronidase activity on day 4.

This investigation has established that a topically applied formulation depressed the acid and alkaline phosphatase and β-glucuronidase activity in rat skin. Since the altered enzyme activities occurred equally in separate skin areas of the same animal, whether treated with the control or active creams, a definite systemic effect was apparent. The air pouch alone significantly depressed the alkaline phosphatase at day 4 in all groups; thus the enzyme levels at this time for Groups D (shaved + control and active cream) reflect the combined effect of the active cream and the formation of an air pouch. In contrast, shaving reduced the β-glucuronidase reaction, while the control cream appeared to lower the acid phosphatase in Group C

* Synalar: Syntex Laboratories, Palo Alto. The control areas were prepared to be similar to the active preparation.

Table 1. *Acid phosphatase, alkaline phosphatase and β-glucuronidase levels in rat skin of four groups of 20 rats*

Group	Acid phosphatase[1]		Alkaline phosphatase[2]		β-Glucuronidase[3]	
	12 h	4 Days	12 h	4 Days	12 h	4 Days
A. Untreated ..	30,189 ±1674	29,543 ±2231	45,512 ±3945	32,132 ±2616§	7906 ±442	7969 ±243
B. Shaved ..	34,731 ±1138	35,892 ±2355	48,627 ±3429	29,835 ±1295§	8982 ±340	7927 ±260§
C. Shaved control cream ..	33,590 ±1858	18,943 ±1920*†§	41,959 ±3496	29,662 ±2603§	7812 ±313†	7055 ±380
D-1. Shaved control cream .. +	21,056 ±2250*†‡	20,004 ±2432*†	19,247 ±1995*†‡	11,575 ±1295*†‡§	5639 ±339*†‡	4848 ±277*†‡
D-2. Shaved, active cream ..	23,639 ±2889†‡	17,356 ±1837*†§	23,275 ±2347*†‡	13,991 ±859*†‡§	6543 ±307*†‡	5136 ±184*†‡§

* Significantly different from untreated control, Group A.
† Significantly different from shaved control, Group B.
‡ Significantly different from control cream, Group C.
§ Significantly different from 12 h.

[1] One unit of acid phosphatase activity liberates 1 μg of α-naphthol from sodium α-naphthyl acid phosphate in 30 min at pH 5·2 and 37° under standard conditions.
[2] One unit of alkaline phosphatase activity liberates 1 μg of phenolphthalein in 30 min from phenolphthalein monophosphate at pH 10·1 and 37° under standard conditions.
[3] One unit of β-glucuronidase activity liberates 1 μg of phenolphthalein in 30 min from phenolphthalein monoglucuronide at pH 4·5 and 37° under standard conditions.

(shaved + control cream) on day 4. This latter effect suggests that cream bases cannot be considered biologically inert.

Since fluocinolone acetonide decreased acid phosphatase and β-glucuronidase, the presented data support the hypothesis that anti-inflammatory agents stabilize the lysosomal membrane (DeDuve, Wattiaux & Wibo, 1962; Weissman & Thomas, 1964; Weissmann, 1965). It is difficult to envisage that any lysosomes, if present, could possibly resist the drastic physical treatment of the tissue samples described. A possible alternative explanation for the decreased hydrolase levels may be that cortisone and its derivatives may directly, or indirectly, partially depress protein-enzyme synthesis resulting in fewer lysosomes (also endoplasmic reticulum and Golgi vesicles) being produced.

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