Dermatologica *156:* 89–96 (1978)

Effect of Topical Salicylic Acid on Animal Epidermopoiesis

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Key Words. Salicylic acid, treatment of normal skin · Epidermopoiesis

Abstract. In contrast to its antihyperplastic effect on pathological proliferation of the epidermis, salicylic acid promotes epidermopoiesis in the normal guinea pig skin. After the application of 1% w/w salicylic acid in acetone-ethanol for 4 weeks, the thickness of the surface epithelium was increased by 40% and that of the deep epithelium by 19%. The mitotic index rose by 17%.

Salicylic acid (SA) in a concentration of 3% w/w in ethanolic solution has been shown to exert a marked antihyperplastic action on artificially induced psoriasiform proliferation of the guinea pig epidermis [5]; the thickness of the surface epithelium, the reactivity of which most closely resembles that of the human epidermis, was reduced to a highly significant extent by comparison with that of hyperplasia controls [5, 7]. An even more potent and deeper-reaching effect was observed when SA was applied in a concentration of only 1% w/w in a special vehicle containing dimethylacetamide that facilitates penetration. In confirmation of clinical experience, these findings in animals demonstrate that the intensity of some of the effects of salicylic acid depends on the vehicle it is applied in. They also make it appear probable that the antihyperkeratotic action displayed by salicylic acid in these concentrations in dermatological practice [3] is due to or associated with an inhibitory effect on exaggerated epidermopoiesis.

Clinical experience has, on the other hand, also shown that when applied

Received: July 4, 1977; acepted: July 28, 1977.

to normal or structurally deficient skin, pastes and ointments containing 0.5–2% w/w of SA have a keratoplastic or stabilizing effect on the stratum corneum and may perhaps also promote epidermopoiesis [refs. in 3]. It consequently appears that in the same range of concentrations, the effect of SA on epidermopoiesis may be altered or reversed, depending on the state of the skin: if the skin is pathologically hyperplastic its effect is antihyperkeratotic and antiepidermoplastic, but on normal or abnormally hypoplastic skin it is keratoplastic and epidermoplastic.

In a study of the keratolytic action of SA, DAVIES and MARKS [1] and MARKS *et al.* [2] attempted to define its effects on the epithelium of the healthy *human* skin. Unfortunately, these investigations produced no conclusive results. In 1975, the same group [2] reported that creams containing 2–12% w/w of SA had no effect whatever on epidermopoiesis in the healthy skin, as determined by reference to epithelial thickness and the ^{3}H-thymidine labeling index, whereas corresponding ointments with ascending SA concentrations caused a progressive increase in both epithelial thickness and the labeling index. In 1976, the same authors [1] found, however, that 1 week's treatment with two applications of SA ointments (2–10% w/w) daily produced no significant change in epidermopoiesis, with regard to either epithelial thickness or the labeling indices of tritiated thymidine, cytidin and histidine. To assess the effects of SA on normal skin and investigate their possible reversal, we have carried out epidermoplasia tests of 4 weeks' duration in guinea pigs [4]. The SA derivative acetylsalicylic acid and the dermatocorticoid hydrocortisone, both of which we recently also evaluated in the hyperplasia inhibition test [6], served as reference compounds.

Material and Methods

Test procedure

The epidermoplasia tests were performed on guinea pigs according to the method described in detail elsewhere [4]. For technical reasons, in particular to facilitate application and avoid the occurrence of irritant effects due to the protracted treatment, a 50:50% w/w mixture of acetone and ethanol (AE 55) was used as vehicle. The single and total doses of the test substances were, however, so chosen as to lie within a readily comparable range between the two skin surface dose levels used in the above-mentioned hyperplasia inhibition test with SA [5]; in that assay 3% w/w SA was applied in ethanol alone in 15 single doses of 0.423 mg/cm^2 skin (total dose over 11 days 6.33 mg/cm^2) and 1% w/w SA in the composite solvent dimethylacetamide-acetone-ethanol in 14 single doses of 0.15 mg/cm^2 skin (total dose over 10 days 2.11 mg/cm^2). In contrast to the procedure followed in the hyperplasia inhibition test, the test substances were applied to the normal skin, altogether 20 times within a period of 4 weeks.

Table I. Test groupings and ponderal development of animals

	Blank controls UDS (3 animals)	Vehicle controls AE 55 (3 animals)	Treatment groups		
			1% HC in AE 55 (3 animals)	1% SA in AE 55 (3 animals)	1% ASA in AE 55 (3 animals)
Mean body weight (g) on day 1	343	331	342	331	335
Mean body weight (g) on day 29 (skin biopsy)	440	472	473	473	468
Mean change in body weight	+97 g +28%	+141 g +43%	+131 g +38%	+142 g +43%	+133 g +40%

UDS = Untreated, depilated skin; AE 55 = acetone-ethanol 50:50% w/w (solvent).

Table II. Dosages of vehicle and test substances

Vehicle Acetone-ethanol 50/50% w/w	*Weight per unit volume* (effective specific gravity) of AE 55: $\bar{x}$ (n = 40) = 73.22±0.17 mg/100 µl = 0.7322 g/ml Single dose volume = 200 µl/animal/5.31 cm² skin Total dose volume = 4 ml/animal/5.31 cm² skin
AE 55	*Single surface dose* = 146.44 mg/animal = 27.58 mg/cm² skin *Total surface dose* (20 applications) = 2,929 mg/animal = *552 mg/cm² skin*
Test substances 1% w/w in AE 55	Single dose volume of solutions = 200 µl/animal/5.31 cm² skin Total dose volume of solutions = 4 ml/animal/5.31 cm² skin
HC SA ASA	*Single surface dose of test substances* = 1.464 mg/animal = 0.276 mg/cm² skin *Total surface dose of test substances* (20 applications in 28 days) = 29.27 mg/animal = *5.51 mg/cm² skin*

Weight per unit volume as determined with Eppendorf B 315 microliter pipette, capacity 100 µl.

Test Substances

Hydrocortisone (HC): cortisol. Salicylic acid (SA): 2-hydroxybenzoic acid. Acetylsalicylic acid (ASA): 2-acetoxybenzoic acid.

Particulars of the test groups and the ponderal development of the animals are given in table I. Table II shows the exact dosages of the vehicle and test substances.

Table III. Epidermal pachometry

Test groups	Number of determinations	Mean epithelial thickness MET ($\bar{x}$), µm	SD of MET		MET, difference from control and significance (c test)	
			absolute µm	relative in % of $\bar{x}$	absolute µm	relative in %
UDS	300	13.41	0.22	1.6	./.	./.
					in relation to UDS	
AE 55	300	17.88	0.30	1.7	+4.47 $p<0.0001$	+33
					in relation to AE 55	
HC	300	17.29	0.38	2.2	−0.59 $p>0.05$	−3
SA	300	26.06	0.44	1.8	+7.18 $p<0.0001$	+40
ASA	300	43.49	0.76	1.8	+25.61 $p<0.0001$	+143

Table IV. Total epithelium planimetry

Test groups	Number of determinations	Mean epithelial surface MES ($\bar{x}$) µm²	SD of MES		MES, difference from control and significance (two-tailed t test)	
			absolute µm²	relative in % of $\bar{x}$	absolute µm²	relative in %
UDS	15	587,722	23,683	4.0	./.	./.
					in relation to UDS	
AE 55	15	656,726	22,628	3.4	+69,004 $p<0.05/p>0.025$	+12
					in relation to AE 55	
HC	15	667,180	32,584	4.9	+10,454 $p>0,05$	+1.6
SA	15	782,724	34,992	4.5	+126,038 $p<0.01/p>0.005$	+19
ASA	15	755,426	23,238	3.0	+98,700 $p<0.005/p>0.001$	+15

Table V. Mitosis rate in stratum germinativum of epidermis

Test groups	Mitosis index per group ($\bar{x}$ of n = 75) per 1,000	SD of MI		MI, difference from control and significance (two-tailed t test)	
		absolute per 1,000	relative in % of $\bar{x}$	absolute per 1,000	relative in %
UDS	43.00	2.87	6.7		
				in relation to UDS	
AE 55	54.00	2 85	5.3	+11.00 $p<0.05/p>0.025$	+26
				in relation to AE 55	
HC	44.33	3.63	8.2	−9.67 $p<0.05/p>0.025$	−18
SA	63.00	3.78	6.0	+9.00 $p>0.05$	+17
ASA	87.00	3.53	4.1	+33.00 $p<0.001$	+61

In the 15 tissue sections of each test group 75 interfollicular segments each comprising 40 contiguous basal cells were evaluated (total 1,000 basal cells per animal and 3,000 per group)

Pharmacometrics

Skin biopsy specimens were examined microhistometrically (epidermal pachometry and planimetry) as described elsewhere [4, 5]. In addition, determinations were made of the mitosis rates in the basal layer of the epidermis after the arrest of cell division in metaphase by the intraperitoneal injection of demecolcine 3.0 mg/kg body weight exactly 5 h before the excision of the skin specimens; five tissue sections were prepared from the biopsy specimen taken from each animal, and in each section five interfollicular segments comprising 40 contiguous basal cells were examined, so that, in all, 3,000 germinal cells were evaluated in each group.

Results

Epidermal pachometry (table III).

Epidermal planimetry (table IV). As has already been pointed out on several occasions [5, 7], the planimetric epithelial values principally reflect

the magnitude of the deep layers (follicular structures and sebaceous glands), which constitute the greater part of the epithelial volume in the guinea pig.

Determination of rates of mitosis in the basal cell layer of the epidermis (table V).

Discussion

As is evident from table I, long-term treatment with the various test substances caused no changes in weight gain or the terminal weights of the animals significantly different from those observed in the animals exposed to the vehicle alone. The general condition and behavior of the animals gave no indication of any percutaneous toxic effects of the substances. Owing to the orexigenic effect of the stress imposed by handling, the food consumption of all the treated animals was actually increased by comparison with that of the untreated controls and their weight gain was correspondingly greater. At the sites of application, no gross deviations from the norm were observed at any time during treatment; in particular, no signs of irritation or degenerative changes of any kind were to be seen.

The long-term application of AE 55 induced a very distinct epidermoplastic reaction comprising an increase of 33% in the width of the surface epithelium, an increase of 12% in the deep epithelium (as reflected in the total epithelial thickness) and an increase of 26% in the mitosis rate, by comparison with untreated controls [cf. 4]. Apart from distinct hyperkeratosis, enlargement of the cells, interstitial edema, and slight spongiosis of the epithelial formations were the predominant histological changes, but there was also a genuine increase in the number of cells and cell layers. Hence, in addition to, or in consequence of the chronic irritant effect of AE 55 there was a significant stimulation of the normal process of epidermopoiesis.

The reference corticoid HC produced the expected results [cf. 4]: it diminished the thickening of the surface epithelium due to the vehicle to a slight and statistically nonsignificant extent, had no appreciable effect on the proliferation of the deep epithelium and reduced the rate of mitosis to the same level as that found in the blank controls. Thus, in accordance with the amply documented clinical and experimental findings, HC did not display any real antiepidermopoietic or epidermal atrophogenic effect in our study, though it did have a suppressant effect on irritation, histologically manifest in the normalization of germinal-cell reproduction. The hyperkeratosis induced by AE 55 likewise appeared upon histodiagnostic comparison to be less pronounced in skin treated with HC.

SA not only had no inhibitory effect on epidermopoiesis in the normal guinea pig skin, but – over and above the effect of the vehicle – caused a highly significant thickening of the surface epithelium (table III), a significant increase in the volume of the deep and hence the total epithelium (table IV), and a distinct, though nonsignificant increase in the mitosis rate in the germinative zone of the epidermis (table V). Histologically, in the surface epithelial zone especially, cellular enlargement slightly greater than that induced by AE was noted, and in places intracellular and interstitial edema or slight spongiosis was present; hyperkeratosis was entirely absent and in fact in most of the sections the horny layer was almost completely detached (this was only seen in the sections of skin treated with SA and was presumably due to the keratolytic action of the substance); there was also a definite increase in the number of cells and cell layers. The epidermometric data hence furnish unequivocal evidence that the effect of SA on the normal guinea pig skin is quite the opposite of its effect on hyperplastic skin; far from having an inhibitory effect, it actually promotes the growth and regeneration of the epidermis. The results of this study accordingly verify the hypothesis that the dermatopharmacodynamics of SA undergo a reversal, depending on the condition of the skin.

The effects of ASA were qualitatively similar, but quantitatively much more potent than those of SA: not only was its stimulant effect on epidermopoiesis more pronounced, but also its irritant action on the epidermis and corium. The irritation caused by ASA – which greatly exceeded that caused by AE – did not, however, lead to any gross morphological alterations in the skin, being perceptible only upon histological examination. Besides distinct irritation of the epidermis (cell enlargement, intracellular and intercellular edema and in many places spongiosis), there was also a notable inflammatory reaction in the corium (moderate vasodilatation and hyperemia; distinct edema and diffuse infiltration of the stratum papillare by a mixed population of cell-types; reactive increase in fibrocytes and connective tissue). A further remarkable qualitative feature of the tissue was the presence of a relatively pronounced hyperkeratosis, even surpassing that seen in the vehicle controls. The significantly greater stimulant effect of ASA on epidermal proliferation compared with that of SA was not only clearly demonstrable in the surface epithelium, but also manifest in the rate of mitosis in the basal cell layer. On the other hand, there was no statistically significant difference between ASA and SA in their effect on the mass of the deep epithelium: in this respect ASA was even slightly less active than SA, perhaps because it penetrates less readily into the deeper epidermal strata. Hence, like that of SA, the effect of

ASA observed in the hyperplasia inhibition test [6] is also reversed when the substance is applied to the normal skin.

Acknowledgements

Thanks are due to Miss M. SÜTTERLIN, Mr. P. RYSER and Mrs. D. BRUNNER for their valuable assistance.

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