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EVALUATION OF TOPICAL ANTI-INFLAMMATORY STEROID FORMULATIONS IN AN ARTHUS MODEL OF INFLAMMATION

MARVIN B. GOLDLUST, PH.D., DOUGLAS M. PALMER, M.S., AND MATTHEW A. AUGUSTINE, PH.D.

Immunology Section, Department of Pharmacology and Department of Pharmaceutical Research, Squibb Institute for Medical Research, Princeton and New Brunswick, New Jersey, U. S. A.

Formulations of a number of steroids were evaluated after topical application in a reversed passive Arthus test (RPA) in rabbits. Four 21-chlorosteroids in the same cream base were investigated. The preparations of SQ 18,566 (halcinonide) and SQ 20,811 showed anti-edema activity, but those of SQ 15,361 and SQ 20,589 were less active. Ointment formulations of halcinonide also reduced edema in the RPA. These results, coupled with previously reported clinical data, suggest that the RPA might be utilized to distinguish good from poor formulations of anti-inflammatory steroids prior to screening tests or clinical trials in humans.

Previous studies by Bagatell and Augustine [1] described the relative effectiveness in the human vasoconstrictor and stripped-skin screens of four 21-chlorosteroids* applied as tinctures. These investigators also reported the relative clinical effectiveness of these steroids when applied as 0.1% creams. Discrepancies were observed in the relative effectiveness of these steroids when the results in the human screens were compared with the data from the clinical trial [1]. As suggested by the physicochemical data [1], factors that influence bioavailability probably account for the observation that the cream formulation, although appropriate for some of the chlorosteroids, was not optimal for every single one.

Topical steroids are screened in the vasoconstrictor and stripped-skin procedures in humans. These tests utilize unformulated, but solubilized (e.g., in ethanol), preparations of newly synthesized steroids. In an attempt to avoid some of the difficulties involved in human studies of large numbers of formulations, we have adapted an animal model of inflammation for the testing of formulated topical steroids, namely, the reversed passive Arthus skin test (RPA) in the rabbit.

The Arthus lesion, which is generated by an immune reaction [2], manifests notable edema. Inhibition of this edema is routinely used in our laboratories to detect nonsteroidal anti-inflammatory agents [3]. In the series of experiments presented here, we obtained data showing that parenteral administration of small doses of corticosteroids, such as triamcinolone acetonide and halcinonide, are effective in this test system. Additional data suggest a rank order of activities in the RPA of the four previously mentioned 21-chlorosteroids [1] when they are applied topically in the same formulation. Our data indicate that SQ 15,361 and SQ 20,589 were less effective topically than halcinonide and SQ 20,811 in anti-edema activity. Ointment formulations of halcinonide were also effective in the RPA.

MATERIALS AND METHODS

The Reversed Passive Arthus Skin Test (RPA)

This test has been described recently [3].

Intradermal administration of steroids. Each rabbit received an intravenous injection of 25 mg of bovine serum albumin (BSA) in 5 ml of saline, followed immediately by intradermal injections of 0.2 ml of rabbit antiserum to BSA (Cappel Laboratories, Downingtown, Pa.) mixed with steroid in saline suspension. Each rabbit received 6 injections, the same dose of steroid being administered to each of 3 sites and the remaining 3 sites serving as controls. The treated and control sites were placed alternately, 3 on each side of the midline of the back. Inhibition of edema by at least 30% was considered to be significant.

Topical application of steroids. A 100-mg quantity of steroid preparation (cream or ointment) was applied around each skin injection site, just after the injection of antiserum. Four sites per rabbit were used. A separate disposable vinyl medical glove (Bard-Parker, Div. of Becton, Dickinson and Co., Rutherford, N.J.) was used for the application of each preparation. The steroids were rubbed gently on the injection blebs but not on the injection sites. No occlusive dressings were used. The edema volumes were measured as previously described [3], 6 hr after application of the steroid. Briefly, a double-fold thickness of skin at the center of each skin site was measured before and after lesions were produced. The diameters of the wheals were measured and the lesion volumes were calculated using the formula for a

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Reprint requests to: Dr. M. B. Goldlust, Squibb Institute for Medical Research, P.O. Box 4000, Princeton, New Jersey 08540.

* Halcinonide (SQ 18,566) is 9α-fluoro-21-chloro-11β, 16α,17α-trihydroxypregn-4-ene-3,20-dione,16,17-acetonide. SQ 15,361 is the $\Delta^{1,4}$ derivative, SQ 20,589 is the $\Delta^{1,4}$, desfluoro derivative, and SQ 20,811 is the desfluoro derivative of halcinonide.

cylinder ($\Pi r^2 \times \Delta$ thickness). Separate treatment and control groups of animals were utilized; inhibition of edema was calculated by comparing the average edema volumes of treated and placebo control groups.

Many of the assays were carried out in the winter, when the rabbits had developed thick seasonal hair coats. In each experiment, we included a sizeable number of rabbits with coats that did not permit the skin to be shaved cleanly; these animals had a thin, uniform layer of coarse hair on the back. Although topically applied creams or placebos were sometimes not fully absorbed, i.e., some residue remained around the Arthus lesions, we could discern no relationship of coarse hair patterns to the presence of residue or to degree of anti-edema activity. Nor were the responses of rabbits with coarse hair patterns obviously different from those of smoothly shaven rabbits within the same treatment group.

Statistical significance was calculated by the Wilcoxon rank sum test, utilizing the table indicated in Dixon and Massey [4]. Each treatment group was compared directly with the placebo-treated group.

Our criteria for significant activity in this assay are $\geq 30\%$ decrease in edema with a p value of ≤ 0.05. These criteria were applied to each experiment.

Vasoconstrictor (VC) and Stripped-Skin (SS) Assays

These procedures were performed as previously described [1].

Steroids

Bulk halcinonide (SQ 18,566), SQ 15,361, SQ 20,589, SQ 20,811, and triamcinolone acetonide were synthesized at the Squibb Institute for Medical Research.

Cream formulations. Preparations of halcinonide, SQ 15,361, SQ 20,589, and SQ 20,811 previously tested in humans [1] consisted of 0.1 gm of steroid, glyceryl monostearate, cetyl alcohol, spermaceti, isopropyl palmitate, and polysorbate 60, and 60 gm of propylene glycol per 100 gm of aqueous cream.

Ointment formulations. Two 0.1% halcinonide preparations, designated WP and WPS, were prepared. WP consisted of steroid plus a mixture of polyethylene glycols (22%) in white petrolatum. WPS was a similar formulation containing 2 gm of Span 80 per 100 gm of ointment. Appropriate ointment placebos were prepared.

RESULTS

Sensitivity of RPA, VC, and SS Assays to Locally Administered Steroids

As previously reported [3], the RPA has been utilized in screening nonsteroidal anti-inflammatory agents via the intradermal administration of these agents at the skin test sites. During these studies, a number of steroids were tested and found to be effective in suppressing edema. Triamcinolone acetonide and halcinonide were evaluated in dose–response studies in an attempt to determine the smallest amount of each steroid that could be detected. The results (Tab. I) indicate that each steroid, applied in a dose of 0.03 μg per site, caused significant inhibition in the RPA test, but did not do so at 0.003 μg per site.

The sensitivity (half-maximal response) of the VC and SS assays to halcinonide (as tincture) was determined from the data of Bagatell and Augustine [1] to be about 0.024 and 0.0012 μg per site, respectively. Since the sensitivity of the RPA test to intradermally administered halcinonide was similar to that for tinctures of these steroids in the VC assay, the possible use of the RPA for assessing the activity of topically applied steroids in formulation was considered.

TABLE I. *Dose-response determinations in the RPA test of intradermally administered steroids*

Steroid[a]	Number of rabbits	Dose (μg/site)	Mean percent decrease in edema[b] (Range)
Halcinonide (SQ 18,566)	4	300	56 (48–62)
	3	30	61 (57–66)
	6	0.3	55 (49–65)
	4	0.03	44 (40–46)
	3	0.003	10 (5–14)
Triamcinolone acetonide	1	300	43
	2	3	53 (46–59)
	4	0.03	43 (37–53)
	3	0.003	17 (9–28)

[a] Each animal received the same dose of one steroid in 3 skin sites. Three other sites were used as controls.

[b] Data are cumulative from 5 experiments.

Topical Application of Steroids in Formulation

The previously described 0.1% cream formulations of halcinonide (SQ 18,566), SQ 15,361, SQ 20,589, and SQ 20,811 [1] were evaluated in a blind fashion in the RPA assay. The results are indicated in Table II. SQ 20,589 and SQ 15,361 were less effective than halcinonide and SQ 20,811. The placebo cream for the 21-chlorosteroids did not cause any significant effect on Arthus edema. In summary, halcinonide in 5 of 6 assays and SQ 20,811 in 2 of 2 assays demonstrated significant activity by our criteria. SQ 20,589 in 4 of 5 assays and SQ 15,361 in 3 of 3 assays were found to be unacceptable.

The low activity of SQ 15,361 cream, applied topically, raised the possibility that this steroid was inherently inactive in the RPA test. However, when applied intradermally, SQ 15,361 was similar in potency to halcinonide (Tab. III, Experiment 1). Additional data obtained at a single dose of 0.3 μg/site indicated that all four chlorosteroids possessed anti-edema activity (Tab. III, Experiment 2).

In addition, Table IV indicates that topically applied ointment formulations of halcinonide were effective in the RPA assay. Appropriate placebos were without effect.

DISCUSSION

The present studies demonstrate that certain topically applied corticosteroid formulations pos-

sess anti-edema activity in the reversed passive Arthus test (RPA) in rabbits. Our results are consistent with the findings of Bagatell and Augustine [1] that SQ 15,361 in a 0.1% cream formulation was inferior to halcinonide when both were compared in a limited clinical trial. However, SQ 20,589 and SQ 20,811, contrary to our RPA data, tended to be equally effective in that clinical trial.

The relative degrees of inhibition in the RPA caused by each of the four chlorosteroid creams show a rank-order correlation with the solubility of the compounds in the solvent compartment of the vehicle. Halcinonide and SQ 20,811, the most soluble of the four steroids in 60% propylene glycol [1], were the most active when topically applied in the RPA. However, use of the method of Katz and Shaikh [5] failed to demonstrate a linear relationship between the product of steroid solubility and partition coefficient and the logarithm of the biologic response.

The observations on SQ 15,361 are of interest. This steroid, as an ethanolic solution, showed good topical activity in the human vasoconstrictor and stripped-skin tests. SQ 15,361 as a cream was less effective clinically than halcinonide [1], presumably because of its poorer vehicle solubility. The solubility of SQ 15,361 in the cream vehicle is only one-tenth that estimated for halcinonide. This is based on solubility in 60% propylene glycol, the concentration used in the cream vehicle. However, the oil/water partition coefficients for SQ 15,361 and halcinonide from the 60% propylene glycol solvent into isopropyl myristate are similar and favor the lipid phase [1]. If the stratum corneum is considered as a lipid barrier, then greater release of the steroid from its vehicle into the stratum corneum should reflect the partition coefficients. Since the coefficients are similar for the two steroids, solubility in the formulation vehicle appears to be more important. These data also suggest the value of a study of formulations that might enhance the topical activity of a steroid both in the RPA and the clinic.

Evaluation by the RPA of various concentrations of a steroid is one approach to gaining further information about the topical effectiveness of that steroid. Because the physicochemical properties of a steroid in a particular formulation, e.g., its

TABLE II. *Effects of topically applied 21-chlorosteroid cream formulations in the RPA in rabbits*

Experiment	Steroid[a, b]	Mean percent decrease in edema
1	Halcinonide	40 (.001)[c]
	SQ 15,361	15 (.16)
	SQ 20,589	33 (.021)
2	Halcinonide	48 (.001)
	SQ 15,361	20 (.021)
3	Halcinonide[b]	41 (.002)
	SQ 20,811	40 (.001)
	SQ 20,589	16 (.16)
4	SQ 18,566[b]	31 (.013)
	Halcinonide	42 (.008)
	SQ 15,361	22 (.20)
5	SQ 20,589	10 (.47)
	Halcinonide	38 (.09)
	SQ 20,589	27 (.12)
6	SQ 20,811	55 (.004)
	Halcinonide	43 (.001)
	SQ 20,589	7 (.29)

[a] Six animals per treatment group or placebo group were used. One hundred mg of 0.1% cream were applied to each of 4 skin sites per rabbit.

[b] Those compounds indicated by SQ numbers were evaluated in a blind fashion. In Experiment #3, halcinonide was tested in both a blind and open fashion.

[c] p value vs placebo group (rank sum test).

TABLE III. *Intradermal activity of four 21-chlorosteroids in the RPA in rabbits*

Experiment	Steroid[a]	Dose (μg/site)	Percent decrease in edema[b]
1	SQ 15,361	3.0	40
		0.3	40
		0.03	30
		0.003	0
	Halcinonide	0.3	50
		0.03	50
		0.003	17
2	Halcinonide	0.3	59; 47
	SQ 15,361	0.3	38; 38
	SQ 20,589	0.3	45; 61
	SQ 20,811	0.3	49; 51

[a] Each animal received one compound at the indicated dose in each of 3 skin sites. Three other sites were used as controls.

[b] Each value represents a single animal.

TABLE IV. *Effects of topically applied halcinonide ointment formulations in the RPA in rabbits*

Experiment	Group size (Treated, Control)	Steroid[a]	Mean percent decrease in edema
1	5,5	Halcinonide (0.1%)-WPS	55 (.004)[b]
2	4,4	Halcinonide (0.1%)-WP	36 (.057)

[a] No significant placebo effects were observed.

[b] p value vs placebo group (rank sum test).

solubility, may vary with concentration, differences in topical potency may reflect not only changes in steroid concentration, but the physicochemical state of the steroid at these concentrations.

The potential usefulness of the RPA model has been demonstrated not only for cream formulations (Tab. II) but also for ointment preparations of halcinonide (Tab. IV). Further studies are needed to correlate topical activities of formulations of other novel steroids in the RPA with those in the screening tests and clinical trials in humans. The authors do not wish to imply that the physicochemical properties of rabbit skin and human skin are comparable, but such studies would help to define the degrees of similarity of the two species. It is probably not possible to discriminate by the RPA between preparations of the same or different steroids that differ only slightly in effectiveness. However, our data do suggest that it is possible to eliminate obviously poor formulations from further evaluation in the more difficult, and perhaps no more informative, clinical trials.

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