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TOPICAL ACTIVITY OF BENDAZAC ON EXPERIMENTAL BURNS

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Bendazac or 1-benzyl-indazole 3-oxyacetic acid is a topical antinflammatory drug which in previous experiments was shown to preferentially inhibit inflammatory processes progressing towards necrosis (1). This activity has been temptatively related to a prevention of protein denaturation (2–4).

The aim of these experiments was to study effects of bendazac and hydrocortisone on heat-induced burns and on acetic acid-induced erythema. Two differently formulated creams have been investigated, in an attempt to find out the most suitable one for topical uses.

METHODS

CFI mice and Wistar rats of both sexes, weighing 20-30 and 130–170 g respectively, were used. The skin areas to be treated were clipped with an electric shaver. When not otherwise indicated, animals were kept under urethane anesthesia (1.7 g/kg i.p. in rats and 1.5 g/kg i.p. in mice) throughout the whole experiment.

Burns were produced in rats by putting on the abdomen for 30 seconds a test tube (weighing 50 g and with a diameter of about 8 mm) in which water was circulated at a temperature of 70°C. In each animal two burns were produced, one on the upper third and the other on the lower third of the abdomen. Two minutes later, the burnt areas were rubbed gently with 2 ml of the creams under investigation. One hour later, the animals were intravenously injected with 1 ml of a water solution containing 0.5% Evans Blue and 0.9% NaCl. Two hours after application of the creams, animals were killed by a blow on the head and the burnt skin was stretched and inserted between two glass slides. The intensity of blueing, resulting from accumulation of Evans Blue in the injured skin, was estimated by a Joyce Loebl mod. II photodensitometer devised for automatic reading of electrophoretic tracings.

Acetic acid erythema was studied in mice. A disc of filter paper, 4 mm in diameter, was soaked with glacial acetic acid and applied on the back of animals taking care that the paper adhered to the skin. After 15 seconds the disc was removed, the excess of acetic acid was wiped and a minute later 0.5 ml of the creams were applied locally. One hour later, the animals were intravenously injected with 0.5 ml of a water solution containing 0.1% Evans Blue and 0.9% NaCl. After 30 minutes animals were killed by a blow on

the head and the skin of the back was examined in the same way as described for heat burns.

The urinary excretion of bendazac was studied in unanesthetized rats. Two ml of a cream containing bendazac were applied on the back skin of the animals. The area treated was covered with a gauze square which was fixed to the skin with elastoplast bandage, thus establishing a waterproof cuff. Immediately after dressing, rats were placed in diuresis cages designed for separate collection of urine and faeces. Throughout the whole experiment the rats had free access to water, but were without food. After 18 hours, urine was collected and its contents in bendazac was determined according to a method already described (2).

Acute local tolerance tests were performed on unanesthetized rats. By means of a glass splinter, 8 longitudinal scarifications of about 1.5 cm, regularly spaced, were carried out on the animals' trunk according to Draize indications (5). The whole scarified area was rubbed with 1 ml of cream, protected with gauze and wrapped with elastoplast bandage fixed on the trunk of the animal. After 24 hours the dressing was removed and rats were examined. Examinations were repeated on the subsequent 2 days.

The drugs were blended in creams having the following formulation:

Type A: 0.6 g Sorbitan monostearate—4.4 g Polyoxyethylenesorbitan--monostearate—3 g Cetylstearyl alcohol—9 g Stearic acid—10 g Paraffin liquid—2 g Isopropyl miristate—6 g Glycerin—0.18 g Nipagin—0.02 g Nipasol—Water q.s. to 100 g-pH 5.5.

Type B: 25 g Xalifin 15®*—6 g Sorbitol 70%—0.18 g Nipagin—0.02 g Nipasol—Water q.s. to 100 g-pH 5.9.

Bendazac and hydrocortisone acetate were used in the highest concentration employed in medical practice. When necessary the pH of the creams was adjusted to the values reported above by means of NaOH.

The control animals were treated with a cream containing only the excipients. Significance of results was evaluated according to the Student's *t* method. In the case of heat burns and of acetic acid erythema, the photodensitometric values were transformed, before the statistic elaboration, in per cent values referring to the highest response obtained in each experiment. Further details regarding this procedure may be found in a previous paper (1).

RESULTS

Results obtained by studying effects of bendazac and hydrocortisone on heat burns are summarized in Table 1.

Bendazac and hydrocortisone were blended, at a 3% concentration, in both type A and type B creams. Bendazac produced a significant protection only when blended in type B formulation, while hydrocortisone was inactive in both formulations. Results obtained by studying effects of bendazac and hydrocortisone, blended both in type A cream and type B cream, on the experimental erythema induced by acetic acid are reported in Table 2.

* Supplied by Croda LTD, Enaith Goole, England.

TABLE 1. Effects of bendazac and hydrocortisone on heat burns in rats.

Treatment	No. of rats	Blue area±S.E.	Percent inhibition	P
Controls (cream A)	31	44.2±2.62	0	N.S.
3% Bendazac (cream A)	23	47.6±3.35		
Controls (cream B)	33	49.0±3.16	23.5	<0.01
3% Bendazac (cream B)	35	35.5±2.37		
Controls (cream A)	10	49.6±6.63	0	N.S.
3% Hydrocortisone (cream A)	10	50.8±6.52		
Controls (cream B)	15	47.8±3.81	6	N.S.
3% Hydrocortisone (cream B)	15	44.7±3.70		

TABLE 2. Effects of bendazac and hydrocortisone on acetic acid-induced erythema in mice.

Treatment	No. of mice	Blue area±S.E.	Percent inhibition	P
Controls (cream A)	33	59.4±5.42	24.4	<0.05
3% Bendazac (cream A)	34	44.9±4.17		
Controls (cream B)	49	53.9±4.64	27.5	<0.01
3% Bendazac (cream B)	73	39.1±2.76		
Controls (cream A)	15	54.7±9.22	42.3	<0.05
3% Hydrocortisone (cream A)	15	31.6±5.74		
Controls (cream B)	68	56.9±3.70	37.5	<0.001
3% Hydrocortisone (cream B)	65	35.4±2.85		

TABLE 3. Urinary excretion of bendazac following topical application on the skin of rats.

Treatment	No. of rats	Percentage found in the urine
3% Bendazac (cream A)	4	0.051
3% Bendazac (cream B)	4	0.047

Bendazac and hydrocortisone, the latter more effectively than the former, prevented the inflammation induced by acetic acid with both excipients.

The above mentioned experiments proved that the activity of bendazac on heat burns was substantially conditioned by the excipients. Therefore it was deemed useful to ascertain whether absorption or local tolerance of the two examined preparations was different. Values of urinary excretion of bendazac after its applications on the skin are reported in Table 3.

The quantity of the drug excreted in the urine proved to be similar with both formulations.

The acute tolerance tests were performed by comparing four formulations, represented by excipients type A and B respectively, with or without 3% bendazac.

The most striking difference among the plain creams was observed 24 hours follow-

ing medication and consisted in an hematic exudation which was produced by the type A, but not by the type B excipients. The same difference among the type A and type B excipients was observed when bendazac was blended into them.

DISCUSSION

Previous experiments have shown that bendazac and hydrocortisone have superimposible antinflammatory effects only in conditions where the vascular response is preminent, while the former is superior against necrotic responses, the latter is superior against proliferative responses (1). Results of these experiments confirm that bendazac and hydrocortisone possess a different spectrum of effects. As a matter of fact heat-induced burns, which are a typical condition evolving toward necrosis, were inhibited by bendazac but not by hydrocortisone; on the other hand the response to acetic acid, which is erythematous in its nature, was inhibited by both drugs though hydrocortisone proved to be more effective than bendazac. On the basis of these results a potential therapeutic interest of bendazac in anti-burn therapy is suggested.

The quality of excipients appeared to be of critical importance with regard to the anti-burn activity of bendazac, which was effective in this test only when the cream containing Xalifin 15® was used. This excipient did not change the absorption of the drug through the skin, but proved to be by itself better tolerated than the conventionally formulated cream. These results suggest that the influence of excipients on the anti-burn activity of bendazac is related to the nature of burns, which involve a severe damage of the skin and probably a hypersensitivity to irritating stimuli; they also suggest that preference should be given in all clinical applications of bendazac, to creams containing Xalifin 15® instead of synthetic surfactants.

SUMMARY

The topical effects of 3% bendazac and hydrocortisone on heat burns and on acetic acid-induced erythema in rats and mice were studied. Moreover a conventionally formulated hydrophilic cream (type A) and a cream containing Xalifin 15® (type B) were compared. Bendazac inhibited acetic acid-induced erythema with both excipients, while its activity against heat burns was shown only by using type B excipients. Hydrocortisone was effective only against acetic acid-induced erythema and its activity was not influenced by excipients.

Urinary excretion of bendazac following application on the skin was similar with both excipients, while its topical tolerance was better with type B excipients than with type A excipients.

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