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THE EFFECT OF SYSTEMICALLY AND TOPICALLY APPLIED DRUGS ON ULTRAVIOLET-INDUCED ERYTHEMA IN THE RAT

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1 Exposure of the skin of rats to u.v. light (>295 nm) for 30 s or longer elicited a delayed erythema response, the rate of onset increasing with the period of irradiation. The erythema was still present at 24 h and was replaced by scab formation in 48 hours.

2 Both topically applied steroidal and non-steroidal anti-inflammatory drugs reduced the erythema formation when administered immediately after u.v. exposure. Propyl gallate, an antioxidant with sun screening properties in man, also possessed topical anti-erythemic activity.

3 Both steroidal and non-steroidal anti-inflammatory drugs, systemically administered 1 h before u.v. exposure, reduced the erythema. However, the steroidal compounds were less effective than the non-steroids and reduced the intensity of erythema by less than 50%. Antagonists of 5-hydroxytryptamine (5-HT) reduced the erythema but several other drugs with different pharmacological activities were ineffective.

4 Neither topical nor systemic treatments of any of the drugs examined suppressed the scab formation at 48 hours.

5 These results and those using other selective blocking agents indicate that in the mediation of the erythema reaction prostaglandins may play a major role and 5-HT perhaps a minor one but that H_1 histamine receptors and α- and β-adrenoceptors have no significant role.

Introduction

The production of erythema in the skin of guinea-pigs exposed to ultraviolet (u.v.) light was described by Wilhelmi (1949; 1950) and has been used as a model of inflammation for the assessment of new anti-inflammatory drugs (Swingle, 1974). Furthermore, Winder, Wax, Burr, Been & Rosiere (1958) demonstrated a good correlation between the erythema-delaying action of systemically administered non-steroidal agents and their anti-rheumatic properties. However, steroidal anti-inflammatory drugs were ineffective in altering this erythema response when administered systemically (Winder *et al.*, 1958), or topically (Lambelin, Vassart-Thys & Roba, 1970).

A similar erythema response in rats has not previously been described although Logan & Wilhelm (1966a) demonstrated biphasic vascular permeability changes in the skin of u.v. irradiated rats in the absence of a significant hyperaemia. The present studies were undertaken to establish whether u.v.-induced erythema could be elicited in the rat using longer exposure times than those used by Logan & Wilhelm (1966a), in an attempt to develop a test for assessment of topically applied steroidal and non-steroidal anti-inflammatory drugs. The effects of a variety of other drugs have also been examined. A part of this work has been demonstrated to the British Pharmacological Society (Lewis, 1976).

Methods

Male Wistar rats (Carworth Europe CE/CFHB) weighing between 80–100 g were used in groups of five. They were prepared for u.v. exposure as previously described by Winder *et al.* (1958) for guinea-pigs. Immediately after clipping, the left flanks were depilated with a cream preparation (Veet-O, Reckitt and Colman Toiletries). Twenty-four hours later three circular areas (6 mm diameter) on the flanks were exposed to u.v. irradiation provided by a Hanovia Model 10 Quartz Lamp fitted with a Kodak 18A glass filter, transmitting light greater than 295 nm wavelength. In preliminary experiments various exposure times were examined. At several intervals after u.v. exposure, the intensity of the erythema of each irradiated spot was assessed visually by a trained observer using the following scoring system: 0, no evident erythema; 0.5, an area of erythema not clearly defined as a circle; 1.0, a full circle of definite redness of any intensity.

Drugs administered topically were dissolved in

absolute ethanol and applied in 0.2 ml volumes into an open-ended cylindrical reservoir held closely over the irradiated skin. They were applied immediately after irradiation. Control irradiated animals received an equal volume of absolute ethanol. The area of treated skin was used to hasten drying of the solution. The area of treated skin was then covered with plastic adhesive (Sleek, Smith and Nephew Ltd.). Drugs were administered systemically (either subcutaneously or orally in a volume of 1 ml/kg) in 5% mulgofen in water, 1 h before u.v. exposure. Animals dosed orally were fasted for 18 h previously. Control animals received an equal volume of 5% mulgofen.

In all drug treatment experiments the groups of animals were randomized and the assessor was unaware of the treatment schedule. Erythema was always assessed at 4 h after irradiation and in some experiments also at 1, 2, 3, 4 and 24 hours. Changes in erythema intensity after drug administration are expressed as percentages of the erythema intensity at 4 h in vehicle-treated rats. All the data were analysed by the randomization test of Siegel (1956). Topical ID_{50} values were calculated after plotting lines of best fit through the data points and expressed as mg drug applied to the skin. After systemic administration the ID_{50}s were calculated for non-steroidal drugs and the ID_{30}s for steroidal drugs.

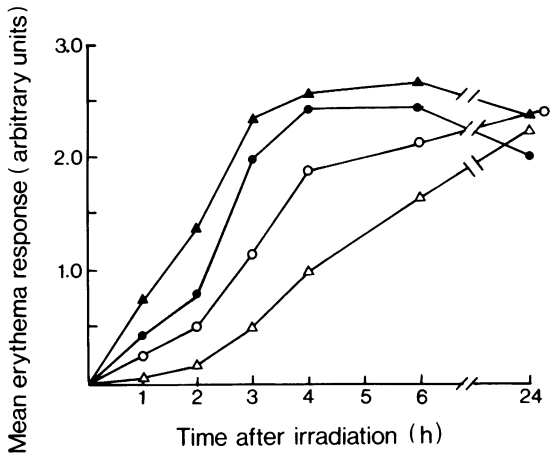

Figure 1 The time course of erythema of rat skin after u.v. irradiation for 30 s (△), 60 s (○), 90 s (●), or 120 s (▲). Each point is the mean of 5 observations.

The following drugs were tested: aspirin, paracetamol, procaine hydrochloride (BDH), betamethasone-17-valerate (Glaxo), cyproheptadine hydrochloride, indomethacin (Merck, Sharp & Dohme), flufenamic acid (Parke Davis), fluocinolone

Table 1 Effect of steroidal anti-inflammatory agents on u.v. erythema at 4 h (90 s exposure)

	Topical			*Subcutaneous*		
	Dose (mg)	*%*	*Approx ID_{50} (mg)*	*Dose (mg/kg)*	*%*	*Approx ID_{30} (mg/kg)*
Betamethasone-17-valerate	1	15				
	3	27*	7.8	N/T		
	10	55*				
Dexamethasone	1	10		0.3	25*	
	3	37*	4.8	1.0	30*	0.5
	10	72*		5.0	48*	
Fluocinolone acetonide	1	30*		0.3	13	
	3	50*	4.4	1.0	26*	1.9
	10	58*		3.0	32*	
Hydrocortisone	1	28		10	10	
	3	40*	9.0	30	20	>100
	10	50*		100	25*	
Org 6216	0.3	28		10	21	
	1.0	53*	1.0	30	33	30.4
	3.0	61*		100	36*	
Prednisolone	1	12		3	21	
	3	45*	7.7	10	32	10.5
	10	48*		30	35*	

Drugs were applied topically immediately after u.v. exposure or subcutaneously 1 h previously. The mean % inhibition by comparison with controls and calculated ID values are shown. Groups of 10 animals were used.
N/T Not tested.
* $P < 0.05$.

acetonide, propranolol (ICI), ibuprofen and flurbiprofen (Boots), mepyramine maleate (May & Baker), methysergide bimaleate (Sandoz), phenylbutazone (Geigy), propyl gallate (Koch-Light), S.C. 19220 [1-acetyl-2(8-chloro-10,11-dihydrodibenz-(b,f)(1,4) oxazpine-10-carbonyl) hydrazine] (Searle), theophylline (Sigma), triprolidine hydrochloride (Wellcome), and other drugs synthesized by Organon Laboratories Limited. Doses refer to free base or acid.

Results

Erythema formation

Skin irradiated for 10 or 20 s showed no erythema formation within 6 hours. Responses to exposure for 30, 60, 90 and 120 s are shown in Figure 1. With 30 s exposure, erythema intensity increased progressively over a period of 24 hours. As the exposure period was increased the rate of onset of erythema was accelerated and the time taken to reach peak erythema was reduced. At 48 h all erythema responses were replaced by scabs which sloughed off after several days. For examination of drug effects an exposure period of 90 s was chosen; this produced a maximal erythema response in about 4 hours.

Effect of drugs on 90 s u.v. exposure

Topical administration. Topically administered anti-inflammatory steroids demonstrated dose-related inhibition of the erythema intensity at 4 h, with the following order of potency: Org 6216 (11β-hydroxy-

Table 2 The effect of various non-steroidal anti-inflammatory agents on u.v. erythema at 4 h (90 s exposure)

	Topical			*Oral*		
	Dose (mg)	*%*	*Approx ID_{50} (mg)*	*Dose (mg/kg)*	*%*	*Approx ID_{50} (mg/kg)*
Aspirin	1	11		100	37*	
	3	25*	9.2	200	59*	148
	10	58*		300	68*	
Bufexamac	1	14		100	10	
	3	33*	8.1	200	15	>200
	10	53*				
Flazalone	N/T			100†	42*	
				200†	54*	154.2
				300†	60*	
Flufenamic acid	3	25		10	17	
	10	46*	10.9	30	46*	38.5
	15	68*		100	69*	
Flurbiprofen	0.1	22				
	0.3	53*	0.28	N/T		
	1.0	68*				
Ibuprofen	0.1	35		3	15	
	0.3	60*	0.3	10	34*	22.4
	1.0	65*		30	57*	
Indomethacin	0.3	27		0.3	23	
	1.0	46*	1.3	1.0	47*	1.3
	3.0	63*		3.0	63*	
Paracetamol	N/T			30	30	
				100	53*	100.0
				300	67*	
Phenylbutazone	1	28		10	43*	
	3	43*	5.6	30	59*	17.2
	10	56*		100	75*	

The drugs were applied topically immediately after u.v. irradiation or orally (or subcutaneouslyt) 1 h previously. The mean % inhibition by comparison with controls and the calculated ID_{50} values, using groups of ten animals, are tabulated.
N/T Not tested.
* $P < 0.05$.

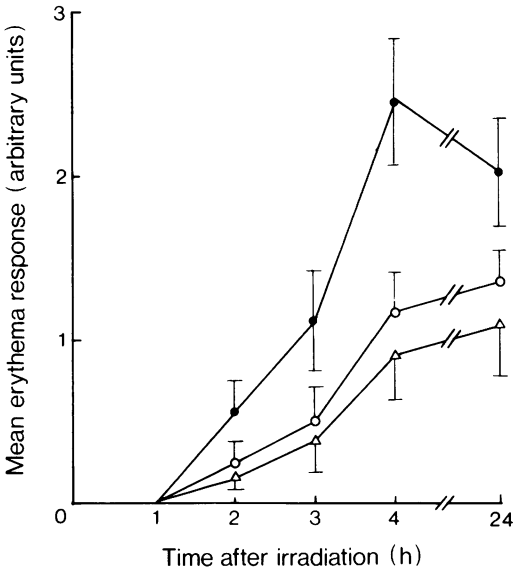

Figure 2 The effect of topically applied drugs on the rate of development of erythema of rat skin induced by u.v. irradiation for 90 seconds. Responses in control (●) and when 3 mg of either fluocinolone acetonide (△) or phenylbutazone (○) was applied in 0.2 ml absolute ethanol immediately after irradiation are shown. Each point is the mean of 10 observations. Vertical lines show s.e. mean.

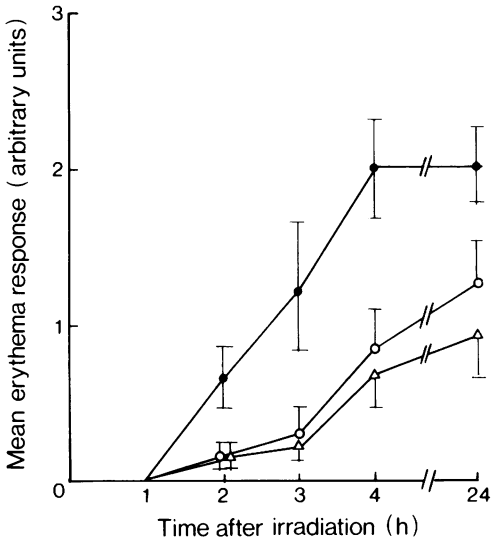

Figure 3 The effect of systemically applied drugs on the rate of development of erythema of rat skin induced by u.v. irradiation for 90 seconds. Responses in controls (●) and in rats given fluocinolone acetonide (10 mg/kg, s.c., △) or phenylbutazone (30 mg/kg, orally, ○) 60 min before irradiation are shown. Each point is the mean of 10 observations. Vertical lines show s.e. mean.

Table 3 Effect of various pharmacological agents on u.v. erythema at 4 h (90 s exposure)

	Topical			*Oral*		
	Dose (mg)	*%*	*Approx ID_{50} (mg)*	*Dose (mg/kg)*	*%*	*Approx ID_{50} (mg/kg)*
Cyproheptadine	3	8		3	0	
	15	12	>15	10	6	>15
				15	25*	
Methysergide bimaleate	3	8	>15	1	36*	
	15	7		3	58*	2.1
				10	75*	
Mianserin	N/T			1	19	
				3	40*	7.2
				10	53*	
Propyl gallate	3	42*				
	10	46*	10	N/T		
	15	70*				

Drugs were applied topically immediately after u.v. exposure or orally 1 h previously. The mean % inhibition by comparison with controls and calculated ID_{50} values are shown. Groups of 10 animals were used.
N/T Not tested.
* $P < 0.05$.

16α,17α,21-trimethyl pregna-1,4-diene-3,20-dione) > fluocinolone acetonide and dexamethasone > betamethasone-17-valerate, hydrocortisone and prednisolone (Table 1). The non-anti-inflammatory steroids, nandrolone decanoate, oestradiol, progesterone and testosterone had no significant effect in amounts of 15 mg per animal ($n = 10$).

Non-steroidal anti-inflammatories also suppressed erythema at 4 h when topically administered (Table 2). The rank order was flurbiprofen = ibuprofen > indomethacin > phenylbutazone > bufexamac ≏ aspirin ≏ flufenamic acid. Propyl gallate was also effective (Table 3).

Cyproheptadine and methysergide were ineffective (Table 3) as also were mepyramine, procaine, propranolol and S.C. 19220 in tests using doses of 3 and 15 mg in groups of 10 animals.

Drugs that reduced the 4 h erythema response were still effective at 24 h although scab formation at 48 h was unaffected. This is shown in Figure 2 for topically applied fluocinolone and phenylbutazone.

Systemic administration. None of the anti-inflammatory steroids, administered subcutaneously, inhibited erythema by more than 48%. However, they all produced dose-related inhibition, except for hydrocortisone which was ineffective at 100 mg/kg. ID_{30} values were determined (Table 1).

All the non-steroidal anti-inflammatory compounds with the exception of bufexamac demonstrated dose-related inhibition of the erythema at 4 h (Table 2) when administered orally, with the following order of potencies: indomethacin > ibuprofen ≏ phenylbutazone > flufenamic acid > paracetamol (4-acetamidophenol) > aspirin. Flazalone was effective subcutaneously but not examined orally.

Methysergide, mianserin and cyproheptadine administered orally reduced the erythema response (Table 3). Mepyramine (10 mg/kg), triprolidine (50 mg/kg), phenoxybenzamine (15 mg/kg), procaine (200 mg/kg), propranolol (20 mg/kg) and theophylline (20 mg/kg) were ineffective when given orally in groups of 10 rats in similar experiments.

The onset and duration of the anti-erythema effect of systemically administered drugs were similar to those described for topical administration as exemplified for fluocinolone acetonide and phenylbutazone in Figure 3. Suppression of the erythema was distinct by 2 h and effects persisted at 24 hours. Scab formation at 48 h was not influenced by either of these drugs or any other drugs that demonstrated anti-erythemic activity at 4 hours.

Discussion

The exposure of rats to u.v. irradiation evoked an erythema in our studies but not in those of Logan & Wilhelm (1966a) who used shorter exposure periods (10 to 30 seconds).

Non-steroidal anti-inflammatory drugs are effective against u.v.-induced erythema in guinea-pigs both systemically (Winder *et al.*, 1958; Adams & Cobb, 1963; Gupta & Levy, 1973) and topically (Graeme, Peters, Maiorana & Cooper, 1975). We have shown a similar effect with these drugs in the rat. Relative effectiveness by the two routes often differed, as expected in view of differences in transport mechanisms and opportunity of inactivation. By topical application, the most potent non-steroidal drugs, ibuprofen and flurbiprofen, were more effective than the most potent steroids. Bufexamac, a drug with reported clinical topical anti-inflammatory efficacy (Lambelin *et al.*, 1970) exhibited anti-erythemic activity in this rat u.v. model.

The topical anti-erythemic activity of propyl gallate, an anti-oxidant, is of interest. In these experiments the drug was applied immediately after irradiation whereas its previously reported protective effect against u.v. irradiation in man (Kahn & Curry, 1974) and the guinea-pig (McDonald-Gibson & Schneider, 1974) could be attributed to radiation absorption.

Systemic pretreatment of irradiated rats with the non-steroidal drugs delayed the onset of erythema. Flazalone, which has been previously demonstrated to be ineffective against the u.v. reaction in the guinea-pig (Draper, Petracek, Klohs, McClure, Levy & Re, 1972), was weakly effective in the rat. The anti-erythemic activity of paracetamol was unexpected since it has been considered devoid of anti-inflammatory properties (Adams, 1960) although it was demonstrated to inhibit the u.v.-induced vascular permeability changes occurring in the mouse (Sim, 1965).

Steroidal anti-inflammatories have been reported to be inactive in the guinea-pig model when administered either topically (Lambelin *et al.*, 1970) or systemically (Winder *et al.*, 1958; Gupta & Levy, 1973). However, applied topically some were as effective as non-steroidal agents in suppressing erythema in the rat. Org 6216, a new non-halogenated steroid with local activity and lacking major systemic and local side effects (Fox, Lewis, Rae, Sim & Woods, 1977) was the most potent steroid examined. None of the non-anti-inflammatory steroids tested was effective after topical application. The steroidal anti-inflammatory drugs were less effective when administered systemically.

The relevance for man of the effects in the rat u.v. model of the steroidal anti-inflammatories is uncertain but topically applied anti-inflammatory corticosteroids can suppress u.v. erythema induced in man (Chanin, 1973; Kaidey & Kurban, 1976; Lewis & Law, unpublished observations); these substances may be of some use in the suppression of human sunburn reactions.

Neither topical nor systemic administration of either non-steroidal or steroidal compounds eliminated the erythema reaction in the rat. This parallels the finding in guinea-pigs when these agents were given systemically (Gupta & Levy, 1973) and those in man where topical and intradermal corticosteroids and topical indomethacin were examined (Kaidey & Kurban, 1976). The residual effects may be associated with necrotic changes in the skin. No major changes in rat skin histology were detected 4 h after a 90 s irradiation but by 24 h degeneration of the epidermis was marked and a large number of inflammatory cells (mainly monocytes, fibroblasts and some neutrophilic leukocytes) had accumulated in the upper dermal region (unpublished observations).

It is possible to formulate a number of theories for mechanisms of anti-erythemic effect.

The erythema induced in the rat was suppressed by the systemic administration of the 5-hydroxytryptamine (5-HT) antagonists, methysergide and mianserin, but not by the antihistaminics, mepyramine and triprolidine. Cyproheptadine, a drug which is both a 5-HT antagonist and histamine antagonist, showed only a weak effect. Bonta (1969) demonstrated a potent anti-erythemic activity of methysergide in the guinea-pig u.v. erythema model and speculated that its mechanism of action could be a result of both its anti-5-HT and its vaso-constrictor effects. In view of the possible dual mode of action of methysergide and possibly other 5-HT antagonists, it is not certain that 5-HT is a mediator of u.v. erythema in the rat.

The early phase of the biphasic vascular permeability changes that occurred after u.v. irradiation in the rat has also been shown to be susceptible to the 5-HT antagonist bromlysergic acid diethylamide (BOL) (Logan & Wilhelm, 1966b). However, only the early phase (5–15 min) of increased vascular permeability appeared to be reduced by BOL. The response corresponding to the erythema production (4 h) was only slightly suppressed by this 5-HT antagonist.

There is mounting evidence that prostaglandins may play a role in the mediation of u.v.-induced erythema. After u.v. irradiation prostaglandin formation occurs in human (Greaves & Sondergaard, 1970; Mathur & Ghandi, 1972), rat (Mathur & Ghandi, 1972) and guinea-pig skin (Sekura Snyder, 1976). Moreover, non-steroidal anti-inflammatory drugs are able to inhibit prostaglandin formation in the skin (Greaves & McDonald-Gibson, 1973) and recently Sekura Snyder (1976) has demonstrated that topical application of indomethacin reduces both the erythema and prostaglandin levels present in the u.v. irradiated guinea-pig skin. There is also evidence that corticosteroids may inhibit synthesis and the release of these substances (Greaves & McDonald-Gibson, 1972; Lewis & Piper, 1975). Propyl gallate at high concentration also inhibits prostaglandin formation (van Dorp, 1967) a factor which may contribute to its topical anti-erythemic efficacy. However, the prostaglandin antagonist, S.C. 19220, was not effective against the erythema although when applied topically it reduced the oedema response to croton oil in the rat (Sanner, 1974).

Ultraviolet damage to the skin causes lysosomal membrane rupture and consequent hydrolytic enzyme release (Johnson & Daniels, 1969; Honigsmann, Wolff & Konrad, 1974). Both steroidal and non-steroidal anti-inflammatory drugs exhibit lysosomal membrane-stabilizing properties (Weissman & Fell, 1962; Ignarro, 1971) that may reduce enzyme release from the lysosomes. Such an effect may play a role in their inhibition of u.v. erythema.

The cutaneous vasoconstrictor activity of the steroids and possibly of certain of the non-steroids, particularly when they are applied topically, may also contribute to suppression of the u.v. erythema. However, steroids with potent vasoconstrictor actions on the cutaneous vasculature in man, such as betamethasone-17-valerate, are not the most potent anti-erythemic compounds in the rat; neither are steroids with high anti-erythemic activity, such as Org 6216, the most active vasoconstrictor agents (Fox *et al.*, 1977).

In conclusion, the mechanism of u.v.-induced erythema induction in the rat and the mode of action of drugs that reduce this response is uncertain, but 5-HT release, prostaglandin formation, lysosomal enzyme release and/or possibly local vasoconstrictor activity may all be contributory factors.

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