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Delayed manifestation of ultraviolet reaction in the guinea-pig caused by anti-inflammatory drugs

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Summary

1. Exposure of depilated skin of guinea-pig to ultraviolet (u.v.) light for 20 s produces a prolonged inflammatory response.

2. The erythaema becomes evident within 15–30 min after the exposure and progressively increases in intensity reaching its maximum by 4–6 hours. The erythaema persists over 24 hours.

3. Increase in vascular permeability is biphasic with an early short-lived rise peaking at 0·5 h and a prolonged secondary response peaking at 9–12 h and lasting over 48 hours.

4. In presence of aspirin, phenylbutazone and indomethacin, administered prior to u.v. exposure, the inflammatory reaction is partially suppressed, depending upon the dose. The drugs are ineffective in aborting or minimizing the response when given after the inflammation is established. Corticosteroids fail to influence the u.v. inflammation in this test. The significance of these findings is discussed.

Introduction

One of the laboratory models of inflammation developed to test the anti-inflammatory properties of drugs is the ultraviolet (u.v.)-induced erythaema of the guinea-pig skin. The studies of Wilhelmi (1949, 1950) and Wilhelmi & Domenjoz (1951) demonstrated that pretreatment of the guinea-pig with certain anti-rheumatic drugs delayed the u.v.-induced erythaema. Winder, Wax, Burr, Been & Rosiere (1958) showed the specificity of this test in detecting non-steroidal anti-rheumatic compounds of clinical value and their method is widely used for evaluating the anti-inflammatory activity of new compounds.

The present studies were undertaken to investigate the temporal response of ultraviolet-induced inflammation of guinea-pig skin in the presence of representative anti-inflammatory drugs.

Methods

American short haired albino guinea-pigs weighing between 280 and 400 g were prepared for u.v. exposure as described by Winder *et al.* (1958). Three circular areas (6 mm diameter) of the right flank were exposed to u.v. radiation from a Hanovia Analytic Model Quartz lamp for 30 seconds.

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At various time intervals (0·25–24 h) after u.v. exposure the intensity of erythaema was visually graded by a trained observer unaware of treatment schedules using an arbitrary scale of 0–3 as follows:

0—an exposed point with no evident erythaema
1—a mild reaction pale pink in colour
3—an intense reaction deep reddish pink in colour
2—a moderate response between 1 and 3.

The maximum score for a 3 point exposure in a single animal is 9.

Following the method of Aschheim & Zweifach (1961, 1962) permeability changes were studied with radio-iodinated human serum albumen (RISA-131^R) given intravenously in 0·5 ml of 0·9% w/v NaCl solution (saline) at various intervals after u.v. exposure. The animals were killed by cervical dislocation 15 min after the i.v. injection of RISA-131^R. Skin samples were punched out from exposed and unexposed control sites. The samples were weighed immediately and after drying overnight in an oven at 100° C, the radioactivity was assayed with a Nuclear Chicago gamma ray spectrometer and the counts per minute per gramme dry weight of tissue were computed.

For the drug studies, the animals were fasted overnight and the test drug was administered intragastrically, half an hour before the u.v. exposure. The following drugs were used: microfined aspirin (15–200 mg/kg), phenylbutazone (5–15 mg/kg), indomethacin (1–4 mg/kg), prednisolone sulphate (5 and 10 mg/kg). All the drugs were suspended in 3% gum arabic and were given in a volume of 0·8 ml/100 g body weight. Animals treated with the vehicle alone served as controls.

In a selected series of experiments, one of the groups was re-dosed two hours after u.v. exposure and the effect on the course of erythaema development was studied in comparison with the other drug-treated group as well as non-treated controls.

Results

Time course of ultraviolet erythaema

Figure 1 shows the time course of development of ultraviolet erythaema. Within 15 min after a 30 s u.v. exposure, erythaema begins to appear in some animals

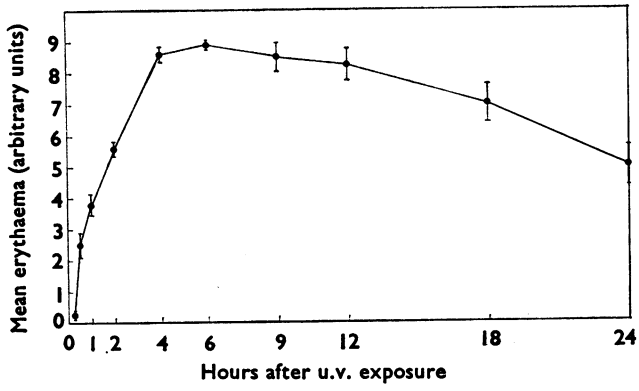


FIG. 1. The time course of development of ultraviolet-induced erythaema of the guinea-pig skin. Each point represents the mean of 12 observations; the vertical bars represent the standard error of mean.

and by 30 min all the animals develop distinct lesions, pale pink in colour. The intensity of the reaction rapidly increases, reaching its maximum by 4 to 6 hours. At the maximum, the lesions appear deep reddish-pink in colour. The erythaema persists with more or less the same intensity for about 12 h when a slow subsidence begins. The reaction is easily noticeable at 24 h, though the lesions are now more dusky in colour and the rapid growth of hair makes evaluation difficult.

Permeability changes after ultraviolet exposure

Figure 2 shows the permeability changes in guinea-pig skin after u.v. irradiation for 30 seconds. An early response of increase in permeability starts within 15 min, reaches a peak at 30 min and sharply declines in the following half hour. A secondary rise in permeability becomes evident 2 to 4 h after u.v. exposure and gradually increases to reach its maximum by 9 to 12 hours. This secondary response rapidly decreases in the following 6 h and then more gradually during the next 2 days.

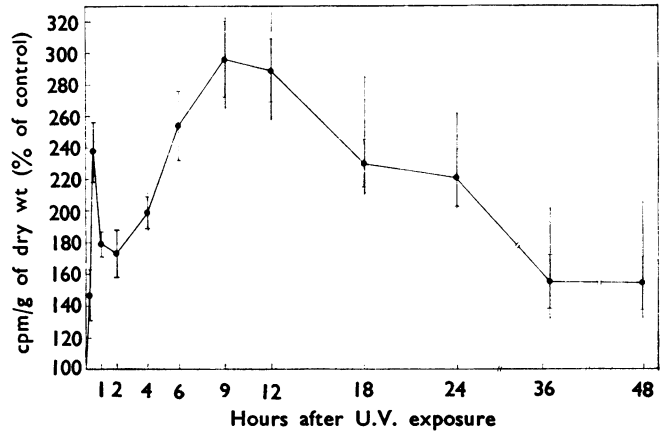


FIG. 2. Biphasic permeability response evoked by local irradiation of guinea-pig skin for 30 seconds. Each point is the mean of 8–10 observations; the vertical bars represent the standard error of mean.

Effect of anti-rheumatic drugs

Ultraviolet erythaema

Pretreatment of animals with a single dose of aspirin, phenylbutazone and indomethacin delays the onset and development of u.v. erythaema (Fig. 3a, b and c). In the drug-treated animals, the erythaema develops much later than the control groups depending upon the dose. The intensity of erythaema progressively increases tending to reach the maximum, especially in low doses. With higher doses the erythaema response is significantly less than the control for the period of observation.

From the graphs in Fig. 3, time to development of 50% of maximum control erythaema was computed and plotted against the dose of the drug on a log scale

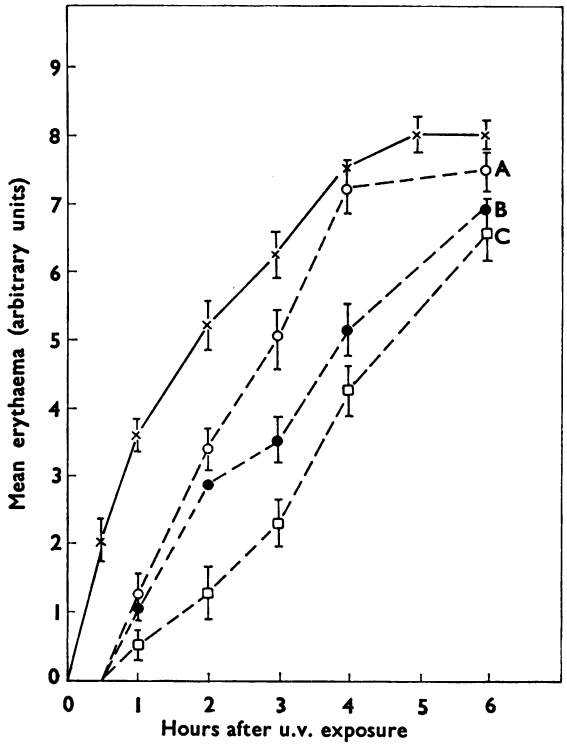

Mean erythaema (arbitrary units)
Hours after u.v. exposure
A
B
C
0
1
2
3
4
5
6
7
8
9

FIG. 3 (*a*)

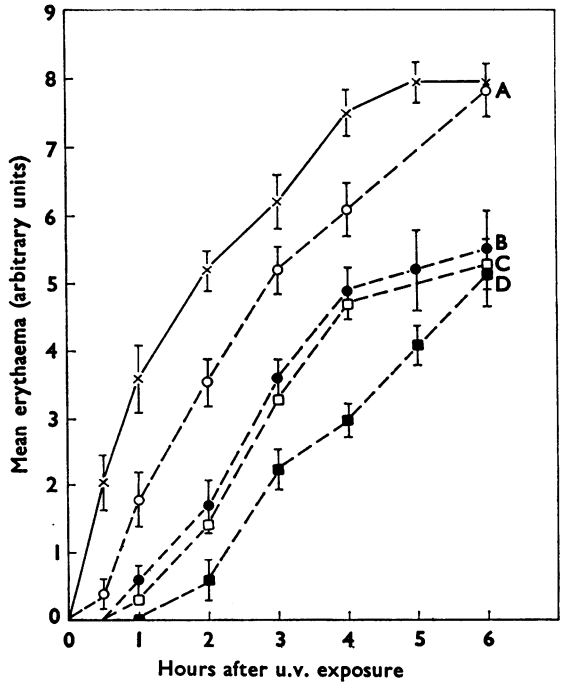
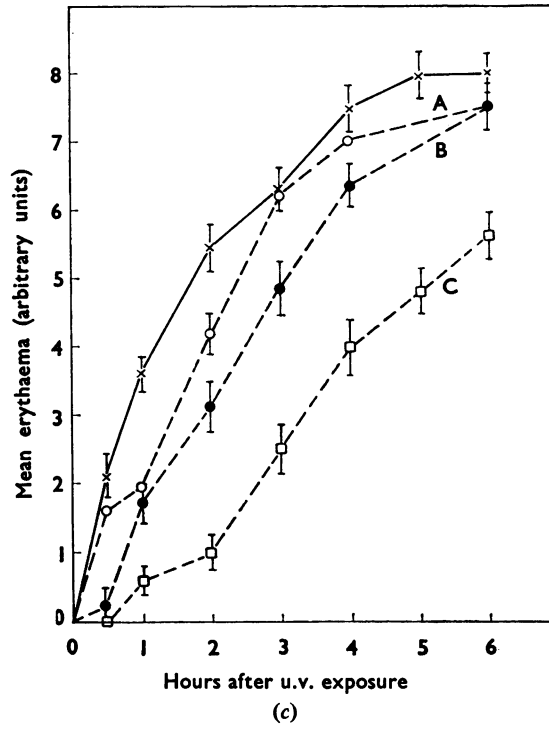

Mean erythaema (arbitrary units)
Hours after u.v. exposure
A
B
C
D
0
1
2
3
4
5
6
7
8
9

FIG. 3 (*b*)



(c)

FIG. 3. Development of u.v. erythaema in control and drug-treated animals. The drugs were administered intragastrically half an hour before u.v. exposure. Controls received the vehicle alone. Each point is the mean of at least 8 observations; the vertical bars represent the standard error of mean. (a) ×——× control – – – aspirin. A. 30 mg/kg; B. 50 mg/kg; C. 100 mg/kg; D. 200 mg/kg. (b) ×——× control – – – phenylbutazone. A. 5 mg/kg; B. 10 mg/kg; C. 15 mg/kg. (c) ×——× control – – – indomethacin. A. 1 mg/kg; B. 2 mg/kg; C. 4 mg/kg.

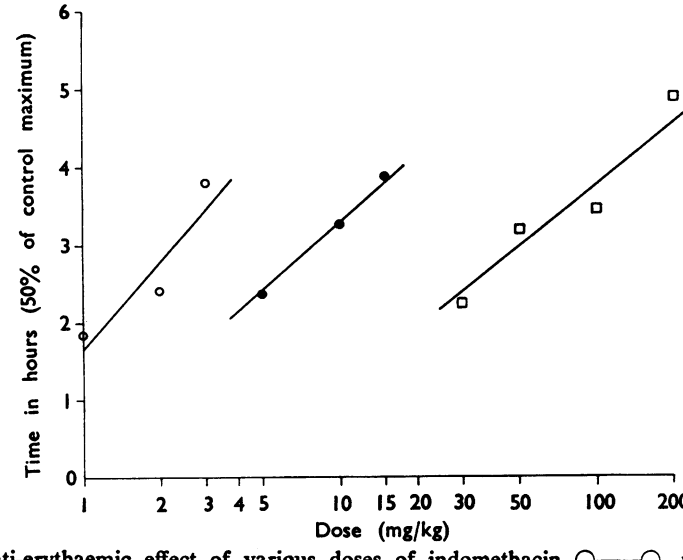


FIG. 4. Anti-erythaemic effect of various doses of indomethacin ○——○, phenylbutazone ●——●, and aspirin □——□. The dose of the drug is plotted on a log scale. The ordinate represents time in hours to development of 50% of control maximum erythaema computed from Figure 3. The average time needed for the vehicle control to achieve 50% maximum erythaema response was 1·13 hours.

as shown in Fig. 4. This depicts the dose-dependent delay in erythaema development and incidentally reflects the relative potency of drugs in this test. In the control animals time to the development of 50% of maximum response was 1–1·5 h (mean 1·13 h).

Permeability changes

Preliminary studies with a single dose administered before u.v. exposure showed that aspirin inhibited the increased permeability induced by exposure of guinea-pig skin to u.v., as shown in Table 1.

TABLE 1. *Effect of aspirin on u.v.-induced permeability changes in guinea-pig skin*

Treatment admin. 0·5 h before u.v. exposure	% Control (non-exposed sites) cpm/g of dry wt. Mean±S.E. Hours after u.v. exposure			
	0·5	4	6	9
Control (3% gum arabic 0·8 ml/100 g i.g.)	236·72±10·63 (*n*=12)	210·64±15·54 (*n*=8)	242·04±17·35 (*n*=6)	288·54±27·01 (*n*=5)
Aspirin 100 mg/kg i.g.	131·08±7·71 (*n*=4)	111·48±16·86 (*n*=5)	201·74±28·16 (*n*=4)	200·98±17·09 (*n*=5)
Aspirin 200 mg/kg i.g.	100·70±18·46 (*n*=4)	119·96±9·31 (*n*=4)	145·00±18·28 (*n*=3)	not done

Radio-iodinated human serum albumen was injected in penal vein 15 min before killing the animals. *n*=number of animals studied.

Effect of repeated doses

Re-dosing the animals with the above drugs early during the course of erythaema development failed to alter significantly the later part of the erythaema curve, even after relatively high doses. Figure 5 shows the results of a typical experiment with indomethacin (4 mg/kg).

Prednisolone sulphate in doses used, failed to influence the u.v. erythaema.

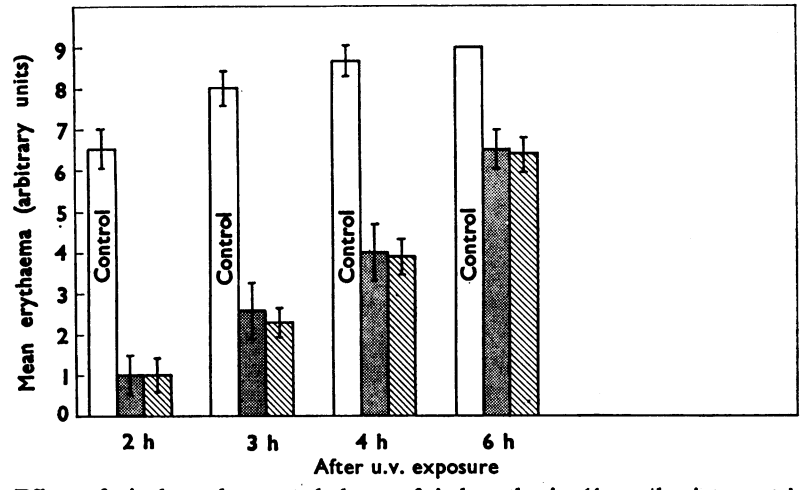


FIG. 5. Effect of single and repeated doses of indomethacin (4 mg/kg intragastrically) on u.v. erythaema response. Indomethacin was given in a single dose one-half hour before u.v. exposure ⁞⁞⁞, or one-half hour before and two hours after u.v. exposure ■. Six animals were used in each group studied. The vertical bars represent standard error of mean.

Discussion

The exposure of guinea-pig skin to ultraviolet radiation evokes a prolonged inflammatory response. A single exposure to u.v. light, at a distance of 18 cm from the skin, induces an erythaematous response and an increase in vascular permeability of more than 48 h duration. The erythaema development seems to proceed to reach a maximum by four hours. However, the time course of the permeability response appears to be different from that of erythaema development. The permeability response is biphasic with an early short-lived response of 1–2 h followed by a prolonged secondary response peaking at 9–12 h after u.v. exposure. At 4 h after u.v. exposure when the exposed sites are deep red in colour, the increase in permeability is only modest. The difference observed in erythaema and permeability responses (Figs. 1 and 2) could be explained by difference in methods of evaluation. The vasodilatation as judged by erythaema reflects cumulative response, whereas permeability was measured by injection of $RISA^{-131}$ at different points during the course of development of the inflammatory response (cf. Logan & Wilhelm, 1966).

The time-course of u.v.-induced inflammation of guinea-pig skin observed in the present studies is somewhat different from that reported by Logan & Wilhelm (1966). The latter authors described a biphasic erythaema as well as a permeability response characterized by an immediate response of 10–15 min duration and a prolonged secondary response. However, the consistent demonstration by Logan & Wilhelm (1966) of the early response required u.v. irradiation for at least 120 seconds. The absence of an early erythaema response and a different time-course of the permeability response in the present studies could be due to use of a milder stimulus, namely, u.v. exposure for 30 seconds. In contrast to the findings of Logan & Wilhelm (1966), we have failed to observe the restoration of vascular permeability to a normal low level after the primary increase but before the appearance of secondary rise in permeability. The cause of this discrepancy could very well lie in different techniques employed for evaluation of permeability changes; Logan & Wilhelm used intravenous Evans blue injection and visual estimation of blueing for recording the permeability changes. However, studies of Udaka, Takeuchi & Movat (1970) have demonstrated a linear relationship between radioactivity of the skin lesions and the amount of Evans blue extracted from the same lesions.

The general pattern of u.v.-induced inflammation is similar to that evoked by other types of injury, e.g. experimental bacterial infection (Burke & Miles, 1958), thermal injury (Wilhelm & Mason, 1960), superficial chemical injury (Steele & Wilhelm, 1966, 1970); though in u.v.-induced inflammation the time-course of the vascular events is relatively slower and the response more prolonged, allowing a better dissection of vascular components of acute inflammation.

Pretreatment of animals with non-steroidal anti-rheumatic drugs modify the u.v.-induced erythaema and permeability response of guinea-pig skin. In doses comparable to those used in clinics, these drugs delay the onset of reaction and slow down the time-course of erythaema development. Studies with aspirin reveal a similar effect on the permeability response. However, even after relatively high doses, the drugs fail to eliminate the reaction. The delayed appearance of the reaction does not seem to be due to decrease in effective drug concentrations in the body since repeated drug administration, early during the course of inflamma-

tion failed to exert an additional suppressant effect. It seems that in the complex biological reaction to u.v. irradiation a component in the early vascular response to the stimulus is sensitive to drug action. After the initial response, the later course of the inflammatory reaction is essentially unaffected by the drug treatment. Adams & Cobb (1963) demonstrated that after the u.v.-induced erythaema is established, treatment with aspirin in oral doses up to 640 mg/kg failed to influence the erythaema. The drugs thus only delay the onset and at best partially suppress the early inflammatory response when administered before exposure to u.v. light. Once the inflammation has developed drugs fail to abort or effectively suppress the reaction. Indeed, as observed in clinics this is the common feature of available anti-rheumatic drugs; while they are useful as palliatives, they do little more than exert modest control over some of the inflammatory manifestations of rheumatic diseases. In fact, the disease process continues even when the patients are receiving the anti-rheumatic drugs and rebound occurs on withdrawal of the drug. It appears that in search of new anti-rheumatic drugs of therapeutic rather than palliative value, the approach of developing test systems based on positive results with known drugs in u.v. erythaema would not be profitable.

Surprisingly, the inflammation in this test system is not influenced by corticosteroids. In his studies with mice, Sim (1965) observed similar ineffectiveness of cortisone in suppressing the u.v.-induced permeability response. A more surprising lack of correlation is the evidence that oxyphenbutazone, a phenolic metabolite of phenylbutazone which has well documented anti-rheumatic activity in clinics, is inactive in u.v. erythaema tests (Adams & Cobb, 1963). Indeed, the correlation observed between anti-rheumatic effect of drugs and their ability to delay the u.v. erythaema in guinea-pig skin is difficult to explain.

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