THE JOURNAL OF INVESTIGATIVE DERMATOLOGY 62: 47-50, 1974

Vol. 62, No. 1
Printed in U.S.A.

INTRADERMAL ANTI-PROSTAGLANDIN AGENTS AND SUNBURN*

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ABSTRACT

Inhibitors of prostaglandin (PG) biosynthesis, indomethacin and aspirin, decrease and delay ultraviolet light-induced erythema when injected intradermally in humans and guinea pigs. Increasing amounts of inhibitor cause a more intense blanch with a longer duration demonstrating a dose response. Indomethacin was approximately 45 times more effective than aspirin. Indomethacin can cause blanching of UV redness if injected at any time from the period of irradiation to 18 hr after UV exposure. Triamcinolone acetonide was effective in preventing erythema in humans, but not in guinea pigs. The ability of anti-PG agents to decrease and delay UV-induced redness lends further support to a role for PG in the mediation of sunburn.

Prostaglandins (PGs) appear to mediate some inflammatory skin reactions. When injected intradermally PGs have been shown to cause long-lasting redness [1-3]. PGs and PG-like substances have been isolated in perfusates from skin traumatized by thermal injury [4], allergic eczematous contact dermatitis [5-7], and ultraviolet light [8-11]. Mammalian epidermis contains the PG synthetase system and is capable of producing PGE_2 and $PGF_{2\alpha}$ from the biosynthetic precursor arachidonic acid [12, 13].

Nonsteroidal anti-inflammatory agents such as aspirin and indomethacin inhibit the PG synthetase system in skin and other tissue homogenates [13-16]. This property may explain their anti-inflammatory action. On the other hand, the effect of corticosteroid anti-inflammatory agents upon PG synthesis is controversial. Some workers [13, 17] have reported no inhibition of PGE_2 biosynthesis from arachidonic acid, while others [18] have reported inhibition of both PGE_2 and $PGF_{2\alpha}$ biosynthesis by steroids.

Systemic corticosteroid [19] and aspirin [20, 21] have both been reported to delay and decrease UV light-induced erythema or sunburn in humans. In guinea pigs, systemic nonsteroidal anti-inflammatory agents including aspirin can delay the development of redness in animals exposed to UV light [22]. When these agents were applied topically they could decrease the temperature of skin treated with UV [23]. Systemic or topical steroids were reported to have no effect upon UV erythema in guinea pigs [22, 23]. In this study we have examined the ability of intradermally injected steroids, PG inhibitors, and a PG antagonist to decrease or blanch UV light-induced redness in the skin of humans and guinea pigs.

Manuscript received August 16, 1973; in revised form September 14, 1973; accepted for publication September 21, 1973.

This paper was presented in part at the meeting of the Society for Investigative Dermatology, Atlantic City, New Jersey, April, 1973.

This work was supported by a grant from Westwood Pharmaceuticals.

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MATERIALS AND METHODS

Ultraviolet irradiation. A Westinghouse FS 20 sunlamp was the light source used throughout the study. The FS 20 emits UV irradiation (UVR) in the region of 280 nm to 350 nm with the major output between 290 nm and 320 nm. A minimal erythema dose (MED) is defined as the time of exposure to the FS 20 sunlamp which produces a discernible redness when the skin is examined 24 hr later. Twenty English short-hair female albino guinea pigs (weighing 300-600 gm) were anesthetized with sodium pentobarbital, clipped, and depilated. One hr after extensive rinsing of the depilated area with warm tap water and drying, an area on the backs of the guinea pigs received 3 MEDs of UVR. Ten human volunteers received 2.5 MEDs of UVR which was confined to a rectangular area on the volar surface of their forearms.

Intradermal injections. From 0 to 18 hr after UVR (including times before and after erythema was apparent) 0.05 ml of test compounds in neutral sterile saline (0.9%) and a sterile saline control were injected intradermally into the irradiated areas. In some experiments injections were also made into nonirradiated adjacent skin. In several cases injections were made before as well as after irradiation. The injection was considered intradermal only if a wheal was produced with the appearance of "peau d'orange." To minimize the effect of regional variation, the injection sites were randomized. Each subject served as his own control.

Grading system. The duration and intensity of the blanch (decrease in redness) which followed injection were recorded as a function of time. Blanch intensity in the injected areas was graded on a 0 to 3+ scale with 0 = no blanch, 1+ = faint blanch, 2+ = definite but not maximal blanch, and 3+ = maximal blanch (equivalent to the color of adjacent nonirradiated skin). To be considered a positive response, the blanch in the test site had to be greater than that at the saline control site. All doubtful responses were scored as zero. The duration of blanching was defined as the time elapsed between when the test site surpassed the saline control until the blanching was no longer detectable.

Measurement of vascular permeability. Two hr after exposure to FS 20 light 30 mg/kg body weight of a 2.5% solution of Evans blue dye in 0.9% saline was injected into the dorsal vein of the thigh of each of four guinea pigs. One hr after the dye injection a small area of nonirradiated skin was exposed to liquid chloroform for 1

min. If this area responded in a positive manner by turning blue, the animal was considered to be successfully injected with dye and is referred to as "blued." The "blued" guinea pigs were then injected intradermally with various test solutions into both nonirradiated skin and UV light-reddened skin. The extent of blanch and the degree of "blueing" were recorded with time.

Antagonism of PGE_2 action. To evaluate the effect of a test agent on the action of PGE_2, 100 μg of test agent in 0.05 ml of saline was injected intradermally into the volar forearm of three volunteers. Immediately afterwards 1.0 μg of PGE_2 in 0.05 ml of saline was injected into the wheal produced by the injection of the test agent and 1.0 μg of PGE_2 in 0.1 ml of saline was injected into a second site on the same forearm. Both sites contained equal amounts of PGE_2 in equal volumes and one site contained the test agent.

To rule out regional variation the experiment was repeated 24 hr later with the two injection sites interchanged. The intensity and diameter of the PGE_2-induced redness was compared between the two sites at 20 and 60 min after injection.

Test agents. The compounds evaluated in this study included: indomethacin (Merck, Sharp & Dohme), sodium acetylsalicylate (Analytical Reagent Grade), 7-oxa-13-prostynoic acid,† triamcinolone acetonide (Squibb Kenalog suspension), and hydrocortisone (Sigma Chemical Co.). The pH of all test solutions was confirmed as neutral before injection.

RESULTS

Effect on UV-reddened skin. The ability of the PG synthetase inhibitors, aspirin and indomethacin, to blanch skin reddened by UVR is demonstrated in both human and guinea pig skin by the data in Tables I and II. The blanched areas were approximately 1 cm in diameter and were apparent as soon as the fluid pressure at the injection site had dissipated—usually within 30 to 60 min after injection, based on the saline control site. The values reported are averages of all experiments performed, which include injections varying from 0 to 18 hr post UVR in humans and from 0 to 5 hr post UVR in the guinea pigs. In two subjects a comparison was made between injections of indomethacin at 3 and 18 hr post UVR. The extent and duration of the resulting blanched areas were greater when injections were made 3 hr after irradiation. When the blanch duration and intensity of all the subjects were plotted as a function of time of injection after UVR no correlation was evident. The average intensity (Tables I, II, and III) at each dose level was calculated by summing the highest blanch score attained for each test site and dividing that sum by the total number of blanched areas. The responses of humans and guinea pigs were strikingly similar. Guinea pig skin consistently showed a stronger blanch but the average duration was almost identical to that of human UV-reddened skin. A dose response is apparent for the PG synthesis inhibitors, with increasing amounts of aspirin and indomethacin causing greater blanching of the reddened skin with a longer duration. Of the two inhibitors indomethacin has the greater ability to blanch irradiated skin with a dose of 10 μg being approximately equivalent to 450 μg of aspirin. UVR erythema was delayed when the inhibitors of PG synthesis were injected before redness had occurred. Neither of these two compounds can completely inhibit erythema. With time the blanched areas became less intense and smaller. Eventually the blanched sites became as red as the surround-

† A gift of Dr. Josef Fried, University of Chicago.

TABLE I

Effect of intradermal indomethacin on UVR erythema

	Dose (μg)	Number of test sites	Number of blanched areas	Average intensity	Average duration (hr)
Human	0.1	5	1	1.0	1.5
Guinea pig	0.1	NT			
Human	1.0	5	5	1.5	3.8
Guinea pig	1.0	7	4	1.8	3.4
Human	10.0	7	7	1.8	5.6
Guinea pig	10.0	9	9	2.2	4.6
Human	50.0	3	3	2.0	5.0
Guinea pig	50.0	4	4	3.0	6.2
Human	100.0	11	11	2.0	5.0
Guinea pig	100.0	30	30	3.0	5.3

NT = Not tested

TABLE II

Effect of intradermal anti-PG agents on UVR erythema

	Dose (μg)	Number of test sites	Number of blanched areas	Average intensity	Average duration (hr)
Sodium Acetylsalicylate					
Guinea pig	4.5	5	2	1.5	2.0
Guinea pig	45	3	3	2.3	2.3
Guinea pig	450	10	10	2.6	3.2
Human	450	6	6	1.6	3.3
7-Oxa-13-Prostynoic Acid					
Guinea pig	1.07	8	4	0.5	1.2
Guinea pig	10.7	10	10	1.6	N
Guinea pig	21.4	7	7	2.3	N

N = Necrosis

TABLE III

Effect of intradermal steroids on UVR erythema

	Dose (μg)	Number of test sites	Number of blanched areas	Average intensity	Average duration (hr)
Triamcinolone Acetonide					
Human	125	10	10	2.7	24+
Guinea pig	125	18	4	1.2	4
Hydrocortisone					
Guinea pig	415	6	2	1.0	2

ing skin. After 24 hr only the needle tracks marked the sites of injection.

The PG antagonist 7-oxa-13-prostynoic acid was evaluated in guinea pigs from 0 to 1 hr after UVR and was found capable of decreasing redness. The antagonist also produced blanching in nonirradiated skin. The blanches induced by the PG antagonist were much smaller than those induced by aspirin and indomethacin. Unlike the inhibitor-induced blanches, these small white areas persisted and some became necrotic.

A species difference is apparent in response to intradermal injections of triamcinolone acetonide (Table III). Only in humans could the UVR-induced redness be decreased by this steroid. If the steroid were injected before redness was apparent the erythema could be totally prevented.

Alteration of vascular permeability. When the "blued" guinea pigs were injected intradermally with 450 μg aspirin or 100 μg indomethacin, a 1-cm blanch developed in the UV-reddened skin. A small deep-blue area was apparent at the center of the blanch where the needle had entered the skin. This small blue area was also present where saline and steroid were injected. There was no blanch associated with the last two-mentioned sites.

Injection of neutralized 7-oxa-13-prostynoic acid led to an immediate blanching of the site which quickly became blue at the 1.07 μg/0.05 ml dose of PG antagonist. At the 10.7 and 21.4 μg doses, the area of immediate blanch became surrounded by a peripheral ring or halo of blue dye. At the higher doses there was central necrosis of the skin.

Antagonism of PGE_2 action. A 100-fold by weight excess of indomethacin had no effect upon the intensity and area of redness induced by PGE_2. In one volunteer there was marked regional variation in the response to injected PGE_2 which averaged out when the experiment was repeated with the sites interchanged.

DISCUSSION AND CONCLUSIONS

This study has demonstrated that known inhibitors of PG synthesis can decrease the intensity and delay the development of UVR-induced erythema in humans and guinea pigs. It is noteworthy that indomethacin was approximately 45 times more effective than aspirin since Vane [14] has shown a similar relative efficacy for these two compounds in their ability to inhibit PG biosynthesis.

PG has been shown to be rapidly metabolized near its site of synthesis [24–26]. If PG is responsible for the UV-induced redness, then either PG must be continually synthesized following the UV trauma to maintain the redness, or the action of PG must be long-lasting so that PG itself need not be present. We could not demonstrate antagonism of the action of PGE_2 by indomethacin. As indomethacin can inhibit the synthesis of PG from arachidonic acid [13, 16], its ability to blanch UVR-induced redness when injected up to 18 hr after UVR suggests that PG is probably synthesized continuously for some time after the skin is exposed to UV light. Since indomethacin is an irreversible inhibitor of the PG synthetase [15], it should be able to stop the production of PG until new synthetase has been made. The duration of the indomethacin-induced blanch would reflect the metabolism and diffusion of indomethacin as well as the amount of time necessary to produce new synthetase.

The PG antagonist 7-oxa-13-prostynoic acid has been shown to competitively inhibit the PG receptor site in smooth muscle [27]. Its ability to blanch UVR-reddened skin as well as nonirradiated skin with resultant necrosis and its ability to increase the skin's vascular permeability are consistent with the actions described for primary irritants [28]. Because the highest concentrations of aspirin, indomethacin, and steroids which we used did not cause an increase in vascular permeability, we believe these agents are not blanching the skin by inducing local edema and fluid pressure artifacts.

It is not yet clear what role steroids play in PG-mediated inflammation. Both the guinea pig and human are considered steroid unresponsive with regard to lymphocytolysis, but there are obvious differences in their response to steroid inhibition of UVR-induced redness as seen in our data and those of others [22, 23]. Steroids have been shown to stabilize lysosomes against UVR damage [29] and to antagonize the action of intradermally injected PG [30], possibly by a direct vasoconstriction of the blood vessels. Steroids may inhibit the release of phospholipases from the lysosome and thus regulate the amount of arachidonic acid liberated from the phospholipid in cell membranes [31, 32]. Our data show that steroids can decrease UVR-induced redness in human skin if they are injected intradermally at any time up to at least 18 hr after UVR.

It is not possible to prove that PG has a role in UVR-induced redness solely by showing that specific PG inhibitors can block or blanch UVR-induced erythema. There are other possible roles which these compounds can play that might account for the data presented here. We have shown that these PG inhibitors do not act by increasing vascular permeability. They could possibly exert their effect by causing vasoconstriction of the blood vessels. The direct measurement of PG in the skin before and after UVR and in the presence and absence of these inhibitors would be more definitive. Until such data are available, our results add to the growing body of evidence which implicates PG as a mediator of the sunburn response of skin to UV light.

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