Ineffectiveness of Aprotinin on Psoralen-UV-A-(PUVA)-Induced Erythema

EILEEN WEINTRAUB, M.D., DENNIS P. WEST, M.S. AND LAWRENCE M. SOLOMON, M.D.

Department of Dermatology, Abraham Lincoln School of Medicine, and the Departments of Pharmacy Practice and Hospital Pharmacy Services, College of Pharmacy (DPW), University of Illinois at the Medical Center, Chicago, Illinois, U.S.A.

Because bradykinin constitutes a possible candidate for mediation of topical 8-methoxypsoralen-UV-A (PUVA)-induced erythema, aprotinin (TrasyloI), inhibitor of kallikrein and interrupter of the cascade leading to kinin production was assessed in guinea pigs. Response was assessed at 24, 48, and 72 hr after topical PUVA and there was no significant difference between normal saline and aprotinin by intradermal or intraperitoneal routes of administration. The results of this study indicate that intradermal and intraperitoneal aprotinin, in the dose and method tested, is not capable of significantly decreasing erythema induced by topical PUVA in guinea pigs.

Indomethacin has been shown to be capable of inhibiting UVB-induced erythema in guinea pigs and humans [1,2]. This finding is significant in that prostaglandins can be implicated as mediators of UVB-induced erythema. Erythema produced by UVB radiation and that produced by PUVA after administration of psoralen (PUVA) are dissimilar in that the latter has a significantly later onset and peaks at approximately 48 hr. Neither topical, intradermal or oral indomethacin is capable of diminishing the inflammatory response produced by PUVA [3]. Therefore, it is probable that substances other than prostaglandin are mediators of this inflammation.

Bradykinin, a nonapeptide, is a vasoactive mediator in some inflammatory reactions. Plasma kinin is derived from the alpha globulin, kininogen, by activation of plasma kallikrein. Similarly, tissue kallidin is formed from a tissue kininogen which is converted to the active kinin by cleavage of its N-terminal lysine. Kallikrein is itself converted from its zymogen, prekallikrein, by the Hageman Factor or a fragment thereof.

Kinins have been associated with arteriolar vasodilation and increased vascular permeability, as well as with increased leukotaxis and contraction of smooth muscle. Dose for dose, bradykinin has been shown to be more potent than a vasoconstrictor than either PGE1 or PGE2 when injected intradermally into guinea pigs and rabbits [4,5], while PGF1 and PGF2 are less potent. Since a major histological change in the dermis of PUVA-treated skin is increased permeability and vasodilation of superficial blood vessels [6], bradykinin constitutes a possible candidate for mediator of the reaction.

Aprotinin (TrasyloI) is a 6,511 MW, 58 amino acid peptide diger derived from bovine lung. Available as 10,000 kallidogenase inactivating units per ml, it is capable of inhibiting the action of a number of proteolytic enzymes, including kallikrein, and thus interrupts the cascade leading to kinin production [7]. It has been primarily studied as an antitrypsin agent in acute pancreatitis, but its anti-inflammatory properties have also been investigated with some success in treatment of anaphylaxis [8], osteoarthritis [9], chronic urticaria [10], and acne [11]. It has been shown to reduce rat paw edema induced by subaponeurotic kaolin injections in rats [12], to inhibit the Schwartzman phenomenon in rabbits [13] and to inhibit inflammation secondary to experimental burns in rats [13].

For these reasons, we felt it would be of interest to investigate the ability of aprotinin to inhibit the erythema induced by topical PUVA.

MATERIALS AND METHODS

Ultraviolet Irradiation

Two Westinghouse F15T8 BLB 40 w bulbs were used as the energy source and mounted in a standard desk top fluorescent fixture covered with aluminum foil. The BLB bulb has a blue filter tube which emits radiation between 310 and 420 nm with an emission peak at 355 nm. The UVA output of the bulbs, as measured by an International Light, Model IL442 radiometer, was 5.2 mw/cm2 at 2 cm below the bulb surface. The UVB output, measured by an International Light, Model IL700 radiometer, was 2 mw/cm2 at the same distance (UVA < 0.39% of UVA).

Preparation of Animals

Sixteen male, short hair, albino guinea pigs were used in the study. Each animal was 6-7 mo old and weighed an average of 650 gm (532-752 gm). The dorsal surface of each animal was clipped and depilated with calcium hydroxide, sodium thioglycolate and calcium thioglycolate cream (Nair, Carter Products) and then thoroughly rinsed and dried. After waiting 24 hr to minimize the effect of any occult tissue irritation, 2 mg of 8-methoxypsoralen (8MOP) was dissolved in 3 ml of 95% ethyl alcohol and evenly distributed over a 4 X 10 cm area. Two hours later, a UVA sunscreen (2-ethoxy-ethyl p-methoxycinnamate 4% and menthol anthranilate 4% cream, Maxafil, Texas Pharmaceutical Co.) was evenly applied to all exposed skin except for 2 rows of three 1 X 1.75 cm areas. The areas were symmetrically placed 0.5 cm from either side of the spine. The animals were immediately placed in thin, chicken-wire cages so that their backs were 2 cm beneath the surface of the bulbs. They were then exposed to light as described.

Determination of the MPD

The minimal phototoxic dose (MPD) is defined as the minimal amount of UVA radiation, given 2 hr after 8MOP, required to produce erythema with definite margins at 48 hr. In order to determine the MPD, 2 animals were prepared as described above. Five areas on each animal were exposed to 2-10 J/cm2 of UVA at increments of 2 J/cm2. After determining the MPD, the response at the other sites was graded as follows: 0 = no detectable erythema, 1* = erythema only, 2* = erythema plus edema, 3* = erythema plus vesiculation, 4* = necrosis. The 6th area on each animal was not exposed to UVA light to insure that nothing in the preparation of the animals was, by itself, capable of producing erythema. Also, a third guinea pig was exposed to UVA light without 8MOP for a period of time equivalent to 2 X MPD of the 2 animals treated with both 8MOP and light.

Intradermal Aprotinin

Five guinea pigs were prepared as described above. Immediately prior to irradiation, 1 row of 3 exposure sites was injected intradermally with 1,000 units (0.1 ml) of aprotinin. The 3 adjacent areas were injected with 0.1 ml of normal saline control. The peau d'orange produced in each case covered approximately 3/4 of the exposure area. Each animal was then positioned under the fluorescent bulbs and exposed to 2 X MPD. Four of the 6 sites were reinjected at 2 hr postexposure and 2 of...
these 4 sites were reinjected at 24 hr postexposure. Response was graded from 0-4* at 24, 48 and 72 hr.

Intraperitoneal Aprotinin

Six animals were depilated and treated with 8MOP and Maxafil as before except that the dorsal surfaces were divided longitudinally along the spine so that only half the area was used at a time. One hour before exposing the animals to UVA light, each received 0.65 ml of normal saline control intraperitoneally. They were reinjected at 2 and 24 hr postexposure. The response was rated as before at 24, 48 and 72 hr.

Once these determinations were made, the other halves of the animals' skins were prepared and 6,500 units (0.65 ml) of aprotinin (10,000 units/kg/dose) was injected one hour prior to and 2 and 24 hr following UVA exposure. The response was rated at 24, 48 and 72 hr. A 7th animal underwent the procedure for intraperitoneal aprotinin but received 2 ml of normal saline and 20,000 units (2 ml) of aprotinin (30,800 units/kg/dose) at appropriate intervals instead of the previous 6,500 units (0.65 ml). In addition to the 2 and 24 hr reinjection times, the animal was also injected at 12 and 36 hr at the increased dose.

RESULTS

The responses produced at the different sites on the guinea pigs' backs at 24, 48 and 72 hr after intradermal or intraperitoneal injection with normal saline versus aprotinin were evaluated (see the Figure). A visible response was produced at all exposure sites at 48 hr, but no statistically significant difference was found between the sites injected with aprotinin and those injected with normal saline. In addition, there was no significant difference at 24 and 72 hr, suggesting that the time course of the erythema production was unaffected by aprotinin. The 2 animals treated with 8MOP and no UVA light and the one animal treated with UVA light and no 8MOP showed no erythema.

DISCUSSION

The results of this study indicate that aprotinin, in the dose and method tested, is not capable of significantly decreasing the erythema induced by UVA radiation after topical 8MOP in guinea pigs. Also, exposure to the radiation source without 8MOP produced no response at any of the 6 sites on one guinea pig and substantiated that the 2 µw of UVB from the radiation source was not a factor in the production of erythema. Likewise, topical 8MOP alone did not induce erythema.

Aprotinin has a rather short serum half-life of 150 min [14]. It is possible that the drug was not present at an effective level at the proper moment to inhibit erythema. However, when the dose and frequency of injection were increased in 1 animal receiving intraperitoneal aprotinin, there was no additional reduction in erythema.

The inability of aprotinin to significantly inhibit PUVA-induced erythema does not entirely rule out kinins as a partici­

Comparison data of responses at paired sites in guinea pigs with aprotinin and 8MOP versus normal saline and 8MOP (mean values are shown with mean ± SD).

REFERENCES


* By Students' paired t-test, not significant at p = 0.05.


