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REPORTS

PROSTAGLANDIN AND DNA SYNTHESIS IN HUMAN SKIN: POSSIBLE RELATIONSHIP TO ULTRAVIOLET LIGHT EFFECTS

WILLIAM H. EAGLSTEIN, M.D., AND GERALD D. WEINSTEIN, M.D.

Department of Dermatology, University of Miami School of Medicine, Miami, Florida

The effect of prostaglandin E_2 (PGE_2) on DNA synthesis in human skin was evaluated. PGE_2 (1 μg) was injected intradermally into normal buttock skin of 15 volunteers followed by tritiated thymidine for autoradiographic quantitation of DNA synthesizing cells. Controls of normal saline, histamine (50 μg), and lower doses of PGE_2 were also injected into 8 of the volunteers.

Forty-eight hours after injection of 1 μg and 0.1 μg PGE_2 there was a 264% and 62% increase, respectively, in the number of DNA synthesizing epidermal cells/high-power field as compared to saline controls. These differences were statistically significant ($p < 0.01$). Histamine (50 μg) produced a statistically significant 36% higher labeling index compared to its saline controls ($p < 0.05$).

Many types of skin injury, including ultraviolet light (UVL) irradiation, produce an increase in the number of DNA synthesizing cells about 48 hr after the stimulus. Our findings suggest that PGE, a putative mediator of UVL-induced inflammation, may be one of the chemical mediators for the UVL-induced increase in DNA synthesizing cells. Histamine may also contribute to the increase in DNA synthesizing cells following UVL-induced inflammation.

The prostaglandins are fatty acids which are found in many tissues and have a wide variety of physiologic actions. Prostaglandins of the E series (PGE) cause long-lasting redness when injected into skin [1]. They are synthesized by human skin [2] and have been implicated as mediators of ultraviolet light (UVL)-induced inflammation. Prostaglandin-like chemicals have been identified in dermal perfusates from UVL-irradiated skin [3] and PGE has been identified in homogenates of UVL-irradiated skin [4]. Drugs such as indomethacin and aspirin, which inhibit the PG synthetase system [5], will delay and decrease UVL-induced inflammation [6].

UVL irradiation of normal skin causes an initial decrease in the number of epidermal DNA synthesizing (S-phase) cells followed by a 3- to 6-fold increase in the number of [^{3}H]thymidine-labeled (S-phase) cells at 48 hr after irradiation compared to unirradiated skin [7].

The relationship between UVL-induced inflammation and UVL-induced changes in DNA synthesis is not understood. If PGE mediates UVL-induced inflammation, does it also mediate UVL-induced changes in DNA synthesis? To study this question, PGE_2 was injected into normal human skin and its effects on DNA synthesis were evaluated by autoradiography and the similarity of these effects to those found following UVL irradiation were compared.

MATERIALS AND METHODS

PGE_2, following confirmation of purity by thin-layer chromatography, was diluted in saline for intradermal injections of 0.05 ml in amounts of 1 μg, 0.1 μg, 0.01 μg, and 0.0001 μg. Intradermal injections of 0.05 ml of saline and histamine (50 μg/0.05 ml saline) were used as controls. Fifteen men and women between 45 and 92 years of age, after signing informed consent forms, participated in the study. The injections were performed with tuberculin syringes and 26-gauge needles on normal-appearing buttock skin. Each injection produced a distinct wheal indicating delivery high in the dermis. The injection test sites were separated from one another by at least 2.5 cm. At the appropriate time after administration of the test agent, [^{3}H]thymidine (sp act 6.0 Ci/mM, 5 μCi/0.1 ml of saline) was injected into each test site. Four-millimeter punch biopsy specimens were obtained from the test sites 30 to 45 min following the injection of the [^{3}H]thymidine. The specimens were fixed in Bouin's solution and prepared for histologic and autoradiographic analysis as previously described [8].

Autoradiographs were examined for heavily labeled

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Reprint requests to: Dr. W. H. Eaglstein, Department of Dermatology, University of Miami School of Medicine, P. O. Box 520875, Biscayne Annex, Miami, Florida 33152.

cells (more than 10 grains) in the interfollicular epidermis. Between 40 and 50 high-power fields (HPF) (ocular 10×, objective 40×) of each specimen were examined. Each HPF was 0.25 mm in surface length and contained approximately 50 basal cells along the interfollicular epidermis. The results are expressed as the number of heavily labeled cells per HPF (labeling index, L. I.)

Three subjects each received 5 injections of 1 μg PGE_2 and 5 injections of saline at different sites. At 0.5, 1.5, 3, 24, and 48 hr after the PGE_2 and saline injections. [^{3}H]thymidine was injected into these test sites followed by biopsies. Two subjects were studied only during the first three time periods. Ten different subjects were injected with saline and 1 μg PGE_2, followed by [^{3}H]-thymidine and biopsy 48 hr later. Eight of these subjects were given additional injections of PGE_2 in lower concentrations (0.1, 0.01, or 0.0001 μg/0.05 ml) and an injection of histamine solution (50 μg/0.05 ml) for testing at the 48-hr time period.

RESULTS

Clinically, all injections of PGE_2 produced redness within 2 to 6 min. The area of redness following 1 μg of PGE_2 was oval, reaching a maximum diameter of 2.5 cm within 15 min and persisting approximately 5 hr. The lower doses of PGE_2 produced a smaller area of redness which resolved sooner than the 1-μg dose. Red streaks or "tails" extended from the oval PGE_2-induced areas of redness. The histamine injections produced immediate redness and marked whealing. The histamine-induced redness was less intense than the PGE_2-induced redness, extended over a larger area (5 cm diameter), and resolved within 2 hr. The saline injections did not produce redness.

Histologically, there was no apparent difference in either the thickness of the epidermis or the configuration of the rete ridges among the specimens injected with PGE_2, histamine, and saline.

The thymidine labeling indices showed no significant differences between PGE_2-injected and saline-injected sites over the first 24 hr. The labeling indices produced by the saline control injection sites were the same over the first 3 hr, but increased slightly at 24 and 48 hr after injection (Tab. I).

Of major interest was the observation that at 48 hr after injection there was a 264% increase in the number of labeled cells per HPF in the 1 μg PGE_2 sites as compared to their saline controls. This difference is statistically significant, $p < 0.01$ (matched-pair comparison). The lower dose of 0.01 μg PGE_2 caused a 62% increase in the labeling index at 48 hr ($p < 0.01$, matched-pair comparison). Histamine (50 μg) produced a 36% higher labeling index compared to its saline controls ($p < 0.05$, matched-pair comparison) (Tab. II).

TABLE I. *Mean labeling indices* in human skin at various hours following intradermal injection of 1 μg PGE_2 and saline control*

	Hours after injection				
	0.5	1.5	3	24	48
Number of subjects	5	5	5	3	13
Mean L.I.: saline	1.7	1.8	1.7	2.5	3.1
Mean L.I.: PGE_2 1 μg	1.4	2.1	1.7	3.2	8.2

* Labeling index (L.I.) = number of tritiated thymidine labeled epidermal cells/high-power field (50 basal cells).

TABLE II. *The effects of intradermal PGE_2, histamine, and saline on labeling indices at 48 hr after injection*

	Number of labeled cells per high-power field					
	Saline	PGE_2				Histamine 50 μg
		1 μg	0.1 μg	0.01 μg	0.0001 μg	
Pt. # 1	4.8	12.1				
2	2.7	20.0				
3	5.1	12.3				
4	2.9	6.4				
5	2.2	8.8				
6	2.1	5.2	5.5			3.5
7	3.1	4.1	3.8	7.7	1.8	3.2
8	4.7	10.7		5.5	4.7	6.8
9	2.9	4.8		4.1	3.4	3.0
10	3.4	7.7		5.5	4.5	2.6
11	2.5	8.5		5.6	1.8	4.9
12	1.8	0.6	6.5	4.0		2.6
13	1.6	5.2	3.0	1.9		3.6
Mean	3.1	8.2	4.7	4.7	3.2	3.8
Standard error	0.32	1.3	0.8	1.1	0.7	0.4

DISCUSSION

We have demonstrated that an erythema-producing intradermal injection of PGE_2 into normal skin is associated with an increase in epidermal DNA synthesizing cells at 48 hr following injection. This is the same time at which an increase in S-phase cells occurs in UVL-irradiated skin. The prostaglandin doses used in this study are well within ranges reported in UVL-damaged skin [4]. The lowest doses which increased the number of S-phase cells (0.1 and 0.01 μg) are below those reported in skin [9, 10].

An increase in human epidermal S-phase cells at 24 to 48 hr can be demonstrated following several different stimuli: UVL irradiation [7], Scotch-tape stripping [11], incisional wounding [12], and friction [13]. A similar increase in the number of S-phase cells also has been noted in certain animal models with specific timed increases following stimuli such as skin friction [14], wounding [15], isoproteranol injections into mice for salivary gland cell proliferation [16], and mouse hepatocellular injury. These observations suggest that cell proliferation in response to certain stimuli requires a finite time period to develop.

One can only speculate at this time on how PGE_2 administration affects the epidermal cell cycle.

Since the labeling index did not decrease but remained relatively normal from 1 to 24 hr following PGE_2 injection (Tab. I), there does not appear to be a block in the movement of cells from G_1 into the S-phase. However, at 48 hr following PGE_2 injection, a "burst" of cells appears in the S phase suggesting either an abrupt shortening of the G_1 period or stimulation of G_1 cells into the S phase as a synchronous group. Theoretically it could also reflect a stimulation of G_0 cells into the S phase at 48 hr, but as yet there is no evidence supporting the existence of G_0 cells in human epidermis.

The failure to find an initial decrease in the number of epidermal DNA synthesizing cells following PGE_2 injection supports the suggestion that the decreased number of S-phase epidermal cells seen within the first few hours after UVL irradiation is due to a direct damaging effect of UVL on epidermal DNA [17].

At the molecular level, the prostaglandins and cyclic nucleotides appear to modulate various cellular functions. In general, prostaglandins of the E series stimulate an increase in the levels of cyclic AMP (cAMP); however, the relationship between cAMP and DNA synthesis is variable. Increased DNA synthesis is associated with increased cAMP levels in rat salivary gland following isoproteranol injection [18], whereas increased DNA synthesis is associated with decreased cAMP levels in fibroblastic cell cultures stimulated with GMP [19]. Cultured fibroblasts are associated with varying cAMP levels throughout the cell cycle but cGMP increases are found in the early G_1 phase [20]. A recent review of the literature [21] with special emphasis on model systems employing thymocytes and regenerating liver cells concludes that increased DNA synthesis follows an increase in cAMP levels.

When normal and psoriatic epidermal cells were studied in vitro, the addition of dibutyryl cAMP in high concentrations inhibited mitoses [22,23]. However, in the rapidly proliferating psoriatic epidermis, both increased [24,25] and decreased [26] levels of cAMP have been reported, leading to conflicting interpretations of the relationship between cAMP and mitotic behavior. Prostaglandins of the E series are reported in in vitro epidermal cell systems to increase cAMP levels [27,28]. Since cAMP concentrations were not measured in the present in vivo studies, we are unable to clarify the relationship between our data and the cAMP effects of DNA synthesizing cells.

As PGE_2 plays a role in the inflammatory response to UVL irradiation, our present findings suggest that PGE_2 may be one of the mediators of the increased number of S-phase cells seen following UVL irradiation. Histamine has also been reported in dermal perfusates of UVL-irradiated skin [29]. Since an increased number of S-phase cells was found 48 hr following histamine injection, it is possible that histamine also contributes to the increased number of DNA synthesizing cells following UVL irradiation. However, the dramatic whealing from our histamine injection of 50 μg is not seen in the skin following UVL irradiation. The demonstration that defined chemical molecules are capable of stimulating proliferative cell activity in the skin may be significant for future biochemical and cytokinetic studies.

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