Effects of Systemic Indomethacin, Meclizine, and BW755C on Chronic Ultraviolet B–Induced Effects in Hairless Mouse Skin

Irene E. Kochevar, Michael Moran, Nancy Lyon, Thomas Flotte, Elizabeth Siebert, and R. William Gange
Wellman Laboratories of Photomedicine, Department of Dermatology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, U.S.A.

Chronic exposure of hairless mice to ultraviolet B (UVB) radiation is associated with inflammation as well as an altered macromolecular composition of the dermis. This study was designed to determine whether or not various systemic anti-inflammatory agents inhibit chronic UVB-induced changes in the macromolecular content of the dermis and, if so, whether each agent had the same or different effects. The agents and doses were chosen for their ability to inhibit the changes induced by a single exposure to UVB radiation (increased vasopermeability, neutrophil accumulation, and skin-fold thickness). Indomethacin, a cyclooxygenase inhibitor, and meclizine, an H_1 histamine receptor antagonist, were administered from slow-release pellets. BW755C, a combined cyclooxygenase and lipoxygenase inhibitor, was administered intraperitoneally 30 min prior to UVB exposure. Animals were exposed to UVB three times per week for 20–26 weeks or were unirradiated. The elastin, glycosaminoglycan and collagen content of the skin were determined by measuring the desmosine, uronic acid, and hydroxyproline levels, respectively. The amount of each macromolecule per area of skin increased after chronic UVB exposure. The increase in desmosine was inhibited by indomethacin; the increase in hydroxyproline was inhibited by meclizine and BW755C. None of the agents inhibited the uronic acid increase. These results suggest that chronic inflammation contributes to the dermal changes seen in chronically UVB-exposed skin and that different inflammatory mediators are involved in the increases observed in elastin, glycosaminoglycans, and collagen. *J Invest Dermatol 100:186–193, 1993*

Age-related changes in skin result from two distinct but concurrent processes. *Intrinsic aging* is assumed to result from a genetically programmed sequence of events, whereas extrinsic aging is dominated by *photoaging,* which is the response to cumulative injury induced by years of exposure to ultraviolet radiation (UVR) from the sun. The changes induced by chronic UVR exposure of human and hairless mouse dermis are similar. Histologically, photoaged dermis shows marked increases in glycosaminoglycans (GAGs) and elastic tissue, degeneration of the elastin into an amorphous mass, decreased staining of collagen, increased numbers of dermal fibroblasts and mast cells, and appearance of inflammatory infiltrate [1–5]. Biochemically, the amount of collagen appears to increase or decrease depending on the amount of exposure to UV radiation [6,7]. Elastin, measured as desmosine, is markedly increased in photoaged human and mouse skin [7,8]. The total uronic acid content of photoaged mouse skin and human skin increases greatly compared to age-matched control skin [7,9]. Action spectrum studies in hairless mice indicate that ultraviolet B (UVB) radiation (290–320 nm) more efficiently produces these changes than longer-wavelength optical radiation [10].

These changes in photoaged skin have been attributed to the inflammation induced by each exposure to UVR. Several observations are consistent with this hypothesis including the presence of inflammatory cells in photoaged skin [1,2,4] and the inhibition of chronic UVB-induced changes in the histology of hairless mouse skin by topical application of the anti-inflammatory agents hydrocortisone and naproxen [11].

This study was designed to determine whether *systemic* anti-inflammatory agents are able to inhibit changes induced by chronic UVB exposure of hairless mouse skin by measuring several dermal macromolecules. In addition, anti-inflammatory agents were selected that inhibit different inflammatory pathways to determine whether selective inhibition of the UVB-induced changes in macromolecular content could be detected.

MATERIALS AND METHODS

Animals Female, hairless albino mice (Skh-hairless-1), 6–7 weeks old, were obtained from Charles River Laboratores. Mice were identified by ear notches and housed in groups of five.

Chemicals Meclizine and indomethacin pellets were obtained from Inovative Research (Toledo, OH). BW755C was a gift of the Burroughs Wellcome Co. (Beckenham, UK). Evans blue and hexadecyltrimethylammonium bromide were obtained from Sigma (St. Louis, MO). Desmosine, antidesmosine antiserum, and Bolton Hunter–labeled desmosine were obtained from Elastin Products

Manuscript received March 3, 1992; accepted for publication November 9, 1992.
This work was presented in part at the Annual Meeting of the Society for Investigative Dermatology, Seattle, WA, May, 1991.
Reprint requests to: Dr. Irene E. Kochevar, Wellman Laboratories of Photomedicine, Massachusetts General Hospital, 37 Fruit Street, Boston, MA 02114.

Abbreviations:
GAG: glycosaminoglycan
ip: intraperitoneal
UVB: ultraviolet B
UVR: ultraviolet radiation

0022-202X/93/$06.00

Table I. Fluences of UVB Radiation (mJ/cm²) Administered at Each Exposure for Chronic Exposure Studies

	Week																		
Experiment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	End
1 (5.1 J/cm², 26 weeks)	12.5	25	37.5	50		→		60	70			→		80		→			100	→
2 (4.8 J/cm², 22 weeks)	12.5	25	40	60	80		→		100						→					
3 and 4 (6.8 J/cm², 20 weeks)	30	40	55	75	→		100			→			130	→	180			→		

Co. (Owensville, MO). Goat anti-rabbit antiserum was from Organon Teknika Corp. (West Chester, PA). Chloramine T, 30% H_2O_2, Bis-Tris buffer, and 4-hydroxyproline were from Sigma (St. Louis, MO). o-dianisidine dihydrochloride was obtained from Eastman Kodak (Rochester, NY) and 4-(dimethylamino)-benzaldehyde was from Aldrich (Milwaukee, WI). Soluene 350 was from Packard Instrument Co. (Downers Grove, IL). Perchloric acid was from J.T. Baker, Inc. (Phillipsburg, NJ).

Administration of Agents Pellets were implanted through an incision just anterior to the scapulae and were placed to rest over the skull. Pellets were implanted every 3 weeks at least 2 d prior to an irradiation. BW755C was administered as an intraperitoneal (ip) injection in 1:5 ethanol:saline 30 min prior to irradiation.

Irradiation Mice were irradiated in separate chambers (3 cm × 1.25 cm plexiglass stalls covered with a wire mesh) to ensure uniform radiation of the dorsal surface and placed under a bank of 12 UVB-Ho-90° bulbs (Elder Pharmaceuticals Inc.). The height of the UVB bulbs was adjusted to provide 0.5 mW/cm² at the dorsal surface of the mice as measured with an IL-1700 radiometer equipped with a SED 240 UVB detector (International Light Inc., Newburyport, MA) prior to each irradiation. The spectrum of the bulbs was measured periodically with a spectroradiometer (Model 742 Optronix Laboratories Inc., Orlando, FL). The spectral output was 76.8% between 290 and 320 nm, 19.7% between 320 and 400 nm, and 3.5% between 200 and 290 nm. Mice were irradiated on Monday, Wednesday, and Friday for periods between 20 and 26 weeks. Three schedules were followed as shown in Table I. Each group contained ten mice.

Assays for Acute UVB-Induced Changes Measurements were made of skin-fold thickness, vascular permeability, and neutrophil accumulation 24 h after a single exposure to UVB radiation. Briefly, the skin along an imaginary line from axilla to hip was pulled up, and its thickness measured with a spring-loaded micrometer (Fowler, Biggsfield, UK). Vascular permeability was quantitatively assayed by the leakage from vessels of an albumin-bound anionic dye, Evans Blue. Evans Blue (1%, 10 μl/g body weight) was injected via the tail vein; 4 h later the animals were killed and the dorsal skin removed and biopsied. Biopsies were solubilized and the optical absorbance of Evans Blue was measured at 620 nm. Neutrophils accumulating in skin were quantified by measuring myeloperoxidase activity in homogenized biopsies with the method of Bradley *et al.* [12].

Preparation of Skin Samples After the mice were killed by ether inhalation, the skins were removed and spread on a sheet of dental wax. All measurements were made on non-tumor-bearing areas of skin. Two punches (3 mm) were taken for light microscopy and frozen sections. For the desmosine and the hydroxyproline assays, a single 4-mm or two 2-mm biopsy punches were taken, weighed, and hydrolyzed (6 N HCl) at 110°C for 24 h. The hydrolysate was evaporated to dryness at 55°C under a stream of N_2, then dried under vacuum overnight. For uronic acid measurements in experiments 1 and 2, the remainder of the skin was weighed and digested overnight at 60°C in a papain solution (1 mg/ml papain, 5 mM cysteine, 5 mM EDTA in sodium acetate buffer, pH 6.0). Samples were centrifuged and the fatty layer was removed and discarded. The aqueous layer was analyzed for uronic acid content. For experiments 3 and 4, three 6-mm punch biopsies were taken and digested in 1 ml of the same mixture overnight. The following morning, an additional 0.5 ml papain solution added and incubation continued for 4 h. The digest was then centrifuged at 500 × *g* and the supernatant was dialyzed against distilled water for 24 h with two changes of water. The dialyzed samples were assayed for uronic acid content.

Desmosine Desmosine was measured by a modification of the radioimmunoassay method of Starcher [13]. In experiments 1 and 2, after incubation of the re-dissolved hydrolysate with probe and antibody solution, the bound probe was immobilized on nitrocellulose filters, washed, and counted in a gamma counter. For experiments 3 and 4, after incubation the mixture was further incubated with 100 μl of goat anti-rabbit antiserum diluted 32 times at 4°C for 2 h. Polyethyleneglycol (MW 8000, 15%, 100 μl), was added and the mixture was centrifuged at 300 × *g* for 1 h. The pellets were counted on a LKB 1272 gamma counter.

Uronic Acid Glycosaminoglycan content of skin was measured by uronic acid analysis according to the method of Bitter and Muir [14].

Hydroxyproline Hydroxyproline was assayed by a modification of the method of Stegemann and Stalder [15].

Histology Biopsy specimens were fixed in formaldehyde-cetylpyridinium chloride. Four-micrometer sections were cut from paraffin blocks. The sections were stained with hematoxylin and eosin for evaluation of the inflammatory infiltrate, the epidermis, and the cellular constitutents of the dermis. Resorcin fuchsin-stained sections were utilized for evaluation of elastic fibers. Colloidal iron stain was used to evaluate GAG content and distribution. The thickness of the epidermis was measured with an ocular micrometer. Mast cells were counted on resorcin fuchsin-stained sections. An eyepiece micrometer with a 10 × 10 square grid (0.25 mm/side) was used. The micrometer was aligned with one edge along the dermal/epidermal junction, and all mast cells falling within the grid were counted. The micrometer was moved along the length of each section so that an average of 10 fields were counted per section. Three sections per group were counted.

Neutrophils were counted on 5-μm frozen sections fixed in acetone, and stained with benzidine dihydrochloride (0.3% in 50% ethanol with 1% sodium acetate, 2.1% H_2O_2, pH 6.0). Safranin O was included in the primary stain. Slides were counterstained with 1% methyl green [16]. Neutrophils were counted as described above for mast cells.

Tumors Any mass with a greater than 1.5-mm diameter was tentatively considered to be a tumor. Histologic identification of tumor type was not made.

Statistics Two-tailed, non-paired Student t test was used to evaluate differences between experimental groups.

RESULTS

Effects of Agents on Acute Responses of Skin to UVB To select the proper doses of anti-inflammatory agents to be used for the chronic UVB-exposure studies, the influence of indomethacin,

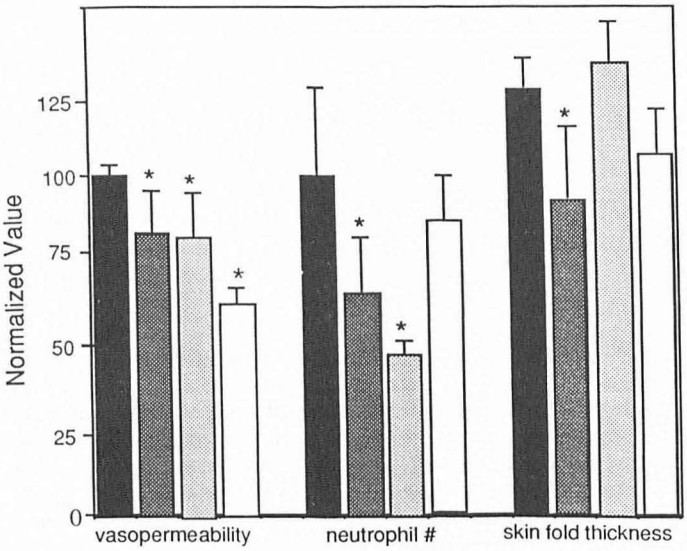

Figure 1. Summary of the effects of anti-inflammatory agents on acute UV-induced changes in hairless mouse skin. The values for vasopermeability, number of neutrophils, and skin-fold thickness are normalized to 100 for the irradiated, non-drug-treated control animals. These control groups are shown as *solid bars*. All values shown are for irradiated animals. Groups treated with 0.10 mg/pellet indomethacin are shown as *dark shaded bars*, groups treated with 1.5 mg/pellet meclizine (or 0.25 mg/pellet for vasopermeability) are shown as *light shaded bars*, and groups treated with 30 mg/kg BW755C are shown as *open bars*. The anti-inflammatory agents did not alter the values for non-UVB-exposed groups compared to the no-drug, no-UVB control groups except for indomethacin, which decreased the skin-fold thickness (see *Results*). The values shown are mean values ± SD values for groups of five mice. *Asterisks*, $p < 0.01$ compared to the irradiated, non-drug-treated control groups.

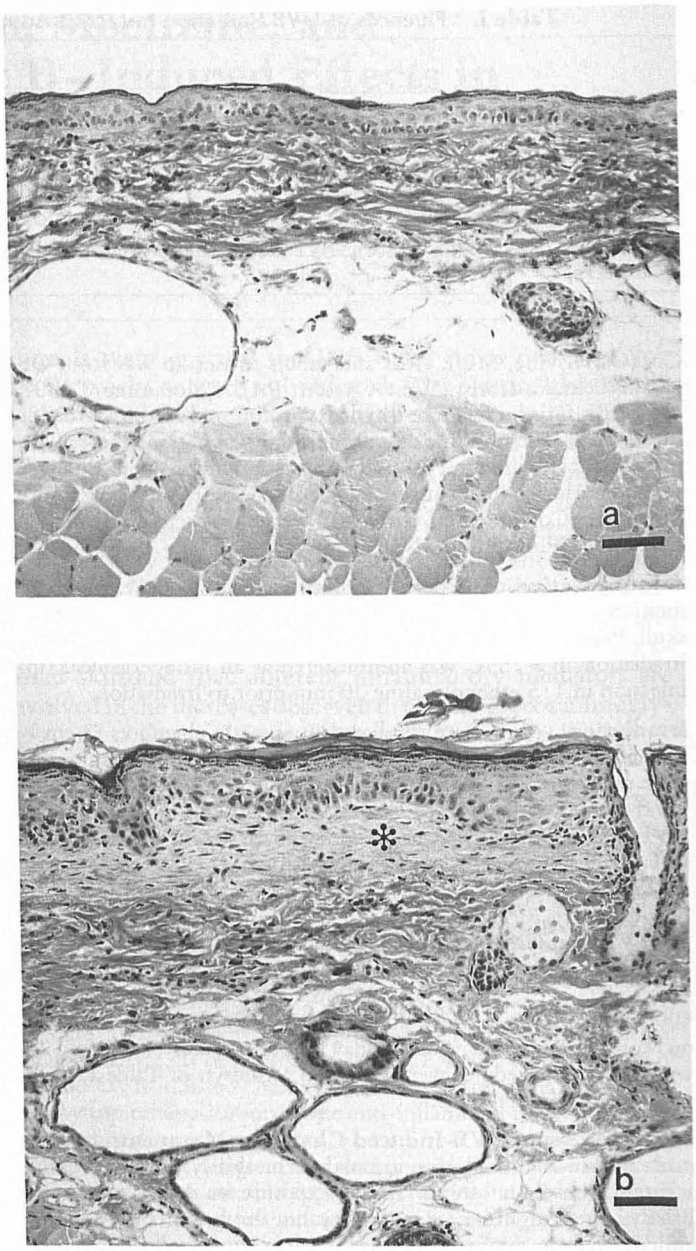

Figure 2. Histology of unirradiated and UVB-irradiated hairless mouse dorsal skin. *a*) Unirradiated, age-matched control mouse (experiment 2, 22 weeks). Note the thin epidermis and dermis. *b*) UVB-irradiated, non-drug-treated control mouse. Note the thickened epidermis and prominent papillary dermis *(asterisk)*. Hematoxylin and eosin; *bar*, 50 μm.

meclizine, and BW755C on skin responses to a single UVB exposure were assessed. Three measurements were made to quantify the inflammation at 24 h, namely, vascular permeability, accumulation of neutrophils, and skin-fold thickness. Based on preliminary measurements made using injections of indomethacin and meclizine, doses of these agents incorporated into slow-release pellets were selected. Experiments were performed 7–10 d after placement of the pellet. BW755C was given as an ip injection 30 min prior to UVB exposure. Preliminary studies indicated that injection times between 2 h and 30 min prior to irradiation gave equivalent results. A series of concentrations of the three anti-inflammatory agents were tested. The results for concentrations of indomethacin, meclizine, and BW755C that were the same as, or close to, those used in the chronic exposures are shown in Fig 1. These are 0.10 mg/pellet for indomethacin, 1.5 mg/pellet for meclizine (except for vasopermeability where only 0.25 mg/pellet was tested), and 30 mg/kg for BW755C.

A single exposure to 75 mJ/cm^2 of UVB radiation induced a 4.6-times increase in the vascular permeability of hairless mouse skin over unirradiated skin as measured by the amount of Evans blue dye accumulated in the skin. All three agents inhibited significantly the UVB-induced increase in vascular permeability when compared to the non-drug-treated, irradiated control group. The drugs alone, without UVB exposure, did not alter the vasopermeability. The myeloperoxidase activity of hairless mouse skin increased 2.4 times at 24 h after a single exposure to 180 mJ/cm^2 of UVB radiation. Indomethacin and meclizine but not BW755C decreased the enzyme activity in irradiated skin compared to the irradiated, no-drug control group. The drugs alone, without UVB exposure, did not alter the myeloperoxidase activity. A single exposure to 180 mJ/cm^2 UVB radiation induced an 0.31-mm increase in skin-fold thickness in the untreated control group. Indomethacin inhibited the UVB-induced increase in skin-fold thickness but meclizine and BW755C were ineffective. Imdomethacin, but not meclizine or BW755C, also decreased the skin-fold thickness of unirradiated animals but the change was not enough to account for the large decrease observed in the irradiated animals.

Histology and Tumors After Chronic UVB Exposure Unirradiated, age-matched control mice as well as meclizine-, indomethacin-, and BW755C-treated mice that were not irradiated showed similar skin morphology (Fig 2). The epidermis was typically 2–3 cells thick. The adventitial dermis was thin. The reticular dermis contained scattered inflammatory cells and mast cells, predominantly located perivascularly in the deep dermis (Fig 3). There were small quantities of glycosaminoglycans identified predominately in the adventitial dermis. UVB-irradiated mice including meclizine-, indomethacin-, and BW755C-treated mice as well as control mice showed similar histologic findings. The epidermis was

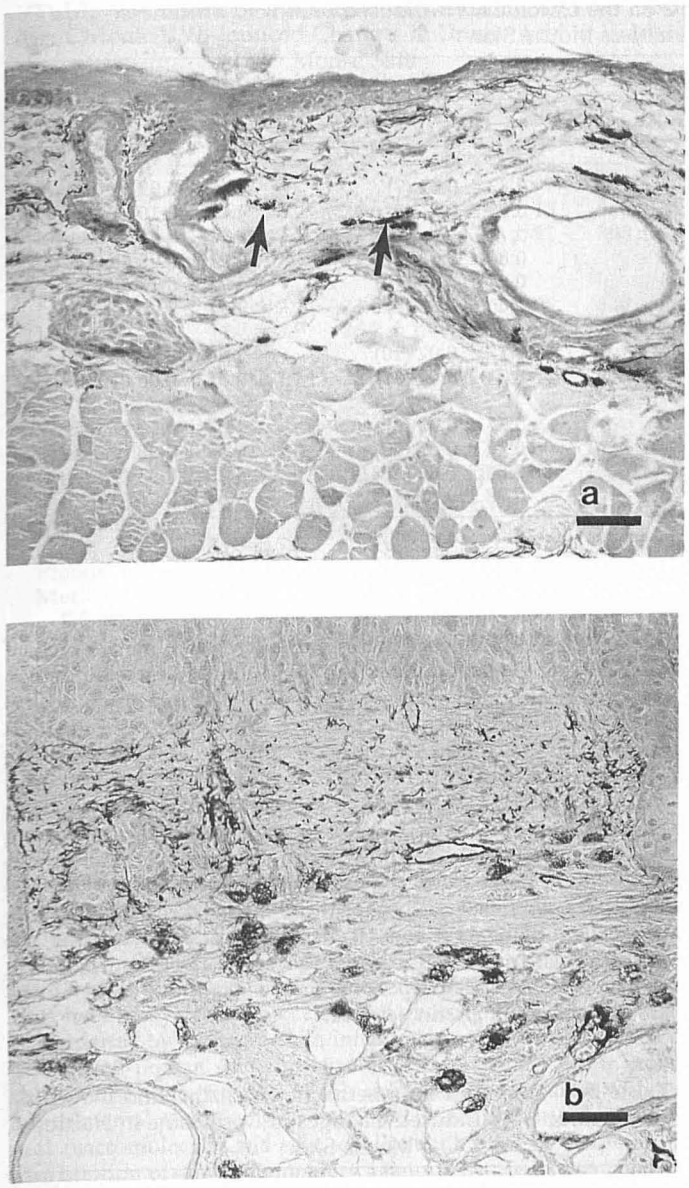

Figure 3. Staining of unirradiated and UVB-irradiated hairless mouse skin for elastin and mast cells. *a*) Unirradiated, age-matched control mouse (experiment 2, 22 weeks). Note the fine fibrillary elastic tissue and the mast cells *(arrows)* in the deep dermis. *b*) UVB-irradiated, non-drug-treated control mouse. Note the increased number of mast cells in the deep dermis. No clumps of elastic tissue are present. Resorcin fuchsin; *bar*, 50 μm.

thickened to approximately 3-4 cell layers. The papillary dermis contained a thicker layer of fine fibrillar collagen. There were more mast cells scattered throughout the dermis, both perivascularly as well as in the interstitium. In addition to the mast cells, there were lymphocytes and scattered neutrophils. The quantity of GAGs was increased with the most marked accumulation being in the adventitial dermis. The elastic fibers appeared slightly thickened and increased in number. There were no clumps of elastic tissue (as may be seen in solar elastosis).

Measurements of dermal thickness made on tissue sections showed a 53-100% increase in animals that are UVB exposed (Table II). This result is supported by measurements of skin-fold thickness showing that the dorsal skin was 50 to 100% thicker in irradiated, non-drug treated animals compared to unirradiated controls. The UVB-induced thickening was only decreased by indomethacin (0.10 mg/pellet).

The average number of dermal mast cells counted in non-irradiated, non-drug-treated control skin from the four experiments was 148 cells/mm^2 (Table III). Exposure to UVB radiation increased the number by factors of 1.9 to 2.3 in the non-treated and placebo-treated groups. None of the agents significantly inhibited the UVB-induced increase in mast cells over the increase seen in unirradiated groups. UVB-exposure increased the number of neutrophils counted in the dermis by factors of 1.6 and 2.1 (Table II). Indomethacin (0.10 mg/pellet) inhibited this increase. Meclizine also inhibited the UVB-induced increase although the placebo pellet had a similar effect. These results are similar to those found for acute UVB-induced changes, namely, indomethacin inhibited the neutrophil increase and the inhibition was produced by one dose of meclizine.

Tumors were counted at the end of the irradiation period for the animals in experiments 3 and 4. In experiment 3, two tumors appeared on the irradiated animals treated with indomethacin. In experiment 4, only one tumor appeared on an unirradiated animal (placebo pellet group). The number of tumors on irradiated animals in experiment 4 were no drug [3]; placebo pellet [3]; meclizine, 0.5 mg [7]; and meclizine, 1.5 mg [13].

Effects of Chronic UVB Exposure on Dermal Macromolecules in the Presence and Absence of Anti-Inflammatory Agents Chronic exposure of mice to UVB radiation employed three different exposure protocols. Mice received either 5.1 J/cm^2 in 26 weeks, 4.8 J/cm^2 in 22 weeks, or 6.8 J/cm^2 in 20 weeks as described in *Materials and Methods* and Table I. The skin of the irradiated groups was visibly red and had more scales than the unirradiated groups.

Chronic exposure of hairless mouse skin to UVB radiation has previously been shown to alter the macromolecules in the dermis. The change in dermal macromolecules will be described primarily using the amount of macromolecule per area of skin. The "amount per area" was chosen over "amount per wet weight" because the measurements of area were more reproducible and the wet weight is highly influenced by the degree of inflammation and GAG content. The results of measurements for elastin, GAGs, and collagen content per area and per weight are shown in Tables IV, V, and VI, respectively. Only the data expressed per area were statistically evaluated.

The elastin content of skin was measured as the amount of the cross-linking amino acid derivative desmosine. In the four experiments, the average amount of desmosine per area of unirradiated skin (4.4 $\pm$ 0.9 nmoles/cm^2) approximately doubled upon chronic UVB exposure to 8.0 $\pm$ 2.3 nmoles/cm^2 (Table IV). In individual experiments the increase in desmosine per skin area increased between 43% and 98%.

Indomethacin at 0.10 mg/pellet inhibited the chronic UVB-induced increase in desmosine per cm^2 compared to the irradiated control. The indomethacin-treated (0.10 mg/pellet), unirradiated animals did not show a significant change in desmosine compared to unirradiated controls. The lower dose of indomethacin also inhibited the increase in desmosine ($p < 0.01$) but the control, unirradiated group showed the same effect ($p < 0.01$). Meclizine (0.5 to 1.5 mg/pellet) and BW755C did not inhibit the chronic UVB-induced increase in desmosine. In fact, the desmosine level in meclizine-treated (0.5 or 1.5 mg/pellet), UVB-exposed animals was higher than that in the groups that were only UVB exposed ($p < 0.05$).

The GAG content of skin was evaluated from the amount of uronic acid present. Unirradiated skin contained 41 $\pm$ 6 μg/cm^2 uronic acid as determined on samples that were dialyzed before analysis (experiments 3 and 4, Table V). As described in *Materials and Methods,* the samples that were not dialyzed before uronic acid analysis gave lower values although these data show the same trends as those from samples for which the samples were dialyzed. Chronic UVB irradiation increased the amount of uronic acid per skin area by 55 to 340%.

Indomethacin (0.05 to 0.10 mg/pellet) and BW755C did not

Table II. Effects of Indomethacin, Meclizine, and BW755C on the Chronic UVB-Induced Skin-Fold Thickness and Dermal Thickness in Hairless Mouse Skin

Group	Dermal Thickness (μm)		Skin-Fold Thickness (mm)[a]	
	No UVB	UVB	No UVB	UVB
Experiment 1				
None	93 ± 13[b]	182 ± 39	0.83 ± 0.02 (9)	1.19 ± 0.07 (8)
Indomethacin, 0.05 mg	108 ± 19	192 ± 7	0.83 ± 0.01 (5)	1.27 ± 0.10 (3)
Experiment 2				
None	120 ± 9	183 ± 38	0.80 ± 0.06 (9)	1.21 ± 0.08 (7)
Meclizine, 0.25 mg	108 ± 13	217 ± 38	0.77 ± 0.06 (10)	1.28 ± 0.15 (9)
BW755C, 37.5 mg/kg	142 ± 52	229 ± 26	0.66 ± 0.06 (3)	1.17 ± 0.06 (7)
Experiment 3				
None	141 ± 25	240 ± 46	0.77 ± 0.02 (10)	1.46 ± 0.03 (10)
Indomethacin, 0.10 mg	154 ± 41	211 ± 36[c]	0.74 ± 0.02 (8)	1.53 ± 0.06 (9)
Experiment 4				
None	142 ± 14	242 ± 35	0.77 ± 0.03 (6)	1.37 ± 0.08 (9)
Placebo	142 ± 10	200 ± 23	0.79 ± 0.02 (10)	1.32 ± 0.06 (9)
Meclizine, 0.5 mg	163 ± 23	264 ± 38	0.80 ± 0.02 (10)	1.29 ± 0.05 (9)
Meclizine, 1.5 mg	179 ± 20	269 ± 45	0.78 ± 0.02 (10)	1.54 ± 0.16 (8)

[a] Numbers in parentheses represent animals at the end of the experiment in that group. All groups had 10 animals initially.
[b] Mean ± SEM.
[c] $p < 0.001$ compared to irradiated, non-drug-treated group.

alter the chronic UVB-induces increased in uronic acid content per skin area. Meclizine (0.5 or 1.5 mg/pellet) decreased the UVB-induced increase in uronic acid but this difference appears to be attributable to the effect of meclizine alone, because in the unirradiated animals meclizine decreased the uronic acid content compared to untreated control groups.

The collagen content of skin is proportional to the amount of hydroxyproline. The average amount of hydroxyproline in unirradiated mouse skin in the four experiments was 0.72 ± 0.08 mg/cm². In each experiment, chronic exposure to UVB radiation increased the amount of collagen per area of skin by 15–52% (Table VI).

In the UVB-exposed groups, meclizine (0.5 and 1.5 mg/pellet) inhibited the UVB-induced increase in hydroxyproline per area of tissue. BW755C also inhibited the UVB-induced increase in hydroxyproline although at a lower level of significance. Indomethacin did not inhibit UVB-induced increase in hydroxyproline because in the unirradiated groups, indomethacin decreased the hydroxyproline content per skin area to the same or greater extent.

As noted above, UVB exposure induces an increase in the wet weight per area of skin that is at least partially due to the increase in GAGs. In collagen, which is only slightly increased per unit surface area upon UVB exposure, the overall concentration (amount per weight) decreases, especially when the UVB-induced GAG increase is larger (experiments 3 and 4). The concentration (amount per weight) of elastin in UVB-exposed skin compared to unirradiated, age-matched skin varied depending on the magnitude of the UVB-induced increase in elastin per area.

Table III. Effects of Indomethacin, Meclizine, and BW755C on Chronic UVB-Induced Increase in Number of Mast Cells and Neutrophils in Hairless Mouse Skin[a]

Group	Mast Cells per mm²		Neutrophils per mm²	
	No UVB	UVB	No UVB	UVB
Experiment 1				
None	165 ± 21[b]	384 ± 21	ND[e]	ND
Indomethacin, 0.05 mg	118 ± 27[c]	330 ± 21[d]	ND	ND
Experiment 2				
None	139 ± 19	302 ± 66	ND	ND
Meclizine, 0.25 mg	129 ± 10	390 ± 22	ND	ND
BW755C, 37.5 mg/kg	96 ± 18	304 ± 82	ND	ND
Experiment 3				
None	130 ± 1	274 ± 21	251 ± 54	390 ± 58
Indomethacin, 0.10 mg	184 ± 1	383 ± 6	251 ± 38	338 ± 1[d]
Experiment 4				
None	157 ± 8	298 ± 21	195 ± 21	412 ± 32
Placebo	150 ± 17	285 ± 18	218 ± 16	286 ± 24[d]
Meclizine, 0.5 mg	115 ± 8[c]	237 ± 14[d]	216 ± 42	349 ± 38[d]
Meclizine, 1.5 mg	131 ± 7[c]	216 ± 18[d]	189 ± 21	258 ± 32[d]

[a] Number of mice in each group at end of experiment is shown in Table II.
[b] Mean ± SEM.
[c] $p < 0.001$ compared to unirradiated, non-drug-treated group.
[d] $p < 0.001$ compared to irradiated, non-drug-treated group.
[e] ND, not determined.

Table IV. Effects of Indomethacin, Meclizine, and BW755C on Chronic UVB-Induced Changes in Desmosine in Hairless Mouse Skin[a]

Group	Desmosine, nmoles/cm²		Desmosine, nmoles/g	
	No UVB	UVB	No UVB	UVB
Experiment 1				
None	4.4 ± 0.5[b]	6.3 ± 0.8	87 ± 15	98 ± 16
Indomethacin, 0.05 mg	3.4 ± 0.6	5.0 ± 0.9	86 ± 16	91 ± 21
Experiment 2				
None	5.3 ± 0.6	10.5 ± 0.9	109 ± 11	143 ± 16
Meclizine, 0.25 mg	7.7 ± 0.1	9.6 ± 1.3	155 ± 21	134 ± 27
BW755C, 37.5 mg/kg	4.0 ± 0.4	9.5 ± 1.4	81 ± 8	138 ± 24
Experiment 3				
None	4.7 ± 0.3	9.3 ± 0.7	110 ± 7	120 ± 10
Indomethacin, 0.10 mg	4.9 ± 0.3	7.1 ± 0.8[c]	152 ± 11	98 ± 11
Experiment 4				
None	3.2 ± 0.2	5.7 ± 0.3	104 ± 7	70 ± 4
Placebo pellet	5.1 ± 0.6	5.8 ± 0.7	127 ± 15	74 ± 8
Meclizine, 0.5 mg	3.1 ± 0.2	7.6 ± 1.0	92 ± 7	107 ± 15
Meclizine, 1.5 mg	4.4 ± 0.3	8.5 ± 0.6	114 ± 8	98 ± 6

[a] Number of mice in each group at end of experiment is shown in Table II.
[b] Mean ± SEM.
[c] $p < 0.005$ compared to irradiated, non-drug-treated group.

Table V. Effects of Indomethacin, Meclizine, and BW755C on Chronic UVB-Induced Changes in Uronic Acid in Hairless Mouse Skin[a]

Group	Uronic Acid, μg/cm²		Uronic Acid, μg/g	
	No UVB	UVB	No UVB	UVB
Experiment 1				
None	15.9 ± 0.7[b]	24.6 ± 1.4	297 ± 13	367 ± 21
Indomethacin, 0.05 mg	12.7 ± 3.5	22.3 ± 0.9	327 ± 91	388 ± 16
Experiment 2				
None	14.0 ± 2.7	23.8 ± 3.2	295 ± 56	343 ± 46
Meclizine, 0.25 mg	14.7 ± 3.9	26.2 ± 1.4	309 ± 82	333 ± 18
BW755C, 37.5 mg/kg	13.3 ± 1.2	22.4 ± 1.7	262 ± 24	323 ± 74
Experiment 3				
None	44.8 ± 5.0	141 ± 6	920 ± 60	1430 ± 50
Indomethacin, 0.10 mg	38.6 ± 4.5	144 ± 9	703 ± 73	1360 ± 43
Experiment 4				
None	36.5 ± 5.8	162 ± 15	701 ± 81	1560 ± 86
Placebo pellet	37.4 ± 4.0	148 ± 8	670 ± 67	1500 ± 61
Meclizine, 0.5 mg	33.9 ± 2.5[c]	146 ± 19[d]	614 ± 42	1420 ± 115
Meclizine, 1.5 mg	25.6 ± 2.3[c]	114 ± 9[d]	463 ± 32	1150 ± 79

[a] Number of mice in each group at the end of the experiment is shown in Table II.
[b] Mean ± SEM.
[c] $p < 0.01$ compared to unirradiated, non-drug-treated control group.
[d] $p < 0.01$ compared to irradiated, drug-treated control group.

DISCUSSION

A single exposure of mammalian skin to UVB radiation causes an increase in several inflammatory mediators including histamine, prostaglandin E_2, 12-HETE, interleukin 1, and tumor necrosis factor-α [17–20]. It has been proposed that chronic inflammation is involved in the skin changes produced by exposure to UVB radiation over many years, a process called photoaging. This hypothesis is supported by the increase in inflammatory cells in the dermis of photoaged human skin and chronically irradiated hairless mouse skin [1,2,4]. The first goal of this study was to determine whether experimental photoaging, as measured by changes in selected dermal macromolecules and cells, could be inhibited by systemic administration of anti-inflammatory agents. The agents were chosen for their abilities to inhibit the formation or action of different inflammatory mediators. Thus, a second question asked was whether any one or all of the agents would selectively inhibit chronic UVB-induced dermal changes, thereby indicating that certain mediators were involved in specific dermal changes.

Anti-inflammatory agents that have been shown to inhibit aspects of acute UV-induced cutaneous inflammation include the cyclooxygenase inhibitor, indomethacin, and H_1 histamine receptor antagonists [21,22]. Several agents tested in preliminary experiments were either ineffective against UVB-induced inflammation or were too toxic to be used in chronic studies. The three agents chosen for further study, indomethacin, meclizine, and BW755C, inhibited signs of acute UVB-induced inflammation, namely, increased vascular permeability, neutrophil accumulation, and increased skin-fold thickness (all measured at 24 h post-irradiation). Our results indicate that indomethacin decreased all three responses of hairless mouse skin to a single UVB exposure. Meclizine, an H_1 histamine receptor antagonist, inhibited the UVB-induced increase in vascular permeability and accumulation of neutrophils. BW755C, a combined cyclooxygenase and lipoxygenase inhibitor, effectively reduced the UVB-induced increase in vascular permeability.

These results on acute UVB-induced skin injury can be interpreted, at least partially, by considering the primary pharmacologic effects of these agents. For example, the reduction by indomethacin in UVB-induced increase of vascular permeability may be attributed to reduced formation of PGE_2, which potentiates the increase in vascular permeability produced by histamine and bradykinin [23]. The reduction of the UVB-induced accumulation of neutrophils by indomethacin may also result from its effect on vasopermeability because formation of chemotactic leukotrienes is not inhibited by indomethacin. The inhibition of UVB-induced vasopermeability by meclizine is attributed to its activity as an H_1-histamine receptor antagonist because binding of histamine via H_1 receptors results in increased vasopermeability in murine skin [24]. The reduction of neutrophil accumulation by meclizine may also be related to its ability to inhibit the UVB-induced increase in vascular permeability. The ability of BW755C to decrease the UVB-induced increase in vascular permeability may be attributed to either its inhibition of lipoxygenase or cyclooxygenase activities although it apparently was not present at high enough concentration or for a long enough period to decrease effectively the production of chemotactic leukotrienes.

Chronic UVB exposure of hairless mouse skin produced increases in the amounts per area of all three of the dermal macromolecules measured; the relative magnitude of the change was GAGs > elastin > collagen. The measurements are made after periods of UVB exposure and represent the net change in macromolecular content compared to age-matched control animals. The chronic

Table VI. Effects of Indomethacin, Meclizine, and BW755C on Chronic UVB-Induced Changes in Hydroxyproline in Hairless Mouse Skin[a]

Group	Hydroxyproline, mg/cm²		Hydroxyproline, mg/g	
	No UVB	UVB	No UVB	UVB
Experiment 1				
None	0.73 ± 0.03[b]	0.84 ± 0.06	13.7 ± 1.5	12.5 ± 0.8
Indomethacin, 0.05 mg	0.62 ± 0.04	0.87 ± 0.08	16.0 ± 2.4	15.2 ± 2.0
Experiment 2				
None	0.93 ± 0.10	1.43 ± 0.25	19.6 ± 2.6	19.9 ± 3.8
Meclizine, 0.25 mg	1.15 ± 0.12	1.48 ± 0.21	24.1 ± 2.9	18.8 ± 2.2
BW755C, 37.5 mg/kg	0.76 ± 0.11[c]	0.95 ± 0.07[e]	15.0 ± 0.7	13.7 ± 1.1
Experiment 3				
None	0.63 ± 0.02	0.72 ± 0.03	14.9 ± 0.8	9.2 ± 0.7
Indomethacin, 0.10 mg	0.49 ± 0.02[d]	0.65 ± 0.03[e]	15.3 ± 1.2	9.0 ± 1.1
Experiment 4				
None	0.60 ± 0.02	0.91 ± 0.06	19.7 ± 0.9	11.1 ± 0.6
Placebo pellet	0.66 ± 0.04	0.86 ± 0.05	16.5 ± 1.0	11.0 ± 0.6
Meclizine, 0.5 mg	0.61 ± 0.04	0.73 ± 0.02[f]	18.0 ± 0.8	10.4 ± 0.9
Meclizine, 1.5 mg	0.57 ± 0.02	0.66 ± 0.02[f]	14.7 ± 0.6	7.5 ± 0.5

[a] Number of mice in each group at the end of the experiments is shown in Table II.
[b] Mean ± SEM.
[c] $p < 0.05$ compared to unirradiated, non-drug-treated group.
[d] $p < 0.01$ compared to unirradiated, non-drug-treated group.
[e] $p < 0.025$ compared to irradiated, non-drug-treated group.
[f] $p < 0.005$ compared to irradiated, non-drug-treated group.

Table VII. Summary of the Effects of Anti-Inflammatory Agents on Chronic UVB-Induced Alterations In Hairless Mouse Skin

Parameter	Indomethacin	Meclizine	BW755C
Dermal Thickness	+[a]	−	−
Mast cells	−	−	−
Neutrophils	−	−	ND[b]
Elastin	+	−	−
Glycosaminoglycans	−	−	−
Collagen	−	+	+

[a] A plus sign indicates that the agent inhibited the UVB-induced increase in that parameter.
[b] ND, not determined.

UVB-induced dermal macromolecular changes found in this study are similar to those reported by others in hairless mouse skin. The increase in GAG content is always greatest. An increase in collagen is not always detected and may vary with the extent of the chronic UVB damage [1]. Further studies are required to determine whether the increases in dermal macromolecules are accompanied by biochemical alterations.

Several mechanisms could lead to these changes in net macromolecular accumulation including activation of macromolecular synthetic pathways in fibroblasts or inhibition of rates of macromolecular degradation. The degree of activation of synthesis or inhibition of degradation must vary between macromolecules because much larger increases are found for GAGs and elastin compared to collagen. An alternative explanation for the UVB-induced increases in macromolecule accumulation assumes that fibroblasts in photoaged skin increase in number but maintain their normal rates of macromolecular synthesis. The number of dermal fibroblasts increases in murine photoaged skin [2]; however, this hypothesis is not consistent with the differing magnitudes by which the three macromolecules are increased in UVB-exposed skin.

Previous work has shown that photoaged skin contains activated fibroblasts [1,4]. Thus, increased synthesis may contribute to the increased net accumulation of dermal macromolecules. Although decreased rates of degradation may also contribute to increased accumulation, one study of chronically UVB-exposed hairless mouse skin did not demonstrate decreases in activity of proteolytic and elastolytic enzymes [8]. Dermal fibroblasts may be directly stimulated by UVB to increase synthesis of macromolecules [25] or indirectly stimulated by mediators elicited from other cells by UVB exposures. For example, synthesis of collagen and GAGs by dermal fibroblasts *in vitro* is stimulated by interleukin 1α and β, and tumor necrosis factor α and β [26]. However, certain cytokines induced by UV radiation also stimulate increased fibroblast collagenase activity.

All three anti-inflammatory agents inhibited the increase in dermal macromolecules induced by chronic UVB exposure indicating, in answer to our first question, that inflammatory mediators are involved in chronic UVB-induced macromolecular alterations and that systemic anti-inflammatory agents can effectively reduce this effect. Interestingly, not all agents affected the dermal content of each macromolecule in the same ways as shown in Table VII. Indomethacin altered the elastin accumulation, meclizine and BW755C inhibited collagen accumulation, but none of the agents altered the GAG increase. Similar but not identical results were obtained in a study of the effects of topically applied anti-inflammatory agents on chronic UVB-induced skin changes evaluated histologically [11]. In that study, the cyclooxygenase inhibitors, naproxen and ibuprofen, inhibited the increase in elastin, similar to our result with indomethacin. Only one of the topical cyclooxygenase inhibitors protected against the increase in GAGs indicating that, similar to our result, the GAG increased accumulation is not as sensitive to inhibition. In contrast, the topical cyclooxygenase inhibitors also inhibited the collagen changes whereas, in our study, BW755C was effective but indomethacin was not. The differences between the two studies may have resulted from the differences in the types of measurements; our study measured net changes in amount of macromolecules whereas the study of Bissett *et al* [11] measured histologic changes. A further contrast between the results of the two studies is that the topical agents suppressed the chronic UVB-induced increase in dermal cellularity whereas the systemic agents did not alter mast cell increases but did decrease UVB-induced neutrophil increases.

Thus, in answer to our second question, different specific mediators appear to be important for the several endpoints measured. For example, the inhibition of the elastin increase by indomethacin may be due to blocking of prostaglandin effects on fibroblast or on endothelial cells. In addition, the mediators may be inducing synthesis or inhibiting degradation of the macromolecules as noted above. Prostaglandins but not histamine appear to be important for chronic UVB-enhanced accumulation of elastin, whereas histamine but not prostaglandins is important for accumulation of collagen. The observation that BW755C but not indomethacin decreased the accumulation of collagen indicates that leukotrienes, rather than prostaglandins, may be important.

In summary, it appears that different inflammatory mediators are involved in the increased accumulation of elastin, GAGs, and collagen in photoaged skin. Further studies are required to describe the detailed interactions and mechanisms involved.

R. William Gange, M.D., died before completion of this study. This research was motivated by his perceptive questions concerning skin damage initiated by UV radiation.

This work was supported in part by National Institutes of Health grant 5 T32 AR07098-16 and the MFEL program under ONR contract N00014-86-K0117.

REFERENCES

1. Kligman LH: The ultraviolet-irradiated hairless mouse: a model for photoaging. J Am Acad Dermatol 21:623–631, 1989
2. Bissett DL, Hannon DP, Orr TV: An animal model of solar-aged skin; histological, physical, and visible changes in UV-irradiated hairless mouse skin. Photochem Photobiol 46:367–378, 1987
3. Kligman AM: Early destructive effects of sunlight on human skin. JAMA 210:2377–2380, 1969
4. Lavker RM, Kligman AM: Chronic heliodermatitis: a morphological evaluation of chronic actinic dermal damage with emphasis on the role of mast cells. J Invest Dermatol 90:325–330, 1988
5. Braverman IM, Fonferko E: Studies in cutaneous aging: I. The elastic fiber network. J Invest Dermatol 78:434–443, 1982
6. Kligman LM, Gebre M, Alper R, Kefalides NA: Collagen metabolism in ultraviolet irradiated hairless mouse kin and its correlation to histochemical observations. J Invest Dermatol 93:210–214, 1989
7. Chatterjee R, Benzinger MJ, Ritter JL, Bissett DL: Chronic ultraviolet B radiation-induced biochemical changes in the skin of hairless mice. Photochem Photobiol 51:91–97, 1990
8. Uitto J, Fazio MJ, Olsen DR: Molecular mechanisms of cutaneous aging. J Am Acad Dermatol 21:614–622, 1989
9. Schwartz E: Connective tissue alterations in the skin of ultraviolet irradiated hairless mice. J Invest Dermatol 91:158–161, 1988
10. Bissett DL, Hannon DP, Orr TV: Wavelength dependence of histological, physical, and visible changes in chronically UV-irradiated hairless mouse skin. Photochem Photobiol 50:763–769, 1989
11. Bissett DL, Chatterjee R, Hannon DP: Photoprotective effect of topical anti-inflammatory agents against ultraviolet radiation-induced chronic skin damage in the hairless mouse. Photodermatol Photoimmunol Photomed 7:153–158, 1990
12. Bradley PP, Priebat DA, Christensen RD, Rothstein G: Measure of cutaneous inflammation: estimation of neutrophil content an enzyme marker. J Invest Dermatol 78:206–209, 1982
13. Starcher BC: Determination of the elastin content of tissues by measuring desmosine and isodesmosine. Anal Biochem 79:11–15, 1977
14. Bitter T, Muir HM: A modified uronic acid carbazole reaction. Analyt Biochem 4:330–334, 1962
15. Stegemann H, Stalder K: Determination of hydroxyproline. Clinica Chimica Acta 18:267–273, 1967
16. Kaplow LS: Simplified myeloperoxidase stain using benzidine dihydrochloride. Blood 26:215–219, 1965

17. Black AK, Barr RM, Wong E, Brain S, Greaves MW, Dickinson R, Shroot B, Hensby CN: Lipoxygenase products of arachidonic acid in human inflamed skin. Br J Clin Pharmacol 20:185–190, 1985

18. Ansel JC, Lugar TA, Green I: The effect of in vitro and in vivo UV irradiation of the production of ETAF activity by human and murine keratinocytes. J Invest Dermatol 81:519–523,1983

19. Gilchrest BA, Soter NA, Stoff JS, Mihm MA: The human sunburn reaction: histologic and biochemical studies. J Am Acad Dermatol 5:411–422, 1981

20. Oxholm A, Oxholm P, Staberg B, Bendtzen K: Immunohistological detection of interleukin I-like molecules and tumour necrosis factor in human epidermis before and after UVB-irradiation in vivo. Br J Dermatol 118:369–376, 1988

21. Black AK, Greaves MW, Hensby CN, Plummer NA, Warin AP: The effects of indomethacin on arachidonic acid and prostaglandins E_2 and $F_2\alpha$ levels in human skin 34 h after u.v.B and u.v.C irradiation. Br J Clin Pharmacol 6:261–266, 1978

22. Logan G, Wilhelm DL: Vascular permeability changes in inflammation: I. The role of endogenous permeability factors in ultraviolet injury. Br J Exp Pathol 47:300–314, 1966

23. Cotran RS, Kumar V, Robbins SL: Robbins Pathologic Basis of Disease. W.B. Saunders, Philadelphia, 1989, pp 54–57

24. Church MK, Moller P: Simple models of anaphylaxis and of histamine and 5-hydroxytryptamine-induced inflammation using the mouse pinna. Br J Pharmacol 55:315P, 1975

25. Schwartz E, Cruickshank FA: Elastin, fibronectin and collagen synthesis in UV irradiated dermal fibroblasts (abstr). J Invest Dermatol 96:586, 1991

26. Duncan MR, Berman B: Differential regulation of collagen, glycosaminoglycan, fibronectin, and collagenase activity production in cultured human adult dermal fibroblasts by interleukin 1-α and β and tumor necrosis factor -α and β. J Invest Dermatol 92:699–706, 1989