Corneal radiofrequency burns: effects of prostaglandins and 48/80

Bernard Nunziata, Richard S. Smith, and Virginia Weimar*

Vascularization of the rat cornea was produced by means of radiofrequency burns adjacent to the limbus. Animals with and without lesions were tested for a vascular response to topical PGE, and compound 48/80, with carbon used as an indicator. There was no response to PGE until 6 hours, at which time dense labeling of limbal vessels was observed. This response gradually decreased and by day 15, when labeling returned to control levels. No further permeability changes were seen for the duration of the experiment to day 90. Compound 48/80 gave four-plus labeling of the limbus and one-plus labeling of corneal vessels at all times during the experiment. The presence or absence of a thermal lesion did not change the degree of labeling. Histamine and bradykinin did not produce vascular labeling either with or without a corneal lesion.

Key words: cornea, vascularization, prostaglandins, histamine.
by the absence of increased IOP after pre-
treatment with indomethacin, an inhibitor
of prostaglandin production. Many inves-
tigators have shown that topical and
intravenous administration of prostaglan-
dins $E_2$, $I_2$, and $F_2$ (PGE$\alpha$, etc.) will also
increase IOP directly by causing an in-
creased production of aqueous humor. 6-11
Polyphloretin phosphate, a prostaglandin
antagonist, blocks these effects in the rab-
bit, 6-7 but not in the monkey. 12 The pro-
posed mechanisms of action which produce
these effects include vasodilation and in-
creased capillary permeability. 6, 13, 14 an
increased permeability of the ciliary epi-
thelium, 15 or both. 16 The iris is also affected
by prostaglandins. Topical PGE$\alpha$ and $E_2$
increase fluorescein leakage in the rabbit iris. 14, 16 In the rat, paracentesis gives the
same result. 17
Without prostaglandin application, nor-
mal limbal blood vessels are impermeable
to carbon but do leak fluorescein in rats. 18
Thorium dioxide escapes from limbal ves-
sels of both control and chemically burned
eyes but does not leak from mature corneal
vessels. 19
Because of the potential importance of
prostaglandins in corneal wound healing,
it was decided to investigate the effects of
PGE$\alpha$ and other drugs on the permeability
of developing corneal vessels to carbon
(200 A).

Methods

Animals. Seventy healthy male Sprague-Dawley
albino rats ranging in weight from 75 to 470
gm. were used in these experiments. Lesions
were made on the corneas of animals anesthetized
with ether, with the use of a radiofrequency le-
dersion generator. 2 The generator was set at a
lethal systemic dose for rats, 48/80 is  apparently
used, after which the tail vein
was cannulated with a 23- or 27-gauge needle.
After cannulation, 1 to 3 drops of the drug
being tested were applied to the cornea and
allowed to sit for 5 minutes before carbon injec-
tion. A dose of 0.2 ml. of carbon (Pelikan Ink,
freshly filtered) per 100 gm. rat weight was
administered through the intravenous cannula.
Approximately 3 hours were allowed for the carbon
to clear from the vascular system before enu-
cleation. Proper clearing of the carbon is essential
to avoid confusing true labeling (carbon particles
cought between the endothelial cells and the base-
ment membrane) with intraluminal carbon.

Enucleation and whole mount preparation. Ani-
males were reanesthetized with pentobarbital,
and the eyes enucleated. In radiofrequency burns less
than 10 days old the cornea frequently ruptured
at the site of injury during enucleation. Since
the new-formed vessels were not included in
perforation, these globes were still useful for
examination.

The entire eye was placed in 4 percent buffered
gluteraldehyde for several hours and then was
frozen and discarded along the equator. The lens was re-
moved and discarded along with the posterior
segment of the eye. The iris and ciliary processes
were also excised. The cornea was cut into tri-
angular sections, and the posterior surface was
scraped clean of any adherent iris vessels. Such
vessels may label with carbon 18 and can be con-
fused with corneal vessels in the whole mounts.
Dissected tissue was left overnight in fixative
and then stored in 0.2M phosphate buffer, pH
7.4. The sections were dehydrated in alcohol and
cleared in xylene for 15 minutes. The degree
of carbon labeling was indicated by a scale
of zero to four plus. A zero value showed no  carbon
deposits on any of the vessels, whereas four
plus showed dense thickening of all vessels along
their entire length.

Chemical agents. Several drugs were tested.
Histamine diphosphate at concentrations from 2.75
to 20 mg./ml. and bradycinin at concentrations
of 100 $\mu$g/ml. to 1 mg./ml. did not cause label-
ing of limbal vessels with topical application.
PGE$\alpha$ (1 mg./ml.) and a histamine liberator, com-
-pound 48/80 (35 mg./ml.) produced labeling of
ocular vessels.

A stock solution of 10 mg./ml. PGE$\alpha$ (The Up-
john Co., Kalamazoo, Mich.) dissolved in 100
percent ethanol was diluted with 0.2M phosphate
buffer, pH 7.4, to a working concentration
of 1.0 mg./ml. Compound 48/80 (Burroughs Well-
come Co., U.S.A.) was used at a concentra-
tion of 35 mg./ml. Although this represents a
lethal systemic dose for rats, 48/80 is apparently
very slowly absorbed by the ocular route. No
animals died during the experiments as a result
of the topical application.

Carbon tracer. Intraperitoneal pentobarbital
anesthesia was used, after which the tail vein
was cannulated with a 23- or 27-gauge needle.
After cannulation, 1 to 3 drops of the drug
being tested were applied to the cornea and
allowed to sit for 5 minutes before carbon injec-
tion. A dose of 0.2 ml. of carbon (Pelikan Ink,
freshly filtered) per 100 gm. rat weight was
administered through the intravenous cannula.
Approximately 3 hours were allowed for the carbon
to clear from the vascular system before enu-
cleation. Proper clearing of the carbon is essential
to avoid confusing true labeling (carbon particles
cought between the endothelial cells and the base-
ment membrane) with intraluminal carbon.

Enucleation and whole mount preparation. Ani-
males were reanesthetized with pentobarbital,
and the eyes enucleated. In radiofrequency burns less
than 10 days old the cornea frequently ruptured
at the site of injury during enucleation. Since
the new-formed vessels were not included in
perforation, these globes were still useful for
examination.

The entire eye was placed in 4 percent buffered
gluteraldehyde for several hours and then was
frozen and discarded along the equator. The lens was re-
moved and discarded along with the posterior
segment of the eye. The iris and ciliary processes
were also excised. The cornea was cut into tri-
angular sections, and the posterior surface was
scraped clean of any adherent iris vessels. Such
vessels may label with carbon 18 and can be con-
fused with corneal vessels in the whole mounts.
Dissected tissue was left overnight in fixative
and then stored in 0.2M phosphate buffer, pH
7.4. The sections were dehydrated in alcohol and
cleared in xylene for 15 minutes. The degree
of carbon labeling was indicated by a scale
of zero to four plus. A zero value showed no  carbon
deposits on any of the vessels, whereas four
plus showed dense thickening of all vessels along
their entire length.

Chemical agents. Several drugs were tested.
Histamine diphosphate at concentrations from 2.75
to 20 mg./ml. and bradycinin at concentrations
of 100 $\mu$g/ml. to 1 mg./ml. did not cause label-
ing of limbal vessels with topical application.
PGE$\alpha$ (1 mg./ml.) and a histamine liberator, com-
-pound 48/80 (35 mg./ml.) produced labeling of
ocular vessels.

A stock solution of 10 mg./ml. PGE$\alpha$ (The Up-
john Co., Kalamazoo, Mich.) dissolved in 100
percent ethanol was diluted with 0.2M phosphate
buffer, pH 7.4, to a working concentration
of 1.0 mg./ml. Compound 48/80 (Burroughs Well-
come Co., U.S.A.) was used at a concentra-
tion of 35 mg./ml. Although this represents a
lethal systemic dose for rats, 48/80 is apparently
very slowly absorbed by the ocular route. No
animals died during the experiments as a result
of the topical application.
Fig. 1. Drug-only animals. A, Small limbal vessels (arrows) show two-plus labeling after exposure to PGE$_1$. A larger vessel (V) is not labeled. Portions of the picture are not in focus because of the thickness of the whole mount. B, Labeling is four plus (arrows) after exposure to 48/80. (Unstained whole mount; x200.)

Controls. Effects from intravenous carbon, the phosphate buffer solvent for PGE$_1$, and the ethanol in the stock solution (controlled for by dissolving PGE$_1$ directly into the buffer) were all sought in control animals. Animals were also tested to establish a control level of PGE$_1$ and compound 48/80 limbal labeling by applications of the drugs to eyes which received no thermal lesion. These controls will be referred to as drug-only animals. A second group of controls, evaluated throughout the experiment, were animals who received the typical thermal
Fig. 2. Limbal capillaries show two-plus labeling when exposed to PGE\textsubscript{i} immediately after the thermal lesion (arrows). (Unstained whole mount; >200.)

lesion but were not exposed to drugs. This group of controls will be referred to as lesion-only animals. The purpose of the lesion-only controls was to assess the effects of the radiofrequency lesion on carbon labeling of limbal and new-formed corneal vessels.

Results

The carbon tracer and phosphate buffer solvent did not cause vascular labeling. In drug-only animals the control level of limbal labeling for PGE\textsubscript{i} was two plus, and that for compound 48/80 was three plus to four plus (Fig. 1).

Limbal labeling immediately following and 6 hours after lesion production was the same for both the PGE\textsubscript{i}-treated and lesion-only control eyes. Those animals injected with carbon immediately after the lesion showed one plus to two plus labeling of the limbus adjacent to the lesion (Fig. 2), whereas a value of four plus was observed at 6 hours (Fig. 3). Limbal labeling at sites remote from the lesion remained at control levels of two plus throughout the experiment regardless of the lesion age.

The labeling in lesion-only control animals immediately after and 6 hours after lesion production must be attributed to some effect of the lesion. The absence of intense labeling in PGE\textsubscript{i}-treated eyes of animals studied immediately after lesion production shows that time is needed before the lesion causes a limbal response to PGE\textsubscript{i}. This same low level of labeling in lesion-only control animals tested immediately after lesion production suggests a gradual increase in labeling after lesion production to a point where at 6 hours labeling is maximal.

A progressive decline from values of four plus at day 1 to the control level of two plus at day 15 was seen at the limbus adjacent to the lesion in PGE\textsubscript{i}-treated eyes. The two plus labeling then remained the same through day 90. A similar effect was noted for the new-formed corneal vessels, ranging from four plus on day 3 to a basal level of one plus at day 15 which was maintained through day 90.

Experiments with topical compound 48/80 consistently showed four plus labeling of the limbus near the lesion regardless
Fig. 3. Limbal capillaries show four-plus labeling when exposed to PGE\(_i\), (arrows) 6 hours after the thermal lesion (X). (Unstained whole mount; ×200.)

of the lesion age. This value is identical with four plus labeling obtained from drug-only control animals treated with topical 48/80 (Fig. 1, B).

At day 1, however, when the lesion-only controls showed zero labeling, the compound 48/80-treated eyes continued to show three plus to four plus labeling of the limbal vessels. This persistence of sensitivity to compound 48/80 continued for the duration of the experiment and may be explained by histamine liberation produced by compound 48/80. The new corneal vessels from day 3 through day 90 labeled one plus, and there was no gradual decrement as noted with the PGE\(_i\). The results for 48/80 and PGE\(_i\) are summarized in the Table I.

Iridial vessels also responded to topical PGE\(_i\). In all animals tested, the iris labeled consistently greater than or equal to three plus. The ciliary processes, the retina, and the choroid did not label.

Comments and conclusions

The principal observations made during this experiment were that limbal and new-formed corneal vessels labeled with carbon shortly after lesion production with topical applications of PGE\(_i\). There was a gradual decrease in labeling as the new blood vessels matured. The return of both corneal
Table I

<table>
<thead>
<tr>
<th></th>
<th>PGE,</th>
<th>48/80</th>
<th>Lesion only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limbal</td>
<td>1-2+</td>
<td>4+</td>
<td>1-2+</td>
</tr>
<tr>
<td>Corneal</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limbal</td>
<td>4+</td>
<td>3-4+</td>
<td>4+</td>
</tr>
<tr>
<td>Corneal</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limbal</td>
<td>4+</td>
<td>3-4+</td>
<td>0</td>
</tr>
<tr>
<td>Corneal</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Day 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limbal</td>
<td>3+</td>
<td>3-4+</td>
<td>0</td>
</tr>
<tr>
<td>Corneal</td>
<td>4+</td>
<td>1+</td>
<td>0</td>
</tr>
<tr>
<td>Day 4-15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limbal</td>
<td>2-3+</td>
<td>3-4+</td>
<td>0</td>
</tr>
<tr>
<td>Corneal</td>
<td>2-3+</td>
<td>1+</td>
<td>0</td>
</tr>
<tr>
<td>Day 15-90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limbal</td>
<td>2+</td>
<td>3-4</td>
<td>0</td>
</tr>
<tr>
<td>Corneal</td>
<td>1+</td>
<td>1+</td>
<td>0</td>
</tr>
</tbody>
</table>

There was a delay in PGE, induced labeling of the limbus adjacent to the site of the corneal lesion. Immediately after lesion production this portion of the limbus labeled only one plus to two plus after PGE, application, a level identical to the control where no PGE, was applied. The possible need for edema to reach the limbus or for the diffusion of some substance responsible for the sensitivity of growing corneal and limbal vessels to PGE, may explain the delay. Cogan22 observed a 2 day latent period in the rabbit, where no changes in the limbus were observed after a corneal lesion. He also pointed out that the closer to the limbus and the more severe the trauma, the shorter this period would be. The distance from the limbus of a thermal lesion has an important bearing on the rate and incidence of vascularization.23

A latent period of approximately 6 hours was seen in these experiments. At 6 hours there was a sudden and marked increase in permeability of the proximal limbus in both lesion-only controls and PGE, treated eyes. By day 1 the basal level of labeling had returned to that of the lesion-only limbus, but topical PGE, continued to elicit strong labeling. This suggests a cessation of release of endogenous prostaglandin by 24 hours.

Although compound 48/80 did not give the same effect as PGE, it was noted that in response to this compound limbal vessels labeled three plus to four plus. This labeling was not seen in lesion-only controls during the acute phase, eliminating the possibility of its being due to endogenous prostaglandin.

The degranulation of limbal mast cells following acute injury has been previously described,24 which may explain the sensitivity to compound 48/80. The degranulation of injured mast cells may also explain the one plus to two plus labeling of the limbus adjacent to the lesion in control animals immediately after the lesion and the four plus labeling of the limbus and corneal vessels in lesion-only controls at 6 hours. Endogenous prostaglandin release...
Corneal radiofrequency burns 291

seems a more likely explanation. The three to four limbal labeling seen in all animals for the balance of the experiment could be explained by release of histamine or other vasoactive substances by mast cells in response to 48/80. The failure of topical histamine to produce the same effect may be due to inability of this drug to penetrate the corneal-conjunctival barrier.

REFERENCES