Modification of 1-tyrosine-induced keratopathy by adrenal corticosteroids

Robert P. Burns, Margaret E. Beard, Virginia L. Weimar, and Edwin L. Squires

A rat fed an excess of tyrosine develops a reproducible, reversible keratopathy. Topical, intramuscular, or oral administration of various glucocorticoids and intramuscular phenobarbital prevent the development of tyrosine-induced corneal opacity, edema, and vascularization in a graduated fashion. Steroid drops placed in the right eye only inhibit development of keratopathy in both eyes. It is suggested that this delaying of keratopathy is due to stimulation of hepatic production of tyrosine aminotransferase.

Key words: rat, cornea, keratopathy, tyrosine, tyrosine aminotransferase, enzyme induction, prednisone, dexamethasone, beta-methasone, phenobarbital.

We have previously introduced a reproducible laboratory technique for biomicroscopic and histologic evaluation of calibrated ocular inflammatory or degenerative disease in the 1-tyrosine-fed rat. This model progresses through well-defined stages of corneal epithelial edema, then stromal edema, leukocytic infiltration, opacity, and vascularization. The keratopathy regresses spontaneously while the initiating tyrosine stimulus is maintained. This is an extremely reliable and reproducible animal model, with the principal variables affecting the degree of corneal disease being the age and/or weight of the rat and the tyrosine content of the diet, when the diet is maintained at sufficient protein, fat, caloric, and vitamin content to prevent concurrent nutritional disease.

Selye found that glucocorticoids, as well as other steroids devoid of glucocorticoid effect, prevent the lethal, ocular, and pedal manifestations of tyrosine toxicity in the rat. However, only mortality rates and gross inspection of the eyes and paws after two weeks of dietary treatment were used as parameters of disease.

Since diverse forms of glucocorticoids are important therapeutic agents in ophthalmology, this study was done to test...
the effect of varying types, dosage levels, and delivery systems of adrenal corticosteroids in the tyrosine-fed rat model of graduated eye inflammation or degeneration.

Materials and methods

Albino female rats (140 to 150 grams) were used because of the previously determined predictability of the onset of keratopathy. The animals were maintained on Purina rat chow until the experiment began. Animals were kept one or two to a cage, and food and water were available ad libitum. The eyes were examined with a Haag-Streit 900 biomicroscope before institution of treatment to make sure that no pre-existing disease was present, then were re-examined daily and eye changes graded according to previously established stages. During the experiment, a fully nutritional 5 per cent tyrosine diet with a potato base was fed. At first, to insure enzyme induction, adrenal corticosteroids were administered for one day before beginning the 5 per cent tyrosine diet. Steroids were given as prednisone, 10 mg. orally, twice daily, via stomach tube; prednisone, 5 mg., intramuscularly, once daily; dexamethasone, 0.1 per cent, one drop three times daily in the right eye only (approximately 0.02 mg. per day); prednisone, 1 per cent, one drop three times daily in the right eye only (approximately 0.02 mg. per day); and prednisone, 0.5 per cent, one drop once daily in the right eye only (approximately 0.01 mg. per day). Therapeutic manipulation controls consisted of normal saline drops in the right eye only or 0.5 ml. of intramuscular normal saline. In later studies, beta-methasone, 0.3 per cent, was given as one drop three times daily in the right eye only (approximately 0.05 mg. per day), starting one day after the tyrosine diet was begun. Phenobarbital, 6 mg., was given intramuscularly twice daily one day before beginning the tyrosine diet. Phenobarbital was employed as a classical nonsteroid hepatic microsomal enzyme inducer. Finally, to study the effect of drug interaction on
Modification of 1-tyrosine-induced keratopathy

Fig. 2. Comparison of degree of keratopathy in 5 per cent tyrosine-treated rats with those given the same diet and dexamethasone 0.1 per cent or beta-methasone 0.3 per cent eyedrops three times daily in the right eye only. Box at bottom of figure indicates duration of steroid treatment.

Summary statistical analysis, comparison of regression lines

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Regression coefficient</th>
<th>Slope</th>
<th>Elevation</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine control animals (5%) (1-11 days) and beta-methasone</td>
<td>0.38</td>
<td>151.91</td>
<td>&lt; 0.001</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tyrosine control animals (5%) (1-11 days) and dexamethasone</td>
<td>0.02</td>
<td>df = 1,508</td>
<td>&lt; 0.001</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tyrosine control animals (5%) (1-6 days) and post-dexamethasone</td>
<td>0.68</td>
<td>27.91</td>
<td>&lt; 0.001</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*F.S. = Not significant.

established, but not irreversible keratopathy, other groups of rats were started on either dexamethasone, 0.1 per cent, drops three times daily in the right eye only, or prednisone, 10 mg., twice daily orally, on the fourth day of tyrosine diet, when both epithelial and stromal opacity was present, but vascularization had not yet occurred.

After the administration of eyedrops to the rats' right eye, the animals frequently went through grooming maneuvers, rubbing both their eyes with their forepaws. It is very possible that they could have removed some of the administered eyedrop and then licked it off the paws later.

Statistical analyses were done by determining the linear regression of stage of disease on days of the 5 per cent tyrosine diet for each set of control animals and for each treatment. The linear regressions for each treatment and its control were compared to determine if statistically significant differences in slope or in elevation occurred. In this test, differences in elevation are tested only if the slopes are parallel, i.e., there is no significant difference in the slopes. The results of the statistical analyses are summarized in the footnotes with each graph.

Animals were killed by decapitation, eyes removed immediately, and processed for histologic examination as described elsewhere.

Results

The control animals fed 5 per cent tyrosine reacted in the predictable manner previously described. On the second day of 5 per cent tyrosine dietary treatment pinpoint corneal epithelial opacities appeared, then enlarged in a snowflake pattern, and involved the stroma by the third day. By the sixth day full-thickness corneal stromal thickening and opacity developed and vascularization began at the limbus. At the twelfth to fifteenth day the cornea was totally vascularized, and in some rats, epithelial ulcers had developed. Beginning of
clearing of corneal edema and vascularization was noted by the sixteenth day. Corneas sometimes became almost totally clear by biomicroscopy, and vessels became invisible, but in other animals slight residual corneal opacity and vascularization were present after three months of 5 per cent tyrosine diet. These degrees of corneal disease were classified as Stages 1 to 5 as previously described.¹

In comparison to the control animals, those treated with 10 mg. of prednisone orally, twice daily, showed no opacity until prednisone treatment was stopped at the eleventh day, then only epithelial opacities appeared (Fig. 1). The difference between the rate of development of the disease in the control animals and in the prednisone (10 mg.)-treated animals was highly significant (P < 0.001). When oral prednisone treatment was stopped, the rate of development of the epithelial opacities was similar to that for control animals during their first six days on the tyrosine diet (Fig. 1).

If a 10 per cent tyrosine diet was fed to rats given 10 mg. of prednisone twice daily, one half the animals showed epithelial opacities within ten days. A 10 per cent tyrosine diet causes total corneal opacity in all animals by ten days,² and is lethal in 60 per cent by fourteen days.³

In rats given prednisone, 5 mg. intramuscularly once daily, epithelial opacities appeared in half the animals by the twelfth day, but no stromal thickening or vascularization occurred. This depression in the rate of onset of the disease is statistically significant (P < 0.001, Fig. 1). After the prednisone treatment was stopped, stromal opacity and edema appeared.

In animals treated in the right eye only
Modification of 1-tyrosine-induced keratopathy

Fig. 4. Comparison of degree of keratopathy in 5 per cent tyrosine-treated rats with those given the same diet and phenobarbital, 6 mg., intramuscularly twice daily. Box at bottom of figure indicates duration of phenobarbital treatment.

Summary statistical analysis, comparison of regression lines

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Regression coefficient</th>
<th>Slope</th>
<th>Elevation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine control animals (5%) and phenobarbital (6 mg. twice daily)</td>
<td>0.47</td>
<td>2.98</td>
<td>154.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N.S. 0.001</td>
</tr>
</tbody>
</table>

*N.S. = Not significant.

with one drop of dexamethasone, 0.1 per cent three times daily, started one day before the tyrosine diet, both eyes remained completely clear on slit lamp examination for eleven days (Fig. 2). When the dexamethasone treatment was stopped corneal epithelial opacities developed rapidly, but the rate of onset of these opacities was significantly less than in the untreated control animals. There was no statistical significance in the degree of prevention of tyrosine keratopathy by oral or intramuscular prednisone or topical dexamethasone.

Rats treated in the right eye only, with one drop of beta-methasone, 0.3 per cent three times daily, started one day after the 5 per cent tyrosine diet, developed corneal epithelial lesions by the second day, which then decreased in degree, and never progressed into stromal opacity, thickening, or vascularity by twelve days of treatment. The rate of development of the lesions was significantly less than that for the control animals (P < 0.001, Fig. 2).

Animals given 1 per cent prednisone, one drop in the right eye three times a day, developed corneal epithelial opacities by the fifth to sixth day of treatment, and progressed to stromal opacity by the tenth to twelfth day of treatment (Fig. 3). There is no difference in the slopes of the two lines, but the degree of development of the disease is significantly depressed (P < 0.001 for difference in elevations of the two curves).

There was no significant difference in either the slopes of the lines or in their elevations between the animals given 5 per cent tyrosine diet alone and those animals on 5 per cent tyrosine diet treated with 1/4 per cent prednisone drops once daily in the right eye, saline eyedrops, or intramuscular saline once daily (Fig. 3).

Intramuscular phenobarbital delayed onset and degree of tyrosine keratopathy. There is no difference between the slopes of the two groups.
Fig. 5. Comparison of degree of keratopathy in 5 per cent tyrosine-treated rats with those given the same diet and prednisone, 10 mg., twice daily by mouth or dexamethasone 0.1 per cent eyedrops three times daily in the right eye only. Box at bottom of figure indicates duration of steroid treatment.

Summary statistical analysis, comparison of regression lines

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Regression coefficient</th>
<th>Slope</th>
<th>Elevation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine control animals (5%) (4-9 days) and prednisone (10 mg.) (4-9 days)</td>
<td>-0.14</td>
<td>df = 1,80</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tyrosine control animals (5%) (4-9 days) and dexamethasone (0.1%) in O.D. (4-9 days)</td>
<td>0.19</td>
<td>15.84</td>
<td>0.19 13.14</td>
</tr>
<tr>
<td>Prednisone (10 mg.) (4-9 days) and dexamethasone (0.1%) (4-9 days)</td>
<td>-0.23</td>
<td>df = 1,80</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tyrosine control animals (5%) (0-3 days) and dexamethasone (0.1%) (4-9 days)</td>
<td>-0.14</td>
<td>1.11</td>
<td>1.10 0.12</td>
</tr>
<tr>
<td>Tyrosine control animals (5%) (0-3 days) and dexamethasone (0.1%) (4-9 days)</td>
<td>-0.23</td>
<td>df = 1,116</td>
<td>1.06 df = 1.52</td>
</tr>
</tbody>
</table>

N.S. = Not significant.

of the regression lines, but there is a significant depression in the degree of development of the disease (P < 0.001 for difference in elevations) (Fig. 4).

In another experiment, to test the effect of systemic and topical steroids on pre-established mild keratopathy, both oral prednisone, 20 mg. daily, and topical 0.1 per cent dexamethasone eyedrops, three times daily in the right eye only, inhibited tyrosine keratopathy in both eyes. There is no difference the three groups of animals on the 5 per cent tyrosine diet for zero to three days. When the animals were then treated with either 0.1 per cent dexamethasone topically in the right eye or with 20 mg. of prednisone orally on the fourth day, the disease was significantly reversed (P < 0.001) and the slopes of the regression lines changed from positive to negative (Fig. 5). There was no significant difference between the prednisone and the dexamethasone treatments under these conditions.

Although some steroid-treated rat’s eyes were graded as biomicroscopically normal, light microscopic examination of selected specimens showed minor alterations in corneal epithelium and stroma qualitatively similar to previously described changes,
Discussion

It is known that tyrosine aminotransferase (TAT) is induced in rat liver cells and in rat hepatoma cells by a high tyrosine diet, and much more effectively by administration of glucocorticoids. This particular effect of adrenal corticosteroids is associated with proliferation of smooth endoplasmic reticulum, increase in liver weight and mitotic index, and accelerated regeneration of the liver after partial hepatectomy. It is postulated, therefore, that hyperstimulation of hepatic TAT by adrenal corticosteroids may delay onset of tyrosine-induced keratopathy in the rat, possibly by lowering blood tyrosine levels. The lag in production of keratopathy in phenobarbital-treated rats would agree with this hypothesis.

Additionally, after discontinuance of the prednisone therapy the more potent steroids delayed the onset of ocular disease more effectively than the less potent steroids. Possibly, this may be related to the half-life of smooth endoplasmic reticulum or induced enzyme.

Proof of the hypothesis that delay of keratopathy is due to increased TAT and decreased serum and aqueous tyrosine levels will depend on direct measurement of serum and corneal tyrosine and liver TAT. These studies are under way.

It must be emphasized that no difference was found between the treated right eye and the untreated left eye in the animals receiving potent corticosteroid drops in the right eye only. Sufficient beta-methasone, dexamethasone, and prednisone eyedrops must have been absorbed systemically either directly through the ocular tissues, by gravity through the lacrimal tract, or by the animal rubbing the eyes and licking its paws to stimulate hepatic TAT activity. Since no consistent biomicroscopic difference between the steroid-treated right eye and untreated left eye was found, local anti-inflammatory effect of steroids is not a factor in this animal model.

Because of the varying effects of different steroids noted consistently here, it is suggested that the tyrosine-fed rat keratopathy may be a useful laboratory tool for indirectly evaluating varying potencies of adrenal corticosteroids, presumably because of an indirect effect on the eye through the liver.

Clinically, because more cases of tyrosine-induced keratopathy are being recognized, it should be noted that doses of corticosteroids employed in this study to reduce corneal disease were very large in comparison to usual human treatment.

REFERENCES