Permeability of blood-ocular barriers of neonatal and adult cats to fluorescein-labeled dextrans of selected molecular sizes

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The permeability of the ocular blood vessels and neuroepithelial layers (the blood-ocular barriers) to fluorescein-labeled dextran of selected molecular sizes was evaluated in neonatal and adult cats by fluorescence microscopy. The iris and ciliary process vessels were permeable to molecules as large as 85Å effective diffusion radius (EDR). In the kitten, the choriocapillaris was permeable to molecules as large as 58Å EDR, and in the adult to molecules only as large as 32Å EDR. The retinal vessels and the retinal pigment epithelium were impermeable to all markers. The role of the cat's iris vessel in aqueous humor formation appears dissimilar to that of other species in that molecules larger than serum proteins traverse the walls of the iris capillaries.

Key words: neonatal kitten, cat, permeability, blood-ocular barriers, fluorescein isothiocyanate labeled dextran, ocular development, vessels, aqueous humor, fluorescence microscopy, tracer molecules

Certain morphologic and physiologic characteristics of the ocular blood vessels and neuroepithelial layers constitute what are known as the blood-ocular barriers. The basic function of those barriers is to control the movement of molecules from the vascular lumen to the paravascular tissues and/or to the ocular fluids. Size is one determinant whereby a molecule may or may not traverse a vascular endothelial lining, and dextran molecules of selected sizes have proved useful in the study of that permeability determinant. Fluorescein isothiocyanate (FITC)-labeled dextran of selected molecular sizes provide the added potential of in vivo as well as in vitro localization of the molecules by fluorescent techniques. We have previously demonstrated the usefulness of FITC-dextran in studies of normal and abnormal rat retinal vessels, the avian pecten, and the rat anterior segment. Fluorescent microscopic localization of FITC-dextran has been utilized in studies of aqueous humor outflow pathways and in blood-ocular barrier permeability determinations.

Fluorescent microscopic evaluations of freeze-dried tissues wherein sodium fluorescein (NaFl) was the permeability marker have been performed for both aqueous humor pathway and blood-ocular barrier investigations. In a study of the blood-ocular barriers of neonatal and adult cat, the iris...
Table I. Penetrance of selected molecular sized FITC-dextrans into ocular tissues of kittens

<table>
<thead>
<tr>
<th>Molecule (EDR)</th>
<th>Circulation time (min)</th>
<th>Iris stroma</th>
<th>Ciliary process stroma</th>
<th>Ciliary process epithelium</th>
<th>Choroidal stroma</th>
<th>Retina via retinal vessels</th>
<th>Retina via RPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC/dex 3 (12Å)</td>
<td>1</td>
<td>++</td>
<td>+</td>
<td>+/−</td>
<td>++</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>FITC-dex 20 (32Å)</td>
<td>2</td>
<td>++</td>
<td>++</td>
<td>+/−</td>
<td>++</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>++</td>
<td>++</td>
<td>+/−</td>
<td>++</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>FITC-dex 40 (45Å)</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>+</td>
<td>++</td>
<td>−</td>
<td>++</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>FITC-dex 70 (58Å)</td>
<td>5</td>
<td>+</td>
<td>++</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>++</td>
<td>++</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>FITC-dex 150 (85Å)</td>
<td>5</td>
<td>+</td>
<td>++</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>+</td>
<td>++</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Relative degree of fluorescence within tissue as indication of permeability of associated barrier vessels or epithelium: ++ + = marked; ++ = moderate; + = minimal; — = negative.

capillaries were found to be markedly permeable to NaFl,14 a finding decidedly dissimilar to that reported for other species.11 12 15 It again brought to mind the question concerning the role of those vessels in aqueous formation.16 Because NaFl is partially bound to serum proteins, it is not known in such studies whether it is the free NaFl (376 mol. wt.; 5Å effective diffusion radius (EDR)) or the protein-bound NaFl (35Å EDR) that does or does not pass the barriers.

To begin assessing that question, FITC-dextrans of selected molecular sizes were utilized to evaluate the permeability of the blood-ocular barriers in the neonatal and adult cat. The results form the basis of this report.

Materials and methods

A total of 16 kittens, ages 5, 13, and 21 days, and 13 adult cats were anesthetized with sodium pentobarbital (35 mg/kg I.P.) and injected intravenously with 0.1 cc of 33% FITC-dextran/0.1M sodium phosphate buffer solution per 200 gm body weight. The compounds utilized were FITC-dex 3 (mol.wt. 3000; EDR 12Å), FITC-dex 20 (mol.wt. 20,000; EDR 32Å), FITC-dex 40 (mol.wt. 40,000; EDR 45Å), FITC-dex 70 (mol.wt. 70,000; EDR 58Å), and FITC-dex 150 (mol.wt. 150,000; EDR 85Å). Details of the properties of these compounds are given in a previous publication. After circulation times from 30 sec to 30 min, depending on the molecular size of the injected FITC-dextran (Table I), the eyes were removed and quick-frozen in isopentane cooled to −105°C by a liquid nitrogen bath. The eyes were freeze-dried for 12 to 18 days in a molecular sieve apparatus in a −35°C environment. After drying, the eyes were grossly dissected, and portions were embedded under vacuum in wax. Sections were cut at 10 μm and examined by epi-illumination fluorescence microscopy with a Zeiss Photomicroscope II equipped with a BG-12 excitor and a Zeiss 50 barrier filter. Photographs were taken with 400 ASA Ektachrome color film.

Results

Neonatal cat. The penetrance of the different FITC-dextran markers into the ocular tissues of the kitten is shown in Table I. Those molecules with an EDR of 58Å or less readily passed the iris capillaries (Figs. 1B, 2A, and 2B) whereas the 85Å molecule did so only minimally. There was evidence of fluorescence in greater concentration in the posterior stroma adjacent to the iris pigment epithelium (Figs. 1B and 2A), and this was interpreted as evidence that the FITC-dextrans entered the iris stroma from the iris capillaries in that region rather than by movement from the anterior chamber aqueous. This gradient was more pronounced in the shorter circulation time specimens (Figs. 2A and 2B). All FITC-dextran molecules up to and including the 85Å molecule were markedly evident in the ciliary process stroma. However, fluorescence was evident in the intercellular spaces of the nonpigmented epithelium of the ciliary processes only with the FITC-dex 3 (Fig. 3) and possibly with the FITC-dex 20 markers. Topographic evalua-
Fig. 1A. Photomicrograph of 5-day-old kitten freeze-dried iris tissue for orientation purposes. Posterior chamber (PC) adjacent to two layers of pigment epithelial cells (PE). Iris stromal capillaries (C) are immediately adjacent to PE layers. Large vessel area (L) lies between capillary area and anterior iris border and anterior chamber (AC). (Freeze-dried preparation; white-light transillumination; ×86.)

Fig. 1B. Photomicrograph of 13-day-old kitten iris after 1 min circulation of FITC-dex 3 (12Å EDR). Presence of dye in posterior iris stroma adjacent to pigment epithelium (PE) is marked. Diminished fluorescence in anterior stroma and marked fluorescence along posterior walls of large vessels (arrowheads) suggest movement of dye from capillaries toward anterior chamber. (Freeze-dried preparation; epi-illumination; wax-embedded; ×25.)
Fig. 2A. Photomicrograph of 5-day-old kitten iris after 5 min circulation of FITC-dex 70 (58Å EDR). Presence of dye within vessels is marked. Note the many small vessels in iris stroma adjacent to pigment epithelial layers (PE). At this early time, marker is more evident in posterior rather than anterior stroma. (Freeze-dried preparation; epi-illumination; wax-embedded; ×125.)

Fig. 2B. Photomicrograph of 5-day-old kitten iris after 20 min circulation of FITC-dex 70 (58Å EDR). Note more diffuse presence of dye throughout iris stroma. (Freeze-dried preparation; epi-illumination; wax-embedded; ×320.)
Fig. 3. Photomicrograph of ciliary process of 13-day-old kitten after 1 min circulation of FITC-dex 3 (12Å EDR). Note marked presence of dye in stroma and presence of dye between nonpigmented epithelial cells (arrows) in this tangential section. PC, Posterior chamber. (Freeze-dried preparation; epi-illumination; wax-embedded; x800.)

Sections of nonembedded pieces of iris and ciliary body also showed an overlying fluorescence on the surface of the ciliary processes in just those specimens receiving FITC-dex 3 and 20.

The choriocapillaris appeared permeable to those markers with an EDR of 58Å or less (Fig. 4), whereas the 85Å marker remained within the vessels (Fig. 5). The retinal blood vessels and pigment epithelium were impermeable to all the FITC-dextrins, even in the very immature (5-day-old) kitten (Fig. 6).

No age-related differences were found in the permeability characteristics of the blood-ocular barriers in the kittens; similarly, the status of the pupil at the time of enucleation had no effect on the results. Depending on the depth of anesthesia, the pupil was either constricted or only partially dilated; in some instances pupil dilation occurred during enucleation.

**Adult cat.** The permeability of the adult cat’s iridal and especially the choroidal capillaries appeared less than that of the kitten. In the iris, the 85Å marker was observed in the stroma adjacent to the pigment epithelial layers, but its presence was markedly less evident than in the kitten. The autofluorescence of the adult cat’s iridal chromatophores could mask to some degree the fluorescein markers, but this did not appear solely responsible for the diminished observance of those markers. Similar lessened fluorescence was also observed in the adult iris with the 45Å and the 58Å markers. The degree of fluorescence in the iris concerning the 32Å and 12Å markers was similar in both neonatal and adult cats.

In the choroid, the 12Å marker was observed to a moderate degree in the adult, and the 32Å marker to a minimal degree. The 45Å, 58Å, and 85Å markers did not appear to...
Fig. 4. Photomicrograph of retina and choroid of 5-day-old kitten after 20 min circulation of FITC-dex 70 (58Å EDR). Note presence of dye within tapetal tissue (T) and retinal vessels (arrows). No dye is present within retinal tissue. (Freeze-dried preparation; epi-illumination; wax-embedded; X320.)

be permeable in the adult cat’s choriocapillaris.

The ciliary processes of the adult cat fluoresced to a similar degree for all markers as in the kitten.

Discussion

A previous study demonstrating the marked permeability of the feline iris capillaries to NaFl raised the question of whether free or protein-bound NaFl was passing the vessel endothelium. It is now apparent that both forms may do so, inasmuch as this present study demonstrated passage of molecules as large as 85Å EDR across the endothelium of the iris capillaries in kittens and adult cats. These data substantiate the finding of Cunha-Vaz et al. that serum protein-bound trypan blue also permeated the iris vessels of kittens. This species, then, is dissimilar to man, monkey and rabbit, and rat in that iris capillaries of those species are impermeable to both the free and protein-bound forms of NaFl. The degree of permeability to larger molecules was less for the adult cat’s iris capillaries than for those of the kitten; this suggests that the maturation of the iris tissues and/or blood-aqueous barriers has caused an alteration in the permeability of the mature iris vessels. This phenomenon of the alteration of vessel permeability by concurrent altered tissue requirements is evidenced in other tissues and under other circumstances.

The role of the iris vessels in aqueous formation in the monkey has been demonstrated to be negligible; however, the role of the feline iris vessels may be significant. A study concerning totally iridectomized cats found a lowered intraocular pressure in those cats. Those authors suggested that the cat’s iris vessels may be necessary to optimal aqueous
humor formation. It is interesting, though, that they found no significant differences in aqueous humor protein content between iridectomized and control cats. If serum proteins passing the iris capillaries are a significant part of the aqueous humor values, a marked lowering of that value could be expected in the iridectomized cats. It is possible, though, that there was a facultative response on the part of the ciliary body epithelium in the iridectomized cats.

In this study, fluorescence was observed on the surface of the ciliary processes in kittens and adult cats that had received FITC-dex 3 and, to a lesser extent, FITC-dex 20. A molecular sieve effect for serum proteins has been demonstrated for the blood-aqueous barrier in man. In that study, serum protein analysis of aqueous humor samples from normal patients showed an inverse relationship between molecular size and aqueous humor concentration. We have demonstrated a similar relationship for the dextran molecules in our in vivo study in rats and in this present study in cats.

The choriocapillaris in the kitten, like the iris capillaries, was more readily permeable to the larger dextrans than in the adult cat. Again it would seem that maturation of the tissues had affected the permeability of those vessels. In the adult cat, molecules with an EDR as large as 32Å passed from the choriocapillaris into the tissue stroma, and this is compatible with a study of the adult rat choriocapillaris wherein heme protein markers of a similar size were permeable whereas larger molecules were not.

It is interesting that there was no apparent effect on permeability of the ciliary process capillaries based on molecular size and/or age. Because studies of this nature have not been performed in other species, it is not
known whether this is a characteristic of the feline. That the ciliary process capillaries appear more permeable than the iris or choroidal capillaries may be a reflection on the amount of bulk-flow necessary for aqueous humor production via the ciliary process epithelium. The stroma of the ciliary process is not extensive; in fact, there is a marked presence of vessels. Therefore it may also be that the high fluorescence of the vessel lumens conveys a feeling of high fluorescence in the ciliary process stroma.

This study also demonstrated the impermeability of the retinal vessels (inner blood-retinal barrier) and the retinal pigment epithelium (outer blood-retinal barrier) to any of the FITC-dextran markers. This was true for both neonatal and adult cats. It is evident that those compounds have no intrinsic adverse effects on the normal permeability of the feline blood-retinal barriers. We have previously demonstrated no adverse in vivo effects in rats, and thus it is apparent that these compounds can be utilized with confidence in experimental studies on the effect of molecular size on blood-retinal barrier permeability.

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REFERENCES