A histopathologic study of retinal lesions inflicted by transscleral iontophoresis *

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Abstract. In the present study, retinal lesions were induced by transscleral iontophoresis (1.5 mA) in rabbits. The size and severity of the lesions increased with the duration of application (2–25 min). No lesion was noted after <1 min application. Immediately after 5 min iontophoresis, the edematous retina exhibited necrotic retinal pigment epithelium (RPE), loss of outer segments, and thinning of the inner and outer nuclear layers. At 5 days after iontophoresis, there was a proliferation of RPE cells and macrophages in the subretinal space, with thinning of the inner and outer retinal layers continuing. By day 14, the retina had been reduced to a glial membrane. Immediately after 15 min iontophoresis, the damaged retina appeared in a mummified form containing no cellular elements. By day 5 thereafter, macrophages and actively proliferating RPE cells had been noted in the necrotic retina. By day 14, a glial membrane had formed.

Introduction

Successful introduction of high intravitreal levels of several classes of drugs by transscleral iontophoresis has been reported in rabbits [2, 6–8, 13, 18] and monkeys [3]. These drugs include antibiotics (gentamicin [1, 2, 3], cefazolin [2], ticarcillin [2], and vancomycin [7]), antifungal agents (ketoconazole [9]), and corticosteroids (dexamethasone [13]). These reports demonstrated that transscleral iontophoresis can deliver high levels of ionizable drugs into the vitreous and suggested that this technique is a promising noninvasive method of intravitreal drug administration. However, some of the investigators [3, 13, 18] observed retinal lesions in rabbits after transscleral iontophoresis. A detailed description of the pathologic features occurring after transscleral iontophoresis is lacking in the literature.

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Before transscleral iontophoresis can be established as an alternative to intravitreal injection of drugs, its potential for causing retinal damage must be investigated. We therefore conducted a study on the pathologic features in rabbit eyes that underwent transscleral iontophoresis. Since most reports of this technique have employed currents of 0.5–3.5 mA for 5–10 min, we decided to use 1.5 mA for 0.5–25 min, conditions that would be most practical in clinical use.

Materials and methods

A total of 15 New Zealand albino rabbits weighing between 1.5 and 2 kg were used in this study. Transscleral iontophoresis was performed as previously described [3]. To summarize briefly, the rabbits were anesthetized by intramuscular injections of ketamine (50 mg/kg) and acepromazine (0.5 mg/kg). An application chamber [3], whose orifice measured 0.6 mm in diameter was equipped with a platinum electrode, which was connected to the negative pole of a d.c. power supply (6209B Constant Current Power Supply; Hewlett-Packard, Berkeley Heights, N.J.). The chamber, filled with 0.01 M phosphate-buffered saline (pH 7.4), was gently pressed against the rabbit eye at a point approx. 2–4 mm posterior to the limbus. The positions of the contact point were marked by 6-0 nylon sutures. The positive pole of the power supply was connected to the rabbit ear.

The effect of increasing the duration of iontophoresis on the retina was studied by applying a 1.5-mA current for 30 s or for 1, 2, 3, 4, 5, 15, or 25 min. Animals were killed 5 days after the procedure. The diameter of the lesions was microscopically estimated using a micrometer disc with serial histologic sections. We examined the histopathologic course of moderately severe and severe retinal lesions secondary to transscleral iontophoresis. Lesions inflicted by the application of 5 or 15–25 min current at 1.5 mA were studied immediately and at 5, 14, and 21 days after iontophoresis. Experiments were performed at least three times under each set of conditions.

Immediately after the animals had been killed, the eyes were enucleated and fixed in 4% paraformaldehyde and 1% glutaraldehyde. The globes were then post-fixed in Dalton's chrome osmium fixative, dehydrated in alcohol, embedded in epoxy resin, sectioned, and studied by light and electron microscopy. The care and maintenance of the rabbits conformed to the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research.
Fig. 1a–f. Increasing morphologic changes in the retina with iontophoresis application time. A current of 1.5 mA was applied for 1, 2, 3, 4, 5, and 15 min and retinal lesions were examined 5 days after the procedure. a 1 min: the retina appears intact. ×210. b 2 min: loss of outer and inner segments, disorganized outer nuclear layer (ONL), and irregular thinning of inner layers are visible. ×220. c 3 min: focally absent RPE (arrowhead) and irregular thinning of the outer nuclear (ONL), inner nuclear (INL), and outer plexiform layers (OPL) can be seen. At d 4 min and e 5 min, reactive proliferation of RPE (arrowhead) and macrophages (M) are visible. ×210. f 15 min: the necrotic retina is infiltrated with macrophages. Actively proliferating RPE cells are visible on Bruch’s membrane (arrowhead). ×210

Results

Effects of increasing application time on retinal lesions

In general, the transscleral iontophoresis-induced retinal lesions examined at 5 days after the procedure increased in severity as the time of application increased from 1 to 25 min (Fig. 1). On gross examination, no retinal lesions were visible after application times of 0.5 and 1 min. The lesions occurring after 2, 3, 4, 5, 15, and 25 min applications appeared as well-circumscribed chorioretinal lesions and were occasionally accompanied by intraretinal hemorrhage.

By light microscopy (Fig. 1a), the sclera, choroid, stroma, choriocapillaris, and large vessels appeared unremarkable, with the retina apparently intact, after 1 min iontophoresis. After 2 min application (Fig. 1b), choriocapillaris appeared to be patent, with some vessels being engorged. Outer and inner segments of photoreceptor cells were lost. The outer nuclear layer was disorganized, and irregular thinning of the inner layers was noted. Following 3 min application, the sclera remained unremarkable. In addition to the features observed after 2 min iontophoresis, the choriocapillaris was occluded. The retinal pigment epithelium (RPE) became focally absent, and irregular thinning of the outer nuclear, inner nuclear, and outer plexiform layers was apparent (Fig. 1c). After 4 or 5 min application, reactive proliferation of RPE was seen. Furthermore, necrotic debris, abundant macrophages in the subretinal space, and thinning of the inner and outer layers were observed (Figs. 1d–e). After prolonged application (15 or 25 min), most of the choriocapillaris and occasionally, large vessels were occluded, and the whole retina was necrotic,