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The effect of topical cyclosporin A on the rabbit cornea

A clinical and electron microscopic study*

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Abstract. To investigate the effect of topical cyclosporin A (CSA) on the rabbit cornea, both eyes of 80 animals were treated with CSA 2% eye drops over a 3-week period under various conditions. CSA was dissolved in castor oil or ethanol 13.8 vol. %. Slit-lamp, scanning and transmission electron microscopic examinations were performed. CSA 2% in castor oil given 5 times daily showed no damage to either the corneal epithelium or endothelium. In contrast the solutions containing ethanol revealed considerable epithelial cell damage.

Introduction

Cyclosporine A (CSA) is a potent immunosuppressive agent and has been shown to prolong survival of allografts in animals and man (Editorial, 1979). In placing corneal grafts experimentally in a rabbit model, the immune reaction produced can be delayed after its intramuscular (Coster et al. 1979; Bell et al. 1982; Shepherd et al. 1980), retrobulbar (Salisbury and Gebhardt 1981), and topical administration (Shepherd et al. 1980; Hunter 1981; Hunter et al. 1981, 1982a, b; Kana et al. 1982). Penetration of topical CSA into the cornea and aqueous humor has been shown by Mosteller et al. (1985) and Behrens-Baumann et al. (1986). Although topical application of the drug avoids its potential systemic side effects, possible local toxic effects on the cornea have not yet been described. Using the rabbit cornea as an animal model, we studied the effects of topical CSA with clinical, scanning and transmission electron microscopic data. In addition, we studied the effect of CSA on epithelial healing after induced corneal abrasions.

Materials and methods

CSA was dissolved in either ethanol or in castor oil. This preparation was then applied to both eyes of outbred male rabbits, ranging in weight from 3.8 to 4.2 kg. Topical saline was used as a control solution. Preparations were given to 80 rabbits as illustrated in Table 1, with 5 animals placed in each group.

All eyes were examined with a slit-lamp to exclude any abnormality of the anterior segment. In 20 eyes (Groups 7 and 8), a corneal abrasion was gently performed with a hockey knife using a microscope, so that the epithelium was completely removed from limbus to limbus. The group-specific drops (without preservatives) were then administered over a period of 3 weeks. The epithelial healing in groups 7 and 8 was further studied with fluorescein staining and documented by photographs.

After 3 weeks the eyes were examined again with a slit-lamp, following which the animals were anesthetized with ketamine (Ketanest) and xylazine (Rompun) for prefixation of the corneas. They were then killed with an overdose of the anesthetics.

Preparation of corneas for electron microscopy

Scanning electron microscopy (SEM). The in vivo corneas were continuously irrigated for 5 min with 4% glutaraldehyde in 1/15 M Sörensen phosphate buffer (pH 7.2) and were then excised with a 7-mm trephine and fixed in the same glutaraldehyde solution for 3 h. The corneas were washed in Sörensen phosphate buffer and postfixed for 1 h in 1% osmium tetroxide. They were dehydrated in ascending concentrations of acetone and were dried at the critical point. All tissue preparations were coated with a 20 nm layer of gold. Observations and photography were performed with a Philips SEM 505 scanning electron microscope.

Transmission electron microscopy (TEM). The in vivo corneas were prefixed in 2% glutaraldehyde and 2% formaldehyde in 1 M cacodylate buffer (pH 7.3). The corneas were then excised and fixed in the same solution for 1.5 h. This was followed by rinsing in cacodylate buffer and postfixing for 1 h in osmium tetroxide in phosphate buffer. Specimens were next dehydrated in a series of ascending alcohol concentrations and were embedded in Epon. Each preparation was sectioned on a LKB 4800 III Ultrotome, stained with uranyl acetate and lead citrate, and observed and photographed with a Siemens Elmiskop 101.

Results

Slit-lamp examination

Epithelial healing after corneal abrasion was not delayed clinically in the CSA group when compared with that of

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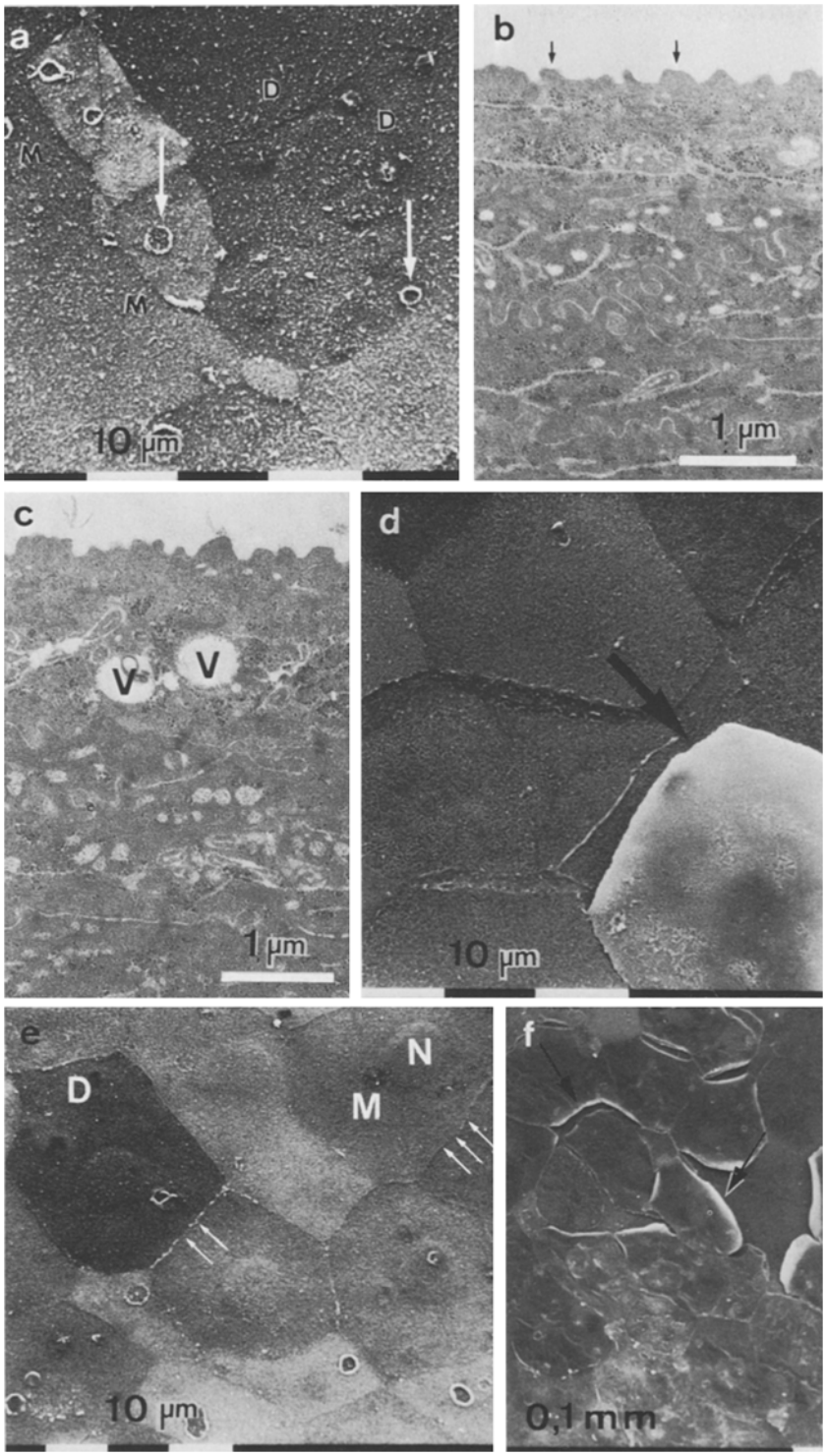

Fig. 1.a, b CSA 2% in castor oil (10 drops daily). **a** Scanning electron micrograph (SEM) of rabbit corneal epithelium. Medium dark (*M*) and dark (*D*) cells, holes (*arrows*), as well as tiny particles on the corneal epithelial surface are readily seen. **b** Transmission electron micrographs (TEM) of regular corneal epithelium, showing reduced surface microprojections without antennulae microvillares (*arrows*). **c, d** CSA 2% in ethanol, 13.8 vol% (10 drops daily). **c** TEM of corneal epithelium with short and reduced microvilli and vacuolization (*V*). **d** SEM of corneal epithelium with mainly medium dark cells and desquamating superficial cells (*arrow*), with pores in the outer plasma membrane. **e** CSA 2% in ethanol, 13.8 vol% (5 drops daily). SEM of corneal epithelium. Medium dark (*M*) and dark (*D*) cells with prominent borders (*arrows*) and cell nuclei (*N*). **f** Ethanol, 13.8 vol% (10 drops daily). SEM of corneal epithelium. Desquamating cells (*arrows*) with holes and pores in their plasma membranes

the castor oil treated animals. Although one eye in each group was lost by infection, all groups as a whole clinically showed no corneal damage or conjunctivitis.

SEM and TEM studies

Group 1 (10 × CSA in castor oil). The corneal epithelium exhibited mainly medium dark cells, as compared with untreated rabbit epithelium (Fig. 1a). The cell borders were unbroken, while microvilli and microplicae were short and sometimes reduced in number. Holes were present, but did not exhibit any visible microstructures. Tiny particles were visible on all preparations and were probably deposits of CSA. SEM revealed the corneal endothelial layer to have no significant pleomorphism. All intracellular borders remained intact, cell nuclei were not recognizable. Small numbers of individual microprojections were present, but their numbers were not more than in normal corneal en-

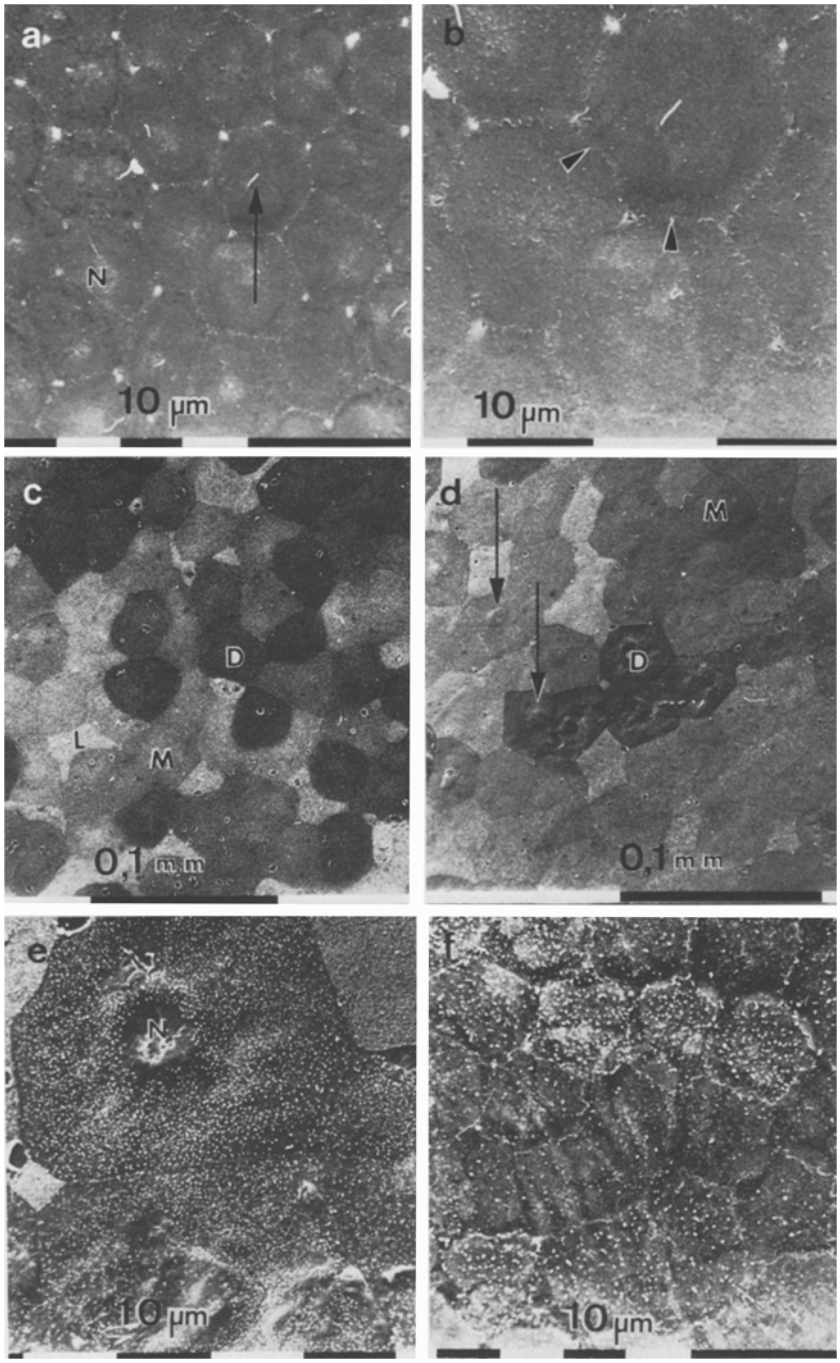

Fig. 2. a, b Ethanol, 13.8vol % (10 drops daily). *a* SEM of corneal endothelium with regular cell mosaic, prominent cell nuclei (*N*), and cilia (*arrow*). **b** High magnification SEM of corneal endothelium with opening borders (*arrowheads*). **c** Castor oil (10 drops daily). SEM of corneal epithelium with light (*L*), medium dark (*M*), and dark (*D*) cells. **d–f** CSA 2% in castor oil 10 drops daily after corneal abrasions. **d** SEM micrograph of healed corneal epithelium: medium dark (*M*) and dark (*D*) cells with reduced surface microprojections and prominent cell nuclei (*arrows*). **e** High magnification SEM. Many dark cells with short and reduced microvilli and prominent cell nuclei (*N*). **f** SEM of corneal endothelium showing more cell projections than on the normal inner corneal surface

Table 1. Treatment of rabbit corneas with either CSA in castor oil or ethanol (eye drops without preservatives)

Group (10 eyes each)	Treatment	Drops daily
1	CSA 2% in castor oil	10
2	CSA 2% in ethanol (13.8 vol.%)	10
3	CSA 2% in castor oil	5
4	CSA 2% in ethanol (13.8 vol.%)	5
5	NaCl 0.9 in ethanol (13.8 vol.%)	10
6	Castor oil	10
7	CSA 2% in castor oil after corneal abrasion	10
8	Castor oil after corneal abrasion	10

dothelium. TEM always revealed an intact histological structure in all parts of the cornea. The epithelial microprojections were slightly shortened and showed no antennulae microvillares (Fig. 1 b).

Group 2 (10 × CSA in ethanol). SEM revealed mainly medium dark corneal epithelial cells equipped with short microvilli and holes with reduced internal microstructures. However, open cell borders in some areas and plasma membrane damage to superficial epithelial cells were demonstrable (Fig. 1 d). TEM further showed that surface cells had curtailed microstructures and vacuolizations which were visible with enlargements of the intercellular spaces (Fig. 1 c). The corneal endothelium had intact cell borders with few micro-

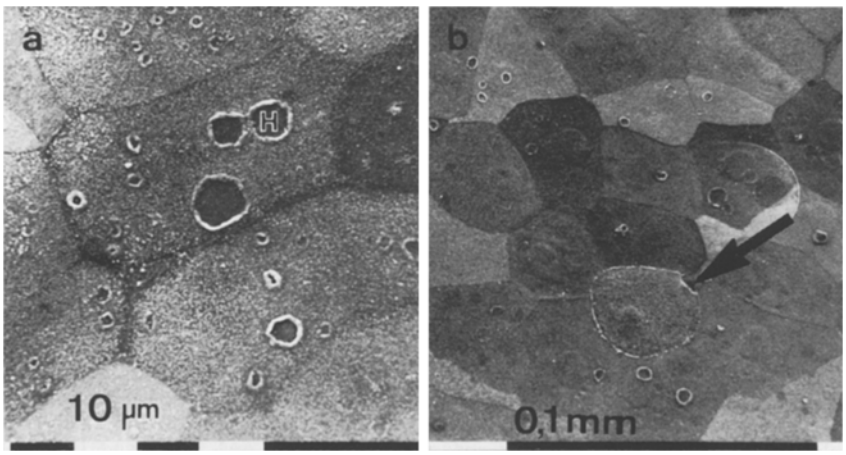

Fig. 3. a, b Castor oil (10 drops daily) after corneal abrasion: **a** SEM of corneal epithelium after abrasion. Healed epithelium with nearly regular surface microstructure, holes (*H*) without normal microprojections. **b** SEM of corneal epithelium after abrasion with desquamating cell (*arrow*)

structures (SEM). Cell nuclei were distinguishable from the rest of the cell surface as light areas.

Group 3 (5 × CSA in castor oil). The corneal epithelium and endothelium were not different from normal tissues inspected with SEM and TEM.

Group 4 (5 × CSA in ethanol). Cell nuclei could be identified as prominent areas, and cell borders appeared as prominent ridges (Fig. 1e). In addition, open cell borders were present. Microvilli with intact cytoplasmic structure were demonstrable for superficial epithelial cells under TEM. An intact corneal endothelial cell pattern with a regular morphology was seen in all SEM preparations.

Group 5 (10 × ethanol). The use of this test solution led to an extensive cell desquamation of the corneal epithelium (Fig. 1f). The regular microstructure could not be detected by SEM in the detaching cells. There was considerable cell damage, with pores and cracks in the outer plasma membrane. Only a few microprocesses could be seen on the surface of the desquamating cells. Although the corneal endothelial cell mosaic was regular in SEM preparations, the cells per se were swollen and the cell nuclei appeared as whitish prominent areas (Fig. 2a). The usually overlapping cell borders were open partially (Fig. 2b).

Group 6 (10 × castor oil). Under SEM, the numbers of medium dark and dark epithelial cells were increased (Fig. 2c), as compared with normal epithelium. The cell borders were intact and the number of holes reduced. In TEM, the corneal endothelial cells revealed an intact pattern.

Group 7 (10 × CSA in castor oil after corneal abrasion). Slit-lamp examination revealed no corneal opacity and no epithelial damage when stained with fluorescein. Mainly medium dark cells with short microvilli were seen under SEM. The migrated corneal epithelium were usually intact. Some migrated cells revealed short microprocesses on their surfaces (Fig. 2d and e). Under SEM, corneal endothelial cells had increased superficial microprocesses and cilia (Fig. 2f).

Group 8 (10 × castor oil after corneal abrasion). The epithelium was usually complete and showed typical cell structures in SEM with light, medium-dark, and dark cells and holes (Fig. 3a). However, some desquamating cells were visible as a sign of the vulnerability of the just-healed epithelium (Fig. 3b). Under SEM, the corneal endothelium revealed an intact cell pattern with more cell microstructures than normal. No relevant alterations could be demonstrated in ultra-thin sections.

Discussion

Topical cyclosporine A (CSA) has been shown to penetrate into the rabbit cornea and aqueous humor (Mosteller et al. 1985; Behrens-Baumann et al. 1986), and will delay the immune reaction after corneal grafting (Shepherd et al. 1980; Hunter 1981; Hunter et al. 1981, 1982a, b; Kana et al. 1982). In its preparation as eye drops, CSA is soluble in either ethanol or castor oil.

An ethanol concentration of more than 45 weight % causes superficial injury to the eye (Grant 1974). In order to get a 2% CSA solution, we reduced the intravenous CSA preparation of Sandimmun to up to a 13.8 weight % concentration of ethanol. Nevertheless, all rabbit corneas treated with eye drops containing ethanol revealed considerable SEM and TEM cell damage despite normal slit-lamp microscopy. This damage showed a dose-dependent relation and resembled corneal changes after hydrochloric acid (Brewitt and Honegger 1979) or benzalkonium chloride (Tønjum 1975; Pfister and Burstein 1976; Burstein 1980; Brewitt and Feuerhake 1980). Our solutions contained no preservatives.

The addition of CSA, however, reduced cell damage of the corneal epithelium. The mechanism of this protective effect is not yet understood. Ahonen et al. (1984) studied the effect of CSA on the formation of granulation tissue in subcutaneously implanted viscose cellulose sponges (which is a commonly used model for studies on wound healing). In CSA treated rats, Ahonen found an increased number of macrophages and accumulation of collagen and therefore suggested that CSA might even enhance wound repair.

In our experiments, CSA in castor oil did not show any negative influences on wound healing after corneal abrasions, when compared with castor oil alone. This further supports the experimental data of Ahonen et al. (1984), although both studies are not directly comparable.

CSA in castor oil caused no significant alteration of corneal cells when applied topically 5 times daily. A more frequent dose (10 times daily) also caused no cell damage.

However, numerous tiny particles could be detected by SEM, which were probably deposits of CSA.

In summary, eye drops of 2% CSA in castor oil given 5 times daily caused no corneal damage detectable clinically or by SEM or TEM. Consequently, our study indicates that there is no contraindication to the topical use of CSA.

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