Original Article

Safety Outcomes of Intrastromal Injection of Sodium Hypochlorite in the Normal Rabbit Cornea

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Abstract

Purpose: To investigate side effects of intrastromal sodium hypochlorite (NaOCl) injection in normal rabbit corneas and its possible use in treatment of fungal corneal infections.
Methods: We conducted a prospective, non-randomized study in a healthy cornea rabbit model. Intrastromal injection of one hundred µl of NaOCl 5 % in one eye and NaOCl 10 % in the other eye was performed in 5 rabbits. Clinical examinations including the study of conjunctival injection, corneal edema, corneal opacity or melting, and limbal ischemia were performed on days 1, 7, 14 and 21after injection. Specular microscopy and pathological studies were also performed three weeks after corneal injections in enucleated eyes.
Results: NaOCl 5 % injection was associated with normal endothelial morphology and cell count in specular microscopy. Some irregularities and drop out was associated with NaOCl 10 % injection.
Conclusion: Intrastromal injection of NaOCl 5 % might be a safe method to treat fungal corneal infections.

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Introduction

Corneal fungal infection may account for nearly half of the total corneal infections in developing countries [1, 2]. The prognosis of fungal corneal infection is very poor compared to bacterial infections and is a major cause of ocular morbidity [1]. The present treatments are topical, intrastromal, intracameral and systemic antifungal medications. Limited penetration into deeper layers of the cornea, limited range of efficacy, and corneal toxicity are the limitations of such treatments. In most cases, the need for tectonic surgical procedures or keratoplasty in fungal infections is higher than bacterial infections [1]. This proves the poor response of the disease to antifungal agents. Also, the success rate of tectonic corneal transplantation surgery for fungal keratitis is low since the graft might be affected by infection recurrence or graft rejection due to the limitation of topical steroid use in early weeks after fungal keratitis keratoplasty.

Sodium hypochlorite (NaOCl) has been suggested as an antifungal agent in different studies [3]; however it has not been used in ophthalmologic setting. In the present study, we examined the ocular effects of intrastromal injection of two different concentrations of sodium hypochlorite (5 % and 10 %) in normal rabbit corneas in order to investigate the possibility of its use to treat fungal infections.

Methods

We conducted a prospective non-randomized study in a healthy cornea rabbit model. Five white male New Zealand rabbits weighting between 2.5 and 3 kilograms were used. This study was approved by ethics committee of Farabi Eye Hospital, Tehran, Iran. The study was done in accordance with the Association for Research in Vision and ophthalmology and the declaration of Helsinki guidelines. Injection of one hundred µl of NaOCl 5 % and NaOCl 10 % was performed in the stromal corneal layer of the right and the left eye of 5 rabbits, respectively, under general anesthesia. Clinical examinations including the study of conjunctival injection, corneal edema, corneal opacity or melting, and limbal ischemia were performed on days 1, 7, 14 and 21after injection.

Rabbits were euthanized three weeks after corneal injections using intracardial injection of phenobarbital, then the eyes were enucleated and confocal microscopy was performed using Konan Eye Bank Kerato-Analyzer specular microscope (Konan Medical, Inc.; Hyogo, Japan). After specular microscopy, the corneas were fixed with 10 % formalin and pathological examination was performed to compare the effects of NaOCl 5 % and NaOCl 10 %.

Results

Rabbit 1: NaOCl 5 % was injected in the right eye and NaOCl 10 % in the left eye. On final slit lamp examination, the right eye had white conjunctiva, clear cornea with mild central haziness and without infiltration. Injection induced total hyphema happened in the left eye because of corneal perforation and made it unsuitable for slit lamp examination. Specular microscopy of the right eye showed normal endothelial distribution and morphology. Specular microscopy was not possible for the left eye because of clot hyphema. The pathology results three weeks post injections are shown in table 1.

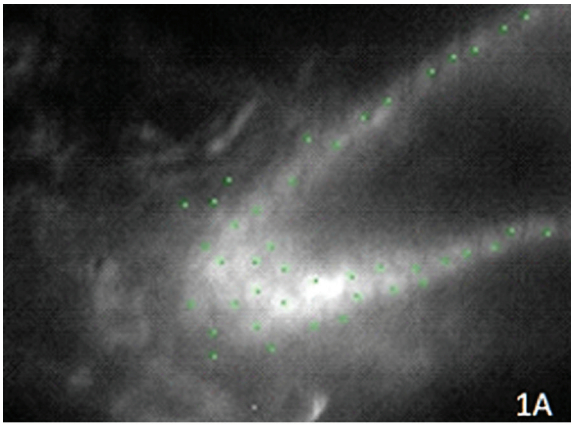
Rabbit 2: NaOCl 5 % was injected in the right eye and NaOCl 10 % in the left eye. On final slit lamp examination of the right eye, the conjunctiva had no injection. Cornea was locally hazy. Corneal vascularization and whitening of injection site was also observed

in this cornea in the first week. Specular microscopy showed normal endothelial cells. The left eye showed corneal total haziness accompanying vascularization and whitening of injection site on the first week. The pathology results three weeks post injections are shown in table 1.

Rabbit 3: NaOCl 5 % was injected in the right eye and NaOCl 10 % in the left eye. On final visit, the right eye conjunctiva was normal with mild corneal haziness in injection site. The left eye had also mild resolving corneal haziness and corneal edema and mild whitening at injection site. The pathology results three weeks post injections are shown in table 1.

Rabbit 4: NaOCl 5 % was injected in the right eye and NaOCl 10 % in the left eye. Three weeks after injection, the right eye conjunctiva was mildly injected and cornea was clear except for whitening of the injection site. The left cornea was mildly chemotic, and had mild haziness with whitening and vascularization of the injection site on the first week post injection. Again, specular microscopy was within normal range in the right eye but endothelial enlargement and decrease in cell count was observed in the left eye. The pathology results three weeks post injections are shown in table 1.

Rabbit 5: NaOCl 5 % was injected in the right eye and NaOCl 10 % in the left eye. On slit lamp examination of the right eye, the conjunctiva and cornea seemed normal except for corneal whitening and vascularization surrounding the injection site. Specular examination was within normal limits in both eyes. NaOCl 5 % injection was associated with normal endothelial morphology and cell count in specular microscopy, but some irregularities and drop out associated with NaOCl 10 % was observed (Figure 1). The pathology results three weeks post injection are shown in table 1.



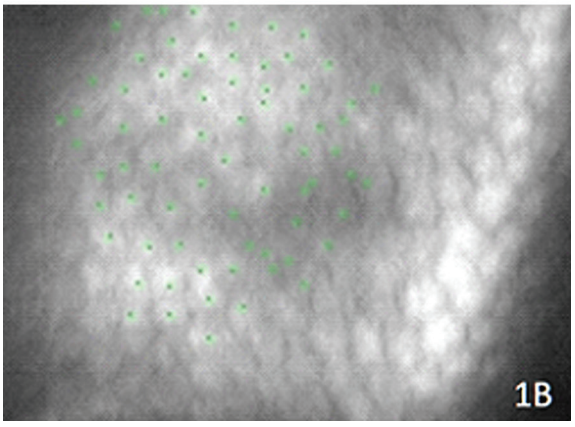


Figure 1: Normal distribution, morphology and size of endothelial cells after NaOCl 5 % injection (1a) compared to decreased endothelial cell count and atypical morphology after NaOCl 10 % injection (1b)

Discussion

Sodium hypochlorite is a solution that has shown some antifungal effects in previous studies [3,4]. A study conducted by Araujo et al.,[5] indicated the susceptibility of all Aspergillus species to this solution. They also stated that a minimal inhibitory concentration of sodium hypochlorite 25-50 µg/ml was effective against Aspergillus Fumigatus, Aspergillus Flavus and Aspergillus Niger. Another similar study was conducted by Mattaei et al., [6] in Brazil. They studied the in vitro fungicidal

Table 1: Pathological findings in rabbits receiving sodium hypochlorite corneal injections

Case	Epithelium	Bowman	Stroma	Descemet	Endothelium
Rabbit 1 (OD)	Focal thinning, Inflammatory cells	Disruption replaced, by fibrosis	Vascularization, Inflammation, Edema, Focal hemorrhage	Folding	Nearly normal with scar
Rabbit 1 (OS)	Focal thickening, Inflammatory cells	Disruption replaced by fibrosis	Vascularization, Inflammation, Severe edema, Focal hemorrhage, Basophilic degeneration	Folding, Thickening, Fragmentation	Depletion
Rabbit 2 (OD)	Thinning Folding	Disruption replaced by fibrosis pannus formation	Vascularization, Inflammation. Edema, Fibrosis	Sub Descemet fibrosis	Scar in injection site
Rabbit 2 (OS)	Thinning, Inflammatory cells	Disruption replaced by fibrosis	Vascularization, Inflammation, Edema, Fibrosis, Epithelial down growth	Descemet fibrosis	Scar in injection site
Rabbit 3 (OD)	Inflammatory cells	Disruption replaced by fibrosis, Pannus formation	Vascularization, Inflammation, Fibrosis	Normal	Localized scar with fibrosis
Rabbit 3 (OS)	Inflammatory cells	Disruption replaced by fibrosis, Pannus formation	Vascularization, Inflammation, Edema, scar formation	Folding	Localized scar with fibrosis
Rabbit4 (OD)	Inflammatory cells	Disruption replaced by fibrosis, Pannus formation	Vascularization, Inflammation, Edema, Scar formation	Mild folding	Localized scar with fibrosis
Rabbit 4(OS)	Inflammatory cells, Acute and chronic ulcer	Disruption replaced by fibrosis	Vascularization, Inflammation Edema, Descemet fibrosis, Perforation	Folding	Depletion
Rabbit 5 (OD)	Normal	Disruption replaced by fibrosis	Vascularization	Normal	Normal
Rabbit 5 (OS)	Normal	Disruption replaced by fibrosis	Vascularization	Normal	Normal

effect and minimal inhibitory concentrations of some antiseptic solutions including NaOCl. They concluded that minimal inhibitory concentration of 55 µg/ml is useful against Aspergillus species. Their study also indicated the resistance of some Aspergillus species to this disinfectant [6]. Another study conducted by Kalkanci et al., [7] found minimal inhibitory concentration 50 of 1024 mg/L solution, and minimal inhibitory concentration 90 of 2048 mg/ L solution of sodium hypochlorite against 77 mold strains.

Cases with fungal keratitis show aggressive and relentless progression and there are only few drugs against fungal keratitis. Corneal transplantation in the early phase to control infection comprises poor optical results, and the rate of the recurrence is high compared

to other types of keratitis. If we are able to postpone corneal transplant in severe extensive fungal ulcers, the prognosis for later rehabilitation measurements will improve. In our study, we injected two different concentrations of intrastromal NaOCl. The eyes with 5 % injection showed nearly normal structural and endothelial findings except for localized scarring. This finding should be further studied in larger and case controlled animal studies to confirm our results.

Conclusion

Intrastromal injection of NaOCl 5 % might be a safe method to treat fungal corneal infections.

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Footnotes and Financial Disclosures

Conflicting of interest:

The Authors have no conflict of interest with the subject matter of the present study.