

Water Soluble Tetrazolium Salt-11 as an Alternative to Riboflavin for Corneal Collagen Cross-linking for the Treatment of Keratoconus

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ABSTRACT

Corneal collagen cross-linking (CXL) is effective in halting progression of keratoconus, nevertheless, it is not without its drawbacks. Water soluble tetrazolium salt (WST) that generates superoxide and hydroxyl radicals following near-infrared (NIR) illumination result in a three-fold increase in corneal stiffness. This seems to be comparable to riboflavin/ultraviolet A (UVA)-mediated cross-linking. Moreover, WST is able to penetrate faster and deep into the cornea while offering protection to endothelial cells and reducing corneal keratocyte loss in the posterior cornea. Early studies suggest good safety and efficacy profile when tested on rabbit eyes *in vivo* and *ex vivo*.

Keywords: Corneal collagen cross-linking, Keratoconus, Palladium bacteriochlorin 13'-(2-sulfoethyl) amide dipotassium salt, Water Soluble Tetrazolium Salt-11, Water soluble tetrazolium Salt-11 formulated with dextran T500.

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INTRODUCTION

Keratoconus is a noninflammatory degenerative ectatic disease of the cornea resulting in progressive stromal thinning and steepening of the cornea. This, in turn, causes abnormal corneal curvature with progressive refractive errors and irregular astigmatism. The pathophysiological mechanism underpinning this disease relates to underlying corneal biomechanical instability. Through stiffening of the cornea, and halting the ectatic process, collagen cross-linking (CXL) is currently the only effective treatment that is capable of arresting progression of keratoconus and has been approved by the Food and Drug Administration for this purpose.¹⁻³

Collagen cross-linking utilizes topical riboflavin on a de-epithelialized cornea irradiated with ultraviolet A

(UVA). This process leads to the elaboration of free radicals and formation of covalent bonds between molecules that promote protein cross-linking.⁴

The toxicity field induced by the collagen cross-linking procedure mandates a minimum corneal thickness of 400 µm to avoid collateral damage to the corneal endothelium, lens, and retina.⁴ The use of hypotonic riboflavin can help increase corneal thickness to levels where CXL can be safely performed.⁵ Still, some patients with keratoconus and other ectasias are therefore ineligible for this treatment.⁴ Although studies demonstrate safety with the above criteria and minimum soaking time of riboflavin of 30 minutes, to ensure adequate riboflavin penetration and therefore UV shielding effect, there is still a risk of localized irreversible UV damage to corneal endothelium and deeper structures if homogenous irradiation is not applied.⁶ Furthermore, there is a possibility that riboflavin in the anterior chamber can lead to free-radical induced damage to anterior segment structures.^{4,7}

Water Soluble Tetrazolium Salt-11

Palladium bacteriochlorin 13'-(2-sulfoethyl) amide dipotassium salt [Water soluble tetrazolium salt (WST)-11, Stakel] is a water-soluble synthetic palladium-bacteriochlorophyll derivative that exhibits photodynamic properties.⁸ It forms hydroxyl and superoxide free-radicals following illumination with near infra-red (NIR) frequencies.⁸⁻¹⁰ Clinically, this property has been exploited in clinical trials for the targeted treatment of prostate cancer¹¹⁻¹⁴ and cutaneous melanoma⁸ via nonthermal tissue ablation using endovascular fiberoptic wires, following parental administration of intravenous (IV) WST-11, with promising results. Histological analysis of liver and kidney specimens from pigs treated, *in vivo*, with IV WST-11 followed by local ablation using endovascular NIR fiberoptic wires demonstrates homogenous well-demarcated zones of necrosis consistent with ischemic injury with preservation of tissue collagen/protein and no collateral damage.¹⁵ Infrared stimulated WST-11 achieves these properties through simulation of an ischemic-reperfusion-type injury following oxidative-free radical formation whilst simultaneously displaying negligible increases in local temperature and no heat-sink effect via

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the microvasculature.¹⁵ Control of ablation depth can also be achieved through varying the illumination intensity.

Ocularly, WST-11 has been investigated for use in the treatment of neovascular choroidal vessels through their photocoagulative effects.¹⁶ In this setting, WST-11 demonstrates limited diffusion and consequent minimal collateral damage to retina and retinal pigment epithelium, on histological analysis, and therefore potentially holds a therapeutic advantage over conventional verteporfin photodynamic therapy.

Thus far, almost exclusively, the literature has focused on the clinical application of targeted localized tissue ablation following parenteral administration of WST-11 and NIR illumination via its photodynamic effects. Liberation of oxidative-free radicals following sensitization with NIR laser could theoretically also be applied in a topical context and WST-11 may thus serve as an alternative agent to the formation of these reactive compounds, which ultimately mediate the CXL process in the cornea.

EXPERIMENTAL USE OF WST-11 AS A CORNEAL STIFFENING AGENT

The rationale behind using WST is to utilize it as a photosensitizer to stiffen the cornea by generating oxygen radicals without inflicting damage to the cornea by illumination at a nonhazardous wavelength – NIR at 755 nm.

Marcovich et al¹⁷ evaluated the use of WST-11 (STEBA Laboratories, Rehovot, Israel) with NIR illumination in corneal stiffening using *ex vivo* and *in vivo* rabbit eye models. They used 2.5 mg/mL WST-11 in saline solution or 2.5 mg/mL in saline with 20% dextran T-500 (WST-D) to limit deep corneal penetration and collateral damage to the endothelium. Rabbits were either treated *in vivo* with WST-11, WST-D, riboflavin, or riboflavin with dextran. The WST-11 and WST-D were applied for 20 minutes followed by 30 minutes of exposure to NIR diode laser (755 nm, 10 mW/cm²) (CeramOptec, Bonn, Germany) whilst riboflavin was applied for 30 minutes followed by 30 minutes UVA irradiation as per established protocol. Polymyxin B, neomycin, and dexamethasone 0.1% ointment was applied twice daily for 2 and 4 weeks and later the rabbits were sacrificed and enucleated. Corneal strips were then placed on a biomechanical analyzer. Results, across all treated groups, show an approximate three-fold increase in mean ultimate stress and tensile modulus; biomarkers of corneal stiffness.

In vivo studies demonstrated complete stromal penetration when exposed to WST-11 for 30 minutes and about 50% penetration with 10 minutes exposure. This resulted in increased corneal thickness from 360–380 to 600–730 µm. Exposure of corneas to WST-D demonstrated penetration only at the outer third of the stroma regardless of exposure time but with higher accumulation levels.

Ex vivo WST-11/NIR treated eyes demonstrated a three-fold increase in corneal stiffness, which is comparable to the riboflavin (RF)/UVA treated corneas. *In vivo* results demonstrated similar stiffening effect between WST-11 and WST-D, while the latter resulting in reduced duration of corneal edema and epithelial defect without development of haze.

Histological examination demonstrated reduced keratocyte density through the stroma, more notably in the anterior half but with preservation of endothelial cell count similar to control eyes.

DISCUSSION

Riboflavin-mediated CXL is an effective method for halting progression of keratoconus. The original CXL protocol is the Drsenden protocol that involves instilling 0.1% riboflavin solution in 20% dextran for 30 minutes, followed by 365 nm UVA illumination with 3 mW/cm² for 30 minutes for a total dose of 5.4 J/cm². Several newer protocols exist now using different riboflavin formulas, frequencies, and duration of instillation, and different UVA irradiation levels and duration.¹⁸

The WST-11 penetrates the cornea in a time-dependent manner; 50% after 10 minutes and 100% after 30 minutes. This is significantly faster than riboflavin corneal penetration of de-epithelialized cornea, while WST-D resulted in reduced penetration. Similar results were observed in the dextran-riboflavin treated group. Nevertheless, WST-D group demonstrated reduced side effects while not compromising stiffening effect. There was reduced duration of corneal edema and faster epithelial defect healing, while simultaneously offering protection to the endothelial cell layer and reducing keratocytes loss in the posterior cornea.¹⁷

Unlike riboflavin/UVA-mediated cross-linking, WST-11 seems to generate only superoxide and hydroxyl radicals and no traces of singlet oxygen after NIR illumination in aqueous solutions. The authors suggest the corneal stiffening is achieved using different mechanisms and suggested the possibility that singlet oxygen and dityrosine formation during RF-D/UVA do not contribute to the cross-linking effect.¹⁷

WST-11 combined with NIR laser resulted in a three-fold increase in corneal stiffness.¹⁷ This is promising as it acts as the basis for halting progression of keratoconus. Although rabbit corneas have served as the useful surgical models for new cataract surgical techniques and laser-refractive procedures, the clinical potential for WST-11-mediated CXL in human corneal ectatic disorders can only be realized through clinical trials on human subjects. Also, WST-11 has been shown to be safe when administered parenterally in human subjects with short half-life, rapid clearance (<60 minutes), and minimal toxicity.

Moreover, NIR lasers are routinely used safely in diagnostic ophthalmic practice in the form of optical coherence tomography. Thus, utilizing WST-11 with dextran to limit penetrance combined with 33% shorter duration of application, possibility of treating patients otherwise excluded from conventional CXL due to corneal thickness requirements, and avoidance of hazardous UVA irradiation, in favor of nonharmful NIR, is a promising candidate as an alternative to the riboflavin-mediated CXL with potentially similar biomechanical results.

Clearly, this is a new agent that is being investigated as an alternative to riboflavin. Studies are limited in the literature and therefore further studies are needed to establish safety and efficacy of this produce to halt progression of keratoconus in cases where riboflavin mediated CXL is not recommended.

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