Riboflavin 0.1% (VibeX) for the treatment of keratoconus

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Introduction: Keratoconus is an ectatic disease of the cornea characterized by biochemical and biomechanical instability of stromal collagen leading to reduction of corneal thickness, variation in posterior and anterior corneal curvatures and progressive deterioration of visual acuity due to irregular astigmatism [1,2]. The recent advent of corneal collagen crosslinking (CXL) in the ophthalmology panorama of the last decade [3,4] transformed the conventional therapy of keratoconus based at best, on a lifetime of rigid contact lens wearing or at worst on corneal transplant, improving its conservative treatment, thus reducing the necessity of lamellar or penetrating corneal graft.

In 2011, the VibeX/KXL™ system promoted by Avedro, Inc. (Waltham, Massachusetts, MA, USA), was granted orphan designation by the Food and Drug Administration (FDA) for the treatment of keratoconus and corneal ectasia following refractive surgeries [5]. Orphan drug designation is granted by the FDA Office of Orphan Products Development to promote the development of new
therapies for rare diseases and disorders. To date, Avedro, Inc., distributes four riboflavin solutions outside the United States: VibeX™, VibeX Rapid™ (Box 1 and 2) (for epithelium-off crosslinking procedure), Paracel™ (for transepithelial or epithelium-on treatment) and VibeX Xtra™ specifically formulated for the use during a Lasik procedure (Lasik Xtra®) and epithelium-on crosslinking. In this manuscript, we will refer to the Avedro, Inc. VibeX and VibeX Rapid solutions.

2. Keratoconus

Krachmer et al. in a 1984 article [1], defined the keratoconus as ‘an ectatic, asymmetric, non-inflammatory, progressive corneal degeneration characterized by thinning, increasing of corneal curvatures and loss of transparency of the central cornea’. The prevalence of keratoconus in the population reported in the literature [2] is 1/2,000 which is within the threshold used by the European Union to define a disease as rare disease (also referred to as an orphan disease) [6], and the incidence is 2 – 3/100,000 [2], although many authors agree that in the coming years there will be an increase of these values due to a more widespread use of corneal topography. According to the experience of Ophthalmology School of Siena, the prevalence of keratoconus amounted to 1 in 1,000 or even 1 in 500 inhabitants [4]. Studies conducted in the precorneal tomography era reported a familiarity of 6 – 8% meaning that about 6 – 8% of patients with keratoconus also has one or more family members suffering from this disease. Still more striking epidemiological studies carried out in the post-corneal tomography era showed that 50% of patients with keratoconus have at least one close relative suffering from the same disease [7]. Keratoconus ectasia is characterized by corneal bulging with thinning typically occurring in the inferior-temporal quadrant that represents the physiological weakest part of the cornea [8,9]. Keratoconus usually starts in the second decade of life (generally at puberty) progressing until the fourth decade of life [2] when it generally stabilizes due to a natural or physiological (age-related) process of cross-linking of stromal collagen [10]. Diabetes seems to be a protective factor against keratoconus onset and progression, due to increased crosslinking process related with the hyperglycemia in the so-called Maillard’s reaction [11,12].

3. Parasurgical crosslinking therapy

Riboflavin ultraviolet type A (UVA) CXL is a relatively new approach for increasing the biomechanical and biochemical stability of the corneal stromal tissue against primary and secondary ectasia. The surgical technique consists in a photopolymerization of stromal collagen fibers by the combined action of a photosensitizing substance (riboflavin, also known as vitamin B2) and UVA. Photopolymerization increases the rigidity of corneal collagen contrasting ectasia progression. Different methods of enhancing the crosslinking of corneal collagen have been comparatively evaluated by Spoerl and Seiler [13] who discovered that the association of riboflavin and UV-A was effective (by significantly increasing corneal stiffening and Young modulus) and less toxic in vivo (by preserving corneal transparency, endothelium, lens and retina). The data reported in literature [14,15] at this time confirm that crosslinking has a main role in the management of early stage progressive keratoconus. In fact, if progression can be reliably documented by means of serial computerized topography, surface aberrometry and differential optical pachymetry, an early intervention is likely to be most beneficial in preventing a visual impairment or at least the development of advanced clinical signs. Spörlein et al. [16] were the first to

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**Box 1. Drug summary.**

<table>
<thead>
<tr>
<th>Drug name</th>
<th>VibeX™</th>
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</thead>
<tbody>
<tr>
<td>Pharmaceutical company</td>
<td>Avedro, Inc. (Waltham, Massachusetts, MA, USA)</td>
</tr>
<tr>
<td>Indication</td>
<td>Keratoconus and secondary corneal ectasia</td>
</tr>
<tr>
<td>Composition</td>
<td>100 mL of solution contains: riboflavin 0.1 g, dextran 500, disodium hydrogen phosphate, sodium phosphate monobasic dihydrate, sodium chloride, water for injectable solution</td>
</tr>
<tr>
<td>Mechanism of action</td>
<td>Ophthalmic medical device used for corneal crosslinking treatment</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Topically</td>
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</tbody>
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**Box 2. Drug summary.**

<table>
<thead>
<tr>
<th>Drug name</th>
<th>VibeX Rapid™</th>
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<tbody>
<tr>
<td>Pharmaceutical company</td>
<td>Avedro, Inc. (Waltham, Massachusetts, MA, USA)</td>
</tr>
<tr>
<td>Indication</td>
<td>Keratoconus and secondary corneal ectasia</td>
</tr>
<tr>
<td>Composition</td>
<td>100 mL of solution contains: riboflavin 0.1 g, HPMC, disodium hydrogen phosphate, sodium phosphate monobasic dihydrate, sodium chloride, water for injectable solution</td>
</tr>
<tr>
<td>Mechanism of action</td>
<td>Ophthalmic medical device used for corneal crosslinking treatment</td>
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investigate the use of riboflavin and UVA irradiation in achieving crosslinking of corneal collagen. Using this method, Wollensak et al. [17] demonstrated a marked positive effect of crosslinking on the biomechanical properties of both porcine and human corneal tissue: crosslinking was shown to increase the rigidity of human corneal tissue (expressed by Young’s modulus at 6% strain) by a factor of 1.5 – 1.7 x [18] (Young’s modulus, also known as the tensile modulus, is a measure of the stiffness of an elastic material). Below we cite the early clinical studies that have documented the effectiveness of the crosslinking treatment in slowing down or arresting the progression of keratoconus. The first report of a series of 23 eyes treated with crosslinking was published in 2003 by the eye school of Dresden [3]. Following Dresden studies, for the first time in Italy in 2006 the researchers of the Siena eye school in Italy [4] published the second international results of a case series of 10 patients affected from progressive keratoconus stabilized through riboflavin UVA-induced CXL.

The in vivo safety and effective penetration of corneal crosslinking induced apoptotic effect correlating with its biomechanical and clinical efficacy; treatment was documented for the first time in humans by Mazzotta et al. [19-21] by using the confocal laser scanning microscopy following the previous Wollensak’s studies on animal model [22]. The presence of stromal edema, the reduction in keratocyte density via cells apoptosis and loss of nerve fibers were observed in the anterior stroma 1 month after crosslinking [20]. In the same studies, the progressive reduction of corneal edema associated with increased stromal density and time-depending cells repopulation of the anterior mid-stromal volume by activated keratocytes between 3 and 6 months after treatment were demonstrated; the deep stroma beyond 300 μm, endothelial cells morphology and count appeared unaffected [20]. Moreover, in a later publication, Mazzotta et al. [21] described numerous needle-shaped reflective striations or bands in the anterior mid-stroma until 2 years after crosslinking, interpreted as new structured collagen following treatment and explaining some morphological- and functional-related aspects of crosslinking itself [19].

4. Riboflavin

Riboflavin, also known as vitamin B2, belongs to the class of water-soluble vitamins. The etymology of the word riboflavin, composed of ribo(se) and flavin, refers to its chemical composition. Riboflavin is composed of a nucleus of dimethyl isoalloxazine (isoalloxazine is the basic structure of all flavin molecules) and ribitol (the reduced form of the sugar ribose): 7,8-dimethyl-10-(1’-d-ribityl) isoalloxazine [23-25]. As suggested by its etymology (flavus in Latin means ‘yellow’), riboflavin crystals have a yellow-orange color, whereas neutral solutions of riboflavin have a typical yellowish-green color. This is why they are used as a food coloring, known as E101. Although riboflavin belongs to the group of water-soluble vitamins, it is in fact one of the least soluble in water (12 mg/100 mL at 25°C) and even less soluble in alcohol. It is more soluble in acidic solutions up to a temperature of 120°C, but unstable in alkaline media and when exposed to ultraviolet and visible light. The spectrum of riboflavin has four absorption peaks at wavelengths 223, 266, 373 and 445 nm. Riboflavin exhibits native fluorescence with an emission peak at 530 nm [23-25]. Vitamin B2 was first discovered in 1879 by the English chemist Alexander Winder Blyth who called it ‘lactoflavin’ because he found it in cow’s milk. Similar substances were called ‘ovoflavin’, ‘hepatoflavin’ and so forth, because they were discovered in eggs, liver and other animal tissues. It was not until 1934 that the Swiss chemist Paul Karrer and the Austrian-born German chemist Richard Kuhn, both Nobel laureates in chemistry, discovered that lacto-, ovo- and hepato-flavin were the same substance, which they called riboflavin [23,24].

Riboflavin plays a fundamental role acting as photosensitizer, favoring the production of free radicals mediating the crosslinking of stromal collagen [26]. The release of free radicals or reactive oxygen species induce (in the presence and/or absence of oxygen) the type I and type II photochemical reactions (oxidative deamination of corneal collagen) leading to the formation of molecular bridges (crosslinks) between and within collagen fibrils. The second role of riboflavin is the UVA absorption, thus allowing the concentration of ultraviolet light A energy delivered by UV source in the anterior half of the corneal stroma. The so-called riboflavin shielding effect reduces the risk of UV-related damage of corneal endothelium, lens and retina [27]. Safety studies have shown that current UVA exposures associated with crosslinking are below the thresholds for endothelial cell damage. There is a risk of endothelial cell toxicity when riboflavin diffuses to the endothelium, and UVA exposures are high enough that they are not adequately attenuated by absorption on transit through a riboflavin-soaked cornea.

5. Expert opinion

Standard riboflavin solution (VibeX) contains the same formulation of the original riboflavin formula used in the first crosslinking studies and protocols (Dresden and Siena); 0.1% riboflavin with 20% dextran T 500 solution has been already used and commonly applied today in the crosslinking therapy for progressive keratoconus. This solution has been CE marked and proven in several published clinical studies to be optimally tolerated in human eyes [20,27-30]. The drops contain 20% dextran 500,000 Da molecular mass in order to make the otherwise hyperosmolar 0.1% riboflavin solution iso-osmolar with the corneal stroma, thus preventing intraoperative corneal swelling [31].

The classic 0.1% riboflavin with 20% dextran T 500 solution was well tolerated and nontoxic, nonirritating and nonallergic for ocular surface during and after epithelium-off crosslinking treatment. Syringe with large cannula provided in the sterile packaging facilitates an easy and
homogeneous distribution of the riboflavin on the corneal surface, Figure 1. Homogeneous distribution of the solution on the corneal surface is essential to avoid the presence of hot spots. Indeed, if hot spots are present, the damage thresholds may be locally exceeded leading to localized endothelial damage [27].

The so-called ‘rapid’ riboflavin solution consists in the presence of hydroxypropyl methylcellulose (HPMC) instead of dextran as excipient. HPMC has long been established for ophthalmic safety and optimal tolerability [32-36]. Methylcellulose has a low surface tension and contact angle, which increases coating ability, having a lack of elasticity, which makes it more ‘viscoadherent’ than viscoelastic. It has been shown not to damage the endothelium [34]. Other advantages are its availability, ability to be autoclaved, low cost and ability to be stored and shipped at room temperature. HPMC prevents the corneal thinning caused by corneal dehydration reported in literature with dextran solution as demonstrated by Kymionis et al. [37] and also experienced by us in the clinical practice (submitted unpublished data).

As reported in literature by Spoerl et al. [27] and by Kamaev et al. [26], riboflavin and fluorescein have similar polarity and molecular mass (376 g/mol) with similar diffusion coefficients of $6 \times 10^{-7}$ cm$^2$/s at 35°C. Since the HPMC significantly increases the penetration of topical fluorescein as compared to other commonly used ophthalmic vehicles [38], it is theoretically reliable that HPMC could be a good vehicle for riboflavin molecules allowing its faster diffusion into the corneal stroma and decreasing the soaking time under 10 min as proposed in the new Avedro, Inc. ‘VibeX Rapid protocol’. The possibility to shorten the riboflavin soaking time is particularly favorable for patients and surgeons, being the entire crosslinking procedure less time-consuming with relative advantages.

Both Avedro, Inc. riboflavin solutions (standard and rapid) were intraoperatively and postoperatively optimally tolerated, with no irritation and allergy and nontoxic for ocular surface having a good distribution and penetration of riboflavin into the corneal stroma and anterior chamber, Figure 2.

In our experience, after epithelium-off crosslinking by using the standard 0.1% – dextran 20% riboflavin solution and the rapid 0.1 % riboflavin with HPMC solution, the reepithelialization was complete after 3 days of soft contact lens bandage and subjectively patients reported a very small degree of discomfort.

In conclusion the Avedro, Inc.’s standard riboflavin solution complies well with other solutions currently available on the market for epithelium-off crosslinking treatment. The ‘rapid’ solution containing 0.1 % riboflavin plus HPMC could become the future device for accelerated (epithelium-off) CXL.

**Declaration of interest**

The authors state no conflict of interest and have received no payment in preparation of this manuscript.
**Bibliography**

Papers of special note have been highlighted as either of interest (●) or of considerable interest (●●) to readers.


23. Ball G. Vitamins in foods: analysis, bioavailability, and stability. CRC Press; Boca Raton; 2006


25. New riboflavin solutions dextran free or with HPMC combined with increased UVA power delivering same energy of standard procedure may represent a future development of crosslinking therapy reducing the time of the procedure and being equally safe. The efficacy of the accelerated crosslinking is now under investigation.


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