Riboflavin, Ultraviolet Light, and the Photochemical Reaction

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The rationale for stiffening of the cornea to arrest the progression of keratoconus came from the observation that, with aging, keratoconus progression is slowing down, and keratoconus is rare in patients with juvenile diabetes, probably due to naturally occurring cross-linking and the resulting stiffening of the collagenous tissue. To produce stiffening of the corneal tissue, a method of cross-linking via photodynamic effect was established using riboflavin (RF) and ultraviolet A (UVA).

Photodynamic therapy (PDT) is based on the excitation of nontoxic light-sensitive compounds (photosensitizers) with light that leads to the production of toxic active radicals. These radicals cause a therapeutic effect (ie, destroy cells and microorganisms) or induce tissue alterations (ie, blood stasis, corneal stiffening). The main components of PDT, which will be discussed in the following sections, are photosensitizer, light, and, in most scenarios, oxygen.

RF was chosen as a photosensitizer due to its nontoxic nature (vitamin B2). Mostly a water-soluble derivative, riboflavin-5’-phosphate is used in RF/UVA cross-linking treatment. It absorbs maximally in blue (445 nm) and UVA (373 nm) light, and has a green fluorescence upon excitation by UVA or blue light at 534 nm. Because of limited availability of light-emitting diodes at that specific wavelength, the first devices used a wavelength of 365 nm.

Light

Irradiation with more energetically strong UVA photons achieves a greater corneal cross-linking (CXL) effect; thus, 365 nm was chosen for the CXL. However, blue light (430 to 445 nm) can be also effective in cross-linking with RF while providing higher penetration depth.

Oxygen

Oxygen plays a major role in most PDT treatments. Reactive oxygen species (ROS) formed via RF photoactivation are responsible for CXL tissue stiffening. The availability of these 3 components of PDT determines the efficiency of the CXL. Corneal strengthening is conditioned by the concentration of each component in the stroma. Corneal penetration of RF is associated with the depth of CXL. Demarcation line, probably indicating CXL extent, was deeper after epithelium-off (300 μm) than after epithelium-on (100 μm) RF/UVA treatment.

UVA in sufficient energy is needed for effective CXL. However, its deep penetration may cause endothelial damage. Transmitted UV energy is attenuated by RF concentration that acts both as a photosensitizer and UVA filter. To shorten irradiation time for RF/UVA CXL, considerations were made based on one of the fundamental laws of photochemistry, the Bunsen-Roscoe law of reciprocity.

Oxygen is a key factor in the biomechanical increase after CXL. Shortening of the irradiation time should take into account the supply of oxygen for efficient photosensi-