The Drug Science of Cross-linking

Research is elucidating how the combination of riboflavin and ultraviolet light strengthens the cornea.

BY GEORGE O. WARING IV, MD, AND DERRICK A. HUEY, MD

The use of chemical cross-linking in materials science as a means of corneal stiffening is well described. Ten years ago, surgeons began cross-linking collagen to stabilize progressive corneal ectatic disorders. During this time, much has been elucidated about the drug science behind this treatment.

RIBOFLAVIN

Riboflavin, also known as vitamin B2, is a naturally occurring photosensitizer. It is the precursor of flavin mononucleotide and flavin adenine dinucleotide, coenzymes that are crucial for the metabolism of carbohydrates, fats, and proteins into energy. Riboflavin is an essential constituent of all living cells. It is water soluble and nontoxic, and it is used as a coloring agent in food and pharmaceuticals. The intake of riboflavin from food and dietary supplements ranges from 4 to 10 mg per day. No adverse effects have been associated with high intakes of riboflavin from food or supplements.

In the cornea, collagen cross-linking occurs naturally with aging due to an oxidative deamination reaction that occurs within the end chains of the collagen. The standard concentration of riboflavin used in the riboflavin-ultraviolet A (UVA) treatment for ectatic disorders is 0.1%. The maximum amount of riboflavin to which a patient is exposed during corneal collagen cross-linking with the standard Dresden protocol is estimated to be 1.6 mg, if the volume of one drop is 0.05 mL and one drop is being instilled every 2 minutes for 30 minutes before and 30 minutes during UVA light irradiation (32 drops total). This amount is considered to be well within a safe dosage.[1]

CYTOTOXICITY

A series of in vitro experiments has characterized the potential cytotoxicity of UVA light and the UVA-riboflavin exposure on keratocytes and the function of endothelial cells. Riboflavin alone has not been shown to have a cytotoxic effect on keratocytes or endothelial cells. The cytotoxic threshold for inducing cellular necrosis or apoptosis was 5 mW/cm^2 for UVA light alone and 0.5 mW/cm^2 for the UVA-riboflavin treatment. Using the Lambert-Beer equation, in human corneas, the cytotoxic keratocyte UVA irradiance of 0.5 mW/cm^2 is reached at a stromal depth of 300 µm.[2] Researchers believe that damage to endothelial cells is due to oxidative injury caused by the reactive oxygen free radicals (singlet oxygen, superoxide anion, hydrogen peroxide) that are generated by the interaction of riboflavin with UV light. Ninety-four percent of incident UVA light is absorbed by the anterior 400 µm of the corneal stroma in the presence of riboflavin (Figure), whereas only 32% is absorbed within that depth in the absence of riboflavin.[3,4] Riboflavin therefore serves the

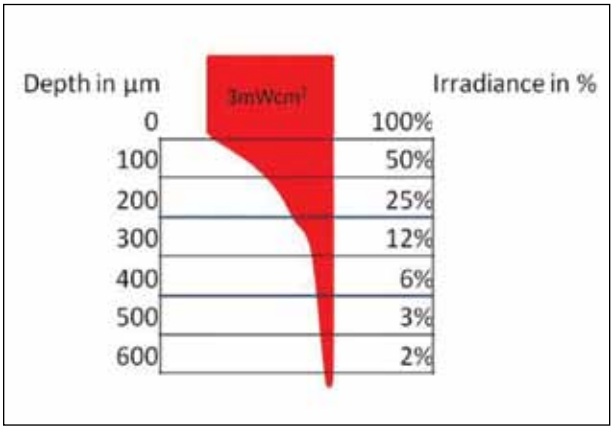

Figure. Irradiance in percentage relative to depth in microns of UVA light (3 mW/cm^2) in the presence of riboflavin. Adapted from Spoerl et al.[4]

"Studies characterizing the nature and interaction of riboflavin and UVA light have revolutionized the treatment of corneal ectatic disorders."

dual purpose of sensitizing the stroma to the effect of UV light and protecting the corneal endothelium from UVA damage.

MECHANISM OF ACTION

Researchers have evaluated the use of riboflavin for the inactivation of blood-borne pathogens through the production of reactive oxygen species (ROS),[5] which can be created by the triplet excited state of riboflavin.[6] In the cornea, ROS can create advanced glycation end products,[7] which, in turn, increase collagen diameter.[8] Investigators have suggested that the advanced glycation end products may stiffen the cornea[9] and, in so doing, may serve as the mechanism underlying the relatively protective effect that diabetes has on the development of keratoconus.[10]

The majority of cross-linking occurs between collagen α and β chains. These cross-link into γ chains and larger aggregates. Corneal proteoglycans also cross-link with themselves to form larger polymers, but collagen and proteoglycans show only limited cross-linking with one another.[11] This suggests that cross-linking creates collagen-to-collagen cross-links as well as proteoglycan-to-proteoglycan cross-links but only a small amount of collagen-to-proteoglycan cross-links. Although the concept is poorly understood, investigators suspect that ROS induce stabilization, either between collagen fibers or to their surrounding matrix. Corneal collagen cross-linking is the first surgical procedure that appears to halt the progression of corneal ectatic disorders such as keratoconus and ectasia after refractive surgery.[1,12]

The corneal epithelium permits the passage of small, lipophilic molecules, whereas the stroma forms a barrier against the passage of lipophilic compounds while readily allowing the movement of hydrophilic molecules.[13] Riboflavin is a highly hydrophilic molecule that absorbs UV light at a wavelength of 370 nm.[14] Intact, tight junctions of the corneal epithelium block the absorption of riboflavin and the transmittance of UVA light into the corneal stroma.[15,16] Investigators have developed numerous techniques for the transepithelial delivery of riboflavin, although the efficacy of the traditional epithelium-off technique is currently better supported by the literature.[17]

The aforementioned studies characterizing the nature and interaction of riboflavin and UVA light have revolutionized the treatment of corneal ectatic disorders and paved the way for the next generation of cross-linking treatment modalities. ■

Derrick A. Huey, MD, is a resident physician in the Department of Ophthalmology at the Medical University of South Carolina in Charleston and at Magill Vision Center in Mt. Pleasant, South Carolina. He acknowledged no financial interest in the material presented herein.

George O. Waring IV, MD, is the director of refractive surgery at the Storm Eye Institute and assistant professor of ophthalmology at the Medical University of South Carolina in Charleston. Dr. Waring is also the medical director of Magill Vision Center in Mt. Pleasant, South Carolina. He is a consultant to Topcon Medical Systems, Inc., and serves as chairman of the scientific advisory board for the company's cross-linking technologies. Dr. Waring may be reached at georgewaringiv@gmail.com.

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