Persistent corneal epithelial defects heal with topical peptide treatment

Cheryl Guttman
in Vienna

TO PIC AL drops containing tetrapeptides derived from substance P and insulin-like growth factor 1 (IGF-1) appears to be a promising therapeutic modality for promoting repair of persistent corneal epithelial defects associated with neurotrophic keratopathy, reported Teruo Nishida MD, DSc, at the 2007 Joint Congress of the European Society of Ophthalmology and American Academy of Ophthalmology.

Dr Nishida reported outcomes from an IRB-approved, open-label, uncontrolled study including 20 eyes of 19 patients with a mean age of 67 years. The investigational peptide solution was formulated with FGLM-amide (Phe-Gly-Leu-Met-amide) derived from substance P and SSR (Ser-Ser-Ser-Arg) derived from IGF-1.

Patients were instructed to use the eye drops four times a day for up to 28 days. At the end of the study, complete epithelial resurfacing was achieved in 14 (70 per cent) eyes. Mean time to healing was 10.1 days. Presence of intact stem cells was associated with a favourable response. The treatment was well tolerated and there were no treatment-related adverse events.

"Currently, there are no specific treatments for persistent corneal epithelial defects in eyes with neurotrophic keratopathy. Our experience with the peptide solution is very encouraging, and we are now planning to perform a double-masked, randomised clinical trial to better characterise the efficacy and safety of this novel treatment for persistent epithelial defects in neurotrophic keratopathy," said Dr Nishida, professor and chairman, Yamaguchi Graduate School of Medicine, Japan.

The dual peptide solution was developed based on a series of previous studies investigating neurotrophic mediators of corneal healing. Interest in the use of substance P, which is present in the sensory nerve fibres of the cornea, derives from the observation that its level in the cornea is reduced in eyes with neurotrophic keratopathy and persistent epithelial defects.

In initial studies, Dr Nishida and colleagues reported that substance P had no effect by itself on corneal epithelial migration. However, they found substance P and IGF-1 acted synergistically to stimulate corneal epithelial migration in vitro in a rabbit cornea organ-culture system. In vivo studies, they found the combination facilitated epithelial wound closure in both an animal model and humans with neurotrophic keratopathy-related persistent corneal epithelial defects.

Subsequently, Dr Nishida and co-workers investigated the minimal amino acid sequences necessary for obtaining the desired biological effects on wound healing for each of the two polypeptides. "We found that the tetrapeptide FGLM-amide derived from the carboxyl terminus of substance P mimics the function of the entire molecule and that SSR, a tetrapeptide derived from the C domain of IGF-1, was the minimum essential sequence for stimulating corneal epithelial migration combined with substance P. Use of these minimal amino acid sequences may be preferred as they would be expected to have little antigenicity as opposed to the full-length polypeptides and also avoid the unwanted biologic effects of the parent molecules," he said.

The patients included in the study were all treated at Yamaguchi University Hospital between January 2004 and June 2006. All were patients with persistent corneal epithelial defects present for at least two weeks despite conventional treatment. The mean ± SD duration for presence of the epithelial defect was 3.7 ± 4.7 months. Mean ± SD corneal sensation measured with the Cochet-Bonnet esthesiometer was 1.8 ± 4.2 mm and the mean Schirmer test result was 12.6 mm per five minutes.

The peptide solution was formulated by the hospital pharmacy under sterile conditions and contained FGLM-amide 1 mg/ml and SSR 56.3 mcg/ml with phosphate buffered saline as the vehicle. Treatment response was assessed using digital analysis of the defect size identified on slit-lamp photographs after fluorescein staining. At baseline, the epithelial defect represented 13 per cent of the total area of the cornea.

Among the 14 eyes that responded, healing occurred within three to 23 days of starting treatment (median 7.1). Seven eyes (50 per cent) achieved complete resurfacing within seven days of starting the topical peptide treatment, the defect healed between days seven and 14 in four eyes (28 per cent), and the three (15 per cent) remaining responders had healed by 23 days.

Additional analyses indicated that presence of intact stem cells was significantly associated with treatment response.

"Of the 14 patients who achieved complete resurfacing, only two (14 per cent) had stem cell deficiency compared with three (50 per cent) of the six patients who had not achieved healing within 28 days," Dr Nishida reported.

On the way to in vivo histology

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at the surgical interface, including cell growth, cell differentiation at the interface, and interface fibrosis. Dr Guthoff suggested that femtosecond laser ablation sites be examined with this technology to determine the actual effect of the laser in the cornea.

Dr Guthoff believes that the information won through in vivo microscopy can help surgeons understand why things happen as they do in the cornea and provide important insights into therapeutic options in refractive surgical patients.

He uses this in vivo technology to study cell differentiation, as well, amplifying pathologic changes on the cellular level in various strata of the cornea. Leukocytes and dendritic cells are easily visible in the corneal epithelium in eyes with radial keratotomy. Cell movement within capillaries and other bits of functional information can be derived from the live, moving microscopic pictures, he noted.

Dr Guthoff described cell populations hardly known before, such as the corneal, antigen-presenting Langerhans cells. The RCM reveals sub-types of this cell in varying degrees of maturity, such as small, non-dendritic cells, big cells with long dendrites, and a net pattern formed between them. These cells play an important role in the immunologic processes of the cornea, as shown by their increased number in contact lens wearers.

Eyes that had penetrating keratoplasty show a high number of infiltrates within the corneal graft. In vivo microscopy can help show which cells are active in this process, such as white blood cells and Langerhans cells, as well as the genesis of this and other inflammatory processes.

He reported that Langerhans cells increase in number in response to preservatives used in eye drops. Although their exact role is still not clear, he feels that this technology will allow researchers to break through this still-unknown area. Again, in vivo Langerhans images corresponded to impression cytological images made of these cells from other studies.

The technique relies on a confocal image, which Dr Guthoff compared to the image from the slit lamp, which eye doctors knew from everyday practice. As with slit-lamp imaging, the investigator precisely focuses the confocal microscope on a certain area, blocking out all other light, to concentrate on the illuminated area, only with the advantage of 800-fold image magnification.

The eye comes into direct contact with the machine, using anaesthesia, artificial tears and a small PMMA cap, which covers the objective and helps stabilise the image seen through the microscope. A newer cap design features a thin groove along the contact side, which helps reduce the ocular pressure and allows investigators to examine the corneal epithelium without side effects.

Dr Guthoff believes that researchers could easily take this technology a step further to perform in vivo biopsies, to check for corneal pathologies. Also, the ‘activated’ keratocytes that are thought to cause corneal net formation in keratoconus patients could be amplified using the RCM 3-D imaging technology to reveal and possibly explain the complex network of interacting cells.

Trishida@yamaguchi-u.ac.jp

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rudolf.guthoff@medizin.uni-rostock.de