J Membrane Biol (2012) 245:381–388
DOI 10.1007/s00232-012-9460-4

Regulation of Connexin43 Gap Junction Protein Triggers Vascular Recovery and Healing in Human Ocular Persistent Epithelial Defect Wounds

Susan Ormonde · Chi-Ying Chou · Lucy Goold · Con Petsoglou · Rasha Al-Taie · Trevor Sherwin · Charles N. J. McGhee · Colin R. Green

Received: 30 March 2012 / Accepted: 20 June 2012 / Published online: 15 July 2012

Abstract Transiently blocking the expression of the gap junction protein connexin43 using antisense oligodeoxynucleotides or blocking hemichannels with connexin mimetic peptides has been shown to significantly improve outcomes in a range of acute wound models. Less is known about their likely effects in nonhealing wounds. In the eye, prolonged inflammation and lack of epithelial recovery in nonhealing corneal epithelial wounds may lead to corneal opacity, blindness or enucleation. We report here the first human applications of antisense oligodeoxynucleotides that transiently block translation of connexin43 in a prospective study of five eyes with severe ocular surface burns (persistent epithelial defects), which were unresponsive to established therapy for 7 days to 8 weeks prior to treatment. Connexin43-specific antisense oligodeoxynucleotide was delivered in cold, thermoreversible Poloxamer407 gel under either an amniotic membrane graft or a bandage contact lens. The connexin43-specific antisense application reduced inflammation within 1–2 days, and in all five eyes complete and stable corneal reepithelialization was obtained. Recovery of the vascular bed and limbal reperfusion appeared to precede corneal epithelial recovery. We conclude that connexin modulation provides a number of benefits for nonhealing ocular burn wounds, one of which is to promote vascular recovery.

S. Ormonde · C.-Y. Chou · R. Al-Taie · T. Sherwin · C. N. J. McGhee · C. R. Green (✉)
Department of Ophthalmology, Faculty of Medical and Health Sciences, New Zealand National Eye Centre, University of Auckland, Private Bag 92019, Auckland, New Zealand
e-mail: c.green@auckland.ac.nz

L. Goold · C. Petsoglou
Save Sight Institute, GPO Box 4337, Sydney 2001, Australia

Keywords Gap junction · Connexin · Hemichannel · Wound healing · Cornea

Introduction

Gap junctional communication and hemichannel opening have been associated with the spread of cell death signals and lesion spread after tissue injury (Frantseva et al. 2002; Garcia-Dorado et al. 1997; Lin et al. 1998; Rawanduzy et al. 2009). In a number of preclinical models of tissue damage and wound repair, reducing protein translation of connexin43 (Cx43) with antisense oligodeoxynucleotides (Cx43AsODN) or hemichannel blockade has been demonstrated to limit inflammation, edema and lesion spread and to provide improved healing or functional outcomes. This has been noted in skin incision and excision wounds (Mori et al. 2006; Qiu et al. 2003), skin burns (Coutinho et al. 2005), the cornea of the eye (Grupcheva et al. 2012), cardiac ischemia (Hawat et al. 2010) and the central nervous system. Central nervous system models have encompassed injuries to the spinal cord (Cronin et al. 2008; Huang et al. 2012), optic nerve (Danesh-Meyer et al. 2008), retina (Danesh-Meyer et al. 2012a) and brain (Davidson et al. 2012). In the cornea of the eye, Cx43 AsODN treatment after epithelial scrape wounding or excimer laser ablation (as used for photorefractive keratectomy) significantly reduces edema and inflammation (myofibroblast differentiation) and speeds the rate of epithelial recovery (Grupcheva et al. 2012).

In the eye, chemical and thermal ocular burns can lead to nonhealing corneal epithelial wounds that pose a significant clinical challenge. Although the management of ocular surface burns has improved with the application of amniotic membrane grafts (Fernandes et al. 2005) and autologous

limbal tissue transplant (the limbus surrounding the cornea of the eye is said to contain stem cells) (Dua et al. 2010), severe chemical burns still have a poor visual prognosis. In this prospective observational study we report the first human use of gap junction Cx43-specific antisense oligodeoxynucleotides to treat five subjects with severe, nonhealing, ocular chemical or thermal burns. All subjects were treated under compassionate-use status when no corneal recovery was apparent 1–8 weeks postinjury, despite accepted best-practice clinical management. Secondary inflammatory changes in the vascular bed of the ocular surface were evident in all cases, and Cx43AsODN treatment resulted in a rapid reduction in inflammation and recovery of the vascular bed and limbal reperfusion. In all five subjects full restoration of the ocular surface integrity was achieved.

Subjects and Methods

Five subjects presenting with severe chemical or combined chemical and thermal burns (Table 1) had extensive 1- to 2-h normal saline irrigation of the ocular surface and fornices (the regions connecting the conjunctival membrane lining the inside of the eyelid with the conjunctival membrane covering the eyeball itself) on presentation until a stable, neutral pH was established. Subjects 1 and 2 in addition had cement debris removed under sedation in conjunction with other treatments. Subsequently, all subjects received the maximum current standard of care treatment for chemical injury, including, as appropriate, g. sodium ascorbate 10 %, g. sodium citrate 10 %, topical corticosteroid (g. prednisone phosphate 0.1 %, g. prednisone acetate 1.0 % or g. dexamethasone 0.1 %), g. chloramphenicol 0.5 %, g. cyclopentolate 1 %, g. atropine 1.0 % (all drops preservative-free where possible), oral doxycycline 100 mg and oral sodium ascorbate (vitamin C).

In all five subjects the initial ocular prognosis was extremely poor with an injury grade V–VI on the Dua classification of ocular burns scale at time of treatment (Dua et al. 2001). This grading was used as it has been shown to be of more predictive value in the case of severe ocular burns (Gupta et al. 2011). A grade V–VI burn is defined as having the entire limbus (12 clock hours) and total surface conjunctiva involved. After consulting Medsafe, the New Zealand Medicines and Medical Devices Safety Authority or the Australian Therapeutic Goods Administration as appropriate, Cx43AsODN was considered as a possible last resort and "urgent innovative treatment." An extensive informed consent process was undertaken with each patient.

Cx43AsODN (5-GTAATTGCGGCAGGAGGAATTG TTTCTGTC-3) has been shown to block translation of the

Table 1 Subject demographics, brief injury details and treatment regimes for each of the five patients treated with Cx43AsODN

	Patient demographics, injury and treatment details	Dua injury grade
1	25-year-old male. Cement from high-pressure hose into left eye. Complete corneal epithelial loss, 90 % bulbar and tarsal conjunctival epithelial loss, 360-degree moderate to severe limbal ischemia. Moderate full-thickness corneal haze, moderate cells in the anterior chamber. Contralateral eye had keratoconus with 6/15 spectacle-aided visual acuity.	V–VI
	Amniotic membrane applied day 5, Cx43AsODN treated day 9.	
2	27-year-old male. Cement from high-pressure hose into both eyes (right eye responded to conventional treatment). Severe conjunctival ischemia, 360-degree limbal ischemia in left eye.	VI
	Superficial tenonectomy day 5, amniotic membrane applied day 9, Cx43AsODN treated day 15.	
3	29-year-old male. Unknown chemical burn to both eyes. Presented 12 h after incident with 60 % corneal epithelial loss and 50 % of bulbar conjunctival injury. No response to intensive standard treatment and deteriorated further, with >90 % corneal epithelium and >75 % of bulbar conjunctival epithelium lost with 270 degrees of limbal ischemia.	IV at presentation but continued to deteriorate to VI
	Consecutive Cx43AsODN treatment given under a bandage contact lens on days 7 and 8.	
4	42-year-old male. Caustic alkali burn to left eye. Complete epithelial loss with severe 360-degree limbal ischemia. Slow recovery with protective Botox ptosis at day 14 and discharged with early corneal epithelial healing under way. Deteriorated and amniotic membrane applied at days 34 and 45 but both underwent rapid proteolytic degradation.	VI
	Double amniotic membrane and Cx43AsODN treated at day 58, amniotic membrane and second Cx43AsODN treatment at day 90.	
5	17-year-old male. Severe chemical and thermal burn from exploding firework. Complete epithelial loss, fusion of eyelids to ocular surface, significant globe injury with some sparing of inferior fornix only.	VI
	Amniotic membrane with Cx43AsODN treatment at days 8 and 42.	

Injury grade is based on the Dua classification of ocular burns scale. A grade of V or VI has very poor prognosis

Cx43 gap junction protein in a number of models (see e.g., Cronin et al. 2006; Green et al. 2001; Qiu et al. 2003), and the antisense oligomers were modified to match the equivalent human sequence and delivered (2 or 20 μM final concentration) in 100 μl ice-cold, filter-sterilized 25 % w/v Pluronic F-127 (Poloxamer407; BASF, Freeport, TX) gel, a thermoreversible gel that sets as it warms to physiological temperature. The gel provides sustained delivery for the unmodified oligonucleotides, which have a half-life of <20 min in cells (Wagner 1994). The half-life for unmodified oligonucleotides in serum is 1–2 min (Phillips and Zhang 1999), thus limiting possible spread of effect from the treatment site.

In each case the eye was prepared with local anesthesia, either topical benoxinate 0.4 %, subconjunctival lignocaine or sub-Tenons marcaine. To maximize and sustain surface contact, in four of the five subjects the gel (2 μM concentration) was injected beneath an amniotic membrane graft sutured to the corneal surface, using a precooled Rycroft cannula and syringe; in the remaining case the gel at 20 μM was applied under a 14-mm corneoscleral bandage contact lens. The eyelids were then closed and padded.

All other topical treatments were stopped after application of Cx43AsODN (except in the subject who had a bandage contact lens and hourly drops were continued) and then resumed 8–12 h later. The extent and rate of epithelial recovery were assessed by measuring the total area of corneal fluorescein dye staining.

Results

Prior to Cx43AsODN treatment the anterior segments of all eyes were ischemic with associated inflammation from the chemical/thermal burn (Fig. 1a, b). Figure 1a shows subject 1 after application of an amniotic membrane at day 5 after injury and prior to connexin antisense treatment on day 9. The blood vessels on the eye surface are poorly defined, and blood flow was stagnant, with 360 degrees of corneal limbus ischemia. In Fig. 1b the eye of subject 3 is shown on the third day after injury. The bulbar conjunctiva is inflamed and has lost blood vessel definition, and the limbus had 270 degrees of ischemia, with only a small (top left) segment retaining blood flow. Slit-lamp examination with fluorescein dye staining in this same eye (Fig. 1c) shows >60 % corneal epithelial loss with fluorescein penetration beneath the remaining epithelium, indicating poor adhesion and viability. This epithelium continued to deteriorate to >90 % loss by day 7 postinjury. There was in fact virtually no epithelial recovery on any of the corneas prior to Cx43AsODN treatment, and in two subjects the eyes were continuing to deteriorate clinically, despite maximal conventional treatment.

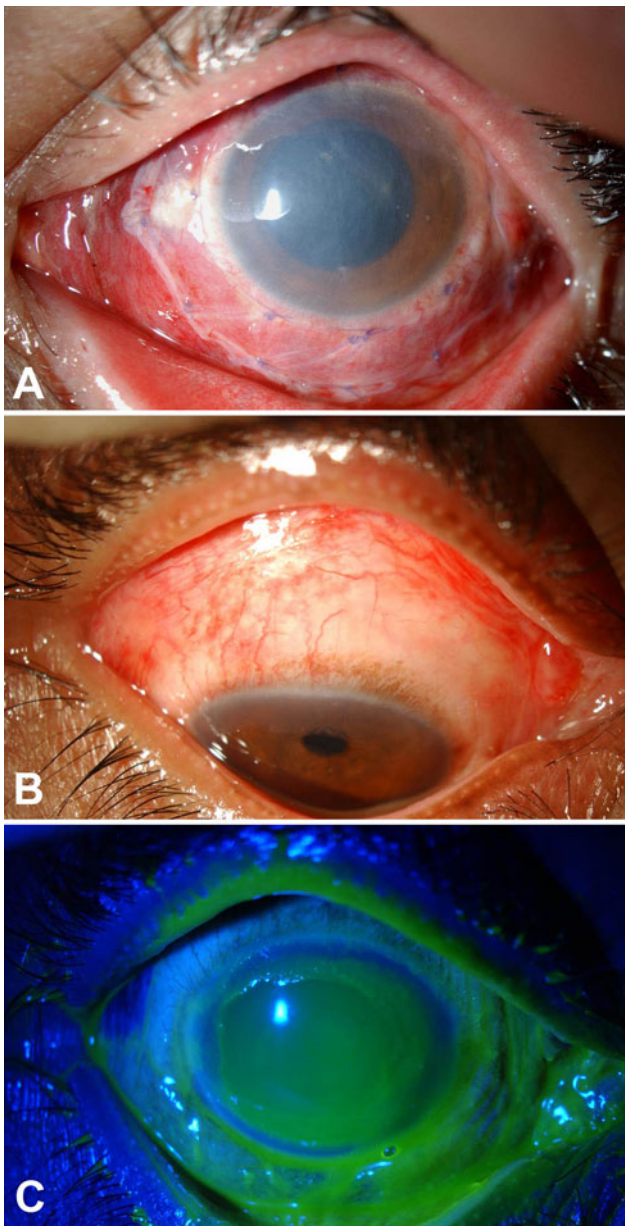
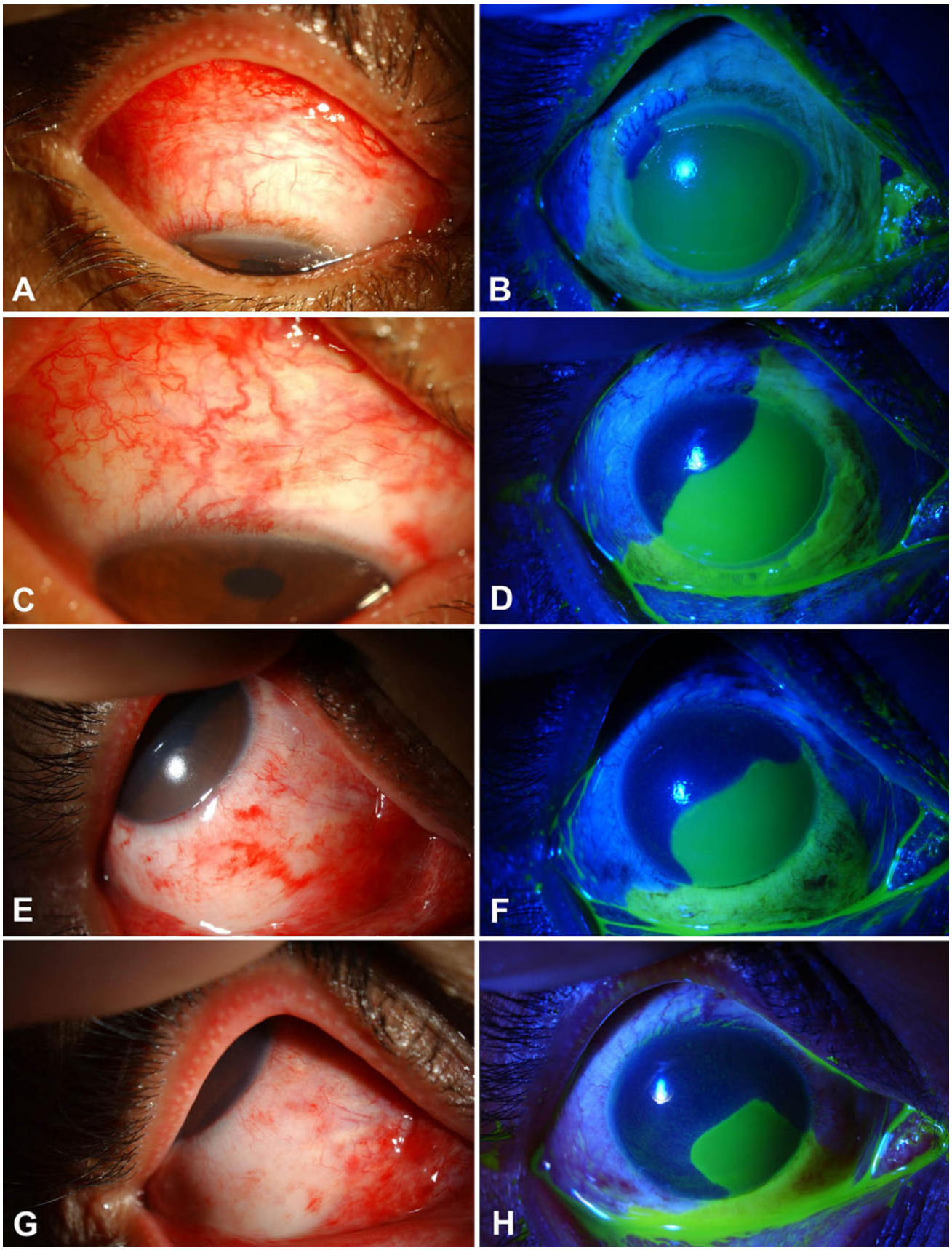


Fig. 1 Subjects presenting with severe ocular burns had ischemic or inflamed ocular surface and poor vascular definition. **a** Subject 1 after application of an amniotic membrane at day 5 after injury and prior to connexin antisense treatment on day 9. Blood vessels are poorly defined, and blood flow is stagnant, with 360 degrees of corneal limbus ischemia. **b** The eye of subject 3 on the third day after injury. The bulbar conjunctiva is inflamed but has lost blood vessel definition, and the limbus has 270 degrees of ischemia, with only a small supranasal segment retaining blood flow. Fluorescein staining (**c**) in this same eye shows >60 % corneal epithelial loss with fluorescein penetration beneath remaining epithelium, indicating poor adhesion and viability. This epithelium continued to deteriorate to over 90 % loss by day 7 postinjury, and the eye was then treated by Cx43AsODN application

In all five severe nonhealing corneal burns, treatment with Cx43AsODN reduced inflammation and a degree of limbal reperfusion was identified within 24–48 h. The most


A
B
C
D
E
F
G
H

◀**Fig. 2** Recovery of the eye of subject 3 after connexin antisense treatment. On day 5 after presentation, a region of supranasal limbal perfusion is seen (**a**), and fluorescein drops revealed only a small area of epithelium remained viable (**b**). After Cx43AsODN treatment on days 7 and 8, more widespread limbal reperfusion occurred, preceding epithelial recovery in the nasal and supratemporal segments (**c** and **d** at day 2 after treatment, **e** and **f** 6 days after treatment). The last portion of the cornea to reepithelialize was the inferior-temporal region, where the limbus remained ischemic longest (**g** and **h** 8 days after treatment). Full epithelial recovery occurred within 11 days after treatment

severely injured eye (subject 5) showed a more delayed initial vascular recovery and epithelial healing 8 days after treatment. Corneal epithelial recovery typically appeared to parallel or follow limbal reperfusion in all eyes. This was highlighted by the response of subject 3. In this case limbal reperfusion and revascularization occurred in a stepwise fashion around the limbus over 8–11 days, with epithelial recovery apparent in successive corneal regions adjacent to areas where limbal reperfusion had first been restored (Fig. 2a–h). In Fig. 2a, b vessel recovery and epithelial healing are beginning at the top left (10 o'clock), but by Fig. 2c, d they have progressed between clock hours 9 and 1. In Fig. 2e and f vascular recovery and epithelial healing are evident between clock hours 7 and 2, and in Fig. 2g, h the last segment not yet showing epithelial recovery (clock hours 4–5) is also the region that remains ischemic at this point in time.

In all five cases complete ocular surface epithelial healing was attained with subsequent surface stability. Specific, brief details for each subject are given in Table 2, and epithelial recovery for each patient (based upon fluorescein staining) is graphed in Fig. 3. In all cases the connexin-specific antisense, applied at times ranging from 7 days to 8 weeks after the initial injury, is seen to have a rapid and positive effect on epithelial recovery. In two subjects reepithelialization reached a plateau, at approximately 80 % cover in one and 20 % in the other, but was retriggered with a second application of the connexin antisense, which then led to 100 % reepithelialization.

Discussion

Cx43 antisense oligonucleotides are designed to achieve transient knockdown in connexin expression followed by recovery of cell–cell coupling in order to allow normal tissue patterning and homeostasis to occur. Prior to the compassionate use in these human subjects animal studies had demonstrated efficacy for the Cx43AsODNs in aiding wound repair and improving epithelial recovery (Coutinho et al. 2005; Qiu et al. 2003).

Table 2 Specific outcomes for each subject treated with Cx43AsODN

Treatment outcomes for subjects 1–5
1 Within 18 h of a single Cx43AsODN treatment evidence of early limbal vessel recovery through 360 degrees and corneal reepithelialization (Fig. 3a) extending 1 mm onto the cornea nasally, superiorly and temporally. Within 42 h 360 degrees of peripheral reepithelialization extending on average 3 mm onto the cornea. The eye was less inflamed, and the anterior chamber cellular reaction reduced. Six days after treatment with Cx43AsODN full reepithelialization of the cornea was attained. The ocular surface remained stable at 18 months, with the patient maintaining an unaided visual acuity of 6/9.
2 Dilatation of limbal vessels superiorly was evident within 24 h of Cx43AsODN application with commencement of local corneal reepithelialization. Corneal reepithelialization increased to 50 % by day 4 and 80 % by day 7 after treatment, healing slowing to reach 90 % by day 17 (Fig. 3b). Full corneal epithelial closure was reached 28 days after treatment. At 12 months the ocular surface remained stable, though extensive corneal vascularization limited visual acuity to 6/60 unaided.
3 Case treated by a single Cx43AsODN treatment under a 14-mm-diameter bandage contact lens on day 7 postinjury. Limbal vessel recovery was evident within 16 h, followed by ocular surface reepithelialization. A second application of Cx43AsODN was provided on day 8, and full corneal reepithelialization was achieved within 11 days posttreatment with minor conjunctival tissue growth into the cornea nasally, although the latter was not visually significant (Fig. 3c). Final visual acuity was 6/6 unaided.
4 Treated by Cx43AsODN 58 days after a severe ocular burn; limbal reperfusion was evident in the inferior 50 % of the limbus, associated with improvement to superior perfusion and early recovery of corneal epithelium within 48 h (Fig. 3d). By 18 days posttreatment the corneal epithelium had healed 70–80 %. However, 11 days later the defect size was unchanged but had developed rolled edges consistent with a persistent epithelial defect; therefore, a second Cx43AsODN treatment was given. Epithelial recovery progressed, and the defect proceeded to complete closure over the next 2 weeks. At 3 months after epithelial closure the corneal epithelial surface remained stable. Although some superficial blood vessel ingrowth onto the peripheral cornea was evident, there appeared to be moderate recovery of the limbal barrier. Final visual acuity was hand movements at 18 months.
5 Due to particularly extensive, severe and deep ocular surface damage and nonresponse to conventional treatment, Cx43AsODN was applied 9 days after injury. Progressive limbal reperfusion was noted from 8 days after treatment and reepithelialization followed. Corneal epithelial recovery reached a plateau at about 20 % coverage, and a second treatment was applied at day 42 with a rapid response (Fig. 3e). Recovery again slowed after about 80 % epithelial coverage but progressed to full, but rather conjunctivalized, corneal epithelial closure over the following 6 weeks. At 18 months postinjury, surgery to release tarsal attachment revealed a clear cornea beneath, and a good final visual outcome is expected.
The rate of epithelial recovery for each is shown graphically in Fig. 3

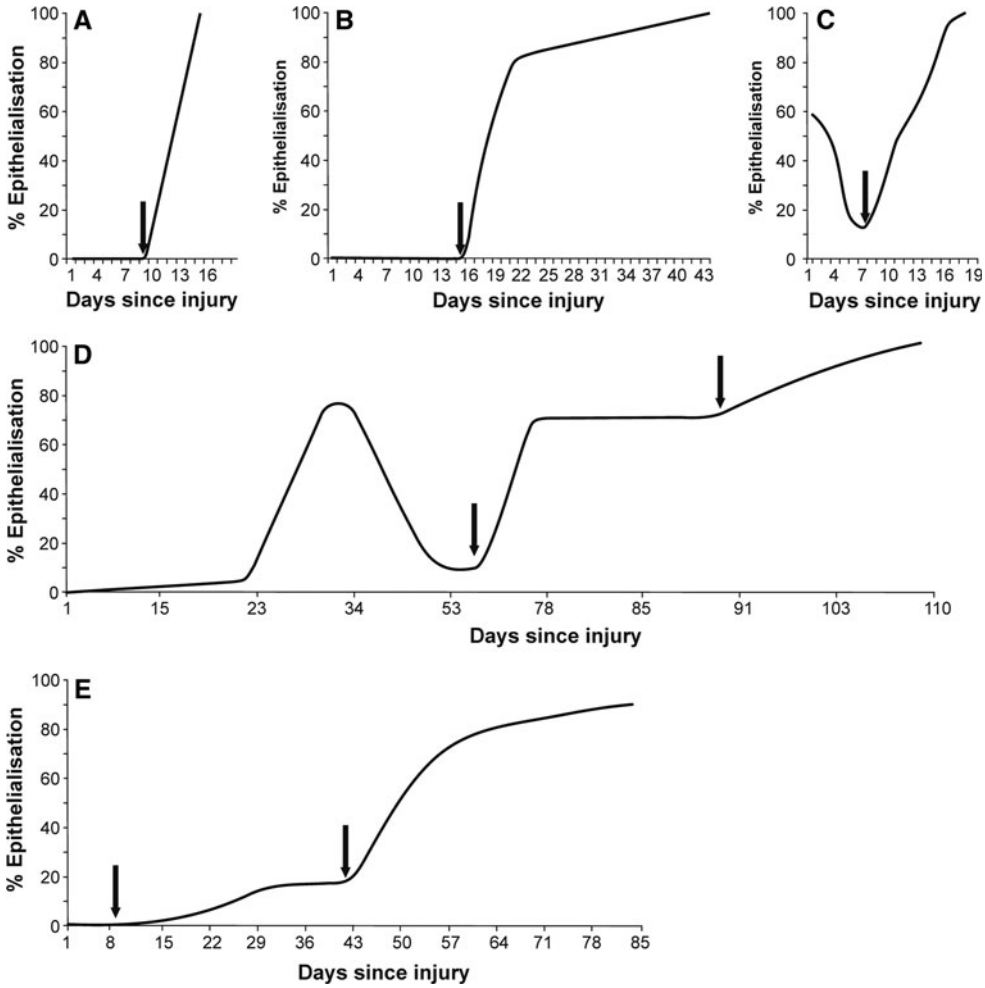


Fig. 3 Graphical representation of corneal epithelium recovery in the five subjects. In each case, points at which the antisense treatment was applied are shown by *arrows*. As subjects were not necessarily seen daily, the *graphs* are interpolated between visits. In all cases the connexin-specific antisense is seen to have a rapid and positive effect on epithelial recovery. Subject 3 (**c**) received two doses of connexin antisense 1 day apart. In subjects 4 (**d**) and 5 (**e**) reepithelialization reached a plateau but was retriggered with a second application of the connexin antisense

In skin wounds keratinocytes at the wound edge downregulate Cx43 in the first 24–48 h as they become migratory in order to close the wound. Cx43AsODNs speed this natural downregulation of Cx43, resulting in faster wound closure (Qiu et al. 2003). The same applies in the cornea of the eye where the corneal epithelium and underlying stroma are analogous to skin epithelium and dermis (Grupcheva et al. 2012). In the dermis of wounded skin and in spinal cord wounds Cx43 is rapidly upregulated after injury, notably in activated neutrophils and glial cells as part of the inflammatory response to injury (Coutinho et al. 2005; Cronin et al. 2008; Qiu et al. 2003). In the corneal stroma, Cx43 is also upregulated, correlating with the inflammatory response typified by differentiation and proliferation of myofibroblasts (Grupcheva et al. 2012). Cx43AsODN treatment in all cases dampens down the inflammatory response with benefits throughout the rest of the healing process.

In this study Cx43AsODNs were applied under compassionate use to one eye of five subjects presenting over a 3-year period with severe nonhealing chemical or thermal burns. Dua grade V–VI ocular surface chemical burns involving the entire limbus and conjunctiva have a very poor prognosis (Dua et al. 2001). All the injured eyes in this study were classified grade V–VI at the time of treatment. The presence of ocular surface inflammation affects the success of limbal transplant or amniotic membrane transplant in grade-VI burns (Gupta et al. 2011); for example, subject 4 had two amniotic membranes applied prior to connexin antisense treatment (under a third membrane), with the second membrane actually dissolving within 4 days, in large part due to the highly inflamed condition of the eye. Amniotic membrane (the inner of the three layers forming a fetal membrane) is used as an ocular burn dressing as it has anti-inflammatory and antiscarring effects and is proposed to contain growth factors that promote epithelial wound healing. In three of the five patients, prior treatment with amniotic membranes had provided no benefit, and in one patient a bandage contact lens was used rather than an amniotic membrane.

Prior to Cx43AsODN treatment and after a minimum of 7 days to more than 8 weeks of intensive, hospital-based, best-practice conventional treatment for a severe chemical or thermal burn injury, all five subjects showed marked

inflammation, limbal ischemia and no signs of ocular epithelial or limbal recovery. Indeed, in two subjects the involved eye was continuing to deteriorate clinically prior to treatment. Following a single low-dose or time-separated double application of Cx43AsODN in a slow-release formulation, the ocular surfaces in all five fully recovered, albeit with a degree of corneal vascularization that one might anticipate in such severe injuries.

The healing pattern in subject 3 (Fig. 2) was not unlike that previously reported for healing of ocular surface wounds involving the limbus (Dua et al. 1994), but our study suggests that an additional influence on the healing pattern may be the relationship with limbal reperfusion. In subjects with a grade-VI corneal burn there was no surviving corneal epithelium from which to trigger cell migration and healing by Cx43AsODN application. Instead, the effect of Cx43AsODN application appears to have been to reduce inflammation, wound edema and arterial insufficiency, also known to be features of some nonhealing wounds. Changes in the vascular bed of the ocular surface are readily discernible at the slit lamp, and in all subjects restoration of limbal perfusion and a general reduction in ocular inflammation appeared to precede full ocular epithelial recovery.

It has previously been reported that exogenous addition of proangiogenic growth factors (VEGF, FGF, PDGF) is not able to prevent vascular regression in wounds (Gosain et al. 2006). These authors concluded that "anti-angiogenic signals that mediate vessel regression in wounds are strongly dominant over pro-angiogenic factors" during the later stages of wound healing. One factor may be increased Cx43 expression. Cx43 is upregulated in the vascular bed of rodent skin wounds within 6 h of injury, contributing to a greater inflammatory response and reduced healing (Couthinho et al. 2003; Qiu et al. 2003). In damaged spinal cord upregulated Cx43 expression results in vascular leak up to 4 mm either side of the injury site, leading to edema, exudation, vessel wall permeability and neutrophil invasion (Cronin et al. 2008). The vascular leak is inhibited with Cx43AsODNs, resulting in reduced inflammation and lesion size. After retinal ischemia–reperfusion, an increase in Cx43 expression correlates with vascular leak, leading to downstream inflammation (astrocytosis) and subsequently neuronal (retinal ganglion cell) loss in the following 7–21 days (Danesh-Meyer et al. 2012). In that case, systemic delivery of hemichannel-blocking mimetic peptides ameliorated vascular leak, reduced inflammation and resulted in almost complete neuronal sparing.

In chronic neuroinflammatory diseases such as Alzheimer and Parkinson disease, morphological changes to the microvasculature indicate capillary dysfunction (Farkas and Luiten 2001). Certainly, a robust inflammatory response to injury can be detrimental to wound closure (Dovi et al. 2004; Ueno et al. 2005), and in humans epithelial wound healing frequently stalls in the chronic inflammation stage (Vuorisala et al. 2009). While bacterial load and poor moisture balance are also associated with nonhealing skin wounds, these are less likely to contribute to the nonhealing ocular cases we report here. Instead, somewhat paradoxically, arterial insufficiency and inflammatory edema of the limbus are possibly major causative factors in impaired corneal epithelial healing (in the context of the normally avascular cornea), as is the case for vascular skin ulcers where the quality of arterial flow or a perturbed venous system is prevalent (Gist et al. 2009; Vuorisala et al. 2009).

In this case series report it cannot be excluded that factors other than connexin modulation may be playing a role. As noted above, however, grade V–VI ocular surface chemical burns normally have a very poor prognosis (Dua et al. 2001); in all cases patients responded immediately to treatment, and in two cases the wounds were continuing to deteriorate prior to treatment. This does not exclude the possibility of vehicle effects, although the supporting literature in animal models, where vehicle and/or control oligonucleotides have no significant effect, would suggest a Cx43-specific mode of action and Pluronic gel is commonly used as a pharmaceutical carrier (Escobar-Chávez et al. 2006). Nonetheless, direct controls were not possible for the compasionate-use case series reported here.

We believe that this first therapeutic human use of Cx43 modulation in five subjects with severe, nonhealing ocular surface chemical/thermal injury indicates that modifying reperfusion and reducing inflammation has potential in the treatment of severe, often unresponsive injuries. While connexin modulation provides a number of benefits in wound healing, downregulation in Cx43 to enable vascular recovery may be a key factor in the treatment of wounds.

Acknowledgments We acknowledge support from the Royal Society of New Zealand Marsden Fund and the Maurice and Phyllis Paykel Trust for characterization work with the antisense oligonucleotides. We acknowledge the Wendy and Bruce Hadden Endowment for salary support (to C.R.G.).

References

Coutinho P, Qiu C, Frank S, Tamber K, Becker D (2003) Dynamic changes in connexin expression correlate with key events in the wound healing process. Cell Biol Int 27:525–541

Coutinho P, Qiu C, Frank S, Wang CM, Brown T, Green CR, Becker DL (2005) Limiting burn extension by transient inhibition of connexin43 expression at the site of injury. Br J Plastic Surg 58:658–667

Cronin M, Anderson PN, Green CR, Becker DL (2006) Antisense delivery and protein knockdown within the intact central nervous system. Front Biosci 11:2967–2975

Cronin M, Anderson PN, Cook JE, Green CR, Becker DL (2008) Blocking connexin43 expression reduces inflammation and improves functional recovery after spinal cord injury. Mol Cell Neurosci 39:152–160

Danesh-Meyer HV, Huang R, Nicholson LFB, Green CR (2008) Connexin43 antisense oligodeoxynucleotide treatment down-regulates the inflammatory response in an in vitro interphase organotypic culture model of optic nerve ischaemia. J Clin Neurosci 15:1253–1263

Danesh-Meyer HV, Kerr NM, Zhang J, Eady EK, O'Carroll SJ, Nicholson LF, Johnson CS, Green CR (2012) Connexin43 mimetic peptide reduces vascular leak and retinal ganglion cell death following retinal ischaemia. Brain 135:506–520

Davidson JO, Green CR, Nicholson LF, O'Carroll SJ, Fraser M, Bennet L, Gunn AJ (2012) Connexin hemichannel blockade improves outcomes in a model of fetal ischemia. Ann Neurol 71:121–132

Dovi JV, Szpaderska AM, DiPietro LA (2004) Neutrophil function in the healing wound: adding insult to injury? Thromb Haemost 92:275–280

Dua HS, Gomes JAP, Singh A (1994) Corneal wound healing. Br J Ophthalmol 78:401–408

Dua HS, King AJ, Joseph A (2001) A new classification of ocular surface burns. Br J Ophthalmol 85:1379–1383

Dua HS, Miri A, Said DG (2010) Contemporary limbal stem cell transplantation—a review. Clin Exp Ophthalmol 38:104–117

Escobar-Chávez JJ, López-Cervantes M, Naïk A, Kalia YN, Quintanar-Guerrero D, Ganem-Quintanar A (2006) Applications of thermo-reversible Pluronic F-127 gels in pharmaceutical formulations. J Pharm Pharmaceut Sci 9:339–358

Farkas E, Luiten PG (2001) Cerebral microvascular pathology in aging and Alzheimer's disease. Prog Neurobiol 64:575–611

Fernandes M, Sridhar MS, Sangwan VS, Rao GN (2005) Amniotic membrane transplantation for ocular surface. Cornea 24:643–653

Frantseva MV, Kokarovtseva L, Naus CG, Carlen PL, MacFabe D, Perez Velazquez JL (2002) Specific gap junctions enhance the neuronal vulnerability to brain traumatic injury. J Neurosci 22:644–653

Garcia-Dorado D, Inserte J, Ruiz-Meana M, Gonzalez MA, Solares J, Julia M, Barrabes JA, Soler-Soler J (1997) Gap junction uncoupler heptanol prevents cell-to-cell progression of hypercontracture and limits necrosis during myocardial reperfusion. Circulation 96:3579–3586

Gist S, Tio-Matos I, Falzgraf S, Cameron S, Beebe M (2009) Wound care in the geriatric client. Clin Interv Aging 4:269–287

Gosain A, Matthies AM, Dovi JV, Barbul A, Gamelli RL, DiPietro LA (2006) Exogenous pro-angiogenic stimuli cannot prevent physiologic vessel regression. J Surg Res 135:218–225

Green CR, Law L-Y, Lin JS, Becker DL (2001) Spatiotemporal depletion of connexins using antisense oligonucleotides. In: Bruzzone R, Giaume C (eds) Methods in molecular biology. Connexin methods and protocols, vol 154. Humana Press, Totawa, pp 175–185

Grupcheva CN, Laux WT, Rupenthal ID, McGhee J, McGhee CNJ, Green CR (2012) Improved corneal wound healing through modulation of gap junction communication using connexin43-specific antisense oligodeoxynucleotides. Invest Ophthalmol Vis Sci 53:1130–1138

Gupta N, Kalaivani M, Tandon R (2011) Comparison of prognostic value of Roper Hall and Dua classification systems in acute ocular burns. Br J Ophthalmol 95:194–198

Hawat G, Benderdour M, Rousseau G, Baroudi G (2010) Connexin 43 mimetic peptide Gap26 confers protection to intact heart against myocardial ischemia injury. Pflugers Arch 460:583–592

Huang C, Han X, Li X, Lam E, Peng W, Lou N, Torres A, Yang M, Garre JM, Tian GF, Bennett MV, Nedergaard M, Takano T (2012) Critical role of connexin 43 in secondary expansion of traumatic spinal cord injury. J Neurosci 32:3333–3338

Lin JH, Weigel H, Cotrina ML, Liu S, Bueno E, Hansen AJ, Hansen TW, Goldman S, Nedergaard M (1998) Gap-junction-mediated propagation and amplification of cell injury. Nat Neurosci 1:494–500

Mori R, Power KT, Wang CM, Martin P, Becker DL (2006) Acute downregulation of connexin43 at wound sites leads to reduced inflammatory response, enhanced keratinocyte proliferation and wound fibroblast migration. J Cell Sci 119:5193–5203

Phillips MI, Zhang YC (1999) Basic principles of using antisense oligonucleotides in vivo. Methods Enzymol 313:46–56

Qiu C, Coutinho P, Frank S, Franke S, Law L-y, Martin P, Green CR, Becker DL (2003) Targeting connexin43 expression accelerates the rate of wound repair. Curr Biol 13:1697–1703

Rawanduzy A, Hansen A, Hansen TW, Nedergaard M (2009) Effective reduction of infarct volume by gap junction blockade in a rodent model of stroke. J Neurosurg 87:916–920

Ueno M, Lyons BL, Burzenski LM, Gott B, Shaffer DJ, Roopenian DC, Shultz LD (2005) Accelerated wound healing of alkali-burned corneas in MRL mice is associated with reduced inflammatory signature. Invest Ophthalmol Vis Sci 46:4097–4106

Vuorisala S, Venermo M, Lepantalo M (2009) Treatment of diabetic foot ulcers. J Cardiovasc Surg 50:275–291

Wagner RW (1994) Gene inhibition using antisense oligodeoxynucleotides. Nature 372:333–335