PERSPECTIVE

Targeting the body to protect the brain: inducing neuroprotection with remotely-applied near infrared light

The incidence of intractable age-related neurodegenerative conditions such as Alzheimer's and Parkinson's diseases and age-related macular degeneration is projected to increase substantially over the coming decades with the ageing of the global population. While the burden of disease associated with other chronic conditions has decreased in recent times due to improved diagnosis and treatment, current therapies for neurodegenerative diseases still fall short in that they are only effective in treating signs and symptoms – they do little to slow or prevent disease progress. Thus, there is an urgent need for treatments that address disease progression.

Considerable research has focussed on developing therapies for these neurodegenerative conditions. Because their initiating causes are still the subject of debate, however, the choice of therapeutic targets has been guided by features of the diseases, such as β-amyloid deposition in Alzheimer's disease and dopamine loss in parkinsonism, rather than by definitive knowledge of the causative mechanisms. It is then unsurprising that these potential therapies have at best (for example the use of L-DOPA for parkinsonism) provided remarkable relief of symptoms but have left the progress of the underlying disease unmitigated.

With knowledge of cause being still elusive, a number of research groups are responding to evidence that cells of many, presumably all, body tissues have evolved systems of self-protection and self-repair, which can be activated by non-pharmacological and non-toxic interventions; and that these systems can mitigate and even reverse the course of degenerative disease. Among the many interventions that have been trialled, photobiomodulation (PBM), the irradiation of tissue with low-energy near infrared light (NIr; 600–1,100nm), shows particular promise. Initially trialled as an accelerant for the healing of lesions to the skin and oral mucosa, PBM has now demonstrated efficacy in mitigating pathology and functional deficits in rodent models of retinal degeneration, traumatic brain injury, acute ischaemic stroke, and Alzheimer's and Parkinson's diseases. Small but well-controlled clinical trials have shown efficacy in mitigating and slowing age-related macular degeneration and in mitigating the consequences of mild to moderate stroke. Furthermore, PBM is effective at low energy levels (<10 J/cm^2) and can be

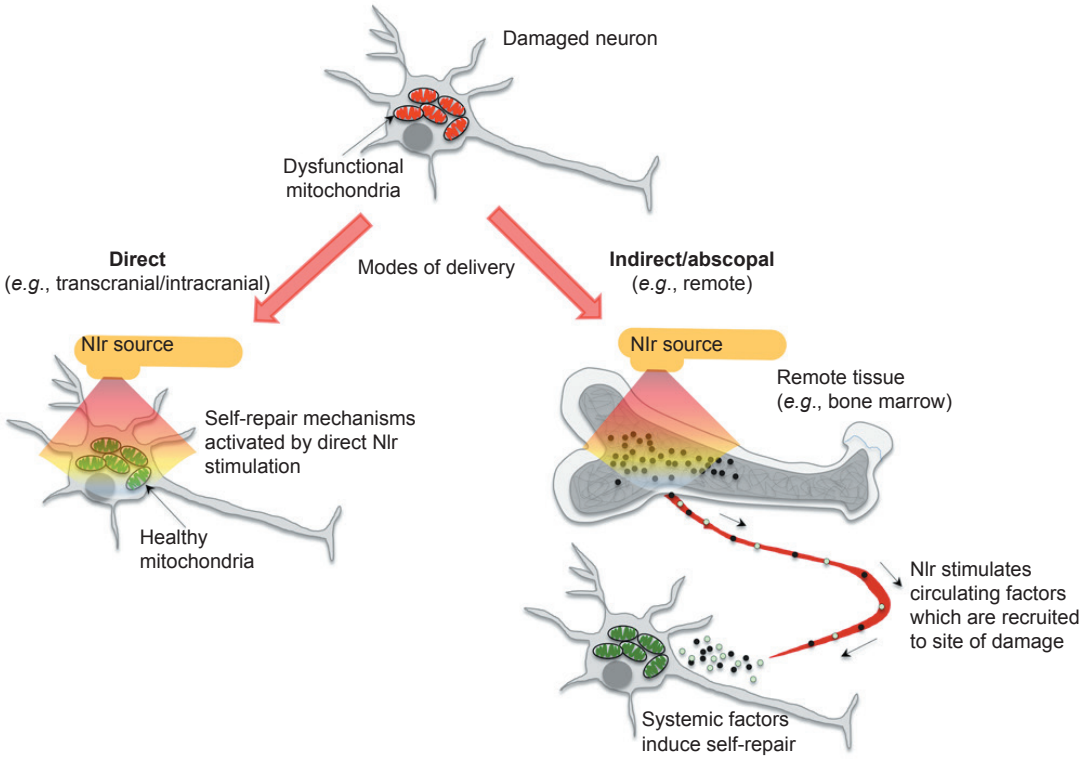

Figure 1 Putative mechanisms underlying near infrared light (NIr)-induced neuroprotection.
Two different mechanisms, one direct and cellular, the other indirect and systemic, appear to be involved in mediating the neuroprotective effects of NIr. Direct NIr irradiation of damaged neuronal tissue (*e.g.*, by transcranial or intracranial irradiation) has been postulated to stimulate mitochondrial activity. This, in turn, triggers a cascade of downstream signalling events that collectively activate endogenous cellular repair pathways. The mechanisms underlying neuroprotection resulting from NIr irradiation of remote tissues (*i.e.*, 'abscopal' effect) presumably involve the stimulation or mobilisation of particular circulating 'factors' (*i.e.*, cells or molecules). These factors can then travel to the site of damage and aid in the repair of dysfunctional neurons, possibly through the release of neurotrophic factors.

sourced from light emitting diodes (LEDs) as well as lasers, making it safe, easy to deliver and stress-free for the subject. LED panels have already received non-significant risk status by the Food and Drug Administration (FDA).

One major barrier to clinical translation for cerebral degeneration is the delivery of sufficient doses of NIr to deep-lying target regions. For example, our recent measurements of 670 nm NIr transmittance across brain tissue indicate a reduction in light energy of ~65% per millimetre. As a result, less than 0.001% of the light aimed at the target will reach tissue 10 mm from the surface of the brain (Moro et al., 2014). As a consequence, transcranially-delivered NIr, although providing sufficient irradiation of damaged tissue in the small rodent brain, appears to be an infeasible approach for treating human diseases in which deep brain structures are affected.

Thus there is a need to develop alternative modes of NIr delivery. One option is to deliver NIr intracranially from an implantable light-emitting device. To this end, a research team headed by Alim Louis-Benabid in Grenoble, France, in collaboration with the authors of this article, has developed an apparatus consisting of an optical fibre linked to an LED or laser. Proof of principle studies in rodents have demonstrated that the optical fibre can be implanted successfully into the brain of rodents, that the device remains stable in freely moving animals and that intracranial irradiation with NIr intensities above normal therapeutic doses produces no evidence of tissue necrosis. Furthermore, intracranially-delivered NIr in rodent protects against dopaminergic cell loss in the substantia nigra pars compacta (SNc) following exposure to the parkinsonian neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Moro et al., 2014). This implantable device is now being tested in a monkey model of MPTP-induced parkinsonism, with early results showing promise.

While the intracranial delivery of NIr using an implantable source is a promising way to target a small, deep-lying region of the brain, such as the substantia nigra in Parkinson's disease, the approach is highly invasive and less useful in treating diseases with more widespread pathology (*e.g.*, Alzheimer's disease). Recent discoveries about the biology of the body's response to NIr have suggested a quite different approach to reaching target tissues; these discoveries have surprised most workers in the field.

Most studies of the protective and reparative effects of PBM have assumed that the NIr should be targeted directly at the damaged tissue. As studies accumulated, however, evidence emerged which challenged this assumption. Studies reported that radiating a lesion on one side of the body accelerated healing of wounds on both sides; or noted that a unilateral effect could never be attained (reviewed by Johnstone et al., 2014). This phenomenon seemed reminiscent of the 'abscopal effect' reported in metastatic cancer, where radiation therapy targeting one tumour occasionally results in regression of distant tumours.

These mostly incidental observations led us to test whether NIr applied to the dorsum of an animal, with the head covered by infrared-opaque foil (to eliminate transcranial irradiation), is protective against MPTP-induced neurodegeneration of the substantia nigra (Stone et al., 2013, Johnstone et al., 2014). Mice were injected with a range of MPTP doses and treated with 'remote' NIr (2x90s) on each day an injection was given, and tissue collected 7 days after the last MPTP injection. Using tyrosine hydroxylase (TH) immunohistochemistry to label functional dopaminergic neurons, remote NIr mitigated loss of TH^+ cells in the SNc resulting from 50 mg/kg MPTP, to a similar degree as achieved with transcranial NIr. At stronger MPTP doses, neither remote nor transcranial NIr treatments were capable of providing significant mitigation of TH^+ cell reduction (Johnstone et al., 2014), consistent with previous findings. This study, the first to test the neuroprotective effects of remote NIr, provides compelling evidence for a systemic mechanism of NIr-induced tissue protection (**Figure 1**). The possibility that elements of NIr-induced neuroprotection can be mediated through a body-wide system involving circulating cellular or molecular factors has clinical as well as fundamental implications, and raises questions as to the identity of these circulating mediators of protection.

The concept of an NIr abscopal effect is also being investigated by the group of Uri Oron at Tel Aviv University, in work focusing on the irradiation of bone marrow for cardioprotection and, more recently, neuroprotection. Through a combination of *in vitro* and *in vivo* experiments, Oron's group demonstrated that NIr treatment increases the proliferation of bone marrow-derived c-kit-positive cells, both in culture and in rats. Furthermore, using a rat model of myocardial infarction, they provided evidence that these cells were mobilised and recruited specifically to the site of damage in the heart, where they were associated with a marked reduction in infarct size and ventricular dilatation (Tuby et al., 2007, 2009, 2011). Targeting NIr treatment to the tibia (easily done with a laser or optic fibre) resulted in more effective protection than targeting the site of damage in the heart (Tuby et al., 2011), providing strong support for our observations of systemic protective effects of NIr. More recently, the same group demonstrated that bone marrow-targeted NIr reduced amyloid pathology and improved cognitive measures in a mouse model of Alzheimer's disease (Farfara et al., 2014).

In the publications describing these studies, the authors suggested that the bone marrow-derived cells activated by NIr treatment are mesenchymal stem cells (MSCs). Although the cell markers available to define MSCs are arguably less than definitive, several observations make this cell type a promising candidate as the mediator of systemic NIr-induced protection. Firstly, studies on cell monolayers and rodent models suggest that MSCs can transmigrate across the blood-brain barrier, binding to activated (*i.e.*, inflamed) endothelium through selectin- and integrin-dependent interactions and releasing proteases (*e.g.*, matrix metalloproteinases) to facilitate transmigration and invasion of the

basement membrane. Secondly, MSCs have the ability to home specifically to areas of pathology and damage through various chemokine-receptor interactions (*e.g.*, monocyte chemoattractant protein-1 and C-C chemokine receptor 2). Thirdly, MSCs release a range of trophic factors that hasten endogenous cellular repair through paracrine actions. Finally, transplantation of exogenous MSCs has already shown neuroprotective efficacy in animal models of various brain diseases, including stroke, traumatic brain injury, multiple sclerosis and neurodegenerative disease (reviewed by Parr et al., 2007, Uccelli et al., 2011, Glavaski-Joksimovic and Bohn, 2013).

MSCs thus appear strong candidates for mediating remote NIr-induced neuroprotection. Even so, considerable further research is required to confirm that MSCs are stimulated by NIr and that they subsequently migrate to damaged tissue to facilitate self-repair. Additional optimisation studies are required to determine the wave length and dose of remote NIr that yields the maximal neuroprotective effect.

In summary, remote NIr-induced tissue protection is emerging as a promising therapeutic intervention. The idea of irradiating the leg to slow brain diseases with the intractability-to-date of dementia and parkinsonism would seem to be counter-intuitive and almost superstitious, had not three substantial biological principles been laid down in the pioneering work. First, the mammalian genome has been shown to include pathways of self-repair and self-protection which are expressed in key organs such as the brain, retina, heart and skin. Second, these pathways can be activated by therapeutic interventions, in the present work by NIr, at safe, low doses. Third, a major component of the therapeutic effect is systemic, involving circulation of a class of protective cells or molecules, and can be activated by irradiation of sites (*e.g.*, bone marrow) well away from the target tissue.

In practical terms, the indirect protective effects of NIr were an unexpected bonus to the value of direct NIr irradiation, for they overcome the problem of tissue penetration, currently a major barrier to the clinical application of light-based therapies to brain disorders. Confirmation of these effects, and identification of the mechanisms of remote NIr-induced neuroprotection will have major implications for our understanding of this treatment and its potential to ignite a new area of therapeutics for neurodegenerative disease.

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