



## Are mitochondria the alpha and omega of retinal disease?

*The physical interactions between light and mitochondrial proteins are beginning to suggest a unified view of disparate retinal pathologies and even the possibility of therapy. Nick Lane PhD looks into the strange goings on between light and mitochondria; curse and cure*

“DON’T worry, nobody understands the mitochondriacs!” was once whispered reassuringly around lecture theatres. And no field is more confusing than the plethora of inherited conditions caused by mutations in mitochondrial DNA, with odd names like LHON, MELAS and MERRF. At least, thankfully, they’re rare.

Ophthalmology has been at the forefront of research into mitochondrial diseases, as so many of them seem to involve the eye. Indeed, some mitochondrial diseases such as LHON typically affect RGC function. This vulnerability is explained by recent research that has uncovered surprising links between the absorption of light by mitochondrial respiratory proteins and the degeneration of retinal photoreceptor and ganglion cells.

“It’s well known that photoreceptor cells are vulnerable to the high energy of blue light, because they’re packed with opsin pigments,” Prof Neville Osborne of the Nuffield Laboratory of Ophthalmology at Oxford, told *EuroTimes*, “but retinal ganglion

cells are also vulnerable to blue light, despite lacking opsins. The reason is they’re rich in mitochondria.”

Unexpectedly, the relationship between light and mitochondria also holds prospects for a remedy, with ramifications that go well beyond the rare mitochondrial disorders, raising hopes of therapies for far more common conditions including glaucoma and AMD.

### The LHON reach of the gene

In his Nobel speech of 1931, the great German biochemist Otto Warburg described his insights into the molecular mechanisms of cellular respiration.

Warburg had made use of an extraordinary titbit of knowledge, which anyone placing their head in a car’s exhaust fumes might care to reflect on in their final moments: carbon monoxide (CO) doesn’t cause irreversible suffocation – it doesn’t permanently block cell respiration. A flash of light can dissociate CO from the

respiratory enzymes and kick-start cellular respiration all over again. But don’t try it at home.

Warburg realised that there must be an interaction between light and the (then) unknown enzyme responsible for consuming oxygen in cellular respiration, now labelled cytochrome oxidase. As pigments don’t absorb light at all wavelengths equally, Warburg predicted the rate of respiration following blockade with CO would depend on the absorption spectrum of the pigment – the more light absorbed, the more CO would dissociate, and the faster the rate of respiration.

So by shining lights of different wavelengths at cells suffocating with CO, and then measuring the ensuing rate of respiration, Warburg could piece together the spectrum of the respiratory enzyme. It turned out to be the spectrum of a haem protein, with properties quite similar to haemoglobin and chlorophyll. Indeed, Warburg went on to suggest that

haemoglobin and chlorophyll evolved from the respiratory pigment. That was a big claim, because it meant that respiration must have evolved before photosynthesis.

**“Unmyelinated axonal transmission is very energy-demanding, reflecting the greater energy required to restore their action potential. It’s this combination of high energy requirement and exposure to light that makes them vulnerable”**

Neville Osborne

## Is the pot half full or half empty?

Cells die by apoptosis in many retinal degenerative diseases caused by point mutations in single genes. But although these causative genes are diverse, the mechanism of cell death is common, involving mitochondria, and offers an important insight into treating more complex degenerative conditions.

Prof Alan Wright and his colleagues at Edinburgh University scrutinised five single-gene disorders, which cause retinal degeneration either as a primary disorder of rods (RHO) or as a disorder of both rods and cones (RPGR, RDS, TULP1, ATXN7).

The biochemical pathways in each case are well known and, importantly, don’t cause oxidative stress, at least not directly.

“Most of the genes known to cause inherited neurodegeneration in the retina do not directly influence free-radical formation, implying they have an indirect effect, at most, on oxidative stress,” Prof Wright told *EuroTimes*.

Nonetheless, the mitochondria are still central: apoptosis takes place via the

intrinsic pathway, which is nearly always triggered by cytochrome c release from mitochondria.

Wright’s team applied a clever trick. They looked at the same point mutation in five different species (mouse, rat, dog, pig, human), each with differing maximum lifespan, and underlying rates of free radical production (*Nature Genetics* 36: 1153-8; 2004).

Perhaps not surprisingly, the onset and progression of disease reflected lifespan. Thus short-lived animals capitulated to retinal degeneration within the span of a few years, longer-lived creatures only after decades. More illuminatingly, though, the onset and progression of disease specifically correlated with the underlying rate of free-radical formation from mitochondria.

“We think that mitochondria are in some way calibrating the overall levels of stress experienced by the cell, and when that level of stress crosses a threshold, the mitochondria force it to commit suicide,” said Prof Wright.

So different genetic or environmental stressors all empty into the same mitochondrial pot, which is already half full of free radicals generated during the vital process of cell respiration – faster in short-lived species, slower in long-lived species.

The faster the pot fills up, the faster the cell crosses the threshold and dies by apoptosis. Better, you might say, for the pot to be half empty than half full.

Critically, any technique that can slow down the rate at which the pot fills – NIR phototherapy, for instance – has the potential to slow the progression of all degenerative diseases, potentially prolonging healthy lifespan.

“We’ve been trying to slow down apoptotic cell death in our five models of retinal degeneration, but if successful our results should, at least in principle, be useful for any disorder characterised by increased apoptotic cell loss – from diabetes to ageing,” Dr Wright told *EuroTimes*.

But for half a century, the very physical relationship between respiration and light languished quietly disregarded in the small print of dusty biochemistry textbooks. Who knows now, or who cares, that cytochrome P450 is so named because light with a wavelength of 450 nanometres is particularly good at dissociating CO from the pigment. But we should care, because Warburg’s long forgotten interactions between light and cytochrome oxidase are beginning to take on central relevance to both retinal diseases and therapies.

One of the clues comes from the rare inherited mitochondrial condition Leber’s hereditary optic neuropathy, or LHON.

LHON is caused by any one of several point mutations in mitochondrial DNA, all of which affect subunits of one of the respiratory complexes in mitochondria (complex I). But the outcome is not a generalised systemic condition, nor even a condition that affects all retinal cell types to the same degree; it is curiously specific to retinal ganglion cells.

Retinal ganglion cells are unusual in that they have unmyelinated axons, which are exposed to light as they traverse the retina.

“Unmyelinated axonal transmission is very energy-demanding, reflecting the

greater energy required to restore their action potential," explains Prof Osborne. "It's this combination of high energy requirement and exposure to light that makes them vulnerable."

It is important to emphasise that light is unlikely to be detrimental to a healthy RGC; it only becomes a factor when the cells are in an already stressed state, he added.

To meet their high-energy demands, RGCs have regular varicosities along their length, which are packed with mitochondria. Several of the mitochondrial pigments, notably those in cytochrome oxidase, absorb blue light. The reactive, energised state is a potent free-radical generator, giving rise to oxidative stress.

In a recent hypothesis paper, Prof Osborne extended these findings to other, more common degenerative conditions such as glaucoma (Osborne et al, *Br J Ophthalmol* 90: 237-41; 2006).

"We hypothesised a few years ago that in glaucoma, a reduced blood flow to the optic-nerve head restricts oxygen delivery to RGCs, especially those in the periphery of the retina," said Osborne. "Again, exposure to light combined with low energy availability causes oxidative stress, making these cells vulnerable to excitotoxicity in exactly the same way as in LHON."

According to Osborne, the differences in the age of onset and the patterns of retinal degeneration depend on the contributing factors (reduced blood flow or mitochondrial DNA mutations). Blue light might then contribute to exacerbate this state to eventually initiate apoptosis.

"We've shown that RGCs in culture are resistant to apoptosis when exposed to a specific quantity of light or low-nutrient medium alone. But it is the combination of

the two factors that induces apoptosis," says Osborne.

Yet surprisingly, while wraparound spectral filter sunglasses might seem an easy answer, they aren't necessarily the best. Light can play other tricks in medicine, and recent work suggests that other, longer wavelengths of light might actually improve the energy balance of cells, saving them from apoptosis.

### Therapeutic photons

Blue light is not the only wavelength absorbed by cytochrome oxidase – the enzyme also absorbs red and near infrared (NIR) rays, which have somewhat less energy, and contrasting effects.

[My own doctoral research, more than a decade ago, made use of the ability of cytochrome oxidase to absorb NIR rays, enabling me to monitor the respiratory function of transplanted organs. But I never suspected that the very act of measurement might have influenced the speed of respiration; that smacks of the uncertainty principle in quantum mechanics, still of rather uncertain relevance to biology. Yet my measurements must have altered mitochondrial function.]

Some of the benefits of light on wound healing and tissue regeneration have been known about since antiquity, but only in the late 1990s, 70 years after Warburg, did Professor Tiina Karu of the Russian Academy of Sciences, show that the chromophore responsible was cytochrome oxidase.

Karu's findings are relevant to any condition in which energetic deficiency and apoptosis play a role, for the effect of NIR is to enhance the energy status of the cell, staving off apoptosis. There is a measurable rise in ATP production and a fall in the rate of apoptosis in cell cultures.

Although NIR penetrates biological tissues to depths of several centimetres, the greatest benefits are likely to be seen in relatively superficial locations, like the retina. And indeed Janis Eells, distinguished professor at the University of Wisconsin, has shown that NIR phototherapy has positive effects in both acute retinal toxicity and in retinal degenerative conditions (ARVO abstract 2006; Eells et al. *Mitochondrion* 4: 559-567; 2004).

"We started out by looking at methanol intoxication, which produces injury to the retina and optic nerve, often causing blindness," Prof Eells told *EuroTimes*. "NIR phototherapy, given as three brief LED treatments, is enough to restore virtually normal retinal function, judging by the ERG in our rodent model," said Dr Eells.

The toxic metabolite of methanol is formic acid, a mitochondrial toxin that inhibits cytochrome oxidase. This offers a clue to what is happening at the molecular level, says Prof Eells – the effect of NIR photons might be to dissociate the formic acid from cytochrome oxidase, just as Warburg had dissociated CO with light in his pioneering experiments.

"We've looked quite closely at another inhibitor of cytochrome oxidase in cell cultures of visual cortical neurons," says Dr Eells, "cyanide." At low doses, cyanide causes apoptosis rather than necrosis. But when Eells and colleagues irradiated their cells with NIR first, they measured a 50% reduction in the rate of apoptosis by standard TUNEL staining.

"The really striking finding is that we irradiated the cells first, before adding the cyanide," says Dr Eells, "yet still there was a protective effect." This sounds eerily like action at a distance, but Eells is ready with a plausible and testable explanation.

"There's a lot of interest today in the signalling role of nitric oxide, especially in mitochondria," says Eells. "NO also binds to cytochrome oxidase, apparently as a physiological regulator, but so far nobody has worked out exactly what the downstream signalling effects are. But it's plausible that NIR could dissociate physiologically bound NO from cytochrome oxidase, and this in turn could have immediate effects on the rate of respiration and ATP availability, as well as important downstream signalling effects."

The idea is appealing. Karu and others have shown that NO donors can reverse the effects of NIR phototherapy; and Eells and her team have shown, using microarrays, that the expression of as many as 80 genes is altered by NIR phototherapy. The most striking differences were observed in genes from the cytochrome oxidase family, the peroxiredoxin family, and genes involved in cell growth and maintenance – upregulating mitochondrial energy production and the stress response.

These differences suggest that NIR phototherapy might show benefits in degenerative conditions as well as in acute toxicity. The signs are promising. At ARVO this year, Eells and her colleagues presented their preliminary findings in a rat model of retinitis pigmentosa (in which the rods and cones die by apoptosis during postnatal development, causing retinal degeneration and blindness).

The findings were very encouraging. Rats treated with NIR phototherapy once per day for five days during postnatal development showed better mitochondrial function (cytochrome oxidase activity) in the photoreceptors. Not only that, but barely half as many cells died by apoptosis.

"The NIR-induced reduction in photoreceptor loss is very impressive," says Prof Eells. "If this translates into improved retinal function in the adult animals it will be a major breakthrough in the treatment of retinal degenerative diseases."

The technique is simple, requiring only an LED (albeit the dosage requires care – in general, short repeated treatments work better than prolonged irradiations – bigger is not better). What's more, NIR phototherapy has the potential to preserve retinal function in any degenerative, or apoptotic, condition including AMD, all the mitochondrial optic neuropathies, and even glaucoma, on the evidence of Prof Osborne.

So will it work? "We desperately need to draw attention to testing this treatment," says Prof Eells. "The trouble is that when you enthuse about light as a therapy, clinicians tend to back away. Maybe if the physical interactions of photons with cytochrome oxidase and NO are better known, people will begin to appreciate the huge potential benefits of this simple technique."

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## Fireflies, mitochondria and nitric oxide

Ever since antiquity, the flashing of fireflies has teased the human imagination. Only today have we got a handle on how they produce flashes of light. The answer is an enzyme called luciferase, which uses ATP to activate luciferin to a luciferyl-adenylate intermediate. This in turn reacts with oxygen to emit a photon, giving rise to bioluminescence.

Yet the luciferase reaction only shows how fireflies produce light. In fact, firefly flashes are finely controlled, occurring in precise and reproducible sequences, with the goal of attracting mates. But how do they switch them on and off so quickly?

The obvious answer – it's all controlled by the nervous system – turns out not to be quite true, as the controlling neurons do not synapse on the lantern cells containing the luciferase enzyme, but instead end on the tracheolar cells, which lie between the tracheolar airway and the lantern cells. This suggests that a chemical mediator must diffuse rapidly, crossing cell membranes, to act on adjacent cells.

In an important paper published in *Science* in 2001 (Trimmer et al, *Science* 292: 2486-8; 2001), Prof Barry Trimmer and his colleagues at Tufts University showed that the missing mediator was none other than nitric oxide (NO). Or at least they showed that NO donors made fireflies flash frantically; inhibitors reversed the effect; and of necessity, NO synthase was abundant right next to the nerve endings.

But their preliminary findings didn't show exactly how nitric oxide worked its magic. The answer was suggested by the morphology of the lantern cells themselves. The luciferase enzyme is tucked away in the centre of the cell, surrounded by a ring of mitochondria, guarding access to that inner sanctum.

These mitochondria, Trimmer now argues, act as oxygen gate-keepers.

By binding to cytochrome oxidase, NO blocks respiration – and the oxygen that would have been consumed in respiration instead passes through the cordon to react with the luciferyl intermediate, producing a flash of light.

And then the crowning glory. The flash of light itself dissociates NO from cytochrome oxidase, kick-starting respiration again. So the flash is its own off switch. Oxygen is now consumed once more by the mitochondrial gate-keepers, allowing the luciferyl intermediate to build up, until the next burst of NO arrives from the nerve endings.

