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Guest Editorial

Near-Infrared Irradiation Photobiomodulation: The Need for Basic Science

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Near-infrared irradiation photobiomodulation (NIR-PBM) has been studied, discussed, and debated now for several decades. PBM is based on the theory that low level light in the NIR range can alter, and improve, cellular function.[1] In particular, it is believed that NIR-PBM functions by improving mitochondrial energy production by stimulating the complex IV enzyme, cytochrome c oxidase (CCO), and increasing adenosine-5′-triphosphate (ATP) synthesis.[2,3]

Cellular effects attributed to NIR-PBM include increased ATP, reduced production of reactive oxygen species, protection against toxins, increased cellular proliferation, and reduction of apoptosis.[2,3] Clinical uses of NIR-PBM have been studied in such diverse areas as wound healing,[4,5] oral mucositis,[6] and retinal toxicity.[7] In addition, NIR-PBM is being considered for study in connection with areas such as aging and neural degenerative diseases (Parkinson's disease in particular).[8]

One thing that is missing in all of these pre-clinical and clinical studies is a proper investigation into the basic science of the NIR-PBM phenomenon. Although there is much discussion of the *uses* of NIR, there is very little on *how* it actually works. As far as explaining what really happens, we are basically left to resort to saying "light enters, then a miracle happens, and good things come out!" Clearly, this is insufficient, if for no other reason than our own intellectual curiosity. But beyond that, we can not hope to truly develop this extremely promising treatment to its highest potential without some understanding of what is actually happening inside the "black box". Therefore, we maintain that the time has come to devote serious effort to the study of the basic science of NIR-PBM.

At the heart of the matter is the question of enzyme kinetics. As it is generally agreed that the cellular target for the NIR is the enzyme CCO,[2,3] an understanding of how the light affects its kinetic properties is the most logical place to start. At this point, there appears to be only one study directly addressing this question.[9] An increase in the observed kinetic constant for the reaction of CCO with cytochrome c was observed at high enzyme/substrate ratios when the enzyme was irradiated with 630-nm laser light. In contrast, a lowering of the kinetic constant occurred at low enzyme/substrate ratios. A mechanistic interpretation of these results was not offered.

Errede et al.[10] have published a detailed study of CCO kinetics, with an analysis of the results in light of several proposed mechanisms. The deduced rate equation for the reaction is complex, and includes many parameters relating to various steps in the proposed mechanism. Pastore's work could be expanded to include a study similar to Errede's, but with the inclusion of NIR. A study of the kinetics along these lines could reveal specifics of the effects of NIR, and lead to mechanistic insights. In particular, it could be possible, eventually, to relate the phenomenon of NIR-PBM to specific steps in the catalytic cycle.

This type of work could also be extended to studies considering other parameters of NIR-PBM application. Most work to date has been using a hodgepodge of wavelengths, intensities, and durations. Wavelengths considered, and promoted, tend to vary from 630 to 880 nm, intensities vary from 10 to 50 mW/cm^2, and fluences vary from 1 to 10 J/cm^2. It appears that the parameters chosen are, in many cases, related more to convenience and practicality than to anything else. Although some investigators have introduced some variability into their experiments,[11] controlled experimental design studies have yet to be performed.

As information regarding the basic mechanisms of the NIR-PBM effect becomes developed, the situation becomes such that a statistical experimental design aimed at optimization would be profitable. As the haphazard choices of NIR parameters may miss, or understate, the benefits to be gained from PBM, a proper designed experiment may lead to a better understanding of how to best use NIR-PBM. Variables such as power and fluence can be studied using factorial designs, while wavelengths can be varied or combined by incorporating mixture design elements into the factorial studies. Not only basic kinetic parameters can be explored this way, but also factors affecting various other downstream *in vitro* and *in vivo* pre-clinical and clinical studies. In this manner, a strong knowledge base can be built up, driving efforts leading to eventual optimal clinical development.

Other factors affecting the basic enzyme kinetics, and therefore the understanding of the mechanism, can also be addressed. In particular, the effect of enzyme inhibitors can be studied in relation to NIR exposure. A great deal of work has been done regarding the effects of NO,[12,13] CO,[14] CN^-,[11] and other inhibitors on the kinetics of CCO. In particular, a role for NO in NIR-PBM has been proposed.[15] A thorough study of the effects of NIR application on the nature of these inhibitions could lead to a better understanding of the mechanistic basis for NIR-PBM.

Further aspects of kinetics that might lead to insights into PBM might include the interactions, if any, of NIR-PBM kinetics with changes in temperature, pH, exposure times, and application sequencing, among others. The information gained

in this regard might relate not only to mechanistic understandings, but could also affect eventual clinical uses of PBM.

Of course, conclusions regarding mechanisms based on kinetics are somewhat speculative, without direct supporting evidence. CCO has been extensively studied spectroscopically, especially using ultraviolet-visible spectroscopy (UV-VIS)[16,17]and electron paramagnetic resonance (EPR)[18] techniques, but there has been very little studied regarding changes caused by exposure to light.[19] As kinetic studies generate new hypotheses regarding mechanisms, new experiments involving spectroscopy, particularly EPR, can be designed to further test these ideas.

All of these studies, of course, presuppose a steady supply of pure, active, cytochrome c oxidase. Fortunately, there is no dearth of useful enzyme preparation procedures published.[20–23] Although involving some initial work and expense, any extensive projects along these lines would benefit from a stable, reliable, in-house source of CCO in quantity.

In sum, we feel that the time is right to move aside from limiting ourselves to studying only the downstream results of NIR-PBM, and aggressively pursue avenues leading to a basic understanding of the underlying science. We have seen basic science projects focusing on enzymes with no proven physiological role criticized as being a "solution in need of a problem." In contrast, here we have a situation that clearly needs an understanding of the basic science, a "problem in need of a solution."

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