

COMBINED MAGNETIC AND PULSED LASER FIELDS PRODUCE SYNERGISTIC ACCELERATION OF CELLULAR ELECTRON TRANSFER

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We have studied the acceleration of cellular electron transfer by the combined magnetic and pulsed laser field at high peak power, but very low average intensity. To monitor the acceleration of electron transfer, the reduction of 2,2,6,6-tetramethyl piperidine-N-oxyl (TEMPO) was followed using the EPR technique. It was shown that the electromagnetic field alone, or the magnetic field alone, produced no reduction of the TEMPO EPR signal. Only a combination between a laser of very low average intensity, but high peak power and a low-intensity magnetic field, reduced the TEMPO signal. The experiment was performed in a medium containing 10^7 *Escherichia coli* (*E. coli*) bacteria per cc. It was verified that at high average intensity the obtained reduction of the TEMPO by electromagnetic radiation was unaffected by the addition of a magnetic field. A possible mechanism underlying the photo-magnetic synergy is proposed.

Introduction:

One of the basic mechanisms of photo-bio-modulation is the acceleration of electron transfer by electromagnetic radiation in the visible and near infra-red region of the spectrum ^{1,2}. In the presence of an electron acceptor such as molecular oxygen (O₂), electron transfer reduces O₂ to the super-oxide anion O₂⁻, which subsequently dismutates into hydrogen peroxide (H₂O₂) ³. Both O₂⁻ and H₂O₂ are highly reactive oxygen species (ROS), which may lead to oxidative stress. At small amounts, ROS have been shown to stimulate signal transduction processes for transcription factor activation, gene expression, muscle contraction, and cell growth ^{4,5}. The regulatory function of ROS was suggested to be connected to the increased activity of GTP-binding proteins like GTPase Rac-1 ⁶, which play a pivotal role in multiple signal-transduction pathways ⁷. The acceleration of electron transfer also increases adenosine triphosphate (ATP) production by the respiratory chain in the mitochondria *via* the reduction of

O₂ to water ^{1,2}. ATP is the fuel that drives protein production, and hence cell proliferation, and regulates ion transport *via* the cell membranes. As a result of electron transfer, the redox state of the cell is displaced to a more oxidized redox state.

We have shown that molecular oxygen can be replaced by 2,2,6,6-tetramethyl piperidine-N-oxyl (TEMPO) ⁸. TEMPO is a stable-free radical, which can readily accept an electron or react with an unstable free radical, thereby losing its free radical, paramagnetic character. This leads to a very sensitive method for measuring electron transfer and free radical production by the observation of the decay of the EPR (electron paramagnetic resonance) signal of TEMPO. A detailed description of the advantages of the nitroxide TEMPO over the more popular EPR spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) was given in our previous publication ⁸. There we have obtained a much better resolved EPR spectrum for TEMPO spin adducts than for the more popular DMPO spin adducts, although the concentration of DMPO was three orders of magnitude higher than that of TEMPO! What gives this surprising advantage to TEMPO? In ordinary spin traps, all the occupied orbitals are occu-

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pied by electron pairs. Therefore, all these orbitals repel additional electrons, such as the unpaired electron of a free radical or a free electron, as a result of Pauli's exclusion principle (see e.g. M. Ben-Nun, T.J. Martinez 1998)⁹). The Pauli repulsion slows down the spin adduct formation reaction by increasing its activation energy. Thus, a considerable amount of free radicals or free electrons will be lost *via* other reactions. TEMPO being itself a free radical has an orbital occupied by an unpaired electron. This orbital does not exert the Pauli repulsion and thus, *via* this orbital, TEMPO reacts rapidly with a free radical or an electron.

In our previous experiment⁸), we have shown that CW red-light illumination of cell samples during 5 minutes with an energy dose of 150 J/cm² and an intensity of 500 mW/cm² produced a reduction of about 20% in the TEMPO EPR signal. It is worth noting that the intensity of the red light used in this experiment was about four times the maximum solar intensity near the equator. In the next section, we shall see how the synergy of a magnetic field of about 35 mT with a pulsed infrared laser with a very low average intensity (0.4-1.4 mW/cm²), but high peak power (25W), together with red and infrared light emitting diodes (LEDs) with a total average intensity of about 50 mW/cm², produces the same TEMPO EPR signal reduction as in the previous experiment, although the intensity and the energy dose were about ten times lower.

Materials and Methods:

We examined three "Handy Cure" devices of "Medical

Quant": (a) pulsed red LEDs (50Hz, 10mW/cm² 635nm) + pulsed infrared LEDs (50Hz, 40mW/cm² 875nm) + short (100ns) infrared laser pulses (50 average intensity: 0.4-1.4 mW/cm², peak power 2' 905nm) + permanent magnet (35 mT); (b) pulsed L (635, 875nm) + infrared laser pulses; (c) pulsed L (635, 875nm) + permanent magnet. We measured modification of the cellular redox state following minutes irradiation with these three devices by monitoring the decay of the characteristic triplet EPR signal of the stable TEMPO radical. Four TEMPO EPR measurements were performed with devices (b) and six measurements with device (a). The decay of the TEMPO EPR signal is ascribed to the reduction of TEMPO to an EPR silent hydroxylamine¹⁰.

The effect of intense CW red light (600-800 nm, 250 mW/cm²) alone or combined with a magnetic field on the TEMPO EPR signal was also examined. TEMPO EPR measurements were carried out by adding 10µL of a 0.1 mM solution of TEMPO to 90µL of a solution containing 10⁷ *E. coli* bacteria per cc. The sample was then drawn into a gas-permeable Teflon capillary (Zeus, NJ) and inserted into a quartz tube. The tube was placed in the cavity of a Bruker 100d X-band spectrometer and measured at the 9.5 GHz frequency. TEMPO spectra were measured with a 50G scan width.

Results:

Unlike the high intensity experiments described in the experiment with device (b), at an average intensity lower by one order of magnitude, yielded no noticeable decay of the TEMPO EPR signal, as expected.

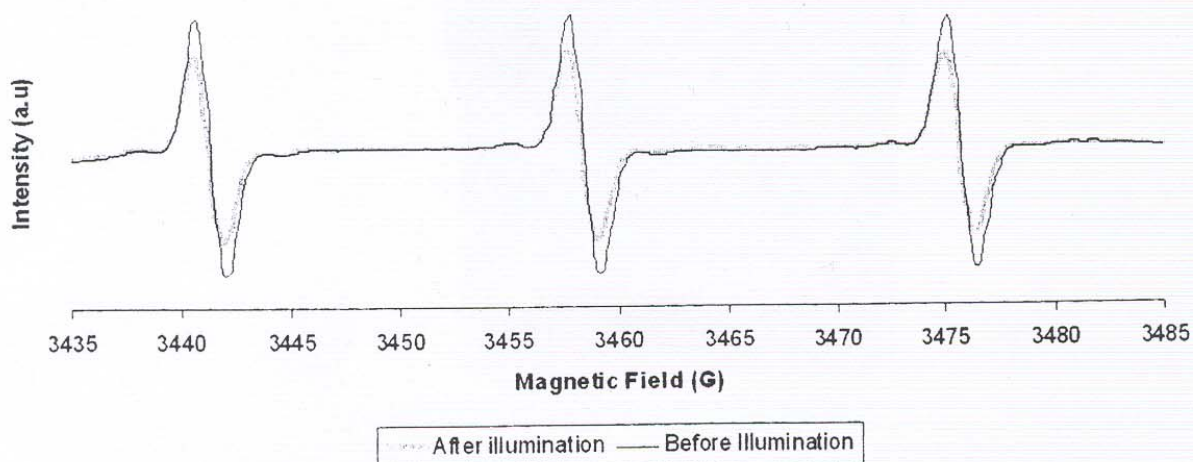


Fig. 1: An EPR spectrum monitoring TEMPO signal reduction following 5 minute illumination with device (a).

addition of the permanent magnet to the LEDs in device (c) did not produce any observable reduction in the TEMPO EPR signal either. Only with device (a), where the permanent magnet and the pulsed infrared laser were simultaneously present, was a $21.8 \pm 2\%$ reduction in the TEMPO EPR signal observed (see Fig. 1).

After acquisition, the spectrum was processed using the Bruker WIN-EPR software version 2.11 for baseline correction. The intensity of the EPR signal was calculated by performing a Double Integration of the peak signals (intensity is expressed in arbitrary units).

At high intensity ($250\text{mW}/\text{cm}^2$) and high fluence ($30\text{J}/\text{cm}^2$), CW red light (600-800nm), a reduction of the TEMPO EPR signal of about 18%, comparable to that with device (a), was obtained. No further reduction was obtained when a magnetic field was inserted. In this case, we have verified that the TEMPO EPR signal reduction is not affected by the magnetic field (Fig. 2)

Discussion:

The bio-photochemical effect of device (a), Fig. 1, is due to the remarkable synergy between a laser of very low average intensity but high peak power, mild LED radiation, and a low-intensity magnetic field. None of the separate components has any effect at all. Adding a magnetic field to an intense CW light had no effect. We speculate that the magnetic field amplifies the effect of the short, intense laser pulse excitation of an endogenous Type I photosensitizer *via* a singlet-triplet transition¹¹⁻¹³). Without the magnet the singlet-triplet transition in the excited state would largely be avoided and the photosensitizer would decay back to the

ground state immediately after the short, intense laser pulse. In the triplet state, the photosensitizer remains excited for a sufficiently long time after the short laser pulse to allow electron transfer on collision with a (paramagnetic) TEMPO molecule. In the case of intense CW radiation, there is sufficient fractional, steady-state occupation of the excited state of the photosensitizer to allow electron transfer to the TEMPO molecule, without the need of replacing one excited (singlet) state by another excited (triplet) state of the photosensitizer by means of a magnet.

The exact light wavelengths are not so important since it should be clear that although the transition probability to the excited state of the photosensitizer may be small at the wavelengths 635nm, 875nm, and 905nm, this is immaterial because of the high intensity of the laser pulse. Thus the effect of the low intensity LEDs is negligible.

The remarkable photo-magnetic synergy enhances electron transfer has important consequences, as discussed in the Introduction. One of these consequences, the activation of the respiratory chain accompanied by enhanced ATP production and cell proliferation, shows that the combination of a magnetic field with a pulsed infrared laser with a very low average intensity, together with red and infrared light-emitting diodes, may improve wound healing by accelerating the replacement of damaged cells. Note that the "Handy Cure" device used in our experiments was approved by the FDA for pain reduction. In the case of auto-immune diseases, pain arises from the aggressive effect of ROS produced by the immune system. Reduction of pain is achieved in this case by the magnet-catalyzed, laser-induced enhancement of electron transfer, which neutralizes the ROS³). We assume here

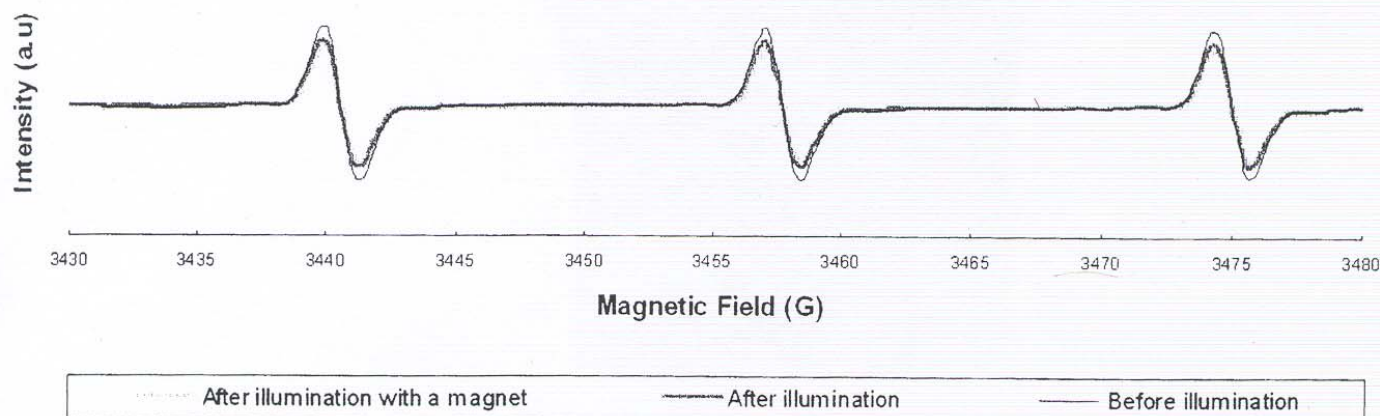


Fig. 2: An EPR spectrum monitoring TEMPO signal reduction following illumination with CW red light. No further reduction was obtained when a magnetic field was inserted.

that universal endogenous photosensitizers such as the flavins and the cytochromes ¹⁴⁾ undergo the same type of photochemical reactions *in vivo* as *in vitro* ¹⁵⁾ It is of course highly desirable to extend the experiments carried out here on bacteria to other cell types and complement these investigations using *in vitro* models by *ex vivo* (tissues of exposed animals) and *in vivo* (exposed animals) ¹⁶⁾.

More generally, pain management may also result from the interruption of anterograde and retrograde transport in the unmyelinated (C-fibers) and the thinly myelinated (A delta fibers) axons by the disruption of microtubules in the cytoskeleton ^{17,18,19)}. The de-polymerization of the microtubules is probably due to oxidative stress ²⁰⁾, another consequence of electron transfer mentioned in the Introduction. The disruption of the microtubules in the axons interrupts the action potential transmitting the pain signal. It interrupts neurogenic inflammation by inhibiting the release of substance P by sensory C-fibers, and of calcitonin gene-related peptide (CGRP) and neurokinin A by both A

delta and C-fibers. These substances have the property of evoking a cascade of events characterized by vasodilatation, plasma protein dilatation and the release of pro-inflammatory mediators such as bradykinin, prostanooids and protons. Neurotransmitter transport is also interrupted, which, in the case of acetylcholine interruption, eliminates muscular spasm ²¹⁾. Note that acetyl choline opens calcium channels, and calcium causes muscle contraction.

In the case of chronic pain, the irradiation by device (a) must be repeated several times per week because of the regeneration (within 24 hours) of the microtubules, until the nervous system is reeducated by down-regulation and depression of the pain syndrome.

Although the energy doses in Refs ¹⁷⁻²¹⁾ were an order of magnitude larger than those needed when using device (a), we have noted in the previous sections that they are in fact equivalent due to the remarkable efficiency achieved by laser-magnet synergy.

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