Primary, secondary and tertiary effects of phototherapy: a review

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ABSTRACT

The classification of the cellular effects of phototherapy into primary, secondary and tertiary types is an aid to understanding variation in the predictability of the events that follow its application. Primary effects are generally restricted to the absorption of photons by cytochromes and catalytic interactions with these and other intracellular molecules. If suprathreshold, they stimulate cell activity, initiating secondary anabolic effects in those cells affected by the photons. These events can also be initiated by non-photonic stimuli. Some of the secondary effects, such as growth factor secretion, can produce effects in cells that did not absorb photons. It is proposed that this group of effects be classified as tertiary. Primary effects are strongly predictable, secondary effects less so, being dependent on cell sensitivity, while tertiary effects are the least predictable, being affected by variation in both the internal and external environment and by intercellular interactions. The investigation of primary and secondary effects of

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phototherapy can be used to determine which irradiation parameters are ineffective *in vitro* and therefore cannot be effective *in vivo*. Since tertiary effects predominate *in vivo* only clinical testing can demonstrate which parameters are most likely to be effective, and with what level of predictability.

It is essential that all relevant exposure conditions be recorded and disseminated if experimental work is to be of clinical value. It is also essential that all relevant information about the target of phototherapy, be it molecule, organelle, cell, healthy volunteer or patient, be recorded and disseminated.

Keywords: Laser therapy – macrophages- fibroblasts – mast cells –keratinocytes- endothelial cells – wound healing – pain relief

INTRODUCTION

Phototherapy is the use of photons to stimulate tissue repair where this is delayed, to improve the quality of reparative tissue, and to relieve pain. It produces cellular effects that, together with extracellular matrix effects, are responsible for its clinical effects. Phototherapy modifies cell activity. Currently red and infrared electromagnetic radiation are the most commonly used [1] although the cellular effects of other parts of the spectrum, e.g. green light, have also been investigated[2]. Generally the effects of phototherapy are classified as **primary** or **secondary**[1]. It is proposed that part of the latter be reclassified as **tertiary** as an aid: (1) to the understanding of the mode of action of phototherapy and (2) to explaining the reduced predictability of efficacy of phototherapy when applied *in vivo* to healthy volunteers or patients, in comparison with *in vitro* treatment of individual types of cells.

COMPARISON OF PRIMARY, SECONDARY AND TERTIARY CELLULAR EFFECTS OF PHOTOTHERAPY

I. Primary effects of phototherapy

Primary cellular effects generally relate to the interaction of photons and the intracellular molecules that absorb them, the cytochromes. Visible light and infrared electromagnetic radiation are absorbed by cytochromes many of which are located in the mitochondria. It has also been postulated that light can act as a catalyst, influencing molecules, organelles and cells without being absorbed [1], although this does not fully explain the attenuation of light as it penetrates tissue. Whether or not the primary effects of light induce a cascade of secondary and tertiary events depends, in part, on the photon density, since the threshold for effectiveness varies in cells according to the internal and external environment.

(1) Speckle formation and polarization

Laser radiation is scattered in tissues because of their heterogeneous nature, producing interference and speckle formation[1], i.e. optical noise. In laser speckles the light is either linearly or partially polarized. Speckle formation occurs regardless of whether the laser device being used emits polarized or non-polarized light. The light radiated within a speckle has a higher intensity than the incident radiation, a result of the constructive interference that occurs when the interfering waves have the same polarization. This, together with attenuation, means that the incident intensity of light applied to tissues differs from the intensity available to cells within tissues, as does polarization.

Cells contain porphyrins, polarization-sensitive molecules. Porphyrins have absorption dipoles; they both absorb and emit polarized light. They are located in mitochondria where they form part of the respiratory chain. They are cytochromes, absorbing blue and red light. When the cytochromes in mitochondria absorb light they interact with it. With the exception of mature erythrocytes, all living cells contain mitochondria and can therefore absorb and be influenced by light[3]. Thus primary effects are inevitable when light of wavelengths that the cytochromes can absorb enters these cells. However, red light also affects mature erythrocytes[4]; therefore there must be other cellular cytochromes external to the mitochondria.

Not all the effects of photon absorption are beneficial. For example, the production of singlet oxygen is stimulated by photon absorption [5]. This introduces lipid hydroperoxides (LHs) into cell membranes, a potentially detrimental effect counteracted by the action of the antioxidant enzyme phospholipid hydroperoxide glutathione peroxidase (PHGPx) which removes LHs from them[6]. Cells are able to survive in an oxygen-rich environment because they contain antioxidant enzymes such as PHGPx and superoxide dismutase which remove free radicals rapidly. Singlet oxygen also has beneficial effects including stimulating the production of energy-rich adenosine triphosphate; it has been proposed that singlet oxygen is produced by the action of light on cellular porphyrins[5]. It is also produced in both the presence and absence of light during cell respiration and phagocytosis. The reported stimulatory effects of photon-induced singlet oxygen production on the healing process[1] may be due to incidences of localized damage which trigger an acute inflammatory response, initiating repair in chronic wounds. It is suggested that the detection of free radical production and/or of fluorescence be used to find out which treatment parameters produce primary effects.

(2) Heating

The excitation of polarization-sensitive cytochromes is only one of the primary effects of

phototherapy. Although most low level phototherapeutic devices produce little heating, it is inevitable that some heating will occur through transduction when the photons are absorbed, and absorption is a major component of phototherapy. A GaAlAs laser with an output in excess of 100 mW can cause the sensation of heating on pigmented skin. However, most phototherapy currently in use and described in the literature involves devices with an output in the 5 – 100 mw range. These devices rarely produce a sensation of heating, but some heat is still generated within the tissues. In tissues, all of which are heterogeneous, local temperature differences will produce local gradients in the distribution of, for example, extracellular water and ions [7]. Small temperature differences can affect cell membrane permeability; a temperature difference of 0.01^{o}C causes a pressure difference of 1.32 atmospheres, which can change the distribution of Na^{+} and K^{+} ions [8]. Changes in cell membrane permeability are fundamental to many of the secondary effects of phototherapy. They are the first of the cascade of secondary and tertiary events that follow the primary effects of phototherapy [9]. Low-energy treatments, involving, for example, light or ultrasound, produce small local increases in temperature which affect the cell membrane, mitochondria and other components of the cell[10]. These increases in temperature can produce secondary events in these cells.

(3) Multi-photon effects

Some molecules including the neurotransmitter serotonin are photochemically transformed when they absorb four photons into a product that produces visible light by two-photon emission[11]. Serotonin is photosensitive and a light-emitter. In its presence localized peaks of high intensity may be produced in cells and tissues by interference. At these peaks more multi-photon effects are likely to occur. Even if monochromatic light is used to irradiate tissues, the production of light of other wavelengths by multi-photon effects and fluorescence should be considered when interpreting the results obtained following such irradiation.

II. Secondary effects of phototherapy

These are defined here as those effects that occur within cells in response to the primary effects within these same cells. The secondary effects are not unique to phototherapy and, because their occurrence depends on the sensitivity of the cells, are less predictable than primary effects, which depend solely upon the presence of specific cytochromes in the cells. They are also more time-consuming and expensive to detect.

The secondary effects of phototherapy occur in response to photoreception. Provided that sufficient photons are absorbed, i.e. that the threshold for the cell is exceeded, photoreception is followed signal transduction and amplification. Increase in ATP and changes in cell membrane permeability to Na^+, K^+ and Ca^{2+} occur in all responsive cells. What follows depends on the cell type and their sensitivity to environmental change. For example, macrophages secrete growth factors[12], fibroblasts proliferate[13] as do endothelial cells [14] and keratinocytes[15]. Other secondary effects include increase in ATP-ase and activation of cAMP and enzymes[1]. Nitric acid formation, a secondary effect of infrared radiation, induces a host of clinically significant secondary effects including the normalization of membrane potential and stimulation of T-cell activity.

The secondary effects of phototherapy should be studied using single cultures of cells grown *in vitro.* Different types of cells must not be mixed, thus preventing tertiary effects. If the cells are grown in an adverse environment they are more sensitive to environmental changes designed to stimulate them than cells maintained under near optimal conditions[15]. The main advantages of studying cells *in vitro* are that mechanisms and treatment parameters can be investigated in a controlled, double-blind fashion on large populations in a reasonably cost-effective and timely fashion. The wavelengths and other parameters to which the cells respond can be determined and optimized.

III. Tertiary effects of phototherapy

Phototherapy produces primary effects in individual cells when it is either absorbed by the cytochromes they contain or acts as a catalyst within these cells. The primary effects lead to secondary effects in these same cells. Other effects, here termed tertiary effects, are induced in cells at a distance from the cells in which the secondary events occurred. Phototherapy induces primary effects in molecules when they absorb or interact with photons. The primary effects induce secondary effects in the organelles, and hence in the metabolic activity, of the cells containing these molecules. Substances such as growth factors, whose secretion can be stimulated by photons, are transported either by diffusion or via the circulation to distant locations where they affect target cells such as fibroblasts and endothelial cells, stimulating their proliferation. This tertiary effect is also known as a systemic effect. It helps to explain why the treatment of one lesion can stimulate the healing of both the directly treated lesion and other lesions that the patient may have. The latter are not affected as much as the lesion to which phototherapy was applied, since this benefits from both local and systemic stimulation. It is clinically important to appreciate that photons need not interact directly with cells in order to affect them. Tertiary effects also occur and it is these which are of predominant importance in the stimulation of tissue repair and the relief of pain by phototherapy. Because of the multiplicity of variable factors in the internal and external environment of a patient, tertiary effects are less predictable than either the primary or secondary effects induced by photons in individual cells *in vitro.*

Many tertiary effects, such as those involving the peripheral and central nervous systems, can only be studied in healthy volunteers and patients *in* vivo. Some, however, can be studied initially under controlled conditions *in vitro.* For example, growth factors secreted into tissue culture medium by macrophages following exposure to phototherapy *in vitro*, can be applied via that medium to fibroblasts in the absence of the treated macrophages. An increase occurs in fibroblast proliferation, induced by the growth

factors secreted by the irradiated macrophages[12]. Although the fibroblasts were not irradiated, their activity is stimulated because of changes induced by photons in the macrophages that had been irradiated. In this experiment it was noted that red light and some wavelengths of infrared stimulated fibroblast proliferation indirectly. When calcium uptake by the macrophages was monitored, following their direct exposure to phototherapy, it was found to be increased by the same wavelengths of light that increased fibroblast proliferation indirectly[16]; permeability of the cell membranes had been increased.

Other tertiary effects of phototherapy produced by the irradiation of blood vessels though the skin have been observed in patients and healthy volunteers. Modification of circulating blood by UV and visible light has been claimed to play a key role in their systemic therapeutic effects [17]. Other *in vivo* experiments have demonstrated that the cells of injured tissue are more sensitive to exposure to light than are the cells of intact tissue. For example, low intensity levels that induce mast cell degranulation in the mast cells of injured skin do not do so in the mast cells of intact skin[18].

Although tertiary effects are the most expensive and time-consuming to investigate, they are also the most clinically significant. Experiments on cell cultures of single types of cells *in vitro* cannot provide full information about how healthy volunteers and patients will respond to phototherapy, because there are many more variables *in vivo* than in the better controlled conditions pertaining *in vitro.* The fundamental reasons for investigating the primary and secondary effects of phototherapy are (a) to determine the mechanisms by which it stimulates tissue repair and relieves pain in patients and (b) to determine which treatment parameters are ineffective *in vitro* and therefore cannot produce anything other than a placebo effect *in vivo.*

The primary, secondary and tertiary effects of phototherapy in patients summate in a manner that can stimulate tissue repair where this is delayed and relieve pain. Because of

the complex and individual nature of each patient, there is variation in the effectiveness of treatment which, ideally, should be modified to produce the optimum effect. To do this, the response of each patient should be monitored regularly. Tissue repair can be monitored non-invasively by high frequency (e.g. 20 MHz) diagnostic ultrasound, which provides 2-dimensional B-scans of skin and subcutaneous soft tissue at a resolution of 65 μm to a depth of approximately 2 cm. Edema, tissue damage and tissue repair can be monitored both qualitatively and quantitatively [9]. The same region can be scanned repeatedly and the response to treatment recorded and acted upon. Pain relief can be assessed by monitoring the patient's need for analgesics; here too the response to treatment should be recorded and acted on.

CONCLUSION

Phototherapy produces primary, secondary and tertiary effects which can collectively enhance tissue repair and relieve pain. Primary effects, due to photoreception, the direct interaction of photons with cytochromes, are very predictable and unique to phototherapy. Photoreception is generally followed by transduction, amplification and photoresponse, the last of which can be classified as either secondary or tertiary. Secondary effects occur in the same cell in which photons produced the primary effects; they are induced by these primary effects. Secondary effects include cell proliferation, protein synthesis, degranulation, growth factor secretion, myofibroblast contraction and neurotransmitter modification, depending on the cell type and its sensitivity. Secondary effects can be initiated by other stimuli as well as light. They are less predictable than primary effects, the sensitivity of the cells being modified by the internal and external environment. Tertiary effects are the indirect responses of distant cells to changes in other cells that have interacted directly with photons. They are the least predictable because they are dependent on both variable environmental factors and intercellular interactions. They are, however, the most clinically significant. Tertiary effects include all the systemic effects of phototherapy. Primary, secondary and tertiary events summate to

produce phototherapeutic activity. Its effectiveness should be monitored non-invasively as the course of treatment progresses and the treatment parameters modified if necessary.

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