Effect of an oxygen pressure injection (OPI) device on the oxygen saturation of patients during dermatological methyl aminolevulinate photodynamic therapy

E. Blake\textsuperscript{a,\ast}, J. Allen\textsuperscript{a}, C. Thorn\textsuperscript{b}, A. Shore\textsuperscript{b} and A. Curnow\textsuperscript{a}

\textsuperscript{a}Clinical Photobiology, European Centre for Environment and Human Health, Peninsula College of Medicine and Dentistry, University of Exeter, Knowledge Spa, Royal Cornwall Hospital, Truro, Cornwall, TR1 3HD, UK.
\textsuperscript{b}Wonford Building, Peninsula College of Medicine and Dentistry, Barrack Road, Royal Devon and Exeter NHS Foundation Trust, Wonford, Exeter, EX2 5DW.

\ast Corresponding author: emma.blake@pms.ac.uk

Telephone: 00 44 (0)1872 256432
Fax: 00 44 (0)1872 256497

James.allen@pms.ac.uk
Claire.thorn@pms.ac.uk
Angela.shore@pms.ac.uk
Alison.curnow@pms.ac.uk

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Abstract

Methyl aminolevulinate photodynamic therapy (MAL-PDT) (a topical treatment used for a number of pre-cancerous skin conditions) utilizes the combined interaction of a photosensitizer (protoporphyrin IX (PpIX)), light of the appropriate wavelength and molecular oxygen to produce singlet oxygen and other reactive oxygen species which induce cell death. During treatment localized oxygen depletion occurs and is thought to contribute to decreased efficacy. The aim of this study was to investigate whether an oxygen pressure injection (OPI) device had an effect on localized oxygen saturation levels and/or PpIX fluorescence of skin lesions during MAL-PDT. This study employed an OPI device to apply oxygen under pressure to the skin lesions of patients undergoing standard MAL-PDT. Optical reflectance spectrometry and fluorescence imaging were used to non-invasively monitor the localized oxygen saturation and PpIX fluorescence of the treatment area respectively. No significant changes in oxygen saturation were observed when these data were combined for the group with OPI and compared to the group that received standard MAL-PDT without OPI. Additionally no significant difference in PpIX photobleaching or clinical outcome at three months between the groups of patients was observed although the group that received standard MAL-PDT demonstrated a significant increase (p<0.05) in PpIX fluorescence initially and both groups produced a significant decrease (p<0.05) after light irradiation. In conclusion, with this sample size this OPI device was not found to be an effective method with which to improve tissue oxygenation during MAL-PDT. Further investigation is therefore required to find a more effective method of MAL-PDT enhancement.
Introduction

Methyl aminolevulinate (MAL)-induced photodynamic therapy (PDT) is a licensed treatment in the UK which may be offered to patients with actinic keratosis (AK), superficial basal cell carcinoma (sBCC) or Bowen’s disease (BD) [1]. The therapy utilizes the combined interaction of three components; a photosensitizer, light of a specific wavelength and molecular oxygen [2]. MAL-PDT treatment involves debulking of the lesion, application of Metvix® (160 mg/g MAL, Galderma, UK), a 3 hour wait, followed by irradiation with visible red light (635 nm, 37 J/cm²). During the 3 hour time interval, MAL, an esterified derivative of aminolaevulinic acid (ALA), enters the haem biosynthesis pathway and is converted into protoporphyrin IX (PpIX), resulting in temporary accumulation of PpIX (the precursor of haem) within diseased cells [3]. PpIX, once activated by light, can undergo photochemical reactions involving molecular oxygen, producing singlet oxygen and other reactive oxygen species (ROS) which induce apoptotic and/or necrotic cell death.

Complete clearance of the lesion is the ultimate goal and although MAL-PDT is effective and produces excellent cosmesis, there is potential for further enhancement [4]. One limiting parameter of the treatment can be explained by the oxygen status of the target tissue, which is considered very heterogeneous. This is thought to be due to differences in inter-capillary distances [5] and to the fact that most tumors present with areas of hypoxia [6], which are thought to be resistant to the therapy. During the photochemical reactions required for successful PDT oxygen is consumed [7] and as the therapy proceeds oxygen levels are also thought to deplete as a result of damage to the microvasculature [8]. The degree to which this occurs depends on the photosensitizer and dose parameters employed. In vitro the efficiency of HpD PDT at varying oxygen concentrations has been measured [9]. The result showed at 1%
oxygen, efficiency was reduced by 50% compared to photoinactivation observed at 20% oxygen [9].

The measurement of oxygen saturation in patients remains particularly difficult. Optical spectroscopy uses non-invasive optical fibers to measure oxygen saturation and was the technique employed in this study. In a previous study of 60 patients undergoing MAL-PDT, and the employment of an optical reflectance spectrometer (ORS), a significant decrease in oxygen saturation was recorded after the first minute of irradiation during MAL-PDT [10]. The evidence to date suggests if oxygen levels can be maintained and/or increased prior to, during or after PDT [5] then the efficacy of PDT might be improved. A number of direct and indirect methods have been investigated to improve tissue oxygenation and potentially allow reoxygenation of the tumor including; hyperthermia [11], normobaric oxygen (NBO), oxygen multistep therapy, hyperbaric oxygen (HBO) [12], perfluorocarbons [13], light dose fractionation [14, 15] and lowering of the light fluence rate [16].

Due to the oxygen dependent nature of wound healing, research into topical oxygen therapy within this field is increasing. Administration of a stream of 100% oxygen bubbles has been shown to improve epithelial wound healing [17] and promote wound angiogenesis [18]. Based on PDT being an oxygen dependent process this pilot clinical study aimed to investigate whether an oxygen pressure injection (OPI) device, applied at the time of light irradiation as an addition to standard MAL-PDT, could act as a method of topical oxygenation and therefore have a positive effect on localized tissue oxygen saturation during PDT. The OPI device employs 100% oxygen, at slightly greater than atmospheric pressure and was designed to increase the penetration of active ingredients in topical creams through the skin, under the action of the pulsed pressure of concentrated oxygen. When previously utilized, during the application of
Metvix cream to nBCC lesions, an increase in the depth of PpIX accumulation was reported [19]. During the treatment here at the time of light delivery, changes in the oxygen saturation and PpIX fluorescence were monitored in the localized treatment area. In addition, clinical outcome at three months was recorded.

Methods

Patients and treatment parameters

In total, twenty patients were included in this study and all were treated with standard MAL-PDT (160 mg/g Metvix, Galderma, UK plus 37 J/cm² red light (Aktilite, Galderma, UK) three hours later), as per the current 2006 National Institute of Clinical Excellence guidelines [20], at the Dermatology Department, Royal Cornwall Hospital Trust (Truro, UK). All patients were provided with verbal and written information about this ethically approved (National Research Ethics Service, UK) non-invasive study prior to providing written consent. Patients who participated had a range of licensed dermatological indications (9 sBCC, 6 BD and 5 AK) and upon entering the study were randomized, to eradicate bias, into one of two groups (with or without application of the OPI device prior to light irradiation).

Measuring oxygen saturation

All patients had the mean blood oxygen saturation of their treatment area measured at several different stages during the PDT treatment procedure; prior to MAL application, following a 3 hour photosensitizing period (after which time the remaining MAL cream was
removed), after 30 squirts of the OPI device to the centre of the lesion (10 patients) or after not having the OPI device applied (10 patients) and finally at the end of the light delivery. The amount of time following application of the OPI device and following light treatment before taking the oxygen saturation measurements was no longer than 2 minutes, this allowed for the removal of the OPI device or Aktilite® machine and the positioning of the ORS. The OPI machine works by concentrating oxygen from the surrounding air and purifying it up to a maximum of 97%. Oxygen was applied to the lesion, released in short squirts at 2.2 bar through a hose system and a pressure-reducing valve which exits through a domed shaped nozzle, designed to allow maximum contact with the skin and minimal oxygen escape.

The oxygen saturation was measured and calculated using an optical reflectance spectrometer (ORS) (School of Physics, University of Exeter, UK) which had been previously validated [21]. The ORS was operated by placing a non-invasive optical fiber (250 µm) on the surface of the lesion. This delivered white light to the skin and returned backscattered light to a spectroscopic detector (SPEX 270M grating spectrometer, recorded by a MK II charge coupled device (CCD) camera (Wright Technologies, UK) equipped with an EEV CCD 30-11-5-219 CCD camera (1024x256 pixel format)). Spectra ranging from 470-1120 nm are acquired within 0.05 seconds and processed with custom written Windows-based software. The computer software calculated the mean values of oxyhemoglobin [HbO₂] and hemoglobin [Hb] across all vessels of the microcirculation of the skin integrated using a four component multi-linear regression algorithm on part of the spectral range (500-600 nm), where a linear relationship between scattering of blood and wavelength is assumed, and thus the oxygen saturation can be derived [21].
The 250 µm probe sampled from the capillary loops and superficial plexus, reaching depths of ~200 µm which was theoretically far enough to investigate the full thickness of licensed PDT lesions. To minimize the effect of other absorbers in the tissue, a reference spectrum was initially taken by applying pressure to the probe sufficiently to occlude the superficial blood vessels. Subtracting this reference spectrum from the subsequent recorded blood-filled spectra produced a spectrum that could be attributed to absorption and scattering by blood alone and not the surrounding interstitium [21, 22]. Due to the heterogeneity of the microvasculature and photosensitizer localization the oxygen saturation measurement was taken at the same point within the lesion on each occasion. In this study, the centre of the lesion was selected for this as it was relatively easy to replicate.

**Measuring PpIX fluorescence**

All patients had their lesions imaged with a non-invasive fluorescence imaging device (Dyaderm, Biocam, Germany) at three different stages during their treatment; prior to MAL application, following the standard application period of 3 hours and at the end of the light delivery. This device delivered white and blue light to the skin (370-440 nm) from a custom filtered Xenon flash light source combined with a 12-bit Sony CCD camera which captured the returning light using a Schott GG 455 filter to exclude the excitation light (370-440 nm). The PpIX excited by blue light emitted fluorescence in the red spectrum and the red pixels of the CCD camera (spectral sensitivity of which at 630 nm is between 85% and 90%) were therefore used to generate a fluorescence image, whilst the white light delivered enabled the collection of a simultaneous normal colored image [23]. Greyscale images were analysed with NIH ImageJ software (http://rsb.info.nih.gov/ij/) and the fluorescence at the centre of each lesion was
recorded. Measuring fluorescence values enabled PpIX accumulation (following MAL application) and PpIX dissipation (following light treatment) to be recorded for each lesion utilizing the previously derived standard operating procedure [23].

**Follow up**

Three months following their final PDT treatment, patients attended a follow-up appointment in which clinical outcome of their lesion was assessed by a Consultant Dermatologist. This is the standard practice of our clinical service. Complete clearance was recorded if no clinical evidence of the tumor remained, partial clearance was recorded if the lesion size had decreased but some disease was still observed and no clearance was reported if the lesion was unaffected following treatment.

**Data analysis**

Twenty patients were recruited based on a POWER calculation which suggested that to detect a change of 10% in oxygen saturation at 90% power ($p<0.05$) ten patients were required in each treatment arm. Statistical significance between data sets was analysed using a one-way ANOVA ($p<0.05$).

**Results**

**Oxygen saturation measurements during treatment**

*For patients without employment of OPI*
The lesions of the ten patients treated with standard MAL-PDT (1A-10A) showed considerable interpatient variation (Fig.1a). This data combined produced a mean oxygen saturation value at each stage of measurement with the ORS (Fig.2). This suggested that the oxygen saturation, monitored pre treatment (prior to MAL), may have been slightly reduced compared to the second measurement taken following the 3 hour MAL application, although this did not reach significance (ANOVA, \(p>0.05\)). Compared to the pre treatment measurement there was no significant change in oxygen saturation prior to light irradiation and in addition, when monitored following light treatment (ANOVA, \(p>0.05\)).

**For patients with employment of OPI**

Examination of the lesions of the ten patients treated with MAL-PDT with addition of the OPI device (1B-10B) again revealed considerable interpatient variability (Fig.1b) and when this data was combined (Fig.2), compared to the pre treatment measurement there was no significant change in oxygen saturation at the three other measurement points; following MAL application, prior to light irradiation or at the end of the light treatment (ANOVA, \(p>0.05\)). Importantly when these data were combined no significant increase in oxygen saturation was observed between the readings taken immediately prior to and after OPI application. However, although not reaching significance, oxygen saturation did actually increase for 5 of the 10 patients individually (1B 5.3%, 2B 1.5%, 3B 6.1%, 7B 8.6% & 8B 2.3%) following the application of the OPI device compared to only 3 of the 10 patients (4A 1%, 9A 1.3% and 10A 3%) treated without the OPI intervention (Fig. 1). Additionally, no significant difference was observed between the pre treatment oxygen saturation values of either group.
**PpIX fluorescence measurements during treatment**

For both groups of patients the individual levels of PpIX fluorescence measured demonstrated interpatient variation throughout the treatment (Fig.3). However, the mean levels of PpIX fluorescence measured for each group indicated a similar trend was occurring. As expected increased PpIX fluorescence was observed 3 hours following MAL application and a reduction in PpIX fluorescence (photobleaching) was observed after irradiation (Fig.4). For the group that received standard MAL-PDT treatment a significant increase in PpIX fluorescence following MAL application ($p<0.05$) was observed. In addition for both treatment groups the level of photobleaching that occurred during light irradiation was significant ($p<0.05$) (Fig.4). At each measurement stage no significant difference in PpIX was detected between either group (ANOVA, $p>0.05$) irrespective of whether the OPI device was employed or not.

**Clinical outcome**

Following follow-up at 3 months, no significant difference in clinical outcome was observed between the two groups of patients. For those treated with standard MAL-PDT without addition of the OPI device, complete clearance was recorded for 7/10 patients, partial clearance was recorded for 1/10 and 2/10 patients did not attend their follow-up appointments. For those treated with MAL-PDT with addition of the OPI device at the time of light application, complete clearance was recorded for 9/10 patients and partial clearance was recorded for 1/10 patient. Audit of our routine clinical service indicates that MAL-PDT complete clearance rates for licensed dermatological lesions were approximately 61% achieved overall in 2008 and 80% in 2009 [10].
Discussion

An intervention to potentially alter oxygen saturation levels during MAL-PDT light delivery was investigated in this clinical pilot study with the employment of an OPI device. Any changes in oxygen saturation during treatment were recorded using ORS. However when the individual data sets were combined, no significant changes in oxygen saturation were observed following application of the OPI device (after Metvix® application and prior to light irradiation). In addition, when the OPI device was employed, oxygen saturation levels did not change significantly compared to patients given standard MAL-PDT without the device. A possible explanation for this could be the amount of time between applying the OPI device and taking the oxygen saturation measurement. This time period was no longer than 2 minutes which allowed for the positioning of the ORS following OPI application. This time period may have been too long to detect a very short lived effect produced by the OPI device, or conversely a longer time period may have been required to allow oxygen diffusion through the skin and thus detect changes in the mean blood oxygen saturation of the localized treatment area. In addition, a greater number of squirts with the OPI device may be necessary to produce a significant clinical effect.

This study was powered to detect a 10% change in oxygen saturation and increasing the sample size could have increased the chance of observing a significant change in oxygen saturation however, based on the data recorded here this appears to be unlikely. Our previous studies have also observed significant interpatient variations as once again observed here. This is thought to be the result of numerous external factors (especially the overall health of the patient), which may potentially alter the capacity for photochemical reactions and therefore the
relationship between PpIX photobleaching and oxygen saturation [24]. For example, the age, body temperature and general well being of a patient can affect their tissue oxygenation status. With reduced oxygenation one could predict that fewer interactions between PpIX and singlet oxygen occur, thus reducing the level of PpIX photobleaching.

To measure changes in PpIX fluorescence during treatment a non-invasive fluorescence imaging system was employed. Our previous studies using fluorescence imaging have indicated that AK, BD and sBCC undergo similar fluorescence changes during MAL-PDT [23]. A positive correlation between PpIX photobleaching and cellular damage, and hence clinical efficacy of the treatment has also been previously demonstrated [25-29]. Therefore, we hypothesized here that if oxygen saturation could be manipulated positively (increased) with the employment of the OPI device, a difference in the level of PpIX photobleaching (greater amount) may have been detected which would ultimately affect clinical outcome (improvement).

Although a significant amount of PpIX photobleaching was observed following the light treatment for both treatment groups ($p<0.05$), as expected, no significant differences in the levels of PpIX accumulation and subsequent photobleaching were observed between the two treatment groups ($p>0.05$). The fact that no significant difference in PpIX accumulation and subsequent photobleaching was observed between the two treatment groups, suggests, that the OPI device was unsuccessful in manipulating the oxygen saturation of the localized treatment area in a significant/positive manner in the small clinical study conducted here. This is based on the fact that if more oxygen was available (following the employment of OPI), a potentially greater amount of PpIX photobleaching would have occurred on irradiation and a significant difference in the level of PpIX reduction following light treatment would have been observed between the two groups (which it was not). In addition, no difference in clinical outcome was
noted suggesting that the intervention of the OPI device at least with the sample size and protocol employed here neither improved nor worsened the efficacy of the MAL-PDT treatment received by this small number of patients.

The literature contains a number of PDT oxygen monitoring studies, which have utilized a number of different techniques. Direct measurements of partial oxygen pressure (pO$_2$) have been monitored with for example, oxygen microelectrodes. This technique has been used in an *in vivo* animal study [14] to monitor the effect of continuous and fractionated ALA-PDT light regimes on the level of oxygen in the colon of normal Wistar rats. Curnow *et al.* (2000) observed a rapid decline in pO$_2$ close to the irradiation fiber as soon as the light dose commenced and with the fractionated regime a partial recovery in pO$_2$ during the dark interval [14]. However the direct measurement of pO$_2$ involves invasive techniques not readily employed in the clinical environment, whereas unless used interstitially, optical spectroscopy techniques (like the one employed in this study) utilize non-invasive optical fibers and data can be acquired quickly and simply [5]. In a recent study which used an ORS system to monitor the oxygen saturation of 60 patients during standard MAL-PDT, a significant decrease was observed following the first minute of irradiation [24]. This decrease was attributed to the consumption of oxygen during the photochemical reactions. A noteworthy limitation of the ORS however, is the fact that the oxygen saturation at a specific point cannot be determined; rather the mean blood oxygen saturation of the localized area is monitored and the irradiation has to be stopped temporarily to enable the measurement to be obtained essentially fractionating the light (something we decided to avoid during this investigation). The requirement for a non-invasive method of measuring tissue oxygen saturation during PDT precisely and in real-time therefore, still remains. A recent method under investigation is photoacoustics [30]. This non-invasive
technique utilizes high-frequency ultrasound and exploits the differential light absorption spectra of hemoglobin. *In vivo* animal experimentation has demonstrated this method, photoacoustics, can be used to visualize the internal mammalian anatomy and measure oxygen saturation allowing the user to determine the location of the signal within a tissue [26]. Nevertheless, although the measurements of blood oxygen saturation below the precancerous lesion can offer an indication of changes within the cellular oxygenation status, more advantageous would be a non-invasive monitoring technique which directly measures the oxygen concentration of the epidermal cells; however such technology does not currently exist [10].

Although our pilot study showed no significant changes in oxygen saturation in either the ten patients monitored during standard MAL-PDT or the ten receiving the OPI intervention, the conclusion made from previous non-interventional oxygen monitoring studies (conducted with larger patient numbers), is that substantial changes in oxygenation can occur during and after PDT [31] and there is little debate surrounding the importance of oxygen during PDT. Oxygen can therefore be consumed in the photochemical reactions required for successful PDT more rapidly than it can be replenished [32]. In one study employing hematoporphyrin-PDT, efficiency was shown to decrease with decreasing oxygen [9]. Therefore, an intervention that could increase and/or maintain oxygen levels during MAL-PDT, to potentially enhance efficacy and ultimately improve clinical outcomes would still be desirable. Both direct and indirect methods of increasing lesion oxygenation have been investigated. For example, one indirect method is the use of light fractionation in which delivery of the red light is interrupted at a particular point for a short period of time [14]. It has been suggested that in the absence of light blood flow is restored [7] and using a lower fluence rate oxygen consumption is slowed [33, 34] both allowing for tissue re-oxygenation [33, 35]. However, it is widely accepted that the tumor
environment is more hypoxic than the surrounding normal tissue, suggesting pre-existing hypoxic cells would be unaffected by reducing fluence rate or light fractionation [36] and thus more direct approaches of oxygenation have also been investigated. For example, mice transplanted with a proven hypoxic tumor model before treatment with Photofrin-PDT were subjected to breathing in room air (RA, control), carbogen or 100% normobaric oxygen (NBO) through a facial mask or subjected to hyperbaric oxygen (HBO) in a custom-built chamber. Results from this study showed that hyperoxygenation could improve tumor response by enhancing direct and secondary cell death mechanisms. Carbogen and NBO breathing were as effective as HBO and all three were more effective in inducing cell death compared to RA. The authors hypothesised that hyperoxygenation oxygenated pre-existing hypoxic cells and compensated for oxygen depletion during PDT [36]. However translating these methods of increasing tissue oxygenation to the clinical environment is not a trivial matter.

The theory of supplying the epidermis with additional oxygen externally is supported by work carried out by Stucker et al. (2002) who demonstrated with an oxygen fluxoptode that under normal conditions atmospheric oxygen can supply the upper skin layers to a depth of 0.25–0.40 mm [37]. It was therefore hypothesized that using the non-invasive, easy to employ and relatively inexpensive OPI device to apply topical hyperbaric oxygen to lesions undergoing MAL-PDT, could affect oxygen saturation levels with a view to combating oxygen depletion known to occur during treatment (but not observed here). One must consider the fundamental challenge of transforming topical gaseous oxygen to its dissolved form enabling the oxygen to be biologically available to cells. For example, it has been demonstrated recently that devices which deliver topical dissolved oxygen are more effective at delivering oxygen through viable skin samples than topical gaseous oxygen devices [38].
Although our study observed no such positive changes in oxygen saturation (or therefore as a result increased PpIX photobleaching or improved clinical outcome) when the OPI device was employed, it is known from an earlier clinical study [19] employing a similar OPI device to apply Metvix cream, that it can be employed at the time of MAL application to achieve greater PpIX accumulation. Therefore, a role for the OPI device may still exist in PDT clinics, but alternative methods of increasing tissue oxygenation topically and/or systemically should be investigated to potentially further enhance dermatological MAL-PDT, particularly in thicker skin lesions.

References


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Figure Captions

**Fig.1** Oxygen saturation measurements (%) of all lesions for patients given: (a) standard MAL-PDT and (b) MAL-PDT with addition of the OPI device (30 squirts applied to the lesion, represented within the figure by the dashed arrow). Error bars represent one standard deviation.

**Fig.2** Bar chart representing mean oxygen saturation (%) at different points in the PDT treatment process, with and without OPI application. Addition of the OPI device is represented within the figure by the dashed arrow. Error bars represent one standard deviation.

**Fig.3** Normalized (to the initial level of PpIX prior to MAL application) levels of PpIX fluorescence, measured at three different stages throughout the dermatological PDT treatment procedure, for the individual patients given (a) standard MAL-PDT and (b) MAL-PDT with
addition of the OPI device (represented within the figure by the dashed arrow). Error bars represent one standard deviation.

**Fig.4** Bar chart representing mean normalized PpIX fluorescence at different points in the PDT treatment process, with and without OPI application. Addition of the OPI device is represented within the figure by the dashed arrow. Error bars represent one standard deviation. * and + indicate significant changes in fluorescence from the pre treatment (p<0.05) and pre light delivery (p<0.05) levels respectively.
Fig. 5
Fig. 2
Fig. 3

(a) Normalized PpIX fluorescence/\% over different measurement stages:
- Prior to MAL
- After 3 h MAL application
- After light

(b) Similar to (a), but with different samples labeled 1A to 10A.

Fig. 3
Fig. 4

The figure shows a bar chart comparing the normalized PpIX fluorescence with and without OPI application at different stages: prior to MAL, after 3 h MAL application, and after light exposure. The chart indicates a significant increase in fluorescence after OPI application, as denoted by the asterisks.