Monitoring of Photobleaching in Photodynamic Therapy Using Fluorescence Spectroscopy

A. Sharwani[1,2,] F.A. Alharbi[3,4]

[1]Department of Oral and Maxillofacial Surgery, Eastman Dental Institute for Oral Healthcare Sciences, London, UK. [2]Department of Oral-Maxillofacial Surgery, King Fahd Central Hospital, Jazan, Saudi Arabia. [3]Department of Otolaryngology, Head & Neck Surgery, Faculty of Medicine, Jazan University, Saudi Arabia. [4]Department of Otolaryngology, Head & Neck Surgery, King Fahd Central Hospital, Jazan, Saudi Arabia

Abstract

Photodynamic therapy (PDT) utilizing aminolevulinic acid (ALA) as a photosensitiser has been used to ablate premalignant/malignant skin conditions including superficial basal cell carcinoma (BCC) with acceptable cosmetic outcome. Clinicians continue however to face difficulties in determining the exact dose that is sufficient to achieve a complete healing from the condition where the experience of the clinician remains the only determinant factor. This inaccuracy sometimes leads to undertreating these lesions. Here, we have clinically evaluated the use of fluorescence imaging system as monitor of protoporphyrin IX (PpIX) photobleaching in patients with BCC and compares this to the clinical outcome. Four readings were acquired from each patient (n=14) "pre-PDT", "peri-PDT" (333secs), "peri-PDT" (660secs) and post-PDT. It was found that red fluorescence values decreased markedly during PDT, and considered to be significant when compared to pre-PDT (P = 0.0018) and post-PDT (P = 0.0025). The red fluorescence value in pre-PDT was found to be higher in BCC of cheek and scalp (where the response rate, RR = 100%) in comparison to the temple (RR = 75%) and nose (RR = 33%). By comparison, the post-PDT red fluorescence values in cheek and scalp were lower than that of the temple and nose, respectively; this may be a useful indicative of the response rate of tissue to therapy.

Keywords:

photodynamic therapy, fluorescence spectroscopy, basal cell carcinoma, aminolevulinic acid, optical monitoring.

Introduction

Head and neck cancer is characterized by its high recurrence rate which plays a major role in the outcome and survival of patients suffering from the disease. The main reason behind recurrence of the disease is the failure to achieve primary complete cure [1]. PDT has been used for the treatment of both potentially malignant and established cancer [2,3]. PDT is based on the interaction between light and photosensitive agent (i.e., ALA), and in the presence of oxygen molecules, free radicals and transient species are produced, which target and destroy the

Corresponding Author: Dr. F. A. Alharbi, Department of Otolaryngology, Head & Neck Surgery, Faculty of Medicine, Jazan University, 2349-Almarjan-Industrial Zone Rd., Unit No. 1, Jazan 82621- 7413, Saudi Arabia. Email: fahdalharbi3@gmail.com

photosensitized tissue with acceptable outcome. The topical application of PDT-ALA has proved to be an adjuvant to surgery in terms of treating premalignant/malignant conditions that affect the skin (i.e. BCC).

ALA is an endogenous precursor of the biosynthetic pathway of haem which transformed enzymatically by ferrochelatase into fluorescing protoporphyrin IX (PpIX). The administration of exogenous ALA can overload the haem biosynthesis pathway and the normal feedback control mechanism is then bypassed, leading to the transient accumulation of PpIX to levels more than those normally present in the body. PpIX is then photo-oxidized (activated) in situ by the illumination with red light at 635nm[8-4]; this process is called photobleaching which is measured as the decrease in fluorescence over

time, since the concentration of photosensitiser and fluoresce intensity are proportional [9]. Once the photosensitiser has been used up, any further irradiation has no extra effect, thus preventing overdosage [10]. It was also suggested that sensitiser photobleaching and the other PDT parameters, such as light fluence and irradiance and the oxygen availability, are considered as the main determinant of PDT efficacy and any misinterpretation, can lead to poorly treated lesions [19-11]. This, of course, carries unfavorable outcome and the patient may need to undergo further treatment however, development of a real-time quantitative method of photodynamic dosimetry is needed. Thus, monitoring the photobleaching and then comparing it to the outcome could result to a successful treatment and better prognosis.

In this study using fluorescence spectroscopy system (FSS) system, we have monitored the photobleaching of PpIX sensitiser in patients with superficial BCC and compared it to clinical outcome.

Materials and Methods

FSS System

The apparatus (Figure 1) consisted of a high optical power output light delivery system that includes a xenon-arc lamp (Medical Light Technologies Ltd, UK) and a fluorescence interference filter at 425($\pm$17) nm (Corion Corp., MA, USA) that was fitted in front of the system. An optical endoscope was used for both illumination and detection of the tissue fluorescence. The fluorescence image of the tissue was taken by high sensitive single chip charge-coupled device (CCD) color camera (Sensicam, Personal Computer Optics, Germany) integrated with red/green/blue (RGB) mosaic filter and coupled to the endoscope to facilitate examination of all oral tissues. The images were captured by a frame-grabber (0.6sec integration time) fitted with an analogue/digital converter (ADC) and analyzed and displayed by computer loaded with software (Sensicam, Personal Computer Optics, Germany). This allowed fast computation and analyzing times. In addition, the RGB system provided wavelength separation
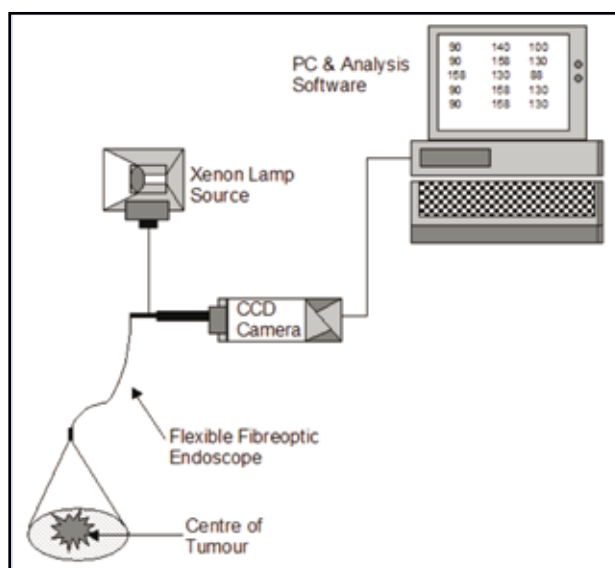
Figure 1: Schematic diagram of the fluorescence ratio imaging apparatus

allowing only red and green detection. The output power of the blue light at the endoscope tip was kept to approximately 2mW/cm; this was found to minimize the photobleaching of the photosensitiser. The tissue accumulated PpIX was excited via an endoscope with a blue light generated by a filtered high optical power output xenon-arc lamp, following excitation, PPIX fluorescence was detected by a sensitive single chip CCD color camera. The image obtained was displayed in black and white and the intensity of whiteness was proportional to the fluorescence intensity.

Patients

Fourteen patients (mean age 66 years, range 39-78 years) with superficial BCC located on the scalp and face took part in this study at the Maxillofacial Unit, University College Hospital (UCH), London. The trial protocol was approved by the joint UCL / UCLH committees of the ethics for human research. An information sheet explaining the aim of our study in simple non-scientific terms was given to each patient who consented prior to examination. Inclusion criteria were patients over 18 years of age diagnosed with superficial BCC.

The Department of Pharmacy/UCH was actively involved in the preparation of the 20% ALA cream; which was applied with a margin of approximately 1cm beyond the skin tumors

and then covered with plastic adhesive dressing. The ALA was left on the skin and the patients were instructed not to expose the creamed area to direct sunlight for 3 h. The cream was then wiped-off, and the tumour demarcated with 5mm margins of macroscopically normal skin. All normal surrounding skin was covered and protected from any light exposure during PDT. The tumour area was illuminated through a fixed perpendicular coupled lens fiber to deliver 100J/cm2 at a fluence rate of 150mW/cm2 and a distance sufficient to cover the whole target area. Four readings were acquired from each patient: "pre-PDT" (5 min before the PDT), "peri-PDT" (333 seconds after starting PDT), "peri-PDT" (660 seconds after starting PDT, marks the end of treatment) and "post-PDT" (15 min after PDT).

Complete response to PDT was defined as the disappearance of the disease (treatment site is macroscopically normal with no evidence of tumour). Partial response was defined as a decrease of at least 50% in the total tumour size relative to pre-treatment size.

Data Processing and Results Analysis

The captured image was intensified and red and green pixels were calculated. Within 0.2sec, the data processing stage was complete; this usually involved digital analyzing of the 12-bit red, green and blue fluorescence images. Dimensionless spatial variable was calculated according to pixel coordination of x and y. The red channel described PpIX fluorescence and red tissue autofluorescence. The green channel recorded the green tissue autofluorescence; while the blue channel showed diffusely back scattered excitation light. Data were expressed as the mean of observation ± standard deviation

(SD). The difference in values was determined using Student's t-test. Results were considered significant when value of $p \leq 0.05$.

Results

PDT Cures Skin Cancer

The response of facial tumors 3-18 months post- PDT was evaluated. The initial obtained data (Table 1) showed that a cosmetic result was achieved in all patients. A complete response was seen in 78.6% (11/14) of the cases; this suggests that no more treatment was required for those patients. Partial response was achieved in the rest of the cases; patients here were required to undergo one or more ALA-PDT to try to achieve a full response and complete healing.

	Cheek	Temple	Nose	Scalp	Mean ±SD	SE	P value
Pre-PDT	160	142	125	154	145.25 ±15.4	7.7	-
333secs	105	91	98	106	100 ±7	3.5	0.0018
660secs	102	91	95	105	98.25 ±6.4	3.2	-
Post-PDT	115	118	124	114	117.75 ±4.5	2.3	0.0025

Table 2: PpIX fluorescence as a function of time

Readings were measured from fluorescence images acquired during and after the irradiation of ALA pr-loaded BCC with red laser. The readings obtained before PDT applications were used as, control

PDT Reduces PpIX Fluorescence

The fluorescence-emission signals of PpIX over time were measured in the red zone of the spectrum after excitation with blue light (Table 2). The pre-PDT red fluorescence from the cheek and scalp was found to be higher than that of the temple and nose in which the response rate to PDT was lower. Post-PDT red fluorescence values were lower in cheek and scalp area when compared to the temple and nose. Significant difference was found when comparing red fluorescence acquired pre-PDT with 333 sec during-PDT (P = 0.0018), and when comparing red fluorescence at 660 sec (end of PDT) with 15 min post-PDT (P = 0.0025).

Anatomical Site	CR (n)	PR (n)	NR (n)	(n)	% of CR
Cheek	4	-	-	4	100%
Temple	3	1	-	4	75%
Nose	1	1	1	3	33%
Scalp	3	-	-	3	100%

Table 1: Clinical responses to PDT

CR= complete response; PR= partial response; NR= no response

Discussion

PDT is a new treatment method for skin cancer. By monitoring the PDT relevant parameters and comparing them to the treatment response, an optimal and reproducible treatment outcome may be achieved. A study made by Ericson et al. included 37 patients undergoing ALA-PDT for actinic keratosis (AK) of the head and neck skin, concluded that both photobleaching rate and primary treatment outcome were dependent on fluence rate. They also suggested that low fluence rate seems preferable when performing PDT on AK using non-coherent light sources[20]. Amelink et al., correlated changes in the local tissue optical properties (absorption and scattering coefficients) during ALA-PDT and changes in PpIX fluorescence by using superficial reflectance spectroscopy which employs a single fiber for the delivery and collection of white light to and from the tissue [12] . The spectra acquired were modulated with blood saturation, relative blood volume fraction, scattering intensity and wavelength dependence of the scattering. Except for blood volume; the study showed a marked correlation between the other parameters and photobleaching of PpIX. This suggests that these parameters can predict PDT-response.

In this study, the red PpIX fluorescence signals were detected after the topical application of PDT. This is in agreement with previous studies which demonstrated that the amount of PpIX synthesised after ALA administration is proportional to its emitted fluorescence[9, 16, 21, 22]. After initiation of PDT, the fluorescence signals were lost over time during PDT which may be due to the fact that most PpIX was photobleached. Also no significant additional photobleaching occurred after a cumulative time of 333 seconds; this may indicate that current PDT time was excessive since slow bleaching may have resulted in higher oxygen levels in tissues during treatment. This is in contrast with the study made by Cubeddu et al., who found an increase in fluorescence when aluminium pthalocyanine (ALS2Pc) sensitiser was used; the increase in fluorescence was suggested as a result of the re-location of sensitiser from vesicles to cytoplasm [23]. Later after PDT, we observed re-fluorescence of lesions. This could be explained partially as PpIX is manufactured by tumour cells and surrounding normal tissue cells and ALA cream was applied to the tumour area with extended margins that was partially shielded during PDT, which in turn results to PpIX manufacturing and its refluorescence.

Next, the relation between PpIX fluorescence/ bleaching and response to PDT was evaluated and a positive relation was observed. The amount of PpIX photobleaching was observed in tumors that showed complete response rate; less bleaching was seen in tumors that showed no response. This in agreement with other studies which have evaluated the PDT-induced damage, the concentration of sensitiser and oxygen availability during exposure to PDT light [24, 11]. In this study, the PDT response was achieved in all tumors, since the same dose of PDT was applied with the same fluence and fluence rate. This is in agreement with previous work [25]. The small percentage with no response or a partial response could be due to the limited depth of ALA penetration. A similar finding was reported in relation to normal rat esophagus [26]. In conclusion, fluorescence spectroscopy has a great potential to monitor 5-ALA induced PpIX fluorescence bleaching during PDT. It can also be used as a valuable tool to predict clinical outcome in particular tumour events. In order to be validated, large data are needed.

Conflict of Interest:

There is no financial relationship with the organization that sponsored the research.

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