

APPENDICES

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K.S. Low, H.K.Chan, A.S. Haji Baba, C.H. Yip, K.W. Chang, Y.L. Lo , P. Jayalakshmi and L.M. Looi. 1995.Simple red light sources for photodynamic therapy of malignant tumours. *SPIE*. 2371: 339-343.

Low, K.S, Chan, H.K.,Haji Baba, A.S. and Sinniah, A. New Approach to photodynamic therapy of malignancies :I. Phamacokinetics of Hematoporphyrin (Hp) and its derivatives (HpD) in mice. Under preparation.

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Photodynamic Therapy in the Practice of Oncology

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Abstract

Photodynamic therapy (PDT) using Hematoporphyrin Derivative (HpD) as the photosensitizer is currently being investigated as an alternative to other modalities such as radiotherapy and chemotherapy for cancer treatment. Three patients were treated with this modality and the results indicate significant response in palliative control of the lesions.

Key words: Photodynamic therapy, Hematoporphyrin Derivatives, mammary carcinoma, squamous cell carcinoma, basal cell carcinoma.

Résumé

La thérapie photodynamique qui utilise les dérivés hématoportrophyriniques comme agent photosensibilisant est actuellement expérimentée comme alternative aux autres modalités thérapeutiques telles que la radiothérapie et la chimiothérapie dans le traitement du cancer. Trois

patients ont été traités avec cette technique, et les résultats montrent une réaction significative dans le contrôle palliatif des lésions.

Mots-clés: Thérapie photodynamique, carcinome mammaire, carcinome à cellules écaillieuses, carcinome à cellules basales, dérivés hématoportrophyriniques.

Riassunto

La terapia fotodinamica che utilizza i derivati ematoporfirinici come agente fotosensibilizzante è attualmente sperimentata come alternativa ad altre modalità terapeutiche come la radioterapia e chemioterapia nel trattamento del cancro. Tre pazienti sono stati trattati con questa tecnica e i risultati indicano una risposta significativa nel controllo palliativo delle lesioni.

Parole chiave: Terapia fotodinamica, carcinoma mammario, carcinoma a cellule squamose, carcinoma a cellule basali, derivati ematoporfirinici.

INTRODUCTION

Photodynamic therapy (PDT) is a method of treating cancer currently under clinical investigation (1, 2, 3). The basis of this technique is the administration of a photosensitizing drug which is retained by the neoplastic lesions. 24-48 hours after the systemic administration of the photosensitizing drug, the tumour is irradiated with light radiation. The chosen wavelength should have good tissue penetration and absorption by the drug. In practice, red laser light at 600-630 nm which photoactivates the drug is used, leading to the generation of singlet oxygen which causes vascular damage leading to tumour necrosis. The photosensitizer in current use is Hematoporphyrin Derivative (HpD) which is a metabolic by-product extracted from calf blood. The photosensitivity of skin to strong light post treatment is the only major side effect of PDT when it is used as the photosensitizer.

This paper reports on initial results in the study of the efficacy of PDT for the treatment of superficial malignant tumours using simple red light sources instead of lasers as the photo-irradiation sources.

MATERIALS AND METHODS

Preparation of HPD Clinical HpD was prepared from commercial Hematoporphyrin dihydrochloride following the procedure of Lipson et al. (1961) (4) as described by Kessel and Cheng (1985) (5). All reagents used were of analytical grade. The preparation was conducted inside a Class 10000 Clean Room to ensure the solution is pyrogen free and sterile for intravenous clinical use. Sterility, pyrogenicity and toxicity tests were carried out. For each batch of preparation, a random test sampling of 20% of the total number of vials was used.

Light source and instrumentation

A red filtered portable surgical head lamp with a fibre optic cable (Schott KL1500-2) and a filtered red light source based on an overhead projector lamp (Sinon 270 A) were used as the light sources for PDT. Both the light sources had a continuous red light with a wavelength of 600-650 nm. The light transmitted by the red filtered portable head lamp had an output power of 120 mW. The optical fibre cable had a diameter of 6 mm.

Modification on the overhead projector lamp was carried out. Part of the projector light output optics were removed. A cold mirror was used to filter out the IR component and the plastic Fresnel lens was covered with a red plastic filter. The light source has an output power of 2 W with approximately half the energy in the wavelength range between 600-650 nm. This light source was found to be effective in the treatment of superficial malignancies.

Patient selection

The patients treated had histologically proven malignancies of the skin, oral cavity and breast. The malignant deposit was accessible to an external light beam. For all three cases, other treatment modalities had failed or were found to be unsuitable. The patients were also screened for contraindications including history of allergic conditions (eg, bronchial asthma, atopic eczema, contact dermatitis), sensitivity to drugs, pregnancy and lactation. Informed consent was obtained after providing detailed information on the nature, aims and potential hazards of the clinical trial, particularly regarding photosensitivity. Patients were specifically warned to keep away from sunlight and strong roomlight, including fluorescent lamps for a period of 4 weeks.

Methodology

Histopathological assessment had been conducted previously to confirm the diagnosis. This was then followed by laboratory investigations which were assessed pre and post PDT. The laboratory investigations were full blood counts, renal function tests, liver function tests, plasma electrolyte levels, blood glucose, ECG and chest X-ray. Clinical HpD that had been stored frozen in darkness was thawed in a beaker of tap water one hour before use. The drug was injected intravenously at a dose of 5 mg/kg. Initially the patient received a test dose of 1 ml of HpD (10 mg/ml) and was observed for 10 minutes. With no indication of allergies or other side effects, the remaining dose was given by slow intravenous push. Heart rate and blood pressure was monitored during the administration. Any side effects or anaphylaxis were recorded. The patient was subsequently kept in a dimly lighted room.

Treatment dosimetry

24 hours after the administration of HpD, the patient was given a test dose of therapeutic light of 50 J/cm² on normal skin to assess possible side effects such as erythema or edema. Following the test dose, the lesion was subjected to a therapeutic dose of red light, the amount of light depended on the area and the depth of the lesion. Superficial tumours about 3 mm in thickness will require a light dosage between 50-150 J/cm². During PDT, black cloth was used to cover normal tissue. For treatment on the face, eyes pads were also used to protect the patients' eyes from reflected light.

Methodology of treatment post PDT

Post treatment, changes at the lesions were recorded. For the first week, changes in the lesions were recorded daily, and subsequently every week for one month. The

assessment of response was monitored by tumour volume based on clinical examination, serial photography and biopsy of the site of the treated lesion. The period of histological assessment was at one month and three months and upon recurrence whichever is earlier.

RESULTS

Case reports

Case 1

A 60 year old woman had a locally advanced right breast tumour involving the skin over the chest bilaterally. The tumour was detected more than a year ago but she did not seek any treatment. The right breast was practically destroyed by the tumour leaving a large ulcer with a crater like hole in the centre with a depth of 2.0 centimeter (Fig. 1). There were many skin nodules (more than 20) over the chest and there was an enlarged fixed lymph node in the right axilla. There were no secondaries in the lungs and bones. A biopsy of the liver showed infiltrative ductal carcinoma. She was initially started on hormonal therapy with tamoxifen 20 mg daily. On review after a month, there was no improvement. She was then started on chemotherapy with the FEC (5-fluorouracil, Epirubicin and Cyclophosphamide) regime 3 weekly. After 2 cycles, no improvement was seen.

PDT was performed for 60 minutes on the large ulcer with red light (600-650 nm) of 1W with a beam diameter of 10 cm from a filtered projector lamp (approximately 50 J/cm²). Subsequently another 15 J/cm² light was added to the periphery of the ulcer using the fibre optic delivery system. Two of the larger skin nodules over the chest wall were also treated using a filtered surgical head lamp with 80 J/cm² and 40 J/cm² respectively with a beam diameter of 1.5 cm. The larger ulcer showed scab formation at the edges with a large amount of serum oozing immediately after PDT. Scab was formed on the nodule which had 80 J/cm² of light. She was continued on her three weekly chemotherapy regime. After 5 weeks, the centre of the large ulcer was clearing up. Thereafter, the wound got smaller in size after 2 months (Fig. 2). Scab has also fallen off from the small nodule with 80 J/cm² of light. The smaller nodule that

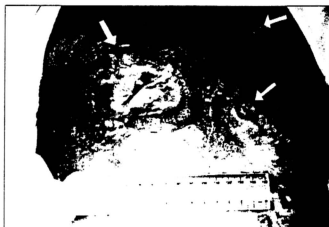


Fig. 1. Advanced breast tumour with metastatic nodules: before treatment (arrows indicate areas selected for treatment)



Fig. 2: Advanced breast tumour with metastatic nodules: two months after treatment.

received 40 J/cm² also diminished in size. She remained in this state for 4 months when she was presented with terminal disease due to tumour infiltration into the bone marrow. Her right breast tumour had begun to ulcerate. She succumbed to her advanced metastatic disease.

Case 2

A 64 year old woman with a non-healing ulcer of the left cheek was also treated. The ulcer was 3 by 4 cm in size (Fig. 3). Biopsy showed a well differentiated squamous cell carcinoma and X-rays did not show any bone involvement. No lymph node metastasis were present. She did not agree to surgical excision. She was treated using a filtered surgical head lamp with a total light irradiation of 100 J/cm². Scab formed over the ulcer 2-3 days after the therapy. This gradually fell off after a month giving a nicely healed area. However, a slightly inflamed area was detected at the lower end of the original tumour site. This was biopsied and showed residual squamous cell carcinoma. She was given a second treatment of PDT (Fig. 4). After a week the patient was discharged. She remained well until two and a half months later when her ulcerating growth started growing back to the original size, she had surgical excision and local flap. Histopathology showed well differentiated squamous cell carcinoma.



Fig. 4: Squamous cell carcinoma: two months after treatment



Fig. 5: Squamous cell carcinoma: before treatment.

Case 3

A 50 year old woman had Bowen's Disease where multiple basal cell carcinoma were found over the face (Fig. 5). She had undergone many surgical excisions of the lesions since 1974. On examination, she had multiple pigmented lesions of varying sizes over the whole of her face and neck. Scars from previous surgery were also present. Seven of her lesions were selected to be treated with PDT. Each lesion was treated using a filtered surgical head lamp with a total light irradiation of 40 J/cm². She went home after 6 days in the ward. She did not take heed of the advice of staying away from the sun and she returned on the 8th day with severe facial oedema, as well as oedema over the exposed parts of her hands and feet. She had sustained second degree burns. Photosensitivity was diagnosed and she was started on steroids and antihistamines. Her swelling subsided after 48 hours. Two weeks after treatment, the lesions had decreased in size (Fig. 6). However, in heavily pigmented areas there did not seem to be much reaction. When reviewed two months later, her lesions had regrown to its original state.

DISCUSSION AND CONCLUSIONS

The cases selected in this clinical trial of PDT were patients who had failed to respond to conventional forms of treatment and were in need of palliation or had refused surgical excision.

In all these cases there were definitely positive response of the tumours to HpD-PDT. Response obtained were significant in case 1 (advanced breast tumour). This patient subsequently died after 4 months post treatment. The cause of death was metastatic tumour infiltration into the bone marrow. Nevertheless, due to HpD-PDT, the patient was spared to some extent the distress associated with an ulcerated lesion. Because the treatment of PDT was short in duration, relatively painless and for the main part noninvasive resulting in significant response of the ulcerating wound, there was definite improvement in the well-being of the patient during the months before death.

Case 2 (squamous cell carcinoma) revealed good response but there was residual tumour at the lower edge of the ulcer

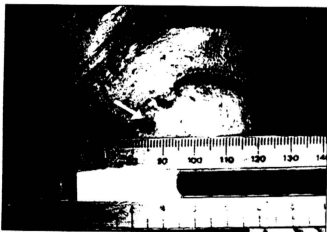


Fig. 5: Basal cell carcinoma: before treatment (arrows indicate nodules selected for treatment)



Fig. 6: Basal cell carcinoma: two weeks after treatment

where there was keratotic skin scab originally. The scab absorbed red light and diminished light penetration into the underlying tumour tissue. However, recurrence occurred about two and a half months post PDT. It does appear that keratotic skin surfaces prevent red light from reaching the tumour. PDT is useful in cytorreduction and may render large lesions available to surgical excision without need for major surgical reconstruction.

Case 3 with basal cell carcinoma, generally showed good response despite skin photosensitivity. Her second degree burn sloughed off her treated as well as untreated lesions. The heavily pigmented areas did not seem to have much reaction. However recurrence occurred about 2 months later, which may be because too low an energy density was used for this patient. She declined further PDT due to her fear of skin photosensitivity.

PDT appears to offer palliative control of the lesions for these patients and all had symptomatic improvement. Although the patients may be mobile, the need to avoid sun exposure limits the patients' activities for at least a month. Keratotic and pigmented skin surfaces also limits light penetration, hampering effective PDT. The filtered red light sources appeared to be as effective as laser sources for such PDT treatment of superficial tumours. These simple light sources are attractive alternatives to expensive sources, which are technically more difficult to operate and to maintain in a hospital environment.

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Note

The clinical protocol was approved by the Ethics Committee of the University Hospital, University of Malaya, Kuala Lumpur. The manufacture and use of this HpD for clinical trials have been registered with the Malaysian Drugs Control Authority. This programme is supported by the Ministry of Science, Technology and the Environment, IRPA vot 2-7-4-17.

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Serum levels of hematoporphyrin derivatives in the photodynamic therapy of malignant tumours

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ABSTRACT

In photodynamic therapy (PDT), red light is administered 24-72 hours post intravenous (i.v.) injection of hematoporphyrin derivatives (HpD). In an earlier animal model study^{1,2}, more effective therapeutic response was obtained when red light irradiation was carried out 15 mins after the injection of HpD. The effectiveness of this immediate PDT protocol has been correlated to the high serum level of HpD immediately after administration and the destruction of the microcirculation system as the dominant tumour destruction mechanism. This study examines the pharmacokinetics and the serum levels of HpD in rats and also in human patients. Such data can assist in defining the optimum time delay for light irradiation in the PDT of cancer.

Keywords: Photodynamic cancer therapy, hematoporphyrin derivatives, serum level, pharmacokinetics.

1. INTRODUCTION

Photodynamic therapy (PDT) is an alternative procedure for treating cancer on clinical investigations³ over the last sixteen years. The present protocol for PDT is the administration of a photosensitizing drug, followed by light irradiation at a delay time of 24-72 hours after the systemic administration of the photosensitizer. The sensitizer in current use is hematoporphyrin Derivative (HpD) which is a metabolic by-product of calf blood. Red light in the wavelength range between 620-630 nm is used to photoactivate the drug, leading to the generation of cytotoxic agents and ultimately tumour necrosis. Photosensitivity of skin to strong light post treatment is the only major side effect of PDT when HpD is used as the sensitizer.

The above protocol of delayed PDT has been derived based mainly on the requirement of serum and normal tissue clearance of HpD prior to light irradiation. However, in a recent study^{1,2}, it was shown that better tumour response can be obtained by light irradiation immediately (15 mins) after the administration of the photosensitizer. The effectiveness of this new immediate PDT protocol has been correlated to the high serum level of HpD leading to the more effective destruction of the microcirculation system. This paper aims to further investigate the pharmacokinetics of HpD in blood serum with a view to extract information on the optimum time of photo-irradiation in HpD-PDT.

2. TREATMENT PROTOCOL OF HPD-PDT

The conventional protocol of delayed PDT was developed mainly from visual inspections of strong tumour tissue fluorescence of HpD⁴ in intraperitoneal injection of HpD and the apparent serum clearance in 3 hours and gastrointestinal clearance by 24 hours. It was also assumed that the primary process of tumour destruction was photocytotoxicity of HpD on tumour cells leading to necrosis.

Based on these assumptions, Diamond *et al.*⁵ adopted a treatment protocol by the delayed photo-irradiation of red light. Hence then, all HpD-PDT animal model and clinical trial studies have been conducted on this procedure of delayed PDT.

While some data had indicated the possibility that tumour destruction is secondary to the destruction of the microcirculation^{6,7} system, and that tumour cells are viable immediately following HpD-PDT⁸, correlation to the PDT treatment protocol has not been recognized prior to work conducted at this centre^{1,2}. Since the primary tumoricidal action stems from the destruction of the microcirculation system, it would be optimum to photoradiate when the serum level of HpD is the

highest, immediately following the i.v. injection of HpD. An alternative treatment procedure referred to as immediate PDT has therefore been proposed, whereby photoradiation was carried out 10-15 mins (allowing time for HpD equilibrium in serum) after the administration of HpD. Animal model results showed that at one-sixth the HpD dosage, immediate PDT achieved better tumour response than the conventional delayed PDT.

Due to the high level of the photosensitizer in the blood serum, immediate PDT causes effective destruction of the microcirculation system. By 24-72 hours after HpD administration, the serum level of HpD is significantly reduced from its peak. The aim of this study is to quantify the blood serum levels of HpD in rats and in also patients.

3. MATERIALS AND METHODS

3.1 HpD assays in serum of rats

It is noted here that clinical trials for delayed PDT have been widely conducted in the past by many centres abroad. The conventional protocol has also been approved by the Ethics Committee of the University Hospital of the University of Malaya for clinical investigations. The manufacture and use of this HpD for clinical trials have also been registered with the Malaysian Drugs Control Authority. Thus far, no report has been made on the use of immediate PDT as a protocol for clinical investigation of cancer therapy. In order to be able to initiate clinical trials on the efficacy of the immediate PDT relative to delayed PDT, various basic data including the serum levels of HpD will have to be acquired. This paper presents some initial data of serum level of HpD as well as an analysis on the pharmacokinetics of HpD with respect to delayed and immediate PDT for cancer treatment.

All experiments were carried out using clinical HpD prepared following the procedure of Lipson *et. al.*⁹ and modified by Kessel and Cheng¹⁰ to increase yield. Each rat received a dose of 10 mg/kg. Blood was drawn at different time intervals for measurement of serum levels of HpD. 1.0 ml of blood was drawn at a series of time intervals at just before HpD injection and at 15 mins, 3 hrs, 12 hrs, 24 hrs and 48 hrs after the administration of HpD. For each set of data, blood was drawn from 4 rats for statistical averaging purposes.

Blood was then left to clot overnight at 4°C before serum was recovered by centrifugation at 10,000 rpm for 10 mins. Samples were stored frozen until assay for total hematoporphyrin, essentially by the method of Kessel and Cheng¹⁰.

To quantify the concentration of HpD in serum, a standard graph with known concentration of clinical HpD in serum was first made. To obtain this standard graph, 0.01 ml of serum was diluted with saline to 1.0 ml with the serial concentration of different known amount of HpD and duplicate samples of 0.4 ml taken. At the same time, 0.01 ml of serum at different time intervals as above was diluted to 1.0 ml with saline and duplicated samples of 0.4 ml taken.

To each was added 1.0 ml chloroform:methanol (1:1) (BDH and Merck). Samples was vortexed for 1 min and centrifuged at 9,000 rpm for 15 mins at room temperature. The upper aqueous layer was discarded and 0.7 ml methanol added to the organic layer (bottom layer). Samples were recentrifuged and the upper layer was again discarded. The bottom layer was then taken to be evaporated under nitrogen for 15 mins at 100 kPa. The residues were dissolved in 0.5 ml 0.5M HCL (Merck), hydrolysed at 100°C for 1 hour in water bath, neutralized with 0.25 ml 1.0 M NaOH and made up to 1.0 ml with 0.02 M cetyltrimethylammonium bromide (CTAB) in 0.2 M of NaOH.

The fluorescence (excitation 400 nm, emission 622.4 nm) was determined in a RF5000 recording spectrofluorophotometer (Shimadzu).

3.2 HpD assay in serum of patients

Assay of HpD level in patient blood serum were similarly carried out. 1 ml of blood was drawn from the patients before HpD administration and also at 15 mins, 3 hours, 12 hours, 24 hours and 48 hours post i.v. administration of HpD. These patients with different types of carcinomas (advanced mammary, squamous cell and basal cell) were treated using the approved delayed PDT protocol. One patient received two cycles of HpD-PDT. Thus, 4 blood samples were drawn and HpD levels in serum were measured and averaged. For each patient, HpD dosage of 5 mg/kg was administered.

4. RESULTS AND DISCUSSIONS

Fig. 1(a) and 1 (b) shows the measured HpD levels in blood serum of rats and human patients respectively. The results show that the maximum concentration of HpD in the serum of rats is around 600 $\mu\text{g/ml}$ as compared to 110 $\mu\text{g/ml}$ in human patients, even though HpD dosage is only twice higher in rats. This implies that the volume of serum per body weight of rat is approximately 2.7 times less than in human being resulting in correspondingly higher serum HpD levels in rats.

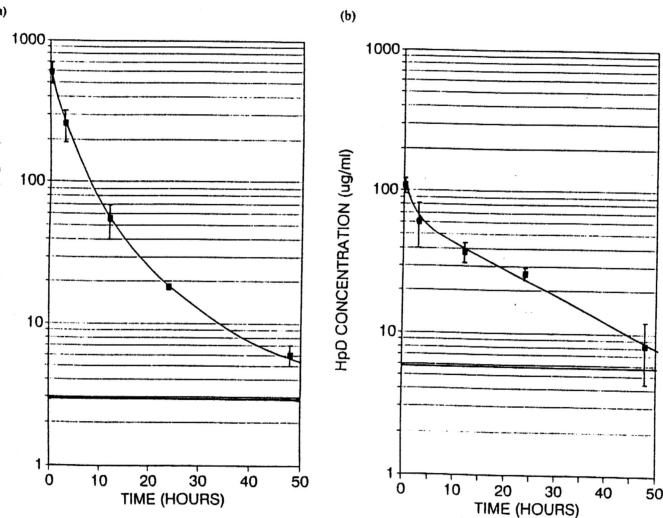


Fig. 1. Serum Level of HpD injected intravenously (a) in rats at 10 mg/kg (b) in patients at 5 mg/kg. Horizontal line indicates endogenous porphyrin levels.

of significance to note is the markedly rapid serum clearance of HpD in rats when compared to human patients. At 48 hours post i.v. administration, serum level of HpD in rats have decreased from almost 600 $\mu\text{g/ml}$ to a mean level of 5 $\mu\text{g/ml}$, a decrease of some 30 fold in concentration. On the other hand, the serum level of HpD in human patients has decreased from around 110 $\mu\text{g/ml}$ to some 8 $\mu\text{g/ml}$, a decrease of 5.8 fold in concentration of HpD. At 48 hours post i.v. administration, serum level of HpD in rats have decreased from almost 600 $\mu\text{g/ml}$ to a mean level of 3 $\mu\text{g/ml}$, a decrease of some 200 fold in concentration. On the other hand, the serum level of HpD in human patients has decreased from around 110 $\mu\text{g/ml}$ to some 3 $\mu\text{g/ml}$, a decrease of 37 fold in concentration of HpD. The metabolism and the excretion rate of HpD is thus many fold higher in rats than in human beings.

We can now attempt to quantify and interpret the response of tumour to PDT on the basis of the measured serum levels of HpD, with respect to the results obtained in immediate PDT^{1,2}. In that work, it was reported that 6 times higher HpD dosage is needed in delayed (24 hr) PDT to produce similar tumour response when compared to immediate PDT. This is to be compared with the 30 fold higher serum concentration of HpD in immediate PDT. It is expected that at such low concentration of HpD in serum, tumour response in delayed PDT is negligible. On the other hand, HpD retained in the vascular endothelium and the stroma may be responsible for tumour response in delayed PDT. This assumption is further supported by the fact that there is no clear distinction in the extent of tumour response for delayed PDT over the period of 24-72 hours, when at 48-hr, there is a 200 fold decrease in serum HpD level. This assessment is also made assuming that direct tumour cell response to PDT is negligible. It can therefore be concluded that in delayed PDT experiments on rats, the PDT response is obtained from the photo activation of HpD retained in the vascular endothelium and stroma. In immediate PDT, it is fairly certain that tumour response is obtained mainly from the high HpD concentration in serum which permeates through the entire vasculature system.

On the other hand, in human patients, serum clearance of HpD is relatively slow. With a factor of 5.8 times less HpD in serum at 24-hr post i.v. injection, tumour response is expected to be at most 5.8 times more significant in immediate PDT than delayed PDT. In fact, with the relatively high concentration of HpD in serum in human patients at 24-hr, it is uncertain if tumour response is due to the high concentration of HpD in serum permeating the entire micro vasculature or due to HpD retained in the vasculature and stroma leading to endothelial cell and stroma destruction. However, at 48-hr, with very low serum level of HpD, tumour response could be mainly due to HpD retained in the endothelium and the stroma. The likely combined effects of both processes in delays PDT of human patients is a process totally different from that deduced for the rats where serum level of HpD is negligible at 24-hr or 48 hr post i.v. administration.

Arriving at the above deductions, it is tacitly assumed that in PDT, there is only one effective HpD component which does not change its bio-chemical properties in-vivo. However, it is well established that HpD is a complex chemical with many components with different uptake and retention properties as well as effect in PDT. The straight forward deductions made in the earlier two paragraphs will have to be correspondingly expanded in complexities when all such factors are taken into considerations.

For this purpose, we have some initial indications obtained of the complex pharmacokinetics of HpD in mice. Based on initial results of a 6-compartment model analysis of the pharmacokinetic data of Gomer and Dougherty obtained from radiolabelled HpD studies injected intraperitoneally in mice, it has been concluded that a multi-compartment as well as multi-hematoporphyrin fractions model will have to be used.

5. CONCLUSIONS

In this paper, we have shown that much more detailed studies on the complex light-photosensitizer-tissue interaction processes remains to be carried out. Notably, our measured serum levels of HpD in rats and human patients indicate very different processes incurred in tumour response to delayed and immediate PDT in animal model and in patients. In immediate PDT, for both rats and human patients, it can be expected that tumour response is a result of high serum level of HpD leading to destruction of the vasculature. On the other hand, in delayed PDT in rats, due to the very low level of serum HpD, tumour response is a result of HpD retained in the endothelium and stroma. For delayed PDT on human patients, it is expected that due to the relatively still high level of serum HpD, both HpD in the serum and HpD retained in the endothelium and stroma contribute to tumour response. However, the relative contributions of these two processes are yet to be established.

This study has raised various fundamental questions conventional on the clinical protocol of HpD-PDT, which is mainly derived from quantitative animal model studies. The complex chemical composition of HpD and the exact role of each fraction in HpD-PDT with respect to time residency in blood serum remains largely unknown. With quantitative data on the pharmacokinetics of the different fractions of HpD and the relative efficiencies of each fraction with time on PDT, the optimum protocol with the right hematoporphyrin fraction can be deduced, minimizing the dosage needed to reduce the problems of the side effects of HpD-PDT.

6. ACKNOWLEDGEMENT

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Simple red light sources for photodynamic therapy of malignant tumours

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ABSTRACT

While laser beams are essential for endoscopic photodynamic therapy (PDT), many types of superficial tumours can be treated effectively using incoherent light sources which are appropriately filtered. This paper reports on clinical trials using simple and effective red light sources modified from an overhead projector and a surgical headlamp with fiber optic output. More than 1 W of effective red light can be obtained and used effectively in the palliative control of large superficial lesions in initial trial cases of three types of malignancies. The use of simple light sources for the treatment of superficial tumours avoids the technical complexities associated with laser sources and enables ready observations of treatment response. Such clinical trials should be conducted extensively in the development of PDT as a viable cancer treatment modality prior to the development of endoscopic procedures.

Keywords: Photodynamic cancer therapy, hematoporphyrin derivatives, simple red light sources.

1. INTRODUCTION

Clinical investigations of photodynamic therapy (PDT) as an alternative modality for treating malignancies have been conducted in many centres around the world over the last sixteen years^{1,2}. The technique involves the administration of Hematoporphyrin Derivatives (HpD), followed by the irradiation of red light over the neoplastic lesions at 24-48 hours after the systemic administration of the photosensitizing drug. While some progress has been made over the last sixteen years, the overall clinical PDT trial programmes are progressing rather slowly, partly due to a general lack of understanding of the complex light-photodynamic-tissue interaction processes and partly due to the general technical difficulties associated with the usage of high power laser systems. High power lasers like the argon ion, copper vapour pumped dye lasers or gold vapour lasers in current use for PDT are all relatively expensive and technically difficult to maintain and operate for routine clinical applications.

While laser beams are essential for endoscopic PDT applications via optical fiber light delivery, many types of superficial malignancies can be treated using incoherent light sources which are appropriately filtered to provide red light for PDT. The treatment of such external lesions facilitate simpler clinical procedures and easier follow-up observations.

In earlier works, a filtered projector lamp has been used in animal model studies and has been shown to yield therapeutic response similar to those obtained using laser sources^{3,4}. This paper reports on extension of these works to clinical trials using a overhead projector lamp with high output power and a surgical headlamp with fiber optical light guide output.

2. MATERIALS AND METHODS

2.1 Light sources and instrumentation

An overhead projector (Sinon 270 A) can be readily modified for PDT clinical trials. A schematic diagram is shown in Fig. 1(a). The projector uses a 400 W halogen lamp. This consists mainly of a voltage step down circuit for the halogen bulb, a cooling fan, a collimating lens and a 14" plastic fresnel lens. The output optics consist of a beam folding mirror and a cold mirror which filters out the infrared radiation with wavelength longer than 720 nm from the output beam. An appropriate red plastic filter paper was placed across the plastic fresnel lens to cut off visible light with wavelength shorter

than 600 nm. Fig. 1(b) shows the spectral output of the overhead projector lamp, showing that approximately 30% of the red light output is in the HpD red absorption wavelength range between 600-650 nm.

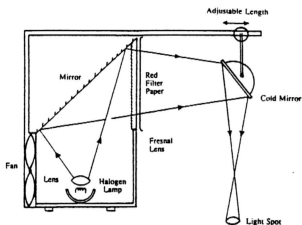


Fig. 1(a). Schematic diagram of a red light source modified from an overhead projector.

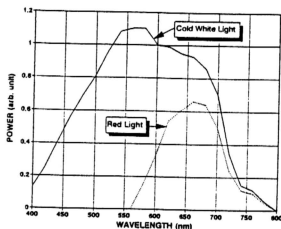


Fig. 1(b). Output spectra of the cold white light and with a red plastic paper filter.

The light source has a red output power of 3.2 W, with approximately 1 W of output between 600-650 nm. The cold mirror used here is an inexpensive standard optics and if possible, a special coated short-pass filter with a cut off wavelength of 650 nm should be used. With an effective output power of 1 W, large superficial lesions can be treated. The modified projector light source is also readily portable and can be conveniently adjusted to provide either a horizontal or a vertical beam with varying spot sizes ranging from a minimum of 3 cm diameter to as large as 10 cm.

For treatment of smaller lesions and particularly to less accessible malignant sites, a surgical headlamp with an optical fiber output is used. A schematic optical output of the surgical headlamp is shown in Fig. 2(a). The light transmitted by the portable surgical headlamp (Schott KL1500-2) is fitted with red filter paper (a newer model Schott EL-2 has a convenient slot for mounting a sharp cut red glass filter). Fig. 2(b) shows the spectral output of the surgical headlamp which has an effective output power of 150 mW between the wavelength range of 600-650 nm. In this case, an internal cold mirror blocks the infrared component from being transmitted by the fiber. The fiber optic cable had a diameter of 6 mm, which then defines the minimum beam diameter and the maximum power density of 500 mW/cm². The output from the optical fiber bundle with an output protective claddings and sheath is 1 cm in diameter but is sufficiently flexible for light irradiation of tumour masses normally not accessible using the projector light output.

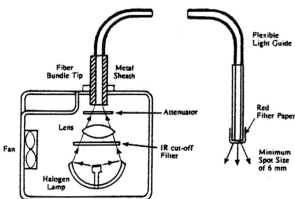


Fig. 2(a). Schematic diagram of a red light source and a red light from a surgical headlamp.

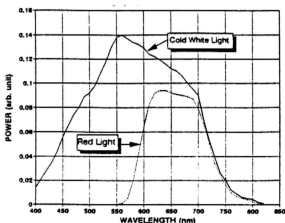


Fig. 2(b). Output spectra of the cold white light and with plastic paper filter.

2.2 Clinical HpD

Clinical HpD was prepared following the procedure of Lipson *et. al.*³ and modified by Kessel and Cheng⁶ to increase the yield. Hematoporphyrin dihydrochloride (Fluka) was used as the starting material and was subjected to a process of acetylation and dehydration. The HpD solid obtained is dissolved in saline solution, made isotonic and then filtered in millipore filters to produce injectable HpD. This last preparation procedure is conducted in a clean room environment. To ensure that the HpD prepared are acceptable for systemic applications, sterility, and pyrogenicity tests were carried out on each batch of HpD prepared. Clinical HpD solution was stored frozen in darkness and thawed in warm water before usage. The usage of this HpD for clinical trials is registered with the Malaysian Drug Control Authority. Approval to conduct clinical trials was also obtained from the Ethics Committee of the University Hospital.

2.3 Clinical cases

Three patients with histologically proven malignancies were treated in this initial trial investigations using the simple red light sources developed. The lesions were all accessible to an external light beam. All patients have undergone other treatment modalities, which were then considered to have failed or were unsuitable to continue further treatment. Informed consent of the patient were obtained. The patients were also specifically warned to keep away from sunlight and strong room for a period of 4-6 weeks post treatment.

Clinical HpD was injected intravenously at a dosage of 5 mg/kg. The patient received 1 ml of drug initially and was observed for 10 mins. When no side-effects were observed, the remaining dose was administered by slow intravenous push. 24 hours after the administration of HpD, the patients were each given a test dose of therapeutic light of 50 J/cm² on a normal skin spot to assess possible side effects such as erythema or edema. Observing no adverse side effects, the lesions were subjected to a therapeutic dose of red light depending on the area and depth of the lesion.

3. CLINICAL RESULTS

3.1 Case 1: Advanced mammary carcinoma

A 60 year old woman with a badly ulcerated right breast tumour, palpable tumour mass over the left breast, enlarged lymph nodes in the right axillae and supraclavicular lymph nodes and with many metastatic nodes was considered for PDT treatment. The right breast was practically destroyed by the tumour leaving a large ulcer with a 10 cm diameter crater like hole in the center with a depth of 2 cm (Fig. 3(a)). She was initially started on hormonal therapy with tamoxifen 20 mg daily but on review after a month, there was no improvement. She was then started on chemotherapy with the FEC regime weekly. After 2 cycles, not much improvement was seen, and it was decided to try PDT on this patient.

PDT was performed on the large ulcer with a 1 W effective red light and a beam diameter of 10 cm from the filtered projector light. Approximately 50 J/cm² of red light was delivered. An additional 15 J/cm² light was irradiated using the flexible optical fiber output from the surgical headlamp to the peripheral side walls of the ulcerated hole, which otherwise may not have received sufficient therapeutic light. Two of the larger metastatic nodules were also treated using the filtered surgical headlamp with 80 J/cm² and 40 J/cm² respectively. During the entire light irradiation procedure, the patient was lying on the bed without any form of anaesthesia. The projector was placed on a trolley across her bed and the beam was directed downwards to irradiate on her chest wall. The fiber was manipulated using a series of clamps mounted on a magnetic base. With the low light intensity used during irradiation, minimal synergistic effects of hyperthermia is expected. During the procedure, the patient had only mentioned of the slight prickly feeling over the treated sites.

The large ulcerated site formed a thick scab with a large amount of body serum oozing from the treated sites during the first 2 days after PDT. A scab was also formed on the nodule which had 80 J/cm² of light. The other nodule with 40 J/cm² did not form a scab but was found to be diminishing in size.

It is noted that, on ethical grounds due to the advanced nature of her lesions, she was still continued on her three weekly chemotherapy regime. After 2 weeks, the entire ulcer was clearing up and after 2 months (Fig. 3(b)), the wound healed with healthy skin gradually forming over the whole tumour site. Scab has also fallen off from the small nodule with

30 J/cm² of light. The smaller nodule receiving 40 J/cm² also diminished in size. No biopsy has been taken on all the sites. Generally, the tissue over the treated area did not indicate palpable tumour mass. However, it was expected that residual tumour mass is still present in the underlying tissue. 4 months after PDT, the patient succumbed to metastasis to the bone marrow.

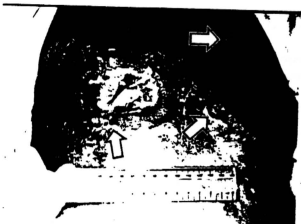


Fig. 3(a). Advanced breast tumour with metastatic nodules before treatment (arrows indicate treated nodules).



Fig. 3(b). Advanced breast tumour with metastatic nodules months after treatment.

2 Case 2: Squamous cell carcinoma

An 84 year old woman with a non-healing ulcer of the left cheek was also treated. The ulcer was 3 by 4 cm in size. Biopsy showed a well differentiated squamous cell carcinoma and no lymph node metastasis were present. She did not agree to surgical excision of the tumour and hence it was decided to treat her with PDT. She was treated using the filtered surgical headlamp with a total light irradiation of 100 J/cm². Scab formed over the ulcer 2-3 days after the therapy. This gradually fell off after a month giving a nicely healed area. However, a slightly inflamed area was detected at the lower end of the original tumour site, which was observed to have some keratotic skin prior to PDT treatment. This was biopsied one month later and showed residual squamous cell carcinoma. The keratotic skin has reduced light penetration into the underlying tumour mass preventing effective response to PDT.

3 Case 3: Basal cell carcinoma

A 50 year old woman had Bowen's Disease where multiple basal cell carcinoma were found over her face. She had undergone many surgical excisions of the lesions since 1974. Scars from previous surgeries were also present. Seven of her lesions were selected to be treated with PDT. Each lesion was treated using the filtered surgical headlamp with a light irradiation of 40 J/cm². She went home after 6 days and did not take heed of the advice of staying away from the sun. She returned on the 8th day with severe facial oedema, as well as oedema over the exposed parts of her hands and feet. She had sustained second degree burns and was started on steroids and antihistamines. Her swelling subsided after 48 hours. Two weeks after treatment, the lesions had decreased in size. However, in heavily pigmented areas, there did not seem to be much therapeutic response. When reviewed two months later, her lesions had regrown to its original state.

4. DISCUSSIONS AND CONCLUSION

In all the cases investigated, there was a demonstrable response of the tumours following PDT using the simple red light sources. Response obtained were significant in the case of the advanced breast tumour. The treated areas showed effective tumour necrosis with the ulcer healing from the edges and has effectively sealed the ulcerated primary tumour site. With the healing of the wound two weeks after PDT, the patient was spared the distress of an ulcerating wound.

Case 2 (squamous cell carcinoma) revealed good response but there was residual tumour at the lower edge of the ulcer where there was keratotic skin scab originally. The scab absorbed red light and diminished light penetration into the

underlying tumour tissue. However, recurrence occurred about two and a half months post PDT. It does appear that keratotic skin surfaces prevented red light from reaching the tumour.

Case 3 (basal cell carcinoma) generally showed good response despite skin photosensitivity. Her second degree burn sloughed off her treated as well as some untreated lesions. The heavily pigmented areas did not seem to have much response. However, recurrence occurred about 2 months later, which may be because too low an energy density was used for this patient. She declined further PDT due to her fear of skin photosensitivity.

The results indicates that significant responses were obtained in these cases involving different types of carcinomas. The simple light sources here enables much simpler treatment procedures and with a minimum of set-up time. The projector light source was highly portable, reliable, inexpensive and its built in focusing devices enable ready adjustment of the beam to a wide range of beam diameters. No hazardous laser radiation is involved and the patient needs only to be screened from the intense red light beam. The operators need only to wear normal sunglasses to avoid the glare during the light irradiation on the tissue masses.

It is noted here that in general, the relative efficacy of PDT as a function of wavelength has not been quantitatively established. The absorption band of HpD in the red is broad covering the spectral range from 600-650 nm. Thus, light with wavelength within this range is expected to be effective for PDT although possibly with varying degrees of efficacies. The relative quantum efficiency in the generation of singlet oxygen via HpD excitation by different wavelength light has also to be quantitatively established.

In two earlier works, it was reported that the broadband red output from a projector light source can be used effectively in animal model works yielding therapeutic responses similar to that observed using narrow band laser light. Significant therapeutic response obtained in this work verified further the advantages in using simple red light sources for the PDT of various superficial lesions. The use of such simple red light sources also offers viable PDT procedures for the palliative control of severe advanced external lesions not amenable to surgery and other treatment modalities. The surgical headlamp with fiber optic output also enables flexible beam delivery for maneuvering the beam into less accessible superficial tumour sites, for example in the oral cavity. Clinical PDT programmes in the treatment of such lesions using simple red light sources should be investigated more thoroughly in order to obtain better understanding of the basic processes in the PDT of malignancies. Endoscopic PDT clinical trial programmes using laser systems need only be initiated at a later stage in the development of PDT as an alternative modality for the treatment of less accessible malignancies.

5. ACKNOWLEDGEMENT

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