CHAPTER ONE

1.0 INTRODUCTION

1.1 A Brief Review Of Photodynamic Therapy

The use of light as a therapeutic tool in surgery is becoming increasingly important, with the routine use of lasers for photocoagulation and in the Photodynamic Therapy (PDT) of cancer. The use of light for therapy in human diseases has a very long history stretching back into antiquity. Phototherapy began in ancient Greece, Egypt and India but disappeared for many centuries only to be rediscovered by western-civilization at the beginning of the twentieth century. Quoting from the review of Daniell and Hill (1991), the concept of PDT for the treatment of patients with malignant tumours dates from 1903, when Tappeiner and Jesionek used eosin and sunlight to treat skin cancer. The following year, 1904, Tappiener and Jodlbauer reported that the presence of oxygen was a requirement for photosensitization. The first photodynamic studies using hematoporphyrin (Hp) were carried out by Hausmann in 1911 for both *in vitro* and *in vivo* investigations. Auler and Banzer (1942) and Figge *et. al.*(1948) demonstrated the affinity of various porphyrins, including Hp, for the malignant tissues, by studying the fluorescence produced when the tissues were exposed to UV light. During the 20 years that followed, the interest in Hp for clinical oncology were concentrated mainly on the detection and localization of malignant tumours by *in-vivo* fluorescence.

In attempting to improve the *in-vivo* localization and photodynamic properties of Hp, Lipson and Baldes in 1960 developed a procedure for the preparation of Hematoporphyrin Derivative (HpD), a complex mixture of porphyrin species. The purportedly superior tumour-localizing and fluorescence properties of HpD were demonstrated clinically by Lipson *et. al*

(1961).and Lipson and Baldes (1961).

The first detailed clinical report of PDT with HpD was published in 1976 by Kelly and Snell who treated a patient with recurrent superficial bladder carcinoma. The clinical development of PDT was then systemically pursued by Dougherty's group, who published results of their clinical studies in 1977 and 1978, at the Roswell Park, Memorial Institute in Buffalo (Dougherty *et. al.*, 1977; 1978). This same group explored the clinical role of PDT, established some of the basic principles, and provided drug and advice to numerous investigations.

Clinical applications of PDT accelerated in the early 1980s, with the original lung cancer trials by Hayata and Kato. At the present time in the United States and Canada alone, there are clinical trials evaluating PDT for cancer in the following fields: otorhinolaryngology, gastroenterology, pulmonology, dermatology, gynecology, urology, neurosurgery, breast cancer, cardiovascular diseases and opthalmology.

Spinelli (1998) reported that a total of more than 3200 lesions were treated with PDT. The photosensitizers most widely used were dihaematoporphyrin ethers and esters (DHE) and HpD. Other modalities of treatment, e.g. radiotherapy, chemotherapy or surgery, have been combined with PDT for advanced lesions. There is a growing interest in the clinical indications of PDT, with a higher number of patients affected by superficial and early stage tumours receiving treatment over the years. PDT appears to have taken a rather long time to become an additional modality for patients with cancer.

1.2 Review Of An Alternative Procedure For Effective PDT

Despite the long history of the clinical use of HpD as a tumour localizer and therapeutic agent, the mechanism of its uptake and retention in malignant and normal tissues remains qualitative and not well understood. The PDT treatment protocol as practised currently was developed based on qualitative fluorescence data (Lipson *et. al.,* 1964 and 1967, Gregorie *et. al.*, 1968, Barker *et. al.*, 1970 and Dougerty *et. al.,* 1975). The idea of administering PDT at a delayed time of 24-72 hrs after the injection of HpD was presumably to allow serum clearance of HpD and also for the selective localization of HpD in the tumour. This was also thought to reduce the risk of unwanted photosensitization of the surrounding subcutaneous tissues.

However, *in-vivo* nuclear imaging studies of radiolabelled HpD by Zainuddin *et. al.* (1987 and 1988) argued that the assumed specificity of HpD for malignant tissue based on tissue fluorescence studies was in dispute. The results showed that the tumour to background ratio rapidly reached a constant value of approximately 5:1 immediately after injection in rats. This ratio remains constant for 24 hrs after which, there was a gradual decline in the level of HpD in the tumour. The results indicated that very high serum concentrations of HpD occurred in tumour bearing rats immediately after the intravenous administration of radiolabelled HpD. That study thus showed that the serum concentration of HpD in the tumour was at least 5 times higher than the normal tissues due to the vascularity of tumour tissues at all times, right after the *i.v.* injection of the photosensitizer.

It is therefore logical to question if the conventional protocol of light irradiation after a delay of 24-72 hrs post-HpD injection is in fact optimal for tumour destruction. With a much higher serum concentration of HpD immediately after the intravenous injection of HpD , it

might in fact be more efficacious to photoirradiate the tumour immediately for more effective destruction of the microvasculature of the tumour. This question is of particular practical relevance in view of the other HpD-PDT data, in which a number of studies have suggested that tumour necrosis occurs as a consequence of damage to the tumour microvasculature leading to vascular stasis and tumour hypoxia rather than a direct tumour cell kill (Bugelski *et. al.*, 1981; Henderson and Dougherty, 1984; Henderson *et. al.*, 1985; Star *et. al.*, 1984 and 1986).

In view of the above, a study was carried out at this centre to determine the efficacy of "immediate" light irradiation post injection PDT as an alternative treatment protocol to the conventional Delayed PDT protocol (Low *et al*, 1988, Olivo *et al*, 1989 and Olivo, 1990). The results of the animal model study with Immediate PDT showed that even with one-sixth the drug dosage, better tumour response was obtained with Immediate PDT when compared to delayed PDT. This suggests that serum HpD levels in the tumour vasculature rather than the intracellular HpD is a key determining factor for the treatment response in Immediate PDT. The poorer tumour response obtained for Delayed PDT may be the result of the much reduced serum HpD levels, which would have fallen considerably from its peak by 24-72 hrs.

This finding suggests that with the possibility of Immediate PDT, tumour localization and retention are no longer relevant requirements for a photosensitizer. Due to the higher vascularity of tumour tissues, higher photosensitizer concentration at all times is assured. With the relaxation of this requirement, it may be more appropriate to adopt immediate PDT protocol and that the photosensitizer used should be rapidly excreted from the body system, thereby reducing prolonged cutaneous photosensitivity. As a result of this, the original photosensitizer, Hp, a monomeric component of HpD was re-examined at this centre. Hp was targeted as the

alternative photosensitizer at this centre due to its most polar nature and being the most essential component compared to the other two monomers of HpD, which is hydroxyethylvinyl deuteroporphyrins (HvD) and protoporphyrin (Pp). Being a much simpler monomeric porphyrin, it allows detailed study of pharmacokinetic and distribution within organs. It has also good photophysical properties *in vivo*, whereby there is a high production of singlet oxygen for each photon absorbed by the drug.

Porphyrins are generally considered among the more difficult compounds to assay accurately. The analytical difficulties encountered are due primarily to the multiplicity of naturally occuring porphyrins and their existence in isomeric forms. A statement has been made in the literature that a pure sample of hematoporphyrin has never been produced (Doiron, 1982). Hence, Hp with 95% purity has been used as the photosensitizer for the studies at this centre. For this study, a purified Hp (95%) was obtained from Porphyrin Products, Inc., U.S.A.

1.3 Objectives Of The Study

The overall aim of this study is to investigate the possibility of more effective PDT with less skin photosensitivity by using the simpler porphyrin, Hematoporphyrin (Hp) as a possible photosensitizer for the treatment of malignancies using the alternative Immediate PDT procedure. The work described the serum and tissues pharmacokinetics of Hp with comparison to its derivatives, HpD as well as their efficacies using mice as an experimental model.

After the intravenous injection, the drugs are immediately present in the serum, and thus their pharmacokinetic behaviours were studied. This gave rise to important pieces of information, including how long the drugs remained in the body and how rapidly the drugs were

cleared. To describe the pharmacokinetics, simple mathematical relationships were required. Thus, a first order compartmental model was developed to quantitatively analyse the serum and tissue distributions of the drugs. Various rate constants between the different compartments were obtained. The analyses showed that Hp has much faster clearance rates from serum and tissues. These rate constants provide means to assess the relative duration of skin photosensitivity of Hp with respect to HpD as photosensitizers for the PDT malignancies.

The therapeutic response of the EMT-6 tumours in mice formed a large part of this thesis; the Immediate PDT using Hp as the photosensitizer is investigated and compared to HpD. As an extension to this, a histomorphological study of the effects of both the photosensitizers at a cellular and vascular level were also discussed. It was carried out to compare the extent of tumour necrosis. In addition, the effects of Hp and HpD as compared to normal tissue adjacent to the tumour (2 mm and 10 mm) were also assessed. At the same time, a comparison of the skin reaction and the duration of skin photosensitivity was made. The experiment was carried out by employing measured dosages of drugs and dose rates of light with known spectrum on normal skin of mice. The violet to deep blue spectrum (390-470nm) was used as a more effective wavelength to determine skin photosensitivity of slight redness to destruction of the epithelium and to avoid preliminary inflamation on the skin photosensitivity conferred by Hp and HpD. It is shown that mice injected with Hp exhibited a much lower skin reaction when compared to HpD. Although in response to tumours, Hp required a somewhat higher drug-light product, one could still minimise considerably the long term skin photosensitivity as exhibited in HpD.

This study also aims to develop a local Hp-Immediate PDT protocol for clinical trials

in the near future. To achieve this, clinical trials using the conventional Delayed PDT procedure was applied using Hematoporphyrin Derivatives(HpD) at this centre, as the necessary ground-work to prepare for the Hp-Immediate PDT clinical investigation programme in the near future. Therefore, this thesis includes the preparation, characterization and analyses of clinical HpD and the development of improved red light sources for routine PDT clinical applications.

Two red light sources were developed to be used as alternatives to the locally available laser systems, a commercial 65mW helium neon laser and a 500mW gold-vapour laser system developed at the University of Malaya for the treatment of clinical superficial malignancies. The simplicity in the construction and the cost-effectiveness of the two new light sources made them attractive alternatives for operation in clinical environment. Futhermore, it has a higher output power as compared to earlier incandescent lamps. This allows for shorter treatment exposure times. For routine clinical use, they have proven to be superior to the laser systems in terms of manoeuvrability in the clinical environment for the treatment of superficial tumours. The clinical results showed significant responses in palliation control over advanced external lesions. On the other hand, the He-Ne laser system was used in the Hp and HpD-Immediate PDT procedure to treat the smaller superficial murine tumours. The animal model studies using Hp-Immediate PDT with local laser system and the clinical trials using the conventional method HpD-Delayed PDT were the first phase of the studies in this programme. It will be extended to endoscopic and interstitial applications for the second phase in clinical trials involving treatment of internal malignancies at this centre at a later stage.

1.4 Outline Of The Chapters

This thesis comprises 7 chapters. Chapter 2 presents the biochemistry and photochemistry of Hp and HpD as photosensitzers in PDT. It also describes the preparation and characterization of both the photosensitizer and the analyses of clinical HpD. Chapter 3 presents possible light sources that are commonly used in HpD-PDT work with emphasis on two simple red light sources developed and modified for the present clinical trials. Also described in this chapter are photo-irradiation techniques employed using both the laser and the simple light sources. Chapter 4 presents the results of three clinical trials on superficial malignancies using the conventional delayed HpD-PDT. The modified red light sources were employed for this purpose. Chapter 5 describes the pharmacokinetics of Hp and HpD in serum and a few selected tissues in the murine system. Lastly, it presents the calculation of an appropriate compartmental model of the pharmacokinetics of Hp and HpD. Chapter 6 developes the use of Hp as a possible sensitizer using the alternative procedure referred to as Immediate PDT. It presents efficacy studies performed on a murine tumour system in which both Hp and HpD are undertaken using the laser system. It also includes a histology study to investigate the differences, if any, between Hp and HpD at cellular and vasculature levels. Lastly, the evaluation of photosensitivity reactions of normal murine skin to various light doses is conducted. Finally, Chapter 7 presents the conclusion of this study and discusses areas for further works and development so as to establish PDT as a suitable alternative or additional modality for cancer treatment.