CHAPTER SIX

6.0 Immediate PDT Using Hp As The Possible Photosensitizer To The Conventional HpD

6.1 Introduction

This chapter focuses on the investigation of the relative efficacy of Hp-Immediate-PDT on EMT-6 tumours. Following this, the next two sections, 6.2 and 6.3 describe the methodology of PDT of EMT-6 tumours and the experimental methodology of treatment for the investigation of Hp. The results of the study are presented in Section 6.4. As an extension of the first study, a histological study to investigate the effects of Hp compared to HpD at a cellular and vascular level are described in Section 6.5. The results of this study is presented in Section 6.6. A study on the comparison on skin reaction and duration of skin photosensitivity between Hp and HpD is presented in Section 6.7 and the results are presented in the following Section, 6.8. Finally, in Section 6.9 and 6.10, the discussion and conclusion for the three studies are presented.

6.2 Methodology Of PDT Of EMT-6 Tumours

6.2.1 *In vivo-In vitro* EMT-6 Tumour Line

The KHJJ tumour line, derived from a primary mammary tumour arising in a Balb/c mouse after implantation of a hyperplastic alveolar nodule, has been maintained for over 100 transplant generations. The 25 th generation of this tumour was grown and selected in cell culture, producing a tumour cell-line, EMT-6 (Experimental Mammary Tumour-6). This line

can be grown and studied either as an animal tumour or a cell culture (Rockwell, 1972 and 1977). It is a rapidly growing, aggressive, transplantable tumour line. This tumour line is sarcomatose with cellular characteristics and anaplastic showing little, if any, histologic structures or biochemical functions suggestive of differentiation EMT-6 cells injected subcutaneously or intradermally into appropriate Balb/c mice form solid tumours. Tumours become palpable approximately 3-4 days after the intradermal injection of 10^5 cells. They reach the experimental size (approximately 50-80 mm^3 in volume) in approximately 10-14 days. At this size, the tumours are homogeneously white, with minimal spontaneous tumour necrosis or absent. EMT-6 tumour rarely metastasizes but kills the host through attaining great size in an approximately one month. It has been observed that EMT-6 cells evoke a immunologic response. Approximately 1-2 % of the Balb/c inoculated with 3-4 X 10^5 EMT-6 cells for routine propagation and experimental use do not develop tumours. Regressions of growing tumours occasionally are also observed.

6.2.2 Method Of Tumour Induction, Propagation, Detection And Determination Of The EMT-6 Tumour

The EMT-6 tumour line was obtained from Cambridge, U.K. The cell lines were cultured at 37^0C in Eagle's MEM with Earles' salt, supplemented with Glutamine (2mM final concentration), antibiotics (penicillin/ streptomycin/ gentamicin) and 20% new born calf serum (Flow Laboratories, North Ryde, Australia). The cells were checked daily to monitor the state of the culture medium and their growth. The culture medium was changed every 2-3 days. Single cell suspensions were then produced by rinsing the cells in a solution of 0.1% Trypsin

in PBS for 2 mins followed by incubating at 37°C for 5 mins. The cells were then spun down at 800 rpm for 5 mins and the supernatant discarded. These were then suspended in PBS and the number of cells was assessed using trypan blue exclusion test. Cells were diluted 1:10 in 0.1% trypan blue solution and counted on a haemacytometer. The cells were then inoculated intradermally into the flank of Balb/c mice. Each mouse received a 0.5 ml suspension containing 2-4 X 10^5 cells. The tumours usually reached a size of 3.5-8.0 mm or more in diameter 10 to 14 days after inoculation of the tumour cell line. To preserve the cells for further propagation of the tumour line, they were placed in the culture medium containing 10% dimethylsulphoxide (DMSO) to a cell concentration of 1X10^6 /ml. These were then aliquoted into 2.5 ml cryovials and kept at -70°C overnight in a polystyrene box. The vials were then transferred to liquid nitrogen until use. In the next cycle of propagation, the cryovials containing cells were removed from liquid nitrogen and immediately thawed in a 37°C water bath. The cells were spun down as mentioned above and were resuspended in the culture medium and introduced into culture flasks and incubated at 37°C.

Each mouse selected for the experiment was 7-8 weeks old. The hair at the site of the tumour was shaven with an animal clipper each time before the inoculation of the cells. Palpable mammary tumours larger than 3.0 mm in diameter were selected for experimental studies. Random samples of the induced tumours were aspirated. The aspirated tissue was processed for light microscopy (cytology processing) and examined microscopically for classification. A representative of tumour examined under light microscopy is shown in Fig 6.1.

Fig. 6.1 Photomicrograph shows an EMT-6 induced malignant tumour. H & E, X200

6.3 Investigation Of Tumour Response Using Hp As The Possible Photosensitizer Compared To The Conventional Photosensitizer, HpD

6.3.1 Treatment Protocol

One day after the random diagnostic aspiration, mice were selected for Hp and HpD-PDT. The mice were divided into 7 groups of 4-13 mice. All the mice in Group 1, 2 and 3 were administered intravenously with Hp at a dose of 20, 25 and 30 mg/kg body weight respectively. The mice in Group 4 and 5 were administered a dose of 10 mg/kg body weight of HpD and Group 6 and 7 were administered with a dose of 15 mg/kg body weight of HpD. Before treatment was given, the mice were anaesthetised with Zoletil 50 (Tiletamine and Zolazepam; 50 mg/ml) at a dose of 41.7 mg/kg body weight.

The He-Ne laser was used for light delivery. The exposed tumour surface was illuminated superficially with a laser beam. The laser beam having a Gaussian profile was expanded using a lens to enclose the whole tumour with near uniform illumination. In this experiment, surface illumination was adopted for tumours between 3.0 - 8.0 mm size along the major axis diameter and depth ranging from 2.5-3.5 mm. Hair over the tumour site was removed prior to light irradiation with an animal clipper.

Light irradiation was done 5 mins after the administration of Hp and HpD. Photodynamic dose is dependent on the local optical fluence, the optical wavelength, the retention of the administered drug at the time of exposure and on the concentration of oxygen in the neoplastic tissue. Assuming conditions where no oxygen limitations are present, clinical and experimental data suggested that tumour destruction was a function of the porphyrin and light product (Konaka and Ono, 1983; Cowled and Forbes, 1985). There is actually a reciprocal relationship between drug (Hp and HpD) and light dose in PDT (Gibson and Hilf, 1985 and Henderson *et al*, 1983). Hence, a product of drug–light dose is expressed in the tumour response and skin photosensitivity.

Tumours subjected to Hp-PDT were irradiated with a drug-light dose product of 6000 J.mg/kg.cm^2, 7500 J.mg/kg.cm^2 and 9000 J.mg/kg.cm^2. Drug-light dose product for Hp-PDT lower than 6000 J.mg/kg.cm^2 does not produce any significant response on EMT-6 murine tumours. On the other hand, tumours subjected to HpD-PDT were irradiated with a drug-light dose product of 1000 J.mg/kg.cm^2, 1500 J.mg/kg.cm^2, 2250 J.mg/kg.cm^2, 3000 J.mg/kg.cm^2.

The therapeutic dose of HpD for DMBA- induced- tumour in rats was 10 mg/kg body weight with an energy of 100 J/cm^2 (Olivo, 1990). However for the EMT-6 tumours, a dose

of 10 mg/kg HpD and a 100 J/cm^2 of light (1000 J.mg/kg.cm^2), were not sufficient to give significant response to the tumours. Hence an increase of drug dose to 15 mg/kg and 150 J/cm^2 of light (2250 J.mg/kg.cm^2), was employed. The latter dosage gave a high therapeutic response to the tumours. This varation in response could be due to different species of animals used and different morphology of tumours.

In order to monitor the surface temperature of the tumour in the mice, a thermistor probe which is connected to a digital readout meter (Fluke 52 $^{K/J\ Thermometer}$) was employed. During irradiation, the thermistor probe was placed on top of the tumour and the temperature was maintained below the hyperthermia effect throughout the experiment.

During illumination, the laser beam was made to cover the whole tumour mass as well as 2 mm beyond the circumference of the boundary / periphery of the palpable tumour mass. In the surface illumination technique, the laser beam spot diameter was varied from 0.4-1.5 cm according to the diameter of the tumour. With the He-Ne output of 65 mW, power densities ranging from 40-300mW/cm^2 were obtained. The tumour exposure time was determined knowing the total energy density required (100-300J/cm^2) and the applied power densities of 50-230mW/cm^2. This corresponded to the tumours being exposed to 7-22 mins of laser light depending on the size of the tumour.

6.3.2 Control Experiments

Control comprised three groups.

Group 1 consisted of 12 mice that were subjected to the four different dosimetry of red light as the test group but were not administered Hp or HpD.

Group 2 consisted of 15 mice that were given Hp or HpD but not irradiated with red light. Hp

dosages of 20, 25 and 30 mg/kg were used. On the other hand, HpD dosages of 10 and 15 mg/kg were used, with three mice subjected to each dosage.

Group 3 consisted of 20 mice that were given neither HpD, Hp nor PDT. This group served as a positive control to ascertain the natural history of the tumour without any interference.

6.3.3.Post Treatment Protocol

After Hp and HpD-PDT, the mice were placed in the dark to prevent photosensitivity for 1 week. After that they were transferred to normal room conditions. The mice were observed everyday during the first week after PDT after which they were observed thrice a week for 1 month.

During these observations, measurements of the dimension of the tumour were recorded, photographs of lesions were taken weekly to make a visual comparison of the tumour before and after it had been treated. A visual description of the tumours and any other pathological description were recorded. Also, any behaviorial pattern changes were recorded.

6.3.4 Tumour Volume Assessment

The tumour size was measured approximately along three orthogonal diameters (D_1, D_2 and D_3). Evensen *et. al.* (1987) calculated the tumour volume based on a spheroidal geometry. The volume of the tumour was assumed to be proportional to the three orthogonal diameters of the tumour mass. In this experiment, the EMT-6 tumours showed variability in dimensions in the xy plane (D_1 and D_2) but the thickness (D_3) remained relatively uniform with an average thickness of 2.5-3.5 mm. For all studies described in this thesis, tumour volumes were

determined based on a spheroidal geometry.

6.3.5 Tumour Response Assessment

Tumour responses were assessed based on a single Hp or HpD-PDT treatment for each mouse. Individual tumours were observed and monitored for a period of 1 month after the treatment. One week after the PDT, an aspiration of the lesion treated was taken with the fine needle aspiration technique (FNA). A substantial amount of tissue was taken and smeared on the glass slide. It was then processed by cytology staining to be viewed under a light microscope by a pathologist.

Apart from a cytological assessment, a gross visual assessment was made, where the tumour response was based on the reduction in tumour volume. The tumour responses were defined as follows:

Complete response (CR): No palpable tumour at the end of the observation period.

Significant response (SR): Tumour volume reduction by 51-99 %

Partial response (PR): Tumour volume reduction by 20-50%

No response (NR): Tumour volume reduction by less than 20%

For a given test group, the average reduction in tumour volume for that sample and the percentage of response were assessed. The average tumour volume reduction is computed for the remaining reduction volume test group of mice whereas the percentage of response was computed for the number of mice with complete or significant response with respect to the total number of mice for the test group. Statistical analyses were also performed to ascertain the level of significance of the data obtained.

6.3.6 Panicolaous Stain For Tumour Tissue Smears (Cytological Processing)

The aspirated tissues from the tumours were prepared and stained using the following procedure:

Chemical	Duration
80% alcohol	10 dips
70% alcohol	10 dips
50% alcohol	10 dips
Distilled water	20 dips
Harris haematoxylin	3 mins
running tap water	till clear (6 mins)
0.5% HCL in distilled water	1 dip
running tap water	6 mins
50% alcohol	10 dips
70% alcohol	10 dips
80% alcohol	10 dips
95% alcohol	10 dips
OG-6	3mins
95% alcohol	4 dips
Ea-50	3 mins
95% alcohol	4 dips

absolute alcohol	5 dips
absolute alcohol	4 mins
absolute alcohol	5 mins
xylene	0 dips (clear)
xylene	5 mins

6.4 Results

Soon after the treatment, all tumours treated within the test group showed darker discolouring as of hematoma. Within 24 hrs of treatment, the tumours were frequently not very palpable because of tumour volume shrinkage and edema surrounding the treatment field. The skin directly overlying the tumour invariably underwent necrosis over a period of 1-2 days. This necrotic process continued until the whole tumour turned into a black necrotic mass. Along with this, there was a noticeable reduction in tumour volume. About three to four days after therapy, a hard brown crust was formed over the tumour. About six days after the treatment, the scab usually dropped off and a residual sore was left behind. By this time, it was considered to be a response to treatment if it is reduced to a nonpalpable mass. The healing process with complete re-epithelialization of the site occured within ten to twelve days post-therapy by which time there was regrowth of hair over the former tumour site. The sequence of changes observed in the treated tumour lesions is depicted in Figs. 6.2, 6.3, 6.4 and 6.5.

Tumours in all the mice were observed for a period of one month following PDT for both the drugs, Hp and HpD. For each group of mice, an average response was calculated. The

results of the various groups are shown in Tables 6.1 to 6.8. Fig. 6.6 shows the percentage of tumour response treated with HpD and Hp with various drug-light dose product. The measurements of tumour size presented in Tables 6.1 to 6.7 were done at one month after post treatment.

In the first experiment (Table 6.1), EMT-6 tumours were treated with red light from the He-Ne laser source using the surface method. The mice were administered with a Hp drug-light dose product of 6000 J.mg/kg.cm^2. 30 % of the tumors treated showed partial response but regrew either from the side or underneath the previous tumour after about two weeks post treatment. Only 10 % responded completely in the treatment and the tumour reduction is 9.43%. The rest of the tumour did not respond. Tumours that did not respond to Hp-Immediate PDT continued to grow in size to exceed 1.5 cm in diameter and about 1.0 cm in thickness.

However, this level of dosimetry was found to be insufficient to bring about good tumour responses, the drug-light dose product was then increased to 7500 J.mg/kg.cm^2. This dosimetry caused a 92.3 % tumour response with a 90.76% average reduction in tumour volume (Table 6.2). The response observed as compared to the controls (Table 6.8) was statistically significant ($p < 0.001$; Fisher's exact probability test). The control group showed only a small average response of 10 % presumably due to spontaneous tumour regression (Table 6.8).

In order to enhance the therapeutic response for Hp, a higher drug dose was administered. The mice were given a Hp drug-light dose product of 9000 J.mg/kg.cm^2. Results in Table 6.3 showed that nine out of ten mice treated showed a complete response which constituted a response of 90 % with an average reduction in tumour volume of 87.33%. The

difference in response between the test group (Table 6.3) and the controls (Table 6.8) was found to be statistically significant ($p < 0.001$; Fisher's exact probability test). Statistical analysis of comparison between treatment with a dose of Hp, 25mg/kg and a dose of Hp, 30 mg/kg was found to be statistically not significant (Fisher's exact probability test). For both the latter doses, an average of 91% of animals responded either completely or significantly to Hp Immediate PDT.

On the other hand, mice given a HpD drug-light dose of 1000 J.mg/kg.cm^2 and 1500 J.mg/kg.cm^2, gave a recorded response of 20% each with a tumour reduction of 20% and 19.08% respectively (Table 6.4 and 6.5). For the drug-light dose product of 2250 J.mg/kg.cm^2 and 3000 J.mg/kg.cm^2, it gave an average in tumour response of 90% and 100% respectively and an average reduction in tumour volume was observed to be 83 % and 100% respectively (Table 6.6 and 6.7). The response observed as compared to the controls (Table 6.8) was statistically significant ($p < 0.001$; Fisher's exact probability test).

The above results are summarized in Fig.6.6 which shows that HpD has a threshold drug-light dose of 1500 J.mg/kg.cm^2 and 2150 J.mg/kg.cm^2 for 50 % tumour regression whereas Hp gives a threshold value of between 6000 J.mg/kg.cm^2 and 7100 J.mg/kg.cm^2. An almost complete response using Hp indicated by the graph is at a drug-light dose product of 7500 J.mg/kg.cm^2 i.e. a drug dose of 25mg/kg and a light dose of 300 J/cm^2. On the other hand, a HpD gives a drug-light dose product of 2250 J.mg/kg.cm^2 with a drug dose of 15 mg/kg body weight and a light dose of 150 J/cm^2 for an almost complete tumour response. The percentage of tumour volume reduction has a similar pattern (Fig.6.7) as the percentage of tumour response shown in Fig.6.6.

The entire duration of the irradiation produced a 1.4 ^{0}C temperature rise at the top of the tumours (i.e. approximately to 37.3 ^{0}C from an initial temperature of 35.9 ^{0}C).

Table 6.1 : Data for EMT-6 murine tumours subjected to Hp-PDT at a drug-light dose product of 6000 J.mg/kg.cm^2

Mouse No.	Tumour size before therapy (cm)	Tumour size after therapy (cm)	Tumour response
M. 1*	0.43 X 0.4	1.2 X 0.9	N.R.
M. 2	0.42 X 0.42	0.1 X 0.1	C.R.
M. 3	0.46 X 0.5	1.16 X 1.72	N.R.
M. 4	0.68 X 0.39	1.68 X 1.42	N.R.
M. 5*	0.5 X 0.37	0.82 X 0.8	N.R.
M. 6*	0.37 X 0.53	1.15 X 1.19	N.R.
M. 7	0.56 X 0.42	2.53 X 2.0	N.R.
M. 8	0.33 X 0.39	1.65 X 2.1	N.R.
M. 9	0.39 X 0.45	0.9 X 1.55	N.R.
M. 10	0.39 X 0.51	1.15 X 1.45	N.R.

Note : All lesions were between 0.25-0.35 cm in thickness before PDT

* Three mice of the test group responded partially after treatment but regrew after 2 weeks.

Table 6.2 :Data for EMT-6 murine tumours subjected to Hp-PDT at a drug-light dose product of 7500 J.mg/kg.cm^2

Mouse No.	Tumour size before therapy (cm)	Tumour size after therapy (cm)	Tumour response
M. 1	0.74 X 0.48	0.0 X 0.0	C.R.
M. 2	0.45 X 0.46	2.09 X 2.23	N.R.
M. 3	0.46 X 0.42	0.0 X 0.0	C.R.
M. 4	0.55 X 0.37	0.0 X 0.0	C.R.
M. 5	0.54 X 0.44	0.0 X 0.0	C.R.
M. 6	0.7 X 0.4	0.0 X 0.0	C.R.
M. 7	0.35 X 1.0	0.0 X 0.0	C.R.
M. 8	0.7 X 0.77	0.0 X 0.0	C.R.
M. 9	0.32 X 0.7	0.0 X 0.0	C.R.
M. 10	0.4 X 0.62	0.1 X 0.1	S.R
M. 11	0.46 X 0.54	0.0 X 0.0	C.R.
M. 12	0.4 X 0.61	0.16 X 0.2	S.R.
M.13	0.55 X 0.69	0.1 X 0.1	S.R.

Note : All lesions were between 0.25-0.35 cm in thickness before PDT

Table 6.3 : Data for EMT-6 murine tumours subjected to Hp-PDT at a drug-light dose product of 9000 J.mg/kg.cm^2

Mouse No.	Tumour size before therapy (cm)	Tumour size after therapy (cm)	Tumour response
M. 1	0.51 X 0.63	0.2 X 0.26	S.R.
M. 2	0.5 X 0.8	0.0 X 0.0	C.R.
M. 3	0.37 X 0.55	0.0 X 0.0	C.R.
M. 4	0.37 X 0.72	1.68 X 1.42	N.R.
M. 5	0.54 X 0.43	0.0 X 0.0	C.R.
M. 6	0.49 X 0.46	0.0 X 0.0	C.R.
M. 7	0.5 X 0.8	0.19 X 0.22	S.R.
M. 8	0.31 X 0.47	0.0 X 0.0	C.R.
M. 9	0.5 X 0.64	0.0 X 0.0	C.R.
M. 10	0.42 X 0.53	0.0 X 0.0	C.R

Note : All lesions were between 0.25-0.35 cm in thickness before PDT

Table 6.4 : Data for EMT-6 murine tumours subjected to HpD-PDT at a drug-light dose product of 1000 J.mg/kg.cm^2

Mouse No.	Tumour size before therapy (cm)	Tumour size after therapy (cm)	Tumour response
M. 1	0.42 X 0.52	1.25 X 2.1	N.R.
M. 2	0.4 X 0.52	0.0 X 0.0	C.R.
M. 3	0.37 X 0.55	1.2 X 1.6	N.R.
M. 4	0.37 X 0.53	1.8 X 1.4	N.R.
M. 5	0.36 X 0.53	0.0 X 0.0	C.R.
M. 6	0.45 X 0.55	1.45 X 1.7	N.R.
M. 7	0.4 X 0.49	0.95 X 1.19	NR.
M. 8	0.49 X 0.36	1.61 X 1.72	NR.
M. 9	0.5 X 0.6	1.42 X 1.68	N.R.
M. 10	0.33 X 0.61	2.03 X 2.12	N.R

Note : All lesions were between 0.25-0.35 cm in thickness before PDT

Table 6.5 :Data for EMT-6 murine tumours subjected to HpD-PDT at a drug-light dose product of 1500 J.mg/kg.cm^2

Mouse No.	Tumour size before therapy (cm)	Tumour size after therapy (cm)	Tumour response
M. 1	0.42X 0.52	0.1 X 0.1	S.R.
M. 2	0.35 X 0.37	0.9 X 1.2	N.R.
M. 3	0.33 X 0.52	1.5 X 1.3	N.R.
M. 4	0.42 X 0.44	2.09 X 1.45	N.R.
M. 5	0.42 X 0.35	1.23 X 2.0	N.R.

Note : All lesions were between 0.25-0.35 cm in thickness before PDT

Table 6.6 : Data for EMT-6 murine tumours subjected to HpD-PDT at a drug-light dose product of 2250 J.mg/kg.cm^2

Mouse No.	Tumour size before therapy (cm)	Tumour size after therapy (cm)	Tumour response
M. 1	0.5 X 0.37	0.0 X 0.0	C.R.
M. 2	0.45 X 0.3	0.0 X 0.0	C.R.
M. 3	0.37 X 0.42	0.0 X 0.0	C.R.
M. 4	0.42 X 0.44	0.0 X 0.0	C.R.

M. 5	0.55 X 0.3	0.0 X 0.0	C.R.
M. 6	0.45 X 0.35	0.0 X 0.0	C.R.
M. 7	0.3 X 0.55	0.2 X 0.1	S.R.
M. 8	0.3 X 0.48	0.1 X 0.15	S.R.
M. 9	0.39 X 0.41	0.3 X 0.25	S.R.
M. 10	0.45 X 0.3	1.2 X 0.9	N.R

Note : All lesions were between 0.25-0.35 cm in thickness before PDT

Table 6.7 : Data for EMT-6 murine tumours subjected to HpD-PDT at a drug-light dose product of 3000 J.mg/kg.cm^2

Mouse No.	Tumour size before therapy (cm)	Tumour size after therapy (cm)	Tumour response
M. 1	0.45 X 0.55	0.0 X 0.0	C.R.
M. 2	0.51 X 0.47	0.0 X 0.0	C.R.
M. 3	0.48 X 0.42	0.0 X 0.0	C.R.
M. 4	0.56 X 0.56	0.0 X 0.0	C.R.

Note : All lesions were between 0.25-0.35 cm in thickness before PDT

Table 6.8 : Average response of control mice using Hp or HpD as the drug (PDT only, Hp or HpD only, neither Hp or HpD nor PDT)

Tumour system	PDT only	HpD or Hp only (average for all Hp or HpD levels)	no HpD or Hp nor PDT
EMT-6	8.3 %	13.3%	10%

Note : All control lesions were between 0.25-0.35 cm in thickness before treatment

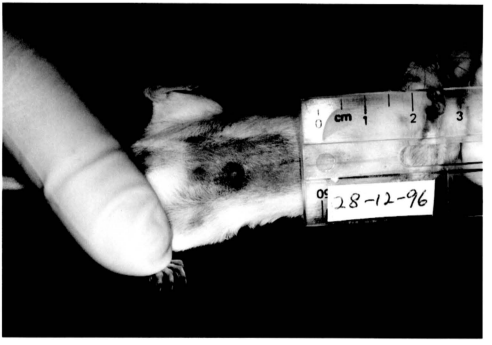

Fig. 6.2 : EMT-6 tumour before PDT

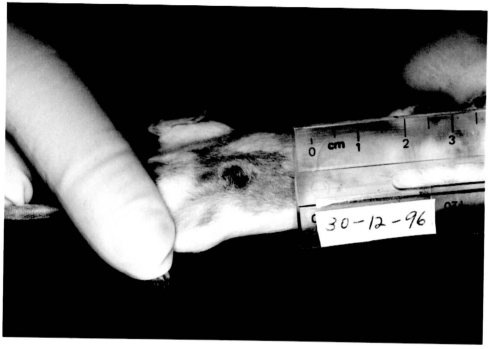

Fig. 6.3 : EMT-6 tumour 2 days after PDT. Tumour has turned necrotic..

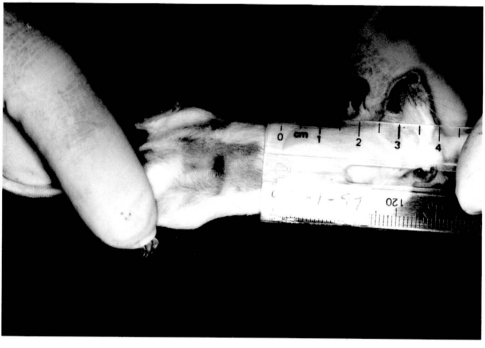

Fig. 6.4 : EMT-6 tumour, 14 days after PDT. A residual sore is left behind.

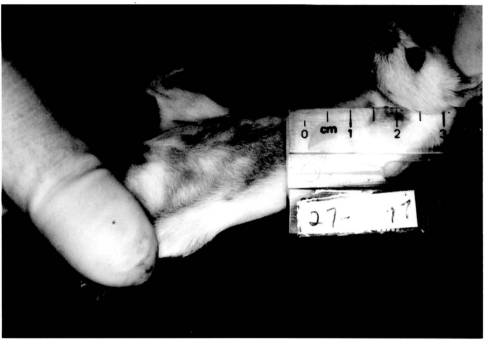

Fig. 6.5 : EMT-6 tumour, 1 month after PDT showing complete tumour regression with no visible sign of the tumour.

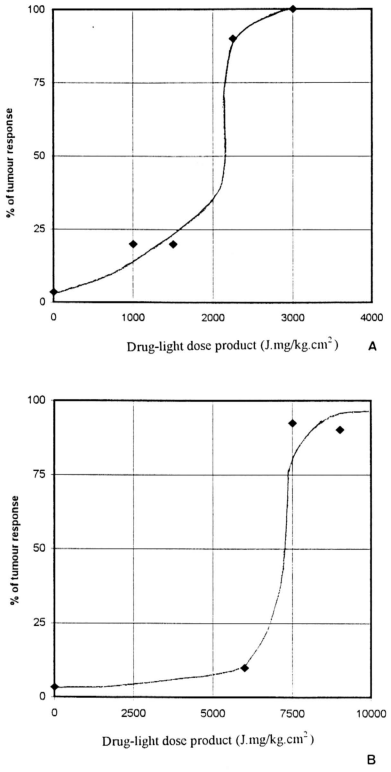

Fig. 6.6 Percentage of tumour response treated with HpD (A) and Hp(B) with respect to various drug-light dose product.

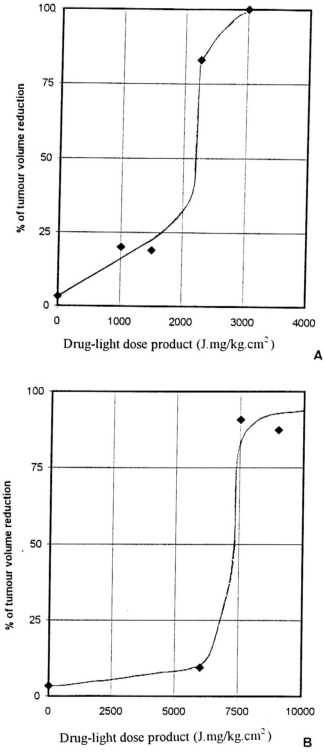

Fig. 6.7 Percentage of tumour volume reduction treated with HpD (A) and Hp(B) with respect to various drug-light dose product.

6.5 A Histomorphologic Study Of The Effects Of Hp As Compared To HpD On EMT-6 Tumours

6.5.1 Introduction

This study investigates the histomorphological changes in EMT-6 (Experimental Mammary Tumour Line-6) tumours as a result of Hp and HpD. It was carried out to compare the extent of tumour necrosis resulting from Hp and HpD. In addition, the effects of Hp as compared to normal tissue adjacent (2 mm and 10mm) to the tumour were also assessed. This study also examines light microscopy of changes in vascular morphology for both Hp and HpD. In view of the fact that Immediate PDT using Hp was found to be comparable with HpD in the earlier study at appropriate drug-light dose product values, it is hoped that this study will contribute towards a better understanding of the underlying mechanism of tumour destruction in PDT.

6.5.2 Experimental Methodology

The EMT-6 tumour line was induced and propagated in Balb/c mice as described. Tumours were induced in eighteen mice comprising 6 groups of three mice. The Hp dose injected was therapeutic dose of 25mg/kg body weight and the HpD therapeutic dose was 15 mg/kg body weight. Following injection, nine mice (Groups 1-3) were administered with Hp. The remaining nine mice (Groups 4-6) were administered with HpD. The dosimetry applied was the same as described earlier in this chapter for both the drugs. The irradiating beam spot had a power density ranging from 40-300 mW/cm^2. The tumours which were administered with Hp were exposed to $300J/cm^2$ of energy fluence and the tumours which were administered with

HpD were exposed to 150J/cm^2 of energy fluence.

Subsequent to either PDT for the drugs, the mice were sacrificed at different time intervals of 1 day, 3 days and 7 days. Groups 1 and 4 were sacrificed 1 day after PDT, Groups 2 and 5 were sacrificed 3 days after PDT, Groups 3 and 6 were sacrificed 7 days after PDT. The tumour and the surrounding normal tissue 2 mm and 10 mm away from the tumour site of each sacrificed mouse were excised and processed for light microscopy, using the methodology as described in Section 6.5.4. Vascular changes were also assessed histologically.

6.5.3 Histopathology Examination And Scoring Systems

(a) **Necrosis**

Necrosis was assessed in the sampled tumour tissue and its surrounding normal tissue at 2 mm and 10 mm away from the tumour by light microscopic examination. In order to objectively assess tumour necrosis the extent of necrosis in the tumour section was scored. A score of 0 denoted no necrosis present in the tumour section. A score of 1 denoted that about 10 -20 % of the tumours were necrotic. A score of 2 denoted necrosis of 30-60% of the tumour and a score of 3 denoted extensive necrosis involving 70-100% of the tumour with hardly any viable tumour present.

Necrosis of the surrounding normal tissue (Connective tissue, fat or muscle) was similarly scored : a score of 0 denoting no evidence of necrosis; a score of 1 denoting patchy necrosis, each necrotic focus being not extensive enough to fill one low power field (100X magnification). A score of 2 denoting extensive necrosis, the necrotic area being large enough to fill one low power field (100 X magnification).

(b) **Vascular changes**

Features of vascular damage, namely, hyperaemia, vessel wall inflamation, fibrin thrombi formation and fibrinoid necrosis were noted. Each vascular feature was scored as follows : a score of 0 denoted no morphological evidence of vascular damage , a score of 1 denoted the vascular change in a few vessels , and a score of 2 denoted the vascular change in most intratumour vessels.

6.5.4 Histophatological Processing

One-half of each tissue excised was cut perpendicular to the skin and fixed in 10% phosphate buffered formalin for 24 hrs. The fixative was prepared by adding 3.5 gm of NaH_2PO_4 (anhydrous) and 6.5 g of Na_2HPO_4 (anhydrous) in 1 litre of 40% formaldehyde solution. After 24 hrs of fixation, each tissue sample was trimmed and fix into cassettes for histopathological processing. The trimmed tissues were then dehydrated with graded methanol, cleared with toulene and infiltrated with paraffin through a Fischer Histomatic Tissue Processor (Model 166) using the schedule:

Chemicals	**Duration**
10% Formalin	0.5h
10% Formalin	1.0h
95% Alcohol	1.0 h

95% Alcohol	1.0h
100% Alcohol	1.0h
100% Alcohol	1.0h
100% Alcohol	1.5h
100% Alcohol:Toulene	1.0h
Toulene	2.0h
Wax	2.0h

After processing, the tissues were embedded in hot liquid paraffin wax using plastic embedding moulds and then cooled. 2-4μm thick section were cut using a Rotary A0 microtome, mounted on microscopic glass slides and routinely stained with haematoxylin and eosin (H and E).

The haematoxylin and eosin stains for light microscopy was prepared using the following procedure:

(i) **Haematoxylin**

Solution A consisted of 10 g of haematoxylin dissolved in 100ml of 10% alcohol. Solution B consisted of 200 g of alum dissolved in 2 litres of hot distilled water. Solutions A and B were mixed throughly and brought to boil. The flame was then withdrawn and 5 g of mercuric oxide added slowly.

(ii) **Eosin**

A working solution of eosin was prepared from a stock solution. The stock solution was prepared by adding 1 g of eosin Y in a mixture of 20 ml distilled water and 80 ml of 95% ethanol. The working solution was prepared by adding 100 ml of stock solution to a mixture of 300 ml of 80% ethanol and 2 ml of glacial acetic acid.

The staining of sections with H and E was carried out according to the schedule given below. After haematoxylin staining (1) the sections were checked microscopically for optimal differentiation before proceeding with eosin staning (2). The sections were mounted in Depex solution and coverslipped. They were then ready for examination under a light microscope.

Chemicals	Duration
(1) Haematoxylin staining	
Xylene	3 mins
Xylene	3 mins
95% Alcohol	2 mins
95% Alcohol	2 mins
95% Alcohol	2 mins
70% Alcohol	2 mins
Tap water to wash well	3 mins
Haematoxylin	10 mins

Tap water to wash well	3 mins
Acid-Alcohol	1-2 dips
Tap water to wash well	3 mins
Potassium acetate	2-3 dips
Tap water to wash well	3 mins
(2) Eosin staning	
80% Alcohol	1 min
Eosin	4 mins
95% Alcohol	5-10 dips
95% Alcohol	5-10 dips
95% Alcohol	5-10 dips
100% Alcohol	3 mins
Xylene	3 mins
Xylene	3 mins
Mounting	3 mins

6.6 Results

Tumour Necrosis

The average tumour necrosis scores for each group of 3 mice subjected to Hp and HpD for various post treatment intervals (2 hrs, 24 hrs, 72 hrs and 168 hrs (7 days)) are charted in Table 6.9. Fig 6.8 illustrates a comparable extent of tumour necrosis observed after the

treatment with Hp and HpD at 168 hrs (7 days) post treatment. There was no difference in tumour necrosis between Hp and HpD at all the time intervals.

Necrosis In Surrounding Tissue

The average surrounding tissue necrosis scores for each group of 3 mice subjected to Hp and HpD for various post-treatment intervals are also charted in Table 6.9. No necrosis was observed in the tissue adjacent (2mm) to the tumour at up to 2 hrs post-treatment for both the drugs. At 24 hr post treatment period, both the drugs resulted in comparable, moderate necrosis (score 1) in the immediate surrounding tissue as illustrated in Fig 6.9. For the period between 72-168 hrs, the immediate surrounding tissue necrosis for drug treated with Hp appeared to be less than for drug treated with HpD. However, this difference was not statistically significant. Necrosis was not observed in normal tissues situated at 10 mm away from the tumour treated with both the drugs, Hp and HpD (Table 6.9).

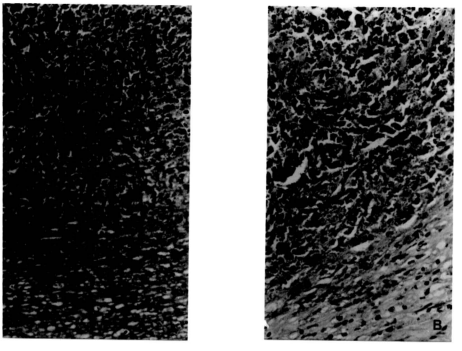

Fig. 6.8 : EMT-6 tumours, 7 days after PDT with Hp (A) and HpD (B) respectively. Score 3 (extensive necrosis). H &E, X100.

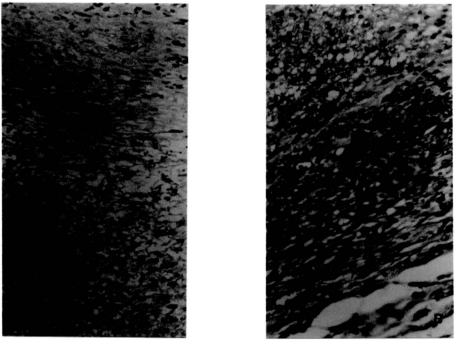

Fig. 6.9 : Tissue adjacent to tumour (2mm), 24 hrs after PDT with Hp (A) and HpD (B) respectively. Score 1 (moderate necrosis). H &E, X100.

Vascular Changes

Apart from necrosis to tumour and normal tissue, both the drugs also caused damage to the microvasculature. Average scores for hyperaemia using Hp and HPD are shown in Figs. 6.10 and 6.11 respectively. Features of vessel wall inflamation, fibrin thrombi formation and fibrinoid necrosis were observed for both the drugs. Table 6.10 charts the cumulated average scores of all vascular changes for each group of 3 to 5 mice subjected to Hp and HpD at various post-treatment intervals.

In general, treatment with the drug Hp achieved a higher score for fibrinoid necrosis and the inflamation of the vessel wall than the drug HpD, the difference observed being not statistically significant. Whereas, treatment with the drug HpD achieved a higher score for hyperanemia and fibrin trombosis but there was no significant difference in those scores as well.

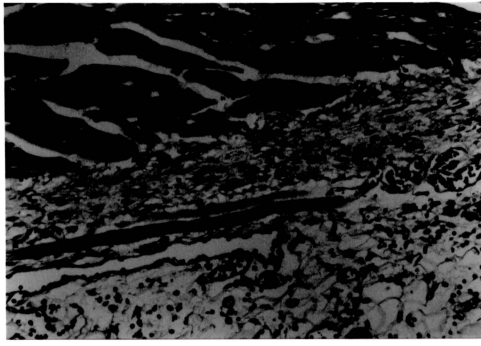

Fig. 6.10 : Mild (score 1.25) hyperaemia of tumour vasculature. H & E, X100

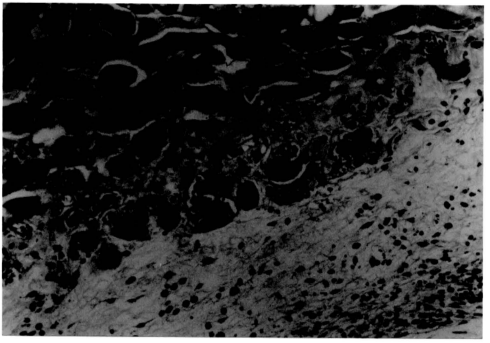

Fig. 6.11 : Severe (score 1.7) hyperaemia of tumour vasculature. H & E, X100

Table 6.9 : Average Histological Scores For Necrosis In Tumour And Adjacent Tissues Following Hp and HpD-PDT (drug-light product) By Post- treatment Intervals

		Average Necrosis Scores (3 mice each group)		
Drug :	Post treatment interval (hrs)	Tumour necrosis	Surrounding tissue necrosis	
Hp (7500 J.mg/kg.cm^2)			2 mm	10 mm
	2	1	0	0
	24	2	1.0	0
	72	3	0.5	0
	168	3	0.6	0
HpD (2250 J.mg/kg.cm^2)				
	2	1	0	0
	24	2	1.0	0
	72	3	1.2	0
	168	3	2.0	0

Table 6.10 : Scores For Vascular Damage In Hp and HpD-PDT

Drug	Average cumulated scores for all the intervals (a group of 3-5 mice)			
	Hyperaemia	Fibrin trombosis	Fibrin necrosis	Inflamation of th vessel wall
Hp	1.25	0.25	0.1	0.2
HpD	1.7	0.35	0.07	0.14

6.7 A Study On Skin Reaction Of Mice Using Hp Compared To HpD

6.7.1 Introduction

Photosensitivity reactions occur in biologic systems as a result of the interaction of appropriate wavelength of electromagnetic radiation with specific chromophores. Typical skin manifestations of acute photosensitivity consists of immediate erythema and edema when expose

to strong light. Lower dosage of light would result in accumulated mild photosensitivity and later accomplished by burning sensation, which is followed by the appearance of delayed erythema and edema 12-24 hrs later. Severe reactions could result in thick eschar formation or deep ulceration. This would heal up within 2 months. Skin is the only organ that is repeatedly exposed to solar and artificial light. Severe generalized photosensitivity of skin to sunlight is the major side effect of PDT when porphyrins are used as the sensitizers. Patients receiving HpD for PDT often suffer from photosensitive reactions and the skin has been thought to remain photosensitive for 4-6 weeks by anecdotal evidence. All patients receiving HpD-PDT were advised to remain out of direct sunlight and to use sunscreen.

The purpose of this investigation was to compare the skin reaction and the duration of skin photosensitivity of Hp to HPD. It was carried out by employing measured dosage and dose rates of light with a known spectrum. The violet to deep blue spectrum (390-470nm) was used as an effective wavelength to determine skin photosensitivity of slight redness to destruction of the epithelium and to provide preliminary information on the duration of skin photosensitivity conferred by Hp and HpD. This wavelength was used because of the absorption spectrum of both the photosensitizers agreed with the action spectrum for cutaneous reactivity (400-420nm) (Blum and Pace, 1937). Moreover, light at this mentioned wavelength is absorbed superficially and would only involve changes to the epidermis without destroying the underlying tissue. In view of the fact that Hp has a rapid clearance from the serum than HpD and a comparable significant tumour response with HpD in the earlier study, it is hoped that this study will contribute towards a better understanding of the underlying pharmacokinetic of the drugs in skin photosensitivity.

6.7.2 Experimental Methodology

Eighteen mice comprising 6 groups of 3 mice were subjected to intravenous dose of both the drug via the tail vein. The Hp dose injected was the therapeutic dose of 25mg/kg body weight, the HpD dose was 15 mg/kg body weight. In order to monitor the surface temperature of the skin, a thermistor probe which is connected to the digital readout meter (Fluke 52 $^{k/J}$ ^{Thermometer}) was employed. During irradiation, the thermistor probe was placed at the surface of the skin and the read out was maintained within $\pm$ 0.1^{0}C. The experiment consists of 6 test groups. Nine mice (Groups 1-3) were administered with Hp. The remaining 9 mice (Groups 4-6) were administered with HpD. The mice were shaved on the back with an electric animal clipper. The shaven skin on the back was marked with 4 spots of 1.0 cm diameter. A masking device was made from aluminium foil and double-sided tape with a 1.0 cm circular hole. Each mice was subjected to four exposure of blue light (390-470nm) using the surgical head lamp (Section 3.3.1.2). The irradiating beam spot had a power density of 40.7 mW/cm^2. Each spot on one mouse was exposed to an energy fluence of 12.52J/cm^2, 25.05J/cm^2, 37.58 J/cm^2 and 75.17J/cm^2 for an exposure time of 5, 10, 15 and 30 min respectively. Subsequent to either Hp and HpD administration, the mice were exposed to blue light at different time intervals such as 1 day, 3 days, and 7 days. Group 1 and 4 were exposed to light 1 day after the administration of the drugs. Group 2 and 5 were exposed to light 3 days after the administration of the drugs. Group 3 and 6 were exposed to light 7 days after the administration of the drugs. The controls comprised of 2 groups of six mice. Group 1 consisted of six mice, three of the mice were injected with Hp and the remaining three were injected with HpD but they were not

administered with the blue light. Groups 2 consisted of 6 mice which were not injected with any drug but were administered with blue light using the same dosimetry as outlined for the test group. Exposed skin areas were examined and photographed at least every other day during the first week, and then at longer intervals depending on the reaction. The final analysis of the animals in the test and control groups were done at the end of third week. Final analysis skin reactions were scored as follows:

0. No damage. No scarring at any time point.
1. Slight damage. Swelling of skin with blanching or redness. Barely visible scar. Intradermal petechial bleeding may occur.
2. Moderate damage. Loss of tissue in exposed area with intact epithelium (depression in exposed area).
3. Severe damage. Destruction of skin but not full thickness, resulting in a superficial scar.

6.8 Results

Within an hour to two hours of a drug dose of HpD at 15 mg/kg and the irradiation of blue light, there was swelling of skin with blanching within the irradiated area at all the time intervals and at all light doses mentioned. By one week there was breakdown of the skin with a brown scab overlying the centre of the irradiated area. By two weeks the scab fell and skin healed from the edges of the necrozed zone. Over the next two and a half weeks to three weeks the only abnormalities visible after healing depended on the post injection interval to exposure were a barely visible scar (score 1), a small centre depressed scar which gives a score of 2 to 2.33 and a resulting superficial scar (score 3) (Table 6.11). The moderate damage was seen at

a dose of 12.52 J/cm^2 and 25.05 J/cm^2 at one day and three days post injection to exposure. It was also seen at a dose of 37.58 J/cm^2 and 75.17 J/cm^2 at the third and seventh day interval post injection to exposure. A barely visible scar is seen at a light dose of 12.52 J/cm^2 and 25.05 J/cm^2 at an interval of seven days and finally a superficial scar (severe damage) at the one day interval at a dose of 37.58 J/cm^2 and 75.17 J/cm^2.

As for Hp at a drug dose of 25 mg/kg and an irradiation of blue light at 25.05 , 37.58 and 75.17 J/cm^2 at an interval of one day and three days with light doses of 37.58 and 75.17 J/cm^2 gave a swelling of the skin with blanching (score 1) within an hour to two hours. The swelling disappeared the following day but the blanching persisted for another few days before turning to pale brown without any formation of scab. By one week the centre of the irradiated area was healed without any scar. As for an interval of seven days, only a light dose of 75.17 J/cm^2 gave a swelling of the skin with blanching or redness (score 1). The other dosages of light at interval of 1, 3, and 7 days did not show any response (score 0).

The entire duration of the irradiation produced no rise in temperature (i.e approximately 35.9 ^{0}C) at the surface of the skin.

The results of this experiment indicate HpD showed an almost moderate to severe skin damage (scores of 1.6 to 3). However, Hp caused no reaction to almost slight damage (scores 0 to 1). This difference was statistically significant (Student's t test, $p < 0.025$). As expected twelve control animals did not show any response. Comparison of skin response at various time intervals between the two drugs is shown in Figs 6.12 and 6.13.

The drug-light dose product computed in Table 6.12 was a multiplication of various blue light doses with the level of drugs at different time interval post injection. The results in

Table 6.12 is summarized in Fig.6.14. The graph shows that Hp and HpD have a similar behaviour in skin photosensitivity. The scores with the drug-light dose product gives a quantitative factor in the scale for skin photosensitvity.

Table 6.11 : Average scores for skin response following time intervals of administration of Hp and HpD to PDT at three weeks

Drug	Time interval (days)	Average skin response scores according to light dose (J/cm^2)			
		12.52	25.05	37.58	75.17
HpD (15 mg/kg)	1.0	2.0	2.0	3.0	3.0
	3.0	2.0	2.0	2.33	2.33
	7.0	1.6	1.6	2.0	2.0
Hp (25 mg/kg)	1.0	0.0	1.0	1.0	1.0
	3.0	0.0	0.0	1.0	1.0
	7.0	0.0	0.0	0.0	1.0

Table 6.12 Skin photosensitivity with respect to drug-light dose product of Hp and HpD

Time interval post injection (day)	Light dose (J/cm^2)	Hp level in the serum (μg) with respect to the time interval post injection	Drug-light dose product of Hp($J.mg/kg.cm^2$)	Average skin response score	HpD level in the serum (μg) with respect to the time interval post injection	Drug-light dose product of HpD($J.mg/kg.cm^2$)	Average skin response score
1	12.52	6	75.12	0	95	1189.4	2
	25.05		150.3	1		2379.75	2
	37.28		225.48	1		3570.1	3
	75.17		4510.2	1		7141.15	3
3	12.52	3	37.56	0	68	851.36	2
	25.05		75.15	0		1703.4	2
	37.28		112.74	1		2555.44	2.33
	75.17		225.51	1		5111.56	2.33
7	12.52	0.8	10.0	0	49	613.48	1.6
	25.05		20.0	0		1227.45	1.6
	37.28		30.0	0		1841.4	2
	75.17		60.0	1		3683.3	2

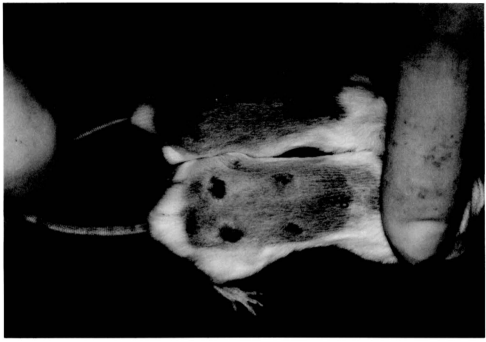

Fig. 6.12 : A comparison of skin response at 1 day interval between the administration of Hp (A) and HpD (B).

Fig. 6.13 : A comparison of skin response at 7 day interval between the administration of Hp (A) and HpD (B).

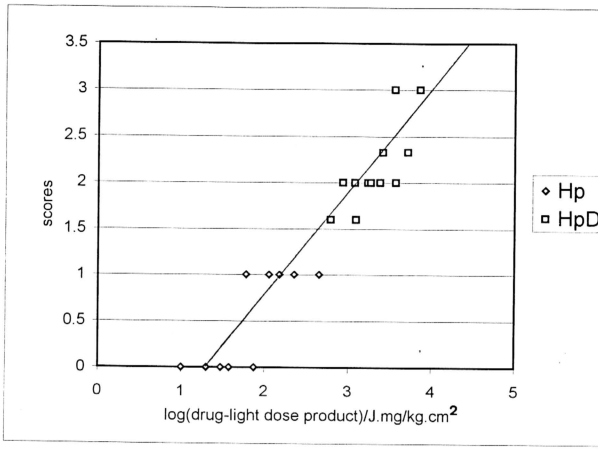

Fig.6.14: Skin photosensitivity with respect to Drug-light dose product of Hp and HpD

6.9 Discussion Of The Results

The first objective of this study was to compare the efficacy of the possibl photosensitizer, Hp with the conventional photosensitizer, HpD in the treatment of murin tumours with reference to Immediate PDT. Hence, tumour response was investigated. This wa followed by histological examination to assess Immediate PDT effects on tumour tissue, it vasculature and the surrounding normal tissue for both the photosensitizers. The secon objective was to investigate on skin photosensitivity. Skin damage was evaluated post injectio

of both the photosensitizers at their therapeutic dose levels.

A Hp dose of 25 mg/kg body weight with a fluence of 300 J/cm^2 (7500 J.mg/kg.cm^2) attained comparable tumour response (92.3%) with HpD (90 %) using a dose of 15 mg/kg body weight with a fluence of 150 J/cm^2 (2250 J.mg/kg.cm^2). The results indicated that the administration of the drug Hp needed a drug-light dose product of 3.5 times more than HpD to achieve an essentially similar tumour destruction (Fig. 6.6 and Fig.6.7).

There are two main reasons for this large differences in drug-light dose product to achieve 50% tumour volume reduction. An obvious reason for the observed result suggests that due to the rapid excretion of Hp from the blood serum in the tumour blood pool, the amount of Hp available in the tumour vasculature is reduced considerably. A much higher drug-light dose product is needed to bring about tumour cell necrosis. The average treatment time for a tumour is 15-20 mins with a 5 mins of waiting post-injection before light is irradiated. To quantify the effective Hp and HpD masses in serum during this light irradiation time period from 5 mins to 20-25 mins, the results of pharmacokinetics studies in Chapter 5 is used. Thus, for Hp and HpD respectively, serum concentration mass in μg are calculated as follows :

$$\text{Hp} : X_1^f(t) = 190\exp(-k_{12}^f.t) + 10\exp(-k_{12}^s.t)$$

$$\text{HpD} : X_1^f(t) = 66\exp(-k_{12}^f.t) + 134\exp(-k_{12}^s.t)$$

Where $k_{12}^f = 5.7$, $k_{12}^s = 1.4$

Based on these expressions, Table 6.13 shows the computed effective porphyrin masses at the different time periods. As shown in the same table, at time period of 20-25 mins would have allowed the serum level of the tumour blood pool to drop to about 17-12 %

Table 6.13 Table showing effective fraction of Hp and HpD remaining at different times and time periods after *i.v.* injection

Time/ mins	X_1^f /200 (Hp)	X_1^s /200 (HpD)
5	0.63	0.80
20	0.17	0.47
25	0.12	0.41
Mean (5-20 mins)	0.400	0.635
Mean (5-25 mins)	0.375	0.605
Mean	0.390	0.62

from its initial value for Hp. On the average, the Hp concentration in serum is 39% of its initi mass when compared to the corresponding HpD concentration of 62% in serum in the tim interval from 5-25 mins post injection of a given amount of drug dosage. Overall, there i effectively a 1.6 times higher concentration of porphyrin in HpD over that of Hp during th period of light irradiation.

Another possible reason for the inequality in dosimetry to produce a similar assessmer in tumour necrosis may be due to differences in phototoxicity of Hp and HpD. One may expe that Hp with a higher amount of monomeric species will yield a higher production of singl oxygen ($^1O_2$) and so is more photoactive (Redmond *et al*, 1985; Moan, 1984; West and Moor 1989) and subsequently enhance the possible mechanisms of photodamage. However, Hp h a higher fluorescence yield and thus a shorter half-life of the excited singlet state (Gomer *et a*

1989). Therefore, these will result in lower yield of excited triplet Hp and thus lower yield o singlet oxygen in the medium. In the absence of quantitative data on the selective phototoxicit of Hp relative to HpD, it can only be speculated that these differences in phototoxicity ca account for the remaining factor of two or so in the drug-light dose product between Hp an HpD for 50% tumour reduction.

Other possible factors resulting in the higher photosensitivity of HpD may be its greate affinity to tumour cells. However, it has already been shown by Henderson and Doughert (1984) and Star *et al.*(1984,1986) that tumour necrosis is secondary to the destruction of th microvasculature, and not a direct process of cell kill. On the other hand, it is more likely tha with their larger molecular size, the oligomers are more readily trapped at the endothelial cell of the capillaries. There is a strong likelihood that HpD could have stained the endothelial ce wall lining in the capillaries resulting in greater photosensitization of the capillary vessels. Th latter seems more logical to have happened, owing to the fact that once a photosensitizer i injected into the blood, it must first be distributed through the vascular space before th sensitizer molecule can reach the tumour cells. These conjectures remain to be verified.

Histopathological assessment of tumour necrosis induced by both the drugs in referenc to Immediate PDT showed that the extent of necrosis achieved were comparable with the drug light dose product for Hp and HpD..The histomorphological study has also shown tha comparable vascular damage occurred following the administration of both the drug Furthermore, Hp did not cause a more severe damage to the normal tissue surrounding th tumour tissue when compared with HpD. Qualitative histopathological assessment showed tha the only noteworthy difference was in hyperaemia. More extensive hyperaemia was observe

following HpD-PDT as compared to Hp-PDT but this has no statistical significance.

The minor differences in results obtained from the studies may be due to the fact that the various parameters used in the histological study could not quantitatively assess the extent of histomorphological changes. In order to verify the extent of histomorphological changes as well as its consequential effects on tumour physiology, a more detailed study on the ultrastructure is required to separate the different possible mechanisms of action in these two photosensitizers.

In the second study, skin photosensitivity was investigated. The HpD reactions on the skin were characterized by a depression in the exposed area, resulting in a superficial scar at the end of three weeks whereas, Hp reactions were milder without any visible scar. The HpD reactions were manifested for considerable longer periods; even at day 7, post injection, the skin reactions gave a score between 1.6 to 2.0. On the other hand, the duration of skin photosensitivity conferred by Hp is shorter. On the seventh day post injection to exposure, only the highest dosage of irradiation evoke a reaction of swelling with blanching of the skin (score 1). The other three dosages did not evoke any reaction to the skin. Hence, it is obvious that Hp cause much less skin photosensitivity than HpD. These observations also suggest that the damage to the skin with HpD is far more severe than with Hp. From Fig.6.14, it could be deduced that Hp and HpD behave in a similar manner in skin photosensitivity. The results give a quantitative assessment of the drug-light dose product of both the drugs which correspond to the various scores in skin photosensitivity. Hp could be increased up to 10 times the drug light dose product for more effective PDT and yet skin photosensitivity would still be manageable. The quantitative factor would be useful as to how much drug-light dose to use in

order to keep skin photosensitivity at a minimum.

There have been various attempts to describe skin photosensitivity using HpD and DHE in animals and patients; some of these will be looked at. DHE and HpD remain detectable in skin for extended periods (Zalar *et al*, 1977; Wilson *et al*, 1988). Severe skin reactions were seen in the animals treated with DHE for up to two months after administration. Zalar *et al* (1977) studied the minimal erythemal dose (MED) in patients treated with HpD (7.5 mg/kg). They found that responses could be evoked at 70 and 85 days after administation of the sensitizer. Gomer and Razum (1984) studied skin response of albino mice treated with HpD or DHE to red light using skin scoring system and found that the skin responses resolved by 28 days post-irradiation with both DHE and HpD senstization. Tralau *et al* (1989) looked at the reaction on the skin of albino mice using solar simulated radiation of DHE. Irradiation evoked a reaction up to two months after the administration of DHE.

The skin damage observed here does not resemble a thermal burn as one would expect a full thickness burn to heal with fibrosis. Barr *et al* (1987) have shown that in animal mucosa thermal burns produced by lasers heal by fibrosis while damage due to photodynamic therapy repairs leaving a relatively normal mucosa. The damage is also different from radiation as necrosis such as this fails to heal in the long term. Possibly, these differences are explained by the mode of action of PDT which is postulated to be through causing vasoconstriction rather than by directly causing cell death (Star *et al*, 1986; Henderson and Dougherty, 1984).

6.10 Conclusion

In view of the findings of this study, by adopting a higher drug-light dose product, Hp is a possible photosensitizer in tumour response with negligible skin photosensitivity when

compared to HpD. This study also implies that both the drugs caused tumour cell death which occurs secondary to the consequence of ischaemia due to the damage of vascular sensitization. Skin photosensitivity exhibited by HpD appeared to be far more severe compared to Hp. Results showed that Hp would exhibit a comparable tumour response as HpD even at a higher dosage without causing skin photosensitivity. The results of the study also indicated a new quantitative means for the measurement of skin photosensitivity with respect to drug-light dose product.