CHAPTER FIVE

5.0 A STUDY OF THE PHARMACOKINETICS OF HEMATOPORPHYRIN AND ITS DERIVATIVES IN MICE

5.1 Introduction

In order to further the understanding of Hp and its derivatives (HpD) in PDT, detailed pharmacokinetic studies are required. This is particularly important with regards to skin photosenstivity, which is related to their retention time or how rapidly the drugs are cleared. The study of the uptake and distribution of both the photosensitizers used in PDT is important to determine optimally efficient PDT. The mass distribution of the photosensitizers in different tissues at various time points were quantitatively reported. This may also help to minimize normal tissue damage, while destroying the tumour.

After an intravenous injection, the drugs are immediately present in the blood and hence distributed to the various organs. A comparison of the serum and tissue uptake, distribution and elimination between the two drugs in animal system were presented. The tissues studied here are liver, kidney, tumour and muscle.

So far, studies on the pharmacokinetics of drugs have been restricted to a single serum/plasma compartment largely for simplicity and to illustrate some basic principles. In practice the behaviour of a drug *in vivo* is often much more complex and a number of additional compartments need to be considered.

Studies of HpD distribution and elimination using solvent extraction technique, radiolabelling and autoradiographic techniques (Gomer and Dougherty, 1979; Bugelski *et .al.*, 1981; Pantelides *et. al.*, 1989; Brown and Vernon, 1991) were reported. Most published analyses have been based either explicitly or implicitly on the validity of two compartmental assumptions. Most of the two compartmental models are consistent with two

exponential processes and are largely defined for fitting the data on serum/plasma pharmacokinetics, which can loosely be interpreted as distribution (from the plasma/serum to the other tissues) and elimination via the plasma/serum. However, two compartments are normally not sufficient to describe the behaviour of a drug satisfactorily. Three or more compartments are required (Holroyd, 1995; Stribbling, 1991).

When the drug is administered intravenously, it is first distributed in the serum and from there to other tissues and subsequently elimination out of the body through the two major organs of the liver and kidneys. The usual two-compartment pharmacokinetic models combine the serum and well perfused tissues (eg. liver and kidney) into a single compartment, whereas the other compartment is poorly perfused tissues. However, the rates of uptake and elimination are different for different organs and tissues. These then behave as separate compartments to the serum. It is necessary therefore to treat the liver and the kidneys as two separate compartments as well as two other compartments of the tumour and the muscle.

Previous works also suggest that the monomeric fraction and a dimeric/oligomeric fraction behave differently following intravenous adminstration. Therefore, considerable care has to be taken to ensure a complete understanding of the fate of the components following its administration. Hence, the pharmacokinetics of the two fractions would be different and models which treat this material as a single compound are inappropriate.

In this study, therefore, they are treated as two separate components; monomers and oligomers and thus the model must take into consideration of multi-compartmental decay curves for two components (multicomponent) model to the serum, liver, kidneys, tumour and muscle data. At times longer than 3 hours, only the oligomeric material is present. It is therefore justifiable to base a pharmacokinetics analysis on the oligomeric and monomeric

as two separate components.

The experimental data used for this study was based on the tissue distribution and elimination of Hp and its derivatives (HpD) which utilized a blood and tissue sampling schedule following the intravenous administration of Hp and HpD. The phamarcokinetics equations are solved using the Laplace transform equations. Thus, quantitative understanding of the pathway of the photosensitizers in the different compartments can be readily interpreted. The results clearly elucidate the origin of the long skin photosensitivity of HpD as a photosensitizer, showing at the same time the suitability of Hp as a photosensitizer for PDT.

5.2 A Review On Tissue Uptake, Distribution And Elimination Of Porphyrins In Murine Model

The study of the distribution of a drug for use in PDT is important. For optimum treatment protocol of PDT, one would wish to quantify the concentration of the photosensitizer in different tissues likely to be included in a given treatment field. This may help to minimize skin photosensitivity leading to normal tissue damage, while destroying the tumour.

It is appropriate at this point to briefly summarize the previous works. Surprisingly, only a few quantitative studies have been carried out concerning the tissue distribution of PDT drugs derived from HpD.

Gomer and Dougherty (1979) used HpD labelled with ^{3}H and ^{14}C for biodistribution studies in tumour mice. They were able to quantify the amount of HpD in various organs and tumour tissues in *i.p.* injection. The tissue levels showed no statistical difference in either form of radiolabelling of HpD. The tissues which were assayed for porphyrin content,

the liver, kidney and spleen (for each time period) contained higher quantities of the radiolabelled derivatives than did the tumour. Based on these results, they queried the reliability of quantification of HpD in tissues using the fluorescence spectroscopic method due to quenching effects by pigmented tissues such as the liver, spleen and kidney.

Tritium-labelled HpD was used by Bugelski *et al* (1981) for autoradiography. Their investigation showed that at 48 hrs, the cells of the reticulo endothelial system (kupffer cells) had a greater level of radiolabelled material present than in parenchymal cells of the same organs.

Kessel and Chou (1983b) and Kessel and Cheng (1985a) reported on tumour localization studies on sarcoma 180 cells *in vivo* and *in vitro* using HpD. The uptake and accumulated of porphyrin was measured at 48 hr. HpD was chemically extracted and its fluorescence were measured. However the poor fluorescence yield of the tumour localizing fraction (dimer, DHE) comprises their data. Subsequently, they found that hydrolysis of the dimer results in quantitative conversion into porphyrin monomers so that fluorescence detection could then be used to monitor drug uptake with a more accurate estimate.

In 1989, Bellnier *et al* studied the distribution of radioisotope of [^{14}C] Photofrin II in a wide variety of tissues for tumour-bearing mice. They also carried out HPLC studies of the material extracted from faeces, liver and spleen. HPLC analyses of fecal extracts showed that mostly monomeric and other low molecular weight of Photofrin II were eliminated rapidly. The higher molecular weight oligomeric fractions of Photofrin II were retained in the liver and the spleen up to 14 days after injection.

Kennedy *et al* (1992) used the technique of *in vivo* fluorescence spectroscopy to monitor the time of maximum fluorescence, first order clearance rate constants and clearance times of 17 different porphyrin derivatives following intraperitoneal injection in

mice. The technique monitors the *in vivo* porphyrins fluorescence observed from the external skin surface. However, it should be noted that surface detected fluorescence represents a summation of emission that may originate from several tissue types, including internal organs.

Vernon *et al* (1995) had developed an accurate and relatively rapid fluorometric assay for Photofrin and polyhematoporphyrin (PHP) in the study of plasma pharmacokinetics in the rat. The method was devised to allow for incomplete hydrolysis by calibration with plasma samples to which known amounts of photosensitizer were added *in vitro*. It was then compared with an "absolute" method in which plasma from animals that have received ^{14}C labelled drug was subjected to radioactivity assay. The two approaches gave almost identical results.

The tissue distribution study reported here was designed in such a way as to clarify some of the results mentioned in the studies cited above. The most important points were to quantitate the levels of Hp and HpD in a variety of tissues using the extraction/fluorescence method and at the same time investigate the pharmacokinetics of the components of the drugs.

5.3 Materials And Methods

5.3.1 Chemicals

Hematoporphyrin in the form of dihydrochloride (Hp, 95% pure) from Porphyrin Products and clinical HpD (prepared from Hematoporphyrin dihydrochloride >60% (Fluka Biochemika) by Lipson's procedure (1961). Hexadecyltrimethyl-ammonium bromide (CTAB) and 4-(2-Hydroxyethyl)-piperazine-1-ethane-sulfonicacid (HEPES) were obtained from Fluka Biochemika, Switzerland. The homogenization buffer, 10 mM HEPES/ 10 mM

CTAB, pH 7.4 was made up thus: 3.64g CTAB and 2.38g HEPES. The correct pH was obtained by adding sodium hydroxide. Chloroform was obtained from Lab-Scan Ltd., Ireland and methanol was obtained from BDH Chemicals Ltd., England. An equal ratio of chloroform:methanol (1:1) was made up to a volume of 1000ml.

5.3.2 Animals

The animals used were 8 weeks old Balb/c mice weighing about 20g each. The mice were implanted with EMT-6 tumour. When the size of the tumour reached between 3-8 mm along the major axis diameter and depth ranging from 2.5-3.5 mm about 10-14 days, they were used for the experiment (refer to section 6.2 and 6.3) . The animals received water and laboratory *chow ad lib*.

5.3.3 Extraction Methods Of HpD And Hp In Serum And Tissues

The mice were divided into two groups. Group 1 was injected with Hp (10 mg/kg) and Group 2 was injected with HpD at 10 mg/kg intravenously via the tail vein. Three animals were divided into 7 groups, at each of the following time intervals post injection: 15 min, 1 hr, 3 hr , 24 hr, 48 hr, 72 hr and 96 hr.

At the appropriate times, the mice were sacrificed and blood samples (1.0 ml) were drawn out from the hearts of the animals by a syringe and was subsequently left to clot overnight at 4 ^{0}C before serum was removed by centrifugation at 10,000rpm for 10 mins. The serum was then stored at -20^{0}C until assay. Various organs from the mice were then removed and were immediately cut to a weight of about 25 mg each. All tissue weights were wet weights when measured. The tissues were then placed into 1 ml cryovials. The tissue samples were kept at -20^{0}C, sealed and protected from light until analysis.

All samples of tissues were extracted by the method described by Kessel and Cheng

(1985b) with a few minor modifications. The tissues studied were liver, kidney, muscle and tumour. All samples of tissue were taken out from the freezer at -20^{0}C and were immediately placed in a teflon sample holder. This was placed in liquid nitrogen for 15 s. The deep frozen tissue sample was then pulverized in the teflon chamber (1600rpm) with a steel ball using a Braun Micro Dismembrator U. The powder was then placed into a homogenization buffer, HEPES/CTAB (1 :1, 1.0 ml) and homogenized using the micro-dismembrator. Aliquots of the homogenate were placed into test tubes, and 1.5 ml of a chloroform : methanol solution (1:1) was added. The contents of the tubes were mixed by vortexing and centrifuged on a Kubota 5100 benchtop centrifuge at 5000 rpm for 5 min. The upper organic layer was removed and discarded.

All samples of serum were also thawed before analysis. To each sample placed into the test tubes, HEPES/CTAB (1:1, 1.0 ml) and chloroform : methanol (1:1, 1.5 ml) solutions were added. The tubes were mixed by vortexing and centrifuged on a Kubota 5100 benchtop centrifuge at 5000 rpm for 5 min. The upper organic layer was removed and discarded.

5.3.4 Hydrolysis Of Hp And HpD Oligomers

For Hp and HpD measurements, the bottom phase was collected and subjected to hydrolysis before the measurement by fluorescence. Serum and tissue blanks were run to corrrect for endogenous porphyrin levels.

The sensitivity of fluorescence measurements can be increased substantially by acid hydrolysis. The upper organic phase of Hp and HpD was removed respectively as described earlier but the bottom phase of Hp and HpD was collected respectively and evaporated under nitrogen gas and the residues were taken up in 0.5 ml of 0.5 M HCl and then

hydrolysed in a boiling water bath for 1 hr in sealed tubes. The tubes were then allowed to return to room temperature, neutralized with 1 M NaOH (0.25ml) and brought to a final volume of 1.0 ml with 20mM CTAB containing 200 mM of NaOH. Thereafter, the mixture was subjected to fluorescence analysis.

According to various reports (Stribbling, 1995; Holroyd, 1995 and Vernon *et al,* 1995), the carbon-carbon bonds are resistant to acid hydrolysis and would lead to incomplete recovery during extractions. Due to this, they embarked on a different procedure which could account for these bonds. As a result of this, a comparison of this method of hydrolysis with theirs on HpD in serum was done.

5.3.5 Measurement Of Fluorescence

Both the porphyrin concentrations were determined using a Perkin Elmer Luminescence Spectrometer (LS50B).The spectra were monitored at room temperature (25 ± 1°C) under the following instrumental conditions: excitation wavelength : 400nm; excitation slit : 5.0 nm; emission wavelength : 550-750nm.

A cuvette was used for tissue fluorescence. The quartz sample cuvette was a normal glass cuvette and could easily be filled and emptied with a Pasteur pipette. The cuvette was then flushed two times with distilled water. Every measurement was carried out three times. The series of measurements began with the lowest and ended with the highest concentration. The blank is poured in again to recheck the repeatability. The cuvette was cleaned with ethanol before proceeding with the new series of measurement. Under the above detailed conditions, porphyrin amounts as low as 0.0625 μg per ml of mixture could be estimated.

5.3.6 Calibration Of The Serum And Tissues Assay For HpD And Hp

Prior to measurements with the fluorescence spectrometer, a hydrolyzed sample of a known amount of HpD has been used as a standard for comparison to the amount extracted from the serum and the other tissues. A similar calibration graph of Hp for comparison to drug extracted from the serum and the tissues with hydrolysis were also obtained. The porphyrins extracted were diluted to a concentration ensuring the monomerization of the porphyrins, as well as a linear relationship between the fluorescence yield and the porphyrin content. Thereafter, the porphyrin content was calculated by interpolation of the data, which is proportional to the emission spectrum of HpD and Hp in the 621-623 nm wavelength range, from the standard calibrated curve.

5.3.7 Mathematical Modelling Of Pharmacokinetics

In order to determine quantitatively the rate of the elimination of the drug, the concentrations of the drug in the body with respect to time were analyzed using a simplified first order compartmental model. Such an analysis will aid in the understanding of the fate of the components of the mixture of the drug which is particularly important with regard to skin photosensitivity.

For a general n-compartmental model, where the drug transport medium is the blood, a schematic model is shown in Fig. 5.1. The elimination of the drug is assumed to be through the liver and the kidney as excretion and urine and designated as compartments y and z only. To first order in the transfer and elimination of the drug, the following equations can be written.

For serum which is designated as compartment 1:

$$dX_1/dt = + \sum_j k_{j1} X_j - \sum_j k_{1j} X_1$$

where j=2 to n.

For the liver which is designated as compartment 2 with excretion channel y:

$dX_2/dt = + k_{12} X_1 - k_{21} X_2 - k_{2y} X_2$.

For the kidney which is designated as compartment 3 and urinary channel z:

$dX_3/dt = + k_{13} X_1 - k_{31} X_3 - k_{3z} X_3$,

For the tumour which is designated as compartment 4:

$dX_4/dt = + k_{14} X_1 - k_{41} X_4$.

For the muscle tissue which is designated as compartment 5:

$dX_5/dt = + k_{15} X_1 - k_{51} X_5$.

Similarly, for all the other compartment n, the equation can be written as:

$dX_n/dt = + k_{1n} X_1 - k_{n1} X_n$.

In these expressions, X is the mass of the drug in the designated compartment, the numerical subscripts for X defining the compartment, the respective k_{ij}'s are rate constants of transfer from the compartment i to the compartment j.

After the drug is injected into the blood stream, there is a rapid transfer from the serum to the liver. During this initial phase of rapid clearance to the liver, the masses of the drug in all the other compartments remain small and can be neglected and from the observation, the reverse transfer of drug from the liver to serum is small, $k_{21} << k_{12}$. Thus, assuming

$dX_1/dt \cong - k_{12} X_1$

Integration of this gives :

$X_1 = X_{10} \exp(-k_{12} t)$

Where X_{10} is the initial mass injected into the blood. Using this expression, k_{12} can be

obtained from the initial slope of the mass distribution graph of the serum. To obtain the mass excretion rate, the n compartment transport equations are added to give :

$dX_T/dt = d(X_1+X_2+...+Xn)/dt = - k_{2y} X_2 - k_{3z} X_3.$

From the experimental results as shown in Fig.5.2 and 5.3, the excretion rates from the different compartments are basically similar to those of the liver compartment. A linear relation between all the masses and the liver is now assumed i.e. $X_1 + X_2 + ... + X_n = aX_2$. The validity of this assumption can be seen quite clearly from the measured concentration of the different masses. Ignoring now the excretion from the kidney which is small due to the smallness of X_3, the above equation can be written as :

$d(aX_2)/dt \cong - k_{2y} X_2$

where a is a mass scaling factor from liver to all the tissues. Integration of this gives:

$aX_2 = X_0 \exp (-k_{2y} t / a)$ and

$X_2 = X_{20} \exp (-k_{2y} t / a)$.

A similar expression can now also be written for all the other compartments:

$X_n = X_{n0} \exp (-k_{2y} t / a)$.

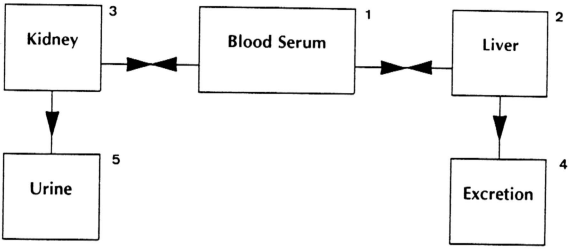

Fig. 5.1: Schematic diagram showing exchange of the drug between the different compartments

5.4 Results

Table 5.1 (a) and 5.1 (b) show the tissue concentration of Hp and HpD respectively in various tissues at different times after injection. As expected, both serum drug levels were initially high, and then dropped rapidly in an exponential fashion. The drug levels in the liver and the kidney were greater than in the tumour and the muscle at all time points. The liver and kidney retain a large amount of the HpD after 96 hrs whereas only a small amount of Hp remains in both these organs.

The results on the uptake and retention of Hp and HpD masses in serum, liver, kidney, tumour tissue and muscle tissue are shown in Table 5.2 (a) and (b) respectively. In converting from the tissue concentrations to the mass distributions, the average weights of these different organs were determined. These values are indicated as footnotes at the bottom of Table 5.2.

Table 5.1(a) : The concentrations of Hp in serum and tissues of EMT6 tumour bearing mice as a function of time post intravenous injection

Drug : Hp					
	Concentration Of Tissues				
Time/h	Serum/μg/ml	Liver/μg/g	kidney/μg/g	Tumour/μg/g	Muscle/μg/g
0.25	79 ± 20	161 ± 31	20 ± 9	13 ± 8	8.5 ± 9
1.00	30 ± 10	121 ± 51	16 ± 4	16 ± 5	7 ± 7
3.00	6 ± 3	20 ± 25	6 ± 2	5 ± 1	2 ± 0.5
24.00	2 ± 3	7 ± 6	4 ± 2.5	3 ± 2	0.7 ± 1
48.00	1 ± 0.7	5 ± 3	2 ± 1	2 ± 3	
72.00	0.8 ± 0.6	4 ± 4	1.4 ± 2	2 ± 1	0.3 ± 1
96.00	0.7 ± 0.5	3 ± 4	1 ± 1	1.2 ± 1	0.2 ± 1

Table 5.1(b) : The concentrations of HpD in serum and tissues of EMT6 tumour bearing mice as a function of time post intravenous injection

DRUG : HpD					
	Concentration Of Tissues				
Time/h	Serum/μg/ml	Liver/μg/g	Kidney/μg/g	Tumour/μg/g	Muscle/μg/g
0.25	174 ± 26	93 ± 33	54 ± 3	29 ± 12	5 ± 3
1.00	61 ± 24	151 ± 32	61 ± 12	32 ± 4	9 ± 3
3.00	30 ± 12	132 ± 21	55 ± 3	23 ± 3	5 ± 5
24.00	10 ± 7	119 ± 22	31 ± 10	28 ± 10	2.5 ± 2
48.00	7 ± 5	114 ± 16	27 ± 9	20 ± 3	
72.00	7 ± 4	85 ± 14	24 ± 5	13 ± 4	3 ± 1
96.00	6 ± 3	78 ± 7	16 ± 7		2 ± 1

Table 5.2(a) : The distribution of Hp mass in serum and tissues of EMT6 tumour bearing mice as a function of time post intravenous injection

DRUG : Hp						
	Mass Of Drug In Tissues/μg					
Time/h	Serum	Liver	kidney	Tumour	Muscle	Total Hp/μ
0.25	48 ± 12	129 ± 25	3 ± 1.4.	6.5 ± 4	17 ± 18	203.5 ± 60
1.00	18 ± 6	97 ± 41	2.6 ± 0.64	8 ± 2.5	14 ± 14	139.6 ± 64
3.00	4 ± 2	16 ± 20	1 ± 0.32	2.5 ± 1	4 ± 1	27.5 ± 24
24.00	1 ± 2	6 ± 5	0.64 ± 0.4	1.5 ± 1	1.4 ± 2	10.5 ± 10
48.00	0.6 ± 0.4	4 ± 3	0.32 ± 0.16	1 ± 1.5	(0.9 ± 1)*	6.8 ± 6
72.00	0.5 ± 0.4	3 ± 3	0.22 ± 0.32	1 ± 0.5	0.6± 2	5.3 ± 6.2
96.00	0.4 ± 0.3	2.4 ± 3	0.16 ± 0.16	0.6 ± 0.5	0.4± 2	3.9 ± 6

Table 5.2 (b): The distribution of HpD mass in serum and tissues of EMT6 tumour bearing mice as a function of time post intravenous injection

DRUG : HpD						
	Mass Of Drug In Tissues/μg					
Time/h	Serum	Liver	Kidney	Tumour	Muscle	Total HpD/μg
0.25	104 ± 16	75 ± 26	8.6 ± 0.5	14.5 ± 12	10 ± 6	212.1 ± 61
1.00	37 ± 15	121 ± 26	6.8 ± 2	16 ± 4	18 ± 6	201.8± 53
3.00	18 ± 7	106 ± 17	9 ± 0.5	11.5 ± 1.5	10 ± 10	154.5± 38
24.00	6 ± 4	95 ± 18	5 ± 1.6	14 ± 5	5 ± 4	125 ± 32
48.00	4 ± 3	91 ± 13	4.3 ± 1.6	10 ± 1.5	(6.5 ± 3)*	115.8 ± 22
72.00	4 ± 3	68 ± 11	4 ± 0.8	6.5 ± 2	6 ± 2	88.5 ± 19
96.00	4 ± 2	62 ± 6	2.6 ± 1.2	(6.0 ± 2)*	4 ± 2	78.6 ± 13

Footnote : average mass - serum : 0.6 g; liver : 0.8 g; kidney : 0.16 g; tumour : 0.5 g; muscle : 0.2 g (estimated)

( )* -- estimated from the log-linear graph

The mass distributions of Hp and HpD are plotted and shown in Fig. 5.2 and 5.3 respectively. To provide a clearer picture of the time sequence of the different processes involved, 2 sets of graphs on different time scales have been plotted, i.e. from 0-6 hr and 0-100 hr respectively. Despite the relatively large error bars, especially for the longer time periods at low concentrations of less than 1 μg, the data generally provide clear indications of the trends in the uptake and retention of Hp as well as HpD.

Fig. 5.4 indicates that there is no statistical difference in the hydrolysis method used here and in Leeds. The values of concentration of HpD in serum from 0.25 hr to 48 hrs are identical in the both procedures.

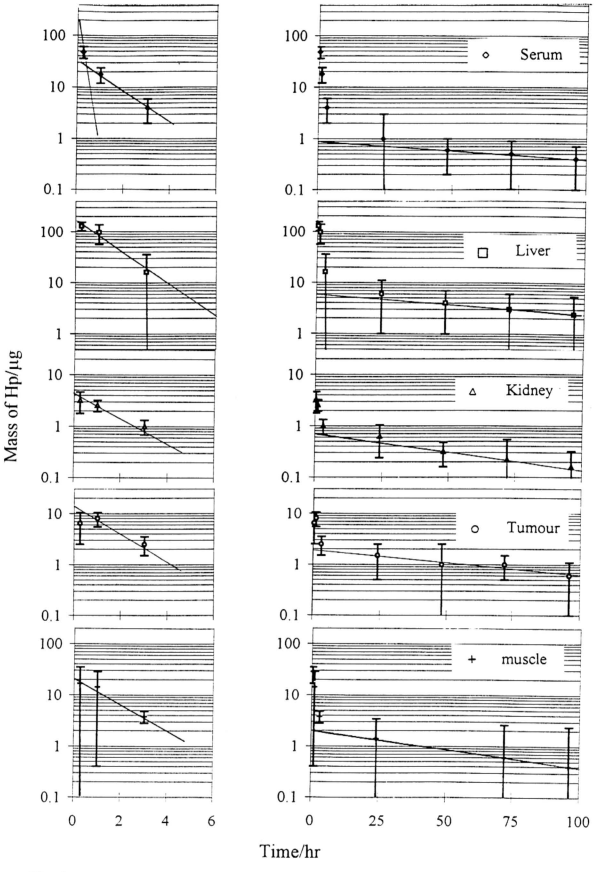

Fig. 5.2 The distribution of Hp mass in serum and tissues of EMT-6 tumour bearing mice as a function of time post injection

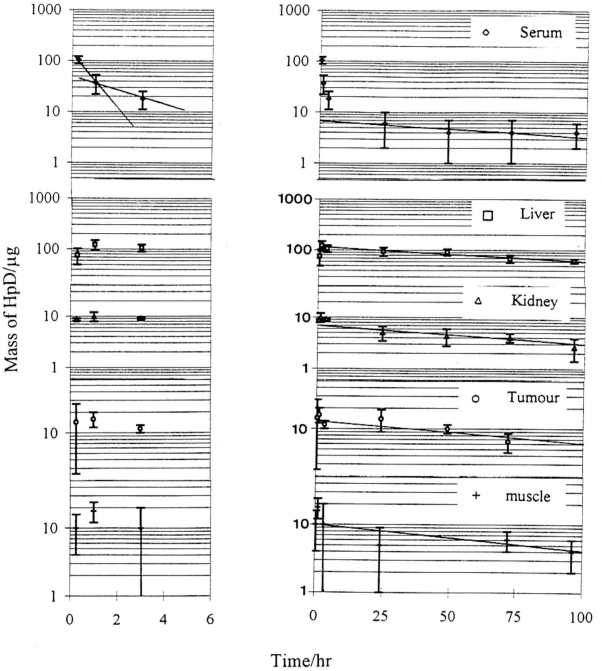

Fig. 5.3 The distribution of HpD mass in serum and tissues of EMT-6 tumour bearing mice as a function of time post intravenous injection

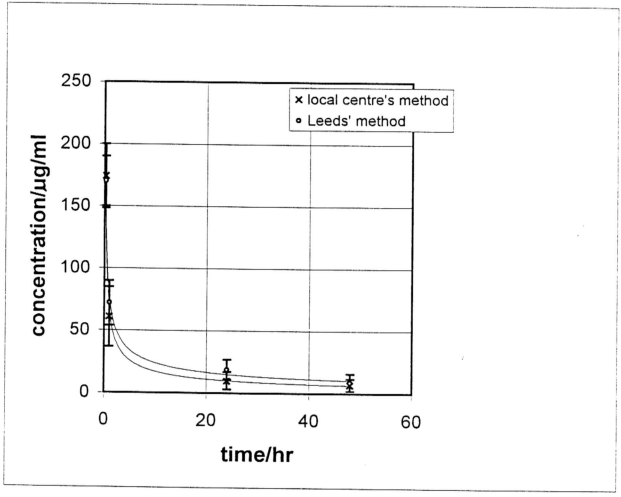

Fig. 5.4 Concentration of HpD in serum as measured using method adapted in this study and compared to another method (Stribbling, 1991 and Vernon *et al*, 1995)

5.4.1 Uptake And Retention Of Hp.

Since the Hp used contains 95 % of the monomer, its uptake and retention is easier to be interpreted. As shown in Fig. 5.2, immediately following the intravenous

administration of the drug, there is a rapid uptake of Hp by the liver. Fifteen minutes (0.25 hr) after the injection of a total of (200 ± 20) μg of Hp into the bloodstream, the serum only retains (48 ± 12) μg, with (129 ± 25) μg taken up by the liver. The uptake of Hp by the kidney, tumour and muscle tissues are relatively low each at less than 20 μg. The total mass at 0.25 hr is (203.5 ± 60) μg indicating small or negligible uptake by the other tissues.

The mass distributions of Hp at 1 hr and 3 hr indicate a rapid excretion from all the tissues. At 1 hr and 3 hr post *iv* injection, the total Hp mass in the 5 compartments remains at 139.6 ± 64 μg and 30 ± 24 μg respectively. As indicated by the data at the first three time intervals of 0.25 hr, 1 hr and 3 hr, the excretion rates from the 5 compartments are logarithmic linear and have approximately the same slopes. All these graphs show that by the time of 24 hr post *iv* injection, the mass distributions of this fast excretion component of Hp will be totally insignificant.

On the other hand, the data at the longer time intervals of 24, 48, 72 and 96 hr after the injection show the presence of a slow excretion fraction. At 24 hr post injection, this slow excretion fraction adds up to a total of (10.5 ± 10.4) μg. By projecting the log-linear graph back to time, t = 0, the mass of this fraction in the different compartments show initial values of 0.9, 6.0, 0.6, 1.9 and 1.9μg respectively. These then add up to a total fraction mass of 11.3μg, which agrees rather well with the 5 % oligomeric fraction as determined by the HPLC analysis. At 96 hr post injection, there is still a high residue mass of (3.9 ± 6.0) μg of the slow excretion fraction from the 5 compartments.

The results on the uptake and retention of Hp indicate the very rapid uptake and excretion of the majority component of the monomer by the liver organ, with relatively small

uptake by the other tissues and organs. Within 3 hours, relatively small quantities of the fast excretion component remain in the body system. However, the 5 % impurity oligomeric component indicate very long retention period and is concentrated mainly in the liver.

5.4.2 Uptake And Retention Of HpD

Table 5.2(b) and Fig. 5.3 indicate the fast uptake of HpD by the liver, although at a slower rate when compared with Hp. The peak concentration of the drug at the liver organ occurs at 1 hr post intravenous injection. While the 1 hr and 3 hr data provide some indication of a rapid excretion process similar to those of Hp. On the other hand, the majority component appears to be taken up by the liver with a slow excretion rate. By projecting back to time t = 0.25hr, at 24 hr post injection, the total porphyrin masses is (125.0 ± 32) μg which is approximately 63% when compared to the 66 % as measured in the HPLC analysis. Of this total mass of (125.0 ± 32) μg measured, (95.0 ± 18) μg is located in the liver organ. At 96 hr, the total quantity of porphyrins remaining in the 5 compartments is (78.6 ± 13) μg indicating a long-retention period in all the organs and tissues.

The results on the uptake and retention of HpD indicate trends in agreement with those of Hp. Due to the higher concentration of 66% oligomeric component in HpD as compared to 5% in Hp, the dominant features of HpD is determined by this slow excretion component. The main uptake of this long retention fraction is by the liver. On the other hand, the presence of the smaller fast excretion monomer (34%) is still apparent in the short time interval of less than 3 hr, much as in the case of the main fraction of Hp.

5.4.3 Calculation Of The Pharmacokinetics Parameters

5.4.3.1 Monomeric And Oligomeric Components Of Hp

From Fig. 5.2 for Hp, the serum clearance parameter, k_{12} of Hp can be estimated from the initial decay slope of Hp during the first 0.25hr. A value of 5.7 or a half life ($t = 0.693/k_{12}$) of 0.12 hr is obtained. From the data at time intervals less than 3 hr, the excretion rates of the fast clearance component (k^f_{2y}) from the different tissues can be assessed. The extracted excretion rates for the fast excretion component are shown in Table 5.3 (a) with a mean value of 1.020 ± 0.118. From Fig. 5.2, the log-linear line is extrapolated to time $t = 0.25$ hr to extract the total amount transferred from the serum to the respective compartments.

Processing similarly the data for time intervals greater than 24 hr, the excretion rates for the different tissues for the slow clearance component (k^s_{2y}) are also tabulated in Table 5.3 (a). A mean excretion rate of 0.0220 ± 0.0052 is obtained

Table 5.3(a): Excretion rates of the fast (k^f) and slow (k^s)component of Hp

TISSUE	Hp ($k^f_{12} = 5.7$)			
	k^s_{2y}	X^s (t=24 h)	K^f_{2y}	X^f (t =0.25 h)
SERUM	0.0161 ± 0.0093	0.75	1.125 ± 0.090	30.0
LIVER	0.0174 ± 0.0010	5.0	1.140 ± 0.105	120.0
KIDNEY	0.0250 ± 0.0091	0.45	0.810 ± 0.195	3.9
TUMOUR	0.0212 ± 0.0053	1.5	1.035 ± 0.735	10.0
MUSCLE	0.0304 ± 0.0020	1.4	0.990 ± 0.600	20.0
MEAN, k_{2y}; k_{2y}	0.0220 ± 0.0052		1.020 ± 0.118	

Table 5.3(b): Excretion rates of the slow (k^s)component of HpD

TISSUE	HpD (k^f_{12} = 1.4)	
	K^s_{2y}	X^s (t=24 h)
SERUM	0.0103 ± 0.0050	6.0
LIVER	0.0093 ± 0.0040	119.0
KIDNEY	0.0122 ± 0.0030	5.8
TUMOUR	0.0102 ± 0.0057	11.0
MUSCLE	0.0121 ± 0.0029	8.0
MEAN, k_{2y}	0.0108 ± 0.0011	

5.4.3.2 Oligomeric Component Of HpD

To extract the serum clearance rate of the oligomeric component from the HpD data, it is first necessary to subtract the contribution of the monomeric component present in HpD. The excretion parameters extracted from the analysis of Hp can be used. Using the information that HpD contains 34% monomeric component, this contribution can accordingly be subtracted. Using this procedure for HpD masses as shown in Fig. 5.3, the serum clearance rate (k_{12}) is estimated to be 1.4. The half life of HpD in serum is calculated to be 0.5 hr.

Processing the data of HpD at time intervals greater than 24 hr, excretion rate of the oligomeric component from the different tissues can be extracted. The results are tabulated in Table 5.3(b). A mean value of 0.0108 ± 0.0011 is obtained.

5.5 Discussion

As expected when employing the intravenous route of drug administration, serum drug levels were initially high, and then dropped rapidly in an exponential fashion. The drug levels in liver and kidneys were greater at each time point than in tumour and muscle tissues analysed (Table 5.1a and 5.1b). This agrees well with all previoulsy published data (Gomer and Dougherty, 1979; Bugelski *et. al.*, 1981; Evensen *et. al.*, 1984; Pantelides *et. al.*, 1989; Bellnier *et. al.*, 1989; Peng *et. al.*,1990). These organs have a very good blood supply with dense vascularity. Vascularity and the degree of porphyrin distribution correlate well. Support for this has come from an experiment in which HpD was injected into rabbits, and various parts of the eye analysed (Gomer *et. al.*, 1985). It was found that the avascular sclera contained negligible amounts of porphyrins, while the vascular choroid contained high amounts.

When comparing the excretion rates for the liver and the kidney for both the drugs, the excretion from the kidney is found to be very small due to the smallness of X_3. (Fig.5.2a and 5.2b). Therefore, excretion mainly comes from the liver, k_{2y}. Hp has a large percentage of monomers which are being excreted out of the liver faster compared to HpD which has a higher percentage of the oligomer. The results showed that the monomers are cleared very rapidly from the serum (i.e. whithin 3 hours to negligible level), while the oligomers are still present after 72 hours (Fig 5.2a and b). The mean value of the k^s_{2y} of Hp and the k^s_{2y} of HpD is expected to be similar, which is 0.0108 ± 0.0011 (Table. 5.3b). The serum half-life of Hp is 4 folds smaller than HpD since the value of k_{12} of Hp is 4 folds higher than HpD. After the intravenous injection of Hp, it became obvious that k^f_{2y}, the excretion rate of the fast clearance monomeric component is about 90 times faster than k^s_{2y}, the excretion rate of the slow clearance oligomeric component (Table 5.3a).

This is in agreement with other reports; Hp, the most polar component of Photofrin II, has been shown previously to be eliminated rapidly from rats in the feces (Kessel, 1986b). Extracts of feces from the 0-24 hr collection contained mostly the low molecular weight porphyrins (monomers) in PII although some DHE was present. Extracts of liver showed retention of the highest molecular weight (oligomers) components of Photofrin II. This is consistent with the fact that the low molecular weight components of Photofrin II were rapidly excreted in feces. This is supported by the observation by Bellnier *et. al.* (1989) and others (Jori *et. al.*, 1979) that oligomeric porphyrins, isolated from Photofrin II using gel exclusion chromatography before injection into mice, retained in tissues such as liver to a greater extent than the low molecular weight porphyrin components of Photofrin II.

Similar behaviour of the drugs has been reported by other workers; hematoporphyrin appeared to be mainly retained in the liver and excreted via the biliary tract. Minor amounts were excreted in the urines, and constantly low hematoporphyrin concentration were found in the kidneys (Jori *et. al.*,1979). Studies in dog (Peck, 1955 and Smetana, 1938) and in man (Zalar *et. al.*, 1977) have led to the same conclusions.

It has been shown that Hp and various components of Hp based drugs such as HpD, Photofrin II and PHP are transported to the tissues by serum following injection. The observed distribution could possibly transported by serum lipoproteins. Serum lipoproteins and albumin, the main components of serum are the highest affinity carriers of Hp and some components of HpD *in vivo*. At the doses normally used in clinical PDT (3-5 mg/kg body weight of HpD), at least 95% of Hp is transported by serum proteins. It appears that the distribution of porphyrins among serum proteins is dependent on their chemical structure. Albumin and globulins serve as a vehicle to deliver hydrophilic compounds (Hp and other monomeric component of HpD), whereas photosensitizers having a high (> 8) octanol /

water partition coefficient (oligomeric components of HpD) are largely delivered by lipoproteins (Jori, 1989). Kessel (1986b) reported that the albumin bound material is predominantly Hp and HvD, whereas the material bound to lipoprotein is primarily the DHE fraction. Similarly, Candide *et. al* (1986) and Morliere *et. al.* (1987) reported that the monomers of PII are transported bound to albumin whereas the oligomers are transported bound to high and low-density lipoproteins (HDL and LDL). Dougherty (1987a) reported that initially, HpD tends to bind to albumin and lipoproteins (nearly equally to LDL and HDL) but at longer periods almost exclusive binding to HDL is found with lesser amount bound to LDL and albumin.

The monomeric component is rapidly cleared from the blood-stream. HPLC analysis of plasma sample from patients administered PHP has shown that the concentration of monomers has fallen negligible levels only one hour after injection, whereas the oligomers are still present after 24 hrs (Brown *et. al.*, 1992; Vernon *et. al.*, 1995). Several reports (Jori, 1987 and Dougherty, 1987b) indicate that the fraction of photosensitizers transported by albumin and globulins is released about 2 hrs of *iv* injection. Similarly, Kessel (1986b) reported that both Hp and HvD can readily be identified in all of the plasma fractions obtained after 0.5 hr, but 48 hr later, no porphyrins could be detected in the albumin fraction but the oligomers were detected in both LDL and HDL.

The compartmental model for multiple components is a better approach than the compartmental model without taking into considerations the complexity of components in the drug. In the case where the data sets do not give good agreement, one may try to fit the data sets to a certain number of compartments. In this case, with the simple mathematical modelling, the number of compartments described by the data can be expanded easily. With the consideration of the various components, one may also need to expand the number of

compartments, in such a case with this model, it become easier to account for each compartment.

The pharmacokinetics of both the drugs component gave consistent time zone. The parameters of the component of Hp in both the drugs were extracted in the first 6 hours, in the short time zone whereas the slow component was extracted based on the other half of the time zone, from 6 hrs to 96 hrs. For the first 3 hrs after *i.v.* injection of both the drugs, it is shown that only the monomeric component were mainly excreted. The slow component is left to be excreted out at a very slow rate over a long period of time. This is the prolonged skin photosensitivity seen after the injection of PDT drugs like HpD and Photofrin II. Therefore Hp with its high polarity component and a rapid excretion rate promises a new approach to PDT.

5.6 Conclusion

Following the *i.v.* injection of Hp, the rate constants, k of the monomers are larger than the respective k values of the oligomers. This supports that Hp has a large percentage of monomers which are more polar and a lower molecule weight being excreted out of the liver and kidneys much faster compared to HpD which has a higher percentage of the oligomer. Thus, Hp promises a new approach to PDT in relation to negligible skin photosensitivity.

The simple model developed allows for multicompartmental approach to determining pharmacokinetics parameters for Hp and HpD. The mathematical modelling gives a quantitative distribution of the drugs in different compartments where the parameters were readily interpreted.