Photodiagnosis and Photodynamic Therapy 26 (2019) 116–123

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Study of pharmacokinetics and cutaneous photosensitization of hemoporfin in healthy volunteers

Junyu Xu[a,1], Xia Zhao[a,1], Yan Wu[b], Ping Tu[b], Peihong Sun[a], Ying Zhou[a], Yan Liang[a], Xiaoyan Chen[c], Yifan Zhang[c], Yimin Cui[a,*], Jining Tao[d,*]

[a] Department of Pharmacy, Peking University First Hospital, Beijing, 100034, China
[b] Department of Dermatology and Venereology, Peking University First Hospital, Beijing, 100034, China
[c] Center for Drug Metabolism and Pharmacokinetics Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, 201203, China
[d] Shanghai Fudan-Zhangjiang Bio-Pharmaceutical Co., Ltd., Shanghai, 201210, China

ARTICLE INFO

Keywords:
Hemoporfin
Pharmacokinetics
Photosensitization
Mass balance
Haematoporphyrin

ABSTRACT

Background: Hemoporfin is a porphyrin-based photosensitizer and has been used for photodynamic therapy of port wine stain birthmarks in China. This study assessed the pharmacokinetics and cutaneous photosensitization of Hemoporfin in healthy volunteers.

Methods: Sixteen healthy subjects received a single intravenous infusion injection of Hemoporfin (5 mg/kg). The concentrations of Hemoporfin (MHD) and its metabolite Haematoporphyrin (HP) in plasma, urine and faeces were determined. The pharmacokinetic parameters were calculated. In addition, the cutaneous photosensitization was evaluated under the irradiation of solar simulator, 532 nm laser, and sunlight.

Results: The C_{max} of MHD and HP were 46.7 ± 8.41 and 1.04 ± 0.265 μg/ml, respectively. The $t_{1/2}$ of MHD and HP were 5.09 ± 0.945 and 5.71 ± 2.65 h, respectively. The $AUC_{0\text{-}24h}$ of MHD and HP were 29.8 ± 6.19 and 0.757 ± 0.285 h·μg/ml, respectively. The $AUC_{0-\infty}$ of MHD and HP were 29.8 ± 6.2 and 0.792 ± 0.308 h·μg/ml, respectively. The cumulative fecal excretion rate of MHD and HP were 45.3% and 1.05% at 96 h, respectively. Whereas, the cumulative urinary excretion rate of MHD was only 0.132% at 96 h. The concentration of HP in urine was less than 10% of MHD. After 52 h of administration, the cutaneous photosensitization associated with the exposure to various light sources was minimal.

Conclusion: MHD and HP were excreted mainly through the faeces after intravenous infusion. Hemoporfin associated cutaneous photosensitization was insignificant.

1. Introduction

Photodynamic therapy (PDT) has been proposed as an alternative for the treatment of hyper vascular dermal lesions in the early 1990's [1]. PDT is based on the selective irradiation of the target tissue (abnormal dilated vessels) which is sensitized by a photosensitizer. A local photochemical reaction is induced which results in the production of oxygen-derived free radicals. These highly reactive singlet oxygen molecules can cause capillary wall damage and vessel closures [1]. Mounting clinical data show that PDT is effective for blanching the reddish colour of port wine stain (PWS) birthmarks [2].

Hemoporfin for Injection (Hemoporfin) is a porphyrin-based second generation photosensitizing drug and recently approved for the treatment of PWS in China. Its photoactive component is a synthesized mixture of two positional isomers of 7(12)-(1-methoxyethyl)-12(7)-(1-hydroxyethyl)-3,8,13,17-tetramethyl-21H,23H-porphin-2,18-dipropionic acid (MHD). It was formerly known as hematoporphyrin monomethyl ether (HMME) [3]. In many ways Hemoporfin ($C_{35}H_{36}N_4O_6$, MW 612.72) appears similar in optical spectral behaviour to other haematoporphyrins in that there are strong Soret band peaks with several Q band peaks in the optical window of PDT treatment [4]. Compared to the first-generation hematoporphyrin derivative (HpD), Hemoporfin has advantages of known structure, rapid metabolism and moderate photodynamic activity [5].

A Phase I clinical trial conducted in health subjects suggested that IV infusion of Hemoporfin was well tolerated [6]. In this early trial, Hemoporfin concentrations in plasma and urine were determined using high-performance liquid chromatography with fluorescence detection

* Corresponding authors.
E-mail addresses: cuiymzy@126.com (Y. Cui), jntao@fd-zj.com (J. Tao).
[1] The first two authors contributed equally to this paper.

https://doi.org/10.1016/j.pdpdt.2019.02.023
Received 13 June 2018; Received in revised form 23 January 2019; Accepted 25 February 2019
Available online 26 February 2019
1572-1000/

(HPLC/FLD). Results suggested that Hemoporfin had a short half-life (< 2 h) after a single IV infusion but urine excretion was not the major route of clearance. The current clinical protocol for adult patients includes the combination of the drug dose of 5 mg/kg and the light source of 532 nm [7]. Available clinical data suggest that Hemoporfin mediated vascular acting PDT is safer and can be repeated in short intervals [8]. Its adverse effects are generally caused by the phototoxicity which is mainly attributed to the overestimate of required light dose [9].

Although the metabolism in animals has been studied [5], only the excretion of Hemoporfin in the urine was measured in a human study. The amount of urine excretion in the first 12-h was only 0.15%. The excretion in the faeces might be a major route. The potential photoreaction of the human skin after the IV injection of the drug has not been studied. It is important to know how long the patients need to avoid the light source after the treatment.

In this study, the concentrations of Hemoporfin and its metabolite Haematoporphyrin (HP) in plasma, urine and faeces were determined in a small group of healthy volunteers after a single IV infusion of Hemoporfin for Injection at a dose level of 5 mg/kg. In addition, the cutaneous photosensitization was also evaluated under the irradiation of various light sources. This study confirmed that Hemoporfin had a short half-life and was mainly excreted in faeces. IV infusion of Hemoprofin did not cause prolonged severe cutaneous photosensitization.

2. Materials and methods

2.1. Study design

This was a single-arm, open-label and single dose pharmacokinetic study. Inclusion criteria: healthy non-smoker subjects of 18–45 years old with the body mass index of 19.0–24.0 kg/m^2 and without signs of clinical abnormalities from clinical examinations and laboratory analyses. Exclusion criteria: history of allergy to any components of Hemoporfin for Injection, female subjects currently pregnant or lactating, habitual drinking of coffee daily, blood donation of more than 400 ml in the past month, history of heart, pulmonary, neurological, endocrine, metabolic or psychiatric disease, taking any drug in the past 30 days, skin test area (back and abdomen) showed obvious skin diseases or other conditions (such as rash, contact dermatitis, scar, hairy) that may affect the evaluation, scar constitution or the formation of scar tendencies, and received extensive sun exposure, existing conditions that might affect drug absorption, distribution, metabolism or excretion drug addiction, untreated mental disorders, drug abuse or alcoholism, hepatitis B/C positive, positive for human immunodeficiency virus, or being considered unsuitable for the study by an investigator.

This study was approved by the Ethics Committee of Peking University First Hospital (China). It was conducted in accordance with the Declaration of Helsinki and GCP. Before participation in the study, written informed consent was provided by all subjects.

2.2. Drug preparation and IV infusion

Hemoporfin for Injection was obtained from Shanghai Fudan-Zhangjiang Bio-Pharmaceutical Co., Ltd. (China). The sterile lyophilized powder contained 100 mg of Hemoporfin per vial. According to the body weight of the subject, the required amount of drug (5 mg/kg) was reconstituted in 50 ml of normal saline. The prepared solution must not be exposed to light and used within 4 h after preparation.

Hemoporfin solution was IV infusion via the median vein of the elbow over a period of 20 min using a syringe pump at a constant speed (2.5 ml/min).Immediately after the completion of drug infusion, 2–4 ml normal saline was injected at the infusion site to prevent the possible phototoxicity induced by the high Hemoporfin concentration at the injection site. After receiving Hemoporfin administration, all subjects were required to stay in the research center for observation for up to 4 days.

2.3. Standard care

The subjects fasted overnight (approximately 10 h) before drug administration and 4 h after Hemoporfin administration. The subjects were asked to avoid strong light exposure after drug administration in order to prevent possible skin phototoxicity that might interfere with the evaluation of skin photosensitization. The subjects were allowed to go outside only before sunrise and after sunset. The subjects continued to avoid sunlight or strong indoor light exposure after discharge and up to 7 days after drug administration. Standardized meals were served during the trial. Caffeine-containing drinks such as tea and coffee were prohibited. Seven days prior to admission and till the end of the follow-up, high intensity and contact sports should be avoided. During photosensitivity evaluation if skin reaction to light irradiation was not return to his or her baseline level the subject would remain hospitalized for observation. On the 14th day after drug administration, the subjects were followed up by a telephone interview to confirm whether the subject's skin returned to normal.

2.4. Sample collection

Venous blood samples (approximately 4 ml) were collected in a vacutainer containing heparin prior to dosing (0 h), 5, 10, and 20 min during dosing, and 5, 10, 20, and 40 min, and 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h and 24 h post-dosing. Blood samples were centrifuged immediately at 4 °C at 3000 rpm for 10 min. Urine samples (10 ml each) were taken prior to dosing and were also collected and pooled for each period at 0–6 h, 6–12 h, 12–24 h, 24–48 h, 48–72 h, and 72–96 h post-dosing, respectively. Faecal samples were collected before dosing and daily till 96 h post-dosing. The samples were weighed, mixed with H_2O of 2 times the weight, and homogenized under ultrasound for 10 min. Plasma samples, urine samples and faecal homogenate were stored at −20 °C until analysis. All sample collection and processing steps were protected from light.

2.5. Pharmacokinetic analysis

Hemoporfin (MHD) and its metabolite Haematoporphyrin (HP) in plasma, urine and faecal samples were analysed using a LC–MS/MS method (Shimadzu LC-20 A HPLC system coupled with TSQ Quantum Vantage triple quadrupole tandem type mass spectrometer, Thermo Finnigan American Company, USA). The quantification limits for MHD were 2.0 ng/ml, 2.0 ng/ml and 1.0 μg/ml for plasma, urine and faeces, respectively, and that for HP were 2.0 ng/ml and 1.0 μg/ml for plasma and faeces, respectively. Both inter-day and intra-day precision (RSD) mean values were less than 13.3%. As the HP concentration in the urine sample is very low (less than 10% of MHD) [6], thus, HP concentration in urine was not determined in this study.

2.6. Pharmacokinetic and statistical analysis

The pharmacokinetic parameters of MHD and HP were calculated by non-compartmental analysis using WinNonlin Version 6.1 (Certara, USA). The measured maximal concentration (C_{max}) and the time to reach maximal concentration (t_{max}) were obtained from the observed values. The elimination rate constant (λ_Z) was calculated by log-linear regression of the terminal portion of the concentration-time curve. The half-life ($t_{1/2}$) was calculated as $t_{1/2} = \ln(2)/\lambda_Z$. The area under the curve from time zero to the time of the last quantifiable concentration ($AUC_{0\text{-}t}$) was calculated by the trapezoidal rule, and the area under the curve from time zero to infinity ($AUC_{0\text{-}\infty}$) was calculated using the formula $AUC_{0\text{-}\infty} = AUC_{0\text{-}t} + C_{last}/\lambda_Z$, where C_{last} is the last measurable concentration. Apparent clearance (Cl) was calculated by dose/$AUC_{0\text{-}\infty}$, and the apparent volume of distribution (V_z) was calculated by Cl/λ_Z.

The cumulative amount of drug excreted into the urine and faeces (Ae) were calculated using urine volume or the faeces weight and drug concentration. The fraction of the drug excreted in urine and faeces (Ae %) was calculated by dividing Ae by the dose. In addition, gender differences in pharmacokinetics were also analysed using SPSS (version 14.0, SPSS Inc., USA). A value of $P < 0.05$ was considered statistically significant.

2.7. Safety evaluation

The physical examinations and laboratory measurements (serum chemistry, haematology, coagulation and urinalysis) were carried out at the screening stage and 24 h and 96 h after dosing. The ECGs and vital signs were obtained at the following time points: screening, 0 h (pre-dosing), 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, 48 h, 72 h and 96 h post-dosing. The injection site reactions were observed and recorded at 0 h (pre-dosing), 2 h, 8 h, 24 h, 48 h and 96 h post-dosing. All adverse events (AEs) were recorded and their severity (mild, moderate or severe) were evaluated.

2.8. Cutaneous photosensitization tests

2.8.1. Using solar simulator

Initially, eight subjects (4 males and 4 females) were randomly selected to receive UVA + UVB light irradiation from a solar simulator (290–400 nm, GS2006 Solar UV Simulator, Beijing QunliAohua Technology Development Co., Ltd., China).To determine the baseline minimal erythema dose (MED) [10,11], before IV injection, six small areas on the dorsum or abdomen were irradiated by the solar simulator in a progressive fashion with an incremental increase of light dose of 1.25 times. The skin reactions at the irradiated site were observed 24 h after irradiation. MED was defined as the minimum light dose that resulted in 1 ° erythema or edema in the irradiated area. The criteria for visually evaluating the degree of skin reaction after irradiation are shown in Table 1 [10–12]. At 24 h after dosing, each received light exposure again from the solar simulator. For safety concern, only ¼ baseline MED was used as the maximal light dose. The light dose for each area was reduced by a multiplier gradient with no overlap in each part of the irradiation site. The skin reaction of the irradiated site was observed 24 h after each irradiation. For those who had more than 1 ° skin reaction would be irradiated with 1 MED again until the reaction returned to 0 or 1 °.

Subsequently, the baseline MED dose of UVA + UVB light irradiation of other eight subjects (4 males and 4 females) were determined before drug injection. They received up to 1 full corresponding baseline MED irradiation at 24 h after dosing. The skin reactions and MEDs were evaluated 24 h after light irradiation. The test was repeated every 24 h and stopped when the minimum amount of erythema was restored to the baseline levels. In addition, these individuals also received controlled light expose to 532 nm laser and daylight - see below.

2.8.2. Using 532 nm laser

The forearm flexion skins of eight subjects, approximately 2 cm spot diameter and no overlap, were irradiated using a 532 nm laser (Wuhan Huagong Laser Engineering Co., Ltd., China) at 100 mW/cm^2 for 20 min before and 24 h after the administration of Hemoporfin. The skin reaction was observed during irradiation, immediately after irradiation, and 24 h after irradiation. The test was repeated every 24 h and stopped when the skin reaction was restored to the baseline levels.

Table 1
Criteria for the degree of skin reaction.

Degree	Erythema	Edema
0	no reaction	no reaction
0.5	trace	barely perceptible
1	minimal with clearly defined borders	easily palpable and confined to the treated area
2	erythema with edema	extending beyond the treated area
3	erythema, edema, and vesiculation	edema and vesiculation

2.8.3. Using outdoor sunlight

The forearm flexion skins of eight subjects, approximately 2 cm spot diameter and no overlap with laser, were exposed to outdoor sunlight for 20 min in the afternoon (12:00-02:00 PM) before and the third day (52 h) after Hemoporfin injection. Sunlight intensity was measured using a power meter (LP-3C, Physcience Opto-electronics Co., Ltd., Beijing, China). The subjects wore sunglasses, protective clothing and held umbrella as during the sunlight exposure. The skin and surrounding areas of the irradiated sites were covered. The skin reactions were observed during the irradiation and 24 h after irradiation. The skin reactions were evaluated 24 h after light irradiation. The test was repeated every 24 h and stopped when the skin reaction was restored to the baseline levels.

3. Results

3.1. Demographic data

A total of forty-two subjects participated in the screening. Sixteen subjects were qualified and other twenty-six subjects were excluded mainly because of abnormal laboratory tests. All recruited subjects (8 males and 8 females) were Chinese Han population. Their mean age was 29.5 years old (20–43 years old), mean body weight 62.1 kg (50–75 kg), and mean body mass index 22.3 kg/m^2 (19.1–23.8 kg/m^2). According to the Fitzpatrick classification, two subjects were skin type II and rest were skin type III. Except one female subject quitted the trial 56 h after drug administration for personal reason (not related to this research) and all remaining 15 subjects completed the trial as planned.

3.2. Hemoporfin pharmacokinetics and mass balance

Blood and urine samples were collected from 16 subjects including the one who quitted later but her urine data were not included. Faecal samples were randomly collected from 8 subjects (4 males and 4 females). MHD and HP concentrations were determined by LC–MS/MS analysis. The pharmacokinetic parameters of MHD and HP were calculated by non-compartmental assay. The mean plasma concentration-time plots for MHD and HP are presented in Fig. 1. The key pharmacokinetic parameters are presented in Tables 2 and 3.

As expected, MHD had the highest plasma concentration (C_{max}) at 20 min following IV infusion (i.e. at the end of 20 min injection). The average MHD concentration was 46.7 μg/ml. The $AUC_{0\text{-}24h}$ value was 29.8 h μg/ml. The plasma elimination half-life ($t_{1/2}$) was approximately 5 h. The metabolite HP was detected in the plasma and its $t_{1/2}$ value was similar to that of MHD. However, its plasma concentration and exposure (AUC) were less than 3% of that of MHD.

In addition to the mean pharmacokinetic parameters of pooled data, that of male and female groups were also analysed separately. The p values (> 0.1) indicated that in term of plasma pharmacokinetic parameters there was no significant difference between male (n = 8) and female (n = 8) for both MHD and HP (see Tables 2 and 3).

Mass balance analysis showed that the excretion of MHD in urine was only 0.132% (n = 15). The urine concentration of metabolite HP was 10% of that of MHD. So the quantitative analysis was not carried out for HP. The total recovery of MHD and HP in faeces was 46.3 ± 10.5% (n = 8) at 96 h. The main excretion route of Hemoporfin was mainly the faecal excretion (45.3 ± 10.4% for MHD and 1.05 ± 0.278% for HP). This suggested that Hemoporfin was mainly excreted through faeces in its original form.

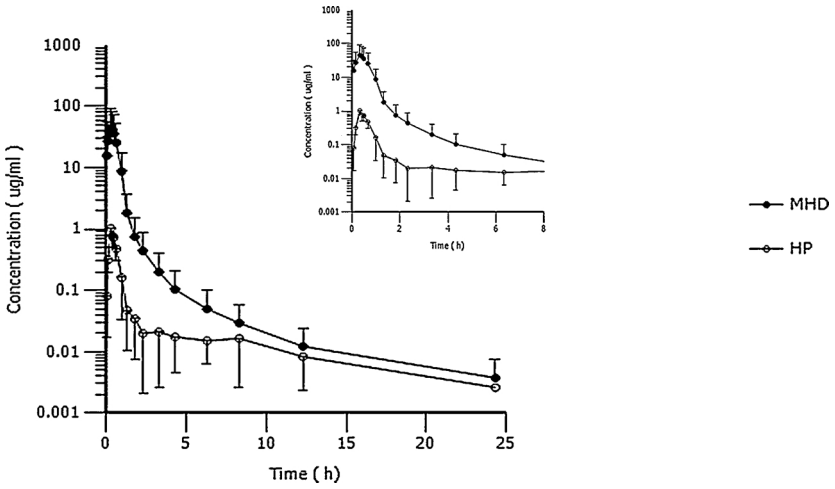

Fig. 1. Mean MHD and HP plasma concentration versus time following single dose injection of Hemoporfin (5 mg/kg).

Table 2
Pharmacokinetic parameters of MHD (mean ± SD).

Parameter	Total (N = 16)	Female (N = 8)	Male (N = 8)	*t*	*p*
T_{max}[a](h)	0.333(0.333,0.417)	0.417(0.333,0.417)	0.333(0.333,0.333)	–	–
C_{max}(μg/ml)	46.7 ± 8.41	44.5 ± 2.92	48.9 ± 11.5	0.880	0.404
AUC_{0-t}(h·μg/ml)	29.8 ± 6.19	29.7 ± 4.01	29.9 ± 8.13	−0.141	0.890
$AUC_{0-\infty}$(h·μg/ml)	29.8 ± 6.2	29.7 ± 4.02	30.0 ± 8.13	−0.133	0.896
$t_{1/2}$(h)	5.09 ± 0.945	4.81 ± 0.938	5.36 ± 0.929	1.174	0.260
Cl(L/h/kg)	0.174 ± 0.0344	0.171 ± 0.0236	0.177 ± 0.0443	0.330	0.746
V_z(L/kg)	1.27 ± 0.339	1.16 ± 0.129	1.38 ± 0.450	1.322	0.222
$Ae(\%)_{urine}$(%)	0.132 ± 0.0829[b]	–	–	–	–
$Ae(\%)_{faeces}$(%)	45.3 ± 10.4[c]	–	–	–	–

[a] Median (range).
[b] N = 15.
[c] N = 8.

Table 3
Pharmacokinetic parameters of HP (mean ± SD).

Parameter	Total (N = 16)	Female (N = 8)	Male (N = 8)	*t*	*p*
T_{max}[a](h)	0.333(0.333,0.500)	0.333(0.333,0.500)	0.333(0.333,0.333)	–	–
C_{max}(μg/ml)	1.04 ± 0.265	1.01 ± 0.202	1.08 ± 0.327	0.284	0.781
AUC_{0-t}(h·μg/ml)	0.757 ± 0.285	0.751 ± 0.179	0.764 ± 0.377	−0.293	0.776
$AUC_{0-\infty}$(h·μg/ml)	0.792 ± 0.308	0.786 ± 0.189	0.798 ± 0.409	−0.350	0.734
$t_{1/2}$(h)	5.71 ± 2.65	6.46 ± 2.55	4.97 ± 2.70	−0.879	0.394
$Ae(\%)_{urine}$(%)	–	–	–	–	–
$Ae(\%)_{faeces}$(%)	1.05 ± 0.278[b]	–	–	–	–

[a] Median (range).
[b] N = 8.

3.3. Safety

There were no serious adverse events or complains noted in this study. Laboratory tests showed that 1 case (male) of elevated triglyceride at 96 h after dosing and 3 cases (two females and one male) of elevated white blood cells in the urine at 24 h after dosing. The AEs reported in the study were carefully evaluated by the investigators and considered as being mild in severity. By the end of the study, all AEs were relieved without medical intervention. No subjects quitted the trial due to adverse events. Subjects had no clinically significant changes in vital signs and ECG examinations during the trial. No subject experienced serious phototoxicity while receiving the skin photosensitization test.

3.4. Cutaneous photosensitization

Skin reactions induced by light irradiation before and after Hemoporfin dosing were evaluated visually. The decrease of MED and/or the increase in the degree of skin reaction after irradiation in the same subject suggested Hemoporfin-related cutaneous photosensitization.

In subjects (n = 8) exposed to UVA + UVB irradiation their baseline MEDs ranged between 700 and 2135 mJ/cm^2. At 24 h after Hemoporfin dosing these subjects were irradiated with UVA + UVB at the dose level of ¼ of their baseline MED, no obvious erythema/edema was developed in these subjects. Then, these individuals received a full baseline MED of irradiation at 48 h after Hemoporfin dosing and the visual evaluation performed 24 h later showed that the skin reaction to UVA + UVB irradiation at corresponding MEDs were at the same levels as their baselines (Fig. 2, top panel).

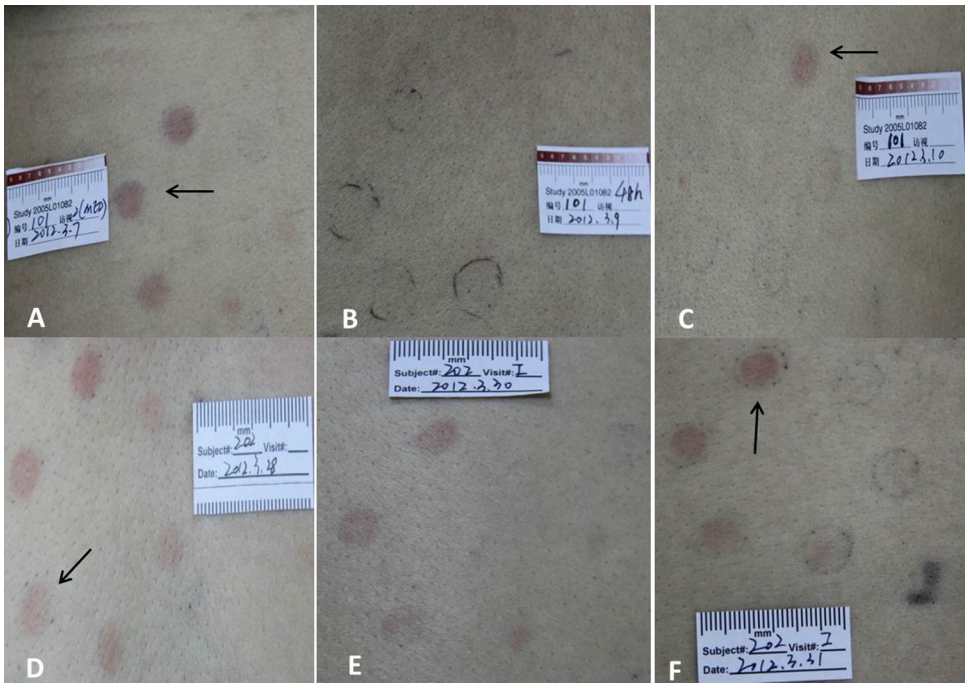

Fig. 2. Representative photographs of skin reactions at 24 h after the subjects receiving gradient doses of UVA + UVB light irradiation. Top panel (female subject No. 101, skin type III): (A) Irradiation at 24 h before Hemoporfin injection, (B) Irradiation at 24 h after Hemoporfin injection (maximal ¼ baseline MED), (C) Irradiation at 48 h after Hemoporfin injection (maximal 1 baseline MED). The arrows indicate the skin erythematic reaction corresponding to 1 MED dose of 1708 mJ/cm^2. Bottom panel (female subject No. 202, skin type II): (D) Irradiation at 24 h before Hemoporfin injection, (E) Irradiation at 24 h after Hemoporfin injection (maxima 1 baseline MED), (F) Irradiation at 48 h after Hemoporfin injection (maxima 1 baseline MED). The arrows indicate the skin erythematic reaction corresponding to 1 MED dose of 1366 mJ/cm^2.

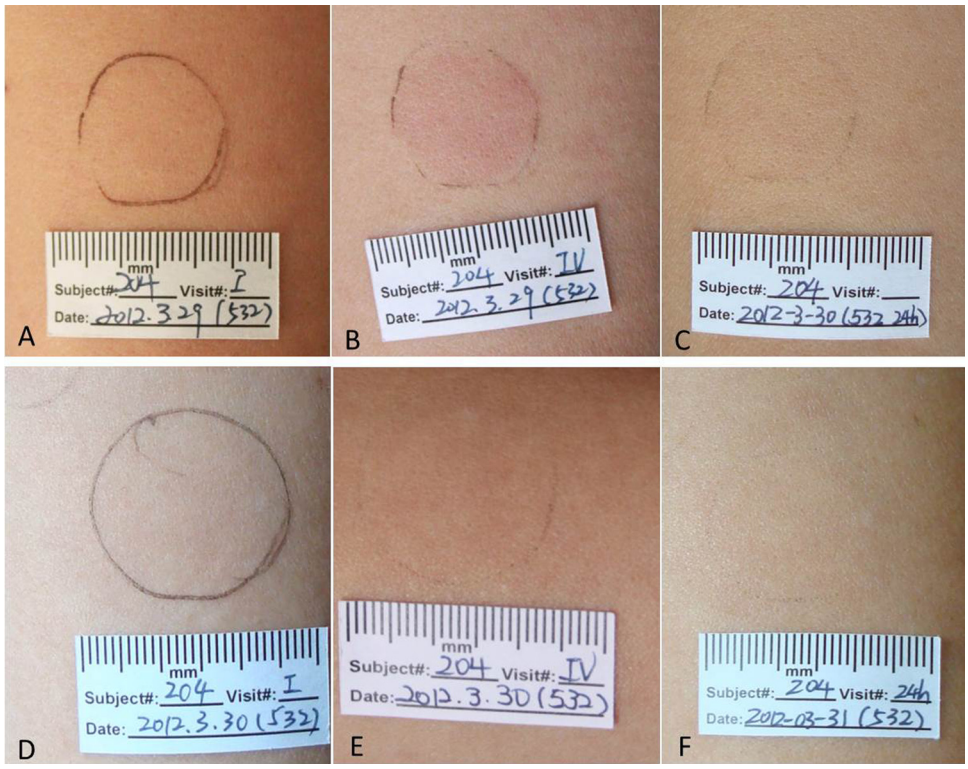

Fig. 3. Representative photographs of a female subject (No. 204, skin type III) showing skin reactions to 532 nm laser light of 120 J/cm^2. Top panel: Irradiation at 24 h after Hemoporfin injection (A) before irradiation. (B) 4–6 h after laser irradiation. (C) 24 h after laser irradiation. Bottom panel: Irradiation at 48 h after Hemoporfin injection (D) before irradiation. (E) 4–6 h after laser irradiation. (F) 24 h after laser irradiation.

Subsequently, another 8 subjects (baseline MEDs ranged between 1366 and 2699 mJ/cm^2) were exposed to the full baseline MED dose of UVA + UVB irradiation at 24 h after Hemoporfin dosing. Three out of 8 (37.5%) showed that their skin reactions were slightly greater than that before the drug administration. This caused a slight decrease in their MEDs. One individual (female, skin type III) changed from 1708 mJ/cm^2 to 1366 mJ/cm^2 and two individuals (one female with skin type II and one male with skin type III) from 1366 mJ/cm^2 to 1093 mJ/cm^2. These three individual received a full baseline MED irradiation again at 48 h after Hemoporfin dosing and the visual evaluation performed 24 h later showed that the skin reaction to UVA + UVB irradiation at corresponding MEDs were at the same levels as their baselines (Fig. 2, Bottom panel).

At 24 h after drug administration, the irradiation of skin with the 532 nm laser at a therapeutic dose level (120 J/cm^2) did not cause conscious symptoms but a warm feeling in the irradiated area during the irradiation. Four out of 8 subjects (50%) showed 1 ° erythema and 1 of them had 0.5 ° edema at the irradiated area between 4–6 h after irradiation. All these skin reactions completely subsided and skin surface returned to normal 24 h after irradiation. Those 4 subjects showing 1 °

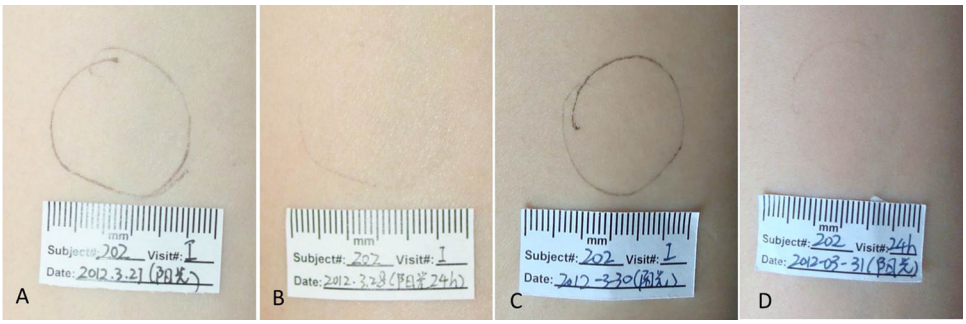

Fig. 4. Representative photographs of a female subject (No. 202, skin type II) taken before and after sunlight exposure. (A) Before Hemoporfin injection and before sunlight exposure. (B) Before Hemoporfin injection and 24 h after sunlight exposure. (C) After Hemoporfin injection and before sunlight exposure. (D) 24 h after sunlight exposure. (Exposure at 52 h after Hemoporfin injection).

Table 4
Photosensitisation of the skin together with $t_{1/2}$ of Hemoporfin in 8 healthy volunteers after Hemoporfin injection.

	$t_{1/2}$	MED0[a]	MED24[b]	MED48[c]	532nm[e] Erythema /Edema	Day light[g] Erythema /Edema
	h	mJ/cm^2	mJ/cm^2	mJ/cm^2	4-6h[f]	24 h[f]
201	5.04	2135	2135	–	0/0	0/0
202	4.98	1366.4	1093.12	1366.4	0/0	0/0
203	6.44	1708	1366.4	1708	1/0	0/0
204	4.84	1708	1708	–	1/0.5	0/0
205	4.16	1708	1708	–	0/0	0/0
206	5.50	1366.4	1366.4	–	1/0	0/0
207	7.32	2669.24[d]	2669.24	–	1/0	0/0
208	5.16	1366.4	1093.12	1366.4	0/0	0/0
Mean	5.43	1753.43	1642.41	1480.27		
SD	1.00	453.54	542.67	197.22		
Min	4.16	1366.40	1093.12	1366.40		
Median	5.1	1708.00	1537.20	1366.40		
Max	7.32	2669.24	2669.24	1708.00		
CV%	18.40	25.87	33.04	13.32		

[a] MED of pre-dose.
[b] MED of 24 h post-dose.
[c] MED of 48 h post-dose.
[d] For subject 207, the area where the illumination energy is 2135 mJ/cm^2 is slightly erythema, and the boundary is clear. Therefore, 1.25 times the dose of this irradiation site is taken as the MED dose.
[e] Subjects received 532 nm laser irradiation 24 h post-dose.
[f] Post irradiation time.
[g] 52 h post-dose.

erythema also received another laser irradiation of the same dose at 48 h after drug administration and no obvious response was detected (Fig. 3).

The individuals received controlled sunlight exposure (20 min at average light intensity of 85 mW/cm^2) at 52 h after drug administration did not show any sign of abnormal symptoms nor noticeable skin reaction at the irradiated areas. The skin reactions to sunlight were classified as 0 ° for all 8 subjects (Fig. 4).

Table 4 lists the half-life and cutaneous reactions of eight individuals. Although three individuals with lower MEDs showed the decrease of MED value after receiving the sensitizer injection, there was no statistical difference between the average MED before sensitizer injection and that after injection ($p = 0.1235$) (Fig. 5a). There was no strong correlation between the half-life and MED (Fig. 5b).

4. Discussion

In PDT, the drug-to-light interval time can significantly affect the primary target of PDT treatment. The microvessels in the light irradiation site are the primary target when light was delivered in a short drug-to-light interval, i.e. during or short after drug injection while sensitizers are mainly retained in the blood vessel compartment [13]. The vascular targeted PDT has been successfully used in the treatment of various ocular vascular diseases [14,15].

Recent study also suggests that vascular targeted PDT can be a useful alternative in the treatment of solid tumor such as prostate cancer [16]. Pharmacokinetic study of photosensitizer used in vascular PDT mode is critical as the plasma concentration of the photosensitizer plays an important role in determining the effectiveness of vessel closure and optimizing the treatment planning.

This study evaluated several key pharmacokinetic parameters that include the C_{max}, $AUC_{0\text{-}24h}$, $t_{1/2}$ and mass balance of Hemoporfin in healthy adults at a clinically relevant dose. In addition, the cutaneous photosensitization after Hemoporfin injection was also examined using various light sources.

Pharmacokinetic data obtained from healthy volunteers suggested that Hemoporfin had a fairly short life time after IV injection (i.e. 5.09 ± 0.945 h, see Table 2) and therefore is more suitable for the treatment of benign vascular diseases than sensitizers with longer half-life. However, in our early study [6], we reported that after a single intravenous injection of Hemoporfin at the same dose in the healthy subjects the $t_{1/2}$ was much shorter (i.e. 1.31 ± 0.33 h). Compared to C_{max} and AUC reported in the early study, the overall exposure was

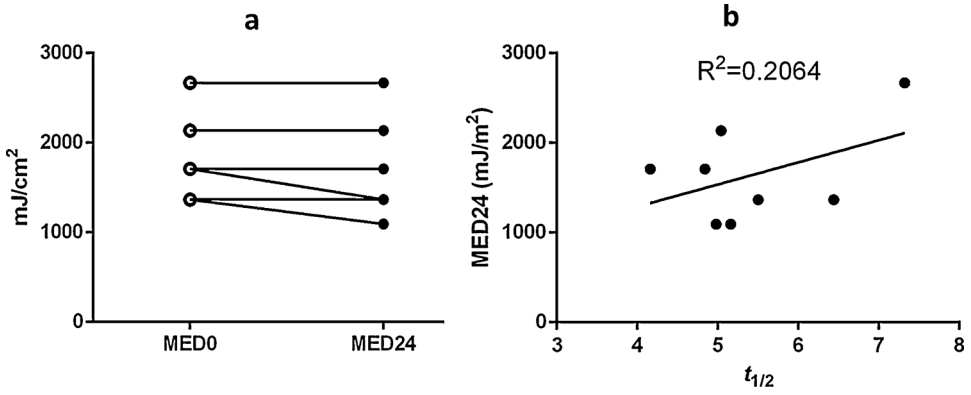

Fig. 5. Compare of MED before and after Hemoporfin injection and its correlation with the half-life of Hemoporfin in 8 healthy volunteers (201–208).

elevated as the C_{max} increased by 31% and AUC by 70%. These differences might be caused by individual variance, improved detection limit and extended blood collection time points. More specifically, the sensitivity and reliability of the mass spectrometry method were higher than that of the fluorescence detection method, and the blood collection time was extended from 8 h in previous study to 24 h in this study.

Compared to other photosensitizers, although longer than Padeliporfin (1.14–1.24 h) the half-life of Hemoporfin is close to Verteporfin (4.92–5.79 h) but shorter than Talapofin (8.63 h) and much shorted than Photofrin (415 h) [17–19]. For sensitizer with the short half-life the light avoidance period can be shortened after PDT treatment and this can be a significant advantage in vascular PDT of benign diseases such as PWS.

Pharmacokinetic data obtained from healthy volunteers suggested that Hemoporfin was mainly excreted from faeces after injection in its original form. The cumulative fecal excretion rate of MHD and HP were 45.3% and 1.05% at 96 h, respectively. Whereas, the cumulative urinary excretion rate of MHD was only 0.132% at 96 h (see Tables 2 & 3). This is consistence with the result obtained in early human and animal study. In the previous human study [6], we reported that the urinary excretion within 12 h was approximately 0.15 ± 0.03% after a single intravenous injection of Hemoporfin at the same dose. An animal study shows that after a single dose of 10 mg/kg IV injection of Hemoporfin in rats [5], the urinary excretion and the faecal excretion within 48 h were 0.19% and 57.5%, respectively. These excretion data indicate that Hemoporfin is mainly cleared by the liver in its original form rather than being metabolized. The urinary excretion might not be the major route for photosensitizers. An early study suggested that prototypes and metabolites of Verteporfin were not detected in urine after 12 h of administration in humans and the cumulative urinary excretion rate was less than 0.01% within 12 h [17]. An excretion study of Padeliporfin showed that in rats the urinary excretion after 96 h of administration was minimal [20].

Early studies show that for commonly used photosensitizers (e.g. Photofrin and Verteporfin) there is no significant gender difference in terms of pharmacokinetics [17,19]. However, although no difference was seen in this study, our early study suggested that there were some differences in C_{max} and AUC_{0-t} values between males and females at the dose level of 7.5 mg·/kg [6]. Both were higher in females than in males - 67.64 ± 7.97 mg/L vs 116.72 ± 20.27 mg/L for C_{max}, 39.99 ± 4.96 mg·h/L vs 88.58 ± 36.00 mg·h/L for AUC_{0-t}. The reason for this gender difference is not clear. We speculate that as Hemoporfin is mainly cleared by the liver and it may be metabolized by p-glycoprotein in the liver. The content of p-glycoprotein is higher in males than females. High concentrations of drugs could saturate the transport capacity of p-glycoprotein and therefore present a gender-specific difference [6,21].

Photosensitizer with slow clearance from body and high retention in the skin tissue can cause prolonged risk of skin phototoxicity and patients have to avoid light exposure for several weeks after a systemic administration of such sensitizer. An early study suggests that the skin phototoxicity associated with Hemoporfin is rare if light avoidance is strictly followed and the skin phototoxicity is mainly caused by over treatment [9]. The post PDT cutaneous photosensitization is likely caused by the presence of photosensitizers in the skin tissue and in the microvessel system as well. This study did not examine the pharmacokinetics of Hemoporfin in the skin tissue due to the difficulties to perform biopsy but examined the cutaneous photosensitization instead, which is directly affected by the concentration of Hemoporfin in the skin tissue. The cutaneous photosensitization at different time points post Hemoporfin injection was examined under controlled exposure using various light sources.

When using the solar simulator that emits both UVA and UVB the decrease of MED values after Hemoporfin injection indicates an elevated cutaneous photosensitization. The tested subjects (n = 16) of this study showed a broad range of the baseline MEDs, i.e. from 700 mJ/cm^2 to 2699 mJ/cm^2, which indicates a good representativeness of skin photosensitivity. At 24 h after Hemoporfin dosing, no signs of phototosensitization were seen when exposed to ¼ MED but approximately 40% showed signs of mild phototosensitization (e.g. MED decrease and skin reaction) when exposed to a full MED. These reactions subsided quickly and another full MED exposure at 48 h after Hemoporfin dosing did not cause detectable phototosensitization (see Fig. 2).

The combination of Hemoporfin and 532 nm light has become the standard approach in the treatment of PWS birthmarks in China [22,23]. Both laser (KTP laser or diode laser) and high power narrow band green LED panel have been used as light sources in PWS PDT. The former is delivered to the lesion site in the form of spherical irradiation spot through optic fiber and the latter in the form of spherical or rectangular irradiation field. To test the possible skin phototoxicity under the therapeutic dose of 532 nm light, a 532 nm laser was used. At 24 h after Hemoporfin dosing, 50% subjects showed 1 ° erythema and 12.5% subjects showed 0.5 ° erythema. But all these reactions completely subsided within 24 h. Those subjects showing 1 ° erythema also received another round of irradiation at 48 h after doing but no one showed noticeable response (see Fig. 3).

The results of solar simulator and laser exposure suggested that the IV injection of Hemoporfin did not cause significant and harmful phototoxicity to the normal skin tissue. To further test the potential skin phototoxicity under natural sunlight exposure, small areas of skin were irradiated directly by sunlight. Due to the limited number of subjects and safety concern this experiment was not carried out until 52 h after dosing. The 20 min of exposure to the sunlight of 85 mW/cm^2 at noon was equivalent to 102 J/cm^2. As expected no subjects showed noticeable skin reaction (see Fig. 4).

In general, those photosensitizers with longer half-life and high plasma concentration might pose high risk of prolonged skin photosensitization. As Hemoporfin had relatively short half-life and its plasma concentration at 24 h after IV injection was already low, these might explain why Hemoporfin showed desired vascular effect under light irradiation but did not cause significant cutaneous phototoxicity to the normal skin under controlled light exposure. Nevertheless, the possible correlation between the half-life and MED was analyzed. Result implied that they had no strong correlation ($p = 0.2064$) (see Fig. 5b). Likely, the Hemoporfin-related non-vascular skin reactions are determined mainly by the drug concentration and intrinsic light sensitivity of the skin.

In conclusion, this pharmacokinetic study demonstrates that Hemoporfin is mainly excreted in faeces in its original form. There are no significant gender difference in terms of C_{max}, AUC_{0-24h}, $t_{1/2}$ and mass balance after the single dose injection of 5 mg/kg. At this dose level Hemoporfin associated cutaneous photosensitization is insignificant after 52 h postdose. However, it should be pointed out that since the research population was dominantly healthy adults and the sample size was small, so for future study larger sample size and younger populations should be considered to investigate the gender difference and skin photosensitization to Hemoporfin.

Conflicts of interest

Jining Tao is an employee of Shanghai Fudan-Zhangjiang Bio-Pharmaceutical Co., Ltd.

Acknowledgements

We thank Professor Zheng Huang (University of Colorado Denver, Denver CO, USA) for valuable discussions. This study was sponsored by Shanghai Fudan-Zhangjiang Bio-Pharmaceutical Co., Ltd.

References

[1] A. Orenstein, J.S. Nelson, L.H. Liaw, R. Kaplan, S. Kimel, M.W. Berns,

Photochemotherapy of hypervascular dermal lesions: a possible alternative to photothermal therapy? Lasers Surg. Med. 10 (4) (1990) 334–343.
[2] W. Yu, G. Ma, Y. Qiu, H. Chen, Y. Jin, X. Yang, X. Hu, L. Chang, T. Wang, H. Zhou, W. Li, X. Lin, 18 years long-term results of facial port-wine stain (PWS) after photodynamic therapy (PDT) - a case report, Photodiagnosis Photodyn. Ther. 12 (1) (2015) 143–145.
[3] Z. Huang, An update on the regulatory status of PDT photosensitizers in China, Photodiagnosis Photodyn. Ther. 5 (4) (2008) 285–287.
[4] T. Lei, G.F. Glazner, M. Duffy, L. Scherrer, S. Pendyala, B. Li, X. Wang, H. Wang, Z. Huang, Optical properties of hematoporphyrin monomethyl ether (HMME), a PDT photosensitizer, Photodiagnosis Photodyn. Ther. 9 (3) (2012) 232–242.
[5] Y. Pu, W. Chen, Z. Yu, Research progress of Hemoporfin - part one: preclinical study, Photodiagnosis Photodyn. Ther. 9 (2) (2012) 180–185.
[6] P. Sun, X. Zhao, Y. Zhou, Y. Liang, H. Zhang, Y. Cui, J. Tao, Tolerance and pharmacokinetics of single-dose intravenous Hemoporfin in healthy volunteers, Acta Pharmacol. Sin. 32 (12) (2011) 1549–1554.
[7] Y. Wang, K. Yuan, W. Gong, J. Zou, Z. Huang, Optimizing light source for photodynamic therapy of port wine stain birthmarks, Photonics Lasers Med. 4 (4) (2015) 375–376.
[8] K. Gao, Z. Huang, K. Yuan, B. Zhang, Z. Hu, Side-by-side comparison of photodynamic therapy and pulsed-dye laser treatment of port-wine stain birthmarks, Br. J. Dermatol. 168 (5) (2013) 1040–1046.
[9] K. Yuan, J. Gao, Z. Huang, Adverse effects associated with photodynamic therapy (PDT) of port-wine stain (PWS) birthmarks, Photodiagnosis Photodyn. Ther. 9 (4) (2012) 332–336.
[10] J.M. Houle, H.A. Strong, Duration of skin photosensitivity and incidence of photosensitivity reactions after administration of verteporfin, Retina 22 (6) (2002) 691–697.
[11] R.A. Weersink, J. Forbes, S. Bisland, J. Trachtenberg, M. Elhilali, P.H. Brún, B.C. Wilson, Assessment of cutaneous photosensitivit of TOOKAD (WST09) in preclinical animal models and in patients, Photochem. Photobiol. 81 (1) (2005) 106–113.
[12] T. Filbeck, M.B. Wimmershoff, U. Pichlmeier, S. Karrer, W.F. Wielan, R.M. Szeimies, W. Rössler, No generalized skin phototoxicity after intravesical application of 5-aminolevulinic acid for fluorescence diagnosis of superficial bladder cancer, Urol. Int. 64 (3) (2000) 126–128.
[13] Z. Huang, H. Xu, A.D. Meyers, A.I. Musani, L. Wang, R. Tagg, A.B. Barqawi, Y.K. Chen, Photodynamic therapy for treatment of solid tumors-potential and technical challenges, Technol. Cancer Res. Treat. 7 (4) (2008) 309–320.
[14] W. Kikushima, Y. Sakurada, A. Sugiyama, S. Yoneyama, N. Tanabe, M. Matsubara, F. Mabuchi, H. Iijima, Comparison of two-year outcomes after photodynamic therapy with ranibizumab or aflibercept for polypoidal choroidal vasculopathy, Sci. Rep. 7 (1) (2017) 16461.
[15] Y. Ho, A. Chao, K. Chen, A. Chao, N. Wang, L. Liu, Y. Chen, Y. Hwang, W. Wu, C. Lai, T. Chen, Clinical outcomes and predictors of response to photodynamic therapy in symptomatic circumscribed choroidal hemangioma: a retrospective case series, PLoS One 13 (5) (2018) e0197088.
[16] A. Kawczyk-Krupka, K. Wawrzyniec, S.K. Musiol, M. Potempa, A.M. Bugaj, A. Sieroń, Treatment of localized prostate cancer using WST-09 and WST-11 mediated vascular targeted photodynamic therapy-a review, Photodiagnosis Photodyn. Ther. 12 (4) (2015) 567–574.
[17] J.M. Houle, A. Strong, Clinical pharmacokinetics of verteporfin, J. Clin. Pharmacol. 42 (2002) 547–557.
[18] A.L. Chan, M. Juarez, R. Allen, W. Volz, T. Albertson, Pharmacokinetics and clinical effects of mono-L-aspartyl chlorin e6 (NPe6) photodynamic therapy in adult patients with primary or secondary cancer of the skin and mucosal surfaces, Photodermatol. Photoimmunol. Photomed. 21 (2) (2005) 72–78.
[19] J.M. Houle, N. Clervoix, S. Bain, J. Spénard, Lack of effect of sex and disease state on the pharmacokinetics of porfimer sodium, Clin. Pharmacokinet. 45 (9) (2006) 923–930.
[20] Committee for Medicinal Products for Human Use (CHMP), European Medicines Agency's committee, Tookad, 2017 EPAR - Public assessment report (Accessed 13 Augst 2018) http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/004182/WC500239529.pdf.
[21] E.G. Schuetz, K.N. Furuya, J.D. Schuetz, Interindividual variation in expression of P-glycoprotein in normal human liver and secondary hepatic neoplasms, J. Pharmacol. Exp. Ther. 275 (2) (1995) 1011–1018.
[22] L. Gan, H. Wang, S.L. Ni, C.H. Tan, A clinical study of HMME-PDT therapy in Chinese pediatric patients with port-wine stain, Photodiagnosis Photodyn. Ther. 23 (2018) 102–105.
[23] Y. Zhang, X. Zou, H. Chen, Y. Yang, H. Lin, X. Guo, Clinical study on clinical operation and post-treatment reactions of HMME-PDT in treatment of PWS, Photodiagnosis Photodyn. Ther. 20 (2017) 253–256.