Comparison of the Concentrations of 8-MOP in both Plasma and Suction Blister Fluid after Oral Ingestion

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Background **: The value of plasma concentration of 8-Methoxypsoralen(8-MOP) in the supervision of photochemotherapy has been recognized. However, plasma levels of 8-MOP were not proportionate to the degree of PUVA induced erythema and couldn't alone predict the degree of PUVA induced erythemal reaction. We made a speculation that the degree of PUVA induced erythema might correlate better with skin tissue levels of 8-MOP than plasma levels. Suction blister fluid(SBF) has been known to represent tissue fluid in the skin. So we performed a study of comparison of 8-MOP concentrations in both plasma and SBF.**

Objective **: Our purpose was to evaluate the correlation of the concentrations of 8-MOP in plasma and SBF 2 hours after oral administration of 0.6 mg/kg of 8-MOP.**

Methods **: Twenty six patients, aged between 16 and 50 years, undergoing suction blister surgery for vitiligo treatment, participated in this open study. Single oral doses of 0.6 mg/kg of body weight of 8-MOP were taken. Blood samples(5ml) and SBF(2ml) were collected at 2 hours after the drug administration, and 8-MOP concentration in plasma and SBF were quantitated by reverse phase high-performance liquid chromatography (HPLC).**

Results **: 8-MOP concentrations in plasma and SBF ranged from 18 to 545 ng/ml and 8 to 179 ng/ml, respectively. On the analysis of linear regression, a close relation could not be observed between two SBF levels; measured and predicted values which were calculated from measured plasma and SBF concentrations (r^2=0.583, $P < 0.001$).**

Conclusion **: The correlation of plasma and SBF concentrations of 8-MOP is weak. So, SBF levels of psoralen are recommended for the study of PUVA erythemal reactions.**

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Key Words : HPLC, Plasma, Psoralen, Suction blister fluid.

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Since the initial work of Pathak et al[1]. on the metabolism of psoralens was reported, it has been demonstrated that psoralen metabolism varies according to individual factor[2-7], the condition of administration[8-10], and even the psoralen preparation used[11,12]. It, therefore, seemed to be indispensable to adjust drug administration as well as the administration of UVA to achieve predictable and desirable PUVA induced erythema and therapeutic effect of PUVA therapy.

However, PUVA induced erythema cannot be predicted from the patient's sun reactive skin

type[10,13] or UVB erythemal sensitivity[14,15]. The value of plasma 8-Methoxypsoralen(8-MOP) concentrations in the surveillance of photochemotherapy has been recognized in the literature[9,10,16-19]. So far, it was suggested that PUVA induced erythemal reaction couldn't be predicted by plasma concentrations of psoralen alone[10,16]. PUVA induced erythemal reaction is suggested to be more likely related with psoralen concentration in the skin tissue rather than that within blood vessels of the skin[20]. If the psoralen concentration in the skin tissue does not coincide with the plasma psoralen concentration, the investigations to find the correlation between PUVA induced erythemal reaction and plasma psoralen concentrations would have no logical basis. This study was designed to investigate the correlation of psoralen concentration in the plasma and suction blister fluid(SBF) which represents tissue fluid in the skin.

SUBJECTS AND METHODS

Subjects and Study Protocol

Twenty six patients of both sexes, aged between 16 and 50 years, undergoing suction blister surgery for vitiligo treatment, participated in this open study. Prior to the study, the experiment protocol had been reviewed and approved by the Ethics Committee of Samsung Medical Center, Seoul, Korea. All participants were prohibited from taking any medicine from 24 hours before until 24 hours after the psoralen administration; especially caffeine, phenytoin and hexobarbital which would affect 8-MOP metabolism[21]. Single oral doses of 0.6mg/kg of the body weight of 8-MOP were taken. These dosimetries are usual dosage for actual treatment condition.

The suction kit is composed of a wall vacuum, connecting tubes and syringes of 10ml. The round aperture of the syringes was applied to the skin of patients. Vacuum pressure was kept around -200mmHg. This suction kit was applied as the patients took 8-MOP. The dermo-epidermal separation could be seen 2 hours later. Blood samples(5ml) and SBF(2ml) were collected at 2 hours after the drug administration. Plasma was immediately separated by centrifugation at 3,000 rpm for 10 minutes and stored duplicate(1ml) at -20℃ until analyzed. SBF was drawn with a mantoux needle and 1ml syringe. SBF was divided into 0.6ml samples in 1.5ml of eppendorf tubes and stored at -78℃ until analysis.

Analytical Methods

Plasma and SBF 8-MOP concentration were quantitated by reverse phase high performance liquid chromatography (HPLC). 8-MOP and the internal standard 5-Methoxypsoralen(5-MOP) were purchased from Sigma(St. Louis, USA). The other chemicals (petroleum ether and absolute ethanol) were analytical grade and methanol was HPLC grade from Burdick & Jackson(Muskegon, USA).

All the chemicals were weighed with a Mettler AE 240 balance. The HPLC system consisted of a WatersTM M600 pump, a M717 Plus Autosampler and a M486E tunable UV detector. Standard stock solutions containing 1mg/ml 8-MOP and 100mg/ml 5-MOP were prepared in absolute ethanol and stored at -4℃ before analysis. A plasma standard for calibration was prepared by diluting stock solution with pooled human plasma and the SBF standard was diluted with plasma aliquot, a mixture of plasma and normal saline in a ratio of 1:2 to bring the protein concentration close to that usually found in SBF[22]. The working internal standard solution was prepared by diluting 150μl of the stock solution of 5-MOP in 250ml of petroleum ether[23].

The standards and samples of plasma and SBF were mixed with 5ml of petroleum ether containing 0.06μg/ml 5-MOP and stirred for 10 minutes in light-protected screw capped glass tubes. After centrifugation for 10 minutes at 3,000 rpm the organic phases (3.5ml) were withdrawn with glass pippette and transfered to test tubes. They were evaporated to dryness under a stream of nitrogen at 37℃ in a heat block. The dry residues were reconstituted in 100μl absolute ethanol and 20μl were injected onto column.

The separation was achieved on Lichrospher™ 100RP-8 (C_8, 4×125mm, 5μm, Merk, USA) column and the mixture of methanol and H_2O (60:40, v/v) was used as the mobile phase. The flow rate was set at 1.0ml/min and the column effluent was monitored by a UV detection at 245nm. All the analysis was performed at room temperature[23]. Waters Millenium™ software was used to measure peak areas.

Table 1. Concentrations of 8-MOP in the plasma and SBF after oral administrations, their ratio, and calculated SBF levels.

Patients No.	Plasma level(x) ng/ml	SBF level (y) ng/ml	Ratio (C_P / C_{SBF})	SBF level (y') Calculated, ng/ml
1	94	51	0.543	40.106
2	132	25	0.189	49.714
3	82	47	0.573	37.072
4	177	59	0.333	61.091
5	172	43	0.250	59.828
6	391	44	0.113	115.199
7	146	49	0.336	53.254
8	277	94	0.339	86.375
9	91	23	0.253	39.348
10	61	27	0.443	31.763
11	545	179	0.328	154.135
12	58	23	0.397	31.004
13	157	116	0.739	56.035
14	312	110	0.353	95.225
15	96	48	0.500	40.612
16	137	36	0.263	50.978
17	176	76	0.432	60.839
18	18	8	0.444	20.891
19	195	34	0.174	65.643
20	72	41	0.569	34.544
21	210	114	0.543	69.435
22	104	60	0.577	42.635
23	23	14	0.609	22.155
24	77	35	0.455	35.808
25	203	49	0.241	67.665
26	195	82	0.421	65.643
Average	161.577	57.192	0.401	57.192
S. D.	114.946	38.077	0.151	29.638

RESULTS

Good linearity with correlation coefficients greater than 0.999 was obtained in a range of 8-MOP concentrations from 0.01 to 2 μg/ml. HPLC chromatograms of 8-MOP and internal standard were shown in figure 1.

The plasma and SBF concentrations for each patient receiving single oral 8-MOP dose of 0.6 mg/kg were shown in table 1. They ranged from 18 to 545 ng/ml and 8 to 179 ng/ml. Mean ± S.D. of them were 161 ± 114 and 57 ± 38 respectively. The ratio of plasma concentration to SBF concentration (C_P/C_{SBF}) in each patient ranged from 0.113 to 0.739 and mean of it was 0.401 ± 0.151. We predicted SBF levels(y') using two parameters of measured plasma(x) and SBF(y) concentrations and there was a weakly positive correlation between predicted SBF levels and measured ones on the analysis of linear regression ($y'=0.253x + 16.34$, $r^2=0.583$, $p<0.001$). Figure 2 showed the relationship between the concentrations in plasma and SBF.

Calculated SBF levels were compared with the measured ones in figure 3. It showed that residual values of SBF levels were diffusely scattered and we thought that two levels had no or low association(s) to each other.

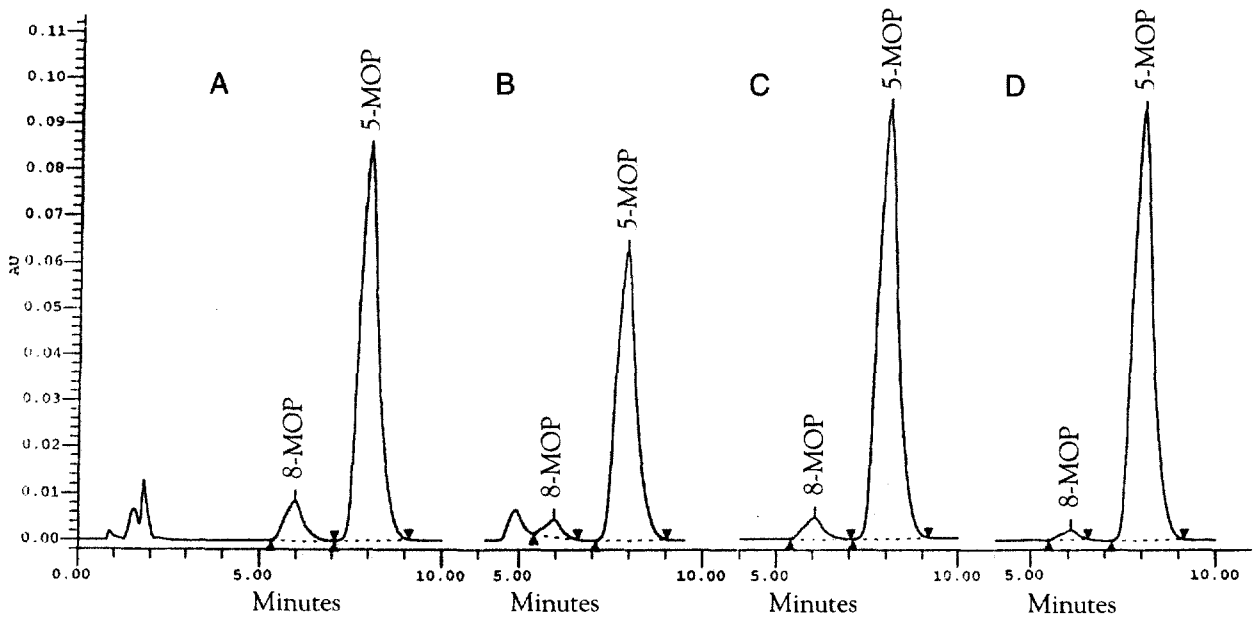

Fig. 1. Representative chromatograms for 8-MOP and 1 μg/ml internal standard (5-MOP): 1 μg/ml plasma standard (A), plasma sample of patients No. 11 (B), 0.5 μg/ml SBF standard (C), and SBF sample of patients No. 11 (D).

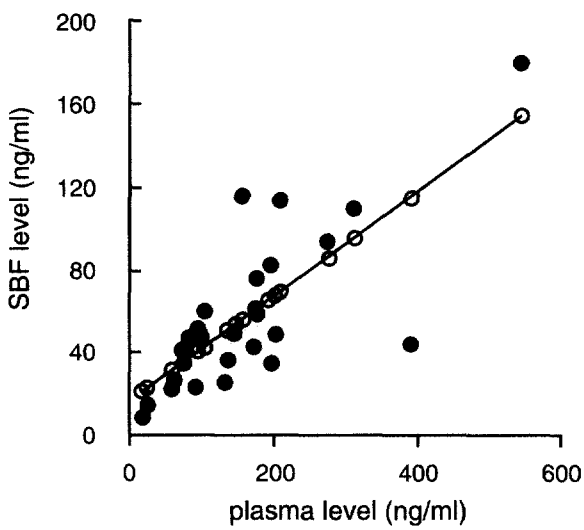

Fig. 2. Relation between SBF levels and plasma levels of 8-MOP (values from table 1). Solid circles: measured SBF levels. Blank circles: calculated SBF levels by the analysis of linear regression ($y'=0.253x+16.34$, $r^2=0.583$, $p<0.001$).

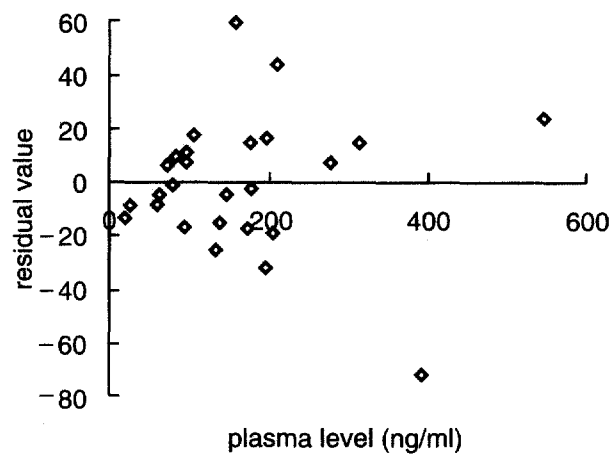

Fig. 3. Residual values (measured - calculated SBF levels, y-y').

DISCUSSION

PUVA is widely used as a therapeutic method in various skin diseases. PUVA erythemal reaction often acts as a very important therapeutic guideline in PUVA therapy. In this case, the concentration of the photosensitizers at the skin is of particular importance to balance beneficial therapeutic effects, for example, the clearing of psoriasis with unwanted side effects such as erythema formation, hyperpigmentation, photoallergy, aging and genotoxic (mutagenic and carcinogenic) effects. Previous studies have revealed marked variation in the

blood levels(50-1,000 ng/ml) of 8-MOP[9,10,16,17,19] and the time needed to reach peak concentration (1-3hr)[6,9,24,25] with therapeutic doses of 8-MOP. This indicates individual differences in absorption and/or metabolism of the drug or differences in the solubility and absorption between various forms of the drug used in these studies.

The large variation in PUVA induced erythemal reaction presents practical problems when choosing a therapeutic dose of UVA. The correlation of psoralen plasma concentration and PUVA induced erythemal sensitivity has been widely investigated[9,10,16,19]. It was shown that the slope of the PUVA erythema dose-response curve, but not the minimal phototoxic dose(MPD), is correlated significantly with the plasma psoralen concentration[10]. Another study done by the same group showed that erythemal sensitivity during PUVA therapy is related to both plasma psoralen concentration and inherent UVA sensitivity and plasma psoralen concentration alone didn't predict PUVA erythemal reaction[16].

The mechanism of the PUVA induced erythemal reaction is not elucidated clearly. It is suggested that psoralen, by administration of UVA, forms photoadducts with proteins, lipids[26,27], and other cellular constituents of the epidermis, dermis and blood capillaries[28]. These photoadducts are considered to provoke toxic reactions in the skin such as erythema, which is suggested to be due to the oxidation of unsaturated fatty acids or damage of proteins in the cell membrane by a free radical mechanism[29]. So PUVA induced erythemal reaction is more likely to be associated with the psoralen concentration in the skin tissue than that in the plasma.

Since several investigations into the kinetics of psoralens in the SBF have been performed, the psoralen concentrations in the SBF might reflect the ones at the skin tissue more accurately than the serum ones[30]. From a pharmacological point of view, SBF obtained by mild suction (-200mmHg) from human skin could be taken as a representative for the interstitial fluid in the skin tissue - which is, similar to human plasma, composed of proteins and lipids[31,32] - and serve as a model for studying pharmacokinetics in the skin[33]. So, we used SBF as a representative of the skin tissue fluid. Also it was reported that the psoralen concentrations reduced more slowly in the SBF than the plasma and that was why skin sensitivity was retained even at markedly decreased serum levels of psoralens[6].

This experiment showed a positive relationship between the concentrations of plasma and SBF as in the previous studies[4,6,25,26,34-36]. However, we could not predict the exact level of SBF 8-MOP concentration through the plasma 8-MOP concentration because the ratio of SBF to plasma levels of 8-MOP ranged diffusely from 0.113 to 0.739. Earlier investigations[4,6,25,35,36] revealed that 8-MOP levels in SBF, as a model for interstitial fluid near the epidermis, amounted to 30% to 40% of the serum concentration and rather consistent SBF to plasma ratio in the concentrations of 8-MOP. Others showed rather inconsistent SBF to plasma ratio like our data[24]. There were many different factors between study designs. Among these studies, Kornhauser et al[35] used peeled off the epidermis in albino guinea pigs of relatively small mass of body as experimental objects and it was thought to result in consistent values of concentration. In the other studies, different factors were as follows; higher negative pressure value (-400 mmHg) for making blisters[25], thin layer chromatography (TLC) as anaytical method[36], different 8-MOP dosage[24], and micronized form of drug (8-MOP)[6] etc. Considering that there were significant differences between the measured SBF levels and the calculated ones presented in both table 1 and figure 3, individual inherent factors seemed to have great influences on the pharmacokinetics in vivo. We recognized that it was very important to measure the concentration of 8-MOP in skin tissue itself, on which therapeutic effect or toxicity were exerted directly, than the plasma concentration after 8-MOP administration.

In conclusion, 8-MOP concentrations in the plasma and SBF after oral administration of 8-MOP have a weak correlation. So, SBF 8-MOP concentration can be used as a more useful value than the plasma 8-MOP concentration for the investigation of PUVA induced erythemal reactions.

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