Yonsei Medical Journal
Vol. 32, No. 3, 1991

Study of the Skin Concentrations after Administration of the Various Phototoxic Drugs

Yoon-Kee Park, Seung Kyung Hann, Moo Yon Cho, Hyung Joo Kim, Sang Chul Shim[1], Seung Ju Choi[1] and Mi Hong Yun[1]

The skin concentrations of 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), and 4, 5', 8-trimethylpsoralen (TMP) were studied in the guinea pig following oral administration and bathing. The skin concentration of phototoxic drugs after oral administration peaked at 1.5 hours, and the concentration of 8-MOP was 3.5 times greater than that of 5-MOP. The skin concentration of TMP was not detected in our study (limit of sensitivity 5ng/ml). The skin concentrations of phototoxic druge after bathing decreased in the order of 5-MOP, TMP, and 8-MOP.

Key Words: Skin concentration, 8-MOP, 5-MOP, TMP

Photochemotherapy(PUVA) with phototoxic drugs and UVA has been widely used and has shown a good therapeutic effect in a variety of skin diseases including psoriasis, vitiligo, mycosis fungoides and atopic dermatitis (Parrish *et al.* 1974; Parrish *et al.* 1976; Lowe *et al.* 1979). The phototoxic drugs mainly used in photochemical treatment are 8-methoxypsoralen (8-MOP). 5-methoxypsoralen (5-MOP), and 4, 5', 8-trimethylpsoralen (TMP). To achieve the maxiumum therapeutic effect in photochemotherapy, it is believed that ultraviolet light should be irradiated when the drug level reaches the highest concentration in the skin (Bersaques *et al.* 1984). Most reports to date are made on the measurement of blood concentration (Siddique *et al.* 1984; Chakrabarti *et al.* 1982). The few made on skin concentration are mostly simple reports on the skin level of one drug (Roelandts *et al.* 1983; Boven *et al.* 1984). Becasuse the blood concentration of TMP was the only drug measured after bathing (Fischer *et al.* 1980), an integrated study of the skin levels of various drugs was thought necessary. Therefore, the authors measured the skin concentrations of 8-MOP, 5-MOP and TMP hourly after ingestion or bathing of the above drugs and found the time and level at which the drug concentration was highest in the skin after biometabolism for the purpose of clinical application to patients.

Received January 22, 1991
Accepted April 29, 1991
Department of Dermatology, Yonsei University College of Medicine, Seoul, Korea
Department of Chemistry[1], Korea Advanced Institute of Science and Technology, Seoul, Korea
This study was supported by CMB-YUHAN Research Fund (1987)
Address reprint requests to Dr. Y-K Park, Department of Dermatology, Yonsei University College of Medicine, CPO Box 8044, Seoul, Korea, 120-752

EXPERIMENTAL MATERIALS AND METHOD

Experimental animals

Seventy guinea pigs, 300-500gm in weight, were divided into the following subgroups:

a. Control-ten guinea pigs
b. Oral administration (10mg/kg)
   Group with 8-MOP-10 guinea pigs
   Group with 5-MOP-10 guinea pigs
   Group with TMP-10 guinea pigs
c. Bathing
   Group with 8-MOP-10 guinea pigs
   Group with 5-MOP-10 guinea pigs
   Group with TMP-10 guinea pigs

Experimental drugs

The pure powders of 8-MOP (Sigma Chemical Co., St. Louis, Missouri, USA), 5-MOP (Sigma Chemical Co., St. Louis, Missouri, USA) and TMP (Sigma Chemical Co., St. Louis, Missouri, USA) were used after being dissolved in absolute alcohol and then diluted in saline. As for the internal standards of 8-MOP and TMP, 5-MOP was used and for that of 5-MOP, 8-MOP was used.

Experimental apparatus

The concentration of the drug was measured with high performance liquid chromatography (HPLC; Waters Associates, M-244, Milford, Conn., USA) equipped with a 254nm UV detector and a Zorbax Sil 4.6mm x 15cm column (Dupont Instrument).

Experimental method

Oral administration of phototoxic drugs: The pure powders of the phototoxic drugs, 8-MOP, 5-MOP and TMP, were separately dissolved in absolute alcohol, diluted in saline, and ingested through the mouth with a tube at a dosage of 10 mg/kg.

Bathing in phototoxic solution: The pure powders of 8-MOP, 5-MOP and TMP were separately dissolved in absolute alcohol and diluted in saline to a concentration of 10 mg/L and the guinea pigs, which were epilated on the back, were bathed for ten minutes.

Skin biopsy: In the group with ingestion of phototoxic drugs, the punch biopsy was performed after removal of the hair on the abdomen in each of the guinea pigs before drug ingestion and at 30 minutes, 1 hour, 1.5 hours, 2 hours, 2.5 hours, 3 hours, 5 hours, and 7 hours after drug ingestion. Also, a skin biopsy was done at 0.5 hours, 1 hour and after removal of the horny layer by repetition of attachment and detachment of scotch tape about 30 times to 1 hour after bathing.

Homogenization of the biopsied specimen: After weighing the biopsied specimen, it was cut into thin sections, which were transferred into a test tube and then mixed with 100ul of the internal standards and homogenized with a polytron homogenizer after grinding for 2 minutes.

To the homogenized tissue, 5cc of extraction solution was added and it was mixed well with the rotator. After centrifugation at 3000 × g for 10 minutes, the supernatant alone was taken, and this supernatant was evaporated using nitrogen gas and vaccum oven.

Measurement of the drug concentration in the tissue using HPLC: After complete evaporization, 40 μl of dichoromethane was added to the test tube to dissolve the residue. This solution was injected into the HPLC column, and the peak on the HPLC recorder was measured with the integrator.

RESULTS

Oral administration of phototoxic drugs

After oral administration of 8-MOP, 5-MOP and TMP at 10 mg/kg, the concentrations of the phototoxic drugs in 1 gram of biopsied tissue at 0.5 hour,

Table 1. The concentration of the phototoxic drugs in guinea pig skin in ng/g of wet weight as a function of time after oral administration (10mg/kg)

Time (hours)	8-MOP*	5-MOP*	TMP
0.5	895.00±166.68	100.00±37.45	ND
1.0	1194.40±384.10	256.20±62.51	ND
1.5	1441.80±173.05	385.80±55.92	ND
2.0	1130.80±144.00	285.40±48.13	ND
2.5	753.00±227.27	264.40±35.50	ND
3.0	505.60±140.24	204.00±46.17	ND
5.0	502.60±87.68	50.90±13.32	ND
7.0	312.20±61.23	66.40±14.86	ND

The values are mean±SEM (ng/g)
ND: Not detected
* $p<0.05$, as compared with Wilcoxon signed-rank test

1 hour, 1.5 hours, 2.5 hours, 3 hours, 5 hours and 7 hours for 8-MOP were 895.00 ± 166.68ng, 1,194.40 ± 384.10ng, 1,441.80 ± 173.05ng, 1,130.80 ± 144.00ng, 753.00 ± 27.27ng, 505.60 ± 140.24 ng, 502.60 ± 87.68ng and 312.20 ± 61.23ng respectively and for 5-MOP , 100.00 ± 37.45ng, 256.20 ± 62.51ng, 385.80 ± 55.92ng, 285.40 ± 48.13ng, 264.40 ± 35.50ng, 204 ± 46.17ng, 59.90 ± 13.32ng and 66.40 ± 14.86ng respectively (Table 1, Fig. 1.) The highest skin concentrations were achieved for both drugs at 1.5 hours, with the level of 8-MOP approximately 3.5 times higher than that of 5-MOP. The level of TMP could not be measured in this experiment with 5ng sensitivity.

Bathing in phototoxic solutions

After bathing for ten minutes in the 10 mg/L solutions of 8-MOP, 5-MOP and TMP skin biopsies were made at 0.5 hour and 1 hour; in the other group skin biopsies were done after removing the horny layers by stripping with scotch tape 30 times at one hour. The concentrations of the phototoxic drugs in 1 gram of biopsied tissue were 1,153.82 ± 85.03ng, 5384.48 ± 84.21ng, and 406.65 ± 78.93 ng for 8-MOP; 4,002.85 ± 675.84ng, 3,111.48 ± 797.73ng, and 2,080.30 ± 501.02ng for 5-MOP; 2,665.94 ± 2,101.41ng, 1,331.36 ± 954.44ng and

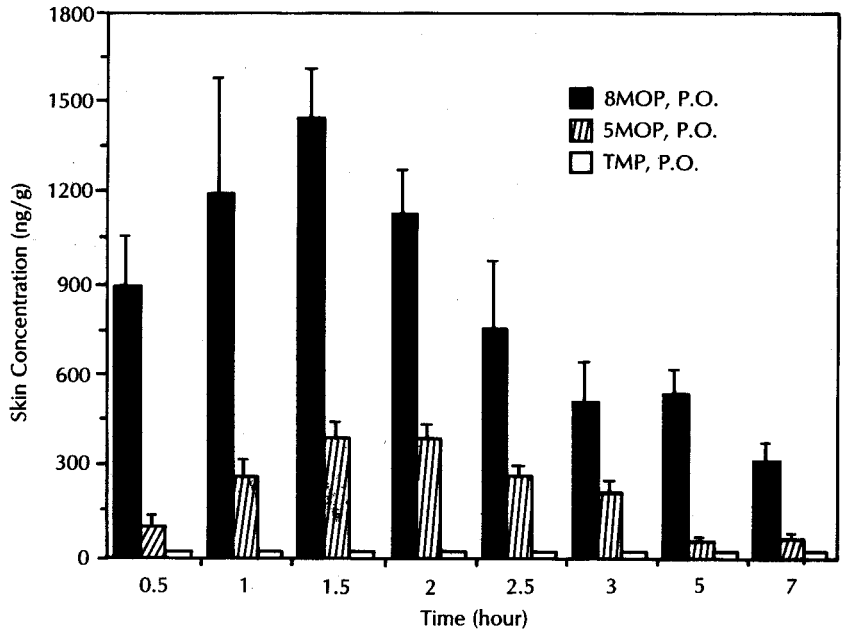

Fig. 1. *The concentration of the drug in guinea pig skin in ng/g wet weight after oral administration.*

Table 2. The concentration of the phototoxic drugs in guinea pig skin in ng/g of wet weight as a function of time after bath (10mg/L)

Time (hours)	8-MOP*	5-MOP*	TMP*
0.5	1153.82 ± 85.03	4002.85 ± 675.84	2665.94 ± 2101.41
1.0	538.48 ± 84.21	3111.48 ± 797.73	1331.36 ± 954.44
stripping	406.65 ± 78.93	2080.30 ± 501.02	1280.56 ± 1184.44

The values are mean ± SEM (ng/g)
* $p < 0.05$, as compared with Wilcoxon signed-rank test

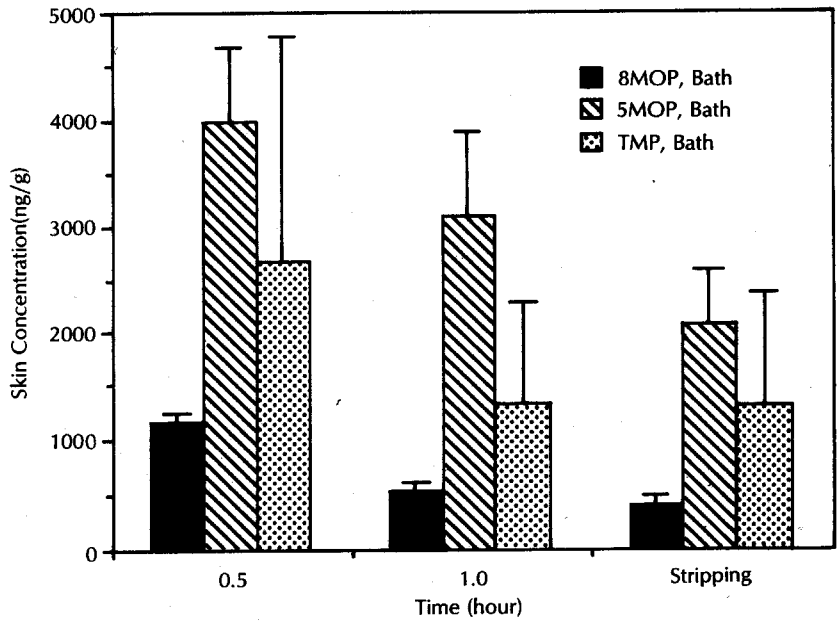

Fig. 2. *The concentration of the drug in guinea pig skin in ng/g of wet weight after bath.*

1,280.56±1,184.44ng for TMP (Table 2, Fig. 2). The concentrations of the drugs in decreasing order were 5-MOP, TMP and 8-MOP.

DISCUSSION

The pharmacologic effect of psoralen was proved scientifically by Lerner (1953) after its first use in vitiligo by El Mofty (1947). Pathak *et al.* (1960) reported the mechanism of erythema formation by phototoxic drugs and ultraviolet light, and Parrish *et al.* (1974) made a report on the first systematic photochemotherapy using psoralen in psorasis and other dermatoses. The principle of photochemotherapy is irradiation of UVA after ingestion of selected phototoxic drugs and to achieve the maximum therapeutic effect, the ultraviolet light should be irradiated when the drug level is highest in the skin. In the past the concentration of the drug was measured with thin layer chromatography (Chakrabarti *et al.* 1978; Stei-ner *et al.* 1978) or gas chromatography (Ehrsson *et al.* 1977; Schmid et al. 1978), but it has been recently shown that HPLC (high performance liquid chromatography) with a few nanograms sensitivity is more accurate (Ljunggren *et al.* 1980).

The blood level of 8-MOP is reported to be highest at 2 hours after oral administration (Thune *et al.* 1977; Kligman *et al.* 1973), and Kornhauser (1982) has reported that the skin concentration is proportional to the blood concentration.

In this study the maximum skin concentration of 8-MOP, 1,441.80±173.05ng was similar to the highest skin concentration reported by Boven *et al.* (1984), 1,595.6±420.4ng; but was greater than the skin concentration reported by Warmer *et al.* (1987), 666±219ng. The reason for the difference in skin concentration is that our study measured concentration of epidermis and dermis but Warmer *et al.* (1987) measured only epidermal concentration.

The time when the highest skin concentration was achieved was 1.5 hours in this study, but in Warmer's study (1987) it was 2 hours, and Boven (1984) reported his result at various peak times. Kornhauser *et al.* (1982) reported that the blood level reached the maximum around 2 hours of ingestion and decreased skin concentration as a function of time, and that the skin and blood concentrations were proportional to each other; this means rapid diffusion from blood to tissue of 8-MOP and 5-MOP. Kornhauser *et al.* (1982) and Warmer *et al.* (1987) have reported that the concentration of 5-MOP was lower than that of 8-MOP.

In this study the level of 5-MOP was conspicuously lower than that of 8-MOP. If taken in equal amounts, because the concentration of 5-MOP is lower than that of 8-MOP, it can be assumed that the risk of skin cancer and damage to DNA that may be caused by photochemotherapy with 8-MOP may be reduced by the use of 5-MOP.

The reason for the low level of 5-MOP is thought to be the decreased absorption from the G-I tract. It is known that phototoxicity by 5-MOP and 8-MOP is induced by the insertion of 5-MOP and 8-MOP into DNA, through formation of a covalent bond with the pyrimidine base, the monofunctional adduct and bifunctional adduct and finally the DNA cross-link. It is also known that the capability of adduct formation is similar to both 5-MOP and 8-MOP (Kornhauser *et al.* 1982). Reports have been made that ingestion of TMP is improper for production of photosensitive reactions (Kligman *et al.* 1973), that the blood concentration of TMP can be measured only after bathing, but not after ingestion (Fisher *et al.* 1980) and that good therapeutic effects can be achieved with irradiation of UVA after bathing with TMP in patients with psoriasis (Salo *et al.* 1981). However, contradictory reports were made on the blood concentration of TMP after ingestion. Murata *et al.* (1970) reported that ingestion of TMP with 200 mg/kg resulted in the skin level of lμg/g, but Chakrabarti *et al.* (1982) said that a much higher blood level, 600 ng/ml, could be detected after oral administration of TMP 30mg/kg.

However, in this study of 5ng/g sensitivity the concentration could not be detected after oral administration of TMP 10mg/kg. The reasons for the above result are poor solubility of TMP, a low absorption rate and rapid metabolism. These reasons also apply to the fact that the level of 5-MOP was found to be lower than that of 8-MOP.

It is known that bathing with 8-MOP is therapeutically more effective than oral ingestion of it, but no reports have been made on systemic absorption and skin concentration on bathing with 8-MOP as well as on bathing with 5-MOP. After bathing with TMP and ultraviolet irradiation, a good therapeutic effect was reported in psoriatic patients, and the blood concentrations in 6 out of 45 psoriatic patients were reported to be 1-9ng/ml (Salo *et al.* 1981). Fisher *et al.* (1980) reported that the blood concentration of TMP in two of four patients who bathed in TMP was 2ng/ml, but no reports on the skin level after bathing were made. The skin concentrations after bathing in solutions dissolved with 10mg/L descended in the order of 5-MOP, TMP and 8-MOP, and there was much variation between individuals.

The skin concentrations measured after stripping 30 times with scotch tape were not much different from those of non-stripped skin after 1 hour of bathing, which means that a larger amount of the phototoxic drug is absorbed through skin than remains in the stratum corneum. In fact, to determine how the degree of photoxicity changes in relation to the change of skin concentration, more research must be carried out on phototoxic reactions after UVA irradiation.

REFERENCES

Bersaques JD, Monbaliu J, Belpaire F: 8-methoxypsoralen plasma levels and phototoxic effect. *Photodermatol 1: 79-84, 1984*

Boven MV, Adriaens P, Roelandts R, Daenens P: Skin determination of the 8-methoxypsoralen concentrations in rat. *Photodermatol 1: 241-244, 1984*

Chakrabarti SG, Gooray DA, Kennery JA: Determination of 8-methoxypsoralen in plasma by scanning fluorometry after thin layer chromatography. *Clin Chem 24: 1155-1157, 1978*

Chakrabarti SG, Grimes PE, Minus HR, Kenney JA, Pradhan TK: Determination of trimethylpsoralen in blood, ophthalmic fluids, and skin. *J Invest Dermatol 79: 374-377, 1982*

Ehrsson H, Eksborg S, Wallin J, Kallberg N, Swanbeck G: Determination of 8-methoxypsoralen in plasma by electron capture gas chromatography. *J Chromatog 140: 157-164, 1977*

El mofty AM: A primary clinical report on the treatment of leukoderma with 9 Ammi majus linn. *J Roy Egypt Med Ass 31: 651-655, 1947*

Fisher T, Hartvig P, Bondersson U: Plasma concentrations after bath treatment and oral administration of trioxsalen. *Acta Derm Venereol (stockh)60: 177-179, 1980*

Kligman AM, Golstein FP: Ineffectiveness of Trioxsalen as an oral photosensitizer. *Arch Dermatol 107: 413-414, 1973*

Kligman AM, Goldstein FP: Oral dosage in methoxalen phototoxicity. *Arch Dermatol 107: 548-550, 1973*

Kornhauser A, Wamer WG, Geles Jr. AR: Psoralen phototoxicity: Correlation with serum and epidermal 8-methoxypsoralen and 5-methoxypsoralen in the guinea pig. *Science 217: 733-735, 1982*

Lerner AB, Denton CR, Fitzpatrick TB: Clinical and experimental studies in vitiligo. *J Invest Dermatol 20: 299-308, 1953*

Ljunggran B, Carter M, Albert J, Reid T: Plasma levels of 8-methoxypsoralen determined by high pressure liquid chromatography in psoriatic patients ingesting

drug from two manufacturers. *J Invest Dermatol 74: 59-62, 1980*

Lowe NJ, Cripps DJ, Dufton PA, Vickers CF: Photochemotherapy for mycosis fungoides. *Arch Dermatol 115: 50-53, 1979*

Murata T, Nobuo T, Hirokazu K: Studies on the absorption, excretion, distribution and metabolism of 3H trioxsalen. *Pharmacometr 4: 577-582, 1970*

Parrish JA, Fitzpatrick TB, Tanenbraum L, Pathak MA: Photochemotherapy of psoriasis with oral methoxalen and longwave ultraviolet lights. *New Engl J Med 291: 1207-1211, 1974*

Parrish JA, Fitzpatric TB,Shea C, Pathak MA: Photochemotherapy of vitiligo using orally administered psoralens and high intensity long-wave ultraviolet light system. *Arch Dermatol 112: 1531-1534, 1976*

Pathak MA, Fellman JM, Kaufman KD: The effect of structural alterations on the erythemal activity of furocoumarins: psoralens. *J Invest Dermatol 35: 165-183, 1960*

Roelandts R, Boven MV, Adriaens P, Schryver FD, Degreef H: The relationship between 8-methoxypsoralen skin and blood levels. *J Invest Dermatol 81: 331-333,1983*

Salo OP, Lassus A, Taskinen J: Trioxsalen bath plus UVA treatment of psoriasis. *Acta Derm Venereol (Stockh) 61: 551-554, 1981*

Schmid J, Koss FW: Rapid, sensitive gas chromatographic analysis of methoxypsoralen in human plasma. *J Chromatog 146: 498-502, 1978*

Siddique AH, Stolk ML, Cormane RH: Comparision of serum levels and clinical results of PUVA therapy with three different dosage forms of 8-methoxypsoralen. *Arch Dermatol Res 276: 343-345, 1984*

Steiner I, Prey T, Gschnait F, Washuttl J, Greiter F: Serum levels of 8-methoxypsoralen levels 2 hours after oral administration. *Acta Derm Venereol (Stockh) 58: 185-188, 1978*

Thune P, Volden G: Photochemotherapy of psoriasis with relevance to 8-methoxypsoralen plasma level and low intensity irradiation. *Acta Derm Venereol (stockh) 57: 351-355, 1977*

Warmer W, Giles Jr. AR, Kornhauser A: Kinetics of 8-methoxypsoralen and 5-methoxypsoralen distribution in guinea pig serum, epidermis and ocular lens. *Photodermatol 4: 236-239, 1987*