Plasma and Skin Concentration of 5-Methoxypsoralen in Psoriatic Patients After Oral Administration

Alfredo Zucchi, Ersilia Raho,* Bettina Marconi,* Sara Nicoli,* Marcello Santini, Fulvio Allegra, Paolo Colombo,* Ruggero Bettini,* and Patrizia Santi*

Institute of Dermatology, Faculty of Medicine, University of Parma, Parma, Italy; *Department of Pharmacy, University of Parma, Parco Area delle Scienze, Parma, Italy

The aim of this work was to investigate the distribution of 5-methoxypsoralen in the skin after oral administration of the drug and to examine the correlation between skin and plasma concentrations. 5-Methoxypsoralen skin concentration was measured in both healthy and psoriatic sites of 10 psoriatic patients after single and multiple oral doses. The results obtained show that 5-methoxypsoralen accumulates at higher levels in the more external layers of the skin after oral administration. The high affinity of drug for the stratum corneum was confirmed by *in vitro* skin affinity measurements. The concentration of 5-methoxypsoralen in the skin was similar in both psoriatic and healthy sites, indicating that the pathology does not influence drug distribution in the skin. After single dose administration, a linear correlation was found between skin and plasma drug concentration. After multiple dose administration, drug concentration in the skin was fairly constant despite the variable plasma concentrations in different subjects. ***Key words: photochemotheray/psoriasis/PUVA therapy/tissue distribution. J Invest Dermatol 117:379–382, 2001***

Photochemotherapy with 5-methoxypsoralen (5-MOP, Bergapten) plus ultraviolet A light irradiation (PUVA) is an effective treatment for several skin diseases, such as psoriasis, vitiligo, and lichen planus (McNeely and Goa, 1998). Moreover, PUVA is effective in the treatment of the T cell lymphoma (Mycosis fungoides) (Zackheim, 1999). In the skin, PUVA produces several species of free radicals and cross-links in DNA (McNeely and Goa, 1998), responsible for its efficacy against vitiligo and psoriasis, respectively.

The standard protocol of the British Photodermatology Group (British Photodermatology Group, 1994) recommends the administration of an oral dose of 1.2 mg per kg of 5-MOP 3 h before UVA irradiation. This time interval allows the drug to reach the skin, after absorption and distribution, where it can reach a level useful for its therapeutic activity.

After oral administration, however, 5-MOP blood concentration showed a certain inter- and intraindividual variability (Stolk *et al*, 1981; Treffel *et al*, 1992; Tanew *et al*, 1998; Shephard *et al*, 1999). In order to monitor the amount of drug in the skin at the time of UVA exposure, the interstitial fluid concentration of 5-MOP given orally was measured, using the skin blister fluid technique (Humbert *et al*, 1991; Treffel *et al*, 1992). Depending on the time of blister formation, 5-MOP concentration in skin blister fluid was 25%–50% of plasma concentration. Additionally, it has been shown, *in vitro*, that 5-MOP binds to epidermis (Artuc *et al*, 1979) and to stratum corneum keratin (Prognon *et al*, 1985), suggesting the possibility of higher tissue concentration compared with plasma. For these reasons, the direct knowledge of skin 5-MOP concentration could give important suggestions for the dermatology practice.

The purpose of this work was to measure 5-MOP skin content in healthy and diseased sites of psoriatic patients after oral single and multiple administration. Drug concentration profile was determined in the epidermis and dermis, and the existence of a correlation between skin and plasma concentration was checked.

MATERIALS AND METHODS

5-MOP and trimethyl psoralen (TMP) were obtained from Galeno s.r.l. (Prato, Italy). 5-MOP capsules were prepared in pharmacy using maize starch, lactose, magnesium stearate, and silicon dioxide as excipients.

All chemicals used were of analytical grade.

Experimental protocol Ten psoriatic patients (seven man and three women, age range 30–60 y; mean initial Psoriasis Area Severity Index (PASI) of 17 ± 8) were enrolled in this study and gave informed authorization. The research followed the tenets of the Declaration of Helsinki (1964) and was approved by the human experimentation committee of the University of Parma.

Because 5-MOP oral bioavailability depends on circadian rhythms (Treffel *et al*, 1990), the patients were instructed to take the drug in the morning, with a low fat breakfast. 5-MOP was given as capsules, at the dose of 1.2 mg per kg (mean amount administered 89 ± 12 mg), 3 h before UVA irradiation.

A blood sample and a skin punch biopsy were taken just before UVA irradiation. After the first PUVA session, therapy continued two times per week for a total of eight sessions. After the last session, a blood sample and a skin biopsy were again taken.

5-MOP analysis The skin biopsies (4 mm in diameter and 1 mm in thickness) were immediately frozen in liquid nitrogen and 20 μm thick slices, parallel to the skin surface, were obtained, using a cryomicrotome (Miles, Elkhart, IN) (Volpato *et al*, 1997). The total thickness analyzed

Manuscript received December 6, 2000; revised March 23, 2001; accepted for publication March 28, 2001.

Reprint requests to: Dr. Patrizia Santi, Department of Pharmacy, Parco area delle Scienze, 43100 Parma, Italy. Email: patrizia.santi@unipr.it

Abbreviations: 5-MOP, 5-methoxypsoralen; PUVA, psoralen UVA; TMP, trimethyl psoralen.

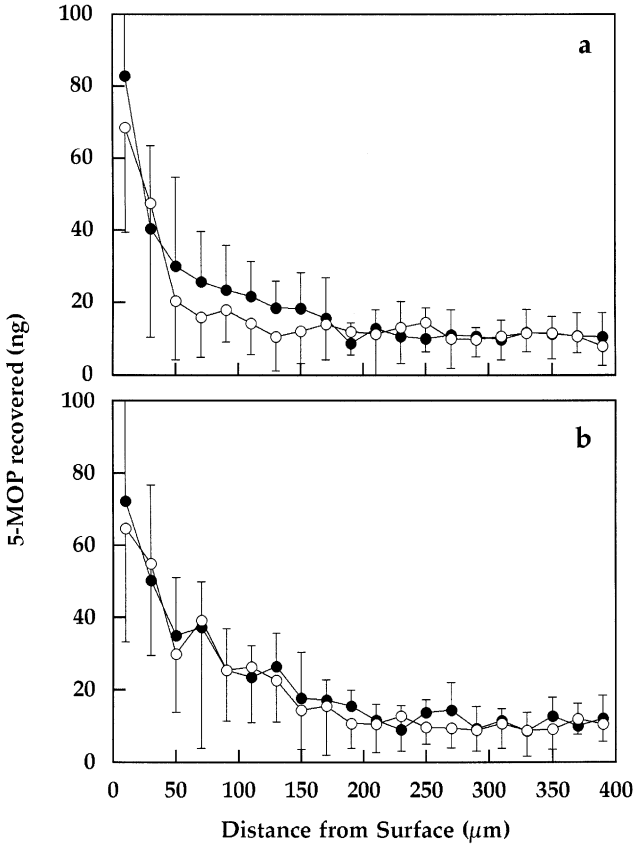

Figure 1. 5-MOP skin content after oral administration. 5-MOP skin content was obtained after oral single administration (*a*) and after multiple administration (*b*) at the dose of 1.2 mg per kg as capsules, in healthy (full symbols) and psoriatic (open symbols) sites of 10 patients (mean values ± SD, n = 10). Each data point represents the average drug content in each skin slice, cut at 20 μm thickness from a 4 mm diameter biopsy.

was 400 μm, corresponding to stratum corneum, epidermis, and part of the dermis.

5-MOP was extracted from each skin slice with 400 μl of methanol (containing trimethyl psoralen 0.5 μg per ml as internal standard), vortexing occasionally for 20 min at room temperature. After the addition of 100 μl of water and centrifugation at 11 000 × *g* for 20 min, the solution was filtered through a 0.45 μm nylon filter (Lida, Kenosha, WI).

The drug was quantitated using reverse-phase high-performance liquid chromatography (HPLC). The isocratic HPLC system (LC 250 pump, Perkin Elmer, Norwalk, CT) was equipped with an UV detector (wavelength 300 nm) and a C_{18} reversed phase column (Nova-PakÆ 3.9 ∞ 150 mm Waters, Milford, MA). The mobile phase was methanol-water (65:35) at a flow rate of 0.8 ml per min. The retention times of 5-MOP and TMP were 3.5 and 8 min, respectively.

The analytical method was validated according to the USP24–System suitability. The limit of quantification of the assay was 0.05 ng per ml, with a relative standard deviation of 2.9%. The tailing factor was 1.35 ± 0.14 and the theoretical plates were 1197. The recoveries of 5-MOP and TMP, from blank skin slices spiked with known amounts of 5-MOP and extracted according to the previously described procedure, were 91.0 ± 2.3 and 88.2 ± 4.3%, respectively.

Blood samples were collected in heparinized test tubes and centrifuged at 700 × *g* for 10 min. A sample of plasma (100 μl) was sonicated with 400 μl of methanol containing TMP 0.5 μg per ml as internal standard. One hundred microliters of water were added and the mixture was centrifuged at 11 000 × *g* for 20 min. The supernatant was filtered through a 0.45 μm nylon filter and analyzed by HPLC as reported before, using methanol-water 55:45 as mobile phase. This eluent allowed the separation of 5-MOP peak from the interfering peaks derived from plasma. The validation parameters did not change significantly.

Drug affinity for skin slices Skin slices obtained from patients not previously treated with 5-MOP, were used to measure *in vitro* affinity of 5-MOP for the skin (Hikima and Tojo, 1997). Each slice (area 0.6 cm^2, thickness 20 μm) was equilibrated with 300 μl of a 0.430 μg per ml water solution of 5-MOP for 2 h. Then, the residual concentration of drug in the aqueous phase was measured by HPLC. The concentration of 5-MOP into each skin slice, considered as an expression of affinity, was calculated as the difference between initial and final drug concentration in the aqueous phase, taking 1.2×10^{-3} cm^3 as the volume of each skin slice.

Statistical analysis The significance of the differences between the values of 5-MOP recovered in the skin was assessed using a test for the comparison of means of related samples (paired-sample, two sided t test).

The correlation between skin and plasma concentration was performed by linear regression, using the least-square method and evaluating the significance of slope and intercept. Both analyzes were performed using Microsoft Excel 8.0 software running on a Macintosh PowerBook G3.

RESULTS AND DISCUSSION

5-MOP skin distribution was studied by measuring its content in 20 μm thick skin slices, as a function of the distance from the surface. Biopsies of normal and diseased skin of the same patients were taken 3 h after oral intake of 5-MOP capsules.

In healthy skin, drug content was higher in the external slices and decreased exponentially from the stratum corneum towards the dermis (**Fig 1*a***). This drug profile was unexpected, considering that 5-MOP was administered orally and reached the skin from the systemic circulation, i.e., from dermal capillaries. Nevertheless, there are reports in the literature indicating that 5-MOP has strong affinity for skin proteins *in vitro* (Artuc *et al*, 1979; Prognon *et al*, 1985). Similarly, a higher concentration of fluconazole was found in the stratum corneum compared with the epidermis and the dermis after oral administration in man (Wildfeuer *et al*, 1994) and similar results were obtained with TMP (Ros *et al*, 1988). In conclusion, this result with 5-MOP is due to the reported high affinity of the drug for the skin

The shape of 5-MOP content profiles in psoriatic skin was quite similar, indicating that drug distribution in skin layers was almost independent of the presence of the psoriatic lesion. A small difference between healthy and psoriatic sites, though not statistically significant, was observed in the range 50–150 μm of depth, where 5-MOP was present at higher concentrations in diseased skin. This small difference could be attributed to increased blood flow and thickness in psoriatic skin compared with normal skin (Hern *et al*, 1999).

After multiple dose administration (twice a week oral administration, 1 mo of therapy; **Fig 1*b***), the profile obtained in healthy skin was practically superimposed to the one obtained in psoriatic skin. Comparing the distribution of 5-MOP in the skin after multiple and single administration, an increase of the amount of drug recovered in the epidermal portion of the skin (0–200 μm from the skin surface), in particular for healthy skin, was found; however, the level of significance of the difference between single and multiple doses was not particularly high (p on average 0.3), due to the large variability of the values.

5-MOP plasma concentration at the time of biopsy (3 h after first dosing) was 378 ± 161 ng per ml (mean ± SD), in agreement with the data reported in the literature (Stolk *et al*, 1981; Treffel *et al*, 1992; Tanew *et al*, 1998; Shephard *et al*, 1999). The average value of plasma concentration obtained after multiple dose administration was practically the same, namely 370 ± 233 ng per ml (mean ± SD). This result indicated that the drug did not accumulate in blood after multiple administration, in agreement with the short plasma elimination half-life (45–105 min according to Stolk *et al*, 1981; 192 min according to Treffel *et al*, 1992).

The total amount of 5-MOP present in the skin was estimated from the amount in the skin biopsy (area 0.1256 cm^2) multiplied for an average body surface area of 1.8 m^2. The percentage of 5-MOP present in the skin was 59 ± 15% of the dose administered 3 h after single administration and 66 ± 13%

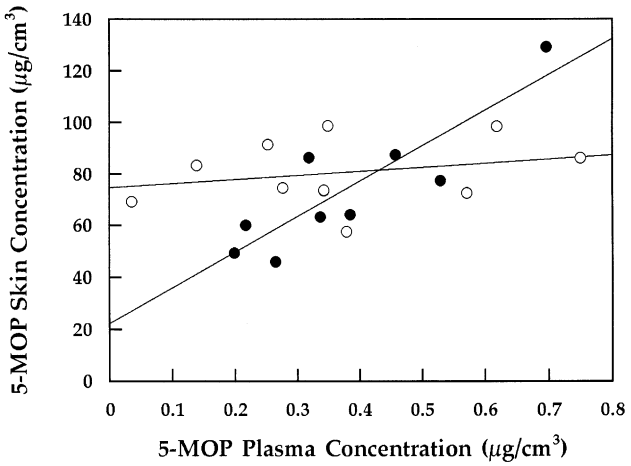

Figure 2. Relationship between 5-MOP skin and plasma concentration. Full symbols: values corresponding to the first administration (regression equation: $y = 22.221 + 137.92x$, $R = 0.87246$); open symbols: values corresponding to multiple administration (regression equation: $y = 74.63 + 16.207x$, $R = 0.26667$).

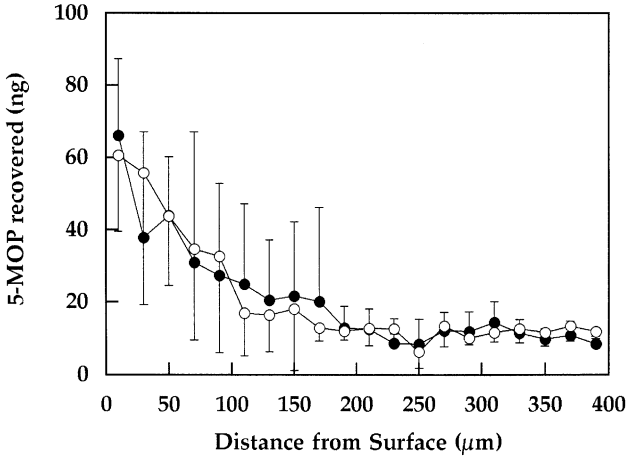

Figure 3. 5-MOP skin content after single oral administration as a function of time. 5-MOP skin content obtained 3 h (full symbols) and 3 d (open symbols) after oral single administration at the dose of 1.2 mg per kg as capsules in four patients (mean values ± SD, n = 4).

after multiple administration. Because more than 50% of the administered dose reached the skin 3 h after administration, skin uptake was very rapid.

In order to study the correlation between 5-MOP skin and plasma concentration at the time of biopsy, the amounts of drug recovered in each skin slice were cumulated and transformed in concentration. A linear correlation between skin and plasma concentration at the time of biopsy was found after the first administration (**Fig 2**). The slope of the regression line was significantly different from zero (mean value ± confidence interval 95%: 137.9 ± 52.9) whereas the intercept was not significantly different from zero (mean value ± confidence interval 95%: 22.1 ± 21.3). 5-MOP skin concentration significantly increased as the plasma concentration increased, indicating an uptake by the skin from the blood compartment.

Multiple administration determined a not significant fluctuation of skin concentration from 60 and 100 μg per cm^3, for plasma concentration varying from 0.05 to 0.75 μg per cm^3. The resulting regression equation showed intercept significantly different from zero (mean value ± confidence interval 95%: 74.6 ± 16.5) and slope not significantly different from zero (mean value ± confidence interval 95%: 16.2 ± 39.8).

In conclusion, the concentration of 5-MOP in the skin was higher than in plasma, after both single and multiple administrations. After multiple administration, the skin content was less dependent on plasma concentration. These results are quite interesting, but due to the limited number of time points taken between the two administrations should be considered as preliminar.

There are indications in the literature that 5-MOP binds to the epidermis (Artuc *et al*, 1979) and to stratum corneum keratin (Prognon *et al*, 1985). To confirm this result, we checked the affinity of 5-MOP for the skin slices, by measuring *in vitro* the uptake of individual slices from an aqueous solution. Blank skin slices were equilibrated with a water solution of 5-MOP at a concentration of 430 ng per ml (in the range of plasma concentrations). 5-MOP showed strong affinity for the first skin slice for which the concentration found was 50–100 times higher than the concentration in aqueous solution. The uptake progressively decreased going into the epidermis, to reach an average value of about 5 μg per cm^3 in the dermis; however, these results indicate a lower affinity for the skin compared with those obtained by Artuc *et al* (1979) and to our *in vivo* experiments, in which the ratio skin to plasma concentration was on average 200. The result can be justified considering the relatively short equilibration time (2 h) used in the affinity experiments and/or by the fact that the skin was frozen before use.

Because skin distribution data obtained in this work support a long drug half-life in the skin, much longer than in plasma, four additional patients received 5-MOP orally (1.2 mg per kg) and skin biopsies of healthy sites were taken 3 h after administration. Three days later, before the following drug administration, a second biopsy was taken. The results obtained (**Fig 3**) show that the concentration profile in the skin was practically unchanged 3 d after the last administration compared with the first biopsy, even if plasma concentration was undetectable in all patients. This experiment suggests a very low clearance of 5-MOP from the epidermal tissue, even if due to the limited number of patients analyzed the statistical significance is limited.

CONCLUSION

From the results obtained it can be concluded that 5-MOP accumulates in the skin after oral administration. The concentration in the skin decreased going from the stratum corneum towards the dermis. The concentration of 5-MOP in the skin was the same in both psoriatic and healthy sites, indicating that the pathology does not influence drug skin distribution. 5-MOP skin distribution was not largely different after single and multiple drug administration. 5-MOP skin content was practically the same 3 h and 3 d after a single oral administration, indicating a low clearance of the drug from the skin. It is perhaps necessary, however, to increase the number of time points in order to confirm the time kinetics through all the layers of the skin.

The linear correlation resulting between skin drug content and plasma concentration after first dose administration, and the absence of correlation found after multiple administration, indicates that a stable level of 5-MOP in the skin is reached after multiple administration. The higher affinity of the drug for the skin compared with plasma, limits the fluctuations of skin concentration in response to variations of plasma concentration.

This work was partially supported by a grant from the Ministry of the University and Scientific research (MURST – Italy).

REFERENCES

Artuc M, Stuettgen G, Schalla W, Schaefer H, Gazith J: Reversible binding of 5- and 8-methoxypsoralen to human serum proteins (albumin) and to epidermis in vitro. *Br J Dermatol* 101:669–677, 1979

British Photodermatology Group. Guidelines for PUVA. *Br J Dermatol* 130:246–255, 1994

Hern S, Stanton AWB, Mellor R, Levick JR, Mortimer PS: Control of cutaneous blood vessels in psoriatic plaques. *J Invest Dermatol* 113:127–132, 1999

Hikima T, Tojo K: Binding of prednisolone and its esters prodrugs in the skin. *Pharm Res* 14:197–202, 1997

Humbert P, Treffel P, Makki S, Millet J, Agache P: Peak blistering point: influence of fluid levels of 5-MOP in human skin in vivo after systemic administration. *Arch Dermatol Res* 283:297–299, 1991

McNeely W, Goa KL: 5-Methoxypsoralen – A review of its effects in psoriasis and vitiligo. *Drugs* 56:667–690, 1998

Prognon P, Font G, Manes J, Postaire M, Zini R, Marty JP, Mahuzier G: Etude in vitro de la fixation des psoralènes à la kératine. *Therapie* 40:459–463, 1985

Ros AM, Wennersten G, Wallin I, Ehrsson H: Concentration of trimethylpsoralen in blood and skin after oral administration. *Photodermatol* 5:121–125, 1988

Shephard SE, Zogg M, Burg G, Panizzon RG: Measurement of 5-methoxypsoralen and 8-methoxypsoralen in saliva of PUVA patients as a noninvasive, clinically relevant alternative to monitoring in blood. *Arch Dermatol Res* 291:491–499, 1999

Stolk LML, Westerhof W, Cormane RH, Wan Zwieten PA: Serum and urine concentrations of 5-methoxypsoralen after oral administration. *Br J Dermatol* 105:415–420, 1981

Tanew A, Ortel B, Rappersburger K, Hönigsmann H: 5-Methoxypsoralen (Bergapten) for photochemotherapy. *J Am Acad Dermatol* 18:333–338, 1998

Treffel P, Makki S, Humbert P, Khaldoun H, Agache P: A new micronized 5-methoxypsoralen preparation. *Acta Derm Venereol* 72:65–67, 1992

Treffel P, Renaud A, Humbert P, Makki S, Faivre B, Agache PG: Chronopharmacokinetics of 5-methoxypsoralen. *Acta Derm Venereol (Stockh)* 70:515–517, 1990

Volpato NM, Santi P, Laureri C, Colombo P: Assay of acyclovir in human skin layers by high-performance liquid chromatography. *J Chrom Biomed Appl* 16:515–520, 1997

Wildfeuer A, Faergemann J, Laufen H, Pfaff G, Zimmermann T, Seidl HP, Lach P: Bioavailability of fluconazole in the skin after oral medication. *Mycoses* 37:127–130, 1994

Zackheim HS: Cutaneous T cell lymphoma: update of treatment,. *Dermatology* 199:102–105, 1999