# Chronopharmacokinetics of 5-Methoxypsoralen*

PIERRE TREFFEL, ALAIN RENAUD, PHILIPPE HUMBERT, SAFWAT MAKKI, BRIGITTE FAIVRE and PIERRE G. AGACHE

*Faculté de Médecine et Pharmacie, 25030 Besancon Cédex, France*

**Diurnal variations in drug pharmacokinetics are a well known phenomenon. Chronopharmacology studies now appear to be attracting increasing interest with a view to establishing an optimum therapeutic prescription. In order to determine possible chronobiological variations of 5-methoxypsoralen (5-MOP) pharmacokinetic, 5-MOP blood concentrations were quantified in 8 healthy subjects after drug ingestion at different times during the day. Stolk's High Performance Liquid Chromatography technique was used to assess the 5-MOP serum concentrations. Each subject underwent three pharmacokinetic studies after oral ingestion of 5-MOP (1.2 mg/kg), in conjunction with a standardized low-lipid meal. The first pharmacokinetic study was started in the morning, the second in the afternoon and the third in the evening. Drug intake was at intervals of 2 days, to avoid drug accumulation. The results showed that the evening intake of the drug induced a higher 5-MOP maximum concentration and a higher 'area under curve' than morning or afternoon ingestion. This study suggests an optimized PUVA therapy, when performed in the evening.**

(Accepted May 14, 1990.)

Acta Derm Venereol (Stockh) 1990; 70: 515–517.

P. Agache, CHU Place St-Jacques, F-25030 Besancon Cedex, France.

5-Methoxypsoralen (5-MOP) is a linear furocoumarin increasingly used for its skin photosensitizing effect, for the treatment of certain dermatological diseases such as psoriasis and vitiligo. 5-MOP photochemotherapy was reported to have several advantages compared with 8-MOP photochemotherapy, most notably for reducing the phototoxic response (1).

Previous studies have shown that the efficacy of PUVA therapy depends on the plasma psoralen concentrations (2, 3). Moreover, it is now well known that pharmacokinetic parameters of drugs may be conditional upon the time of drug intake. The aim of this study was therefore to demonstrate diurnal variations in pharmacokinetic parameters of 5-MOP.

## MATERIAL AND METHODS

### *Subjects*

Eight healthy students (4 males, 4 females, average age 23 years) took part in this experiment. They fasted for 12 h before the administration of 5-MOP (Psoraderm-5® tablets, each containing 20 mg (Bergaderm, Rungis, France)).

### *Drug administration*

Just before oral drug ingestion (1.2 mg/kg), a standardized low-lipid meal was given to each subject. The meal consisted of a glass of orange juice, a jam sandwich and a cup of black coffee or black tea. No additional food was allowed until the end of the pharmacokinetic study. For each participant, the morning pharmacokinetic time started at 8 a.m., the afternoon one at 2 p.m. and the evening at 7 p.m. A minimum washout interval of 2 days was kept between each pharmacokinetic study. Blood samples were collected, after 5-MOP oral administration, at 0 h, 30, 60, 90, 120, 150, 180, 240, 300 and 360 min.

The serum fractions were separated and stored at −20°C until analysed.

### *Assay procedure*

Following extraction, 5-MOP concentrations were quantified by high performance liquid chromatography (HPLC), according to the method published by Stolk (4). This technique was chosen for its sensitivity, reproducibility and easiness.

1. *Extraction of 5-MOP from serum*

*Reagents* (analytical quality)
- dichloromethane (DCM)
- heptane

*Method*

1.0 ml plasma or serum was mixed with 10 µl of the internal standard solution (8-MOP) in a glass test tube. 5 ml heptane-DCM (4 : 1) was added. The mixture was shaken for 5 min and centrifuged for about 7 min at 5000 rpm. Then 4 ml of the upper organic layer was transferred to a clean test tube and evaporated to dryness on a waterbath (50°C),

* This work was presented in part in: Psoralens: Past, Present and Future. International Symposium, Paris, April 1988.

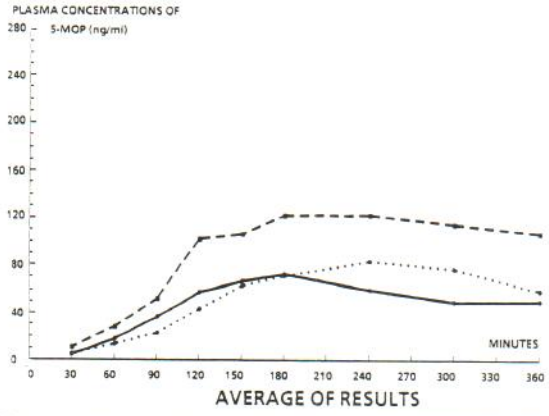


*Fig. 1.* Average of results. 5-MOP plasma concentration versus time. Mean. Drug oral intake in the evening (----), in the afternoon (·····) and in the morning (——).

under a nitrogen stream. After cooling, 50 µl of the mobile phase was added and mixed on a vortex mixer for 30 s. 20 µl was injected into the HPLC.

2. *HPLC analysis*

*Instrumentation*

The liquid chromatograph (Hitachi, Merck, Clevenot) consisted of:

- 655A-11 liquid chromatograph
- L-5000 LC controller for model gradient system
- 655A variable wavelength UV monitor
- D-2000 chromato-integrator

The column used was a reverse phase Rp8 (Merck) and the detection was in UV at 254 nm. The determination of the 5-MOP serum levels was based on peak height measurements.

*Chromatographic conditions*

All chromatographic analyses were done at ambient temperature. The mobile phase consisted of methanol–water (70 : 30), HPLC quality. It was degassed ultrasonically before use. The flow rate was 1.0 ml per minute.

*Standard solutions*

The internal standard was 20 µg/ml : 20 mg 8-MOP per litre of ethanol. The linearity and the precision of the assay were determined by adding 0, 5, 10, 15 and 20 µl of the 5-MOP standard solution (20 mg/l ethanol), respectively to drug-free plasma samples with 10 µl of 8-MOP (the internal standard solution) in each tube. These solutions were treated in the same way as the human serum samples. Peak height ratios of 5-MOP and the internal standard were calculated and the concentrations of the drug in serum were found directly from the standard graph.

*Statistics*

The Mann-Whitney test was used to assess differences in pharmacokinetic parameters, depending on the time when the drug was ingested.

## RESULTS

The 5-MOP pharmacokinetics for 8 subjects were valuated for three intake times during the day. Fig. 1 shows the serum levels achieved with 5-MOP on three occasions with a single oral drug intake. Pharmacokinetic parameters are given in Table I. The mean of the 5-MOP highest concentration peaks for each intake time was 3 h after oral drug intake. These results confirm previously published data (5, 6, 7). The statistically significant highest maximal concentration ($C_{max}$) was obtained after the evening intake of 5-MOP ($p < 0.001$). No differences were observed between the morning and the afternoon $C_{max}$. The area under the curve, calculated for the three periods, gave significantly higher values for oral intake during the evening, as compared with the two other periods. Area under the curve (AUC) was statistically greater in the evening study than in the morning or the afternoon study ($p < 0.001$). The 5-MOP serum concentrations were higher when oral intake had taken place during the evening.

## DISCUSSION

For most drugs, the intensity of a pharmacologic effect is proportional to the drug concentration in extracellular fluid which can enter tissues. Blister fluid resembles interstitial fluid quite closely (8). Relationships between 8-MOP serum levels and 8-MOP cutaneous blister fluid levels have been demonstrated (9). Such a relationship exists with 5-MOP (unpublished data). Moreover, in view of the relationship between serum psoralen concentration and PUVA therapy efficiency, our results suggest that PUVA therapy might be more efficient or might need a lower psoralen posology when performed in the evening. This also implies that the patient will need less UV irradiation, and then less radiation side effects.

Circadian rhythms could be one explanation for

Table I. *Time of maximum concentration ($T_{max}$), maximum concentration ($C_{max}$) and area under curve (AUC) (Mean + SEM) at different oral intake times*

| Parameter | Morning | Afternoon | Evening |
|---|---|---|---|
| $T_{max}$ (min) | 193±27 | 244±26 | 191±30 |
| $C_{max}$ (ng/ml) | 82±5.9 | 89±11.05 | 175±7.45 |
| AUC (ng min. ml$^{-1}$) | 1754±423 | 1757±532 | 3282±449 |

the variation in drug bioavailability observed in our study. Indeed, circadian rhythms are prominent in the rates of drug absorption, distribution, metabolism, and excretion. Statistically significant circadian rhythms have been demonstrated for the various parameters used to characterize classical pharmacokinetics (10). Thus, the determination of pharmacokinetic parameters of 5-MOP for each patient, at different times of the day, may lead to an optimized treatment. Our study suggests that timing of drug dosing is an important therapeutic parameter that usually received too little attention.

## REFERENCES


1. Tanew A, Ortel B, Rappersberger K, Honigsmann M. 5-Methoxypsoralen (Bergapten) for photochemotherapy. J Am Acad Dermatol 1988; 18: 333–338.
2. Swanbeck G, Eriksson H, Ehrneb M, Wallin I, Jonsson L. Serum concentration and phototoxic effect of methoxypsoralen in patients with psoriasis. Clin Pharmacol Ther 1979; 25: 478–480.
3. Andrew E, Nilsen A, Thune P, Wilk I. Photochemotherapy in psoriasis. Clinical response and 8-MOP plasma concentrations at two levels. Clin Exp Dermatol 1981; 6: 591–600.
4. Stolk LML. Determination of 8-Methoxypsoralen in biological fluids by reverse phase HPLC. Pharm Weekblad 1980; 2: 280–284.
5. Stolk LML, Westerhof W, Cormane RH, Van Zwieten PA. Serum and urine concentrations of 5-Methoxypsoralen after oral administration. Br J Dermatol 1981; 105: 415–420.
6. Bonnot D, Beani JC, Boitard M, Reymond JL, Beriel H, Amblard P. Comparaison des cinétiques plasmatiques du Méthoxy-5-psoralène et du Méthoxy-8-psoralène. Rech Dermatol 1988; 1: 515–518.
7. Makki S, Quencez E, Humbert P, Taillard C, Agache P, Guinchard C. 5-Methoxypsoralen pharmacokinetics in psoriatic patients. In: Fitzpatrick TB, Forlot P, Pathak MA, Urbach P, eds. Psoralens: past, present and future of photochemoprotection and other biological activities. Montrouge: John Libbey Eurotext, 1989: 167–174.
8. Volden G, Thorsud A, Bjornson I, Jellun E. Biochemical composition of suction blister fluid determined by high resolution multicomponent analysis (capillary gas chromatography-mass spectrometry and two-dimensional electrophoresis). J Invest Dermatol 1980; 75: 421–424.
9. Lauharanta J, Juvakosdi T, Lassus A. Pharmacokinetics of 8-MOP in serum and succion blister fluid. Arch Dermatol Res 1982; 273: 110–114.
10. Halberg F, Halberg E. Chronopharmacology and further steps toward chronotherapy. In: Benet LZ, ed. Pharmacokinetic basis for drug treatment. New York: Raven Press, 1984: 221–248.