

Bioavailability of a New Oral Methoxsalen Formulation

A Serum Concentration and Photosensitivity Response Study

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A new encapsulated liquid preparation of methoxsalen (soft gelatin capsule) was compared with the currently marketed hard gelatin capsule form and with an oral solution of methoxsalen in a random crossover design. Serial serum samples were collected from 25 healthy adult subjects and assayed by a high-performance liquid chromatography technique. Additionally, photosensitivity was determined by serial testing in 12 subjects. The soft-capsule formulation resulted in a 59% increase in extent of absorption and in a greater rate of absorption of methoxsalen than from the hard capsule. This was demonstrated by consistently higher peak serum concentrations (225 vs. 199 ng/mL), which occurred at earlier times (1.8 vs. 3.0 hours) following administration of the soft capsule. Photosensitivity testing confirmed these results, as photosensitivity was greater (9.53 vs. 12.68 J/cm²) and reached a maximum earlier (1.5 vs. 4.25 hours) following administration of the soft capsule. The soft capsule can be considered equivalent to the oral solution in both serum concentrations (peak concentration, 231 vs. 225 ng/mL, and time of peak, 1.6 vs. 1.8 hours) and photosensitivity response (maximum photosensitivity, 9.24 vs. 9.53 J/cm², and time of maximum photosensitivity, 1.32 vs. 1.53 hours).

(Arch Dermatol 1986;122:768-771)

Administration of methoxsalen (8-methoxypsoralen) followed by irradiation with long-wave ultraviolet light in the A-range (UV-A) is an established method of dermatologic treatment with wide interpatient variability. Numerous studies have documented differences in oral absorption among

methoxsalen products. Stolk et al¹ observed greater absorption of methoxsalen when it was administered as a hydroalcoholic solution than when it was administered as powder in a gelatin capsule. Herfst and De Wolff² observed differences in absorption between

For editorial comment see p 763.

two methoxsalen products marketed in the Netherlands. Menne et al³ observed large variations in plasma concentrations of methoxsalen among seven brands of methoxsalen. Higher plasma concentrations of methoxsalen have been observed when the drug is micronized⁴ or in solution⁵ prior to encapsulation. Recently, it has been suggested that more attention be given to the form of administration of methoxsalen as a means of improving therapy with this drug.

This study examines the bioavailability of a new encapsulated liquid preparation of methoxsalen (soft gelatin capsule) through serial serum concentrations of drug and serial determinations of photosensitivity to UV-A. The new preparation is compared with the currently marketed capsule form of methoxsalen and with an oral solution.

SUBJECTS AND METHODS

Subjects

Twenty-five healthy adult volunteers, weighing between 59 and 116 kg and between the ages of 19 and 37 years, were selected to participate in this study; five were women and 20 were men. All subjects received a physical examination and laboratory screening before participation. Subjects were taking no other medications at the time of the study. Informed consent was obtained.

Drug Administration and Serum Sampling

Each subject received three different methoxsalen preparations as a single dose according to a randomized crossover design. Treatment A was an alcoholic solution of methoxsalen (20% ethanol/water); treatment B was crys-

Accepted for publication Jan 7, 1986.

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talline methoxsalen in a hard gelatin capsule (Oxsoralen (10 mg), Elder Pharmaceuticals, Bryan, Ohio); and treatment C was a liquid preparation of methoxsalen encapsulated in a soft gelatin capsule (10 mg/capsule). Each subject ingested a dose of 0.6 mg/kg of methoxsalen, rounded to the nearest whole capsule (10 mg). Each phase of the study was separated by one week.

Subjects were instructed to fast overnight from 10 PM and for four hours after administration of each dose of methoxsalen. On the mornings that drug was to be administered, each subject was instructed to drink 240 mL of water on arising and 240 mL of low-fat milk (0.5% butterfat) with the medication. No other food or beverage was allowed until four hours after dosing; then, food and beverage were allowed ad libitum.

Blood samples were collected from a forearm vein at the following times relative to methoxsalen administration: 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 hours after dosing. After ample time for clotting, the samples were centrifuged and the serum was collected. Serum samples were frozen until assay.

Analytical Methods

Serum samples were assayed for methoxsalen using Eschbach's high-performance liquid chromatographic (HPLC) method (J. Eschbach, MS, written communication, 1978). Briefly, this method required extraction of the deproteinized serum sample with benzene:ethyl acetate (90:10). The organic layer was evaporated to dryness and reconstituted with methylene chloride. An aliquot was chromatographed on a silica column with methylene chloride:acetonitrile (95:5) as the mobile phase. Detection was by ultraviolet absorbance at 254 nm. Trioxalen (trimethylpsoralen) was used as an internal standard. The minimum detectable concentration of methoxsalen using this method was 4 ng/mL.

Bioavailability Estimation

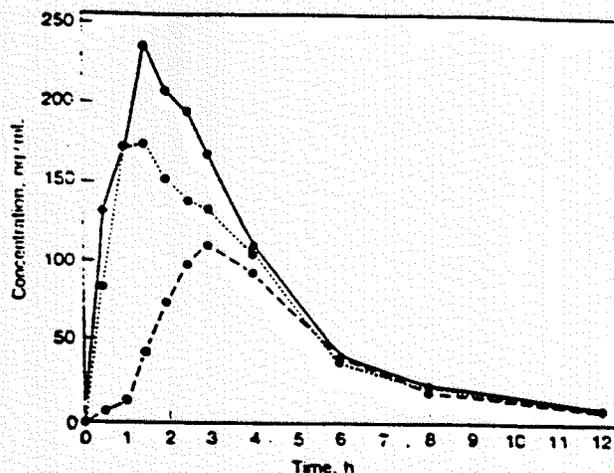
The area under the serum concentration-time curve (AUC) was estimated using the trapezoidal rule. Peak serum concentrations and time of peak serum concentrations were based on observed values. Half-life was estimated from the terminal rate constant of the best-fitting polyexponential equation for each set of serum concentration data. The best-fitting polyexponential equation was determined using a computer program (ESTRIP).

The AUC and the peak serum concentration are functions of the dose of drug administered. Since the subjects received doses of methoxsalen based on body weight, the AUC and the peak serum concentration were normalized to a total dose of 40 mg. This allowed for a better comparison of biopharmaceutic parameters between individual subjects.

Photosensitivity Determinations

Twelve male volunteers agreed to participate in this phase of the study. Each subject received a series of exposures of UV-A equivalent to 1.4, 2.0, 2.8, 3.9, 5.4, 7.6, 10.7, 15, and 21 J/cm² at the following times relative to administration of the methoxsalen preparations: 0.5, 1, 1.5, 2, 3, 4, and 6 hours after dosing, according to a previously described method.¹² The source of ultraviolet radiation was six lamps (F36T12BLHO) powered by a solid-state ballast, using dosimetry for control of exposure. The intensity of erythema was determined 72 hours after dosing.

The dose of UV-A radiation necessary to induce a 1+ erythema (pink erythema filling exposure site) 72 hours



Mean serum concentration of methoxsalen (0.6 mg/kg) following administration of three formulations. Solid line indicates oral solution (treatment A); dashed line, hard-capsule formulation (treatment B); and dotted line, new soft-capsule formulation (treatment C).

after ingestion of methoxsalen and exposure to UV-A was called the minimum erythemic dose. Maximum photosensitivity was defined as the occurrence of the lowest minimum erythemic dose.

Statistical Analysis

Results of the serum concentration phase of the study were analyzed using the analysis of variance for a randomized block design. Four missing values were estimated by Yates' method.¹³ When significant differences were observed among treatment means, Tukey's method was used for pairwise comparisons. Friedmann's test was used to analyze the photosensitivity data.¹⁴

RESULTS

Serum Concentrations

Mean methoxsalen serum concentrations after administration of the oral solution, the hard gelatin capsule, and the soft gelatin capsule are illustrated in the Figure. Mean values of important bioavailability parameters and results of statistical analysis are given in Table 1.

Statistical analysis indicates no significant differences in mean half-life of methoxsalen following administration of the three treatments ($P > .05$). Statistically significant differences exist among treatment means for AUC, normalized AUC, peak serum concentration, normalized peak serum concentration, and time of peak serum concentration. Pairwise comparisons of treatment means indicate significant differences between the solution and the hard capsule and between the hard-capsule and soft-capsule formulations. Such differences occur for each of the bioavailability parameters. When the oral solution and the soft-capsule formulation are compared, no significant differences between treatment means are observed.

The mean AUCs, when normalized for dose, indicate that approximately 59% of the dose is absorbed

Parameter	Treatment†			Analysis of Variance	Tukey's Method		
	A	B	C		A vs B	B vs C	A vs C
AUC, ng·h/mL	521 (± 629)	521 (± 378)	795 (± 452)	<i>P</i> < .01	S	S	NS
Peak concentration, ng/mL	255 (± 174)	129 (± 94.1)	242 (± 129)	<i>P</i> < .01	S	S	NS
Time of peak concentration, h	1.6 (± 0.98)	3.0 (± 0.93)	1.8 (± 0.87)	<i>P</i> < .01	S	S	NS
Half-life, h	2.19 (± 0.77)	2.38 (± 1.13)	2.15 (± 0.78)	NS			
Normalized AUC, ng·h/mL	835 (± 509)	492 (± 345)	726 (± 354)	<i>P</i> < .01	S	S	NS
Normalized peak concentration, ng/mL	231 (± 137)	199 (± 81.0)	225 (± 105)	<i>P</i> < .01	S	S	NS

*AUC indicates area under concentration-time curve; S, significant (*P* < .05); and NS, not significant (*P* > .05).

†Treatment: means (± SDs) for treatment A (alcoholic solution), treatment B (hard capsule), and treatment C (soft capsule).

Parameter	Treatment*		
	A	B	C
Mean maximum photosensitivity, J/cm ²	9.24 (± 5.54)	12.68 (± 4.17)	9.53 (± 5.61)
Median maximum photosensitivity, J/cm ²	10.7	15	7.6
Range maximum photosensitivity, J/cm ²	2-21	7.6->21	3.9-21
Mean time of maximum photosensitivity, h	1.32 (± 0.51)	4.25 (± 1.91)	1.50 (± 0.50)
Median time of maximum photosensitivity, h	1.5	6.0	1.5
Range time of maximum photosensitivity, h	0.5-2	2->6	0.5-2

*Treatment means (± SDs) for treatment A (alcoholic solution), treatment B (hard capsule), and treatment C (soft capsule).

from the hard capsule relative to that absorbed from an oral solution. The observed peak serum concentrations, when normalized for dose, were higher for the solution and the soft capsule compared with the hard capsule. They also occurred at earlier times for the solution and the soft capsule compared with the hard capsule. The later parameter indicates a faster rate of absorption of drug from the solution or the soft capsule than from the hard capsule.

Photosensitivity

A summary of the results of the photosensitivity phase is presented in Table 2. The maximum photosensitivities produced by the oral solution and the soft-capsule formulation were at lower light levels than that of the hard capsule. Maximum photosensitivity occurred at earlier times relative to dosing for the solution and the soft-capsule formulation than for the hard capsule. These differences between formulations appear even more dramatic when the

individual values are considered. The oral solution and the soft capsule both produced maximum photosensitivity within one-half to two hours after dosing in all 12 subjects. The hard capsule produced maximum photosensitivity from two to more than six hours after dosing. Three of 11 subjects did not respond to methoxsalen in the hard-capsule formulation at 21 J/cm² for up to six hours after dosing. While maximum photosensitivity varies widely within the study panel, the solution and the soft-capsule formulation consistently require lower levels of light intensity to produce a response when compared with the hard capsule.

Statistical analysis of the results indicates significant differences among treatments in both maximum photosensitivity (.01 < *P* < .018) and time of maximum photosensitivity (.006 < *P* < .008).

COMMENT

The results of this study are similar to those of many investigations reported previously. Stolk et al¹ observed faster absorption of an aqueous solution compared with a hard-capsule preparation. Herfst and De Wolff² observed delayed absorption from a hard-capsule formulation, with peak serum concentrations occurring between one and four hours (mean, 2.4 hours). Hönigsmann et al³ in a study that compared a hard capsule and a soft capsule manufactured in Europe, observed almost identical results to those reported here. Levins et al⁴ independently compared the identical hard- and soft-capsule formulations and obtained results that are similar to those from the photosensitivity phase of this study.

Herfst and De Wolff² report a half-life of 1.1 hours for methoxsalen after oral doses of 0.5 to 0.7 mg/kg; however, their study sampled blood for only five hours after dosing. Goldstein et al⁵ report a half-life of 2.1 hours, which is very similar to our findings. Both Goldstein et al⁵ and Ehrsson et al⁶ note the biphasic nature of the elimination phase of the serum concentration time curve, which could result in underestimation of the half-life if blood samples are not taken for a long enough period of time after dosing.

Significant intersubject variability in the measured biopharmaceutical parameters was observed during this study. This variability was generally

consistent among the different formulations and was similar in magnitude to that observed in previous studies with methoxsalen.^{11,12} Considerable intersubject variability in maximum photosensitivity was also observed. It would appear that intersubject variability in absorption, disposition, and response is an inherent problem with methoxsalen. Of practical interest is the conservation of reduced intersubject variability in the time of maximum photosensitivity with the solution and the soft gelatin capsule, as described above. The data indicate that maximum response is achieved within a much tighter time frame following administration of the solution or the soft gelatin capsule. This may result in greater assurance that the period of maximum photosensitivity coincides with the period of UV-A irradiation in the clinical setting.

In conclusion, the results of this study indicate that the liquid formulation encapsulated in a soft gelatin capsule is a significant improvement in methoxsalen dosage form compared with the crystalline drug formulated in a hard gelatin capsule. It not only results in more drug being absorbed into the body, but also at a faster rate. This produces higher serum concentrations of drug and a concomitant increase in photosensitivity to UV-A. Use of the soft-capsule formulation will result in more reliable absorption of drug and may result in a lowering of the methoxsalen dose and/or the intensity of UV-A necessary to treat dermatologic disorders.

We would like to acknowledge the invaluable assistance of James Marshall, PhD, in the design of this study. We also thank Paula Kempf and other members of the technical staff for their laboratory assistance.

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ARCHIVES OF INTERNAL MEDICINE

Continuous Ambulatory Peritoneal Dialysis for Psoriasis

Zbylut T, Twardowski, MD; Kenneth D, Lempert, MD; Barry J, Lankhorst, MD; William A, Weiton, MD; Frederick C, Whittier, MD; Philip C, Anderson, MD; Karl D, Nolph, MD; Ramesh Khanna, MD; Barbara F, Prowant, RN; Lois M, Schmidt, RN (*Arch Internal Med* 1985;146:1177-1182)

8-Methoxypsoralen Serum Levels in Poor Responders to Photochemotherapy

Importance of Drug Formulation and Individual Factors

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Abstract: 8-Methoxypsoralen (8-MOP) serum levels of psoriatic patients poorly responsive to photochemotherapy (PUVA) treatment (problem cases) were determined by the HPLC method with 11 single blood probes over 8 hours. Abnormally low or deviated serum levels were found in 7 of 11 PUVA problem patients. There was a great interindividually different first-pass effect for 8-MOP in dependence on the galenic formulation of the 8-MOP brand; therefore, the change to another 8-MOP brand with a modern galenic formulation led only to a slight increase of serum levels, and consequently an increase in the 8-MOP dosage was necessary. It is important to be cautious at this point because patients may show an unproportional increase of 8-MOP serum levels due to the individually limited capacity of biotransformation. The studies of the authors reaffirm the necessity of the determination of 8-MOP serum levels in problem cases of PUVA therapy.

The efficiency of systemic photochemotherapy (PUVA) depends on two main factors, the light source (wavelength and dose) and the photosensitizer (the compound, its formulation, and its concentration in the dermis). A correlation has been reported between the concentration of 8-methoxypsoralen (8-MOP) reaching the dermis and the serum levels,¹⁻³ which can be measured by several methods⁴ including high-pressure liquid chromatography.^{5,6} 8-Methoxypsoralen serum levels exhibit a great interindividual variation,⁷⁻¹¹ depending on the limited biotransformation of the 8-MOP by the first-pass effect in the liver.¹²⁻¹⁴

Therefore, individual serum levels are of interest in therapy of PUVA problem cases,¹⁵ and in research,⁴ to correlate the therapeutic results with the actual serum level of the photosensitizer.

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The aim of this study was to determine serum levels of poor responders in PUVA therapy, and to investigate the influence of various 8-MOP brands, as well as the increase of their dosage, on individual serum curves.

Materials and Methods

- Each patient (n = 11) took one of the 8-MOP brands Geroxalen® or Mopsoralen® without any food or other drugs in therapeutic doses from 0.36 to 1 mg/kg body weight, depending on the brand. At 12 time points after oral administration, 5-ml samples of venous blood were drawn and 8-MOP was extracted from the serum probes on the same day.

The determination was carried out by means of high-pressure liquid chromatography,⁶ with a lowest limit of detection of 10 ng/ml.

From the determined 8-MOP values we calculated c_{max} as the highest serum concentration at the time t_{max} , and the area under the curve (AUC) as a variable of bioavailability.

Results and Discussion

We found abnormally low ($c_{max} \leq 160$ ng/ml) or deviated serum levels in 7 of 11 poor responders, which might be the reason for the ineffective PUVA therapy (Table 1).

One patient (G1) reached a serum concentration below 50 ng of 8-MOP/ml, one (G2) below 100 ng of 8-MOP/ml, and another (M7) had his maximum between the third and fourth hour after application and a serum concentration of only 18 ng at the time of irradiation. The amount of 8-MOP in the dermis would be even lower, as reported in the literature.^{3,16}

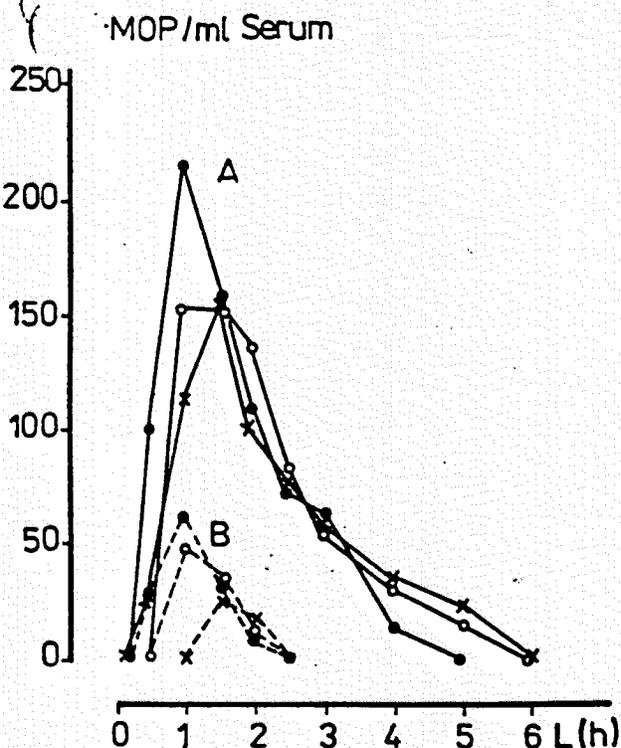
In the past, the 8-MOP brands were often changed in such cases of PUVA nonresponders, for example from Mopsoralen to the liquid capsule form Geroxalen with a modern galenic formulation. Therefore, we determined the intraindividual serum levels in two

8-MOP-Serum Concentration (ng/ml) from 11 Nonresponders to PUVA after a Single Administration of Oxorsoralen in Therapeutic Doses and the Calculated AUC_{0-8} ($0-8$ Hour) Values; C_{max} *

G/M	Dosis (mg · kg ⁻¹)	t Post application (h)										AUC_{0-8} (ng · ml ⁻¹ · h)	
		0,25	0,5	1	1,5	2	2,5	3	4	5	6		8
1	0,36	13	31	47*	46	37	16	8	0	0	0	0	88
2	0,50	0	72*	18	10	0	0	0	0	0	0	0	38
3	0,39	0	119	166*	136	131	89	50	33	16	8	0	396
4	0,46	133	322*	177	131	112	87	58	31	24	18	10	526
1	0,62	18	12	18	59	135*	86	60	21	14	0	0	229
2	1,0	0	8	9	26	144	152*	108	68	35	21	11	395
3	0,69	0	180	272	275*	191	185	95	68	38	32	39	793
4	0,72	87	113	118	153*	126	105	137	71	0	—	—	442
5	0,72	41	32	62	167	273*	197	129	113	129	87	39	875
6	0,71	0	110	147	156*	95	45	35	11	0	0	0	294
7	0,76	0	0	0	15	18	93	160*	158	95	35	22	518

G-MOP: 8-methoxypsoralen; PUVA: photochemotherapy; AUC: area under the curve; G: Geroxalen; M: Mopsoralen.

healthy volunteers after intake of 30 mg of 8-MOP in equivalent doses of Oxorsoralen®, Geroxalen (three capsules), and Mopsoralen (two tablets), as shown in Figure 1, and investigated the relative bioavailability.



1. Individual serum levels after application of Oxorsoralen (O), Geroxalen (●), and Mopsoralen (x) with 30 mg of 8-MOP equivalent doses in two patients (A:—; B:—).

The three brands exhibit different serum profiles, depending on their galenic formulation.

Interestingly, patient A had the highest values after all three brands, and patient B had the lowest ones every time. Patient B had AUC values after Geroxalen ($58 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}$) similar to those for Mopsoralen ($37 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}$) and Oxorsoralen ($49 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}$), but the maximal serum levels differed from 61 ng/ml (Geroxalen) to 26 ng/ml (Mopsoralen). These results show that the change in patient B to another 8-MOP brand with a better galenic formulation (ie, Oxorsoralen to Geroxalen), led to higher serum values of the photosensitizer in blood and probably also in the dermis, but in relation to other volunteers, the same patient had characteristically lower curves.

This is in agreement with the individually determined biotransformation capacity of the liver.^{11,13,17} Therefore, the change to another 8-MOP brand with a better galenic formulation is only one step to higher blood values, and it is also necessary to increase the doses given in the single case.

It is necessary to exercise caution at this point, however, because patients may show an unproportional increase of 8-MOP serum levels due to individually limited biotransformation. Thus, we found an unproportional rise in the serum level by a factor of 5 in patient G1 after intake of 30 mg of Geroxalen (C_{max} 47 ng/ml) and after doubling the amount of 8-MOP (C_{max} 260 ng/ml). Our results emphasize the advantage of evaluating 8-MOP serum levels in PUVA problem cases to make this excellent treatment more efficient in these patients.

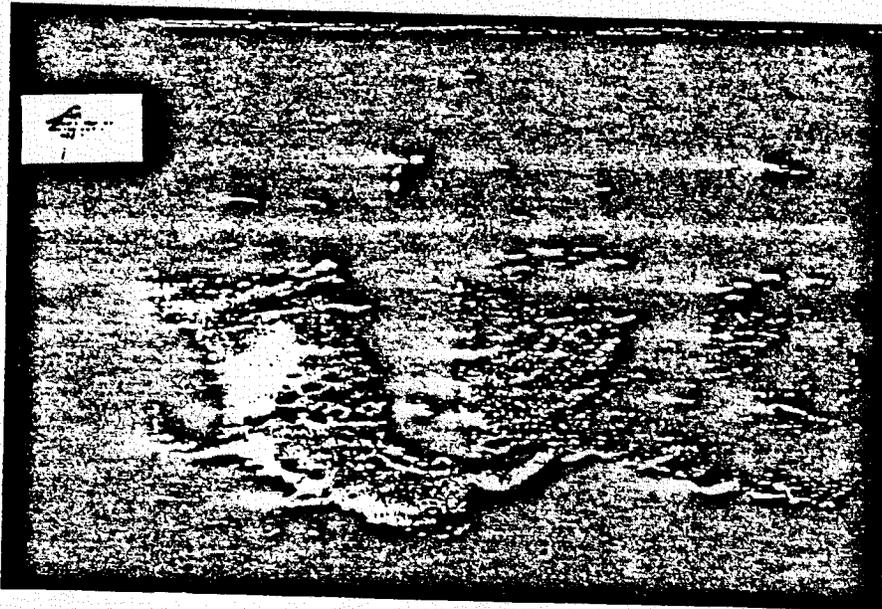
Acknowledgment

B. Quednow, M.D., and H. Walther, M.D. (Institute of Clinical Pharmacology, School of Medicine, Magdeburg, Germany) cooperated in the determination of serum concentrations of 8-MOP.

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"Beach Scene I." Richard C. Gibbs, M.D., New York, NY. Oil painting—second place. American Academy of Dermatology Art Exhibit, Atlanta, GA, 1990. Photograph courtesy of Dermik Laboratories, Inc.

The Use of Stable Isotopes to Prove the Saturable First-pass Effect of Methoxsalen

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Methoxsalen, administered orally shows a strong, albeit saturable first-pass effect, as demonstrated by classical dose linearity testing and by a new method, using stable isotopes and gas chromatographic mass spectrometric analysis. The therapeutic consequences of the first-pass effect are discussed.

INTRODUCTION

Methoxsalen, (8-methoxypsoralen, 8-MOP) is being used increasingly in the treatment of psoriasis, together with ultraviolet (UV) irradiation 'PUVA-therapy'.^{1,2} Only limited kinetic data are available about 8-MOP: i.v. kinetic studies in man have never been undertaken for toxicological reasons and because of the very low solubility of 8-MOP in plasma and suitable solvents. The latter problem was also manifest in i.v. kinetic studies in rats.³ Plasma level evaluations were performed above all to improve therapeutic efficacy. It was shown that crystal size⁴⁻⁷ is of importance for bioavailability. Recently the influence of food-intake on the kinetics of 8-MOP was reported.⁸ It is the aim of this paper, to demonstrate the first-pass effect of methoxsalen, to discuss the therapeutic consequences of this effect and to demonstrate its influence on bioavailability studies with stable isotopes.

EXPERIMENTAL

Three healthy male volunteers were used: R.G. 37 yr, 66 kg; E.H.S. 39 yr, 76 kg; F.B. 43 yr, 59 kg. No other drugs were taken during the test.

Substances

8-MOP was used in tablet form, Meladinine[®], Baso-therm Biberach (GFR).

[8, 8, 8-²H₃]Methoxsalen (= [²H₃]-8-MOP): the synthesis was performed by methylation of 8-hydroxypsoralen with CD₃I. The isotopic purity was greater than 99%.

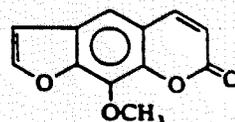
The internal standard was 8-ethoxypsoralen (8-EOP), synthesized from 8-hydroxypsoralen by ethylation.

Details of the study

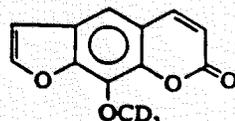
The study was performed on the following dates with formulations as indicated: 04.04.79 (L 10); 21.03.79

Abbreviations: 8-MOP = 8-methoxypsoralen; 8-EOP = 8-ethoxypsoralen; PUVA = psoralen and long-wave ultraviolet light.

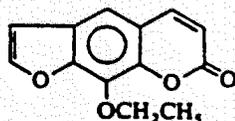
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[M]⁺ = 216



[M]⁺ = 219



[M]⁺ = 230

(L 20); 04.04.78 (L 40); 28.11.78 (LS 40); 08.12.78 (LF 40); 07.03.79 (LT 40). L 10, L 20 and L 40 were solutions of 10, 20 and 40 mg 8-MOP per person. LS 40 was a mixture of the solutions of 20 mg 8-MOP and 20 mg [²H₃]-8-MOP. LT 40 was a solution of 20 mg 8-MOP, followed 37.5 min later by a solution of 20 mg [²H₃]-8-MOP. LF 40 was a solution of 20 mg [²H₃]-8-MOP and simultaneous 20 mg as tablets.

The formulations were prepared by dissolving 32, 64, or 128 mg of 8-MOP or [²H₃]-8-MOP in 30 ml of ethanol and 30 ml of Solketal[®] (2, 2-dimethyl-1, 3-dioxalane-4-methanol (Givaudan, CH)) and dilution with water to 160 ml. Each volunteer was given 50 ml of the appropriate formulation, followed by 50 ml of water to rinse down all the substance. Two hours later the volunteers received a standard breakfast (tea and white bread with ham). Four hours later a normal lunch was consumed.

Samples

At each time interval 10 ml blood samples were collected in heparinized tubes. Centrifugation was performed in glass tubes. The plasma samples were stored at -25 °C also in glass tubes.

Analytical procedures

Instrumentation Gas chromatograph: Hewlett Packard type 5840, with electron capture detector type 8803 B

and autosampler, type 1672. 1.2 m OV 17 column, temperature 220 °C, injector 250 °C, detector 250 °C, carrier gas Ar/CH₄ 95:5.

Gas chromatograph mass spectrometer Finnigan 3300, with 6015 data system. The gas chromatograph was equipped with a 12 m capillary column SP 2100 (Bebjak, Kissing GFR), and helium was used as carrier gas (0.6 bar). Injector 260 °C, splitless injection, column temperature initially 70 °C, after 45 s programmed to 230 °C at 10 °C min⁻¹.

Chemicals: All solvents used were from Byk-Mallinkrodt, and were nanograde, (Wesel, GFR). All other chemicals were from Merck (Darmstadt, GFR).

Extraction procedures

Our simple electron capture detection procedure⁹ was used for all formulations containing only 8-MOP. For the gas chromatographic mass spectrometric (GCMS) simultaneous detection of 8-MOP and [²H₃]-8-MOP variations of the more complicated procedure of Ehrsson¹⁰ to remove cholesterol had to be employed. To 1 ml plasma, diluted with 1 ml water, 5 ml toluene containing the internal standard 8-EOP (20 ng ml⁻¹ = 100 ng) was added. After extraction and phase separation the toluene was evaporated, and redissolved with 100 µl of methanol. After adding 1 ml of 0.1 N NaOH, it was left for 20 min until the opening of the lactone ring was completed. The water phase was extracted once with 5 ml CH₂Cl₂ and twice with 5 ml toluene. The organic phases (containing cholesterol, for example) were discarded. Then 0.5 ml of 1 N HCl was added to form the lactone ring again. After extraction with 4 ml toluene, the toluene was evaporated, redissolved in 100 µl CHCl₃ and 1 µl injected into the gas chromatograph mass spectrometer.

The extractions were performed by shaking for 10 min with a shaking machine. Phase separation was carried out by centrifuging and freezing out the water phase (when toluene was used). All evaporation steps were performed with a Vortex^R evaporator. The calibration curves were constructed by adding the appropriate amounts of 8-MOP and [²H₃]-8-MOP to drug free plasma.

RESULTS

Analytical details

In Fig. 1 the mass spectra of 8-MOP, [²H₃]-8-MOP and 8-EOP are shown. Starting with the elimination of a methyl radical from the 8-methoxy group, 8-MOP exhibits a rather simple fragmentation, and a series of consecutive CO losses follows. In the case of [²H₃]-8-MOP the corresponding elimination of 18 mass units from the molecular ion was observed. In contrast to 8-EOP the molecular ion was not the base peak in the spectrum and fragments by the elimination of 28 mass units corresponding to the loss of C₂H₄ and CO. In Fig. 2 selected ion monitoring (SIM) traces of 8-MOP, [²H₃]-8-MOP and 8-EOP for about 100 pg pure substance are shown. They demonstrate the good performance of the

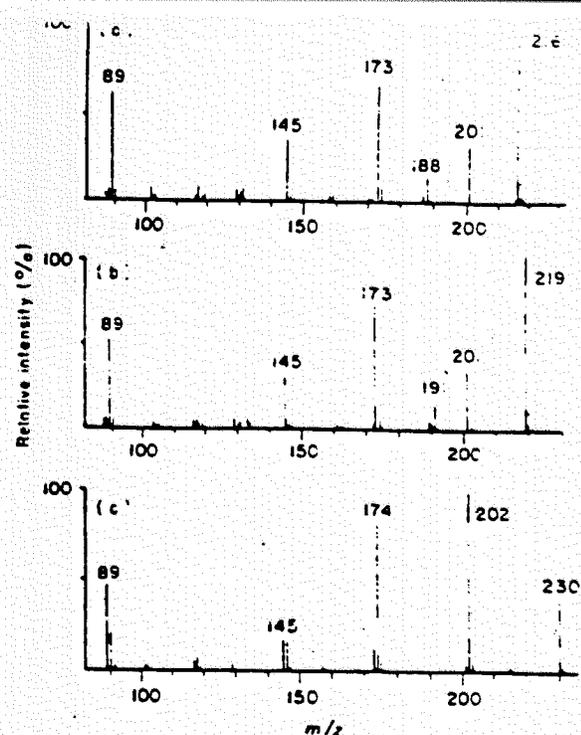


Figure 1. Electron impact mass spectra of: (a) 8-MOP; (b) [²H₃]-8-MOP; (c) 8-EOP.

gas chromatograph mass spectrometer. The analyt methods applied for estimating 8-MOP and [²H₃]-8-MOP correspond to the International Federation Clinical Chemistry recommendations¹¹ concern

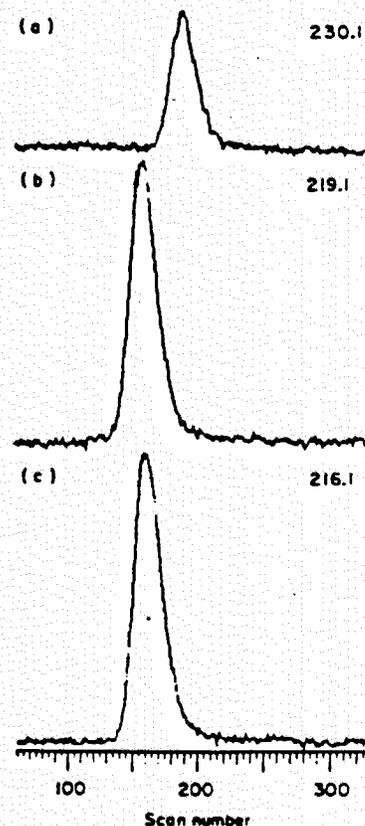


Figure 2. SIM analysis of about 100 pg of standards: (a) 8-MOP; (b) [²H₃]-8-MOP; (c) 8-EOP.

precision, accuracy, specificity and sensitivity.^{9,10} Blanks are very low ($< 3 \text{ ng ml}^{-1}$), and the variation of these blanks is small. The *detection limit* of the assay is about 5 ng ml^{-1} , and the detection limit of the pure substance about 20 pg .

Linear calibration curves (Fig. 3) in the range $25\text{--}500 \text{ ng 8-MOP ml}^{-1}$ (the range after therapeutic doses) were obtained. They are expressed by the following equations: $y = b \cdot x + a$. 8-MOP: $a = 0.049$; $b = 0.0214$; $B = 0.9989$. [$^2\text{H}_3$]-8-MOP: $a = 0.023$; $b = 0.0208$; $B = 0.995$.

The *reproducibility* of the assay was studied by preparing plasma samples containing different amounts of 8-MOP and [$^2\text{H}_3$]-8-MOP and analysing them on different days. The results are shown in Table 1.

By liquid scintillation counting, the *recovery* of ^{14}C labelled 8-MOP was $91.1\% \pm 2.4$ ($n = 5$) at a concentration of 200 ng ml^{-1} .

The *selectivity* of the method is revealed by the very low blanks in the range of $1\text{--}3 \text{ ng ml}^{-1}$. As the metabolic pattern and the metabolites from human plasma are known^{4,12,13} we are sure that no metabolites interfere with the quantification of the parent compound. The complicated extraction procedure is strongly recommended, since our first experiments were performed with a single extraction step using toluene without further clean-up and after three injections into the gas chromatograph we had strong interferences from cholesterol.

Studies in humans

The advantages of the simultaneous administration of labelled and unlabelled compounds were demonstrated by Strong *et al.*,¹⁴ and Sullivan and McMahon.¹⁵ They compared the i.v. and oral routes of administration.

However, we dosed oral 8-MOP tablets and a solution simultaneously for the following reasons: first, our toxicological experiences were not sufficient for i.v. administration; second, the US Food and Drug Administration proposes to check the bioavailability of a formulation by comparing it with a solution. Surprisingly, the 8-MOP tablets exhibited a greater bioavailability than the solution in two out of three volunteers (Fig. 4). This result has three theoretical explanations: isotopic effects,¹⁴ the content uniformity of the solid formulation or a saturable first-pass effect.^{18,19}

First, we ruled out isotopic effects in the metabolism of 8-MOP and [$^2\text{H}_3$]-8-MOP by administering the substances simultaneously as a solution. Both levels were identical. In Fig. 5 the regression line of 8-MOP and [$^2\text{H}_3$]-8-MOP levels of all three volunteers is shown.

Table 1. Reproducibility of 8-MOP and [$^2\text{H}_3$]-8-MOP determinations on one day (A) and between days (B)

	Plasma level (ng ml^{-1})	Mean peak ratio		No. of determinations	CV %	
		8-MOP	[$^2\text{H}_3$]-8-MOP		8-MOP	[$^2\text{H}_3$]-8-MOP
A	25	0.53	0.54	4	5.5	5.1
	100	2.22	2.09	4	0.7	0.9
	400	8.62	8.35	4	4.2	2.3
B	100	—	2.11	6	—	2.1

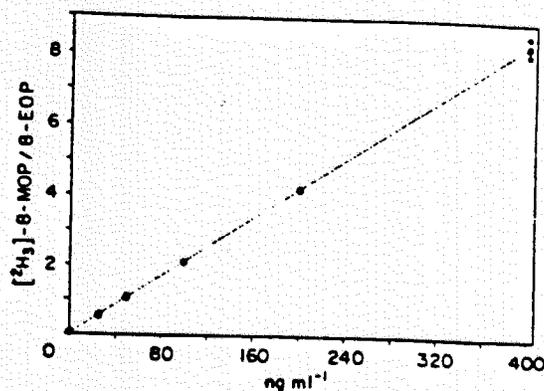


Figure 3. Calibration curves for [$^2\text{H}_3$]-8-MOP with 8-EOP as internal standard from human plasma. $n = 4$ for each value.

The regression coefficient, the intercept and the slope are 0.9995, 1.9 and 0.97 respectively. The latter corresponds exactly to the quotient of the slopes of the calibration curves of [$^2\text{H}_3$]-8-MOP and 8-MOP. Second, we rechecked the content uniformity of the tablets employed. It was suggested⁵ that the poor content uniformity of other 8-MOP formulations was responsible for differences in plasma levels. The weight ($n = 10$) of our 10 mg tablets was 9.906 mg ; the variation coefficient 1.43%. This is an excellent result.

To check the first-pass effect, we administered 20 mg 8-MOP and 20 mg [$^2\text{H}_3$]-8-MOP with a time interval of 37.5 min . The result is depicted in Fig. 6. The second dosing shows a two to threefold higher plasma level.

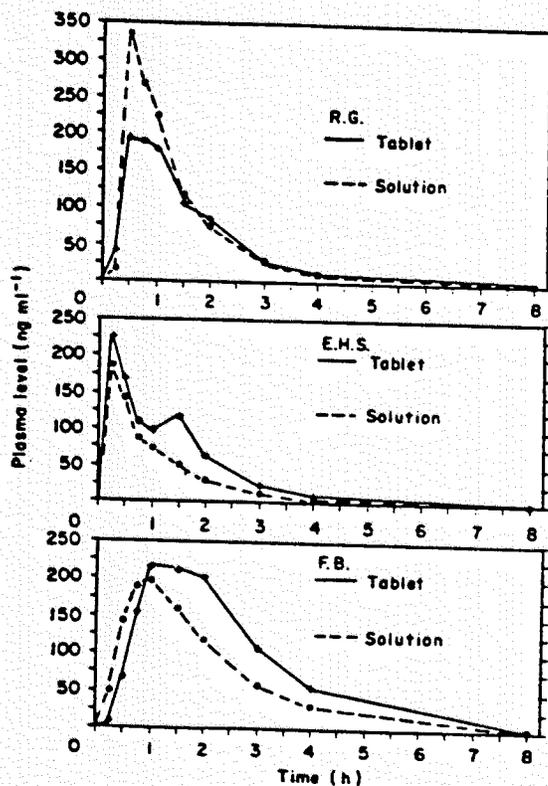


Figure 4. Plasma levels of 8-MOP and [$^2\text{H}_3$]-8-MOP of three volunteers after administration of 8-MOP as tablets (20 mg) and [$^2\text{H}_3$]-8-MOP (20 mg) as a solution.

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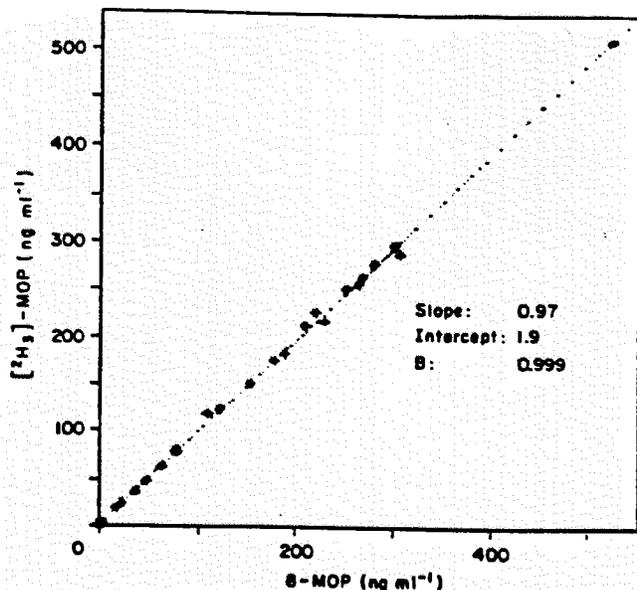


Figure 5. Regression line of 8-MOP and $[^2\text{H}_3]$ -8-MOP plasma levels after simultaneous administration of both substances as a solution.

Two further points are worth mentioning. The small humps in the 8-MOP plasma level after administration of the $[^2\text{H}_3]$ -8-MOP are caused presumably by displacement of the 8-MOP in some compartments. Second, if we compare the two initial levels, we notice a sigmoid shape in the 8-MOP curve. This is an indication that certain processes do not follow first-order kinetics.

Apart from the saturable first-pass effect, these results could also be explained by the influence of the first dose

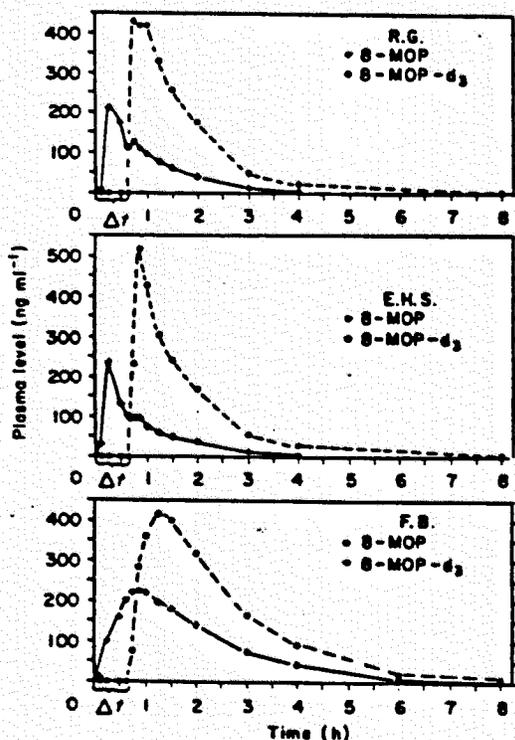


Figure 6. Plasma levels of 8-MOP and $[^2\text{H}_3]$ -8-MOP of the three volunteers after administration of both substances as a solution but with a time interval Δt of 37.5 min.

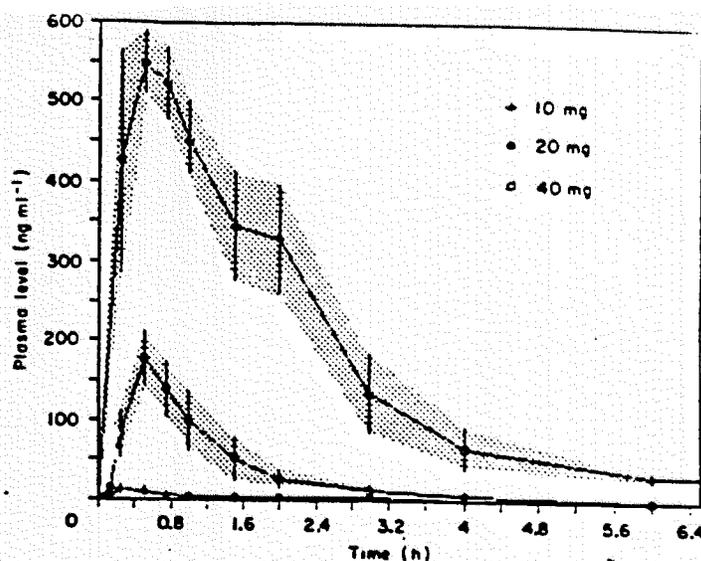


Figure 7. Plasma levels of 8-MOP after administration of 40, 20, 10 mg of 8-MOP as a solution to three volunteers; mean values \pm standard deviation of the mean.

on the absorption of the second. This does not pertain, however, since time-dependent kinetics¹⁶ were ruled out by a classical dose linearity testing procedure. The plasma levels of 8-MOP after administration of 40 mg, 20 mg or 10 mg, respectively are shown in Fig. 7. A plot of the AUC values against the dose yields a threshold dose¹⁷ of about 0.23 mg kg^{-1} .

DISCUSSION

Employing a classical method, involving the administration of different doses at different times as well as a new method with stable isotopes, we demonstrated a saturable first-pass effect^{18,19} for 8-MOP. We did not establish whether it is a gut or a hepatic first-pass effect.¹⁹ Since 8-MOP is metabolized in man mainly by oxidative processes,¹² the latter is much more likely. Our results suggest that the low plasma levels in rabbit²⁰ and dog²¹ after oral doses were not caused exclusively by the low solubility of 8-MOP in the solid formulations employed. In analogy to the calculations of Gibaldi *et al.*¹⁸ we compute that about 60–80% of the

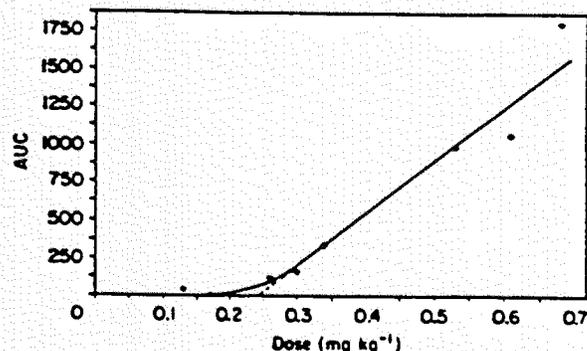


Figure 8. Plot of area under the curve (AUC) vs dose after administration of 40, 20, 10 mg of 8-MOP to three volunteers; the threshold dose is about 0.23 mg kg^{-1} .

administered 40 mg of 8-MOP as a solution are systemically available.

This saturable first-pass effect means that small variations in the dose administered, as well as the absolute levels or rate of absorption, cause great differences in the systemic bioavailability. This effect has consequences for the therapy and the method.

Consequences for therapy

Sound PUVA therapy can be attained only with a formulation which avoids the influence of the first-pass effect. From this point of view we suggest the administration of 8-MOP by infusion or orally, as a solution, since 8-MOP is absorbed from a solution very rapidly. Consequently, high quantities can pass the liver without being metabolized. The topical application is even more reasonable. It avoids the first-pass effects as well as the systemic effects which can be observed at high plasma levels.

Insufficient, low 8-MOP plasma levels during therapy should be compensated initially by higher 8-MOP doses rather than by higher irradiation doses.

Consequences for the method

The use of stable isotopes for bioavailability studies rules out—as known—individual variations. However, the non-existence of any isotope effect must be demonstrated by simultaneous administration of labelled and non-labelled compounds both in the same formulation. Dose linearity testing must be performed. We suggest the administration of labelled and non-labelled compounds with a small time interval in between.

If we observe non-linear kinetic processes, the co-administration of a labelled drug influences the kinetics of the unlabelled drug. In such a case this design of the experiment cannot be used for bioavailability studies.

Acknowledgements

We thank Reini Krug, Stefan Weigle, Peter Köster and Heinz Switek for excellent technical assistance.

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Received 13 June 1980

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Paper presented at the Third International Symposium on Quantitative Mass Spectrometry in Life Sciences, Gent, Belgium, June 1980