The pharmacokinetics of 8-methoxypsoralen following i.v. administration in humans

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1 8-methoxypsoralen (8-MOP) is a naturally occurring photoreactive substance which, in the presence of u.v. light, forms covalent adducts with pyrimidine bases in nucleic acids. For many years, 8-MOP has been used in PUVA therapy for treatment of psoriasis. Recently, the drug has been found to inactivate effectively bacteria spiked into platelet concentrates. The purpose of this study was to determine the pharmacokinetics and safety of 8-MOP administered intravenously in the bactericidal dosage range.

2 Eighteen volunteers were divided into three treatment groups to receive, respectively, 5, 10, and 15 mg 8-MOP infused over 60 min. Frequent arterial samples were gathered, and the blood and plasma were assayed for 8-MOP concentration. The pharmacokinetic parameters were determined by moment and compartmental population analysis, the latter performed with the program NONMEM. Haemodynamics, ventilatory pattern, and subjective effects were recorded throughout the study.

3 The intravenously administered 8-MOP was well tolerated in all individuals, and no acute toxicity was observed.

4 The pharmacokinetics of 8-MOP were best described by a three-compartment mammillary model in which the volumes and clearances were proportional to weight. The mean pharmacokinetic parameters for the plasma concentrations were: $V_1 = 0.045 \ 1 \ kg^{-1}$, $V_2 = 0.57 \ 1 \ kg^{-1}$, $V_3 = 0.15 \ 1 \ kg^{-1}$, CL_1 (systemic) = $0.010 \ 1 \ kg^{-1} \ min^{-1}$, $CL_2 = 0.0067 \ 1 \ kg^{-1} \ min^{-1}$, $CL_3 = 0.012 \ 1 \ kg^{-1} \ min^{-1}$. The mean pharmacokinetic parameters for the blood concentrations were: $V_1 = 0.061 \ 1 \ kg^{-1}$, $V_2 = 1.15 \ 1 \ kg^{-1}$, $V_3 = 0.21 \ 1 \ kg^{-1}$, CL_1 (systemic) = $0.015 \ 1 \ kg^{-1} \ min^{-1}$, $CL_2 = 0.011 \ 1 \ kg^{-1} \ min^{-1}$ and $CL_3 = 0.015 \ 1 \ kg^{-1} \ min^{-1}$.

5 The plasma pharmacokinetic model described the observations with a median absolute error of 17%, and the blood pharmacokinetic model described the observations with a median absolute error of 18%. Analysis of the relative concentration of 8-MOP between plasma and red blood cells suggested concentration-dependent partitioning.

6 The addition of 7.5 mg 8-MOP to 300 ml platelet concentrate would produce bactericidal concentrations of 25 μg ml^{-1}. Simulations based upon our data show that intravenous administration of 7.5 mg over 60 min would result in systemic concentrations of 8-MOP similar to those observed with conventional PUVA therapy. We conclude that the extensive safety history established in PUVA therapy will be applicable to this new application of 8-MOP.

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Introduction

8-methoxypsoralen (8-MOP) is a naturally occurring photoreactive substance found in common foods such as parsley, celery, and limes. In the absence of long wavelength u.v. light (UVA), 8-MOP reversibly intercalates into double-stranded regions of DNA and RNA. Photoactivation, effected by addition of UVA light (320–400 nm), results in formation of covalent adducts between 8-MOP and pyrimidine bases. This property is the basis of PUVA therapy used by dermatologists which slows the renewal of the skin cells in the treatment of chronic refractory psoriasis. [1].

Lin *et al.* [2] have shown recently that photoactivation of 8-MOP at a concentration of 25.0 $\mu g\ ml^{-1}$ produces inactivation of pathogenic bacteria in platelets that are suspended in 15% plasma and 85% synthetic media [2]. Lin's findings suggest that any bacteria present in platelet concentrates would be inactivated, prior to infusion, by exposure to 8-MOP and UVA light. The total amount of 8-MOP in a pooled transfusion of six random donor units of platelets would be 7.5 mg (300 ml × 25 $\mu g\ ml^{-1}$).

A few pharmacokinetic studies based upon oral administration of 8-MOP have been performed in humans [3–6]. Data from these studies are of limited value in understanding the systemic exposure expected after intravenous administration, because the absolute bioavailability and rate of absorption of 8-MOP are not known when the drug is administered orally. While the pharmacokinetics in humans of intravenously administered 8-MOP are not known, animal studies using intravenous 8-MOP have been done. In animals, 8-MOP has extensive tissue distribution, rapid clearance and substantial interindividual variability [7].

The current investigation was performed to determine the pharmacokinetics of 8-MOP in humans following intravenous administration. These results permit an assessment of the time course of exposure to 8-MOP that can be anticipated following the intravenous administration of platelets photochemically treated with 8-MOP and UVA light.

Methods

Pretreatment, treatment and post-treatment

After obtaining Institutional Review Board approval and informed consent, 18 healthy adult volunteers were included in the study. Men and women between the ages of 18 and 40 years, without a history of significant medical illness, were eligible. Volunteers were ineligible if they chronically used tobacco, alcohol, medications or illicit drugs, or if their laboratory blood and urine tests (including serum hepatitis B surface antigen and HIV antibody test) were abnormal. Before the study day, volunteers were instructed to abstain from ingesting any medication or alcohol or from using tobacco for at least 24 h before the study time. On the study day, volunteers

fasted from midnight before entering the study facility.

At the study site, an 18 G catheter was placed in a large forearm vein for fluid and drug administration. Normal saline was infused at the rate of 1.5 ml kg^{-1} h^{-1} before and after the drug infusion. A 20 G radial artery catheter was placed for blood sampling and continuous blood pressure monitoring. Electrocardiogram (5 lead), pulse oximetry and nasal end-tidal CO_2 were also continuously monitored.

Wrap-around UVA protection including glasses were worn by the subjects on the study day until the sunset. Subjects stayed over night in a hospital room at the Palo Alto Veterans Administration Medical Center; they were discharged the next day after having a physical examination and the last blood sample.

Potential side effects were assessed every 30 min during the first 6 h, then at 10 and 24 h after the beginning of the infusion and were graded as mild, moderate or severe.

Drug administration

Formulation   The test article dosage form was provided in amber vials containing 2500 μg 8-MOP in 100 ml 0.9% sodium chloride USP (25 $\mu g\ ml^{-1}$). The formulation was manufactured under GMP conditions by Chesapeake Biological Laboratories (Baltimore, MD) using bulk GMP 8-MOP purchased from ICN Pharmaceuticals (Costa Mesa, CA).

Administration   Each subject received one of the three doses (5 mg, 10 mg or 15 mg) of 8-MOP as a constant rate infusion over 60 min. The amount of drug to be infused was diluted into 600 ml total volume by the addition of sterile saline just before starting the infusion. The drug was infused intravenously over 60 min (rate of infusion: 10 ml min^{-1}), using a volumetric pump. The infusion rate and duration were selected to be similar to the dose and time course of 8-MOP likely to be seen with a standard platelet transfusion (300 ml).

8-methoxypsoralen assay

Sample handling   Arterial blood samples (10 ml) were drawn at baseline and at 2, 5, 10, 15, 20, 30, 40, 50, 60 min after the beginning of the infusion, and at 2, 5, 10, 15, 20, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240, 270, and 300 min after the end of the infusion. The arterial catheter was removed 6 h after the beginning of the infusion. Two additional venous blood samples were drawn at 600 and 1440 min after start of infusion.

Initial studies suggested that 5–7% of 8-MOP was adsorbed onto the tubing during the infusion. To determine properly the infusate concentration of 8-MOP, two 5 ml samples of the infusate were drawn immediately prior and immediately after the infusion from the distal infusion line immediately proximal to the intravenous catheter. The 8-MOP concentration in

the infusate was calculated as the average between pre- and post-infusion 8-MOP concentrations in the sampled infusate.

Sample processing Blood samples were drawn into heparinized tubes, mixed, and placed on ice until processing. Sample processing took place within 2 h of blood drawing. Three millilitre aliquots were drawn for whole blood assay and placed on ice. Then the tubes containing the remaining whole blood were centrifuged at 2000 g for 10 min at 4° C to prepare and isolate the plasma phase. The plasma phase was drawn off and placed on ice.

H.p.l.c. analysis Plasma and whole blood samples were assayed by Steritech, Inc. (Concord, CA) using modifications of the method developed by Gasparro *et al.* [8]. Before loading the whole blood samples onto solid phase extraction (SPE) cartridges, 1 ml was lysed by the addition of 4 ml Isoton II (Coulter) followed by addition of two drops of Zapoglobin (Coulter). After 5 min, the treated samples were centrifuged at 5900 g and the supernatant loaded onto SPE cartridges. A haematocrit determination was also performed on whole blood samples. Eluted samples were injected onto a C-18 column (Rainin Instruments, Emeryville, CA), 4.6 mm × 25 cm column with 4.6 mm × 3 cm guard column, 5 μm particle, 60 A pore size. A Beckman (Fullerton, CA) h.p.l.c. system was used with autoinjector, a variable wavelength detector, a solid phase ^3H detector, and the System Gold computer integration software. The h.p.l.c. was run isocratically with a mobile phase of 45% acetonitrile and 55% 0.1 M ammonium acetate. 8-MOP eluted at 9.3 min with this system and was detected by absorption at 300 nm. Samples were injected using a 100 μl sample loop injector. Eight-MOP levels were determined by comparing the integrated absorption peak of 8-MOP with a standard curve of 8-MOP concentrations. Standard curves were generated weekly by injecting freshly prepared standard samples. Samples were also spiked with a small amount of $[^3H]$-8-MOP as an internal control of recovery. Final concentrations were corrected for the fraction of tritium recovered relative to a calibration sample and for the amount of 8-MOP contributed by the internal standard. Accuracy of the h.p.l.c. system was evaluated by injecting standards between sets of experimental samples and comparing the recovery to a set of control standards run at the beginning of each week. The limit of detection was 10 ng ml^{-1} with a coefficient of variation of less than 10%.

Pharmacokinetic analysis

Linearity analysis The dose-normalized concentration *vs* time curves were obtained for plasma and whole blood by dividing each concentration value by the dose of 8-MOP administered. The linearity of the concentration time course with the dose was visually verified by plotting the dose-normalized concentration *vs* time for each dose and verifying that the shape and scale of the dose-normalized curves were

independent of dose. The dose normalized AUC (e.g. AUC/Dose) values were then compared for both plasma and whole blood between the three doses by a one way ANOVA. A statistical significance level $P < 0.05$ was required to demonstrate the lack of linearity of AUC with the dose.

Moment analysis Clearance, mean residence time, and apparent volume of distribution at steady state were calculated non-parametrically using standard moment analysis [9]. The area under the concentration *vs* time curve (AUC) was calculated for each patient using linear trapezoids when concentrations were increasing, and log-linear trapezoids when concentrations were decreasing. The terminal slope was estimated using log-linear regression of the terminal portion of each curve. The clearance was calculated as

$$CL = \frac{Dose}{AUC} \quad (1)$$

The first moment curve (concentration × time *vs* time) was calculated for each data set and the area under the first moment curve (AUMC) calculated using also an interpolation-integration method. The mean residence time (MRT) of 8-MOP was calculated as

$$MRT = \frac{AUMC}{AUC} - MRT_{infusion} \quad (2)$$

where $MRT_{infusion}$ was the mean residence time of the infusion input (i.e. duration/2). The apparent volume of distribution at steady state (V_{ss}) was calculated as

$$V_{ss} = CL \times MRT \quad (3)$$

The population parameter estimates were calculated as the average of the individual values. The moment analysis of the pharmacokinetics were performed for both the plasma and the whole blood concentrations.

Compartmental analysis The population pharmacokinetic parameters were determined using the mixed-effects non-linear regression program NONMEM, version IV level 1.1 [10]. NONMEM estimated the typical value of each volume and clearance parameter for two and three compartment mammillary compartmental models in the population. For the two compartment model, NONMEM estimated V_1, the volume of the central compartment, V_2 the volume of the peripheral compartment, CL_1, the irreversible systemic clearance of drug from the central compartment (combining both renal and metabolic components), and CL_2, the distribution clearance to the peripheral volume. For the three compartment model, NONMEM estimated V_1 and CL_1, defined as for the two compartment model, V_2 and CL_2, the volume and distribution clearance for the rapidly equilibrating compartment, and V_3 and CL_3, the volume and distribution clearance for the slowly equilibrating compartment.

NONMEM provided estimates of the interindividual variability about each pharmacokinetic parameter. Each

parameter, P_i, in the ith individual, was modelled as a function of the typical value of P in the population, P_{TV}, and η_i, as follows:

$$P_i = P_{TV} \, e^{\eta_i} \qquad (4)$$

where P_{TV} is the median value of P in the population and η is a random variable with a mean of 0 and a variance of ω^2. While ω is thus the standard deviation of the parameter in the log domain, for values less than 0.40 it will be quite similar to the coefficient of variation used in classical descriptive statistics, and thus will be referred to as the 'CV' of the parameter estimate.

NONMEM was also used to estimate the residual intraindividual variability. A log normal residual error model was assumed, in which the jth observation in the ith subject, O_{ij}, was assumed to be related to the predicted concentration, Y_{ij}, as follows:

$$O_{ij} = Y_{ij} \, e^{\varepsilon_{ij}} \qquad (5)$$

where ε is a random variable with a mean of 0 and a variance of σ^2. Thus, σ is the standard deviation in the log domain, which approximately corresponds to the coefficient of variation in the standard domain for small values of σ.

The NONMEM analysis included incorporation of patient covariates in the model. The specific covariates investigated were weight, age, body surface area [11] (BSA^A), lean body mass [12] (LBM^B), haematocrit, and serum albumin. The covariates were generally incorporated into the model as a scalar times the covariate plus a constant, in which the scalar and constant were simultaneously estimated by NONMEM. This structure permitted the scalar to become 0 if the covariate added no information to the model, and permitted the constant to become 0 if the pharmacokinetic parameter was simply proportional to the covariate (e.g. clearance was proportional to weight).

We systematically tested each volume and clearance term as a linear function of each patient covariate, as well as a 'weight scaled' model in which all volumes and clearances were assumed to be a linear function of weight. Models were considered better if the decrease in the NONMEM objective function, −2 log likelihood (−2LL) with the more complex model, with the addition of a single term, exceeded 10 (χ^2 0.998[1]) [13]. The severe significance level of 0.998 was selected to compensate for the large number of models considered.

Calculation and representation of results From the estimates of the pharmacokinetic parameters provided by NONMEM, at the time of each observation we calculated the predicted concentration, C_P. We calculated the weighted residual (WR) as the difference between the measured concentration, C_M, and the predicted concentration, as a fraction of the predicted concentration:

^{A}BSA = $weight^{0.425} \times height^{0.725} \times 0.007184$
^{B}LBM = $1.1 \times weight - 128 \times (weight/height)^2$ for men
     = $1.07 \times weight - 148 \times (weight/height)^2$ for women

$$WR = \frac{C_M - C_P}{C_P} \qquad (6)$$

For each fit we also plotted the WR over time for all individuals.

To measure the bias of the prediction from each model estimated by NONMEM we calculated the median weighted residual (MDWR):

$$MDWR = median \, \{WR_1, WR_2, ..., Wr_n\} \qquad (7)$$

where n is the number of data points in the study ($n = 308$).

To measure the overall accuracy of the model we calculated the median absolute weighted residual (MDAWR):

$$MDAWR = median \, \{|WR_1|, |WR_2|, ..., |Wr_n|\} \qquad (8)$$

where n is the number of data points in the entire study ($n = 308$).

To calculate the accuracy of the model in each individual subject we calculated the individual mean absolute weighted residual (MAWR) as:

$$MAWR = \frac{\sum_n |WR|}{n} \qquad (9)$$

where n is the number of data points for each subject (range: $n = 12$–20, median = 17.5).

To display how accurately the pharmacokinetic parameters provided by NONMEM described the observed data, we plotted the fit in the best, median and the worst case individuals, according to the individual MAWR value. These graphs provided a visual interpretation of the goodness of fit for the pharmacokinetic models.

From the volumes and clearances estimated by NONMEM we calculated the hybrid rate constants and fractional coefficients using standard pharmacokinetic equations [14, 15].

Partition coefficient The partition coefficient (PC) between plasma and red cells has been calculated for each data point of each patient.

$$partition \; coefficient = \frac{C_{red\;cells}}{C_{plasma}} \qquad (10)$$

Recognizing that $C_{blood} = C_{red\;cells} \times$ haematocrit $+ C_{plasma} \times (1 - $ haematocrit$)$, and substituting for $C_{red\;cells}$ in the definition of PC above, we get the following definition of PC in terms of C_{blood}, C_{plasma} and haematocrit, all of which were measured in this study:

partition coefficient

$$= \frac{C_{blood} - C_{plasma} \times (1 - \text{haematocrit})}{\text{haematocrit} \times C_{plasma}} \qquad (11)$$

The above equation yielded a measurement of the partition coefficient at the time of each observation.

Visual inspection of the data suggested a possible linear relationship between partition coefficient and time, and a possible inverse biexponential relationship between the partition coefficient and the plasma 8-MOP concentration. We used NONMEM to estimate the relationship between partition coefficient and time using the following model:

$$PC_{ij} = \theta_1 + \theta_2 t + \theta_3 e^{-\theta_4 C_P} + \theta_5 e^{-\theta_6 C_P} \qquad (12)$$

where θ_1 is the constant component of PC, θ_2 is the slope of the PC vs time relationship, θ_3 and θ_5 are coefficients of the relationship between PC and C_P, and θ_4 and θ_6 are the exponents of the relationship between PC and C_P. The interindividual variability (η vector) was modelled as log-normally distributed about each value of θ, and the residual variability, ε_{ij}, was modelled as log-normally distributed as well. Whether the slope factor relating PC to time, θ_2, was statistically significant was determined by comparing the NONMEM objective function (−2 log likelihood) obtained with the model shown above with a model in which θ_2 was fixed at 0. A difference greater than 4 was considered significant at $P < 0.05$.

Results

Pretreatment, treatment and post-treatment

Eighteen volunteers completed the study. The volunteers received 5 mg, 10 mg or 15 mg 8-MOP ($n = 6$ in each dose group). Four additional volunteers were enrolled but did not complete the study or were not evaluable. There was no difference between the three dose groups in any demographic parameter (Table 1).

None of the doses of 8-MOP studied altered the vital signs monitored (Table 2). One subject developed a slight headache, five felt tiredness or drowsiness. Three subjects reported hunger or thirst, and five complained of intestinal gas, all of which we attributed to the prolonged fast required for the study. No adverse reactions were observed that could be attributed to the 8-MOP.

Pharmacokinetic analysis

Linearity analysis Figures 1 and 2 (top graphs) show individual plasma and whole blood concentrations of 8-MOP vs time, respectively. The shape of

Table 1 Volunteer demographics (mean ± s.d.)

	5 mg (n = 6)	8-MOP 10 mg (n = 6)	15 mg (n = 6)	Global (n = 18)
Weight (kg)	85.4 ± 14.2	79.8 ± 11.8	74.2 ± 15.0	79.8 ± 13.7
Height (cm)	186.6 ± 9	177.7 ± 11.1	177.4 ± 7.3	180.6 ± 9.8
Age (years)	30.7 ± 8	35.8 ± 8	31.2 ± 9.7	32.6 ± 8.4
Body surface area (m²)	2.10 ± 0.2	1.98 ± 0.2	1.91 ± 0.21	2.00 ± 0.21
Lean body mass (kg)	66.9 ± 8.4	60.8 ± 9.3	58.0 ± 9.4	61.9 ± 9.3
Haematocrit (%)	44.2 ± 1.6	43.3 ± 4.7	42.8 ± 2.8	43.5 ± 3.2
Serum albumin (g dl⁻¹)	5.07 ± 0.38	4.77 ± 0.19	5.03 ± 0.47	4.96 ± 0.37

Table 2 Mean arterial blood pressure (MAP), heart rate (HR), respiratory rate (RR), and arterial saturation (SpO₂) vs time during and after the infusion of 8-methoxypsoralen. Values are expressed as mean ± s.d.

	Baseline	30 min*	60 min*	120 min*	180 min*
5 mg dose					
MAP (mm Hg)	90.8 ± 16.7	89.8 ± 12.3	86.8 ± 16.2	83.8 ± 9.4	83.7 ± 6.9
HR (beats min⁻¹)	51.6 ± 6.2	52.3 ± 9.9	59 ± 10.8	50 ± 4.2	51 ± 4.4
RR (min⁻¹)	14.8 ± 6.1	16.8 ± 1.9	19.3 ± 4.8	13.3 ± 6.1	16.7 ± 4.1
SpO₂ (%)	97.7 ± 1.5	98 ± 2.1	98.3 ± 1.9	98 ± 2.1	97.8 ± 2.3
10 mg dose					
MAP (mm Hg)	95.7 ± 12.1	98.7 ± 16.1	97.3 ± 14.2	91.7 ± 14.2	94.3 ± 9.4
HR (beats min⁻¹)	58.5 ± 11.9	61.3 ± 12.4	59.2 ± 8.1	58.7 ± 7.1	60.2 ± 6.8
RR (min⁻¹)	16.3 ± 4.5	17.7 ± 2.3	17.3 ± 6.2	16.7 ± 3.6	17.5 ± 3.5
SpO₂ (%)	98.2 ± 1	98.2 ± 1.5	98.5 ± 1.2	97.8 ± 1.5	98 ± 1.4
15 mg dose					
MAP (mm Hg)	88.8 ± 6.3	89 ± 7.3	88.8 ± 7.8	85.5 ± 7.3	86 ± 11.5
HR (beats min⁻¹)	60.5 ± 11.3	61.3 ± 8	61.5 ± 9.8	62.3 ± 12.6	60.8 ± 11
RR (min⁻¹)	19.3 ± 6.4	19.7 ± 4.3	19.8 ± 4.7	19.3 ± 5.3	19.2 ± 4.1
SpO₂ (%)	97.3 ± 1	97.2 ± 1.7	97 ± 1.8	96.7 ± 1.9	97.5 ± 1.8

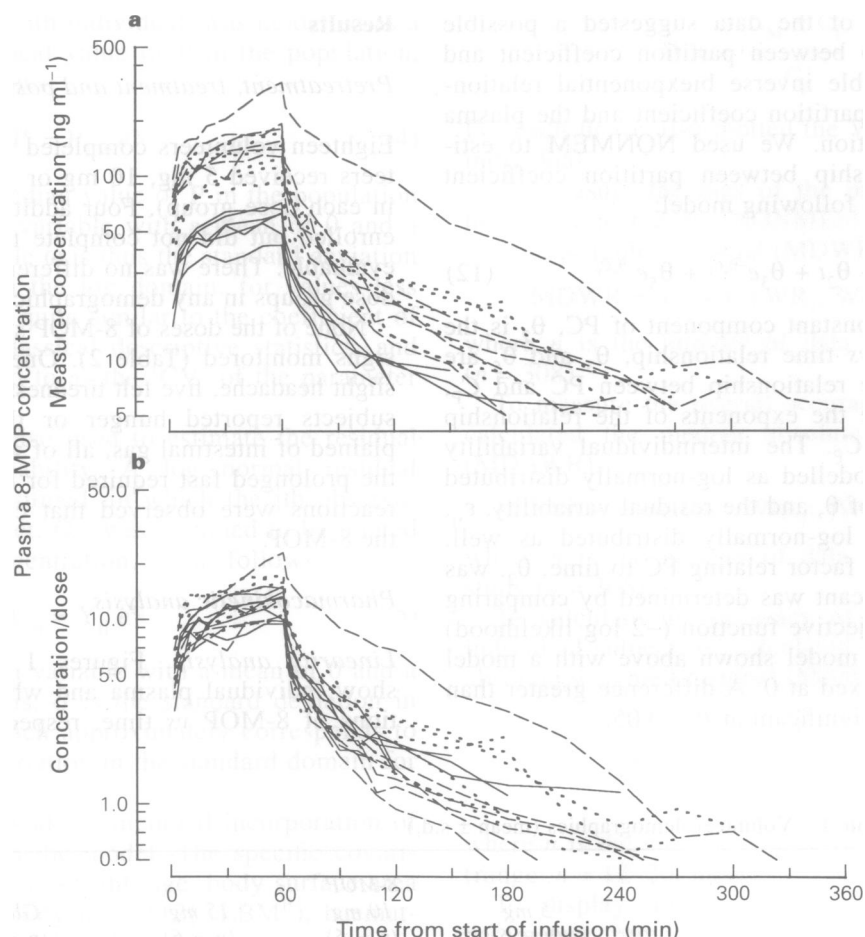*Elapsed time following start of 60 min 8-MOP infusion.

Figure 1 Individual time course of 8-MOP concentrations (a) and dose normalized concentrations (b) in plasma. The dose normalized concentrations are defined by the concentration at each time divided by the total dose (i.e. the concentration that would be achieved with a unit dose infused over 60 min). — 5 mg, --- 10 mg, – – – 15 mg.

the curves was consistent from volunteer to volunteer and was independent of the dose of drug administered. The absolute magnitude of the curves was proportional to the infusion rate, as is demonstrated when the concentrations are normalized to the infusion rate (Figures 1b and 2b). The peak plasma and whole blood concentrations at the end of the infusion were directly proportional to the dose (Tables 3 and 4). The AUC/dose ratio was not statistically different by ANOVA when increasing the dose.

Moment analysis Average values for clearance, mean residence time and apparent volume of distribution at steady state for plasma (Table 3) and whole blood (Table 4) were determined in each dose group. Although whole blood 8-MOP concentration decreased below the limit of detection earlier than plasma, the percentage of AUC and AUMC under the observed data was similar for whole blood and plasma. When compared with the plasma, the whole blood analysis showed a higher clearance (18 *vs* 12 ml kg^{-1} min^{-1}) and a shorter MRT (41.3 *vs* 52.6 min), resulting in a similar large V_{ss} ($\approx$600 ml kg^{-1}). Neither clearance, MRT, nor V_{ss} demonstrated a relationship to dose.

Compartmental analysis The three compartment model (Table 5) was preferred to the two compartment model (not shown) as demonstrated by a decrease of more than 100 in the NONMEM objective function ($-2LL$) for both the plasma and whole blood models.

The only covariate that significantly improved the pharmacokinetic model was weight, applied to the volumes and clearances in simple proportion. The addition of weight as a proportional scalar decreased the $-2LL$ by 77 for the plasma model, and by 10 for the blood model. When weight was applied to individual volume and clearance terms there was no significant improvement compared with the model with weight applied to all parameters. Thus, the 'weight scaled' model (i.e. weight covariate for all parameters) is the preferred model from this analysis.

The weighted residuals over time for each subject were calculated (Figure 3). The model accurately predicted the observations during the infusion period, but the accuracy decreased during post-infusion period, especially for the plasma concentrations. This result is supported by plotting the median predicted concentration, the best predicted and the worst predicted concentrations using the pharmacokinetic para-
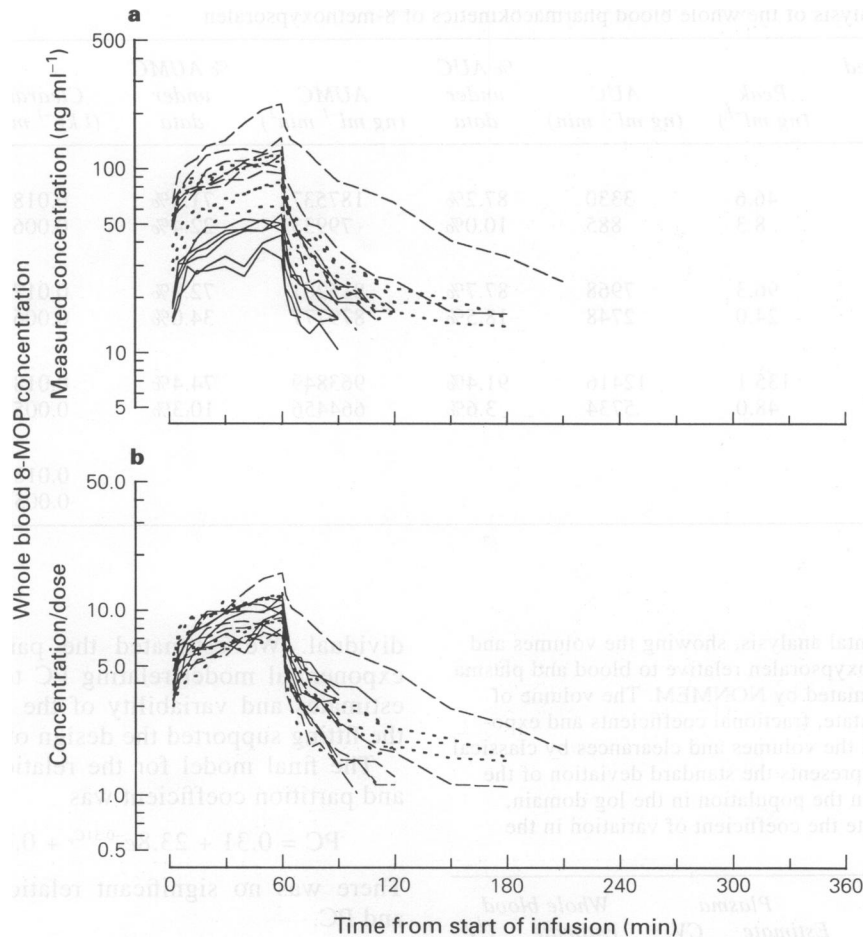
Figure 2 Individual time course of 8-MOP concentrations (a) and dose normalized concentrations (b) in whole blood.
— 5 mg, --- 10 mg, – – – 15 mg.

Table 3 Moment analysis of the plasma pharmacokinetics of 8-methoxypsoralen

	Measured dose (mg)	Peak (ng ml^{-1})	AUC (ng ml^{-1} min)	% AUC under data	AUMC (ng ml^{-1} min^2)	% AUMC under data	Clearance (l kg^{-1} min^{-1})	MRT (min)	V$_{ss}$ (l kg^{-1})
5 mg dose	(n = 6)								
Mean	4.6	60.2	4756	87.4%	402908	62.9%	0.012	50.4	0.52
s.d.	0.17	10.4	978	8.0%	238355	20.4%	0.0035	35.1	0.22
10 mg dose	(n = 6)								
Mean	9.5	138.7	11626	90.9%	1039448	67.4%	0.011	56.8	0.61
s.d.	0.26	33.3	3366	7.0%	441795	16.3%	0.0018	16.5	0.09
15 mg dose	(n = 6)								
Mean	14	195.8	16340	94.4%	1502020	74.8%	0.014	58.5	0.81
s.d.	0.67	89.2	8474	3.8%	894246	17.1%	0.0034	23.9	0.34
Average									
Mean							0.012	52.6	0.61
s.d.							0.0031	23.9	0.23

meters obtained from compartmental analysis (Figure 4). In plasma, the MAWR ranged from 8% to 13% to 42% for the best, median, and worst case predictions of the pharmacokinetic model. In whole blood the MAWR ranged from 6% to 19% to 82% for the best, median, and worst case predictions of the pharma-

cokinetic model. The average value of individual MAWR values was 17% for plasma and 18% for whole blood.

Partition coefficient calculation The partition coefficient analysis was done as follows: we first plot-

Table 4   Moment analysis of the whole blood pharmacokinetics of 8-methoxypsoralen

	Measured dose (mg)	*Peak (ng ml⁻¹)*	*AUC (ng ml⁻¹ min)*	*% AUC under data*	*AUMC (ng ml⁻¹ min²)*	*% AUMC under data*	*Clearance (l kg⁻¹ min⁻¹)*	*MRT (min)*	*V_{ss} (l kg⁻¹)*
5 mg dose (*n* = 6)									
Mean	4.6	46.6	3330	87.2%	187537	71.5%	0.018	24.1	0.38
s.d.	0.17	8.3	885	10.0%	79933	22.4%	0.0064	12.1	0.13
10 mg dose (*n* = 6)									
Mean	9.5	96.3	7968	87.7%	815201	72.9%	0.017	58.7	0.74
s.d.	0.26	24.0	2748	18.5%	879777	34.0%	0.0062	73.3	0.64
15 mg dose (*n* = 6)									
Mean	14	135.1	12416	91.4%	963849	74.4%	0.018	41.0	0.66
s.d.	0.67	48.0	5734	3.6%	664456	10.3%	0.0053	18.3	0.20
Average									
Mean							0.018	41.3	0.59
s.d.							0.0056	44.0	0.40

Table 5   Compartmental analysis, showing the volumes and clearances for 8-methoxypsoralen relative to blood and plasma concentrations, as estimated by NONMEM. The volume of distribution at steady state, fractional coefficients and exponents are derived from the volumes and clearances by classical formulae. The 'CV' represents the standard deviation of the estimated parameters in the population in the log domain, which only approximate the coefficient of variation in the standard domain

	Plasma		*Whole blood*	
	Estimate	*CV*	*Estimate*	*CV*
Estimated parameters				
Volumes (l kg⁻¹)				
Central	0.045	5%	0.061	7%
Rapid distribution	0.57	54%	1.15	69%
Slow distribution	0.15	34%	0.21	28%
Clearances (l kg⁻¹ min⁻¹)				
Systemic	0.010	25%	0.015	42%
Rapid distribution	0.0067	14%	0.011	3%
Slow distribution	0.012	40%	0.015	51%
Derived parameters				
Volumes (l kg⁻¹)				
Steady state	0.76		1.42	
Fractional coefficients				
A	0.94		0.95	
B	0.046		0.036	
C	0.014		0.011	
Hybrid rate constants (min⁻¹)				
α	0.66		0.70	
β	0.047		0.045	
γ	0.0070		0.0052	
Measures of goodness of fit				
Residual CV (σ)	8%		8%	
MDWR	−10%		+5%	
MDAWR	17%		18%	

ted partition coefficient (PC) *vs* time and PC *vs* C_P. The graph of PC *vs* time was nearly random and suggested no relationship. The graph of PC *vs* C_P was polyexponential in appearance. Figure 5 shows the partition coefficient over concentration in each in-

dividual. We estimated the parameters of a polyexponential model relating PC to concentration. The estimates and variability of the parameters given by the fitting supported the design of the model.

The final model for the relationship between time and partition coefficient was

$$PC = 0.31 + 23.8e^{-0.31C_P} + 0.60e^{-0.026C_P} \qquad (12)$$

There was no significant relationship between time and PC.

Discussion

This study characterized the arterial pharmacokinetics of 8-MOP administered intravenously to human volunteers. Arterial samples were used instead of venous samples because the concentration in arterial blood accurately reflects the time-course of systemic exposure during treatment, while the concentration in venous blood is affected by the uptake of drug into the tissues of the hand and the forearm. The study was designed from prior human studies with oral dosing, as described below.

Pretreatment, treatment and post-treatment

During treatment of psoriasis, oral doses of 0.4 to 0.7 mg kg⁻¹ of 8-MOP are commonly used, and such doses are given three times a week for several weeks. The peak plasma concentration has been observed approximately 1.5 h [3] to 3 h [4] after oral administration, depending on the formulation. For this reason UVA therapy is usually performed 2 h after 8-MOP administration. The peak plasma concentration achieved has been reported to range from 155 ng ml⁻¹ [3] to 325 ng ml⁻¹ [4]. In this study we administered a smaller dose of 8-MOP (0.06 to 0.2 mg kg⁻¹), but administered it intravenously with the intention of achieving a similar range of peak plasma concentration to that reported with psoriasis therapy. In this
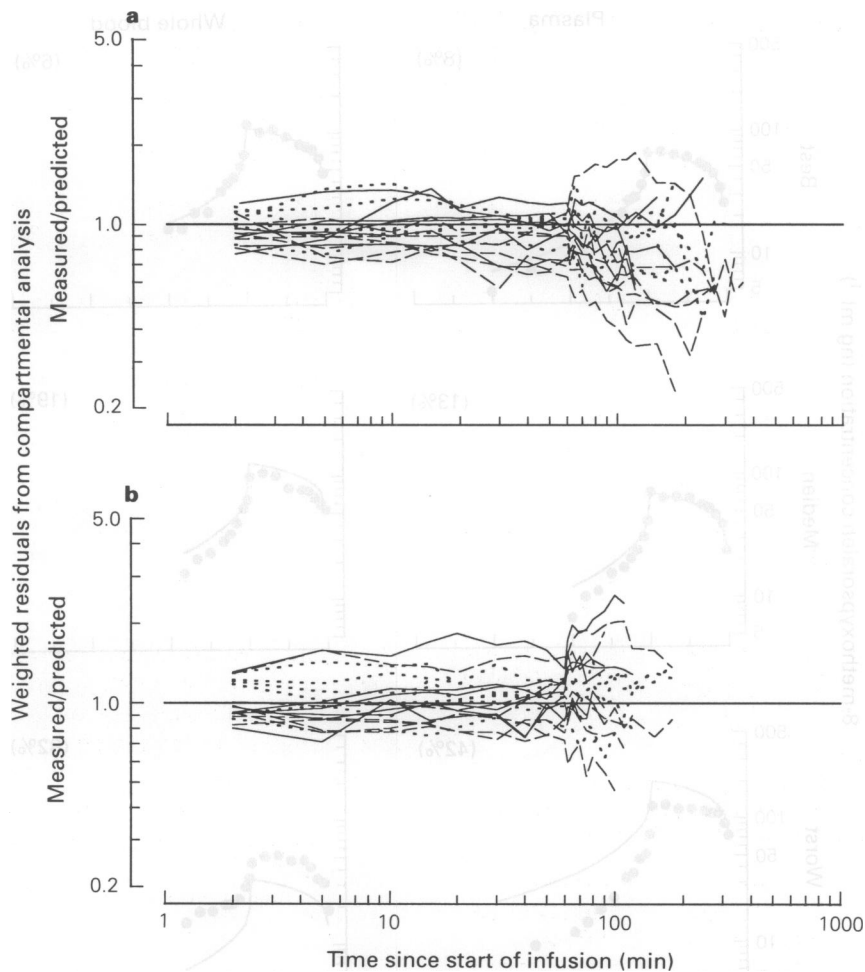
Figure 3   Individual weighted residual (WR) in plasma (a) and whole blood (b), for the three compartment model in which volumes and clearances were proportional to weight. When displayed in log scale, after centring the residuals about 1, WR is equivalent to C_M/C_P since

$$\log(\mathrm{WR}) = \log\left(\frac{C_M - C_P}{C_P} + 1\right) = \log\left(\frac{C_M}{C_P}\right)$$

Thus, a subject whose concentration were perfectly described by the model would be represented by a straight horizontal line at Y = 1.

study, the peak plasma concentrations in one subject reached 322 ng ml^{-1}. In all other subjects the peak plasma concentrations were less than 200 ng ml^{-1}.

None of our subjects experienced significant side effects or vital signs changes. None received UVA therapy, and all wore u.v. protective glasses. In PUVA therapy, some acute minor effects have been reported, depending on the 8-MOP formulation and dose administered: nausea (3% to 51%) [16–18], headache (2%) [16], dizziness (1.5%) [16], pruritus (14% to 71%) [16, 17]. A higher and earlier peak concentration has been observed when side effects were present (417 ng ml^{-1} vs 280 ng ml^{-1}) [19]. In primates receiving 2–18 mg kg^{-1} 8-MOP orally, only dose-dependent emesis, but no organ damage was observed [20]. Chronic surveillance of PUVA therapy has failed to identify any clinically relevant toxicity [16, 21]. Also, a prospective study over 5 years examining

ophthalmological findings in patients receiving PUVA indicated a small risk of ocular damage in patients who received at least 100 PUVA treatments [22]. When it occurred, ocular damage was thought to be associated with exposure to the combination of UVA light and 8-MOP.

Pharmacokinetic analysis

Linearity analysis   In the range of doses studied, we found the concentration *vs* time curve to be linearly correlated to the dose. Different findings have been described by two authors: Schmid after oral administration reported large differences in bioavailability and shape of the concentration *vs* time curve with dose and formulation [5]. Schmid explained this on the basis of a significant first pass effect. Giving higher dose (1–10 mg kg^{-1} i.v.) to dogs, Monbaliu
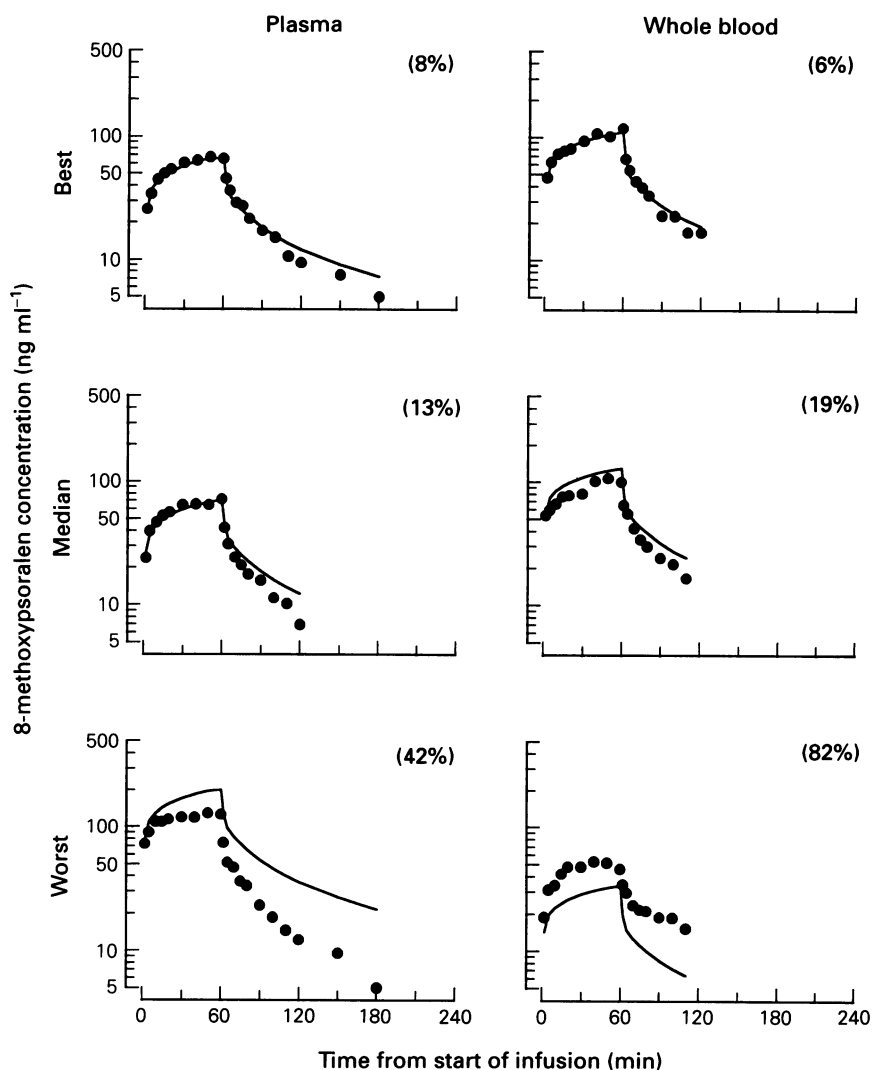
Figure 4 The best, median and worst performance of the three compartment population weight proportional model applied to the individual data set in plasma (left column) and in whole blood (right column). The dots represent measured 8-MOP concentrations, the solid line represents the concentrations predicted by the population models. % in brackets = MAWR.

described a decreasing clearance when increasing the dose, suggesting saturable metabolism [7]. Our data here suggest that the doses administered were too small to saturate the metabolic pathway. Although we conclude that the pharmacokinetics of 8-MOP are linear, this may be not true at higher doses.

Moment analysis The moment analysis is often considered as a reference method, its main advantage being that its only assumptions are simply linearity and elimination of all drug from the central compartment [23]. It estimates only the fundamental parameters clearance, volume of distribution at steady state, and mean residence time, and does not provide predicted concentrations over time. The inability of moment analysis to predict concentrations limits its application as a modelling tool. Additionally, it is highly dependent on the accuracy of the extrapolated portion of the curve (as is compartmental modelling, of course). The extrapolated portion of the curve will affect the AUMC more than the AUC. As clearance is a function of AUC, while V_{ss} depends on both AUC

and AUMC, it would be expected that the clearance estimated by moment analysis would agree with the clearance estimated by compartmental analysis, while the V_{ss} might differ more between the two techniques because of intrinsic differences in how the terminal slope, and thus the areas under the moment curves, is calculated.

In this analysis the plasma clearance of 8-MOP was estimated to be 0.012 l kg^{-1} min^{-1} by moment analysis, which is in very reasonable agreement with the clearance of 0.010 l kg^{-1} min^{-1} estimated by compartmental analysis. The whole blood clearance of 8-MOP was estimated to be 0.018 l kg^{-1} min^{-1} by moment analysis, and 0.015 l kg^{-1} min^{-1} by compartment analysis, again showing excellent agreement. By contrast, using moment analysis the V_{ss} was 0.61 and 0.59 l kg^{-1} in plasma and whole blood respectively, while using compartmental analysis the V_{ss} was 0.76 and 1.42 l kg^{-1} in plasma and blood respectively. This likely reflects the fact that only 60–75% of the AUMC calculation was derived directly from the observed data, while the remaining 25–40% was
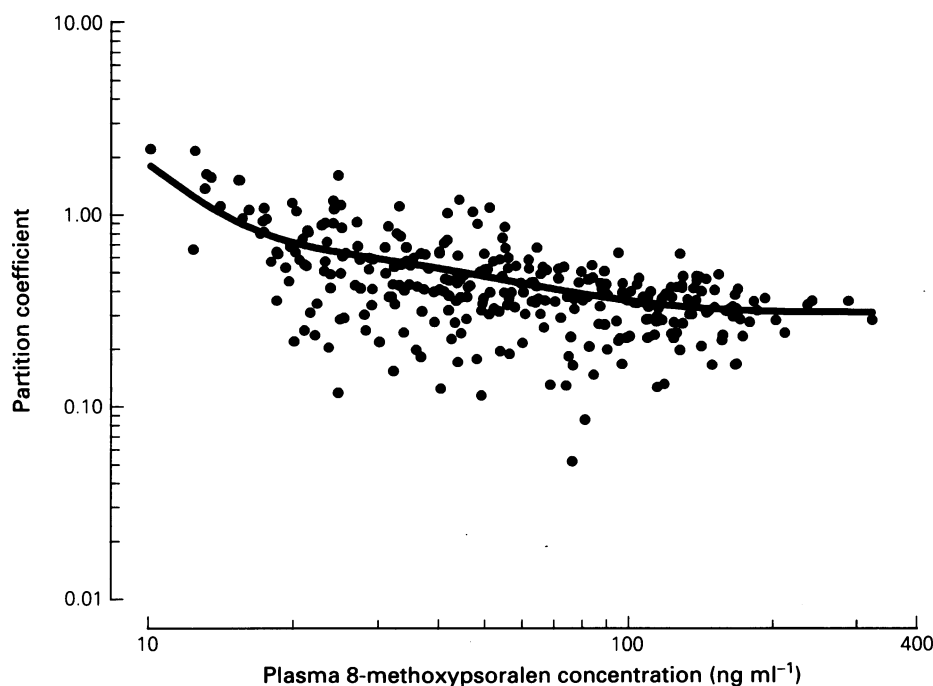
Figure 5 Individual values of the red blood cell/plasma partition coefficient *vs* plasma 8-methoxypsoralen concentration (C_p), and the line described by the regression equation relating them: PC = 0.31 + 23.8$e^{-0.31C_p}$ + 0.06$e^{-0.026C_p}$. — regression line.

entirely extrapolated. This difference in V_{ss} between the two approaches does not indicate that one approach was wrong, and the other was correct, but simply that the data were not sampled long enough to give confidence in the estimates of the V_{ss}. However, for the purpose of estimating the peak concentration and the total exposure (e.g. AUC) to 8-MOP following intravenous administration, the agreement of the moment and compartmental calculations of clearance indicates that the study design was entirely adequate.

Compartmental analysis Several approaches to compartmental analysis have been described [24] and recently compared for multicompartmental models [25]. We chose a mixed-effects population pharmacokinetic analysis as the best available method to assess the interindividual variability of the parameters. The three compartment model was preferable to the two compartment model. Inclusion of the weight as a covariate provided a significant improvement in the log likelihood when compared with a non-proportional model. Models in which the volumes and clearances were proportional to body surface area or lean body mass resulted in similar, but not better, log likelihood values to the weight-proportional model. The other covariates did not show further improvement in the log likelihood beyond that obtained with the weight-proportional model.

As it can be visually estimated on the plot of WR (Figure 3)[C], the pharmacokinetic parameters estimated

[C]Personal communication: Young L, *et al.* The influence of model misspecification on pharmacokinetic parameter estimation. Manuscript in preparation.

by NONMEM accurately described the observed data. A moderate bias appeared for the last post-infusion samples in plasma (i.e. the model slightly overestimated the late concentrations). This was not observed for whole blood partly because the limit of detection was achieved earlier.

Only plasma pharmacokinetic data are available from the literature. Herfst and colleagues [3] used a two compartment model for pharmacokinetic analysis in patients receiving oral 8-MOP for treatment of psoriasis. They found higher values for both volume of distribution at steady state (3.2 l kg^{-1} *vs* 0.76 l kg^{-1} in our study) and elimination clearance (154–219 l h^{-1}, compared with 48 l h^{-1}). These differences with our results are probably due to the oral route of administration and the assumption by Herfst and colleagues that 8-MOP is completely bioavailable when taken orally. When a drug is administered orally, clearance must be calculated as:

$$CL = \frac{Dose \times F}{AUC} \tag{1'}$$

where F is the fraction bioavailable. What Herfst and colleagues have therefore calculated is not clearance, but CL/F. If we assume that the true metabolic clearance is 48 l h^{-1}, as calculated in our study, and that CL/F as estimated by Herfst *et al.* was 186.5 l h^{-1} (the average of their range of values), then we can infer by combining these results that F, the fraction bioavailable, is approximately 26%. However, Herfst *et al.* studied chronically treated patients, and it may be that chronic treatment increases clearance [26]. Thus, the true F may be somewhat higher than suggested by this calculation.

Mays and colleagues gave variable doses of 8-MOP i.v. to rats and found a similar V_{ss} (0.84 l kg^{-1}) to what we report [27]. Their estimates of clearance decreased with increasing doses of 8-MOP, and for high doses they report similar clearance to what we report herein. In dogs given intravenous 8-MOP Monbaliu and colleagues found a similar clearance to what we report herein, but a higher volume of distribution [7].

Partition coefficient analysis   Pibouin and colleagues directly measured the partitioning of 8-MOP between red blood cells and serum [28]. They found a partition coefficient of approximately 20% (RBC:serum ratio). They also reported that 8-MOP binding in serum was high (91.4%) and was constant within the therapeutic range, suggesting concentration-independent pharmacokinetics for conventional doses. Our results are consistent with theirs. We found a greater partitioning within red blood cells (31% intercept at $C_p = 0$). The difference may be because the red blood cell concentration was calculated from whole blood and plasma concentration in our study, and not directly measured. Additionally, we found a concentration dependence of the partition coefficient that was not reported by Pibouin and colleagues. Pibouin and colleagues did observe that the concentration in red blood cells was three-fold higher than would have been predicted by passive diffusion alone, suggesting active transport into the cells. Our results showing concentration dependence suggest that this transport mechanism is saturable. Additionally, the presence of two different rate constants suggests that there may be more than one site of 8-MOP transport. Of critical significance to our pharmacokinetic modelling, however, is that dependence of the partition coefficient on

plasma concentration would not be expected to alter significantly the linearity of the pharmacokinetics within the observed range of 8-MOP concentrations.

In that the purpose of this study is to assess the likely mean and range of 8-MOP concentrations resulting from an intravenous administration of 8-MOP to patients receiving a platelet transfusion, we performed a final analysis of our data. For the 18 subjects studied, we normalized their plasma concentrations to a dose of 1.0 mg administered over 60 min (Figure 1, lower graph), and then multiplied the resulting concentrations by 7.5 mg to reflect their likely concentrations had they received a dose of 7.5 mg in the study. We then calculated, in the log domain (reflecting the log-normal distribution of plasma concentrations) the mean and 90% confidence bounds. The 90% confidence bounds are based on the standard deviation times 1.71, the *t* statistic for 10% probability with 17 degrees of freedom. The mean and upper and lower confidence bounds were transformed back to the standard domain for graphic representation (Figure 6). This analysis suggests that with a dose of 7.5 mg infused over 1 h the concentration of 8-MOP in the plasma will be considerably less than that achieved with oral doses during routine PUVA therapy. Thus, the long history of safety associated with oral 8-MOP is likely to be clinically applicable to 7.5 mg of intravenously administered 8-MOP.

To conclude, at doses that effectively inactivate bacteria in platelet concentrates, 8-MOP plasma concentrations were similar to those routinely observed when 8-MOP is administered orally in PUVA therapy. We observed no acute toxicity after intravenous doses of 5 to 15 mg administered over 60 min. The concentration was linearly related to the dose in the range of
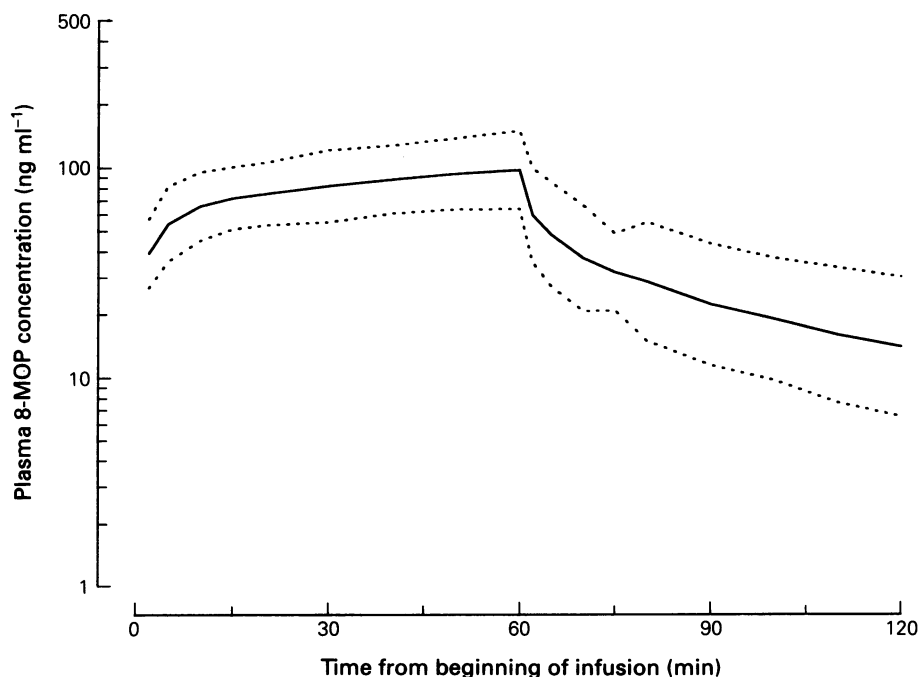

Figure 6   Predicted 8-MOP concentration and 90% confidence bounds in plasma after a 7.5 mg i.v. infusion of 8-MOP given over 60 min. —log mean concentration, ···· 90% confidence bounds.

doses studied. The pharmacokinetics of 8-MOP were characterized by a rapid decay of both plasma and whole blood concentration after the end of infusion, a large volume of distribution, and rapid elimination. Using a population pharmacokinetic analysis, we found that a three compartment weight-proportional model accurately described the observed data. These results were consistent with the non-parametric moment analysis and also with the data from the literature for plasma 8-MOP pharmacokinetics. We anticipate that 7.5 mg 8-MOP, administered over 60 min, would not be associated with excessive plasma concentrations or systemic exposure. By extrapolation from our own results and the extensive safety history of 8-MOP in PUVA studies, we anticipate that 7.5 mg 8-MOP administered intravenously over 60 min would be associated with neither acute nor chronic adverse effects.

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