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Correlation of 8-MOP plasma levels and skin response to UV-A irradiation in PUVA patients

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Abstract

Plasma levels of 8-methoxypsoralen (8-MOP) and photosensitivity to long wave ultraviolet irradiation (UV-A) have been simultaneously measured over a time scale from 1-8 hours in 20 non-fasting volunteers undergoing photochemotherapy for psoriasis with 8-MOP and UV-A (PUVA). Five 4 cm^2 patches of skin on the buttocks were irradiated (365 nm, 2-28 joules) with fluorescent lamps (Sylvania FR72T12) at each of four times from 2-6 hours after ingestion of the 8-MOP. The skin reaction was measured as the minimal phototoxic dose (MPD) for each of the four exposures at 48 hours after irradiation. Four patients' 8-MOP plasma profiles were studied under fasting conditions. 8-MOP was extracted from plasma by a benzene extraction method. 8-MOP plasma levels were determined by UV absorbance after seperation by high pressure liquid chromatography (HPLC) in a silica particle column eluted with methylene chloride:acetonitrile (95::5).

Two volunteers displayed unusual biphasic time course patterns under the non-fasting conditions. Monophasic patterns were observed for both patients under fasting conditions. Two volunteers under fasting conditions demonstrated reproducible time course patterns on 4 occasions each. All subjects received approximately the same 8-MOP dose (mg/kg), but absorption patterns and peak plasma levels of the drug varied widely from person to person. There was no correlation between dose and peak plasma

level. Elimination rates obeyed first order kinetics and were consistent in the population for fasting and non-fasting subjects. For each subject there was a correlation between 8-MOP plasma level and MPD such that an inverse proportionality existed. In our population of 20 volunteers no erythema was observed in four patients whose peak 8-MOP plasma levels were below 30 ng/ml. In ten patients maximum sensitivity to irradiation occurred simultaneously with development of peak levels of 8-MOP observed with time. On the average there was a 0 to 1 hour delay between peak 8-MOP plasma concentration and maximum sensitivity to irradiation. Knowledge of a patient's 8-MOP plasma profile over time is a valuable tool for evaluating patients who respond poorly to PUVA therapy.

Introduction

Treatment of psoriasis with psoralens (furocoumarins) and long wave ultraviolet light (PUVA) has been a well recognized and effective therapeutic modality (1,2,3,4,5,6). The first reported medical use of furocoumarins was documented in the ancient Indian scriptures, the Atharva Veda (circa 1400 B.C.). Extracts from Psoralea corylifolia applied topically in conjunction with exposure to sunlight were used in the treatment of vitiligo (7). This regimen was found to induce repigmentation of the white, pigmentless patches of skin which characterize the disease. Egyptian medical lore describes a similar treatment. The Arab physician Ibn El-Bitar documented the use of an extract from the plant Ammi majus in the treatment of vitiligo in the thirteenth century A.D. (8).

In 1948, A.M. El Mofty (9) reported the clinical use of pure extracts from Ammi majus in the treatment of patients with vitiligo. These extracts were isolated and described by Fahmy and Abu-Shady (10) as the furocoumarins psoralen, the parent molecule, and its derivatives 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP) and 8-isoamyleneoxypsoralen(Ammidin) (Fig.1). Over 28 naturally occurring furocoumarins from five major plant families have been isolated (7). The synthesis of new derivatives of psoralen generally involves methylation of the parent molecule. 4,5',8-trimethylpsoralen (TMP) is one such compound.

In 1953 Lerner, et. al. (11) described the use of 20-50 mg.

of oral 8-MOP and GE sunlamp therapy in the treatment of nine patients with vitiligo with overall good results. This regimen was noted to induce erythema 48-72 hours after administration with induction of pigmentation after repeated treatments. Long wave ultraviolet light (UVA) alone will not produce erythema except at very high intensity (12). Stegmaier (13) employed 20 mg. of oral 8-MOP followed by exposure to sunlight in 25 fair skinned people for induction of tanning and protection against sunburn. Both authors suggested the use of this therapeutic modality on an experimental basis in the treatment of "those disorders in which ultraviolet light acts therapeutically, such as psoriasis."(11)

Furocoumarins have been found to exert their skin photosensitizing properties in a unique manner. The erythema produced by most photosensitizing dyes appears immediately after irradiation while the erythematous response with psoralens occurs 48-72 hours after irradiation. Most photodynamic drugs operate through a singlet oxygen mechanism. They absorb radiant energy and transfer it to a metastable excited state of oxygen. This is a potent oxidizing agent which acts to inactivate and disrupt normal metabolic processes. In 1957 Musajo, et.al.(14) found that furocoumarins possessed no photooxidative activity in several chemical and biologic systems. Oginsky, et.al.(15) showed that the presence of oxygen actually decreased the lethal effectiveness of 8-MOP and UV irradiation in bacterial cultures.

DNA has been shown to be the primary molecular target of psoralen photoreactivity. In 1965 Musajo, et. al.(16) noted a

strong modification of the fluorescence spectra of native DNA irradiated in the presence of psoralen. They suggested that psoralens formed a covalent linkage with DNA. Furocoumarins have been shown to intercalate between base pairs in the DNA double helix and form covalent crosslinks with pyrimidine bases (17,18). A C4 cyclobutane ring is formed between furocoumarins either at the 3,4 pyrone double bond (Fig. 2A) or with the 4'5' furan double bond (Fig 2B) and the 5,6 double bond of the pyrimidine bases. The occurrence of these DNA crosslinks have been verified in vitro by electron microscopy visualization in chromatin (19) and in cells from PUVA treated psoriatic patients (20).

The combination of psoralens and UVA irradiation has been shown to inhibit nucleic acid synthesis in vitro (21,22,23) and to inhibit macromolecular synthesis in vivo (24). The relative photoreactivity of a broad range of furocoumarins with nucleic acids was shown to correlate with the relative ability to inhibit nucleic acid synthesis in a DNA virus (25). Relative furocoumarin photoreactivity correlated with relative photosensitization in guinea pig skin (26). The reaction of psoralens with DNA explains many of the biological effects of the light active furocoumarins.

Psoriasis has been characterized as a disease in which abnormal epidermal cell proliferation occurs (27). Numerous cytotoxic, chemotherapeutic agents have been employed in the treatment of severe psoriasis. Nitrogen mustard (28) and 5-fluorouracil (29) have been used topically. Methotrexate (30) hydroxyurea (31) and azaribine(32) have been used systemically.

Methotrexate is known to be particularly effective in inhibiting epidermal cell proliferation. As a folate antagonist it kills cells during the S phase of the cell cycle, during the peak of DNA replication. It is much more effective when cells are in the logarithmic phase of growth (33). Methotrexate has been shown to inhibit DNA synthesis in psoriasis (34).

Because of its ability to inhibit DNA synthesis, the combination of psoralens and UVA irradiation were employed in the treatment of psoriasis. Initial trials with topical psoralens and UVA irradiation by Tronner and Schule (35), Walter and Voorhees (36) and Weber (37) demonstrated that this method could improve psoriatic lesions. Parrish, et. al. (1) and Wolff, et. al. (2) demonstrated that oral methoxsalen in combination with UVA irradiation was effective in complete clearing of severe psoriasis. The term "photochemotherapy" was coined to describe the crucial interaction of light and drug which resulted in the beneficial cytotoxic effects of this regimen. Multicenter clinical cooperative studies in the U.S. (4, 5) and in Europe (6) have shown conclusively the effectiveness of PUVA photochemotherapy.

The standard PUVA protocol (38) calls for irradiation two hours after oral ingestion of the drug. Oral doses range from .5 to .7 mg/kg body mass. Special fluorescent lamp systems which emit in the UVA range (320 - 400 nm) are designed for whole body, uniform irradiation. Initial irradiation doses are determined by skin type (4) or by minimal phototoxic dose (MPD) (38). Six skin types (types I - VI) are defined (Table I).

These are based on tanning histories and assessment of skin pigmentation. The MPD is determined by phototesting. The phototoxic reactions of PUVA include erythema, edema and blistering as an extreme reaction. These reactions peak at 48 to 96 hours after exposure; the most severe reactions peak later. The MPD represents the individual's minimally detectable erythematous response to irradiation at sensitive sites. It is expressed in joules/cm^2. Phototesting is performed by irradiating a series of small patches of skin with gradual increments of known amounts of UVA irradiation (Fig. 3). The patches are read at 48-72 hours. The initial UVA dose is the patient's MPD. The clearing phase requires from two to four treatments per week. Tolerance to irradiation develops with the increased pigmentation which occurs during therapy. Gradual increments (0.5 to 2.0 J/cm^2) in UVA exposure are made in the course of therapy. Relapses frequently occur within weeks to months following initial clearing. A maintenance phase of 1 to 4 treatments per month is often employed.

In three major clinical cooperative studies (4,5,6) PUVA has resulted in complete clearing in 70-90% of psoriatic patients. Most patients cleared in 20 to 30 treatments. Some patients showed only moderate improvement and/or required a significantly greater number of treatments. Three to ten percent failed to clear altogether. Some patients exhibited flares during therapy (Kobner reaction). Patients with plaque or guttate psoriasis responded better than those with erythrodermic psoriasis. From 40 to 50% of patients had relapses

within one year. The efficacy of maintanance therapy in the prevention of recurrences has been questioned (5,6).

Side effects from PUVA therapy included erythema, nausea, pruritus, headache, dizziness and the Kobner reaction. Irreversible lenticular binding of 8-MOP following UVA exposure has been demonstrated in vitro. This could be a hypothetical factor in cataract development(39). Case reports of systemic lupus erythematosus (40, 41), discoid lupus (42), bullous pemphigoid (43), epidermal dystrophy (44) and multiple basal cell epitheliomas over non-sun-exposed areas (45) have been associated with PUVA therapy. An increased risk of basal cell and squamous cell carcinoma of the skin has been documented in the U.S. cooperative clinical trial (46). This risk was associated with a history of previous exposure to ionizing radiation and previous cutaneous carcinoma. Many of the patients undergoing photochemotherapy have had other forms of therapy which leave them at high risk for developing cutaneous neoplasms. It was not clear from this initial report what role if any PUVA therapy may play as an initiator or promotor of carcinogenesis in humans. It has been suggested that psoralens plus ultraviolet irradiation may act as promotors by inhibiting the immunologic surveillence for neoplastic cells which has been hypothesized to occur in skin (47). PUVA has been shown to cause abnormal cell mediated immunity reactions in the skin (48) and to affect circulating lymphocytes (49, 50, 51).

Minimal psoralen intake and radiation load without compromising therapeutic benefit would be desirable in preventing any

possible long term side-effects from PUVA therapy. Knowledge of the pharmacokinetics of 8-MOP and of the relation of skin response to 8-MOP plasma concentration would be important in achieving maximal therapeutic effect with minimal quantities of drug and UVA exposure. Such knowledge would also be useful in evaluating the wide variety of therapeutic responses to photochemotherapy.

Numerous methods have been developed for determination of 8-MOP plasma or serum concentration. All of these involve extraction of the drug in an organic phase. Separation and resolution of the extract and detection of 8-MOP has been performed using a variety of systems. Thin layer chromatography (TLC) and scanning fluorometry (52, 53, 54); gas-liquid chromatography (GLC) and flame ionization detection (55) or electron capture detection (56, 57); and high pressure liquid chromatography (HPLC) and UV absorption spectrometry (58, 59, 60, 61, 62) have been employed. One extremely sensitive and complex system involves HPLC followed by glass capillary GLC and mass spectrometry detection (63). Many of these methods are unsuitable for rapid and repeated determinations of 8-MOP plasma profiles because they are time consuming, expensive, require excessive sample size or lack sensitivity. A rapid and inexpensive HPLC system which is able to measure 8-MOP plasma concentrations of 10 ng/ml from one ml. of plasma has been developed at the Yale Department of Dermatology by Ljunggren, Carter and Albert (64). This technique has been further refined with the use of an Altex model 330 HPLC to achieve a sensitivity of 4 ng/ml.

In this study minimal phototoxic doses (MPD's) and 8-MOP plasma concentrations have been simultaneously measured at several times during an 8 hour period following drug ingestion in 20 psoriatic patients. The sensitivity of the skin to UVA irradiation in the presence of 8-MOP may provide a relative estimation of 8-MOP levels in the skin over a given time course. The 8-MOP absorption patterns have been analyzed for correlation with skin sensitivity to irradiation. The pharmacokinetic behaviour of 8-MOP has been evaluated. Four patients have been studied under fasting and non-fasting conditions to determine the effect of food on 8-MOP absortion patterns.

Materials and Methods

Patients: Twenty-two volunteers with severe psoriasis currently undergoing PUVA therapy were studied after informed consent had been obtained. The experimental protocol had prior approval of the Human Investigation Committee, Yale University School of Medicine. (HIC no. 946) The patients ranged in age from 26 to 72. Ten patients were males, twelve were female. Twenty patients were studied for the correlation between skin photosensitivity and 8-MOP blood levels. Eleven volunteers were of skin type III, seven of skin type II and two of skin type I (Table I). All patients were advised to follow their normal diet preceding a PUVA therapy session. Most patients ingested a small amount of bland food with their 8-MOP capsules to offset the nausea-producing effect of the drug. Four patients fasted for 10 hours preceding ingestion of the drug. All patients ingested 8-MOP (Oxsoralen, Paul B. Elder Co., Bryan, Ohio) in 10 mg capsules. Dosage levels ranged from .40 to .82 mg of 8-MOP/kg body mass with an average dose of .62 mg/kg. One exception is a patient with an extreme dose at 1.77 mg/kg. This patient mistakenly ingested a second 60 mg dose two hours after ingesting the first dose.

Irradiation: Twenty volunteers were irradiated beginning 2,3,4 and 6 hours after ingestion of the 8-MOP. At each irradiation five patches each 4 cm^2 of plaque free skin on the buttocks,

thighs or lower back were exposed to progresseve increments of UVA irradiation (Fig. 3). The irradiation doses varied from 2 to 28 joules/cm^2 in the population. The dosage for each patient was chosen on the basis of skin type and current treatment levels so as to assure erythema in at least the highest energy patch. The dose increments progressed semi-logarithmically. For example, one typical patient who had type III skin and was receiving 12 J/cm^2 for psoriasis treatment was phototested with 7, 10, 14, 19 and 25 J/cm^2. All other area of the body were shielded from irradiation. We used a bank of 8 fluorescent lamps (Sylvania FR72T12). The output of the lamp was constant during each session as monitored at 5 minute intervals with the International Light model IL441 UVA detector (reading at 365 nm). Output decreased slightly from 13 to 10 milliwatts/$cm^2$with aging of the lamps. Duration of irradiation and distance from the lamps were adjusted to produce the desired energy exposure. Erythematous response was judged at 48 hours after exposure.

Materials: Benzene employed for the extraction was analytical reagent grade (Fisher Scientific, Fairlawn, N.J.). The methylene chloride and acetonitrile were HPLC grade (Burdick & Jackson, Muskegon, Mich.). For the borate buffer 61.8 gm boric acid (H_3BO_3) and 74.6 gm potassium chloride (KCl) were mixed in 1 L distilled water (solution #1); and 106 gm of sodium carbonate (Na_2CO_3) was dissolved in 1 L distilled water (solution #2). One liter of the 1M borate buffer was made by combining 630 ml of the H_3BO_3 - KCl solution #1 with 370 ml of the Na_2CO_3 solution #2. The mixture was buffered up to pH 9.0 with the Na_2CO_3 solution #2.

Standards: The 8-MOP used as external standard in the HPLC analysis was obtained from Sigma Chemical Co., St. Louis, Missouri and was recrystallized twice from methanol. Ammidin, a psoralen derivative (Fig. 1), was employed as internal standard and external standard in each analysis and was a gift provided by AB Draco, Lund, Sweden. Standard solutions were made from 1mg 8-MOP or ammidin/10 ml methylene chloride stock solutions. Methylene chloride: acetonitrile (95::5) was added to 100 ul of the stock solution in a 10 ml volumetric flask up to volume. All standard solutions were 1 ng/ul. Standard solutions were made fresh every 2 to 3 days and stock solutions were replaced every month owing to the volatility of the solvents. All solutions were stored refrigerated.

Glassware: All glassware was cleansed with Alconox detergent (Scientific Products, McGaw Park, Illinois) and rinsed thoroughly with tap water followed by two rinses with distilled water. Two 100% ethanol rinses and one methylene chloride (scientific grade) rinse guaranteed the removal of all organic residues. The extraction was performed in 13 ml conical glass centrifuge tubes with ground glass stoppered tops (Kimble Kimax 6945-B). Teflon stoppers were used for a tight seal during extraction. These tubes were treated with polyethylene glycol (PEG). This was found to enhance 8-MOP recovery (64). The tubes were filled with a 2# PEG/chloroform solution (2 gm PEG 6000/100 ml chloroform) for 5 minutes and the solution was poured back for repeated use. The tubes were then baked in an oven (150° - 200°C) for two hours.

Extraction: Blood samples were obtained at 1, 2, 3, 4, 6 and 8 hours after ingestion of the 8-MOP. All blood samples were obtained by venipuncture in heparinized tubes and the plasma was seperated immediately. The extraction procedure was modified in this laboratory by Ljunggren, et. al. (64) from a technique originally described by Puglisi, et. al. (58). To 1 ml samples of plasma were added: 200 ng of ammidin as internal standard and 2.5 ml of the 1 M borate buffer. Samples were then extracted with 8 ml of benzene at room temperature on a reciprocating shaker for 20 minutes at 60 cycles per minute. The samples were then centrifuged at 5°C for 15 minutes on a Sorvall General Lab Centrifuge (model GLC 2B) at 2000 RPM. The organic layer was removed by glass pipette and evaporated at 45°C under dry nitrogen. Approximately 95% of the benzene was removed after cold room centrifugation. Drying time was 30 minutes. The residue was placed under vacuum for an additional 30 minutes. The residue was redissolved for chromatographic analysis in 200 ul of 95::5 minture of methylene chloride: acetonitrile.

Chromatographic procedure: The high pressure liquid chromatograph (Altex, model 330, Berkeley, California) was equipped with an Altex 254 nm UV detector and a Laboratory Control Data recorder. The stationary phase in the system was a microparticulate silica gel column (Partisil 10, I.D. 4.6 mm, length 250 mm, Whatman Inc, Clifton, New Jersey). The mobile phase was the 95::5 mixture of methylene chloride: acetonitrile. The flow rate was 2.2.ml/min. Best results were

obtained with pressures below 2000 psi. A 20 ul sample of the redissolved sample was injected onto the column.

Calculations: Calculations of 8-MOP plasma concentrations were based on the relative peak heights from the chromatogram: of the plasma 8-MOP and the ammidin internal standard, and of 20 ng of 8-MOP and ammidin as external standard. The external standard peak heights were determined daily. Calculations are based on the formula:

$$\text{8-MOP } \left(\frac{ng}{ml}\right) = \frac{\text{External Std. Ammidin}}{\text{Internal Std. Ammidin}} \times \frac{\text{Patient 8-MOP}}{\text{External Std. 8-MOP}} \times 200 \left(\frac{ng}{ml}\right)$$

plasma conc. | peak heights | peak heights | internal std. conc.

The internal standard accounted for any loss of the patients' plasma 8-MOP during the extraction procedure. The validity of this approach was tested with known amounts of 8-MOP and ammidin that were added to control plasma samples (64).

Elimination half lives of 8-MOP time courses were calculated by the method of least squares linear regression analysis using a Hewlett-Packard computer (model 9810A) and plotter (model (9862A).

Results

Plasma levels of 8-MOP as low as 4 ng/ml were measured using 1 ml of plasma. The extraction and chromatography could be performed on 10 to 20 samples in 2 to 3 hours. Retention time was approximately 2'45" for ammidin and 3'45" for 8-MOP (Fig. 4). A broad band of plasma impurities had an elution time of 1 to 2 minutes. The 8-MOP peak height was generally 75% ± 10% the peak height of ammidin for equal concentrations. This ratio was constant over a wide range of controlled plasma concentrations as shown in the standard curve (Fig. 5).

A total of 31 8-MOP time courses were obtained in 22 volunteers (Tables II & III). There was marked variability in absorptive patterns. Peak concentrations ranged from 13 ng/ml to 782 ng/ml. Time of peak concentration varied from 1 to 6 hours after ingestion. There was no correlation between oral dosage of 8-MOP as adjusted for body mass and peak levels of the drug (Table IV, Fig. 6).

Elimination rates obeyed first order kinetics. All half-lives (Table IV) were calculated from either 3, 4 or 5 data points from each patients time course. Several patients had inadequate correlation coefficients ($P > 0.10$) or failed the 95% confidence limit ($z = 1.96$) for a normal population. In the non-fasting population the 13 valid half lives ranged from 1.3 to 2.8 hours with an average of 2.1 ± 0.5 hours. In fasting

subjects half lives ranged from 1.3 to 2.3 hours with an average of 1.7 $\pm$ 0.3 hours.

Twenty one patients were studied under non-fasting conditions. Two patients demonstrated unusual biphasic absorption patterns (Pts. 8 & 13, Fig. 7). When these two patients were studied under fasting conditions, they both exhibited the typical monophasic time course with greater peak levels of the drug. Three additional non-fasting patients had less pronounced biphasic patterns (Pts. 5, 18 & 19). Two patients were studied under fasting conditions on four successive occassions (Pts. 15 & 22). Both patients demonstrated consistent 8-MOP patterns (Fig. 8). There was one hour variation in time of peak levels, 0.2 hour variation in half life and a 135-206 ng/ml and a 108-217 ng/ml variation in peak plasma levels respectively.

Twenty patients were irradiated to determine minimum phototoxic dose (MPD) while 8-MOP plasma concentrations were monitored (Table II). Four patients (Pts. 1-4) with peak 8-MOP concentrations below 30 ng/ml demonstrated no erythema at any of the energy levels irradiated (Group I). The remaining sixteen patients with peak 8-MOP concentrations between 48 and 782 ng/ml developed erythema. The lowest MPD for each patient (LMPD) was the time of greatest skin sensitivity to irradiation. Ten patients demonstrated simultaneous peak 8-MOP plasma concentration and an LMPD (Group II). Seven of these patients maintained maximum skin sensitivity one hour after peak levels had fallen off. Three patients with rapid 8-MOP absorption

demonstrated 1 to 3 hour delays in maximum skin sensitivity (Group III). Maximum skin sensitivity preceded peak 8-MOP levels in three patients with slow 8-MOP absorption (Group IV). The data from thirteen patients with complete profiles at all times of irradiation was summarized (Table V). The lowest average MPD and the greatest number of LMPD's for these patients occurred at 3 hours after ingestion. The greatest average 8-MOP plasma concentration occurred at 2 hours after ingestion.

There was no correlation between peak 8-MOP levels and LMPD's for the population as a whole. A general trend in all the time courses was the inverse relation between 8-MOP plasma concentrations and MPD's. The cumulative data from the 13 patients (Table V) demonstrated that the 8-MOP level falls as the MPD rises. With adjustment for delays in distribution the converse also held. This relation was best described as an inverse proportionality between the $\log_e(\text{MPD})$ and the $\log_e(\text{8-MOP})_{\text{plasma}}$. The product of these two parameters yielded a constant K_t at time t after ingestion and an average value K_T over all times:

$$\log_e \text{MPD} \approx \frac{1}{\log_e(\text{8-MOP})_{\text{plasma}}}$$

$$K_t = \log_e \text{MPD}_t \times \log_e(\text{8-MOP})_t$$

Each patients plasma profiles were evaluated on an individual basis (Table VI). In instances where peak 8-MOP concentration did not correspond with an LMPD time curves were shifted so as to account for variations in distribution time.

Discussion

This HPLC analysis of 8-MOP plasma concentration has been previously discussed by Ljunggren, et. al. (64). A simple one step extraction of the 8-MOP with benzene was sufficient for excellent recovery. Multiple organic phase extractions (54, 55), acidification for release from plasma proteins (54, 57) and complex clean up procedures (56) were not necessary. The thin layer chromatography systems with fluorodensitometric determination could be sensitive to within 5ng/ml with as little as 1 ml of serum (53), but all such systems (52, 53, 54) lacked specificity for detection of 8-MOP alone. The gas chromatography systems required purification steps (55, 56) and all had detection limits of 10 ng/ml (55, 56, 57). The sensitivity of this HPLC system was comparable to others (59, 60, 61, 62). Multiple venipunctures in one patient were possible as only 1 ml of plasma was required for each determination.

Treatment of glassware with polyethylene glycol was found to be necessary to prevent possible binding of 8-MOP to glassware. Ammidin was added as an internal standard before the extraction. It accounted for any variations in extraction efficiency between samples. Ammidin is an isoamylene derivative of 8-oxypsoralen (Fig. 1) with UV absorption characteristics similar to 8-MOP (Fig. 9), but it has a more rapid elution time on this HPLC system. It was ideally suited as an internal standard. Plasma

extraction of 8-MOP has been shown to be slightly more efficient than serum extraction using this technique (64).

There was great variability in 8-MOP plasma profiles, peak 8-MOP levels and time of peak level. The recommended oral dosage from the U.S. cooperative clinical trial protocol is .50 to .70 mg/kg body mass (38). The majority of the patients in this study fell within this range. Drug dosage has been based on a dose response experiment on 5 subjects which had shown that 40-60 mg oral doses were required to produce a perceptible erythematous response, but 80-100 mg oral doses resulted in edema and blistering.(65). Steiner, et. al.(52) has shown in 37 patients that there is no correlation between 2 hour 8-MOP serum levels, oral dose or response to irradiation. Wagner, et. al. (66) performed complete 8-MOP plasma profiles on 21 patients ingesting comparable amounts of drug and could not account for variations in peak 8-MOP levels or area under the curve (AUC) measurements. The oral dose of 8-MOP cannot be used to predict what plasma levels of the drug will be achieved.

Elimination rates of the parent molecule obeyed first order kinetics. Average elimination half lives ranging from 1.1 to 1.9 hours (62, 67, 68) have been reported on populations of 4 or 5 patients. The ingestion of food had no statistically significant effect on elimination rate, as in agreement with Ehrsson, et. al. (68). Busch, et. al. (69) studied radiolabelled 8-MOP elimination and found that the decline of radioactivity was first order and biphasic with a rapid initial half life and a terminal half life of about 200 hours. Small quantities (~1%)

of 8-MOP metabolites may linger much longer than the parent molecule (67, 69). Organ distribution studies of radiolabelled 8-MOP in rats (70) have shown a high concentration in liver, kidney and adrenals soon after oral or intravenous administration. This coincides with the predominant organ damage distribution when lethal 8-MOP doses are given to guinea pigs (11). Psoralens are extensively transformed to polar metabolites by hydroxylation and glucuronidation (69, 71, 72). In man 80% of orally administered 8-MOP is excreted in the urine within 8 hours (71), but lesser quantities of polar metabolites are eliminated in the urine and feces over several days (69). 8-MOP is rapidly metabolized following absorption with relatively consistent elimination rates in the population.

Absorption patterns show great variation in the population. The time course patterns for two individuals may be markedly different even though oral dose and elimination rates are similar (Fig. 10). Other investigators (53, 62, 64, 66, 67, 68, 73, 74) have also observed wide variation of absorption patterns.

Food intake may account for some of this variation. Five patients in this study and subjects in other studies (69, 74) demonstrated biphasic absorptive patterns. When food intake was restricted in two of the patients in this study absorption patterns were monophasic, with earlier and greater peak levels of the drug. Two patients repeatedly demonstrated consistent absorption patterns under fasting conditions. Gazith, et. al. (67) reported that fasting subjects had significantly greater overall 8-MOP levels than non-fasting subjects ingesting the same amount of drug. Ehrrson, et. al. (68) reported that food ingestion

enhances absorption of the drug in five subjects. Food intake is often practiced with 8-MOP administration in order to counter the nausea associated with ingestion of the drug. A consistent plasma profile would be crucial in order to insure a favorable therapeutic response. This study would suggest that minimum food intake and consistent dietary habits be maintained.

Individual differences in ability to dissolve the drug formulation may account for some of this variation in absorption patterns. Thune and Volden (59) suggested that dissolution rate may affect the plasma level of the drug. Absorption rate constants have a much greater variation than elimination rate constants in the same population of patients (68). Food content of the upper gastrointestinal tract would be likely to affect dissolution rates. Several studies (57, 74, 75) have shown that liquid preparations of 8-MOP have greater and more rapid absorption than crystalline preparations. Busch, et. al. (69) have shown in man that a course crystalline preparation of radiolabelled 8-MOP has a much greater fecal elimination than a similar amount of liquid preparation. Seventy percent of ingested 8-MOP was absorbed in the liquid preparation, whereas only 30% was absorbed in the crystalline preparation as measured by urinary elimination. The fecal radioactive content was found to be predominantly polar metabolites of 8-MOP. Dissolution and opening of the lactone ring occurs in basic solutions (56, 57). Such conditions exist in the small intestine and may account for this finding. Variable GI transit times may cause much of the variation in absorption patterns seen in

this and other studies. Variations in the bioavailability of different manufacturers' crystalline preparations have been shown in this (64) and other laboratories (76). Investigators in numerous countries using different 8-MOP crystalline preparations report wide differences in 8-MOP plasma concentrations (52 - 62). A preparation which insures rapid and consistent dissolution and absorption of 8-MOP would be most desirable.

The roles of early metabolism and drug distribution in accounting for inter-individual variations of absorption patterns have not been clarified. Substantial concentrations of 8-MOP and its metabolites have appeared in the plasma within 15 minutes of ingestion (69). Both the metabolites and the parent molecule followed a parallel absorption and elimination pattern (67, 69). This suggested a first pass phenomenon for 8-MOP metabolism. Stolk, et. al. (74) suggested that the significantly greater bioavailability of the liquid vs. solid capsules may be due to greater first pass liver metabolism of the latter because of longer dissolution times in the GI tract. No studies have yet been published documenting individual differences of metabolism of the drug. 8-MOP was preferentially distributed to liver, kidney, adrenal, skin and blood in that order in laboratory animals (70, 71). Skin has been shown to have 1.5 X the blood concentration of 8-MOP, though this factor was variable as a function of time after ingestion (70). Herfst, et. al. (61) has compared 8-MOP serum concentrations to suction blister concentrations obtained just after irradiation in 20 PUVA psoriatic patients. Serum to suction blister fluid ratios

of 8-MOP concentration ranged from .06 to 1.54. Individual differences in skin and perhaps other organ distributions may account for some of these variations in 8-MOP plasma profiles.

Psoralens reversibly bind to plasma proteins *in vitro* (77, 78). 8-MOP was 75 to 84% reversibly bound to serum albumin with 2.4 (77) and 0.7 (78) binding sites per albumin molecule reported. Chakrabati, *et. al.* (54) acidified all plasma samples inorder to release 8-MOP from plasma proteins prior to extraction. Investigators in this (64) and all other laboratories which performed 8-MOP analysis found almost complete 8-MOP extraction from plasma or serum into the organic phase without performing such a step. Individual differences in plasma protein binding may affect drug distribution but it cannot account for differences in plasma profiles.

In twenty patients a profile of skin response to irradiation was obtained while 8-MOP plasma concentrations were monitored. Four patients (Pts. 1-4, Group I, Table II) with peak 8-MOP concentrations below 30 ng/ml demonstrated no erythema at any of the energy levels irradiated. Patients 5 and 6 with peak 8-MOP levels of 48 and 68 ng/ml did develop a skin response at all times of irradiation. This suggests that a peak level of 8-MOP between 30 and 50 ng/ml is required to produce an erythematous response. Such a threshold might vary for each individual as a function of skin type, degree of pigmentation and the extent of metabolism and distribution of the drug. All four patients were maintained on an oral dose of 8-MOP that was adequate by current standards. These patients did not

respond to sensitizing doses of irradiation, and they gave a history of poor response to therapy. Low plasma levels may be the norm in these individuals and the reason for their poor therapeutic response. Herfst, et. al. (53) found that patients with peak levels greater than 65 ng/ml had a history of a good therapeutic response, whereas one patient with a peak 8-MOP level of 29 ng/ml had a moderate therapeutic response. One patient with little to no absorption had totally failed PUVA therapy. Wagner, et. al. (66) found lower-than-average 8-MOP plasma levels and low area under the curve (AUC) values in PUVA problem cases with psoriasis. This suggests that the 8-MOP threshold for an erythematous response may approximate the 8-MOP therapeutic threshold. Analysis of 8-MOP plasma profiles may be useful in evaluating patients who respond poorly to PUVA therapy.

Of the sixteen patients who demonstrated erythema, thirteen had complete skin response profiles. The summary data from these patients (Table V) shows that the skin response to irradiation was greatest at 3 hours and significant at 2 hours, whereas the 8-MOP plasma levels were greatest at 2 hours. Thune(76) found that skin response maxima occurred at 1 hour and 8-MOP plasma maxima occurred from 1/2 to 1 hour in 8 patients on a new formulation of the drug. The time of greatest skin sensitivity may reflect the time of greatest skin concentration of the drug. For the population as a whole a 0 to 1 hour delay in 8-MOP plasma-to-skin equilibrium can be expected.

Inorder to maximize therapeutic response each patient must

be evaluated on an individual basis. Ten patients demonstrated simultaneous peak 8-MOP plasma concentration and at least one LMPD (Group II, Fig. 11). Seven of these patients had maximum skin sensitivity one hour after peak levels, as would be expected for a 0 to 1 hour delay in plasma to skin equilibration. Patients 16 and 6 with peak 8-MOP concentrations at 1 hour after ingestion demonstrated 2 and 3 hour respective delays in maximum skin sensitivity. In some patients plasma-to-skin equilibration may be significantly slower than 8-MOP absorption (Group III, Fig. 11). Three patients had LMPD's which preceded maximum plasma levels of the drug. When slow, steady absorption of 8-MOP occurs over a period of several hours plasma-to-skin equilibration may precede maximum plasma concentration of the drug (Group IV, Fig. 11).

Only seven of the sixteen patients who developed photosensitive reactions demonstrated maximum skin sensitivity at 2 hours. Only eight of the sixteen patients had peak 8-MOP levels at one or two hours. Two hours after 8-MOP ingestion is the recommended time for irradiation in the PUVA protocol (38). The wide variation of 8-MOP absorptive patterns and plasma-to-skin distribution times may account for many of the poor responses to PUVA therapy observed in clinical trials. Wagner, et. al. (66) found 8-MOP plasma maxima at 2 hours in most patients with a history of an adequate therapeutic responses; whereas 7 of 14 PUVA problem cases had significant time deviations in plasma profiles. Stevenson, et. al. (62) found that 3 out of 6 poor responders to PUVA therapy had abnormal plasma profiles with

later peak levels (5.1 to 8 hours). Determination of 8-MOP plasma profiles or analysis of MPD profiles may be useful in evaluating patients who respond poorly to therapy because of rapid or slow absorption and distribution of adequate plasma concentrations of drug. Wagner, et. al. (66) found that improvement in some PUVA problem cases could be achieved by shifting the therapeutic UVA irradiation to the peak of the 8-MOP plasma level. As a consequence of this study several patients were irradiated at later times after ingestion with beneficial results.

In accordance with other investigators (52, 53)there was no correlation between 8-MOP peak levels and the amount of radiant energy required to produce an LMPD in the population as a whole. The variation in skin type and in absorption and distribution of the drug may account for this. A general trend in all the profiles is the inverse proportionality between 8-MOP levels and MPD's. Swanbeck, et. al. (79) noted a significant negative linear correlation between the logarithm of serum concentration and MPD in the cumulative profile data from 5 volunteers. To account for variations in skin response this inverse relation was examined for each patient on an individual basis. This relation was best described as a direct inverse proportionality between the $\log_e$(MPD) and the $\log_e$(8-MOP). The product of these two parameters yielded a constant K_t at each time t, which was averaged over all times K_T. It was necessary to shift the profiles of 6 patients such that peak 8-MOP levels corresponded to an LMPD. This accounted for time

variations in absorption and distribution of the drug. The value of K varied from individual to individual. The average value of K_T in the population is greater for darker skin types: K_T values averaged 11.9 for skin type III patients, 9.4 for skin type II patients and 8.4 for skin type I patients. K values would be expected to deviate with changes in pigmentation. The value of K describes a relation between 8-MOP plasma levels and skin response to irradiation for each individual that would be useful in predicting one parameter from a knowledge of the other.

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TABLE I: Skin types and criteria.*

Skin type	Criteria
I	Always burn, never tan
II	Always burn, sometimes tan
III	Sometimes burn, always tan
IV	Never burn, always tan
V	Moderately pigmented patients
VI	Heavily pigmented patients

* The criteria for patients with skin types I, II, III, and IV, generally of European extraction, are based on the history of the usual reaction to the first hour of full sun exposure in early summer. Skin type V generally includes patients of Asiatic, American Indian, Mexican, and Puerto Rican extraction. Skin type VI generally includes patients of African extraction. From reference (4).

TABLE II: 8-MOP plasma levels and simultaneous skin response time course studies of 20 PUVA patients.

PT	Dose	Skin Type	Hours After Ingestion 1 hrs (8-MOP)	2 hr (8-MOP)	2 hr MPD	3 hr (8-MOP)	3 hr MPD	4 hr (8-MOP)	4 hr MPD	5 hr	6 hr (8-MOP)	6 hr MPD	8 hr (8-MOP)	Group
1	.68	II	4	6	>22	11	>22	13	>22		4	>22	0	
2	.76	III	13	14	>27	8	>27	8	>27		4	>27	0	I
3	.57	II	4	5	>25	8	>25	16	>25		10	>25	4	
4	.60	III	16	28	>26	23	>26	16	>26		8	>26	0	
7	.64	III	4	7	26	68	13	*79	13+		22	13	13	
8	.53	I	49	22	>13	8	>13	38	13		*85	6+	69	
10	.69	III	0	19	>28	110	>28	*111	17+		77	17	20	
11	.73	III	91	121	15	*129	12+	109	15		39	19	27	
12	.74	II	9	*137	9+	120	9	89	12		41	12	19	
14	.79	I	20	*163	5+	160	5	76	8		25	11	8	II
15	.69	III	84	140	7	*167	7+	113	7		68	10	47	
17	.48	III	48	*179	12+	102	12	47	15		37	15	13	
18	.58	III	172	*200	7+	139	14	147	14		104	14	70	
20	.61	II	140	*347	15+	157	15	100	18		27	18	25	
6	.40	II	*68	40	11	28	11	24	8+		14	15	18	
16	.48	II	*171	137	5	134	3+	93	3		43	8	32	III
19	.82	III	*222	133	11+	147	15	180	19		90	25	57	
5	.60	II	23	30	11	41	7+	*48	11		17	11	39	
9	.63	III	24	57	13	73	9+	*103	13		29	23	13	IV
21	1.77	III	-	187	-	562	13	571	10+	*782	567	13	314	

Oral dose 8-MOP in mg/Kg body mass. (8-MOP) indicates 8-MOP plasma concentration in ng/ml. MPD is the minimal phototoxic dose. Group I: Patients with 8-MOP plasma levels <30 ng/ml and no skin response to irradiation. Group II: Patients with simultaneous peak 8-MOP levels and LMPD. Group III: Patients with peak 8-MOP levels preceding LMPD by 1 or more hours. Group IV: Patients with peak 8-MOP levels occurring 1 hour after LMPD.

* indicates peak 8-MOP plasma concentration

\+ indicates a LMPD

TABLE III: 8-MOP plasma levels in fasting subjects

PT	Dose	1 hr (8-MOP)	2 hrs (8-MOP)	3 hrs (8-MOP)	4 hrs (8-MOP)	6 hrs (8-MOP)	8 hrs (8-MOP)
13	.50	80	*174	88	50	17	11
8	.53	110	*178	142	72	53	27
15(#1)	.69	117	*206	121	71	23	14
15(#2)	.69	29	139	*145	90	28	10
15(#3)	.69	76	123	*148	91	33	16
15(#4)	.69	32	126	*135	102	38	16
22(#1)	.64	63	*109	63	41	17	12
22(#2)	.64	167	*180	134	88	37	25
22(#3)	.64	*217	213	135	66	37	17
22(#4)	.64	81	*158	128	76	43	18

Oral dose 8-MOP in mg/kg body mass. (8-MOP) indicates 8-MOP plasma concentration in ng/ml. * indicates peak 8-MOP plasma concentration.

TABLE IV: Elimination rate half lives under non-fasting and fasting conditions

pt.	peak conc. (ng/ml)	time of peak (hrs.)	dose (mg.kg)	$t_{1/2}$	R	# of significant observations
		NON-FASTING				
1	13	4	0.68	—	—	2
2	14	2	0.76	—	—	1
3	16	4	0.57	2.1	.9925	3
4	28	2	0.60	2.0	.9999	3
5	48	4	0.60	—	—	2
6	68	1	0.40	2.8	.9940	4
7	79	4	0.64	1.5	.9722*	3
8	85	6	0.53	—	—	0
9	103	3	0.63	1.3	.9917	3
10	110	3	0.69	1.6	.9494*	3
11	129	3	0.73	2.0	.9786	4
12	137	2	0.74	1.9	.9993	4
13	144	2	0.50	—	—	1
14	163	2	0.79	1.4	.9875	4
15	167	3	0.69	2.8	.9900	4
16	171	1	0.48	2.4	.9807	4
17	179	2	0.48	1.7	.9747	5
18	200	2	0.58	3.7	.9993+	3
19	222	1	0.82	2.4	.9917	3
20	347	2	0.61	1.7	.9473	4
21	782	5	1.77	2.3	.9998	3
		FASTING				
13	174	2	0.50	1.5	.9799	5
8	178	2	0.53	2.3	.9719	4
15 (day 1)	206	2	0.69	1.5	.9895	5
15 (day 2)	145	3	0.69	1.3	.9995	4
15 (day 3)	147	3	0.69	1.5	.9970	4
15 (day 4)	135	3	0.69	1.5	.9993	3
22 (day 1)	108	2	0.64	1.9	.9834	5
22 (day 2)	180	2	0.64	2.0	.9908	5
22 (day 3)	217	1	0.64	1.8	.9906	5
22 (day 4)	157	2	0.64	1.8	.9952	4

$t_{1/2}$ calculated by method of least squares linear regression assuming first order elimination rates.

For $P \leq 0.10$: $R > .9880$ for 3 observations
$R > .9000$ for 4 observations
$R > .8050$ for 5 observations

* indicates insufficient correlation coefficient

\+ indicates failure of 95% confidence limit (z=1.96) for a normal population distribution.

TABLE V: Composite time course of 13 patients*

	1 hr	2 hr	3 hr	4 hr	6 hr	8 hr
8-MOP average ± S.D.	83±72	130±89	113±46	93±42	43±28	29±19
MPD average ± S.D.	_	11±6	10±4	12±5	15±5	_
number of LMPD's	_	7	10	4	1	_

*Average values of the data from thirteen patients with complete drug level and skin response time courses.

TABLE VI: Product of log MPD x log (8-MOP) plasma at 2, 3, 4 and 6 hours after ingestion (K_t) and average value of product over all times (K_T)

Patient	Skin Type	K_2	K_3	K_4	K_6	K_T
			Group II			
7	III	6.3	10.8	11.2	7.9	9.0±2.3
8	I	—	—	9.3	8.0	8.6±
10	III	—	—	13.3	12.3	12.8±
11	III	13.0	12.1	12.7	10.8	12.1±1.0
12	II	10.8	10.5	11.2	7.7	10.0±1.6
14	I	8.2	8.2	9.0	7.7	8.3±0.5
15	III	9.6	10.0	9.2	9.7	9.6±0.3
17	III	12.9	11.5	10.4	9.8	11.1±1.4
18	III	10.3	13.0	13.2	12.3	12.2±1.3
20	II	15.8	13.7	13.3	9.5	13.1±2.6
			Group III			
6	II	—	—	8.8	9.0	8.9±0.2
16	II	—	5.6	5.4	9.4	6.8±2,3
19	III	13.0	13.2	14.7	15.8	14.2±1.3
			Group IV			
5	II	8.9	7.5	8.3	7.9	8.2±0.6
9	III	11.0	10.2	10.2	9.5	10.4±0.6
21	III	—	16.3	15.3	15.6	15.7±0.5

Constant is based on the observation of the inverse relation between minimal phototoxic dose (MPD) and 8-MOP plasma concentration for each individual:

$$\log \text{MPD} \approx \frac{1}{\log \text{(8-MOP) plasma}}$$

log MPD x log (8-MOP) plasma = constant K

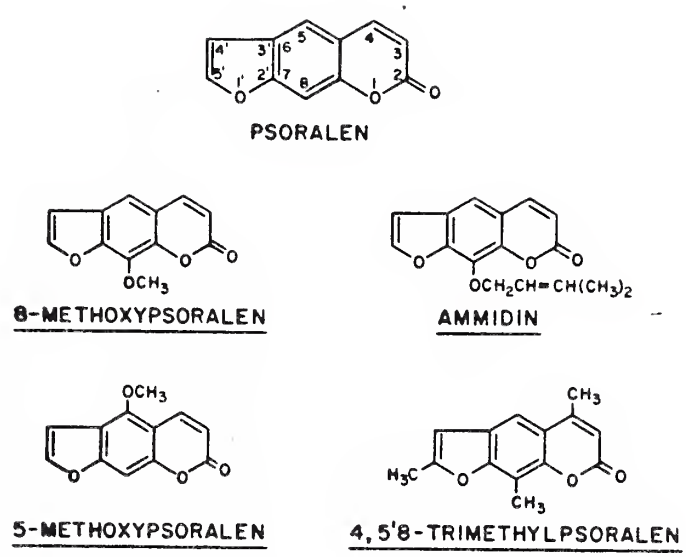

Figure 1: Molecular structure of psoralen 8-methoxypsoralen, 5-methoxypsoralen, ammidin and 4,5'8 trimethylpsoralen.

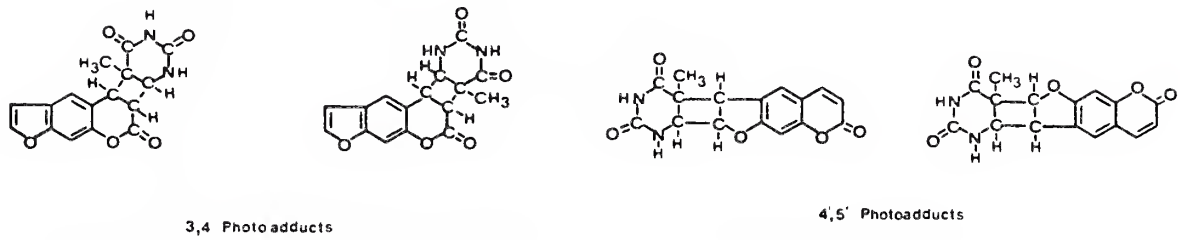

Figure 2: Molecular structure of the 3,4 (a) and 4'5' (B) photoadducts between psoralen and a pyrimidine base.

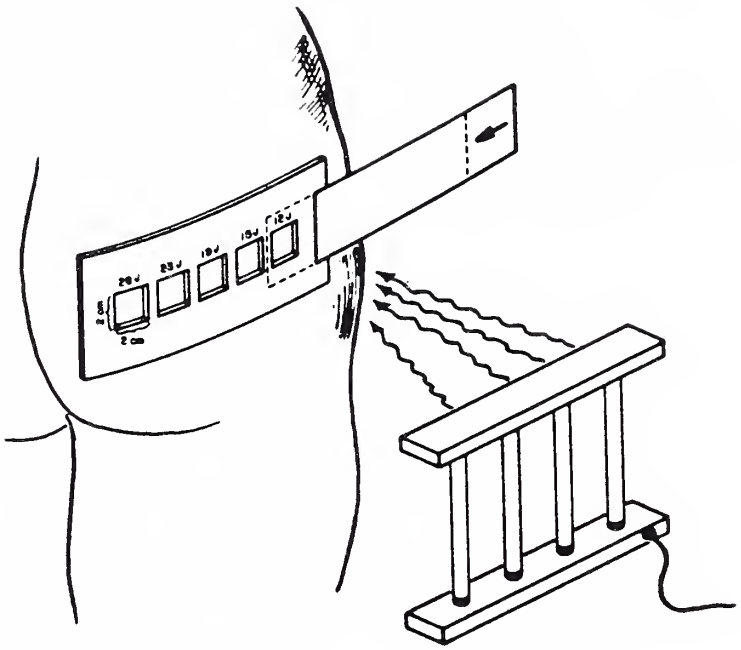

Figure 3: Determination of the minimum phototoxic dose (MPD) on the buttocks.

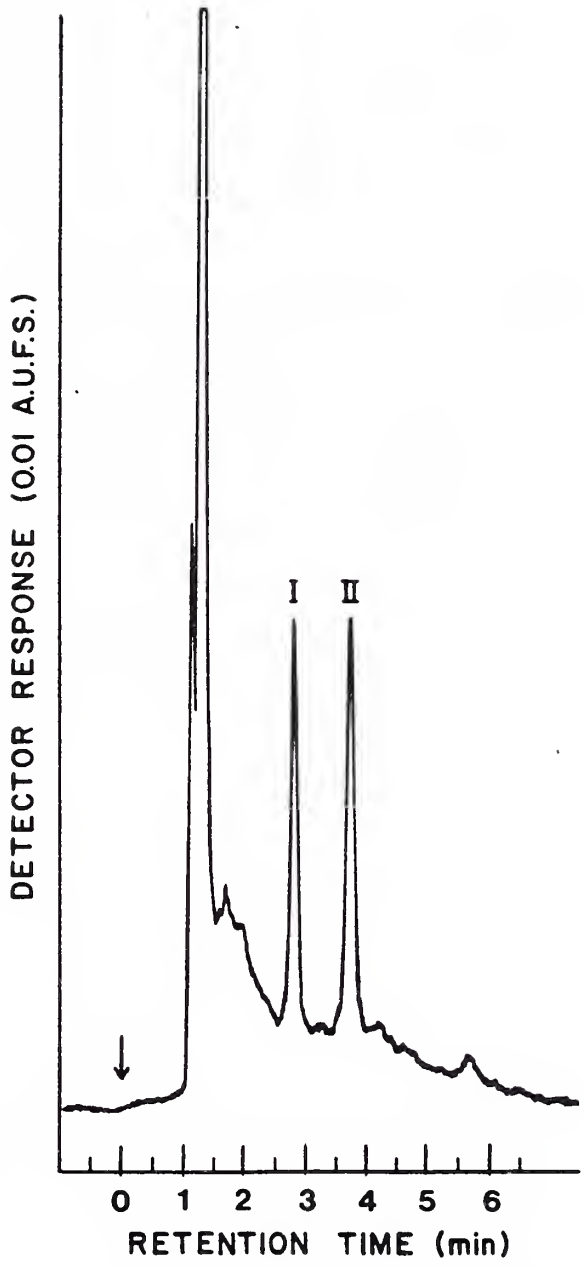

Figure 4: A sample chromatogram. Plasma impurities elute from 1 to 2 minutes after injection (arrow). Ammidin retention time: 2'45" (I). 8-MOP retention time: 3'45"(II).

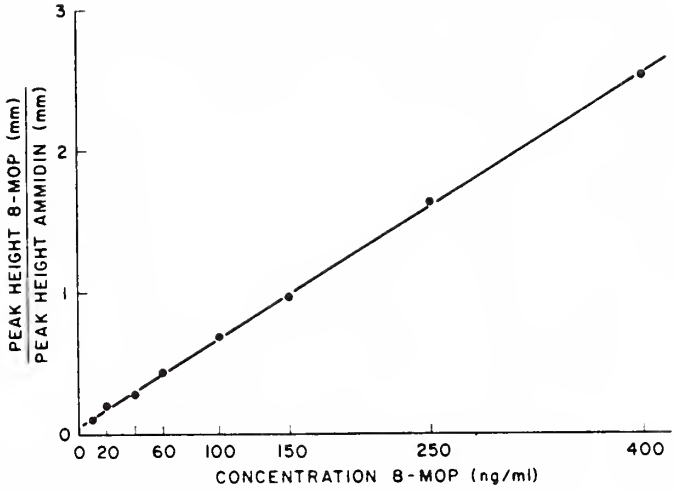

Figure 5: Standard curve for 8-methoxypsoralen and ammidin. 8-MOP (10-400 ng) was added to 1 ml of human plasma with 200 ng ammidin as internal standard.

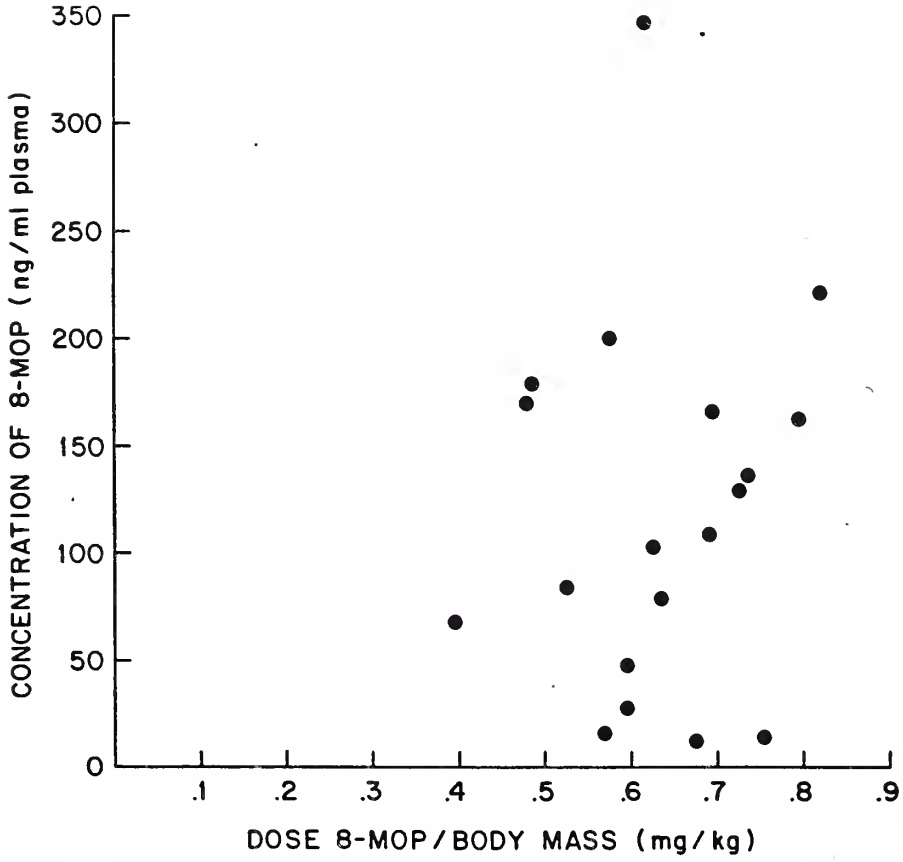

Figure 6: Scatter plot of 8-MOP plasma concentration as a function of 8-MOP oral dose. No correlation was observed.

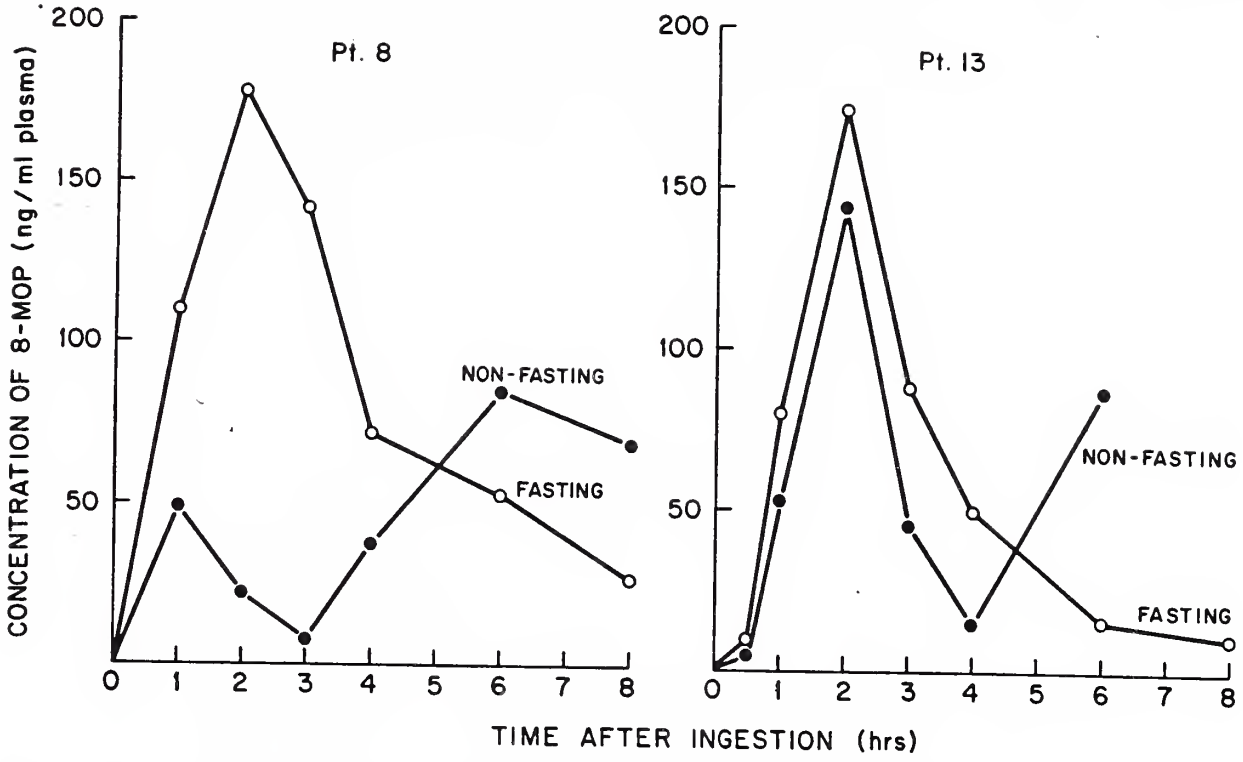

Figure 7: Biphasic time course studies in 2 PUVA patients under non-fasting conditions and monophasic patterns in the same patients under fasting conditions.

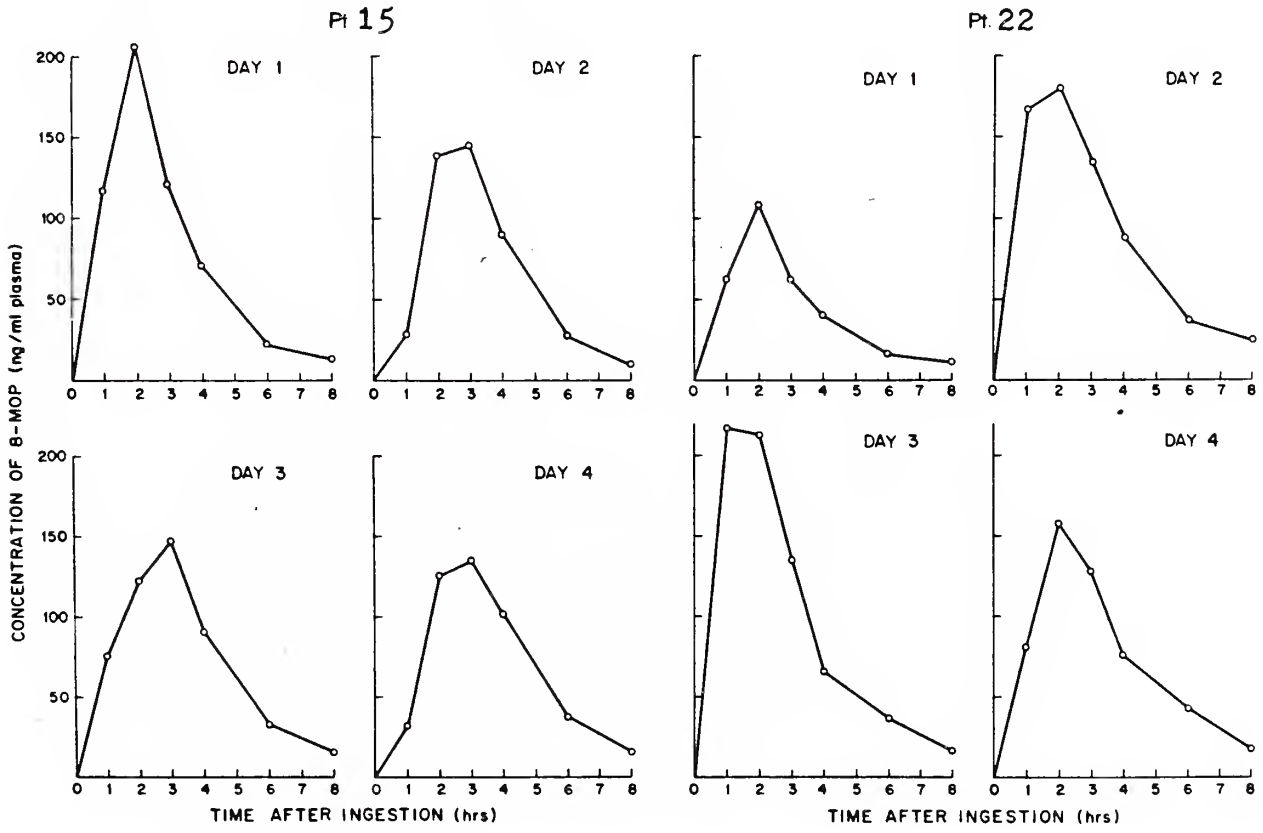

Figure 8: Repeated 8-MOP profiles in 2 patients under fasting conditions. Each individual displayed similar patterns.

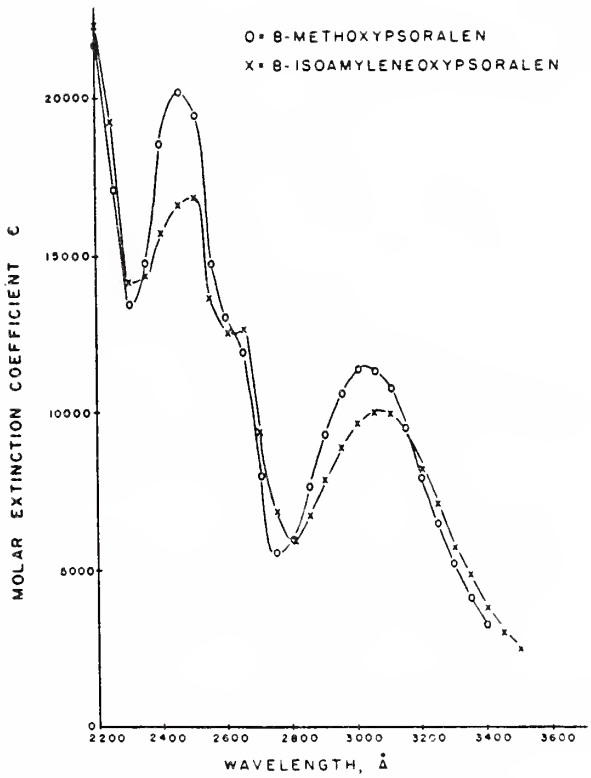

Figure 9: Absorption spectra of 8-methoxypsoralen (8-MOP) and 8-isoamyleneoxypsoralen (ammidin).

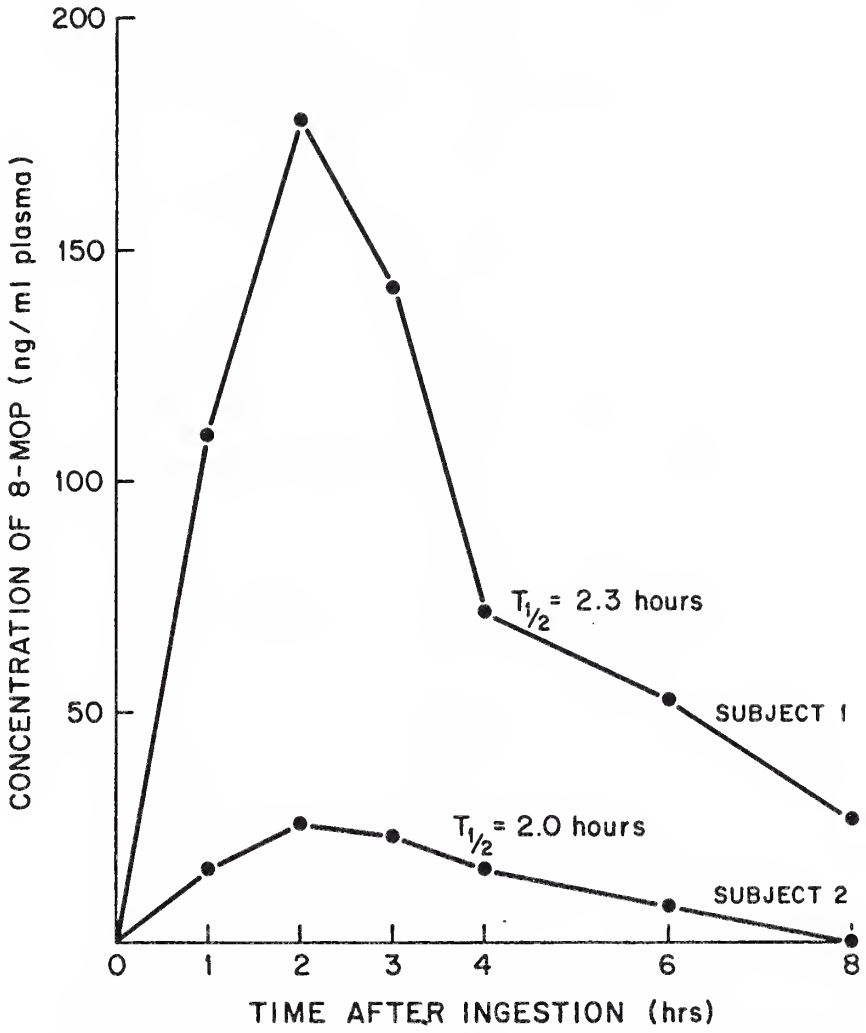

Figure 10: Time course studies of 2 patients with similar elimination rates and markedly different plasma levels.

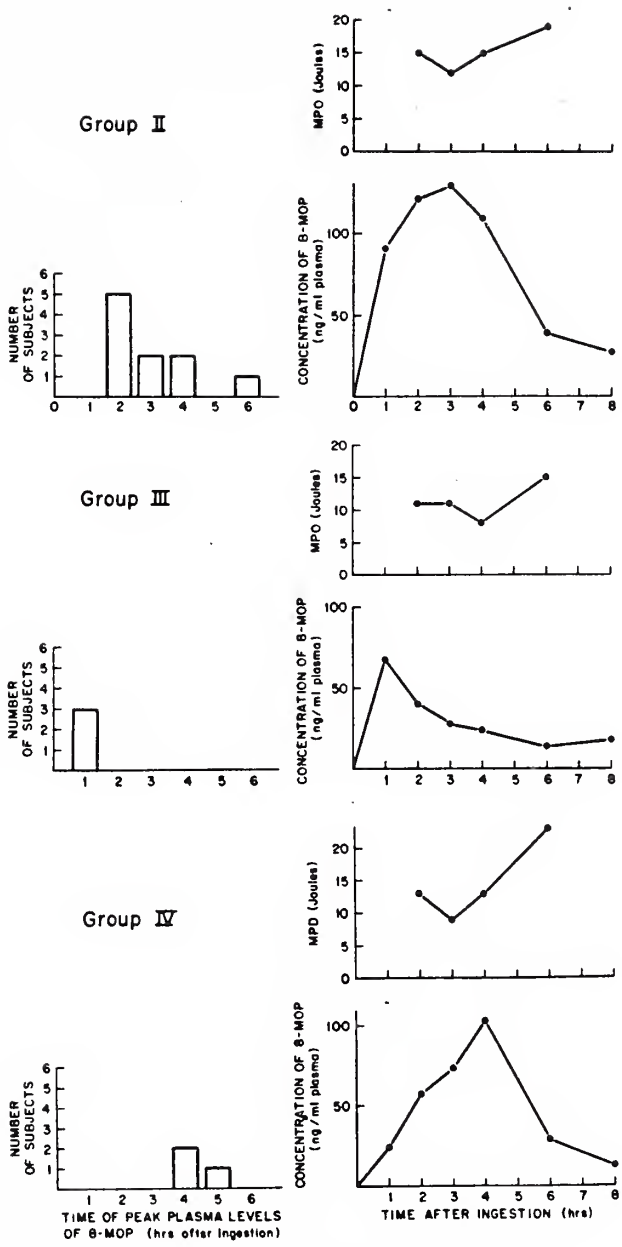

Figure 11: LMPD and peak 8-MOP occurred simultaneously in 10 patients (Group II). LMPD occurred 2 or 3 hours after 1hr peak 8-MOP levels in 3 patients (Group III). LMPD preceded peak 8-MOP levels by 1 hour in 3 patients with delayed absorption (Group IV). Representative profiles at right. Note: Four patients did not develop any phototoxic reaction (Group I).

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