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The Effect of Psoralens on Hepatic and Cutaneous Drug Metabolizing Enzymes and Cytochrome P-450

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Psoralens are tricyclic furocoumarins with potent photosensitizing properties in the skin and are now widely used in the treatment of several dermatologic diseases. In this study the effect of 3 different psoralens 8-methoxypsoralen (8-MOP), 4,5′,8-trimethylpsoralen (TMP) and isopsoralen on hepatic microsomal drug-metabolizing enzymes and cytochrome P-450 has been assessed in mice and rats. 8-MOP administered orally to CD-1 mice daily for 6 days caused 2-3 fold increases in hepatic aryl hydrocarbon hydroxylase (AHH), ethylmorphine N-demethylase and cytochrome P-450. The absorbance maximum of the induced cytochrome was at 450 nm. Aniline hydroxylase activity was unchanged. Chronic administration of 8-MOP to hairless mice caused significant enhancement of hepatic ethylmorphine N-demethylase and cytochrome P-450 but had no effect on AHH; whereas chronically administered TMP had no significant effect on any of these parameters. Isopsoralen and TMP administered orally to CD-1 mice daily for 6 days had no effect on any of these liver enzymes or on hepatic P-450. 8-MOP administered daily for 6 days to rats caused a greater than 4-fold enhancement of AHH and greater than 2-fold enhancement of ethylmorphine N-demethylase and cytochrome P-450. These studies indicate that orally administered 8-MOP induces hepatic drug-metabolizing enzymes and cytochrome P-450 to a lesser extent than do the barbituates and suggest that this drug could influence the rate of biotransformation of concomitantly administered drugs in patients undergoing PUVA therapy.

The psoralens are naturally occurring furocoumarin compounds that are used clinically in the treatment of selected dermatologic diseases including vitiligo [1], psoriasis [2,3], and mycosis fungoides [4–6]. Despite their widespread use very little is known concerning the metabolism of these compounds. Mandula, Pathak, and Dudek demonstrated that one of the psoralens, trimethylpsoralen (TMP), is metabolized to 4,8-dimethyl, 5′-carboxypsoralen in mouse liver and that this metabolite is also found in the urine of human subjects [7].

Further studies have shown that TMP is converted into at least 3 separate metabolites by mouse liver microsomes *in vitro* [8]. This metabolic biotransformation of TMP is dependent on NADPH and fulfills the co-factor requirements for a mixed-function oxidase reaction. An important characteristic of the mixed-function oxidase system is the ability of many different chemicals including drugs to induce its metabolic activity in various tissues including liver and skin [9,10]. No studies have rigorously evaluated the possible induction effects of various psoralens on hepatic and cutaneous drug metabolism and cytochrome P-450. Mandula, Pathak, and Dudek did show that a single acute dose of psoralens had slight induction effects in the liver but no evaluation of the effect of chronic administration of these compounds on the heme-protein cytochrome P-450 was performed.

In the study reported here we assessed the effects of systemically administered psoralen compounds on the *in vitro* hepatic microsomal metabolism of model substrates and on cytochrome P-450. We further compared the induction effects of psoralens to those of model inducers such as phenobarbital and 3-methylcholanthrene in mice and rats. Finally, the effect of topically applied psoralens on cutaneous drug metabolism was also evaluated.

MATERIALS AND METHODS

Materials

4,5′,8-trimethylpsoralen (TMP) and 8-methoxypsoralen (8-MOP) were gifts from the Paul B. Elder Company (Bryan, Ohio). Isopsoralen was isolated from Psoralea corylifolia (leguminosae) and purified and characterized by high pressure liquid chromatography, NMR and mass spectroscopy in our laboratories. All of the psoralens were repeatedly recrystallized in hot methanol prior to use in order to maximize purity. This was confirmed by thin-layer chromatography, NADPH, 3-methylcholanthrene, and benzo(a)pyrene were obtained from Sigma Chemical Company (St. Louis, MO). All other chemicals were of the highest grade commercially available.

Treatment of Animals

Male mice (strain CD-1) from the Charles River Breeding Laboratories weighing 25–30 gm were treated daily for 6 days by oral administration of 8-MOP, TMP or isopsoralen at a dose of 0.8 mg/kg/day. Since all of these drugs are highly water insoluble (50 mg/L) they were suspended in 10% gum acacia (USP) and administered p.o. by stomach tube. Controls received the vehicle alone. In other experiments the effects of orally administered 8-MOP were compared with those of typical inducers of drug metabolism such as phenobarbital (75 mg/kg/day × 3 in saline) and 3-methylcholanthrene (40 mg/kg/day × 3 in corn oil) in the CD-1 mice. The effects of chronically administered 8-MOP (1.2 mg/kg/day) 5 days per week for 6 mo were also evaluated using hairless mice (Strain SKH:hr-1 obtained from Dr. G Mann, Genetics Division, Skin and Cancer Hospital, Temple University, Philadelphia, Pennsylvania).

The effects of orally administered 8-MOP were also assessed in male Sprague-Dawley rats (Holtzman, Madison Wisconsin). 8-MOP was suspended in corn oil by sonication and administered orally at a dose of 0.8 mg/kg/day for 6 days. On day 7, the animals were sacrificed. In other studies the effects of topical application of 8-MOP and 3-MC on

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Abbreviations:
AHH: aryl hydrocarbon hydroxylase
DMeCP: 4,8-dimethyl, 5′-carboxy-psoralen
3-OHBP: 3-hydroxybenzo(a)pyrene
8-MOP: 8-methoxypsoralen
PCBs: polychlorinated biphenyls
TMP: 4,5′,8-trimethylpsoralen
3-MC: 3-methylcholanthrene
PAP: *p*-aminophenol

cutaneous AHH was compared in rat skin. The nuchal region of the animals was shaved with clippers and treated with topically applied 8-MOP (50 mg/kg in acetone) or 3-MC (50 mg/kg) once daily for 3 days and the animals sacrified on day 4.

Preparation of Microsomes

Twenty-four hours after the last treatment the animals were killed by decapitation and washed hepatic microsomes were prepared as described previously [12]. Whole skin homogenates were prepared by techniques routinely used in these laboratories [13].

Enzyme Assays

Cytochrome P-450 concentrations in hepatic microsomes were analyzed from the dithionite plus carbon monoxide minus dithionite difference spectrum [14] employing a DW-2a spectrophotometer. Baseline correction was obtained using a Midan Microprocessor. The molar extinction coefficient of 91,000 was used for the absorbance change between 450–490 nm to determine the cytochrome P-450 concentrations. Ethylmorphine N-demethylase activity was determined as described by Alvares and Mannering [15]. The formaldehyde formed was estimated colorimetrically as described by Nash [16]. Aniline hydroxylase was assayed by measuring the formation of *p*-aminophenol by the phenol indophenol method of Imai, Ito, and Sato [17]. Aryl hydrocarbon hydroxylase activity was determined by a modification of the method of Nebert and Gelboin [18] as described previously [12]. The quantitation of phenolic BP metabolites was based on comparison of fluorescence to a standard of 3-hydroxybenzo(a)pyrene (3-OHBP). Protein was estimated according to Lowry et al [19], using bovine serum albumin as a reference standard.

Spectral Binding Studies

Microsomal suspensions containing microsomes equivalent to 100 mg of liver, wet weight, per ml of 0.1 M K_2HPO_4-KH_2PO_4 buffer, pH 7.4, were used. Spectral changes caused by the addition of 8-MOP to liver microsomes were carried out as described by Schenkman, Remmer, and Estabrook [20].

RESULTS

Comparative Effects of 3 Different Psoralens on Hepatic Drug Metabolism and Cytochrome P-450 in Mice

As shown in Table I, oral administration of isopsoralen and 8-MOP both caused significant increases in hepatic microsomal protein while TMP had no such effect. 8-MOP also caused a 3-fold increase in AHH activity, a 2-fold increase in ethylmorphine N-demethylase activity and a 50% increase in cytochrome P-450. The absorbance maximum of the 8-MOP-induced hepatic microsomal heme-protein was at 450 nm (Fig 1). In contrast, isopsoralen and TMP evoked no statistically significant increases in any of these latter measurements.

Comparative Effect of 8-MOP with Other Typical Inducers of Hepatic Drug-metabolizing Enzymes

Inducers of cytochrome P-450 and hepatic drug-metabolizing enzymes can be divided into at least 2 broad categories [10]. One group, of which phenobarbital is a typical example, enhances the biotransformation of a large number of substrates and increases a microsomal heme-protein with an absorbance maximum at 450 nm. The second group, of which 3-methylcholanthrene is a typical example, enhances the metabolism of a much smaller number of substrates and induces a microsomal heme-protein known as P-448 or P_1-450 [19] that is spectrally and catalytically distinct from P-450. The enzyme induction effect of 8-MOP was compared to that of phenobarbital and 3-methylcholanthrene in an effort to identify the category of inducer to which it belongs.

As shown in Table I, both 8-MOP and phenobarbital caused significant increases in hepatic microsomal protein content and in AHH activity while 3-MC had no such effect. Both phenobarbital and 3-MC significantly enhanced aniline hydroxylase activity while 8-MOP did not. Phenobarbital and 8-MOP each caused significant increases in ethylmorphine N-demethylase activity and in the levels of cytochrome P-450. These data indicate that 8-MOP is a phenobarbital type of inducer in CD-1 mouse liver. In further studies the spectral characteristics of the cytochrome P-450 induced by 8-MOP were compared with those evoked by phenobarbital and 3-MC. The CO-difference spectra of liver microsomes from control mice and from mice treated with 8-MOP, phenobarbital and 3-MC are shown in Fig 1. As would be expected liver microsomes from controls and phenobarbital-treated animals showed an absorption maximum at 450 nm, whereas microsomes from 3-MC treated animals showed an absorption maximum at 448 nm. Liver microsomes isolated from 8-MOP treated mice demonstrated a peak absorbance at 450 nm. These data indicate that 8-MOP is a phenobarbital type of heme-protein inducer though its potency

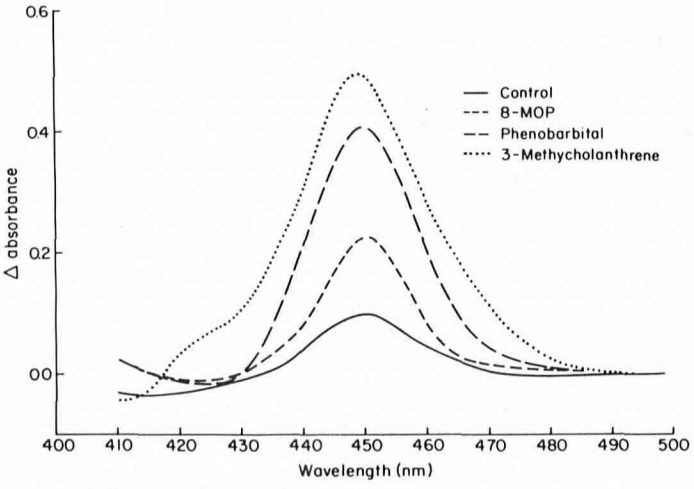

FIG 1. Comparative CO-difference spectra of rat liver microsomes in animals treated with various inducers of hepatic drug-metabolizing enzyme activity.

TABLE I. *Comparative effects of orally administered isopsoralen, trimethylpsoralen and 8-methoxypsoralen (0.8 mg/kg/day × 6) to CD-1 mice on hepatic cytochrome P-450 levels and drug metabolizing enzyme activities*

Treatment	Microsomal protein (mg/gram wet weight)	Aryl hydrocarbon hydroxylase pmol 3-OH BP/min/mg protein	Aniline hydroxylase (nmol PAP/min/mg protein)	Ethylmorphine N-demethylase nmol HCHO/min/mg protein)	Cytochrome P-450 (nmole/mg protein)
Control	15.8 ± 0.5[a]	122 ± 7	1.83 ± 0.03	8.43 ± 1.00	1.10 ± 0.04
Isopsoralen	19.6 ± 0.4	117 ± 10	1.68 ± 0.09	7.03 ± 1.33	1.04 ± 0.05
Trimethylpsoralen	16.0 ± 0.2	150 ± 10	1.83 ± 0.08	7.13 ± 0.04	1.16 ± 0.02
8-Methoxypsoralen	18.0 ± 0.5[b]	345 ± 4[b]	2.03 ± 0.15	16.60 ± 1.17[b]	1.58 ± 0.06[b]
Phenobarbital	23.2 ± 1.6[b]	245 ± 16[b]	2.77 ± 0.18[b]	27.22 ± 1.33[b]	2.48 ± 0.06[b]
3-Methylcholanthrene	18.4 ± 1.3	1140 ± 42	2.32 ± 0.13[b]	8.18 ± 1.17	1.26 ± 0.03

[a] Data represent mean ± SE of at least 3 separate sets of pooled livers from 3 mice.
[b] Significantly different from controls ($p < 0.05$).

as an inducer appears to be considerably less than that of the barbiturate.

Effects of Chronic Administration of 8-MOP and TMP on Hepatic Drug Metabolism in Hairless Mice

As shown in Table II, oral administration of 8-MOP for 6 mo evoked a significant increase in ethylmorphine N-demethylase activity and in cytochrome P-450 levels but had not effect on AHH activity. Chronically administered TMP caused no detectable changes in any of these measurements.

Effect of 8-MOP on Hepatic Drug Metabolism in Sprague-Dawley Rats

These studies were carried out in Sprague-Dawley rats to further characterize the enzyme induction effects of 8-MOP in another widely studied animal species. As shown in Table III, 8-MOP caused significant increases in liver microsomal AHH and ethylmorphine N-demethylase activities and in cytochrome P-450 levels but had no effect on microsomal protein content, or on aniline hydroxylase activity. CO-difference spectra of hepatic microsomes prepared from these animals also confirmed that 8-MOP induced a heme-protein with an absorbance maximum at 450 nm. In addition, the ethyl isocyanide difference spectra of microsomes from control and 8-MOP treated rats showed 455:430 peak ratios of 0.64 and 0.66 respectively (Table III). This is also consistent with a phenobarbital type of induction response.

Binding Spectrum of 8-MOP to Control Liver Microsomes

Compounds interact with hepatic microsomal suspensions to give a Type I difference spectrum, characterized by a peak at about 385 nm and a trough at about 420 nm, or a Type II difference spectrum, characterized by a peak at about 430 nm and a trough at about 390 nm. As shown in Fig 2, addition of 8-MOP resulted in a Type I difference spectrum which is identical to that of hexabarbital. This finding indicates that 8-MOP interacts with liver microsomes similar to other Type I compounds.

Effect of Topical Application of 8-MOP and 3-MC on AHH, a Drug-metabolizing Enzyme in Rat Skin

In these studies an effort was made to determine whether topically applied 8-MOP had any induction effect on cutaneous drug metabolism. The activity of the carcinogen-metabolizing enzyme, AHH, was assessed as shown in Table IV. 3-MC caused a 20-fold enhancement of the skin enzyme whereas 8-MOP had no induction effect whatsoever. This is of interest since it is consistent with our data in liver indicating that 8-MOP is an inducer of the barbiturate type which characteristically has little or no induction effect upon drug metabolizing enzyme activity in the skin.

DISCUSSION

Psoralens (8-methoxypsoralen and psoralen) have been used since antiquity in the treatment of the amelanotic skin disease known as vitiligo [22] and more recently 8-MOP has been used in the treatment of psoriasis [3], mycosis fungoides [4] and several other skin disorders. The linearly annulated photoreactive psoralens are furocoumarin compounds useful in dermatologic therapy because of their ability to function as photosensitizers and as inhibitors of DNA synthesis. In the presence of longwave ultraviolet radiation (UV-A or 320-400 nm) the photoactivated psoralens form covalent bonds with pyrimidine

TABLE III. *Effect of orally administered 8-methoxypsoralen to Sprague-Dawley rats on hepatic cytochrome P-450 levels and drug metabolizing enzyme activities*

Measurement	Controls	8-Methoxypsoralen	% Change
Liver weight (grams)	5.66 ± 0.39[a]	5.47 ± 0.26	−3.4
Microsomal protein (mg/gram wet weight)	22.5 ± 0.5	23.8 ± 0.6	+5
AHH (pmol 3-OHBP/min/mg protein)	83.5 ± 6.2	334.5 ± 19.5[b]	+400
Aniline hydroxylase (nmol PAP/min/mg protein)	0.80 ± 0.05	0.73 ± 0.03	−8.4
Ethylmorphine N-demethylase (nmol HCHO/min/mg protein)	6.53 ± 0.25	15.22 ± 0.05[b]	+232
Cytochrome P-450 (nmol/mg protein)	0.74 ± 0.02	1.52 ± 0.09[b]	+204
Ethyl isocyanide difference spectra (Ratio of 455:430 Peaks)	0.64 ± 0.01	0.66 ± 0.04	—

[a] Data represent mean ± SE of 5 rats.
[b] Significantly different from control ($p < 0.05$).

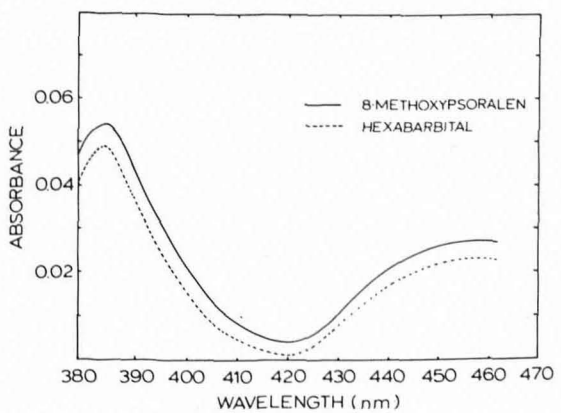

FIG 2. Binding spectrum of 8-MOP to control rat liver microsomes.

TABLE IV. *Comparative effect of topical application of 3-methylcholanthrene and 8-methoxypsoralen on cutaneous aryl hydrocarbon hydroxylase in neonatal rats*

Treatment	Aryl hydrocarbon hydroxylase (pmol 3-OHBP/min/mg protein)
Control	0.58 ± 0.06[a]
3-Methylcholanthrene	10.36 ± 0.17[b]
8-Methoxypsoralen	0.60 ± 0.08

[a] Data represent mean ± SE of 5 experiments.
[b] Significantly different from control ($p < 0.05$).

TABLE II. *Effect of chronic oral administration of 8-methoxypsoralen and trimethylpsoralen (1.2 mg/kg/day-5 days/week for 6 mo) to hairless mice on hepatic cytochrome P-450 levels and drug-metabolizing enzyme activities*

Treatment	Microsomal protein mg/g wet tissue weight	Aryl hydrocarbon hydroxylase pmol 3-OHBP/min/mg protein	Ethylmorphine N-demethylase nmol HCHO/min/mg protein	Cytochrome P-450 nmol/mg protein
Control	12.6 ± 1.4[a]	124 ± 13	13.2 ± 0.8	0.78 ± 0.03
8-Methoxypsoralen	13.4 ± 0.9	122 ± 14	2.22 ± 1.5[b]	1.23 ± 0.14[b]
Trimethylpsoralen	12.2 ± 1.0	117 ± 15	12.8 ± 0.7	0.08 ± 0.05

[a] Data represent mean ± SE of at least 3 separate sets of pooled livers from 2 mice.
[b] Significantly different from control ($p < 0.05$).

bases in DNA [23,24]. These light-activated cycloaddition products are formed between the 3,4, or 4',5', position of the psoralen molecule and the 5,6 position of pyrimidine bases [24,26]. Crosslinks between opposite strands of DNA are subsequently formed which in turn may account for the known ability of psoralens to inhibit DNA synthesis and replication. Previous studies have also shown that topically applied or orally administered psoralens in combination with UV-A can form cycloaddition products and crosslinks with DNA of skin cells in experimental animals and in humans receiving photochemotherapy with psoralens [27].

Despite the increasing clinical use of the psoralens in treating a variety of dermatologic diseases, relatively little is known concerning the absorption, distribution and metabolism of these compounds either in experimental animals or in humans. Orally administered psoralens are almost totally absorbed in the gastrointestinal tract and less than 5% can be detected in the feces [29,30]. Within 12 hr more than 90% of an orally administered dose of 8-methoxypsoralen can be found in the urine primarily as glucuronides or as hydroxylated moieties. Maximum blood levels of these drugs occur 2–3 hours following oral administration after which they appear to be rapidly excreted in the urine.

Little is known concerning the metabolism of psoralens by the liver or other tissues. Mandula, Pathak, and Dudek have shown that TMP is apparently metabolized in part to 4,8-dimethyl, 5'-carboxy-psoralen (DMeCP), a nonphotosensitizing metabolite which can be detected in the urine of mice and of humans to whom TMP is administered orally [7]. Recent studies have shown that microsomes obtained from untreated CD-1 mice are capable of metabolizing TMP to DMeCP as well as to selected other metabolites *in vitro* [8]. These studies also showed that this reaction required NADPH and oxygen for optimal catalytic activity. Thus TMP appears to be metabolized at least in part by the hepatic microsomal cytochrome P-450-dependent mixed-function oxidase system.

8-MOP, on the other hand, does not appear to be appreciably metabolized in mice or in humans although at least 4 metabolites are observed in the urine of mice and of human subjects receiving oral 8-MOP [31]. Careful analysis of urine from animals and humans receiving 8-MOP showed that only a small percentage (<5%) is excreted as the unmetabolized parent compound. Kolis et al studied the metabolism of ^{14}C-8-MOP in dogs [32]. They isolated several metabolites from the urine including 7-hydroxy-8-methoxy-2-oxo-2-H-1 benzopyran-6-acetic acid, α,7-dihydroxy-8-methoxy-2-oxo-2H-1-benzopyran-6 acetic acid and (Z)-3-(6-hydroxy-7-methoxybenzofuran-5-yl)-2 propenoic acid.

We attempted all manner of variation of standard methods of *in vitro* incubation of 8-MOP with microsomes from untreated CD-1 mice and could find no evidence of metabolite formation. In other studies we have attempted without success to demonstrate *in vitro* metabolism of 8-MOP by microsomal fractions obtained from rats and mice (either untreated or treated with phenobarbital, 3-methylcholanthrene, or the polychlorinated biphenyls (PCBs), unpublished observations). A major limiting factor in these *in vitro* assay systems is the very poor water solubility (approximately 50 mg/L) of the 8-MOP which may inhibit its ability to penetrate the microsomal membranes.

Data presented in this paper indicate that treatment of mice and rats with psoralens can cause enhancement of hepatic microsomal drug metabolism and the heme-protein cytochrome P-450. Of the 3 psoralens studied 8-MOP was the only one which had consistently significant effects of drug-metabolizing enzymes and cytochrome P-450. Thus, short-term (daily for 6 days) 8-MOP administration caused significant increases in microsomal protein concentration, AHH and ethylmorphine N-demethylase activities and cytochrome P-450 concentrations in mouse liver. Chronic administration (5x weekly for 6 mo) of 8-MOP also caused significant increases in ethylmorphine N-demethylase and cytochrome P-450. Short-term administration of 8-MOP to Sprague-Dawley rats caused significant increases in hepatic microsomal AHH, ethylmorphine N-demethylase and cytochrome P-450 while TMP had no effect upon any of these measurements.

Spectral studies of the 8-MOP-induced heme-protein indicated that the absorbance maximum was at 450 nm, similar to that of control or phenobarbital-treated animals. Ethyl isocyanide difference spectra also showed that microsomes prepared from 8-MOP treated animals demonstrated a 455:430 peak ratio similar to that of control or phenobarbital-treated animals. The addition of 8-MOP to liver microsomes resulted in a Type I difference spectrum. These findings, in aggregate, suggest that 8-MOP may be a substrate for liver microsomes although we were unable to verify this possibility in the studies described here.

Skin application of 8-MOP to neonatal rats had no effect upon cutaneous AHH activity an enzyme which in this tissue normally increases in activity following treatment with polycyclic aromatic hydrocarbon carcinogens such as 3-MC. Thus, 8-MOP does not appear to influence this microsomal enzyme system in the skin.

In summary, our studies indicate that 8-MOP is a moderately potent inducer of hepatic microsomal drug-metabolizing enzymes and cytochrome P-450 and that the induction response to this drug is analagous to that which occurs in animals treated with barbiturates.

Our data indicate that administration of 8-MOP to patients undergoing PUVA therapy may alter rates of hepatic metabolism of concomitantly administered drugs. While the enzyme induction effect of 8-MOP is qualitatively similar to that evoked by the barbiturates, it is quantitatively much less effective in this regard. These findings suggest that the interaction of psoralens with the hepatic metabolism of other drugs is likely to pose a less serious problem than that which has been attributed to the barbiturates.

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Reduction of the Fraction of Circulating Helper-Inducer T Cells Identified by Monoclonal Antibodies in Psoriatic Patients Treated with Long-term Psoralen/Ultraviolet-A Radiation (PUVA)

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Ultraviolet radiation has been found to alter the distribution and function of human lymphocytes. To determine whether photochemotherapy (PUVA) alters circulating levels of T cell subset marker-bearing lymphocytes, cells from 9 patients with psoriasis undergoing PUVA therapy for several years (mean 4.6 ± 1.4 yr), 17 patients with active untreated psoriasis, and 20 healthy volunteers were reacted with monoclonal antibodies to T cell surface markers, including OKT3 (all peripheral blood T cells), OKT4 (helper/inducer T cells), OKT6 (common thymocytes), and OKT8 (suppressor/cytotoxic T cells), and analyzed by flow cytometry. There were no differences in the distribution of T cell subsets between healthy volunteers and patients with active psoriasis. In contrast, the percentages of lymphocytes reacting with OKT3 and OKT4 were lower (by 16% and 12% percent respectively, $p < 0.0025$) in the PUVA-treated patients compared to healthy volunteers or patients with active psoriasis that had not received PUVA therapy. There was no difference in the percentage of OKT8 and OKT6 bearing cells. Squamous cell carcinoma of the skin subsequently developed in 2 of 3 PUVA-treated patients with the lowest percentages of T4-bearing cells. These findings indicate that long-term PUVA therapy is associated with a reduction in circulating helper/inducer T cells. This reduction may have a role in the altered immune function reported in PUVA-treated patients.

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Abbreviations:
Con A: concanavalin A
PSS: phosphate-buffered saline
PHA: phytohemagglutinin

Ultraviolet radiation can alter the distribution and function of lymphocytes both in animals and humans [1–6]. Oral psoralen and subsequent exposure to ultraviolet-A radiation (PUVA)