Molecular Aspects of Extracorporeal Photochemotherapy

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8-methoxypsoralen (8-MOP), activated upon exposure to long-wavelength ultraviolet radiation, is used therapeutically to treat the diseased blood cells of cutaneous T-cell lymphoma patients. The factors responsible for the efficacy of this therapy are reviewed. Primary among these are the plasma level of 8-MOP at the time of irradiation and the effective dose of UVA. 8-MOP plasma levels determined in a series of six patients demonstrated that the drug is absorbed at a highly variable rate (122 ng/ml ± 67). A new liquid form of 8-MOP is absorbed with a modest increase in plasma levels (170 ng/ml) but with no improvement in the variability (±163). An examination of the dose-response relationship between 8-MOP concentration and UVA dose indicated that properties such as 8-MOP photoadduct formation and PHA response are proportional to the combined doses of these two factors. A new molecular target for 8-MOP photomodification, cell membrane DNA, is described.

HISTORY

The curative powers of natural products containing the class of drugs known as furocoumarins (psoralens and angelicins) have been known to mankind since biblical times [1]. The ancient Egyptians ingested the leaves of ammi majus, a plant that grew by the Nile River, to treat depigmented areas of skin. Although 5-methoxypsoralen was first isolated in 1834, it was not until almost a century later, in 1931, that Phyladelphy demonstrated that sunlight was a necessary co-factor for the activation of the compound [2]. In 1948, El Mofty isolated and characterized the active ingredient of the ammi majus plant, 8-methoxypsoralen (8-MOP). Using the purified compound, he showed that exposure of the skin to sunlight after its ingestion led to repigmentation [3]. Aaron Lerner and Thomas Fitzpatrick, then at the University of Michigan, were intrigued by these findings and began their own studies on 8-MOP. Their research established that 8-MOP could be safely administered to humans and that a relatively low dose was effective for the treatment of vitiligo [4]. By recording the excitation spectra of 8-MOP, Lerner, now at Yale, observed that the optimal wavelength for the activation of 8-MOP was 365 nm, which corresponds to an intense band in the low-pressure mercury spectrum. In the 1950s, an Italian research team led by L. Musajo at the University of Padua initiated studies on the molecular aspects of the biological effects of psoralens after an unpleasant personal experience with the potent photosensitizing effects of the psoralen-containing fig leaf [5]. These pioneering

photochemical studies first demonstrated that a 2 + 2 photocycloaddition occurred between psoralens and pyrimidines in DNA [6]. At the time the characterization of these DNA reactions provided a satisfactory explanation for the biological effects of 8-MOP and ultraviolet A light (UVA).

In the early 1970s, John Parrish led another Harvard team in the development of 8-MOP and UVA (PUVA: psoralen/ultraviolet A treatment) for the treatment of psoriasis, a hyperproliferative disease of the skin in which the epidermal cells reproduce themselves at an accelerated rate [7]. The formation of 8-MOP photoadducts slowed the process and restored the proliferation to near-normal rates. Studies also suggested at that time that this property appeared to be secondary to the ability of 8-MOP to cross-link DNA [8]. Angelicins, however, which are incapable of cross-link formation, also inhibited DNA synthesis [9]. To treat psoriasis effectively, it was necessary to develop high-intensity lamps so that clinically effective doses could be delivered in a reasonable time period. Kraemer et al. also showed that T cells circulating through the skin were affected by PUVA [10].

The success of 8-MOP plus UVA in the treatment of psoriasis led investigators to test its potential efficacy in other disorders of the skin. In 1979, Gilchrest showed that PUVA was effective in the treatment of cutaneous T-cell lymphoma (CTCL), an epidermotropic neoplasm [11]. Even though the beneficial effects were only palliative, the encouraging results stimulated the next quantum leap for 8-MOP and UVA photochemotherapy. Specifically, Edelson, then at Columbia, reasoned that the direct exposure of the diseased T cells of CTCL patients to 8-MOP and UVA might enhance their therapeutic efficacy. In collaboration with scientists at Johnson & Johnson, Edelson and his colleagues, Gasparro and Berger, developed a system which permitted 8-MOP-containing blood to be irradiated outside the patient's body [12]. In the first-generation prototype system, six sterile plastic bags connected in series were sandwiched between rigid plastic water-jacket plates, which were then exposed to UVA from both the top and bottom. The rigid structure maintained the blood thickness at 1 mm. Irradiation from both sides enhanced the probability that a given lymphocyte rising to the surface would encounter a therapeutic dose of UVA. Using monoclonal antibodies developed by Santella and Gasparro, it was shown that even though the hematocrit was reduced to approximately 5 percent, the shielding effects of red blood cells and plasma reduced the efficiency of the psoralen-DNA photoreaction by nearly two orders of magnitude [13]. Despite this drastic reduction, the doses of UVA delivered under these conditions were sufficient to induce clinical responses (see [13]; article by Berger). The use of psoralens and UVA to affect T cells directly forms the basis of extracorporeal photochemotherapy, which is the focus of this issue.

PSORALEN PHOTOADDUCTS

The furocoumarins are a class of tricyclic aromatic compounds formed by the fusion of a 2,3 furan bond to the 6,7 coumarin bond. The linear compound that results is known as a psoralen (Fig. 1, upper diagram). If the 2,3 furan bond is fused to the 7,8 bond of the coumarin, an angular furocoumarin, angelicin, is formed (Fig. 1, lower diagram). The extended aromatic structure of psoralens is responsible for their ability to absorb long-wavelength ultraviolet A radiation. Figure 2 shows the UV spectrum of 8-MOP. Absorption bands near 250 and 300 nm are characteristic of all furocoumarins. It is interesting to note that the optimal wavelengths for activation of 8-MOP (that is, 320–400 nm) do not coincide with the absorption peak at 300 nm. In fact,
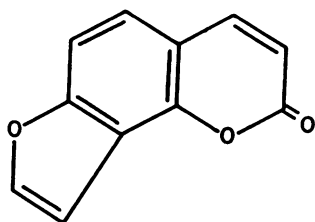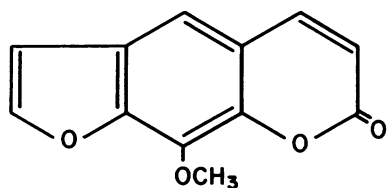
FIG. 1. Furocoumarin structures: 8-MOP (*upper diagram*), angelicin (*lower diagram*).

irradiation of psoralens with 300 nm radiation leads to very efficient photodegradation of the compound. Various substitutions such as methyl, methoxy, hydroxyl, carbethoxy, and amino groups, when added to the furocoumarin ring, alter DNA binding, photochemical reactivity, and the specific biological effects of these drugs (Table 1). In general, methyl groups make the psoralen compound less water-soluble but increase its ability to associate with DNA base pairs by intercalation. A greater degree of intercalation leads to greater photochemical efficiency for photoadduct formation. For example, compare the data for 8-MOP and 4'-amino-methyl-4,5',8-trimethylpsoralen (AMT). The binding constant for AMT is roughly 200 times greater than that for 8-MOP. The biological effectiveness of AMT, as measured by tritiated thymidine incorporation after mitogen stimulation, is at least an order of magnitude greater than that for 8-MOP. The development of new forms of psoralen which are more water-soluble and which interact more strongly with DNA could lead to significant improvements in extracorporeal photochemotherapy.
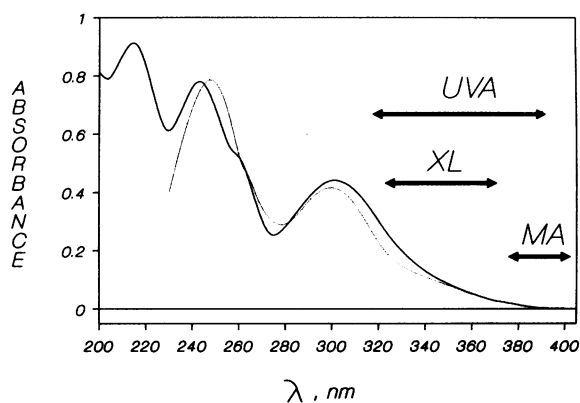
FIG. 2. UV spectrum of 8-MOP. *Solid line:* 10 μg/ml 8-MOP in ethanol recorded by the author, using a Pharmacia-LKB spectrophotometer interfaced with an IBM PC; *dashed line:* 8 μg/ml in ethanol recorded by Aaron Lerner in 1958, using a Beckmann DU spectrophotometer. UVA indicates the spectral output of the UVA lamps used to activate psoralens. MA indicates the wavelength region in which monoadducts are the sole photoproduct, and XL indicates wavelengths capable of inducing cross-link formation.

TABLE 1
Physical Chemical Properties of Psoralens[a]

Compound	Solubility μg/ml	DNA Binding Constant M^{-1}	ng/ml for 50% Activity[b]
8-MOP[c]	38	770	15
AMT	10^4	150,000	<1
TMA	3	10,100	—

[a]For a more complete listing of psoralen properties, see [35].
[b]In combination with 1 J/cm² UVA in a mitogen response assay
[c]Abbreviations: 8-MOP, 8-methoxypsoralen; AMT, 4'-amino-4,5', 8-trimethylpsoralen; TMA, 4,6,4'-trimethylpsoralen

UVA photoactivation of furocoumarins results in the creation of specific excited states. Molecular orbital calculations have shown that the furocoumarin 3,4 bond would be expected to be the most reactive position because this is the site of the greatest electron density [14]. In fact, in a solution of 8-MOP and thymine, the most prevalent photoproduct is the 3,4-monoadduct [15]. In double-stranded DNA, however, the 4'5' adduct becomes the primary photoproduct (Fig. 3). The change in photoproduct yield is due to the particular suitability of intercalation sites for photochemical reactions between the DNA base, thymine, and the psoralen molecule. Intercalation forces cause the psoralen to be properly oriented for thymine photoaddition when excited by incident UVA radiation. In addition, the psoralen-base pair association minimizes the importance of excited-state lifetimes, because the encounter between a photoexcited psoralen molecule and a reaction partner is no longer diffusion-controlled.

Thus, psoralen derivatives can react at either the 3,4 bond of the pyrone ring or the 4,5 bond of the furan ring. Multiple methyl groups, as in 4'-amino-methyl-4,5',8-
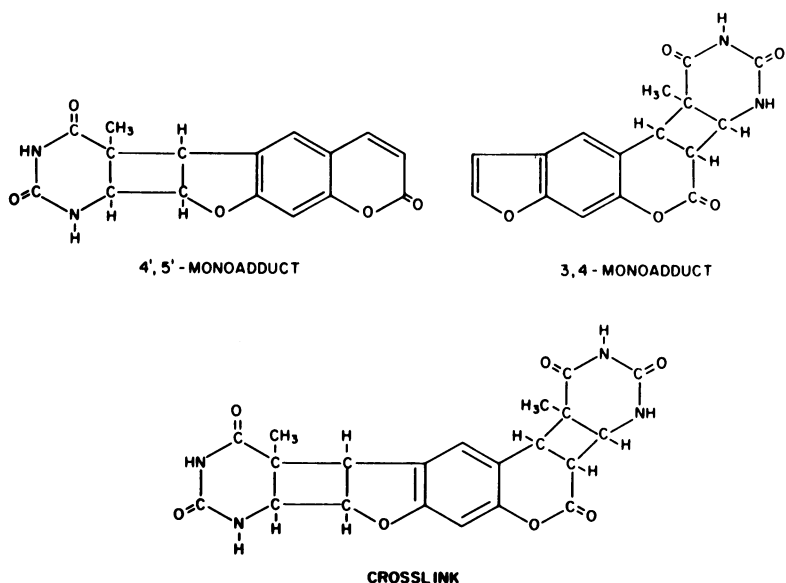

FIG. 3. Psoralen photoadducts. *Upper diagram*: 4',5'-monoadduct (*left*), 3,4-monoadduct (*right*); *Lower diagram*: cross-link.
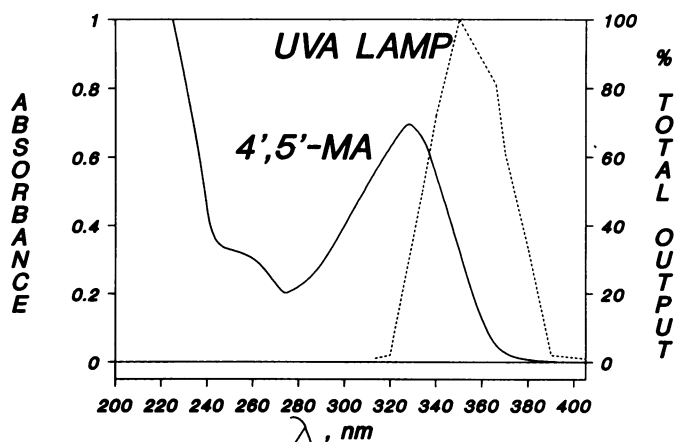
FIG. 4. UV spectrum of the 4',5'-monoadduct (*solid line*) and the relative output of UVA lamps (*dotted line*).

trimethylpsoralen (AMT), cause the 4',5'-bond to be the principal site of photoadduct formation [16]. Furocoumarins capable of reacting at both sites are termed "bifunctional." In double-stranded DNA, these bifunctional compounds can form interstrand cross-links (Fig. 3). Photoadduct formation is wavelength-dependent. Irradiation of furocoumarin-DNA solutions with monochromatic wavelengths as long as 400 nm can lead to photoadduct formation (MA in Fig. 2) with yields being proportional to the extinction coefficient at a given wavelength. Irradiation with wavelengths in the range, 320–370 nm, leads to the efficient formation of cross-links (XL in Fig. 2). Wavelengths in this range are a major component of UVA lamps and overlap strongly with the absorption spectrum of the 4',5'-monoadduct (Fig. 4). Wavelengths less than 320 nm cause photoreversal of previously formed adducts and degradation of molecules that are not intercalated between base pairs.

Angelicins (angular furocoumarins) cannot cross-link DNA because the isomeric arrangement of their aromatic rings does not permit the necessary alignment with properly opposed thymines on the two DNA strands. In addition, bulky side groups in some psoralens such as 3-carbethoxypsoralen block activity at one site and, hence, these compounds can only form DNA monoadducts (e.g., carbethoxypsoralen) [17].

PARAMETERS AFFECTING EXTRACORPOREAL PHOTOCHEMOTHERAPY

The impressive clinical effects of 8-MOP and UVA in photomedicine are much more apparent than the mechanisms by which they are achieved. Cellular DNA (nuclear, mitochondrial, and cell membrane) may be the targets of 8-MOP and UVA. In addition, the combination of 8-MOP and UVA may alter enzymes, receptors, and membrane components. Affecting these structures could alter how a cell is processed by the immune system. Whatever the ultimate mechanism for the efficacy of photopheresis, there remains one inescapable conclusion, *8-MOP and UVA act in concert to produce therapeutic effects.* For the treatment to be optimally effective, substantial levels of 8-MOP must be present and an effective dose of UVA must be delivered to the 8-MOP-containing cells.

HPLC Analysis of 8-MOP in Plasma

Samples are obtained from patients in two ways: either by venipuncture or by direct removal from the photopheresis collection bag. In either case, the specimen is then

centrifuged at 2,000 rpm for ten minutes to obtain a plasma sample that is free of any sediment. For each 1 ml of plasma to be analyzed, 1.25 μL [^{3}H]8-MOP (Amersham, 2.6 ng/μL) is added as an internal standard. In 1986 as the use of photopheresis became more widespread, our laboratory developed the more efficient solid-phase extraction method (SPE) for the isolation of 8-MOP from plasma samples [18]. Existing methods for the isolation of 8-MOP from plasma involved extraction with organic solvents (e.g., hexane or benzene), followed by evaporation to re-concentrate the sample and then resuspension in a solvent suitable for reversed-phase HPLC analysis. This protocol required two hours to prepare HPLC-ready samples (five specimens in duplicate). In the SPE method, on the other hand, the multi-step organic extraction method was replaced with a single-step extraction using a solid-phase extraction cartridge. In essence, these cartridges are "mini"-HPLC columns in which a vacuum is used to apply the sample and to draw the eluting solvent through the "mini-column." The columns are first "activated" with 10 ml HPLC-grade methanol and then primed for sample application with 10 ml PBS; 1.00 ml of plasma containing the [^{3}H]8-MOP internal standard is then applied. HPLC-interfering proteins and salts are removed with a 10 ml PBS wash. The 8-MOP is then eluted by application of a 1.00 ml 70 percent methanol-water solution. The specimen is next analyzed by two independent methods. First, to assure that complete recovery has been achieved, duplicate samples of the eluted specimen are assayed by liquid scintillometry. Second, each sample is analyzed by reversed-phase HPLC. 200 μL is applied to a reversed-phase phenyl column (4.6 mm $\times$ 15 cm). Using a mobile phase consisting of 50 percent acetonitrile and 50 percent 0.05 M ammonium acetate (pH 4.6), the column eluant is monitored at 300 nm. Under these conditions, 8-MOP elutes at 265 seconds. A typical chromatogram is shown in Fig. 5 (left panel). The peak area is used to compute the 8-MOP concentration in the plasma sample. In the right panel of Fig. 5, the ultraviolet spectra of the 8-MOP containing peak (lower) and a presumed metabolite (upper) are shown.

In the photopheresis procedure, several centrifuge cycles are used to obtain fractions for exposure to UVA. Incremental volumes of plasma and white blood cells are obtained over a 90-minute period during a typical procedure. Figure 6 shows that the 8-MOP concentration increases with each additional plasma fraction. Once addition of plasma is discontinued, there is no further increase in 8-MOP concentration. It is also important to note that subsequent addition of white cells alone does not appreciably dilute the 8-MOP and that the subsequent exposure to 90 minutes of UVA does not photodegrade the 8-MOP.

8-MOP Absorption

8-MOP and UVA acting synergistically are responsible for the clinical efficacy of photopheresis. In the initial development of 8-MOP-UVA phototherapy for psoriasis, the dose of UVA required for clinical efficacy was unequivocally demonstrated. Clear dose-response relationships between potency and the amounts of UVA and 8-MOP have been demonstrated in cellular systems (see below). It is assumed that a similar relationship exists *in vivo*. Thus, physicians administering PUVA routinely use devices to monitor and adjust the UVA doses delivered to patient skin. It is equally important to measure the amount of 8-MOP present in the target tissue. Two factors must be addressed in photopheresis. One is the individual ability of a patient to absorb the drug, and the second is the time at which plasma fractions are added to the collection bag
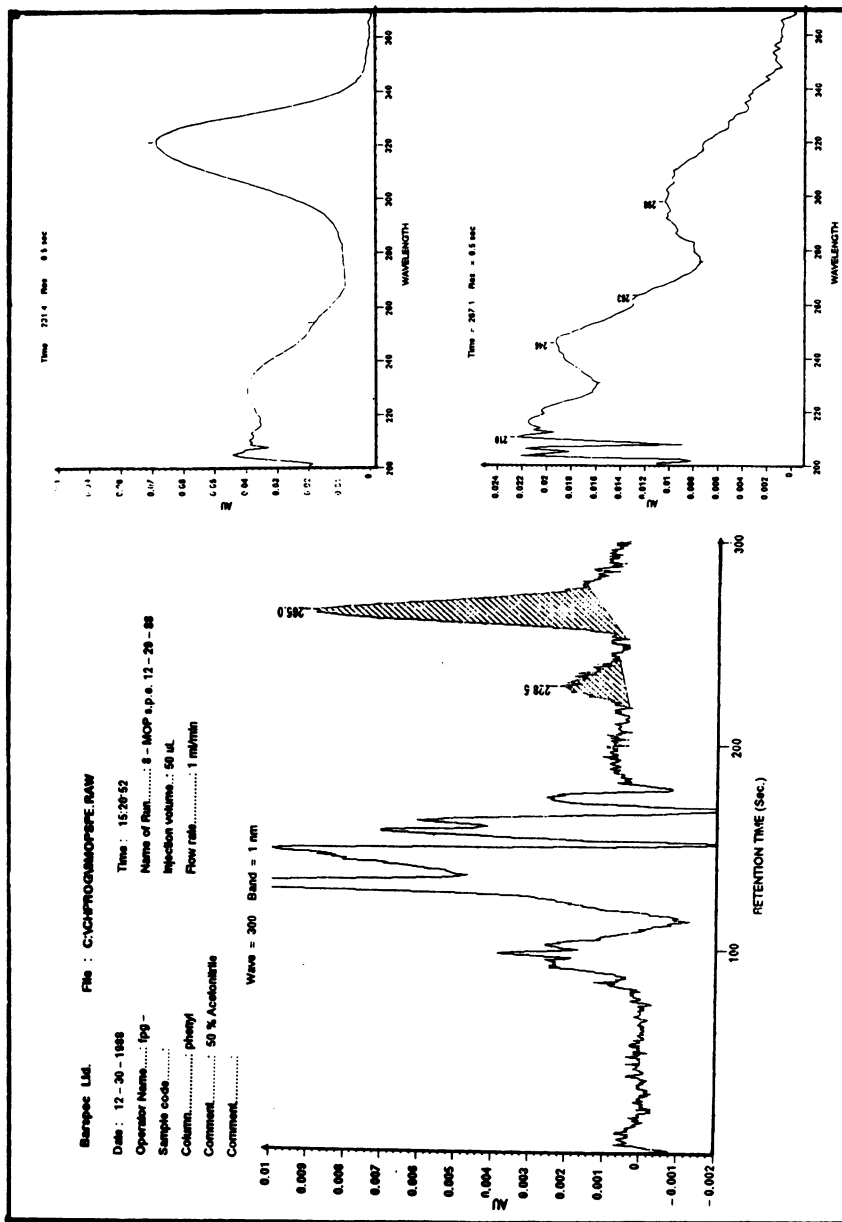
FIG. 5. HPLC chromatographic results. *Left panel*: Chromatogram showing 8-MOP at 265 seconds. *Right upper panel*: UV spectrum of the material eluting at 265 seconds; *right lower panel*: UV spectrum of species eluting at 240 seconds.
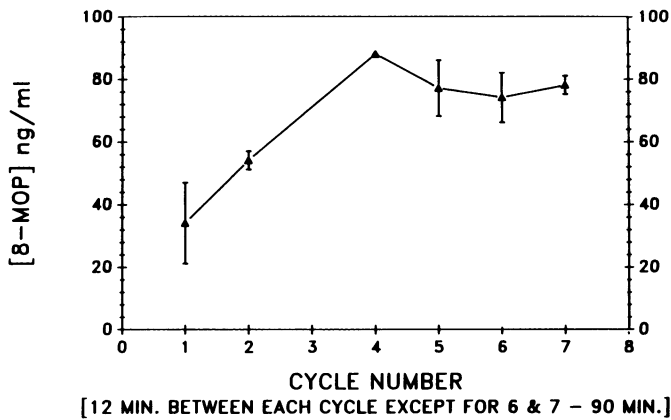
FIG. 6. Effect of photopheresis cycle number on 8-MOP concentration.

during photopheresis. In Fig. 7, the absorption characteristics of two forms of 8-MOP (crystalline and liquid) are illustrated for five individuals. Two of these curves are prototypical. Curve A illustrates the rapid absorption of oxsoralen ultra. In two other individuals, however, the oxsoralen ultra did not demonstrate such characteristics (curves B and C). Curves D and E show the kinetics that are characteristic of crystalline 8-MOP, namely, a relatively broad absorption requiring two to three hours to reach a maximum level between 100 and 200 ng/ml. Prior to the availability of liquid 8-MOP, we had analyzed more than 400 samples and found an average 8-MOP level of 122 ng/ml (±67, with a range of 0 to 440 ng/ml). The samples were always obtained from patients two hours after ingestion of the capsules which had been taken one half hour before eating a light breakfast. Even under these controlled conditions, intra-individual variation was quite pronounced (Fig. 8). On any given day of treatment there was a significant probability that the patient could have a sub-optimal level of 8-MOP present in his or her plasma. Oxsoralen ultra demonstrated a similar pattern in a series of 27 patients: the average level was 170 ng/ml (± 163, with a range of 0 to 591 ng/ml). Although the average 8-MOP level was somewhat greater in the patients who ingested the ultra form, the values are distributed over a very wide range.
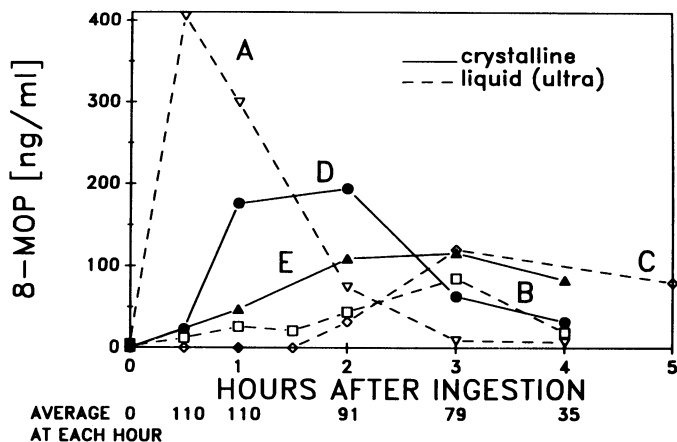

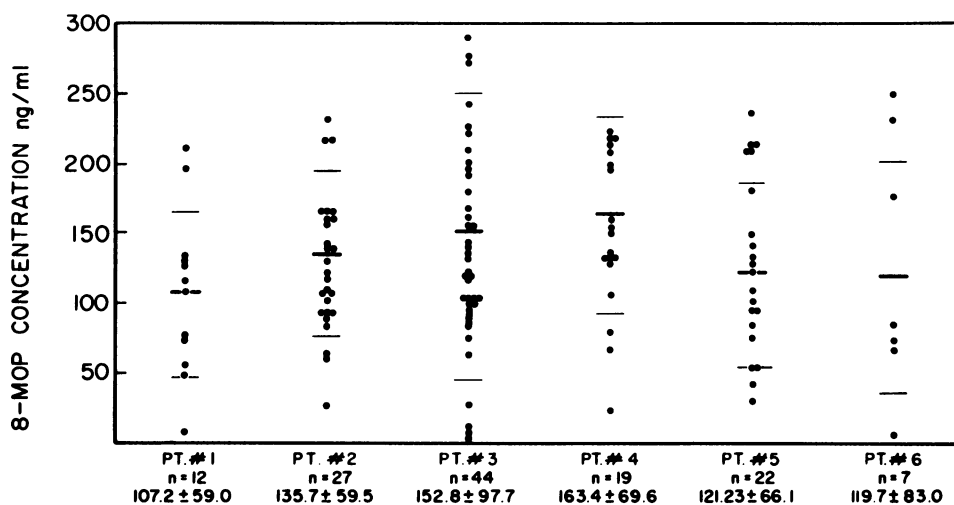FIG. 7. Time course for 8-MOP absorption.

FIG. 8. Intra-individual variation in 8-MOP plasma levels.

In a small percentage of patients who ingest 8-MOP (either form) for the first time, the first-pass effect is observed [19]. This effect is a phenomenon in which first exposure to a drug induces a high level of liver activity that effectively prevents any significant level of accumulation in the plasma. In the vast majority of these patients, the second ingestion of the drug leads to normal or near-normal levels; however, a small proportion never achieve the normal levels or, if they do, much higher doses of the drug are required. If the patients tolerate the ingestion of the drug (minimal nausea, for example), there would appear to be no problem with these increased doses.

Many investigators have attempted to elucidate the physiological parameters underlying the variable absorption of 8-MOP [20]. The absorption data for the two forms of 8-MOP indicate that the actual physical formulation of the drug, crystalline or liquid, has very little effect on the ultimate bioavailability of the drug. A simple chemical principle provides the explanation. The water solubility of 8-MOP is very low—38 μg/ml [21]. A typical patient may ingest 40 mg of the drug. If all of this drug dissolved instantly, it would lead to a stomach concentration of 40 mg in 500 ml of gastric contents (80 μg/ml). This quantity is almost twice its solubility. Thus, once in the stomach, the drug is expected to do what is thermodynamically impossible. One could argue that the ultimate concentration might be determined by the total volume of fluid compartments of the body. In a 70 kg, adult, 60 percent of the body weight, or 40 liters, is an approximation of the sum of plasma water, interstitial fluid, and intracellular fluid. The volume of distribution (V_d) of a drug is computed by dividing the total amount of the ingested drug by the plasma concentration. Using a drug dose of 40 mg of 8-MOP (0.6 × 70) and a plasma level of 120 ng/ml, V_d would be greater than 300,000 ml, which means that very low concentrations of 8-MOP are expected to be found in the blood [19].

Furthermore, one must contend with the reality of the kinetics of the solubility process. To gain an appreciation of this process, a single oxsoralen ultra capsule was added to 500 ml of distilled water (at pH 3). To mimic any possible role of gastric agitation, the mixture was stirred gently. Complete dissolving of the contents of the capsule (10 mg) would yield a concentration of 20 μg/ml (10 mg in 500 ml). The
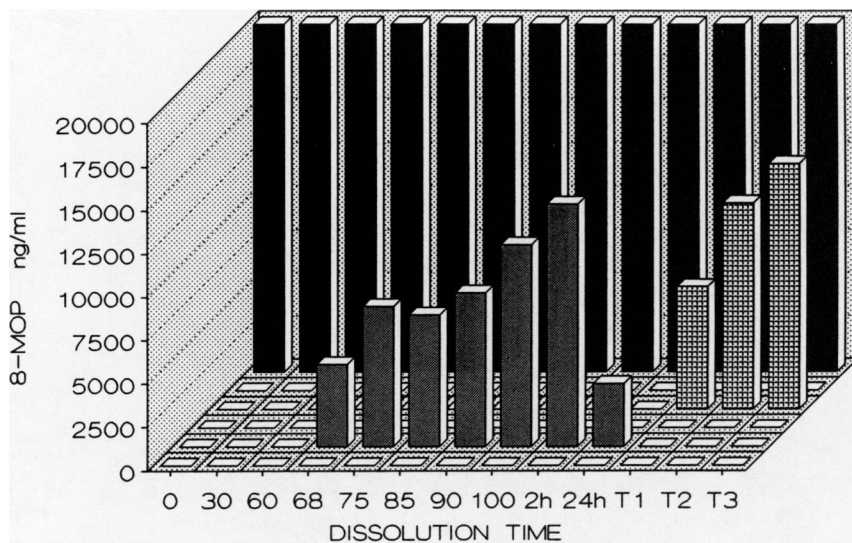
FIG. 9. Dissolution of oxsoralen ultra.

results of this experiment are portrayed in Fig. 9. The solution started out at room temperature, but with stirring the temperature gradually increased to 32°C over the first 30 minutes. To measure the amount of 8-MOP present, 1 ml samples were taken at the points in time indicated in Fig. 9 and immediately centrifuged; 50 μL was then applied to a phenyl reversed-phase column and analyzed as described above. The capsule did not rupture until 68 minutes had elapsed. At that point, the 8-MOP concentration was 4.6 μg/ml. Since it was anticipated that, once the capsule was open, the 8-MOP might rapidly dissolve, another sample was taken at 75 minutes, at which point the concentration was 7.5 μg/ml. Close visual inspection of the solution at this time revealed that it consisted of a suspension of small crystals. Stirring for another 145 minutes did lead to a final concentration of ~14 μg/ml, which was still 30 percent below the expected value. *The 8-MOP, which had been completely solubilized within the capsule, precipitated once it was exposed to an aqueous environment.* Visual inspection showed that constant stirring led to virtually complete dissolution. When the solution was allowed to sit without stirring until the next day and then tested, however, the concentration had dropped to 5.1 μg/ml. To test whether the insoluble material could be dissolved more rapidly, the solution was heated to 50°C and tested for its concentration at half-hour periods (indicated as T1, T2, and T3 in Fig. 9). Even under these highly idealized conditions, 90 minutes was required to redissolve the 8-MOP. In a separate trial, the contents of another capsule dissolved instantly in absolute ethanol.

Thermodynamics and kinetics are the factors governing the dissolution of 8-MOP and, no matter how the drug is prepared in a capsule, the bottom line is that it is an extremely insoluble material and hence will always be absorbed in an unpredictable manner. From this perspective, it is easy to see how depending on stomach contents, patient health, and perhaps a myriad of other parameters, a patient could end up with very different blood levels on different days.
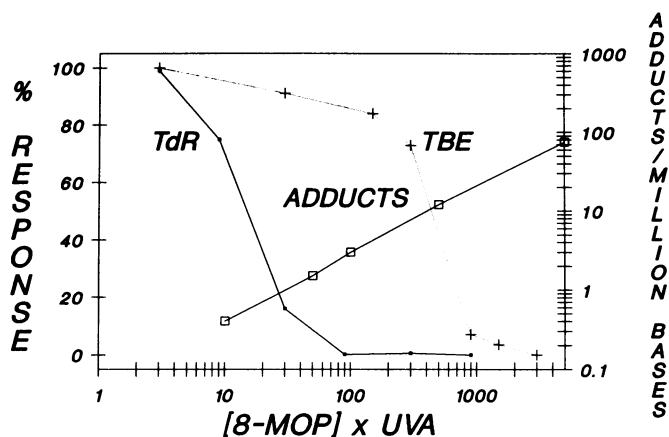
FIG. 10. Effects of 8-MOP and UVA on lymphocytes.

Effective UVA Dose During Photopheresis

The presence of red blood cells and plasma lead to a significant attenuation of the UVA dose that is delivered to lymphocytes during photopheresis. DNA treated with 100 ng/ml 8-MOP 1 J/cm² UVA results in the formation of 55 adducts per million bases. If lymphocytes suspended in PBS are treated with the same doses of 8-MOP and UVA, 4 adducts per million bases are formed—nearly a fourteenfold reduction in photoadduct yield. If red blood cells are present, there is another sixfold reduction in adduct formation (~1 adduct per million bases). *In vitro* studies of lymphocytes treated with 8-MOP and UVA showed that the formation of 1 adduct per million bases correspond to an effective UVA dose of 1–2 J/cm².

The *in vitro* dose-response effects of 8-MOP and UVA on lymphocytes are summarized in Fig. 10, in which the various properties are plotted versus the combined doses of 8-MOP (in ng/ml) and UVA (in J/cm²). Gasparro et al. have demonstrated that tritiated thymidine incorporation depended only on this product [22]. These data clearly indicate that the nuclear processes represented by tritiated thymidine incorporation (TdR in Fig. 10) are completely suppressed when the combined doses of 8-MOP and UVA reach 50. Another nuclear process for which there is a similar dose-response effect is the repair of 8-MOP photoadducts and the recovery of cells after 8-MOP/UVA treatment [Goldminz D, et al: manuscript in preparation]. The effects of 8-MOP and UVA on cell viability (TBE) are affected to the same extent only when the combined doses of 8-MOP and UVA reach 300–400. Trypan blue exclusion provides a measure of membrane integrity. Thus, the dose-response curves shown in Fig. 10 correspond to two extreme processes. In one case, low concentrations of 8-MOP and UVA induce relatively few 8-MOP photoadducts, which have a drastic effect on nuclear events (tritiated thymidine incorporation and repair of adducts, for example). At much higher concentrations, another cellular site is critically damaged, namely the cell membrane. Given the 8-MOP concentrations typically achieved in patients and the low effective UVA doses that are actually delivered during photopheresis, it is likely that most of the time lymphocytes are exposed to doses that fall in an intermediate region on this curve; that is, 100–200. Under these conditions, nuclear processes which control cell activity would be crippled. The cell membrane would remain intact, however, even though molecular modifications, which are the precursors for the

ultimate disintegration of the membrane, may begin to occur. These cells, when re-infused in a patient, would be able to circulate for a significant period of time. During this period, the immune system would have the opportunity to respond to changes that might have been induced at the cell surface. These changes could be any one of the following (or some combination): protein photoadducts [23], cell surface DNA modification (see below), and/or alteration of cytoskeleton rigidity (see below).

Although the biological effects of 8-MOP plus UVA on cells have usually been focused on genotoxic events, its successful use in photopheresis raises the question of whether these effects alone could be responsible. Does the photomodification of cell surface structures alter the immunological status of cells? In the next section, little-appreciated effects of 8-MOP and UVA on cellular components other than nuclear DNA are reviewed.

CELL SURFACE DNA AS A TARGET FOR 8-MOP AND UVA

One additional cellular target on which we have initiated studies is DNA bound to specific receptors [24] on the surface of human lymphocytes. The surface-bound DNA (cmDNA) is detectable using selective extraction methods as well as immunofluorescence assays. Bennett showed that DNA extracted from the cell membrane constitutes about 2 percent of total cellular DNA. The 260–280 absorbance ratio ranges from 1.48 to 1.60, which is significantly lower than that observed for nuclear DNA (1.8–2.0). cmDNA isolated from lymphocytes of healthy volunteers and from a cultured lymphoblastic cell line was 1.7 percent to 2.1 percent of all DNA with a 260/280 ratio of 1.48 [Dall'Amico R, Gasparro FP: manuscript in preparation]. At this time, the origin and the function of cmDNA are unknown; however, the observation that anti-DNA antibodies interact with the surface of cells from patients with systemic lupus erythematosus suggests a possible role in autoimmune diseases [25]. We have studied cmDNA because it is a potential target for modification by photoexcited psoralen molecules. The binding with psoralens may alter the antigenicity of cmDNA and lead to immunologically mediated events in patients treated with 8-MOP and UVA. cmDNA is photomodified by 8-MOP but to a lesser extent than chromosomal DNA [Dall'Amico R, Gasparro FP: manuscript in preparation]. Lymphocytes incubated for 40 minutes with 100 ng/ml of 8-MOP and exposed to 5 J/cm^2 at room temperature were found to contain 1.8 adducts per million bases in cmDNA and 15.9 in nuclear DNA. The same experiment performed at 4°C showed a greater number of adducts: 7 in cmDNA and 34.4 in nuclear DNA. Shortening the incubation time to only one minute at 4°C did not change the number of adducts in cmDNA (6 adducts/million bases) but halved the number in nuclear DNA (18 adducts/million bases). Interactions between cmDNA and proteins, which also might explain the low 260–280 ratio, may reduce its ability to be photomodified by psoralens. We have also used a highly specific monoclonal antibody (8G1) which was previously shown to be specific for 8-MOP 4′,5′-monoadducts to demonstrate that 8-MOP photoadducts occurred on the surface of lymphocytes [13].

TARGETS OTHER THAN DNA

Microspectrofluorometry of cells incubated with psoralens indicated that psoralen is distributed throughout the cell [26]. Although mutagenesis can be attributed to direct modification of DNA, many of these other biological phenomena cannot be linked to

DNA modification. Numerous studies support the idea that reactions with many other target sites account for a large number of these effects. Non-DNA effects of psoralens and UVA have been reviewed recently [27].

Joshi and Pathak have suggested that lipid oxidation is most likely responsible for the resultant erythema, inflammation, edema, and skin vesiculation [28]. More recently, however, Ortel and Gange have shown that sub-erythemic doses of UVA can be administered to skin [29]. After a 24-hour period which allowed the removal of any free 8-MOP, an additional UVA exposure induced erythema. This delayed effect was attributed to the formation of cross-links by persistent monoadducts formed by the initial exposure to UVA. Although many studies seem to support the idea that cytotoxicity, mutagenicity, and antiproliferative effects are more likely to arise from cycloaddition with pyrimidines in DNA, there is still some controversy about the possible role of other target molecules.

In our laboratory we have shown that high doses of 8-MOP and UVA lead to photomodification of membrane-bound proteins. In a polyacrylamide gel analysis of membrane proteins after treatment with 8-MOP and UVA, 15 radiolabeled bands were detected. No changes in band position were observed, which would suggest that protein-protein cross-links were not formed [Dall'Amico R: preliminary results].

Laskin et al. described a specific saturable, high-affinity binding site for psoralens on the membranes of HeLa cells [30]. It appears that 8-MOP photomodification of this psoralen receptor leads to an alteration of cell growth and differentiation. These investigators have proposed a model in which photoalkylation of the psoralen receptor modulates the epidermal growth factor receptor by inducing the phosphorylation of the latter.

Photomodification of lipid components may alter membrane fluidity and lead to changes in the nature of immunological recognition of membrane structures [31]. Such changes could play a role in the immune recognition of 8-MOP/UVA-modified cells.

NEW PHOTOPHARMACOLOGIC AGENTS

Today two major forms of photochemotherapy are being used clinically. Psoralen plus UVA, long used to treat dermatologic disorders such as vitiligo and psoriasis, is now being applied to cutaneous T-cell lymphoma (Heald et al. in this issue), scleroderma (Rook et al. in this issue), and rheumatoid arthritis (Edelson in this issue). In oncology, hematoporphyrin derivatives (HpD) form the basis of photodynamic therapy (PDT) for various solid tumors (e.g., esophageal, bladder, and so on [32]. Both of these modalities are based on clinical principles developed over the last two decades. Advances in molecular biology (specifically, the facile production of monoclonal antibodies and the convenient synthesis of oligonucleotides) are directing research for additional photochemotherapies in new and exciting areas. Monoclonal antibodies tagged with photoactivatable moieties offer the potential of specifically eliminating a discrete population of cells [33]. Limitless opportunities exist for therapies using antisense oligonucleotides coupled with photoactivatable groups ([34]; [Gasparro FP: submitted for publication]). The specificity of DNA base pairing is used to guide photoactivatable molecules to susceptible sites within specific genes. Once properly situated, a dose of UVA light is used to activate the chromophore and simultaneously to inactivate the gene. The ability to select the site of drug activity within a specific gene with the flick of an electrical switch clearly offers the potential for an infinite degree of specificity.

SUMMARY

As demonstrated by microspectrofluorometry, psoralens are ubiquitous molecules. Scattered throughout the cell as they are, they are potential modifiers of various biological entities when exposed to UVA radiation. Thus, it is not surprising that a panoply of effects would be observed in a cellular system. Dissecting the respective roles of these effects will ultimately lead to a deeper understanding of disease processes, which in turn can result in the development of more precise, scalpel-like photopharmacologic agents.

REFERENCES

1. Fitzpatrick TB, Pathak MA: Historical aspects of methoxsalen and other furocoumarins. J Invest Dermatol 32:229–231, 1959
2. Phyladelphy A: Zur Atiologie der wiesenpflanzen-(Bade) Dermatitis. Dermatol Woch Schr 92:713–715, 1931
3. Fahmy IR, Abu-Shady H: *Ammi majus linn:* Pharmacological study and isolation of a crystalline constituent, ammoidin. Q J Pharm Pharmacol 20:281–291, 1947
4. Lerner AB, Denton CR, Fitzpatrick TB: Clinical and experimental studies with 8-methoxypsoralen in vitiligo. J Invest Derm 20:299–314, 1953
5. Musajo L: Interessante proprieta della furcoumarine naturali. Farmaco 10:529–558, 1955
6. Musajo L, Rodighiero G, Dall'Acqua F: Evidence of a photoreaction of the photosensitizer furocoumarin with DNA and with pyrimidine nucleosides and nucleotides. Experientia 21:24–25, 1965
7. Parrish JA, Fitzpatrick TB, Tannenbaum L, et al: Photochemotherapy of psoriasis with oral methoxsalen and long wavelength ultraviolet light. N Engl J Med 29:1207–1211, 1974
8. Cole RS: Light induced cross-linking of DNA in the presence of furocoumarin (psoralen). Biochim Biophys Acta 217:30–39, 1979
9. Rodighiero G, Dall'Acqua F, Averbeck D: New psoralen and angelicin derivatives. In Psoralen-DNA Photobiology, Volume I. Edited by FP Gasparro. Boca Raton, FL, CRC Press, 1988
10. Kraemer KH, Waters HL, Ellingson OL, Tarone RE: Psoralen plus ultraviolet radiation induces inhibition of DNA synthesis and viability in human lymphoid cells in vitro. Photochem Photobiol 30:263–270, 1979
11. Gilchrest BA: Methoxsalen photochemotherapy for mycosis fungoides. Cancer Treat Rep 63:663–667, 1979
12. Edelson RL, Berger CL, Gasparro FP, et al: Treatment of cutaneous T-cell lymphoma by extracorporeal photochemotherapy. N Engl J Med 316:297–303, 1987
13. Santella RM, Dharmaraja N, Gasparro FP, Edelson RL: Monoclonal antibodies that recognize 8-MOP-modified DNA. Nucleic Acids Res 14:2533–2544, 1985
14. Song PS: Photoreactive States of Furocoumarins. Natl Cancer Inst Monograph 66. 1984, pp 15–19
15. Joshi PC, Wang SY, Midden WR, Voiturez L, Cadet J: Heterodimers of 8-methoxypsoralens and thymine. Photobiochem Photobiophys 8:51–60, 1984
16. Kanne D, Rapoport H, Hearst JE: 8-methoxypsoralen-nucleic acid photoreaction: Effect of methyl substitution on pyrone vs. furan photoaddition. J Med Chem 27:531–534, 1984
17. Papadopoulo D, Sagliocco F, Averbeck D: Mutagenic effects of 3-carbethoxypsoralen and 8-methoxypsoralen plus 365 nm irradiation in mammalian cells. Mut Res 124:287–297, 1983
18. Gasparro FP, Battista J, Song J, Edelson RL: Rapid and sensitive analysis of 8-methoxypsoralen in plasma. J Invest Dermatol 90:234–236, 1988
19. Craig CR, Stitzel RE: Modern Pharmacology. Boston, Little, Brown and Co, 1986
20. Brickl R, Schmid J, Koss FW: Pharmacokinetics and Pharmacodynamics of Psoralens after Oral Administration. Natl Canc Inst Monograph 66. 1984, pp 63–67
21. Hearst JE: Psoralen photochemistry and nucleic acid structure. J Invest Dermatol 77:39–44, 1981
22. Gasparro FP: Proceedings of the 10th International Symposium on Photochemistry. New York, Plenum, 1989
23. Veronese F, Schiavon O, Bevilacqua R, Bordin F, Rodighiero G: The effect of psoralens and angelicins on proteins in the presence of UV-A irradiation. Photochem Photobiol 34:351–354, 1981
24. Bennett RM, Gabor RT, Merritt MM: DNA binding to human leukocytes: Evidence for a receptor mediated association, internalization and degradation of DNA. J Clin Invest 76:2182–2190, 1985

25. Bennett RM, Kotzin BL, Merritt MM: DNA receptor dysfunction in systemic lupus erythematosus and kindred diseases. J Exp Med 166:850–863, 1987

26. Moreno G, Salet C, Kohen C, Kohen E: Penetration and localization of furocoumarins in single living cells by microspectrofluorometry. Biochim Biophys Acta 721:109–111, 1982

27. Midden WR: Chemical mechanisms of the bioeffects of furocoumarins. In Psoralen-DNA Photobiology, Volume I. Edited by FP Gasparro. Boca Raton, FL, CRC Press, 1988

28. Joshi PC, Pathak MA: Production of singlet oxygen and superoxide radicals by psoralens and their biological significance. Biochem Biophys Res Comm 112:638–646, 1983

29. Ortel B, Gange RW: An action spectrum for psoralen crosslink formation in human skin in vivo? J Invest Dermatol 92:495A, 1989

30. Laskin JD, Lee E, Yurkow EJ, Laskin DL, Gallo MA: A possible mechanism of phototoxicity not involving direct interaction with DNA. Proc Natl Acad Sci USA 82:6158–6162, 1985

31. Weinreb A, Deutsch M: Cancer diagnosis by fluorescence polarization. Abstracts. 10th meeting of the International Congress for Photobiology, Jerusalem, 1988

32. Gomer CJ, Dougherty TJ: Determination of [^{3}H]- and [^{14}C]-hematoporphyrin derivative distribution in malignant and normal cells. Cancer Res 39:146–151, 1979

33. Oseroff AR, Ara G, Ohuoha D, Aprille J, Bommer JC, Yarmush ML, Foley J, Cincotta L: Strategies for selective cancer photochemotherapy: Antibody-targeted and selective carcinoma cell photolysis. Photochem Photobiol 46:83–96, 1987

34. Smith CC, Aurelian L, Pawameswara Reddy M, Miller PS, Ts'o POP: Antiviral effect of an oligo(nucleoside methylphosphonate) complementary to the splice junction of herpes simplex virus type 1 immediate early pre-mRNAs 4 and 5. Proc Natl Acad Sci USA 83:2787–2791, 1986

35. Gasparro FP: Psoralen-DNA interactions: Thermodynamics and photochemistry. In Psoralen-DNA Photobiology, Volume I. Edited by FP Gasparro. Boca Raton, FL, CRC Press, 1988