8-Methoxypsoralen and Longwave Ultraviolet Irradiation Are a Novel Antiproliferative Combination for Vascular Smooth Muscle

Keith L. March, MD, PhD; Brian L. Patton; Robert L. Wilensky, MD; and David R. Hathaway, MD

Background. Smooth muscle cell proliferation plays a major role in the genesis of restenosis after angioplasty or vascular injury. Although the effects of arterial exposure to high-energy radiation sources such as laser have been investigated in detail, the effects on vascular cells of low-intensity radiant energy in combination with phototoxic agents have not been extensively characterized. Psoralens are phototoxic agents that are known to be well tolerated when used in conjunction with local exposure to ultraviolet light in the A band (UVA) for the treatment of various dermatologic proliferative disorders.

Methods and Results. We have investigated the effects of psoralen/UVA (PUVA) exposure on the proliferation of bovine aortic smooth muscle cells. Proliferation and viability were assessed over a 14-day period by trypsin blue exclusion counts. Cell cycle effects were evaluated by thymidine incorporation and flow cytometry with DNA quantitation after addition of serum or platelet-derived growth factor B-chain (PDGF-BB) to subconfluent cells synchronized by serum withdrawal. No effect was observed after exposure to 8-methoxypsoralen (8-MOP) at concentrations up to 10 μM or UVA irradiation at energies up to 2.5 J/cm². Longwave ultraviolet light and 8-MOP were found to behave synergistically as potent inhibitors of DNA synthesis in bovine aortic smooth muscle cells with the EC₅₀ in combination ranging from 7 μM at 0.35 J/cm² to 0.2 μM at 2.1 J/cm². Similar antiproliferative effects were obtained by an inverse variation of dose and energy delivered. After serum stimulation, inhibition of DNA synthesis was found with either an immediate or delayed (16-hour) application of PUVA. This effect was independent of subsequent 8-MOP washout. Flow cytometry of cells treated with PUVA at several times after serum stimulation demonstrated for each time point a block in further cell cycle progression for cells in all phases of the cell cycle. Evaluation of [¹²⁵I]-labeled PDGF and epidermal growth factor (EGF) binding revealed no effect of PUVA on the apparent number or affinity of PDGF binding sites present but did reveal a dose-dependent inhibition by PUVA of EGF binding. This inhibition of EGF binding occurred increasingly at higher PUVA doses than the cell cycle inhibition and accordingly did not appear to represent a critical mechanism for the antiproliferative effect. Cell counting after a single exposure to PUVA (1 μM, 1.5 J/cm²) revealed complete stasis of cell proliferation over a 28-day period without recurrent exposure. No increase in trypsin-positive cells was noted over this period.

Conclusions. PUVA treatment represents a novel method for locally inhibiting proliferation of vascular smooth muscle cells without producing cytolsis. (Circulation 1993;87:184–191)

Key Words • restenosis • proliferation • cytostasis • photoactivation

Since its introduction, percutaneous transluminal angioplasty has been used for the nonsurgical recanalization of atherosclerotic blood vessels with an increasingly high rate of procedural success.¹ Unfortunately, the long-term benefit after percutaneous transluminal angioplasty is significantly limited by the recurrence of vascular occlusion or restenosis at the site of the angioplasty that is seen in approximately 30–40% of recanalized vessels in the 6 months after the procedure.²,³ The primary cause of the restenotic lesion has been identified as an increased mass of vascular smooth muscle cells and surrounding extracellular matrix resulting from markedly accelerated proliferation and protein synthesis by these cells.⁴ It has been recognized that the stimuli for this proliferation, which include both local increases of stimulatory factors and decreases in inhibitory factors, originate from multiple sources over an extended period of time after the initial vascular injury.⁵ This suggests that effective treatment to modulate this response would include either protracted exposure to active agents or use of agents with extended effects.

In conjunction with the need for sustained antiproliferative effect, an additional prerequisite for successful treatment is limitation of systemic activity either

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From the Krannert Institute of Cardiology, Indianapolis, Ind.; the Department of Medicine, Indiana University School of Medicine, Indianapolis; and Richard L. Roudebush Veterans Administration Medical Center, Indianapolis.

Supported in part by grants from the National Institutes of Health, the American Heart Association, and the Herman C. Krannert Fund.

Address for correspondence: Keith L. March, MD, PhD, Krannert Institute of Cardiology, 1111 West 10th Street, Indianapolis, IN 46202-4800.

Received February 18, 1992; revision accepted September 15, 1992.
achieved by superior specificity of the pharmacological agent used or localized delivery of therapy. Several mechanical methods for the local application of active pharmacological agents at the site of potential restenosis have been described. Local application of formulations intended for sustained release has been described as a method to achieve this goal. An alternative approach to localization of therapy is the restricted activation of a systemically administered drug that possesses low intrinsic activity before its activation. The hematoporphyrins have been described as a set of compounds potentially useful in this manner using photoactivation to achieve efficacy at the desired location. Unfortunately, the cutaneous photosensitivity seen for up to several weeks after the administration of these compounds renders their widespread clinical use potentially cumbersome. Derivatives of psoralens (furocoumarins) have achieved substantial clinical utility in the photoactivated inhibition of the hyperproliferation associated with psoriasis and cutaneous T cell lymphoma. In contrast to the hematoporphyrins, psoralens exhibit approximately 90% excetration within 12 hours of administration, rendering phototoxicity minimal after this time period.

This study characterizes the ability of 8-methoxypsoralen (8-MOP) in conjunction with ultraviolet light in the A range (UVA) to inhibit the growth of vascular smooth muscle cells in culture.

Methods

Cell Culture

Bovine aortic smooth muscle cells (BASMCs) were obtained by outgrowth from medial explants of thoracic aortas of cows within 4 hours of slaughter. Initial outgrowth as well as standard maintenance growth medium was Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS) added. Media were renewed every 2–3 days. All growth was in a humidified incubator equilibrated with a 5% CO₂ atmosphere. Cultures were passaged immediately before full confluence by brief exposure to Hank’s balanced salt solution (HBSS) containing trypsin (0.5 mg/ml) and ethylenediaminetetra-acetic acid (EDTA) (0.5 mM); all experiments were performed using cells of passage seven or less. Cells were counted and assessed for trypan blue exclusion with a hemocytometer at each passage and at selected times during time-course experiments, routinely showing >95% of the population to exclude trypan blue. Most subcultures and all experiments were plated at a density of 10,000 cells/cm² regardless of container.

These cells exhibited typical morphological characteristics of vascular smooth muscle in vitro including a pattern of variably multilayered growth and demonstrated specific immunoperoxidase staining by a monoclonal antibody selective for muscle α-actin (HHF-35), which did not stain endothelial cells or fibroblasts.

[³H]-Thymidine Incorporation

Cells were seeded as above in 24-well microtiter plates and allowed to attach overnight, after which the cultures were washed with phosphate-buffered saline (PBS) and placed in serum-free medium consisting of DMEM with 1 μM insulin and 5 μg/ml transferrin added for a total of 48–52 hours (more than one doubling time for actively cycling cells). Serum-free medium was then removed and replaced by DMEM with 10% FBS or else platelet-derived growth factor B-homodimer (PDGF-BB) was added to a final concentration of 10 ng/ml. 8-MOP was added at variable concentrations and times as described in dimethyl sulfoxide (DMSO) carrier to a final DMSO concentration of <0.015 vol%. At the indicated times relative to serum or PDGF stimulation, cells were exposed to UVA; this exposure began after at least 30 minutes of 8-MOP treatment, a time period more than sufficient for diffusion of 8-MOP into cells. 8-MOP was removed when indicated. [³H]-Thymidine was added 18 hours after serum repletion to a concentration of 2 μCi/ml, and cells were incubated for 6 additional hours or as described in the figure legends. At the end of the incubation, cells were released from the wells, and unincorporated precursor was removed by washing with distilled water cell residues collected on a glass mesh filter by an automated cell harvester. Radioactivity was measured by liquid scintillation spectroscopy.

Light Source and Irradiation

Irradiation was performed at 22°C on monolayer cultures with lids removed, using a light source sensitizer of a TM-36 bank of four T8 15-W bulbs filtered to eliminate UVB radiation (UV Products). The flux rate was measured at the media plane using a UVX-36 digital radiometer (UV Products) and was typically 1.2 mW/cm². Control cells were placed in the field of irradiation with opaque covers (sham-irradiated) for a length of time equivalent to that for cells receiving the maximum UVA exposure for each experiment (e.g., 21 minutes for a total of 1.5 J/cm²). Cells designated to receive shorter exposure times (e.g., 7 minutes for a total of 0.5 J/cm²) were likewise covered after exposure but maintained in the environment until all irradiations were complete. No detectable heat was produced during irradiation. After irradiation, cultures were incubated as indicated for each experiment.

Flow Cytometric Assays

Cells were plated, allowed to attach, and placed in serum-free medium for 48–52 hours as described above. 8-MOP was added at the designated concentrations in either serum-free medium or serum-containing medium followed by UVA irradiation at various times, also as above. At the specified times after serum addition, these cells were harvested by trypsin/EDTA, washed with HBSS/5% bovine serum albumin (BSA), pelleted, and resuspended in PBS. For single-color analysis, the PBS contained 0.6% NP-40 and 0.1 mg/ml propidium iodide, to which was added RNAse to a final concentration of 2 mg/ml. Flow cytometric analyses were done using a Becton-Dickinson FACScan exciting at 488 nm and sensing at 585 nm. Events were gated on a fluorescence area versus fluorescence width map to eliminate potential clumped nuclei. Such events represented less than 3% of the total in general.

Growth Factor Binding Assays

Cells were seeded as above in 24-well microtiter plates and allowed to grow to confluence in DMEM/10% FBS (approximately 5–7 days). The cell layers were
then exposed to 8-MOP for 30 minutes at 1 μm followed by UVA radiation of the total energy input indicated at room temperature. Binding assays were immediately performed at 4°C. Cells were washed twice with DMEM/1% BSA and then incubated for 4.5 hours with rocking in DMEM/1% BSA containing [\textsuperscript{35}S]-PDGF-BB (human) at the depicted concentrations. Parallel wells were incubated with an additional 100-fold excess of unlabeled PDGF-BB. Binding was terminated by washing cells four times with DMEM/1% BSA, and cells were harvested in 1 ml 1% Triton X-100/0.1% BSA. Radioactivity of this material was measured by gamma counting, and specific binding was taken as the difference between binding found without and with excess cold competitor.

Epidermal growth factor (EGF) binding experiments were performed in a similar fashion except that the binding incubation was performed in DMEM/25 mM N-(2-hydroxyethyl)piperazine-N’-2-ethanesulfonic acid (HEPES) (pH 7.2)/0.1% BSA containing [\textsuperscript{35}S]-EGF (human) for 2 hours, and harvesting was performed in 0.2 M NaOH.

[\textsuperscript{35}S]-Amino Acid Incorporation

Cells were seeded in 24-well microtiter plates and synchronized by serum deprivation as above. Serum was added to 10% at the time of psoralen/UVA (PUVA) treatment, also as above. At 22 hours after serum addition, the medium was switched to methionine-free serum, to which \textsuperscript{35}S-labeled amino mixture (Translabel) had been added to an activity of 2 μCi/ml, and cells were incubated for 2 additional hours. At the end of labeling, cells were released from the wells, and unincorporated precursor was removed by washing with normal saline. Cell residues were collected on a glass wash filter by an automated cell harvester. Radioactivity was measured by liquid scintillation spectroscopy.

Results

DNA Synthesis and Cellular Proliferation

BASMCs obtained as above have been characterized with respect to their synchronization by serum deprivation and their cell cycle behavior upon serum repletion.\textsuperscript{12} It has been found that after a 48-hour exposure to serum-free medium as above, no \textsuperscript{3}H incorporation into DNA is present, and approximately 90% of cells have a 2n DNA complement consistent with location in the \textit{G}_0 or \textit{G}_1 phase of the cell cycle. Synchronous emergence of cells into the S-phase beginning about 18 hours after serum repletion is evident by flow cytometry using quantitative DNA staining with propidium iodide and by \textsuperscript{3}H-thymidine incorporation. The effect of UVA radiation and 8-MOP exposure on cycling in these cells was characterized by using protocols in which synchronized subconfluent cells were treated with 8-MOP starting 1 hour before serum addition. These cells were subsequently exposed to variable doses of UVA irradiation either one-half hour before or 17 hours after serum addition. The serum addition was performed either with or without a concomitant medium change and consequent washout of remaining 8-MOP. The combination of 8-MOP and UVA was seen to inhibit \textsuperscript{3}H-thymidine incorporation, and this inhibitory effect was found to be insensitive to the variation in PUVA timing relative to serum stimulation as well as to the continued presence or absence of 8-MOP in solution (Figure 1). Additional experiments (data not shown) confirmed essentially equivalent inhibition achieved by PUVA treatment at the time preceding serum addition by up to 30 hours. No inhibition of thymidine incorporation was detectable after exposure to either 10 μM 8-MOP or UVA at 2 J/cm\textsuperscript{2} given individually. Accordingly, subsequent experiments were performed in synchronized cell populations by routine addition of 8-MOP to the desired concentration at the time of FBS addition to 10% and administration of ultraviolet light at the time desired for the effect.

The effect of variable combinations of 8-MOP concentrations and UVA energy density on DNA synthesis in synchronized cells was evaluated with administration of ultraviolet light 30 minutes after addition of 8-MOP concentration. Figure 2A depicts the results as a function of 8-MOP concentration. It is apparent that similar inhibition of DNA synthesis may be achieved by inverse variation of the psoralen dose and light energy used. The EC\textsubscript{50} values for 8-MOP at the tested irradiance levels were 0.3 μM at 1.5 J/cm\textsuperscript{2}, 2.5 μM at 0.68 J/cm\textsuperscript{2}, and 5 μM at 0.34 J/cm\textsuperscript{2}. Figure 2B shows this data as a function of radiant energy delivered. A nearly linear variation in DNA synthesis inhibition with UVA energy is seen. Similar effects were seen in DNA synthesis as stimulated by PDGF-BB (data not shown).

The strongly synergistic interaction between 8-MOP and UVA radiation is illustrated by the upward concavity of curves depicting complementary pairs of values.
yielding equivalent inhibition of [3H]-thymidine incorporation (Figure 3).

Cellular proliferation and viability over time were studied in unsynchronized subconfluent populations exposed to 1 or 10 μM 8-MOP and 1.5 J/cm² UVA radiation alone or in combination (Figure 4). Consistent with the lack of observed effect on thymidine incorporation, exposure to either drug or light alone in these dosage ranges resulted in no detectable effect on either initial experimental proliferation or plateau density relative to controls. 8-MOP at 1 μM with UVA at 1.5 J/cm² followed by 8-MOP washout resulted in complete stasis of growth over a 14-day period in the absence of any increase in trypan-positive cells or noticeable cell detachment. However, 8-MOP at 10 μM with UVA at 1.5 J/cm² followed by 8-MOP washout produced cell

**FIGURE 3.** Isobologram depicting curves of equal inhibition of [3H]-thymidine incorporation by various 8-methoxypsoralen (8-MOP)/ultraviolet light (A band) (UVA) combinations in synchronized subconfluent bovine aortic smooth muscle cells. EC₅₀ and EC₈₀ values are calculated as 50% or 80% inhibition relative to untreated controls. The upward concavity of these curves reflects the strong interdependence of 8-MOP and UVA exposure for inhibitory effect.

**FIGURE 4.** Plot of synergistic effect of 8-methoxypsoralen (8-MOP) and ultraviolet light (A band) (UVA) exposure on proliferation of bovine aortic smooth muscle cells. Cells were seeded at 10,000 cells/cm² and allowed to attach for 12–24 hours. Cells were then exposed to 8-MOP at the indicated concentrations followed in 30 minutes by exposure to UVA radiation at 1.5 J/cm² where indicated and subsequent 8-MOP washout. Cell counts were subsequently performed at intervals on duplicate samples and are represented as averages, with error bars depicting SEM. No increase in trypan-positive cells was seen after treatment with 8-MOP at 1 μM and UVA at 1.5 J/cm², but 8-MOP at 10 μM with UVA at 1.5 J/cm² produced cell lifting and death detectable over several days after treatment.
lifting and death ensuing over several days after treatment. The observation of a prolonged cytostatic effect and minimal cytotoxicity with the dose combination (1 μM, 1.5 J/cm²) was repeated in several experiments with smooth muscle cells obtained from different animals and at differing passages as well as in experiments with cells synchronized by serum deprivation before dosing. Serial observations of treated cells revealed preservation of microscopic morphology and attachments to the substrate (Figure 5) while maintaining a subconfluent density equivalent to that at the time of treatment. No mitotic figures were present. Untreated control cultures plated at the same time and density are shown to have reached confluence at the time of the photograph. Flow cytometry was performed to evaluate the DNA content distribution of cells treated with this cytostatic regimen after 14 days of incubation (Figure 6). This revealed substantial numbers of cells with DNA content consistent with location in the S and G₂/M compartments as might be seen for subconfluent cells in exponential growth, suggesting the possibility of cell cycle arrest in all cell cycle phases at the time of initial treatment.

**Cell Cycle Block by 8-MOP and UVA Irradiation**

The location of cell cycle block caused by the cytostatic combination of 8-MOP and UVA was further evaluated in subconfluent populations of BASMCs synchronized by serum deprivation as described in “Methods.” Initiation of the G₂/G₁→S phase transition by addition of FBS to 10% was accompanied by addition of 8-MOP to 1 μM or 10 μM. Cultures were then exposed to UVA totaling 1.5 J/cm² at 0, 18, 21, 24, or 27 hours after serum addition and harvested for flow cytometric analysis at 18, 21, 24, 27, or 30 hours after serum addition to monitor changes in cell cycle distributions in each treatment group at 3-hour intervals (Figure 7). The control population (left column) shows synchronized entry into and through the S-phase into the G₂/M compartment by 30 hours after serum addition. It was found that PUVA treatment but not 8-MOP alone blocks cell cycle progression at or near the point at which the cells are subjected to the treatment. At the time of serum addition, this is a point before S-phase entry; at subsequent times after serum addition, populations of cells exhibit block in S-phase. This is manifest as lack of substantial change within each treatment group of cells with time from stimulation (Figure 7, columns) in comparison with the control column. Instead, the histograms for cells treated with PUVA at
these doses parallel the findings in the control population at the time of treatment (10 μM and 1.5 J/cm² shown; 1 μM and 1.5 J/cm², essentially the same, not shown).

**Effects of 8-MOP and UVA Irradiation on Growth Factor Binding and Protein Synthesis**

To evaluate the possible role of PUVA-mediated modulation of growth factor binding and consequent transmembrane signaling in the ability of PUVA to inhibit proliferation, the effects of PUVA on equilibrium binding of PDGF-BB and EGF to BASMCs were studied. These two factors are derived both from platelets and cells of the arterial wall and represent important mitogens for arterial smooth muscle cells.

Confluent cultures of BASMCs were treated with 1 μM 8-MOP and 1.5 J/cm² UVA independently or in combination, and PDGF-BB or EGF binding was determined. Less than a 5% change in apparent receptor number or affinity was observed after this combined PUVA treatment dose known to be effective in inhibiting proliferation. Likewise, no effect was detected upon exposure to either UVA or 8-MOP alone at these doses. Further experiments were performed using 1 μM 8-MOP and a UVA dose titration to 4 J/cm², but no change in PDGF-BB binding was noted (data not shown). A similar study of EGF binding, however, revealed an inhibition of specific EGF binding by UVA either alone or in conjunction with 8-MOP exposure: This inhibition demonstrated UVA dose dependence between 1 and 4 J/cm², with an approximately 25% decrease in EGF binding noted at 4 J/cm².

Incorporation of 35S-methionine and -cysteine into total protein upon entry into the cell cycle was studied with relation to PUVA treatment in a population of smooth muscle cells synchronized as above. During labeling from 22 to 24 hours after serum stimulation, protein synthesis was found to be increased approximately twofold relative to the baseline without serum addition. This increase was not prevented by the treatment of cells with 8-MOP concentrations of 1 μM or less with UVA energy of 1.5 J/cm² at the time of serum stimulation. It was prevented, however, by treatment with a dose combination (10 μM, 1.5 J/cm²) at the same time (data not shown).

**Discussion**

This study describes the ability of relatively low-intensity light in the ultraviolet A band to inhibit smooth muscle proliferation in vitro in the presence of the photoactive agent 8-MOP. The protracted cytostatic effect of intermediate doses of psoralen and ultraviolet treatment suggests that this combination might be used effectively as a single-dose treatment approach to the
inhibition of neointimal proliferation after arterial injury in vivo, provided that a mechanism could be developed for the delivery of ultraviolet light of the desired intensity to the cells that give rise to the proliferative lesion. The specifically cytostatic effect of this treatment with preservation of long-term cellular viability represents a theoretical advantage over therapies that would use cytotoxicity as the means to inhibition of cellular proliferation. This is so because actual destruction of cells comprising the arterial wall might be expected to predispose to loss of mechanical integrity of the wall and subsequent aneurysmal dilation or perforation. Additionally, the release of intracellular contents upon necrosis would form a stimulus for a local inflammatory and thrombotic response with associated cytokine release and possible exacerbation of subsequent cellular proliferation.

The excellent tolerance of 8-MOP in current clinical use for psoriasis or other dermatologic diseases and the lack of danger of tissue perforation by light energies in the ranges used supports the feasibility of this combination of therapies for potential intravascular use. This is particularly so because the typical peak serum concentration of 8-MOP after standard dosing for dermatologic applications ranges from 1 to 2 μM, exactly the midrange of doses found to function as cytostatic in these studies. Although administration of hematoporphyrin compounds and subsequent photoactivation have been described in the vasculature of animals and in smooth muscle culture, systemic use of these drugs is limited by cutaneous photosensitivity lasting for up to several weeks after their use. Accordingly, their use has generally been restricted to the experimental treatment of certain malignant diseases.

The observed dependence of the inhibition of thymidine incorporation on 8-MOP concentration as modulated in a linear fashion by the intensity of subsequent ultraviolet radiation is consistent with observations in other systems showing a direct relation of 8-MOP adduct formation to 8-MOP concentration and the total energy of light exposure. This in turn suggests that the inhibition of DNA synthesis is related directly to the density of cross-links or adducts. A threshold phenomenon for the inhibition of subsequent proliferation by such cross-links has been demonstrated in which a density of about one 8-MOP cross-link per 300,000 base pairs is required for such inhibition. Doses resulting in lower frequency of adduct formation have been seen to slow cell cycle progression and induce cell cycle–specific block in the G2 phase. Eventual repair of DNA-based injury of this more limited degree apparently allows for renewed cellular proliferation. The mechanism of cell cycle specificity of block at lower doses is not clear but may involve inhibition of chromosomal disjunction. On the other hand, the phase-non–specific inhibition of cell cycle progression observed in an intermediate dosage range may result from irreparable inhibition of semiconservative DNA replication in addition to chromosomal disjunction.

The cellular mechanisms involved in the cytotoxic effect observed at higher doses of PUVA treatment have not been clarified. It has been suggested that certain effects of PUVA treatment might be explained by interactions with proteins or other molecules distinct from those interactions primarily involving DNA. For example, the release of diacylglycerol by UVA light has been demonstrated to result in the activation of protein kinase C with subsequent phosphorylation and inactivation of the EGF receptor. Psoralens potentiate an effect of UVA on the EGF receptor leading to inhibition of EGF binding. A psoralen binding protein demonstrating saturable, high-affinity binding sites has been described, but its role in these effects is not yet known. Protein synthesis inhibition has also been reported to occur at PUVA doses higher than those required for DNA synthesis inhibition in mammalian cells. The effects of PUVA exposure on EGF and PDGF binding as well as protein synthesis were accordingly studied in BASMCs. Our findings support the existence of such effects in high-dosage ranges of ultraviolet light as indicated by a progressive inhibition of EGF binding seen in smooth muscle cells as ultraviolet energy dosage increases with slight additional inhibition in the presence of 8-MOP. However, this effect is relatively minor in the dose range of 1 μM 8-MOP, 1.5 J/cm², which was found to be cytostatic for BASMCs, and it therefore seems unlikely to contribute substantially to the inhibition of proliferation found at this dosage. Because of the key role perceived for PDGF as a mitogen for smooth muscle cells, the possibility of a similar inhibition of its binding as a result of PUVA exposure was evaluated, but no evidence of such an effect was seen in the dosage range used. This is consistent with the suggestion that activation of protein kinase C by diacylglycerol released by UVA light may be responsible for the inactivation of the EGF receptor by phosphorylation since comparable protein kinase C–mediated inactivation is not a prominent feature in the regulation of the PDGF receptor. Measurement of BASMC protein synthesis after PUVA exposure revealed no inhibition at cytostatic doses but showed measurable suppression in the cytotoxic dosage range. The mechanisms involved in this inhibition have not been defined but may relate to the cytotoxicity observed in the high-PUVA dosage range distinct from that causing cytostatic effects.

One possible limitation to the application of this technique to modulation of the in vivo vascular proliferative response is suggested by the prolonged nature of the observed cytostatic effects. It is a possible concern that the treated cells will gradually die some time after the initial treatment, although any such gradual cell loss would probably be less undesirable than more widespread cytolyis after generally cytotoxic regimens. Cell ingrowth to replenish such damaged cells may in fact take place from the lateral boundaries of the treated area, where ultraviolet exposure would not have occurred. The processes of DNA repair could also allow for the eventual return of proliferative capacity. Such issues will best be addressed by in vivo experiments evaluating the effects of PUVA exposure on induced vascular proliferation by using the general parameters of dosage validated in cell culture. Such experiments are currently in progress.

Conclusions

In primary cultures of BASMCs, the combination of 8-MOP and UVA radiation has been found to inhibit DNA synthesis synergistically. It is possible with this combination to achieve stasis without cell death over a
2-week period. Such stasis occurs without clear dependence on the growth stage of the cells at the time of treatment. Local illumination of an atherosclerotic plaque at the time of angioplasty after psoralen administration may represent a novel approach to modulation of smooth muscle cell proliferation.

Acknowledgments

The graphics support of Phil Wilson and technical support of Denise Ford are appreciated.

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8-Methoxypsoralen and longwave ultraviolet irradiation are a novel antiproliferative combination for vascular smooth muscle.
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Circulation. 1993;87:184-191
doi: 10.1161/01.CIR.87.1.184
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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