Lasers in Surgery and Medicine 20:111–118 (1997)

Cell Damage Induced by Angiovist-370 and 308nm Excimer Laser Radiation

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Background and Objective: **Radiographic contrast media containing iodine-labeled organic compounds can be present in the irradiated field during laser angioplasty using 308 nm excimer laser radiation. These compounds absorb light at 308 nm and may undergo photochemical reactions that produce products that damage cells.**

Study Design/Materials and Methods: **This study was undertaken to determine whether photoproducts that damage human lymphocytes in vitro are formed when Angiovist 370 (AV), a contrast medium containing triiodinated aromatic compounds, is exposed to 308 nm radiation.**

Results: **The absorption spectrum of AV developed a new peak at 360 nm that extended to wavelengths greater than 500 nm when dilute AV solutions were exposed to 308 nm radiation indicating that photoproducts were formed. Irradiating dilute AV solutions above a layer of human lymphocytes caused a dose-dependent decrease in thymidine incorporation using fluence rates between 5.2×10^6 and 1.0×10^8 W/cm^2. Decreased DNA synthesis was independent of the pulse length (10 ns vs. 230 ns) but was lower, at a given dose, when the highest fluence rate was used. Incubation of lymphocytes with preirradiated AV solutions also decreased incorporation of thymidine in a radiation dose-dependent manner. The cell damaging photoproducts in preirradiated AV solutions were unstable; within 15 min, the effectiveness had decreased by ~85%.**

Conclusions: **These results indicate that exposure of AV to 308 nm excimer laser radiation produces photochemical products that damage human cells in vitro. Lasers Surg. Med. 20:111–118, 1997.**

Key words: laser angioplasty; radiographic contrast media; cytotoxic photoproducts

INTRODUCTION

Excimer laser radiation at 308 nm is used currently for ablation of plaque in arteries [1,2]. To date, over 5,000 patients have had atherosclerotic coronary lesions treated with excimer laser angioplasty. In this procedure, tissue is exposed to multiple 230-ns long 308 nm excimer laser pulses. Typical fluences out of the laser catheter tip range from 35 to 80 mJ/mm^2 and pulses are generally delivered at 20 to 30 Hz. The peak fluence rates incident onto the plaque surface are 3×10^7 W/cm^2. During ablation, the catheter is advanced at a rate of 1 to 2 mm per 3 sec and multiple (1 to 5) passes are usually made. Based on these numbers, an estimate on the delivered dose ranges from 1.5 J to 25 J for a 10 mm-long lesion.

During laser angioplasty using a 308 nm ex-

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Contract grant sponsor: NIH; Contract grant number: ROI GM 30755; Contract grant sponsor: Medical Free Electron Laser Program under ONR; Contract grant number: N00014-86-K0117.

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Accepted for publication 8 February 1996.

cimer laser, radiographic contrast media is used to visualize the artery by angiography. Without special precautions, the contrast media can be present during the laser procedure. In fact, until recently, excimer laser angioplasty was frequently performed with contrast media in the field of excimer laser energy delivery at the catheter tip. We have reported preliminary results on the adverse interactions of excimer laser energy with iodinated contrast describing high pressure shock wave transients [3] as well as the potential for producing toxic photoproducts [4].

All commonly utilized radiographic contrast media including Hexabrix, Isovue, Renographin, and Angiovist, have nearly identical absorption spectra and are likely to have similar interactions with 308 nm light [5]. These compounds absorb at 308 nm and are likely to undergo carbon-iodine bond cleavage since photons at 308 nm possess greater energy (93 kcal/mol) than typical carbon-iodine bonds (57 kcal/mol). Cleavage of carbon-iodine bonds produces free radicals and, ultimately, stable photoproducts [6] both of which can be toxic to cells. Iodinated aromatic drugs, in particular amiodarone, are well known to cause cutaneous phototoxicity in patients exposed to sunlight [7]. The underlying chemical mechanism for the phototoxicity of amiodarone is believed to involve cleavage of carbon-iodine bonds by the ultraviolet wavelengths in sunlight [8,9].

This study was undertaken to determine whether stable photoproducts or unstable intermediates produced by 308 nm irradiation of radiographic contrast media caused a decrease DNA synthesis in mammalian cells. Angiovist-370, which contains triiodinated aromatic compounds (Fig. 1), was chosen as the contrast media to be studied. Photosensitization of human lymphocytes by 308 nm excimer laser irradiation of Angiovist was investigated.

MATERIALS AND METHODS

Angiovist-370 (AV) is a commercially available radiographic contrast medium containing diatrizoate meglumine (660 mg/ml), diatrizoate sodium (100 mg/ml), and calcium disodium EDTA (0.1 mg/ml). The preparation was obtained from Berlex Imaging Inc. and contained 370 mg/ml of inorganic iodine. AV was diluted 1:20 with Hank's buffered saline (HBS; GIBCO, Grand Island, NY) for irradiations.

Cell Preparation

Lymphocytes were prepared using a standard clinical laboratory protocol [10]. Briefly, after obtaining informed consent, blood was collected from human volunteers in heparin free of preservatives, diluted 1:3 in HBS, placed on a Ficoll-hypaque density gradient (Pharmacia, Inc., Piscataway, NJ), and centrifuged at 400*g* for 30 min at 25°C. The separated lymphocytes were collected from the interface, washed three times with HBS, then suspended in HBS at a concentration of 2.5×10^6 cells per ml determined with a Coulter counter (model ZF, Coulter Electronics, Inc., Woburn, MA).

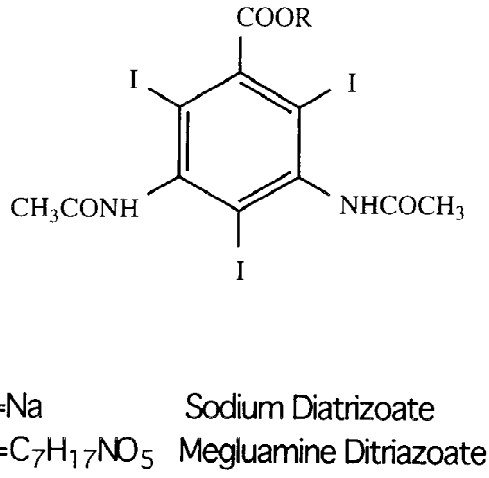

Fig. 1. Structure of diatrizoate salts in Angiovist-370.

Laser Irradiation

Irradiations were performed with a laboratory excimer laser (EMG-103-MSC, Lambda-Physik, Acton, MA) and a clinical excimer laser system (Dymer 200, Advanced Interventional Systems, Irvine, CA). The laboratory laser emits 308 nm, 10-ns pulses at 100 Hz. The laser beam was focused with a 18 cm focal length lens and reflected down perpendicular to the table with a 308-nm laser mirror (Acton Research Corp, Acton, MA). The sample was placed approximately 10 cm away from the lens giving a 0.1 cm × 0.4 cm rectangular irradiation field (area = 0.04 cm^2). The pulse energy was measured with a calibrated pyroelectric detector (J25, Molectron Detector, Inc., Campbell, CA). The energy was varied using a series of CaF_2 optical windows which attenuate the beam by approximately 30% per window. Fluence rates of 5.2×10^6, 1.0×10^7, and 1.0×10^8 W/cm^2 were used.

The clinical excimer laser emits 308 nm, 230 ns pulses at 20 Hz and its output was delivered to the sample via a 300 μm fused silica optical fiber.

The output end of the 0.22 NA fiber was held 4 mm from the sample giving a 0.13 cm diameter irradiation field (area = 0.013 cm^2). The pulse energy was determined with an internal energy meter supplied with this laser system. The output energy was set to 30 mJ/pulse which equates to a fluence of 23 mJ/mm^2. Typical fluences used for clinical arterial plaque ablation range from 35 to 80 mJ/mm^2. The fluence rate was 1.0×10^7 W/cm^2. The cumulative dose for both laser systems was controlled by varying the number of pulses delivered to the sample.

Irradiations of AV were performed with and without lymphocytes present. When irradiating AV with cells present, 180 μl of 1:20 diluted AV was added to 20 μl of the suspended lymphocyte solution in 96-well plates (Fisher Scientific, Boston, MA). Direct laser-induced cell killing was eliminated by centrifugation of the plates which forces the cells to the bottom. The amount of energy reaching the cells is negligible due to the shallow 1/e-penetration depth of 308 nm light in 1:20 diluted AV (see Results). For each predetermined dose, the appropriate number of laser pulses were delivered, then the cells were washed three times with HBS. The elapsed time from irradiation to washing was approximately 15 min. When irradiating AV without cells, 180 μl of 1:20 diluted AV was placed in 96-well plates and irradiated with the appropriate number of pulses. Twenty μl of suspended lymphocytes were added to the irradiated solution and left for 1 hr. The cells were then washed three times with HBS. The elapsed time from irradiation to placing the solution on cells was approximately 15 min. All irradiations were performed in triplicate.

Absorption Measurements

Absorption of pre- and post-irradiated AV samples was measured with a diode array spectrophotometer (8451A, Hewlett Packard, Inc., Palo Alto, CA). Two hundred and fifty microliters of 1:20 diluted AV was placed in 96-well plates and given 20 J of 308 nm radiation at a fluence rate of 1.0×10^8 W/cm^2. Absorption was determined in the 200 to 300 nm or 300 to 500 nm wavelength ranges using dilutions of 1:30,000 or 1:200, respectively, in HBS and a 1 cm pathlength quartz cuvette. The measurements were referenced to HBS.

DNA Synthesis Assay

After washing, all cells were resuspended in complete media (RPMI 1640), containing 20% fetal calf serum, 200 I.U./ml penicillin, and 200 μg/ml streptomycin (all from GIBCO) in round-bottom microtiter plates (Fisher Scientific). The mitogen phytohemagglutinin, PHA, (Ha 17, Burroughs Wellcome, Bechenham, UK) was added at 0.25 μg/culture and the cells incubated for three days at 37°C in an atmosphere of 5% CO_2. DNA synthesis was determined by measuring ^{3}[H]-thymidine incorporated into DNA during a 6-hr pulse. Briefly, 0.5 μCi of ^{3}[H]-thymidine (sp. ac. 6.7 Ci/mM; New England Nuclear, Boston, MA) was added to each well and the cells incubated for 6 hr. The contents of individual wells were collected on a glass fiber filter strip and washed freely using an automated harvester (model 200A PHD Cell Harvester, Cambridge Technology, Inc., Watertown, MA). Dried filter paper circles from each well were placed in scintillation fluid and radioactivity measured with a scintillation counter (LS3801, Beckman Instruments, Inc., Irvine CA). The mean and standard deviation of the triplicate samples at each dose and their nonirradiated controls were determined. Thymidine incorporation is expressed as percentage of counts in a sample relative to nonirradiated controls. Standard deviations were determined from error propagation techniques for the division of two means [11]. The inherent limit of sensitivity of this assay was 0.1%. Valid results are not obtained below this level. *P*-values are determined using a two-tailed, unpaired t-test.

RESULTS

Laser-Induced Decomposition of AV Solutions

The absorption spectrum of AV diluted 1:30,000 with HBS exhibits an absorption maximum at 240 nm and a low absorption between 280 and 320 nm (Fig. 2A). The extinction coefficients for the diatrizoate chromophore at 240 nm and 308 nm are 26,000 and 490 M^{-1} cm^{-1}, respectively, assuming that this chromophore is the only light absorbing species at these two wavelengths. The absorption spectrum between 300 and 500 nm of a less highly diluted AV solution (1:200 dilution) is shown in Figure 2B. The strong absorption at 308 nm indicates that this wavelength penetrates poorly into the 1:20 dilutions of AV used for phototoxicity experiments. The 1/e penetration depth at 308 nm for the 1:20 dilution of AV used in phototoxicity experiments is 180 μm; 99% of the incident 308 nm radiation is absorbed within 830 μm from the surface. The layer of AV solution over the cells is 6.3 mm deep (200

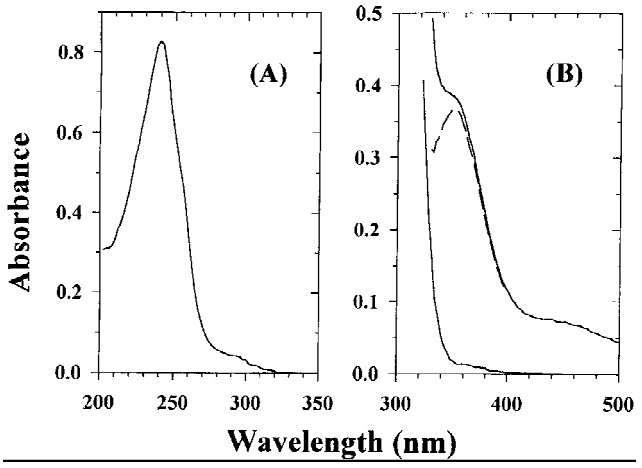

Fig. 2. Absorption spectra of Angiovist solutions before and after irradiation with 308 nm radiation. **A:** Absorption of a 1:30,000 dilution of Angiovist in a 1 cm cuvette prior to irradiation. **B:** Absorption of 1:200 dilution of Angiovist before (left solid curve) and after (right solid curve) irradiating with 500 pulses of 308 nm at 1×10^8 W/cm^2 for a total dose of 20 J. The difference spectrum between these two curves is shown as a dashed line.

μl in a 0.25 in diameter cylinder); thus, the cells are not exposed to significant 308 nm radiation.

A 1:20 diluted solution of AV was exposed to 500 pulses of 308 nm radiation at a fluence rate of 1.0×10^8 W/cm^2 and the spectrum of the irradiated solution (1:200 dilution of the original AV solution) is also shown in Figure 2B. Irradiation resulted in an increase in the absorption at all wavelengths and an extension on the absorption spectrum to >500 nm indicating that AV was altered during the irradiation. The original clear solution was pale yellow after irradiation. The difference spectrum found by subtracting the spectrum of the nonirradiated solution from that of the irradiated (dashed line, Fig. 2B) shows a new absorption maximum at 350 nm. Over a 30-min period after the irradiation, the yellow color faded.

Solutions of AV (diluted 1:20; 250 μl samples) were exposed to varying numbers of pulses of 308 nm laser radiation at three pulse energies (2, 4, and 40 mJ) which correspond to fluence rates of 5.2×10^6, 1.0×10^7, and 1.0×10^8 W/cm^2. The change in absorption was measured on AV solutions with a final dilution of 1:200 and the results are shown in Figure 3. At all three fluence rates, the absorption at 350 nm increased with increasing dose. The increase was greater when the 308 nm radiation was delivered at 1.0×10^8 W/cm^2 than at the two lower fluence rates suggesting that an intensity-dependent process was occurring. An occasional spark was observed in air above the solution suggesting the ignition of gases generated when irradiating at 1.0×10^8 W/cm^2. When an exhaust fan was used to disperse the vapor above the solution, the ignition rate was greatly reduced. The absorption spectra of irradiated samples with and without the fan were similar.

Treatment of Cells With Angiovist Exposed to 308-nm Excimer Laser Irradiation

In the first series of experiments, samples containing 1:20-diluted AV above a layer of lymphocytes were exposed to 308 nm laser radiation (10 ns pulses). The samples were irradiated with varying numbers of pulses at the three pulse energies used for the photodecomposition studies corresponding to fluence rates of 5.2×10^6, 1.0×10^7, and 1.0×10^8 W/cm^2. Cells were washed and cytotoxicity assessed 3 days later. The results are shown in Figure 4A. The dose response curves often showed enhanced incorporation of thymidine at the lowest doses and a linear decrease in thymidine incorporation at the intermediate doses. At the highest doses, the curves appeared to plateau indicating either the presence of ~0.1% of a resistant population or the limit of sensitivity of this assay technique. Incorporation of thymidine was reduced to 10% (D_{10} value) by doses of 11.0 ± 1.4 J and 12.9 ± 3.3 J when fluence rates

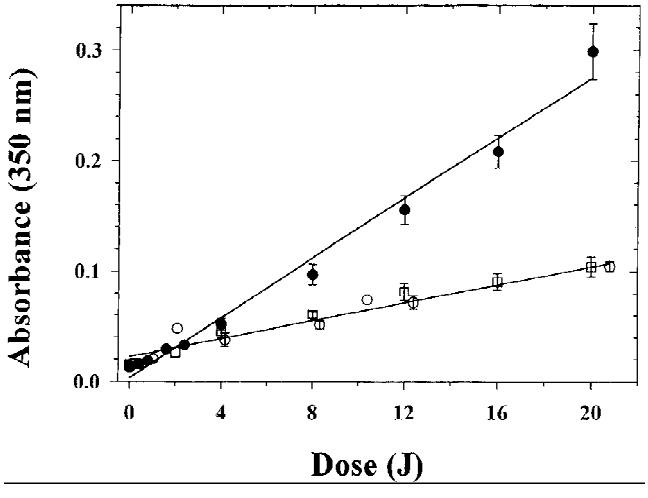

Fig. 3. Dependence of the absorbance at 350 nm of irradiated AV solutions on the cumulative dose for three fluence rates. The open circles, open squares, and filled circles correspond to fluence rates of 5.2×10^6, 1.0×10^7, and 1.0×10^8 W/cm^2, respectively. The lines correspond to linear fits to the 5.2×10^6 and 1.0×10^8 W/cm^2 data. Error bars are SEM.

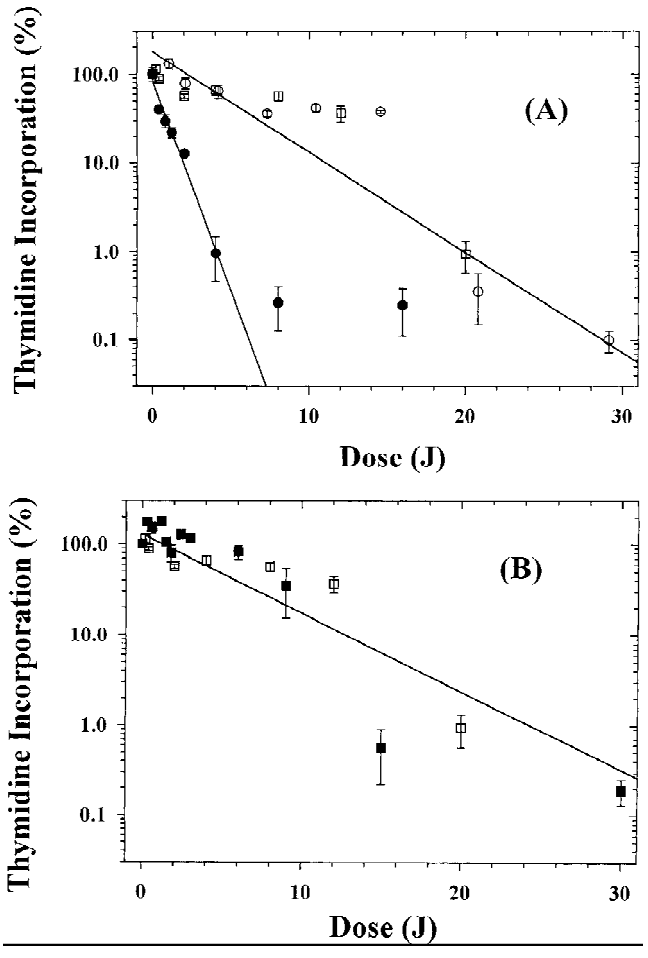

Fig. 4. Photosensitization caused by Angiovist solutions irradiated at 308 nm in the presence of cells. **A:** Thymidine incorporation for lymphocytes present in AV solutions (1:20 dilution) irradiated with a laboratory excimer laser with a 10 ns pulse duration. The open circles, open squares, and solid circles refer to fluence rates of 5.2×10^6, 1.0×10^7, and 1.0×10^8 W/cm^2, respectively. The straight lines are linear fits to the 5.2×10^6 and 1.0×10^8 W/cm^2 data. The slopes, intercepts and calculated D_{10} values for these data are given in Table 1. **B:** Dose-response curves from a comparison between irradiation with 10 ns pulses (open squares) and 230 ns pulses (clinical excimer laser; filled squares). The 10 ns excimer laser data are the same set given in A. The fluence rate was 1.0×10^7 W/cm^2. The straight line is a linear fit to both sets of data; the parameters are given in Table 1. Error bars are SEM.

of 5.2×10^6 and 1.0×10^7 W/cm^2 were used, respectively. Exposure to 308 nm radiation at 1.0×10^8 W/cm^2 was more effective; the D_{10} value was reduced to 1.9 ± 0.2 J. The D_{10} values for all of the dose response experiments are summarized in Table 1.

Lasers used in the clinical setting using fiber optics generally have pulse durations longer than 10 ns to avoid fiber damage. To determine whether the pulse duration altered the phototoxicity of AV, a direct comparison was made using the laboratory excimer laser (pulse duration = 10 ns) and a clinical excimer laser system (pulse duration = 230 ns) delivering 1.0×10^7 W/cm^2 under otherwise identical conditions. The results are shown in Figure 4B. The D_{10} values for laser radiation delivered in 230 ns pulses and 10 ns pulses were identical within experimental error (Table 1, $P = 0.21$).

A second series of experiments tested the hypothesis that stable photoproducts, rather than short-lived excited states or free radicals, formed from AV damaged cells. In these experiments, 1:20 dilutions of AV were exposed to varying numbers of pulses at the same three pulse energies used above. These solutions were added to the cells 15 min after irradiation and the sample was incubated 1 hr before washing. The results are shown in Figure 5A; the D_{10} values (~2 J) were the same within experimental error (Table 1).

A comparison of the effects of pre-irradiated solutions formed using the 10-ns and 230-ns pulse duration excimer lasers is shown in Figure 5B. Again, the effect of pulse duration on thymidine incorporation is negligible (Table 1, $P = 0.30$).

Experiments were performed to further explore the observation that pre-irradiated AV appeared to cause a greater decrease in DNA synthesis than AV irradiated with cells present. An AV solution (180 μl, 1:20 dilution) was irradiated with 1.2 J at 1.0×10^7 W/cm^2 and added to 20 μl of cell suspension immediately (ca. 1 min), 15, 30, 45, and 60 min after the irradiation. Each sample was incubated for 60 min before washing. In a similar experiment, an AV solution was exposed to 2.0 J at 1.0×10^7 W/cm^2 and immediately placed on cells. The irradiated solution was removed after 15, 30, 45, and 60 min of incubation time. The results shown in Figure 6 indicate that increasing the time between irradiation and addition to the cells decreases the effectiveness of the photoproduct mixture. Also, the longer the time cells were incubated with the irradiated mixture, the greater the reduction in DNA synthesis.

DISCUSSION

Our results demonstrate that AV causes cell damage in vitro when solutions are exposed to 308 nm excimer laser radiation. The results in

TABLE 1. Effect of the Pulse Duration and Fluence Rate Used to Irradiate Angiovist Solutions on Incorporation of Thymidine

Pulse duration (ns)	Fluence rate (W/cm^2)	Cells present during irradiation?	Slope[a]	Intercept[a]	D_{10} values (J)
10	5.2×10^6	Yes	0.26 ± 0.02	177 ± 21	11.0 ± 1.4
10	1.0×10^7	Yes	0.20 ± 0.04	129 ± 19	12.9 ± 3.3
10	1.0×10^8	Yes	1.09 ± 0.08	82 ± 5	1.9 ± 0.2
230	1.0×10^7	Yes	0.25 ± 0.03	174 ± 26	11.5 ± 1.9
10	5.2×10^6	No	1.64 ± 0.28	156 ± 32	1.7 ± 0.4
10	1.0×10^7	No	1.79 ± 0.24	256 ± 45	1.8 ± 0.3
10	1.0×10^8	No	1.05 ± 0.05	108 ± 4	2.3 ± 0.2
230	1.0×10^7	No	1.23 ± 0.68	359 ± 104	2.9 ± 2.6

[a]Linear fit to $\ln(y) = \ln(A) - kD$, where y is thymidine incorporation in %, A is the exponential prefactor in %, k is the dose rate in J^{-1}, and D_{10} is the dose delivered in J that reduces thymidine incorporation to 10% of the control, unirradiated value. All errors are SEM.

Figures 4 and 5 indicate that the decrease in DNA synthesis is directly proportional to the number of cell damaging events produced by irradiation of AV solutions. Angiovist-370 solutions were approximately 6-fold more damaging to cells when the radiation was delivered at the highest fluence rate (1.0×10^8 W/cm^2) rather than the lower fluence rates (Fig. 4A) in the presence of cells. Most other studies of the effect of fluence rate on phototoxicity have shown a decrease in the phototoxicity of a drug or dye with an increase in fluence rate [12,13]. The inverse relationship between phototoxicity and fluence rate has been ascribed to depletion of ground state molecules [12] or to depletion of the oxygen required for the phototoxicity mechanism at high fluences [13]. The enhancement of AV photosensitization at the highest fluence rate correlates with the increased rate for formation of photoproduct(s) observed when AV solutions are exposed to this same high fluence rate compared to fluence rates 10- and 20-fold lower (Fig. 3). A mechanism involving absorption of two photons may account for the enhanced photosensitization and photodecomposition of AV at high fluence rates. Absorption of one photon at 308 nm by AV produces an excited state of the AV molecule. The excited state, or a short-lived species formed from it, may absorb another photon to form highly energetic species that proceeds to form photoproducts that are damaging to cells. Because two photons must be absorbed by the same molecule during the laser pulse, the yield of the highly damaging photoproduct will increase with the square of the fluence rate. A more extensive study will be needed to establish the mechanism for the fluence-rate dependence of AV photosensitization. In a related study of the effect of fluence rate of 308 nm radiation on cells, it was shown that the yield of DNA photoproducts which cause cell toxicity was not affected by a 200-fold change in fluence rate; the yield of these photoproducts was proportional to the total fluence delivered and not to the fluence rate over the range 2.9×10^5 to 5.9×10^7 W/cm^2 [14].

Both short-lived species and stable photoproducts appear to be involved in decreased DNA synthesis induced by AV. The short-lived species exist for less than 15 min since the incorporation of thymidine increases from approximately 4% when the photoproduct mixture is added 1 min post-irradiation to approximately 83% when the photoproduct mixture added at 15 min post-irradiation. The identity of the short-lived species is not known. Homolytic cleavage of a carbon-iodine bond in AV to yield a substituted phenyl radical and a iodine radical is the most likely photochemical reaction based on the known photochemistry of iodinated aromatic compounds [6]. Iodine radicals combine to form I_2 which may account for the yellowish-brown color of the irradiated solution which fades with time. The substituted phenyl radical is highly unstable and will react rapidly with other molecules in the solution. The highest fluence rate produced a lower D_{10} value than the lower fluence rates when cells were present during the irradiation but not when preirradiated solutions were added to cells (see Table 1). This result suggests that the highly damaging product is too short-lived to be transferred (about 2-3 min) to the cells.

The observation that DNA synthesis decreases as an irradiated AV solution is left on cells longer (Fig. 6) indicates that a stable toxic photoproduct is present. Stable photoproducts have been implicated in the phototoxicity associated with other phototoxic drugs including chlor-

promazine [15], benoxaprofen [16], and non-steroidal anti-inflammatory drugs [17] although the phototoxicity mechanism for amiodarone, a highly phototoxic drug with a iodinated structure similar to that of AV, does not involve a stable toxic photoproduct [9]. The identity of the stable photoproduct cannot be determined from our results but, as described above, it is likely to be formed by a mechanism involving initial loss of iodine. The cell damaging photoproducts formed from AV by 308 nm irradiation appear to exist long enough to travel to sites distant from the site of laser ablation and possibly cause cell damage. Clinical data to assess this possibility are, to our knowledge, unavailable.

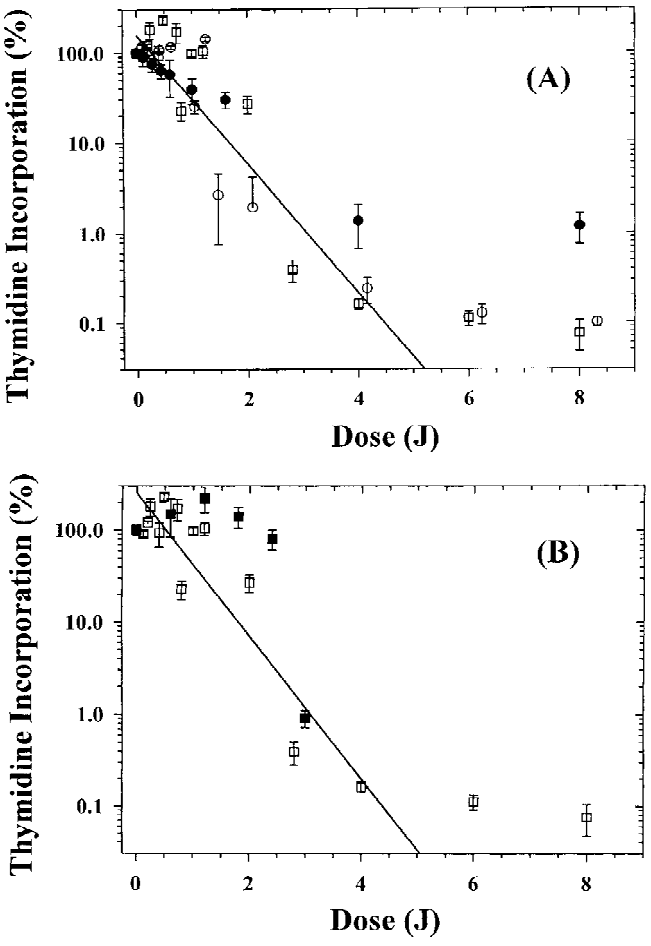

Fig. 5. Effect of pre-irradiated Angiovist solutions. **A:** Incorporation of thymidine into lymphocytes after addition of AV solutions (1:20 dilution) irradiated with a 308 nm excimer laser having a 10 ns pulse duration. The open circles, open squares, and solid circles refer to fluence rates of 5.2×10^6, 1.0×10^7, and 1.0×10^8 W/cm^2. The straight line is a linear fit to the 5.2×10^6 W/cm^2 data. The slopes, intercepts, and calculated D_{10} values for these data are given in Table 1. **B:** Comparison of response to AV solutions irradiated with 10 ns pulse duration laser (open squares) with solutions exposed to the clinical excimer laser having a 230 ns pulse duration (filled squares). The 10 ns excimer laser data is the same set given in A. The fluence rate was 1.0×10^7 W/cm^2. The straight line is a linear fit to both sets of data; the parameters are given in Table 1. Error bars are SEM.

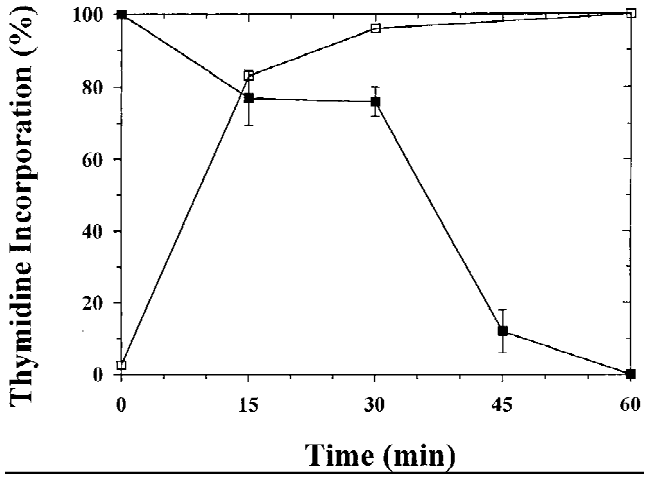

Fig. 6. Effect on synthesis of DNA from combining irradiated Angiovist solutions with cells at various times. Open squares show incorporation of thymidine into cells treated with AV solution that was irradiated with 1.2 J at 1.0×10^7 W/cm^2 and then added at various times. Each sample was incubated 60 minutes before washing. Filled squares show thymidine incorporation into cells treated with an AV solution that was exposed to 2.0 J at 1.0×10^7 W/cm^2 and immediately placed on cells. The irradiated solution was removed after the times shown. Error bars are SEM.

The 6.5-fold lower D_{10} values for Angiovist solutions irradiated in the absence of cells than those irradiated in the presence of cells maybe attributed to longer incubation times. The cells which received preirradiated Angiovist solutions were incubated for 60 min, whereas the cells that were present during the irradiation of Angiovist were only incubated for 15 min. The solid squares in Figure 6 show a drastically enhanced response when the incubation time is increased from 15 to 60 min.

The relationship of the in vitro photosensitization by AV to possible effects on endothelial cells or other vascular cells during 308 nm excimer laser angioplasty is not known. The fluence rates and doses used approximate those employed in laser ablation procedures [1]. Although the pulse duration of the excimer laser used (10 ns) are shorter than those used clinically (230 ns), our results (Fig. 4B) indicate that a 23-fold increase in pulse duration did not alter the D_{10} value. It was difficult to approximate the concentration of

AV in arteries during angioplasty since it is injected only periodically to observe the blood vessel, thus, the AV concentration varies greatly during the procedure. The AV solution used (1:20 dilution) will only approximate the concentration found in arteries a small portion of the time. In addition, the length of time that any area of a vessel is exposed to AV photoproducts may be much shorter than in these experiments. The incubation times used after irradiation with cells present or not were chosen in order to test the toxicity effect rather than to be a close mimic of the clinical situation. Despite these approximations, our results suggest that cell damage may be a potential problem when contrast media containing iodinated aromatic compounds are present during excimer laser angioplasty.

In summary, these studies indicate that exposure of AV solutions to 308 nm excimer laser radiation in the fluence rate and dose range used in laser ablation of arterial plaque produces photoproducts that cause cell injury in vitro. Whether these photoproducts are important during clinical procedures utilizing AV cannot be determined from these results.

ACKNOWLEDGMENTS

The authors acknowledge support for this research by a NIH grant RO1 GM30755 and the Medical Free Electron Laser Program under ONR contract N00014-86-K0117.

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