Gas Chromatographic-Light Microscopic Correlative Analysis of Excimer Laser Photoablation of Cardiovascular Tissues: Evidence for a Thermal Mechanism

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The present series of experiments used gas chromatography to identify vapor-phase photoproducts liberated during excimer laser irradiation of cardiovascular tissues in air and blood. In air, laser beams produced from ArF (193 nm) and XeF (351 nm) excimer laser gas mixtures were delivered to samples of myocardium and atherosclerotic coronary arterial segments through the wall of a quartz cell, using 8–40 mJ/pulse. In blood, 351 nm were delivered via an optical fiber, using 14 mJ/pulse. When the experiments were performed using an air–tissue interface, the dominant photoproducts identified in order of elution from the gas chromatographic column were methane, acetylene, ethylene, ethane, propyne, allene, propylene, propane, and butene. When a fiberoptic was used to accomplish 351-nm excimer laser tissue ablation in a blood field, a similar gas chromatographic spectral distribution was observed. These vapor-phase photoproducts are indistinguishable from those observed following continuous wave laser irradiation or flame torching of cardiovascular tissues. Thus, despite the fact that excimer laser ablation of cardiovascular tissues is characterized by the absence of signs of thermal injury, the results of these experiments suggest that the predominant mechanism of excimer ablation is, like continuous-wave laser irradiation, a thermal process. (Circulation Research 1987;60:429–437)

Previous investigations have demonstrated in vitro that the excimer laser may be used to accomplish cardiovascular tissue ablation without causing thermal injury to boundary sites. It has subsequently been demonstrated that the excimer laser, unlike most biomedical continuous wave systems, can be used successfully to ablate heavily calcified lesions. Both thermal injury-free tissue ablation and enhanced ablation of calcified lesions constitute potential advantages over continuous wave systems for the application of laser irradiation to the treatment of cardiovascular disease. Srinivasan and associates have suggested that the basis for the results achieved with the excimer laser is related to the high photon energy of the excimer ultraviolet beam, which results in photochemical breaking of molecular bonds, rather than thermal degradation of the target tissue. More recent investigations, however, have suggested that the mechanism of excimer tissue ablation may not be unique. Elimination of thermal injury has been shown to be wavelength-independent: results indistinguishable from those accomplished with the excimer laser have been demonstrated using not only a variety of visible but infrared wavelengths as well. In contrast to the high photon energy of the excimer beam, the low photon energy of the infrared carbon dioxide laser virtually precludes the possibility of photochemical ablation.

On the basis of these findings, we have pursued the hypothesis that the tissue effects achieved with all pulsed lasers are the result of energy delivery that is sufficiently discrete to vaporize tissue by a thermal mechanism without allowing thermal diffusion — and consequent thermal injury — to boundary tissue sites. The present series of experiments was designed to determine whether the specific vapor-phase photoproducts liberated as a result of excimer laser photoablation further support this hypothesis.

Materials and Methods

Tissue

Tissue samples included specimens of human myocardium and atherosclerotic segments of human coronary arteries obtained in the fresh state at necropsy examination and preserved in saline at 4°C for less than 24 hours prior to each experiment. Myocardial specimens were used for these experiments only if gross inspection disclosed no foci of fibrosis or necrosis; coronary arterial segments were used only if gross inspection disclosed greater than 75% cross-sectional area narrowing by atherosclerotic plaque. Both of these findings were subsequently confirmed by light microscopic examination of adjacent tissue sections following completion of laser irradiation.
Laser Irradiation

Laser irradiation was accomplished using an excimer laser (Lambda Physik, Acton, Mass., model EMG 52 MSC) modified to run on fluorine gas mixtures. Two of the 4 principal excimer wavelengths were selected for the present series of experiments as representative of the extremes in photon energy available for excimer laser ablation: 193 nm from an ArF gas mixture and 351 nm from a XeF gas mixture.

The excimer emission was delivered to the tissue specimens in one of two configurations. For experiments carried out using an air-tissue interface, both 193 nm and 351 nm were delivered to the tissue specimen as a focused beam through the walls of a quartz sample holder. The focal length of the lens was 100 mm. The illuminated face of the sample holder consisted of an optically flat quartz plate, which consequently did not alter the focal spot of the laser beam. For experiments carried out in a blood field, the excimer beam was delivered via a 600-μm core ultraviolet-transmitting optical fiber (SpecTran Inc., Sturbridge, Mass.) (Figure 1) interfaced with the laser by means of a specially designed coupling system. The distal tip of the fiber was positioned approximately 1 mm above the surface of the tissue specimen. Experiments were carried out either with aerated samples or with the cell chamber first flushed with an inert gas (pure argon or pure nitrogen) prior to laser irradiation. No differences were observed in pilot experiments in which the cells were purged with an inert gas or carried out under atmospheric conditions. The end point for laser irradiation in each experiment was vaporization of a portion of the specimen sufficient to generate a quantity of photoproducts for analysis by gas chromatography.

To identify vapor-phase photoproducts generated during nonfocused excimer laser irradiation, one tissue specimen (myocardium) was positioned 3 cm proximal to the focal point of the lens and irradiated using XeF (351 nm).

To compare the vapor-phase photoproducts generated during excimer laser irradiation to those generated during continuous-wave laser irradiation, an additional 7 specimens of myocardium (4) and atherosclerotic coronary arterial segments were irradiated using a 20-watt argon ion gas laser (Coherent, Inc., Santa Clara, Calif.). All were carried out using the focused laser beam with a spot size 1.0 mm in diameter as described previously.12 Fluence was constant at 75 J/cm² (5 watts for 120 seconds).

One specimen (myocardium) was heated using a Bunsen burner flame, with an air-tissue interface, through the walls of a quartz sample holder.

Gas Collection and Analysis

Gas-phase photoproducts were collected by withdrawing 1-ml gas samples into a syringe inserted through a rubber septum at the top of the quartz cell. The gas phase analyses were performed on a Varian 3700 gas chromatograph equipped with a flame ionization detector and a Hewlett-Packard 3380A electronic integrator for determination of product peak areas. The separation column was maintained at 185° C, with helium used as the carrier gas. Multiple gas chromatographic columns were used to sample for photoproducts of varying molecular size and configuration. Identity of photoprodut determination was confirmed by gas coinjection of the gas chromatographic columns. The total quantity of vapor-phase photoproducts liberated in each experiment was variable, depending on pulse energy, repetition rate, and cumulative exposure. While the variability in total gas production among the group of experiments obviates quantitative comparison of gas chromatographic peaks from one experiment to another, within an individual experiment the peak heights of the columns are indicative of the relative amounts of liberated photoproducts.

Tissue Analysis

Each specimen was examined in the fresh state, immediately following completion of laser irradiation, for gross evidence of tissue charring, defined as gross blackening or apparent carbonization at the perimeter of the tissue crater. The specimen was then fixed in toto in 10% buffered formalin for 24–72 hours, cleared with xylene, impregnated with and then embedded in paraffin, and cut at 4-μm intervals. Sections stained with hematoxylin and eosin, as well as sections stained with Richardson’s elastic-tissue trichrome stain, were then examined by light microscopy.
A total of 14 tissue specimens were irradiated with excimer laser focused beam. These included 8 experiments involving myocardium (4 at 193 nm; 4 at 351 nm) and 6 experiments involving segments of atherosclerotic coronary arteries (3 at 193 nm; 3 at 351 nm). Pulse duration was consistent at 20 nanoseconds. Repetition rate was constant at 20 Hz for ArF or 40 Hz for XeF. Pulse energy for the focused beam varied from 8-40 mJ/pulse; corresponding fluence varied from 3.2 J/cm² to 16 J/cm². Pulse energy for the optical fiber was 14 mJ/pulse. Cumulative exposure varied from 15 to 88 seconds.

Representative findings from these experiments are illustrated in Figures 2-4. The dominant vapor-phase photoproducts identified in order of their elution from the gas chromatographic column included methane, acetylene, ethylene, and ethane; a lesser amount of the 3-carbon products propyne, allene, propylene, and propane; and trace amounts of the 4-carbon products butene and butene derivatives.

Light microscopic analysis of the results obtained using an air–tissue interface disclosed two disparate types of results. When the tissue specimen was irradiated at the focal spot of the lens through which the laser beam was transmitted, the characteristic excimer incision devoid of thermal injury was observed (Figures 2–4). In contrast, when the tissue specimen was irradiated 3 cm proximal to the focal point of the lens, typical signs of thermal injury, including coagulation necrosis and polymorphous lacunae, were routinely observed (Figure 5). Nevertheless, the results of gas chromatographic analysis of vapor-phase photoproducts remained independent of the results of light microscopic analysis. As is illustrated in Figure 5, a similar profile of vapor phase photoproducts was observed when fluence was diminished (by defocusing the beam) to produce light microscopic signs of thermal injury.

The results of experiments carried out using a blood–tissue interface were indistinguishable from those described above. This is evident in comparing a representative gas chromatographic analysis obtained with a blood field (Figure 6) to one obtained with an
air–tissue interface (Figures 2–4). Once again, light microscopic analysis confirmed that signs of thermal injury were absent when the tissue specimen was irradiated with a focused beam (Figure 6). Likewise, use of a nonfocused beam produced typical signs of thermal injury. As with an air–tissue interface, the profile of vapor phase photoproducts obtained in a blood environment was similar, independent of the presence or absence of light microscopic signs of thermal injury.

Finally, when the various gas chromatographic spectra were evaluated at the highest gain experimentally possible, several previously undetected peaks were observed in the regions between the 2-carbon and 3-carbon products; these peaks, however, were considered minor inasmuch as the peak area in each case constituted less than 1% of the smallest identifiable 3-carbon peak. Theoretically, these small peaks might represent a wavelength-dependent side mechanism, different from the dominant thermal mechanism that accounts for most of the vapor-phase photoproducts. In the present series of experiments, however, the finding of these minor peaks was too inconsistent to allow for a statistically meaningful interpretation.

The results of gas chromatographic analysis of vapor-phase photoproducts in the experiments described above were similar to results obtained following continuous-wave laser irradiation of myocardium (Figure 7) and atherosclerotic coronary arterial segments (de-
Excimer (193 nm) irradiation of atherosclerotic coronary arterial segment: thermal injury absent.

**Figure 4.** Gas chromatographic analysis (left) of vapor-phase photoproducts collected during ArF excimer (193 nm) laser irradiation of atherosclerotic coronary arterial segment. Light microscopic examination (right) disclosed no signs of thermal injury. (Hematoxylin and eosin, ×25).

scribed previously in greater detail\(^2\) and simple flame-torching of myocardium (Figure 7). In all of these latter cases, gross examination disclosed a rim of gross charring at the periphery of the laser crater, and light microscopic examination disclosed typical histologic signs of thermal injury.

**Discussion**

Nearly all biomedical applications of laser irradiation reported to date have utilized continuous wave lasers.\(^3\) Light microscopic analyses of tissues irradiated with laser energy delivered in the continuous mode have consistently indicated histologic and ultrastructural alterations indicative of thermal injury: a superficial zone of frank charring and a subjacent zone of polymorphous lacunae.\(^4\)–\(^7\) These findings have been observed regardless of wavelength and organ system.\(^8\)

Furthermore, gas chromatographic analyses of the vapor-phase photoproducts liberated during continuous-wave laser irradiation\(^12,16\) have confirmed that continuous-wave laser irradiation accomplishes tissue vaporization by means of a thermal process.

In contrast, the observation that ultraviolet radiation from an excimer laser could accomplish photo-etching of organic polymer films unassociated with signs of thermal injury\(^5,6\) has stimulated investigation of this laser for biomedical purposes. Results similar to those described with organic polymer films have been demonstrated in a variety of organ systems.\(^20,21\) In particular, in vitro\(^2,6,12\) and in vivo\(^2\) experiments have documented that excimer ablation of coronary arterial atherosclerotic plaque, myocardium, and aortic valve calcific deposits can be achieved without accompanying thermal injury.

While the anatomic results of excimer laser irradiation have been reproduced by a number of investigators, the underlying mechanism of excimer laser photoblation has been the subject of controversy. Srinivasan and colleagues\(^5\) have suggested that excimer laser photoablation proceeds on a nonthermal basis. Based on the high absorption coefficient of organic materials for ultraviolet light in combination with the peak energies attainable with the excimer laser, calculations based on theoretical modeling have demonstrated that excimer-derived energy input exceeds individual bond energies for most organic materials. As a result, the term "photoablative decomposition" was coined to describe a process by which direct bond breaking, unassociated with preliminary vibrational transitions, resulted in the production of "... small, volatile fragment molecules ...\(^6\) A model system in which such molecules could be reproducibly generated and identified has thus far proved elusive.

Lane et al,\(^21\) however, have suggested that three empirical observations favor a photochemical process as the mechanism responsible for excimer laser tissue ablation: 1) anatomically evident thermal injury is absent; 2) the velocity of fragments directed away from tissue surface exceeds that expected for thermally evaporated fragments; and 3) temperature calculations based on measurements of velocity spread and internal degrees of excitation yield a temperature below that corresponding to thermal evaporation.

More recent experimental data have suggested that despite the fact that anatomic results typical of excimer laser tissue ablation are distinct from those achieved by continuous-wave laser irradiation, the underlying mechanism may not be unique. First, anatomic results identical to those described using the excimer laser have been produced with visible\(^9\) and infrared\(^11\) wavelengths. In particular, the demonstration that excimer-like results can be accomplished using a high peak-power, pulsed CO\(_2\) laser\(^11\) indicates that the absence of thermal injury does not exclude a thermal mechanism.
The energy of a single infrared photon (less than 0.1 eV) is far less than that of most biomolecular bonds; as a result, direct photochemical bond disruption is unlikely to occur with CO$_2$ laser irradiation. Furthermore, it is statistically unlikely that multiphoton processes explain excimer-like CO$_2$ ablation, due to the large number of low-energy CO$_2$ photons that would be required. Consequently, the results achieved with the high-energy CO$_2$ laser suggest that the absence of thermal injury is the result of a thermal process in which thermal diffusion has been optimized. Such "spatial confinement of laser-induced ablative effects" has been demonstrated previously in experiments in which the combination of a sufficiently brief pulse duration and adequately long pulse interval do not exceed the thermal relaxation time of the irradiated tissue.$^{13,24}$

**FIGURE 5.** Gas chromatographic analysis (top) of vapor-phase photoproducts collected during nonfocused XeF excimer (351 nm) laser irradiation of myocardium; tissue specimen in this case was irradiated proximal to the focal point of the lens. Light microscopic examination (bottom) disclosed typical signs of thermal injury, including superficial coagulation necrosis (CN) and subjacent polymorphous lacunae (PL). (Hematoxylin and eosin, $\times 25$).
Second, results observed with excimer laser (or high peak-energy CO₂ laser) irradiation that is either protracted or nonfocused also contradict the notion that excimer tissue ablation is a nonthermal event. Previous in vitro studies, for example, have demonstrated both gross and classic histologic signs of thermal energy when KrF excimer (focused) irradiation of cardiovascular tissues was extended beyond 200 seconds, suggesting that such protracted exposure ultimately overwhelmed the capability of the irradiated tissue to allow efficient thermal diffusion. Similar results were observed when fluence was reduced by using the excimer or high peak-energy CO₂ laser in a nonfocused manner.

The results observed in the present series of experiments further support the hypothesis that the process of excimer laser tissue ablation is not different from that of continuous-wave laser irradiation. In every case, the gas chromatographic spectrum of organic vapor-phase products liberated during laser irradiation of cardiovascular tissues was dominated by photoproducts indicative of a thermal process. Specifically, the spectrum of organic vapor-phase photoproducts liberated during focused excimer laser irradiation was indistinguishable from those observed during continuous-wave laser irradiation or simple flame torching. Photoproduct profile was unrelated to the results of light microscopic tissue analysis: When the excimer laser was used in a nonfocused manner, classic signs of thermal injury were observed, but the profile of liberated photoproducts remained unchanged. Photoproducts generated were not a function of the specific type of cardiovascular tissue: Similar results were observed following laser irradiation of myocardium or atherosclerotic coronary arterial segments.

Furthermore, the results of gas chromatography did not vary as a function of wavelength. The excimer wavelengths employed in the present series of experiments were selected as representative of the extremes in photon energy available for excimer ablation of cardiovascular tissues. Srinivasan et al and Puliafito et al have also suggested that the high quantum yield for peptide bond cleavage at 193 nm produces a superior anatomic result compared to other excimer wavelengths. In contrast to 193 nm, 351 nm represents the excimer wavelength of lowest photon energy; this fact is of practical significance inasmuch as 351 nm may be successfully transmitted via conventional fiberoptics, even at short (20-nanosecond) pulse durations. Despite these considerations, similar photoproducts were identified by gas chromatography using both of these excimer wavelengths. Similar results have also been reported recently using a KrF excimer laser (248 nm), although histologic analysis to document absence of thermal injury was not part of the experimental protocol. The finding that individual excimer wavelengths are not associated with unique photoproduct profiles is consistent with the finding that ultrastructural results accomplished in cardiovascular tissues using 193 nm have been duplicated with 248, 308, and 351 nm; 355, 532, and 1064 nm; and 10,600 nm.

The experiments in the present series that were performed using an optical fiber to transmit 351 nm and thereby irradiate tissue specimens immersed under a 3-
cm column of blood are useful for assessing the role of a blood field in modulating the mechanism of excimer photoablation. The finding that photoproduct profile is not significantly altered by a blood field suggests that blood does not directly participate in the photoreaction.

Earlier work from the 1960s on the effect of pulsed ruby laser light (693 nm) on aromatic organic compounds disclosed gas-phase photoproducts similar to those observed in the present experiments: methane, acetylene, and lesser amounts of ethane, ethylene, pro-

pane, and allene. It was emphasized, however, that such apparent thermal degradation mediated by pulsed lasers should not be equated with conventional pyrolysis of organic material. Instead, because the experiments are performed under conditions far from chemical equilibrium, a pulsed laser experiment is more similar to a temperature-jump experiment in which all the heating processes are carried out in conditions to which ordinary equilibrium thermodynamics do not apply. Therefore, simple labels such as "photothermal" or "photochemical" and the analogies drawn to these types of reactions may not fully characterize pulsed laser photoablation of tissue samples.

In this respect, the photoproducts analyzed in the gas phase may not represent all the products formed during excimer laser photoablation; nonvolatile components, for example, expected to be of greater mass than the gas-phase products, may in fact accompany the vapor-phase products indicated here. Furthermore, when the gas chromatographic spectra are evaluated at the highest gain experimentally possible, new minor peaks (peaks less than 1% of the smallest identifiable 3-carbon peaks) are observed in the regions between the 2-carbon and 3-carbon peaks. These may represent photoproducts formed by a wavelength-dependent side mechanism, different from the thermal process accounting for the predominant photoproducts.

Finally, it is conceivable that excimer laser ablation produces an initial set of photoproducts indicative of a nonthermal process (e.g., radicals and radical-recombination products), which then undergo subsequent photoreaction with the impending laser beam photons to account for the thermal products identified by gas chromatography. The present experimental design does not exclude this possibility. Photoemission spectroscopy, not employed in the experimental protocol described above, is currently being used in our laboratory in an attempt to resolve this point. Nevertheless, the photoproducts identified in these experiments suggest that the dominant mechanism responsible for excimer laser ablation of cardiovascular tissues is a thermal one.

References


KEY WORDS • laser • angioplasty • thermal injury
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