A New Model to Study Quantitative Effects of Laser Angioplasty on Human Atherosclerotic Plaque

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A new model for analyzing the major effects of the use of any laser angioplasty system is described. Changes in any of the six major determinants of effect (energy, duration, wavelength, medium, absorption, geometry) can be evaluated. In this report a neodymium: yttrium aluminum garnet (Nd:YAG) laser was used to make 408 laser exposures in vitro on segments of human cadaveric atherosclerotic aorta. Energy, medium (air, human blood, perfluorochemical and saline), geometry and duration were varied. The depth and width of the resultant plaque craters were measured.

A large amount of exposure to exposure variability was found in all groups of experiments, even when conditions were held as constant as possible in this rigidly controlled laboratory setting. This variability is attributable to differences in energy absorption by the plaque. Changes in media and fiber optic tip to plaque distance also markedly altered exposure outcome. For example, the average depth of the hole created by a 15 W, 2 second blast with the fiber tip adjacent to the plaque in blood was 1.7 ± 0.1 mm (n = 27), but the range was between 0.5 and 2.7 mm. Under the same conditions, except with the fiber tip 1 mm away from the plaque, the average hole depth was 0.4 ± 0.1 mm (n = 12) and the range was 0.0 to 1.7 mm.

The use of this model to analyze the major determinants of lasing effects in different laser angioplasty systems should help to select the best conditions for lasing and allow assessment of the variability of outcome.

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laser exposures to determine the contribution of changes in medium, geometry, energy and duration of exposure to the outcome of each exposure. The effect of variable absorption of energy by the tissue was assessed by making multiple exposures with the same energy, duration, geometry, medium and wavelength on different target sites in different tissues.

Methods

Laser apparatus. A Nd:YAG laser (model 100 Fiberlase Medical Laser) with built-in laser to fiber couple was used in these experiments. Power output was selected in the range of 15 to 80 W with a variation of ± 10% of the power output from exposure to exposure. Duration of each exposure was instrument controlled at ± 0.1 second. The fiber optic was a 3 m long flexible glass fiber with a 0.6 mm diameter core. Power output at the fiber tip was checked with the instrument’s built-in power meter, and the instrument was recalibrated before and after each change in power setting.

Sample preparation. Samples of moderately atherosclerotic, distal human abdominal aorta were obtained from nine separate autopsies, rinsed in saline solution and frozen. The sample from each autopsy was divided into four equal parts and the pieces were blindly randomized into one of four treatment groups: 1) air, 2) 0.9% aqueous sodium chloride, 3) whole, citrated human blood from random donors, and 4) perfluorochemical (FC-43 emulsion, The Green Cross Corporation). A short beaker was half filled with paraffin, and the pieces of aorta were pinned flat to the bottom of the flask with the atherosclerotic intimal surface opened outward. The fiber optic tip was then oriented perpendicular to the plaque surface with a three-dimensional stereotactic device (David Kopf Instruments). The tip was then positioned either immediately adjacent to the plaque for some determinations, or at 1.0 ± 0.1 mm above the plaque for other determinations. The device had three purposes: 1) to hold the fiber tip as steady as possible, 2) to assure the same perpendicular orientation from exposure to exposure, and 3) to provide an accurate measurement of the distance from the fiber tip to the plaque surface. After the positioning of the tip was achieved, the vessel and tip were submerged in the predetermined medium before lasing.

Measurements. Each laser exposure was assessed in three ways:

1. Either effect or no effect (±).
2. Direct measurement of the diameter and depth of a cross section of the blast with a digital caliper (Fowler Ultracal). Accuracy and reproducibility of these measurements were assessed when six measurements of a standard width (0.6 mm) were made by three independent observers and all values were within ± 0.1 mm around a mean of 0.65 with an SE of 0.01 mm. Because the shape of the holes approximated cylinders, the volume of plaque and vessel destroyed could be calculated by volume = π r²h. In all experiments there were no differences between the trends of the widths and depths of the exposures with the calculated volumes. To simplify presentation, only the volume of the blasts will be presented.

3. Histologic slides were prepared from the cross sections of each blast after the initial measurements. The tissues were stained with both hematoxylin-eosin and elastic stains before microscopic analysis.

Energy-response curves. Two hundred seventy-six exposures were analyzed in these experiments. Distal aortas from five different autopsies were each divided into four sections and randomized to one of the four media. Multiple laser exposures were made on each section. Energy-response curves were generated with multiple 2 second exposures at each power (over a range of 15 to 90 W), in each medium and at each distance (0 and 1 mm).

Time-response curves. In these experiments, 132 total laser exposures were analyzed. Distal aortas from four different autopsies were each divided into four sections and randomized to one of the four media. Multiple laser exposures were made on each section. Time-response curves were generated with 15 W exposures over a range of 0.5 to 8 seconds with the fiber tip adjacent to the plaque.

Statistical analysis. The mean value ± SE was calculated for each treatment group. All comparisons between treatment groups were made by unpaired t test using two-tailed Student’s t distribution. Differences among groups were considered statistically significant if the probability (p) value was less than 0.05.

Results

Effect of the medium. A. Energy-response with fiber tip adjacent to the plaque and with constant power. Multiple exposures were made in each medium (on sections of different aortas) and all variables (time = 2 seconds, power = 15 W, fiber tip adjacent) were held constant (Table 1A). There was a large range of potential effects in each medium. There was a significant statistical difference (p < 0.05) between the effects of these types of exposures in air and blood versus perfluorochemical and saline solution. The difference in percent of exposures with any effect was striking. Equally important was the marked range of effects with a given medium as judged by volume (mm³) of tissue destroyed. For example, exposures in air had a 90-fold difference, and exposures in blood had a 7-fold difference. The average volume of plaque destroyed is presented in Figure 1. A large difference attributable to medium is again demonstrated.

B. Energy-response with fiber tip 1 mm from plaque with constant power. In this experiment, multiple exposures were
made on sections of different aortas in each medium using 15 W of power with 2 second duration with the fiber tip 1 mm away from the plaque surface. Table 1B demonstrates the wide variation related to the effect of the medium. The effects of air and blood are statistically different (p < 0.05), as well as the effects of air and blood versus perfluorochemical and saline solution. When a comparison was made between the effects of exposures with the fiber tip at 1 mm versus the effects of exposures with the tip adjacent to the plaque (Table 1A and 1B, respectively), a statistical difference (p < 0.05) was found in all media.

Effect of energy change 1 mm from the plaque over different powers. In Figure 2 the power versus percent of the exposures resulting in measurable plaque destruction are compared. All determinations were made with 2 second exposures and with the fiber tip 1 mm from the plaque. The wide difference in percent of positive effects over a large power range is obvious. Quantification of the different power effects is represented in Figure 3. From these data it is possible to estimate that the power to destroy 1 mm³ of plaque under these conditions would be about 15 W in air, 20 W in blood and between 60 and 70 W in saline solution and perfluorochemical. It is also evident from this experiment that increasing energy generally increases the average amount of plaque destroyed.

Time-response: keeping power constant and altering duration. In this experiment, the power was constant at 15 W, and the duration was varied. Figure 4 shows the effects of increasing the duration of laser exposure. There was an increase in the average volume of plaque destroyed with increasing duration, but considerable exposure to exposure variation is evident from the ranges in each group presented in Table 2.

Histologic results. Light microscopic examination of the exposures revealed histologic findings similar to those previously described (9,10). Laser exposures created roughly cylindrical craters in the atherosclerotic plaque intima. Deeper exposures penetrated through the intima into the media. The
crater edges uniformly showed a thin layer of charring overlying a zone of thermal necrosis. No histologic difference was noted when craters made in the four media were compared.

The possibility that the depth of plaque penetration may vary depending on the relative concentration of the lipid, calcium or hyaline material in the complicated atherosclerotic lesions was noted by Lee et al. (11). It would be difficult, if not impossible, to quantitatively assess the composition of the target site before the exposure. After the exposure, it is impossible to histologically quantify the type of material that was vaporized, although predictions might be made based on the type of material that remains in the surrounding heterogeneous plaque. We were therefore unable to quantitatively relate histologic characteristics to the marked variable effects observed with similar exposures. Qualitatively, histologic sections with increased calcium did seem to correlate with decreased resultant effect. By making multiple sets of determinations on multiple sections of atherosclerotic plaque in a randomized fashion, our model closely approximated the different types of tissue target areas that will be found when laser angioplasty is used in vivo (where the exact calcium, lipid or hyaline concentration of the plaque will also be unknown before the exposure).
Discussion

Our experimental model allows analysis of the contribution of each of the six major determinants of effect (energy, duration, wavelength, medium, absorption, geometry) in any given laser angioplasty system. The data describe specific relations between five of the six determinants (energy, duration, absorption, medium and geometry) when an Nd:YAG laser source is used to lase moderately atherosclerotic distal aorta. Because complicated atherosclerotic plaques are histologically the same in the aorta, the coronary arteries and other medium-sized vessels, the trends of effects of laser exposures noted on aortic plaques should be comparable in any of these vessels. The Nd:YAG (near infrared 1,064 nm) is one of several potential laser sources currently available for convenient use in fiber optic laser angioplasty systems. Other readily available sources include the argon (blue-green visible light 488 to 514 nm) and the carbon dioxide lasers (infrared 10,640 nm). Many of the effects on plaques from laser exposures from Nd:YAG laser angioplasty sources are likely to be comparable with effects from carbon dioxide or argon laser angioplasty sources, especially when high-energy levels are used in each exposure. In our study, we chose to use four optically and thermodynamically different media in order to point out the importance that media can have on lasing outcome. From these studies, it is evident that comparison of results from any model used to study laser angioplasty must take this factor into account.

Variability of laser effect. One of the most important findings in our studies was the large amount of exposure to exposure variability. This variability was evident in all groups of experiments, even when conditions were held as constant as possible in a rigidly controlled laboratory setting. For example, the average depth of the hole created by a 15 W, 2 second blast with the fiber tip adjacent to the plaque in blood was 1.7 ± 0.1 mm (n = 27), but the range was between 0.5 and 2.7 mm. Under the same conditions, except with the fiber tip 1 mm away from the plaque, the average hole depth was 0.4 ± 0.1 mm (n = 12) and the range was 0 to 1.7 mm. By looking at the wide ranges of effect in similar groups of the experiments, one could easily predict that perforations of some small vessels would occasionally occur under conditions similar to those necessary to cause a minimal effect in other vessels.

Table 2. Range of Values of Plaque Destroyed (mm$^2$) in Time-Response Experiment

<table>
<thead>
<tr>
<th>Medium</th>
<th>Time (seconds)</th>
<th>0.5</th>
<th>1.0</th>
<th>1.5</th>
<th>2.0</th>
<th>4.0</th>
<th>8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>0.5 to 3.3</td>
<td>2.0 to 5.3</td>
<td>2.0 to 9.5</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>0.0 to 3.8</td>
<td>0.0 to 4.6</td>
<td>1.3 to 6.1</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.0 to 1.0</td>
<td>0.0 to 6.1</td>
<td>0.1 to 6.3</td>
<td>-</td>
<td>-</td>
<td></td>
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N = 12 exposures in each set of conditions.
herent source of variability whatever manipulations are made with the other, more readily changeable determinants of effect. Although the variability of effect using carbon dioxide and argon laser sources remains to be rigorously explored in the same manner as in this study, the implications are that the predictability of the outcome of any given laser exposure may be limited by the individual characteristics of the targeted plaque.

One can increase the absorption of energy by the plaque after treating the surface with dyes that preferentially absorb the given laser energy source. However, there have been no complete reports in which either the dyes or the vehicle of dissolution of the dyes were nontoxic to humans. It also remains to be shown whether the use of dyes will alter the blast to blast variability of effect on different plaques. It is possible that the absorption of the dye by the heterogeneous plaques will be variable enough that the blast to blast effect will continue to have large variation. The study of the different laser angioplasty systems using different dyes could be done using the model presented. Such studies could define the variation of effects of each of the dyes in a fashion that would allow comparison from study to study.

The energy-response and time-response data confirm that increasing the energy or duration of the exposures will increase the resultant effect. These are not surprising findings, and should prove to be true regardless of which laser energy is chosen. The large exposure to exposure variability of effect of similar blasts proved consistent in our data throughout the tested ranges of energy duration of exposures and is an important principle. There was no clear "therapeutic window" of conditions in our system; we could not get consistently predictable results.

Distance from fiber tip to plaque. Another important conclusion from our experiments is that there is a large difference in effects caused by different distances from the fiber tip to the plaque. A significant difference in effect was shown in all four media when 15 W, 2 second adjacent exposures were compared with 15 W, 2 second exposures with the fiber tip 1 mm away from the plaque. For example, the average volume of effect in blood of the adjacent exposures was 2.6 ± 0.3 mm³ (n = 27, Table 1A) with a range of 0.9 to 6.1 mm³ compared with 0.2 ± 0.1 mm³ (n = 12, Table 1B) and a range of 0.0 to 1.1 mm³ for the 15 W, 2 second exposure with the fiber tip 1 mm away from the target.

There are probably two main reasons why the exposures are smaller when the fiber tip is at a greater distance from the plaque. First, there will always be some component of absorption or reflection of the laser energy by the medium. The amount of this energy loss will depend on the wavelength of energy light used as a source, the wavelength of light absorbed by the medium and the amount of medium between source and target. In our experiments with Nd:YAG laser source (principal wavelength of admission in the near infrared 1,064 nm), the greatest amount of energy was transmitted through air, followed fairly closely by blood, with saline and artificial blood a distant third. Although these findings should hold for any laser angioplasty system using Nd:YAG as an energy source, they do not necessarily predict absorption characteristics when lasers emitting other light energies are used. One would expect a carbon dioxide (infrared) laser source to act in a qualitatively similar fashion to the Nd:YAG system in the media used in this report, although the amount of energy necessary to cause a specific effect would probably be different. The argon laser source should be similarly transmitted in air, perfluorochemical and saline solution, but when blood is used as a medium, there may be a proportional decrease in the translation of energy through the media secondary to the intense absorption of blue-green light by the hemoglobin. The decrease in medium transmission with argon in blood may be partially offset by the black "charring" that is noted around craters, which would be expected to enhance absorption of any visible light in that region.

The second reason for the energy difference between adjacent versus 1 mm exposures is that the light existing at the fiber tip is not parallel. The energy density of light from the fiber tip therefore varies with the distance to the target. A simple analogy is the way light disperses as it exits a flashlight. This principle may be very beneficial to users of fiber optics in laser angioplasty, as the energy intensity drops off greatly with distance from the fiber tip, and remote targets may be spared damage. Conversely, this principle makes the fiber tip to target distance exquisitely important to exposure outcome, and this must be considered when any attempt is made to predict the effect of any given exposure. This factor should prove to be important when carbon dioxide or argon lasers are used for laser angioplasty, as they will use the same type of optics that were tested in our Nd:YAG laser system.

Implications. Although laser angioplasty has received a great deal of attention, rigorous studies of the basic effects of lasing atherosclerotic plaque under different conditions are just being considered. The best way to approach the overall problem may be to initially study each of the possible determinants of lasing outcome. If we understood the contributions of the different lasing conditions, we could more easily predict the outcome of each blast. Our study indicates the importance of medium, time, energy, distance and target absorption-reflection characteristics to the outcome of each exposure. The discovery of the large contribution of target characteristics implies that this may ultimately be the limiting factor in our ability to consistently predict results. The wide variability in exposure effects is disturbing, and implies that it may not be possible to obtain controlled uniform effects with the high energy type of exposures used with the Nd:YAG laser. Similar studies using other wavelengths could be different, although one would predict that the best
results will be obtained if methods can be found to selectively dye the plaque to enhance specific absorption of the laser light. Alternatively, the normal intima could be dyed and a laser wavelength chosen that would be preferentially reflected by the normal intima. The model presented in this study represents one way to analyze the six major determinants of lasing effect in a laser angioplasty system. Extension of the use of this model with other laser sources would allow meaningful comparison among the different systems.

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References