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Photoacoustic Fibrinolysis: Pulsed-Wave, Mid-Infrared Laser–Clot Interaction

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Abstract. **Objectives: The purpose of this study was to determine whether a mid-infrared laser can induce selective fibrinolysis and to analyze the effect of altered fibrin structure (thin vs. thick fibers) on laser–clot interaction. Background: Mechanical disruption of thrombus can be achieved with balloon angioplasty, sonication, and thermal energy. Thrombi avidly absorb light in the mid-infrared optical spectrum due to their high water content. This phenomenon provides a potential for mid-infrared lasers as a source for selective thrombolysis. As fibrin is the essential component of clot, a study of mid-infrared laser-fibrin interaction is warranted. Methods: Clots of varying fibrin structure were lased in cuvettes with a solid-state, pulsed-wave, mid-infrared laser (2.1 micron, 500 mJ/pulse, 250 msec pulse length). Total pulse energies of 5 Joules (J), 10 J, 37.5 J, 75 J, and 112.5 J were tested. Protein content of the extruded fluid was measured by optical density absorbance at 280 nm. The amount of released material was studied as a function of lasing energy and clot structure. SDS-polyacrylamide gel electrophoresis was applied for analysis of protein bands in order to identify unique protein bands released by the selective effect of laser fibrinolysis. Results: A threshold for mid-infrared laser induced fibrinolysis was found; application of up to 20 J of energy did not result in dissolution. As lasing energy was increased above 37.5 J, the structure of these gels was mechanically destroyed and 12.4 ± 6.7% (mean ± SEM) of the original content of protein was released. Electrophoresis revealed that lased gels did not release any unique protein band. Lased, thin fibers released significantly less protein than thick fibers, indicating that they are more resistant to the effect of this wavelength of energy. Conclusions: Mid-infrared laser can induce in-vitro photoacoustic dissolution of fibrin clots. However, this wavelength laser achieves fibrinolysis by mechanical destruction of the target clot rather than by a selective effect, as induced by the pulsed-dye laser. A threshold exists for energy levels required. Thin fibrin fibers, with their high elastic modulus (i.e., gel rigidity) appear more resistant than thick fibers to the effect of lasing at this wavelength.**

Key Words. **laser, thrombolysis, fibrinolysis, infrared**

Thrombi are known to have a high water content, which results in a large thermal sink and, consequently, dissipation of laser thermal energy [1]. They absorb a wide range of laser wavelengths within the optical spectrum. Between 1000 and 3000 nm, light absorption in thrombi dramatically increases as water molecules avidly absorb laser energy at 1900 nm [2]. This phenomenon suggests a potential for mid-infrared lasers as a device for dissolution of occlusive thrombi. In limited clinical experience with mid-infrared laser irradiation (Ho:YAG, 2100 nm) of intracoronary thrombus during acute myocardial infarction [3–5], we have observed that this laser appears to induce thrombolysis. This has created interest in evaluating the mechanisms by which this laser interacts with thrombus.

We herein report on in vitro studies of the structural changes in fibrin clot composition induced by exposure to a 2100 nm laser wavelength. The goals of these studies were (1) to examine the hypothesis that the mid-infrared laser can induce selective fibrinolysis; (2) to define the extent of fibrinolysis induced by mid-infrared laser emission and to analyze the composition of materials released from the lased clot; and (3) to assess the effect of altered fibrin structure on the extent of fibrinolysis elicited by laser energy. To our knowledge, this is the first analysis of the effects of pulsed-wave, mid-infrared laser irradiation on the in vitro fibrin clot.

Methods

Laser equipment

A pulsed-wave, solid-state, mid-infrared (holmium: YAG) laser (Eclipse 2100, Eclipse Surgical Technologies, Palo Alto, CA) was utilized for in vitro fibrinolysis. The device is capable of emitting 250–600 mJ/pulse, with a pulse length of 250 msec and a repetition rate of 5 Hz. Laser light with a 2.1 μm wavelength

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(mid-infrared zone) was transmitted through a flexible catheter composed of multiple optical low-hydroxide silicon fibers. The optic fibers are arranged in a close-packed circular array surrounding a central lumen. The laser catheter has an atraumatic outer wrap and a soft tip, which assist in positioning the laser within the target clot and in easy maintenance of coaxial alignment. The laser beam is emitted in a front-firing mode, thus ablating along the coaxial length of the catheter. A 1.5 mm laser catheter was utilized, emitting 500 mJ/pulse (33 J/cm^2). It contains 26 optic fibers, 100 microns each, with a fiber/catheter tip area ratio of 0.196. The effects on clots after exposure to 10, 20, 75, 150, and 225 pulses corresponding to pulse energies of 5 J, 10 J, 37.5 J, 75 J, and 112.5 J, respectively, were analyzed. Pulses were counted by a pulse-counter mounted on the laser device.

Formation of Clots

Fibrin gels were prepared by a previously described method [6–8] in 10 mm polystyrene cuvettes (Fisher Scientific Co.) from purified fibrinogen solutions that were buffered to pH 7.4 (Tris 0.05 M) and supplemented with 10 mM calcium at 37°C. For purified gels, clotting was initiated by the addition of thrombin (final concentration 1 NIH unit/ml) to buffered fibrinogen solutions of 1 mg/ml. Human thrombin consisting of greater than 90% alpha thrombin was purchased as a lyophilized powder (Sigma Chemical, St. Louis, MO). The material, which has a specific initial activity of 4300 units/ml, was dissolved in water and diluted with 0.10 M NaCl to a final concentration of 20 NIH units/ml. This solution was divided into 1 ml aliquots and frozen at −90°C. Thrombin was free of plasmin and plasminogen. Nanopure water was prepared utilizing a Nanopure II system (Sybron-Barnstead, Boston, MA) and was used in the preparation of all solutions. Clots formed in this manner are covalently cross-linked by trace amounts of factor XIII found in the fibrinogen preparation and are activated upon addition of thrombin.

Formation and assessment of clots of varying fibrin structure

Clots of varying fibrin structure were prepared by increasing the concentration of NaCl so as to shift the ionic strength from 0.15 to 0.30 in steps of 0.05. Clots were allowed to form for 1 hour, after which time the fiber mass/length ratio was measured and lasing was initiated. All gels were formed in triplicate. Turbidity measurements were made at 37°C utilizing a Cary 2290 spectrophotometer (Varian Instrument Division, Sunnyvale, CA). After the addition of thrombin, the increase in sample turbidity was used to monitor the rate and extent of clotting. Measurements were taken at the HeNe laser line, 633 nm. The moment of thrombin addition was taken as time zero. Turbidity development was monitored for 10 minutes, after which time gelation was allowed to be completed unobserved. After 1 hour the fibrin structure was assessed by scanning gels from 400 to 800 nm. The mass/length ratios of the fibrin fibers were determined according to the following equation [8]:

$$\tau = [(88/15)\pi^3 n(dn/dc)^2 C\mu]/N\lambda^3, \quad (1)$$

where τ is the turbidity, n is the solution refractive index, dn/dc the refractive index increment, λ is the wavelength, C is the concentration of fibrinogen in g/ml, N is Avogadro's number, and μ is the mass/length ratio. For clear gels, μ was determined from the slope of a plot of τ versus $1/\lambda^3$. For more turbid gels, where the radius of the fibers is no longer small relative to the incident wavelength, μ was obtained from the inverse of the intercept of a plot of $C/\tau\lambda^2$ versus $1/\lambda^3$ [6].

Lasing technique

After determination of the fiber mass/length ratio, the gel-filled cuvette (cross-sectional area 1 × 1 cm) was mounted on a clamp and sealed at the top with parafilm. Gels were lased at ambient room temperature (~27°C) 1 hour after thrombin addition. The laser catheter tip was advanced through a hole in the parafilm (no guidewire involved) and positioned above the center of the gel top. Just prior to lasing, the tip was advanced 5 mm into the upper surface of the clot. Energy was delivered by a controlled lasing technique [9] to a predetermined total energy level as described earlier. The catheter was not advanced manually during lasing in order to avoid mechanical probing of the clot. When lasing was complete, the catheter tip was withdrawn.

Determination of extent of fibrinolysis

The remaining postlasing clot was removed from the cuvette by winding on a thin glass rod. The extruded fluid was analyzed for material released from the clot. The amount of released material was studied as a function of lasing energy and clot structure (μ). Extruded fluid was tested for total protein content by measurement of absorbance at 280 nm. Percent change in protein content was calculated based upon comparison with fluid from a control, nonlased gel.

Analysis of protein fragments released during laser irradiation

Molecular weights of material released from purified fibrin gels during laser exposure were estimated utilizing SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Fifty microliters of each supernatant was subjected to SDS-PAGE according to the method of Laemmli [10]. Six percent polyacrylamide slab gels were used. Electrophoresis was continued until the dye front reached the bottom of the gel. A standard curve was prepared by plotting molecular weights versus migration distance for a group of high molecular weight SDS-PAGE standards (Bio-Rad Laboratories, Hercules, CA). Molecular weights of frag-

ments were estimated by comparing fragment migration distances to the standard curve.

Data analysis

Data are expressed as means ± SEM. Comparisons of the effects of number of pulses on optical density of the supernatant were made by use of Student's t test. $p < 0.05$ was considered to be statistically significant.

Results

Effect of laser energy on in vitro clots

The optical density at 280 nm was increased in supernatants obtained after removal of lased clot. This implies that proteinaceous material was released during the lasing process, and it indicates a fibrinolytic effect of in vitro lasing. Increasing optical density from gel supernatants exposed to higher energy levels indicated increasing protein release. As the lasing energy was increased, the amount of protein released from the fibrin clot increased (Figure 1). At low energy (10 J), 4.49 ± 0% of the original protein was released. At the highest energy (111 J), 12.4 ± 6.7% of the original protein content was released. Statistically significant amounts of protein were released at energy levels above 37.5 J.

The effect of increasing lasing energy on the mechanical disruption of fibrin clots is documented in Figure 2. Gel #1 is an undisturbed control gel. Gel #2 is a sham control into which the catheter was inserted but no lasing was performed. Gels #3–8 were lased with increasing numbers of pulses in order to deliver increasing amounts of energy. Energy increased from 5 J for gel #3 to 112.5 J for gel #8. At the lowest energies, gas bubble formation was noted (gel #3). Photoacoustic shockwaves were seen emanating from the laser catheter tip and propagating through the clot. Gels #4 and #5 were lased with the same amounts of energy (37.5 J). Gel #5 was formed

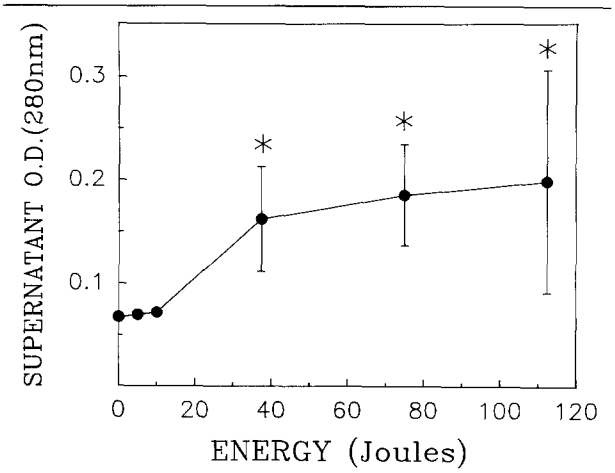

Fig. 1. Effect of increasing energy on the amount of proteinaceous material released into the supernatant during lasing. Statistically significant ($p < 0.05$, marked by asterisk) amounts of protein are released at energies at and above 37.5 J.

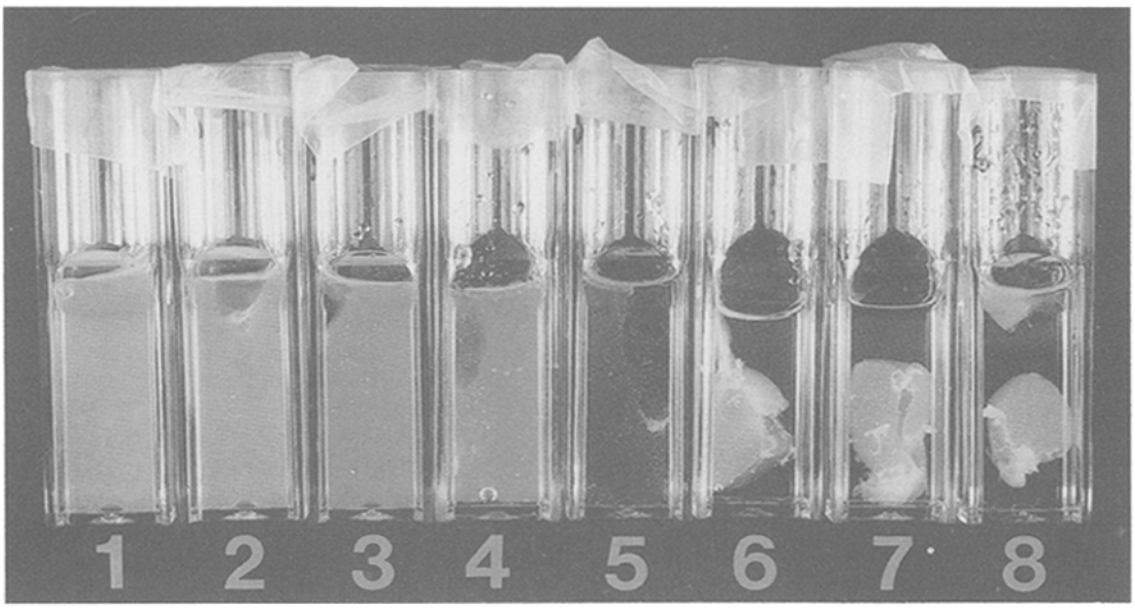

Fig. 2. Effect of increasing laser energy on the mechanical disruption of fibrin clots. With the exception of gel #5, all gels were formed under identical conditions: fibrinogen 2 mg/ml, thrombin 1.0 NIHu/ml, pH 7.4, ionic strength 0.15 and 10 mM $CaCl_2$. Gel #5 contained no added calcium, which results in a weaker, less turbid gel. Gel #1 is an undisturbed control, and gel #2 is a sham control into which the laser catheter was introduced but in which no lasing performed. The amount of energy delivered to the clot increased from gel #3 through gel #8, with the exception of gels #4 and #5, which were lased with the same energy.

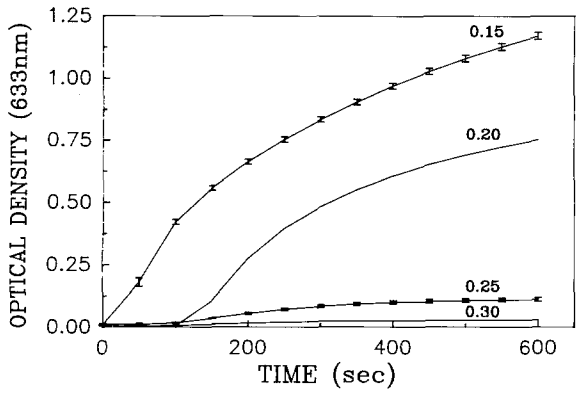

Fig. 3. Altered fibrin assembly and structure induced by increasing ionic strength. All conditions other than ionic strength were identical in these gels. Such gels contain identical amounts of protein but a radically altered structure. Decreased gel optical density is due to thinner, less massive fiber formation. These gels were subjected to identical lasing energies to investigate the effect of fibrin structure on the extent of laser-induced thrombolysis. When lased with equal energies, less material was released from clear gels.

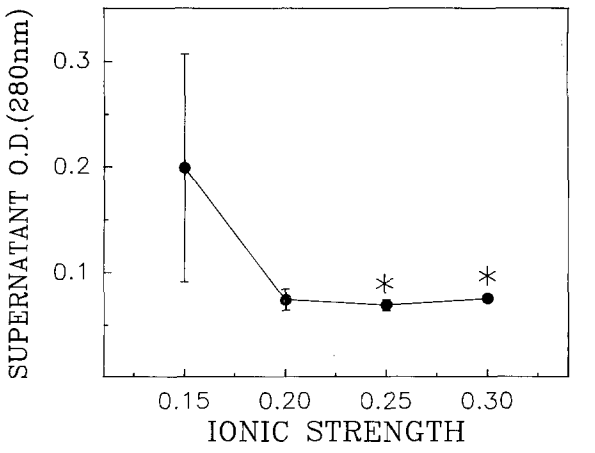

Fig. 4. Effect of altered fibrin structure on the amount of protein released during laser thrombolysis. Protein release was significantly reduced ($p < 0.05$) at ionic strengths above 0.20.

under conditions that produce clear gels (see later) to better illustrate bubble formation. As the lasing energy was increased, mechanical disruption of the gels increased and the quantity of material released into the supernatant increased. At energy levels above 37.5 J (gels #6–8), gross disruption of gel structure was noted.

Influence of varying fibrin structure

Figure 3 shows the effect of increasing ionic strength on fibrin polymerization. At higher ionic strengths, fibrin gels are composed of thinner fibers, which scatter less light, resulting in decreased optical density at baseline. This was confirmed in the present study by measuring gel optical density as a function of wavelength and by calculating the gel fiber mass/length ratio. This parameter represents the average mass of gel fibers per unit of fiber and decreases as gel fibers decrease in cross-sectional size. For the gels depicted in Figure 3, the fiber mass/length ratio declined from 5.77 to 2.11 to 0.446 to 0.273 $\times$ 10^{13} Da/cm as the ionic strength rose from 0.15 to 0.20 to 0.25 to 0.30, respectively. When these gels were lased at energy levels of 112.5 J, the amount of protein released declined as the ionic strength increased (Figure 4). When compared with the amount of protein released at ionic strength 0.15, protein release at ionic strength 0.25 and 0.30 was significantly reduced ($p < 0.05$). Thus, the thinner the fiber, the less fibrinolysis occurred.

Characterization of clot fragments released during laser exposure

Molecular weights of standards were 45,000, 66,000, 97,400, 116,000, and 200,000. Based on these standards, protein bands were noted at weights of 40,000, 48,000, 90,000, 98,000, 111,000, 200,000, and $\geq$230,000. The band at 48,000 was a doublet, and the band at 230,000 was a triplet. While the protein content of supernatants from unlased gels was less than 29.7% of that seen in lased gels, the electrophoretic pattern was strikingly similar, attesting to the fact that no small, unique protein fragment is generated during mid-infrared laser fibrinolysis, and, therefore, only mechanical disruption accounts for the clot dissolution.

Discussion

There are two major findings in this study: (1) Solid-state, pulsed-wave, mid-infrared laser irradiation can induce fibrinolysis by mechanical disruption of the lased clot, in contrast to selective thrombolysis, which is achieved by other wavelength lasers, such as the pulsed dye-laser [11]; (2) thin fibrin fibers are more resistant than thick fibers to the photoacoustic effect of this wavelength energy.

Effect of laser on in vitro clots

A fresh thrombus is known to contain a high water content, which results in a large thermal sink and, consequently, dissipation of laser energy [1]. Since absorption of laser emission is a function of both the pigmented component and aqueous content of blood, the holmium laser, whose wavelength (2.1 micron) coincides with strong water absorption peaks, appears to be fit for thrombolysis. In this study, the effect of increased lasing energy on the release of proteinaceous material from clot structure was measured as changes in supernatant absorbance at 280 nm. The mechanical, nonselective disruption of the gel structure was associated with increased supernatant absorbance at 280 nm, indicating release of protein from

the gel (i.e., fibrinolysis). A strong association between mechanical disruption of the gel structure and high energy level was demonstrated.

Laser tissue photomechanical interaction is associated with buildup of acoustic fields. As these powerful acoustical waves propagate through the biotissue, they are transformed into shock waves, giving rise to great pulse pressure gradients within the tissue [12]. It has been shown that several hundred bars of pressure are generated during pulsed-wave laser ablation of atherosclerotic plaque [12]. Apparently, the induction and radial distribution of a shock-wave front creates local tissue fragmentation [13]. Thus, it is likely that the nonselective yet significant mechanical destruction of lased clots that we observed in our experiment was due in large part to the formation and propagation of shock waves.

Our study demonstrates the existence of a certain threshold for the nonselective fibrinolysis by mid-infrared photoacoustic energy. Application of up to 20 J of energy did not result in significant fibrinolysis. With energy levels exceeding 37.5 J, the mechanical structure of fibrin gels was totally destroyed, as judged by visual inspection and spectrophotometry analysis. This finding is consistent with the report of Lee et al. [14], who found that laser energies transmitted directly from an argon-ion source produce vaporization and penetration of human thrombus in a linear dose-response fashion—the longer the thrombus, the greater the power intensity or time exposure necessary to penetrate the clot.

Effect of varying fibrin structure

Previous studies have indicated that clots composed of thick fibers dissolve rapidly when exposed to plasmin [15,16]. Conversely, clots composed of thin fibers are relatively resistant to enzymatic digestion. Thin fiber formation is induced by hyperglycosylated fibrinogen and by high immunoglobulin levels [17,18]. It has been proposed that thick fibers offer a better environment for plasminogen activation [15], are a better substrate for plasmin [16], or both. Conditions that favor thick fiber formation enhance fibrinolysis, while those that favor thin fiber formation delay clot dissolution [19, 20]. The situation appears to hold true in both purified gels [15] and plasma systems [21]. Thin fibrin fibers are known to occur in pathologic conditions such as diabetes mellitus [22] and multiple myeloma [23, 24]. In the current study, we examined the effects of photoacoustic rather than enzymatic fibrinolysis. Nevertheless, our results are very similar to the results observed with plasminogen activation. We have recently demonstrated [23] that gels composed of thin fibers exhibit higher elastic modules (i.e., gel rigidity) than normal plasma gels and therefore are much more resistant to deformation by externally applied forces. The present study suggests that conditions that induce thin fiber formation may result in delayed or unsuccessful laser induced fibrinolysis.

Clinical implications

Most cases of acute myocardial infarction are due to sudden obstruction of coronary arteries by a thrombus at the site of ruptured or fissured atherosclerotic plaque [25]. Although immediate survival has significantly improved through pharmacologic thrombolysis, its success is not complete. Approximately 20–30% of infarct related arteries fail to open with thrombolytic regimens, and 15% of reperfused arteries occlude in the subsequent hours and days [26]. Furthermore, in a large number of patients with acute myocardial infarction, thrombolytic drugs cannot be administered, either because the patients present too late to receive these agents or because patients have contraindications to their use. Similarly, primary balloon angioplasty can achieve excellent clinical results in acute myocardial infarction [27]. However, in the presence of residual thrombus, mechanical recanalization by balloon can result in distal embolization and enhanced platelet stimulation and thrombosis.

Laser thrombolysis has several potential clinical advantages, including rapid removal of the target clot, vaporization of procoagulant reactants, absence of a systemic lytic state, and facilitation of adjunct balloon angioplasty. Clinically, our limited experience [28] with holmium laser application during acute myocardial infarction is encouraging; 25 patients who sustained complicated acute myocardial infarction, whose lesions deemed unsuitable for balloon angioplasty, were treated with this modality. Following laser thrombolysis and adjunct balloon angioplasty, mean flow increased from TIMI grade 0.7 to 2.8 ($p < 0.001$). Clinical success (including restoration of TIMI III flow, elimination of chest pain and ischemia, <50% residual stenosis, no death, coronary artery bypass graft surgery, perforation, major dissection, or extension of infarction) was achieved in 24 of 25 patients (95%) who survived and were discharged.

Study limitations

The test method we used is limited to fibrinolysis because laser was not applied on platelet-rich plasma. The presence of hemoglobin, red blood cells, and leukocytes can affect the laser interaction, as can the normal concentration of factor XIIIa and endogenous fibrinolytic factors, including α-2 antiplasmin and PAL-1. Nevertheless, in this project we sought to investigate the basic mechanisms affecting fibrin, the essential component of clot. This in vitro approach is supported in the literature [29]; thus, despite the limitation of testing platelet-poor plasma, our observations illuminate the mechanism of interaction between laser and fibrin clot.

Conclusions

The results from this in vitro study indicate that mid-infrared laser energy has a fibrinolytic effect, yet this

effect is achieved by nonselective mechanical disruption of the irradiated clot. It is hoped that understanding the in vitro mechanism(s) involved in laser–fibrin clot interaction will provide a better conceptual basis for the clinical use of this technology.

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