XeCl EXCIMER LASER-INDUCED FLUORESCENCE FOR SELECTIVE ABLATION OF ATHEROMATOUS TISSUE

KOH ARAKAWA, M.D., THEODORE PAPAZOGLU, M.S., THANASSIS PAPAIOANNOU, M.S.
WEI QIANG SHI, M.S., MICHAEL FISHBEIN, M.D., FRANK LITVACK, M.D.
JAMES S. FORRESTER, M.D., AND WARREN S. GRUNDFEST, M.D.

In order to develop a reliable laser-induced fluorescence (LIF) guided laser angioplasty system, real time, pulse-by-pulse fluorescence spectra were recorded and the same fiberoptic was used for both detection of the fluorescence and for atheromatous tissue ablation. A 308 nm XeCl excimer laser served as the laser source for both the induction of fluorescence and the ablation. The fluorescence signal was induced at high laser energies during ablation without any serious change in the fluorescence pattern. A new characteristic fluorescence peak at 540 nm for atheromatous tissue was observed after treatment with chlortetracycline hydrochloride (CTC). This allowed the development of an algorithm and a subsequent index to discriminate the atheromatous tissue from the normal tissue. During atheromatous tissue ablation, this index changed as normal tissue was approached, thereby avoiding vessel perforation. Our results suggest that monitoring of this index through the catheter delivering the laser energy enhances selective ablation while simultaneously reducing the risk of vessel perforation.

SELECTIVE ablation of atherosclerotic plaque without risk of vessel perforation is a primary goal of laser angioplasty. To achieve this, the fluorescence spectra from arterial vessels recently have been investigated as a possible means of catheter guidance during laser angioplasty. Attempts of a laser-induced autofluorescence discrimination of normal and atheromatous tissue have been reported with differing degrees of success!¹⁻⁹ Since a variety of factors, such as the excitation wave length and the

delivery/collection geometry of the detection system, can affect the fluorescence lineshape and the guidance capability of laser-induced fluorescence-based systems!¹⁰ a diagnostic algorithm defined for a particular collection system may be valid only for that system. Further, the diversity in the composition of the atheromatous tissue (fatty, fibrous and/or calcified) could also increase the complexity of fluorescence-aided tissue identification?⁹

In the search of a tissue identification method based on fluorescence discrimination, tetracycline has been shown to bind preferentially to atherosclerotic plaque!¹¹⁻¹³ As tetracycline-stained plaque yields a characteristic yellow fluorescence under UV

Key words:

Atherosclerosis
Excimer laser
Ablation
Fluorescence spectroscopy
Exogenous fluorescence probe

(Received October 24, 1990; accepted March 4, 1991)

The Departments of Medicine (Cardiology), Surgery and Pathology, Cedars-Sinai Medical Center and The University of California Los Angeles School of Medicine, Los Angeles, California

This study was presented at the 39th Annual Meeting of the American College of Cardiology, New Orleans, Louisiana, March 1990.

Mailing address: Koh Arakawa M.D. First Department of Medicine, National Defence Medical College. 3-2 Namiki, Tokorozawa, Saitama 359, Japan

Fig.1. The experimental set up.

excitation, it was thought that tetracycline might be useful as a fluorescence probe for discriminating between normal and atherosclerotic tissue and that its application to this task was worth investigating.

Since currently used laser angioplasty systems incorporate 308 nm excimer lasers¹⁴⁻¹⁸ it was further thought desirable that a practical system should use this same wavelength (308 nm). Therefore, the purpose of this study was to evaluate whether 308 nm excimer laser-induced fluorescence could help to discriminate atheromatous from normal tissue stained with chlortetracyclin hydrochloride (CTC), and whether laser-induced fluorescence (LIF) could detect plaque from normal media during the transition of the fiberoptic for the ablation. Throughout this study, a single fiber was employed for the ablation, excitation, and the detection of the fluorescence signal.

METHODS

Tissue specimens

Segments of the human femoral artery and aorta containing atherosclerotic plaque and normal vessel walls were obtained from 9 autopsied cadavers within 12h of death. The vessels were opened longitudinally to expose the intimal surface. In a subset of normal arterial samples, the media was exposed by removing the intima and several layers of media with surgical scissors.

Thick, atheromatous plaque was selected for analysis, complex plaque (ulcerated and hemorrhagic specimens) and flattened heavy calcified lesions were excluded. Each sample was classified by gross inspection as being normal intima, normal media, or atheromatous plaque. The fluorescence spectra of 50 sample points from normal intima, 36 from normal media, and 56 from atheromatous

Fig.2. The plaque index.

Fig.3. Representative fluorescence spectra from a normal area of the aorta before and after treatment with CTC. A: before treatment with CTC; B: after treatment with CTC.

plaque were recorded.

Arterial segments of each of three tissue groups were incubated in a saline bath containing 1 mg/ml of chlortetracycline hydrochloride, CTC (Sigma Chemical Company) for an hour and then rinsed with saline

for an hour. By varying the concentration of CTC (100 μ g/ml–2 mg/ml), the incubation time (10 min–24h) and the rinse time (0–24h), it was found that the combination of 1 mg/ml of CTC and an hour of incubation and an hour of rinsing was the

Fig.4. Representative fluorescence spectra from the media. A: before treatment with CTC; B: after treatment with CTC.

Fig.5. Representative fluorescence spectra from atherosclerotic plaque before and after treatment with CTC. A: before treatment with CTC; B: after treatment with CTC.

procedural treatment for optimal fluorescence identification. Each sample was placed in a tissue holder and submerged in a saline bath (Fig. 1). During irradiation, the fiber tip was kept in constant contact with the tissue by a specially constructed appar-

atus that allowed us to vary the force applied to the catheter from 1 to 4g.

Laser source

An adapted xenon chloride (XeCl) excimer laser (Advanced Interventional Systems;

Fig. 6. Variations in fluorescence spectra of atherosclerotic plaque treated with CTC (A–C)

Costa Mesa) was used as the 308 nm laser light source. The laser emitted light pulses with a frequency of 10 Hz and a pulse width of approximately 130 nanoseconds. For the fluorescence recordings, an energy density of 10 mJ/mm² was used; for ablation, 35 to 50 mJ/mm² were used. The laser energy was delivered through a fused silica optical fiber with a core diameter of 600 μ m.

With the addition of a homemade module, the fluorescence spectra were collected through the same fiber and sent from a dif-

ferent port of the clinical laser unit to the entrance of the detector. At the same time, via a second homemade module, the intensity of the laser output pulses was monitored on a pulse-by-pulse basis and recorded by the same detector.

Optical signals were collected at the entrance slit of a Jarrel Ash 0.25m Monochromator. The tissue fluorescence was spectrally dispersed by a diffraction grating (150 grooves/mm) and imaged on the detector of an Optical Multichannel Analyzer (OMA III, Model 1422G, EG&G). The detector consisted of 700 discrete recording elements through which the signal was directed to a microchannel intensifier. The analog to digital conversion was made by an independent OMA board (Controller, 1463 EG&G) and the final results were displayed on the OMA main module screen and saved on a hard disk (Winchester, 20 Mb). Further, in the optical path of the fluorescence signal various filters were placed in order to attenuate the intense 308 nm laser line (Schott Glass High Pass Filters 335 nm, 345, Melles Griot Neutral Density Filter O.D.2).

To improve the signal to noise ratio, background spectra that were recorded before the initiation of irradiation were subtracted from the acquired data during the experiment. In this way, background fluorescence from other sources was minimized and chances of dark noise artifacts from the detector were reduced. The detector was triggered by an electrical signal taken from the laser pulser, and the time between one complete scan period and the period of laser pulses permitted some experimentation with various acquisition strategies. The one we finally chose was to obtain the average of ten scans per pulse for the tissue surface fluorescence study and one scan per pulse for the ablation study.

Development of a plaque index (PI)

From the fluorescence spectra before and after treatment with CTC, a plaque index was devised (Fig. 2):

$$\text{Plaque index (t)} = \frac{(I(t)_{540} - I(t)_{512})}{(I(t)_{540} + I(t)_{512})} \times 100,$$

whereby I_{540} and I_{512} are the measured fluorescence intensity at 540 and 512 nm, respectively. The numerator expresses the dif-

Fig. 7. The PIs of a normal area of the aorta, media and plaque were -18.2 ± 4.4 , -21.9 ± 4.3 , and -15.8 ± 5.7 before treatment with CTC, and -11.5 ± 4.1 , -12.0 ± 4.5 , and 10.0 ± 10.1 after treatment with CTC.

ference in spectra; the denominator normalizes the variations in the overall spectral intensity.

Changes in laser-induced fluorescence during ablation

To evaluate the ability of the index to guide the selective ablation of atherosclerotic plaque, 11 atherosclerotic aortic segments tagged with CTC were irradiated until perforation as identified by an abrupt decrease in the fluorescence intensity at 430 nm. The fluorescence spectra were recorded over a range from 300 to 700 nm during ablation, and from the data stored on the OMA system diskette, time-resolved curves of the intensities at 540 ± 5 nm, 512 ± 5 nm and 430 ± 5 nm and of PI were derived.

The accuracy of changes in the plaque index for detecting the plaque-media boundary during ablation was monitored, and an atheromatous pattern was identified when the index had positive or zero values, whereas a normal pattern was recognized when the index was negative. The number of pulses corresponding to a positive (atheroma) index was determined in relation to the total number of pulses needed for the perforation of an atherosclerotic aorta. This ratio was

considered as being the plaque to total arterial wall thickness ratio (PT ratio).

Histology

After ablation, each specimen was fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin-eosin. The thickness of the plaque and the total arterial wall were measured by quantitative ocular micrometry with a calibrated, reticular eyepiece. The plaque to total arterial wall thickness ratio was also calculated.

Statistical analysis

All values are expressed in means $\pm$ S.D., and the significance of difference among the groups was assessed by an analysis of variance. The significance of differences between unpaired measurements was assessed by the Bonferroni *t*-test. A simple linear regression analysis was used for comparisons of the plaque to the total arterial wall thickness ratio on the basis of the histology and plaque index.

RESULTS

Laser-induced fluorescence spectra

Fig. 3 shows the typical fluorescence

Fig.8. Time-resolved LIF spectra recorded pulse-by-pulse during ablation of the CTC-treated normal aorta (A). The X-axis is the fluorescence wavelength, The Y-axis is the fluorescence intensity, and the Z-axis is the number of pulses or the elapsed ablation time ($\times 100$ msec). Figure 8B shows the histology of the ablation site. I: intima; M: media; Ad: adventitia. Figures 8C and 8D show the time-resolved variations of PI (C) and fluorescence intensity at 430 nm (D) during ablation. The X-axis is the number of pulses. The Y-axis is the PI in Fig. 8C, the fluorescence intensity at 430 nm (D). From the start of ablation to perforation (arrow), the PI is negative.

spectra from a normal aorta before and after exposure to CTC. The normal pretreatment spectrum had a broad emission band between 350 and 700 nm, and was characterized by two fluorescence intensity peaks at 390 and 430 nm.

After treatment with CTC, the fluorescence intensity of the 390–450 nm bands decreased and that of the 500–600 nm bands

increased slightly. We identified this as being the normal pattern.

Figure 4 shows similar pre and post CTC spectra from the normal media, which are similar to the spectra from the intimal surface of the normal aorta before and after CTC.

Fig. 5 shows typical spectra from an atherosclerotic plaque before treatment with CTC. The two peaks of fluorescence intensities at 390 and 430 nm also were present in the plaque spectra before CTC exposure. After treatment with CTC, the plaque spectra changed significantly. A new peak occurred at 540 nm, and the fluorescence intensity at 390 and 400 nm decreased.

Fig.9. Time-resolved LIF spectra recorded pulse-by-pulse during ablation of CTC-tagged plaque (A). X, Y and Z-axes are same as in Fig. 8A. Initially, the LIF spectra showed the plaque pattern, but as tissue was ablated, the LIF spectra changed to a normal vessel pattern. Figure 9B shows the histology of the plaque close to the ablated section. P: plaque; M: media; Ad: adventitia. Figures 9C and 9D show the time-resolved variations of the PI (C) and the fluorescence intensity at 430 nm (D) which were derived from the spectra of Fig. 9A. X-axis is the number of pulses or the elapsed ablation time ($\times 100$ msec). Initially, the PI showed positive values (plaque pattern). During ablation, but before perforation occurred, the PI changed to negative values (normal vessel pattern).

(Fig. 6B).

Fig. 7 summarizes the values for the plaque indices derived from the normal intima, the media, and the atheromatous tissue before and after treatment with CTC. The indices from the normal intima and media were negative before (-18.2 ± 4.4 and -21.9 ± 4.3) and after (-11.5 ± 4.1 and -12.0 ± 4.5) CTC. In contrast, the atheromatous tissue developed a positive index (10.0 ± 10.1) after CTC (before CTC, -15.8 ± 5.7).

Our index classification criterion correctly identified 50 out of 50 normal samples and

Variations occurred in the fluorescence spectra of atheromatous plaque after CTC (Fig. 6). In addition to the typical pattern (Fig. 6A), the variations included a 3rd, small fluorescence peak at 540 nm with no decrease in the fluorescence intensity at 390 and 430 nm (Fig. 6C), and the emergence of an intermediate pattern between the two

TABLE I THE PLAQUE TO TOTAL ARTERIAL WALL THICKNESS RATIO BY PI AND HISTOLOGY

Perforation study (n)	No. of perforation (n)	No. of plaque pattern (n)
1	14	9
2	45	19
3	23	6
4	43	32
5	13	11
6	43	11
7	30	13
8	16	7
9	37	18
10	20	9
11	38	32

Perforation study	PT ratio by PI index	PT ratio by histology
1	0.64	0.73
2	0.42	0.63
3	0.26	0.50
4	0.74	0.78
5	0.85	0.73
6	0.26	0.41
7	0.43	0.44
8	0.44	0.59
9	0.49	0.54
10	0.45	0.48
11	0.84	0.79

The number of the pulses corresponding to the plaque pattern (No. of plaque pattern) was compared in relation to the total number of pulses needed for perforation (No. of perforation). The index indicated the plaque pattern presence before perforation occurred. The plaque to total arterial wall thickness ratio (PT ratio) by PI was almost similar (correlation coefficient=0.85, $p<0.01$) to that obtained by histology (PT ratio by histology).

47 out of 56 samples of the atheromatous tissue (specificity 100%, sensitivity 84%).

Plaque index during ablation

Fig. 8A shows the sequence of LIF spectra recorded at 100 msec intervals (pulse to pulse) during the ablation of the CTC-treated normal aorta. Initially, the fluorescence spectra showed a normal pattern. During

ablation the spectral pattern did not change, except for intensity, and the index was always negative (Fig. 8C).

In contrast, the plaque pattern of the spectra from the CTC-treated atheromatous tissue was recognized at the beginning of ablation (the 1st fluorescence spectrum in Fig. 9A). As the tissue was ablated, however, the fluorescence spectra changed from a plaque pattern to a normal pattern (Fig. 9A), and the change in the index corresponded to the change of the fluorescence spectra and to the subsequent histologic findings (Figs. 9B,C).

The number of pulses corresponding to the plaque pattern in the PI index was compared in relation to the total number of pulses needed for perforation (Table I). The index indicated the plaque pattern presence before the perforation occurred. The plaque to total arterial wall thickness ratio, obtained from the plaque index, was also compared with that obtained from the histologic findings. This ratio correlated with that obtained from the histology with a correlation coefficient of 0.85 ($y = -0.23 + 1.27x$, $p < 0.01$).

DISCUSSION

This study demonstrates that CTC-treated atherosclerotic plaque can be discriminated from normal areas of the aorta by the laser-induced fluorescence spectra and by an index derived from the spectra. In addition, the fluorescence spectra changed from an abnormal pattern to a normal pattern during the ablation of the atheromatous tissue before the danger of vessel perforation.

A major technical advantage is that we were able to obtain these results by using a single fiberoptic for both the LIF spectral analysis and the atheromatous tissue ablation. In the most of other studies, different fibers (at least of two fibers) were employed for the fluorescence delivery/collection. Richards-Kortum et al¹⁰ demonstrated that systems with different delivery/collecting geometries will not achieve equivalent fluorescence spectra for a given tissue sample. To resolve this problem, they have suggested that fluorescence should be collected only from the directly illuminated region of the tissue. Since our system uses a single fiber for delivery/collection of fluorescence

while maintaining constant contact with the tissue, it minimizes the geometrical problems.

In addition, only CTC-tagged atherosclerotic tissue emitted the characteristic fluorescence spectra of CTC, which made it easy to discriminate from normal intima and media. Thus the plaque index that we have developed has proven reasonably effective in discriminating between the plaque and normal vessel tissue. As a potential laser guidance mechanism, it was found to be particularly effective since we had no false positive identifications.

CTC

While the question of CTC binding to plaque constituents was not addressed in this study, CTC, being lipophilic, localizes in the same area that phospholipid stains do,¹¹ and acid phospholipids are thought to promote precipitation of calcium phosphate in the extracellular matrix.^{19,20} CTC binds to divalent cations like Ca^{2+} and Mg^{2+} , yielding fluorescent chalets and creating a characteristic emission band which is enhanced in the presence of lipids at around 540 nm.²¹ Therefore, CTC may bind selectively to atherosclerotic plaque that contains Ca^{2+} and lipids, resulting in similar fluorescence behavior. In our study, CTC binding to calcified lesions as well as to non-calcified lesions was observed.

LIF spectra of tissue surface

Studies have identified atherosclerotic tissue though autofluorescence spectra analysis and some success has been achieved in discriminating normal tissue from atherosclerotic tissue!¹⁻⁹ Further, it has been found that the percentage of calcification in the plaque results in a significant variation in the autofluorescence spectra.^{2,9} Thus, this variability of the plaque composition has required the use of several and sometimes complex identification algorithms^{2,3,5} with only partial success.⁷ It is known that CTC binds preferentially to calcified lesions in vitro and in vivo!^{1,12} There is also clinical evidence that an intravenous administration of CTC helps in identifying atheroma.¹² Therefore, if the fluorescence spectra of the atheromatous CTC-tagged tissue were to have a more characteristic pattern, this would de-

crease the spectrum variability and therefore increase the reliability of atherosclerotic tissue identification. In this regard, our data has shown that CTC-tagged plaque spectra are dominated by a characteristic CTC fluorescence pattern, and so has resulted in the constitution of a simple identification algorithm.

The characteristics that have been identified are a new peak at 540 nm, with a relative decrease in the intensity of the normal 390 and 430 nm peaks. A possible explanation for this behavior may be the overlap seen between the tissue autofluorescence emission band and the absorption band of the tetracycline¹² that leads to partial absorption of the autofluorescence by the CTC and a consequent emission to longer wavelengths. Therefore, this observed variation in the fluorescence pattern (Fig. 6A–C) may be related to the concentration and/or transmural localization of CTC in the atheromatous tissue which, in turn is related to the variable CTC-tagging affinity in the biochemical compositions of the plaque. The relationship between fluorescence pattern and macroscopical classification of plaque was found in this study. A slight increase in the fluorescence intensity at 500–540 nm in normal intima and media after CTC tagging was also observed, although this minor change in the fluorescence intensity did not affect the discrimination between normal and atheromatous specimens by the indexing method.

The concentration of CTC used in this study was high (1 mg/ml), and the question of what amount constitutes the optimal CTC dosage for in vivo use was not addressed. However, results of a clinical study indicated that a 1g i.v. administration of tetracycline appears sufficient for tissue identification!²

LIF spectra during ablation

Most investigators have used low laser energy to induce the LIF spectra!^{1-8,22} one reason being that a high laser energy bleaches the fluorescence dye²² as well as the native chromophores.^{6,8} In our study CTC fluorescence was observed even at ablative energy densities (35–50 mJ/mm²/pulse), and so we were able to use high laser energy densities for both tissue ablation and excitation.

Some investigators^{8,9} have reported successful discrimination of normal and atherosclerotic tissue at ablative energy densities with the KrF excimer laser (248 nm wavelength) or the Er:YAG laser. These laser sources, however, cannot be delivered through a quartz fiber whereas the long-pulsed XeCl excimer laser can. During ablation of a normal artery by a 308 nm excimer laser, no appreciable change in the fluorescence spectral pattern was observed (Fig. 6A), and only small intensity changes. On the other hand, during atheromatous ablation, we were able to identify the uncovering of normal tissue by the fluorescence spectra.

Thus our results suggest that a 308 nm XeCl excimer laser is well suited for use in a dual capacity, for ablation and excitation for fluorescence analysis. This single wavelength approach is more practical than the use of separate wave lengths for ablation and excitation that have been reported in the literature⁸

An advantage of our system is the use of a single fiber for both purposes. This design avoids problems resulting from the use of multiple fibers (one for ablation, another for detection). The use of a single fiber also reduces the fluorescence spectral fluctuations attributed to radiation scattering inside the tissue.

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