CARDIOVASCULAR NEAR-INFRARED IMAGING
L.A. Cassis, J. Yates, W.C. Symons¹, and R.A. Lodder*

College of Pharmacy
University of Kentucky Medical Center
Lexington, KY 40536-0082

*Author to whom correspondence should be addressed.
Telephone: 606-257-9232
Email: Lodder@pop.uky.edu

¹Present address: Department of Electrical Engineering, College of Engineering,
University of Kentucky, Lexington, KY 40506.

ABSTRACT

This research uses near-infrared spectrometric imaging to nondestructively locate and determine Low-Density Lipoprotein (LDL) cholesterol that may serve as an in vivo marker for vulnerable atherosclerotic plaques. Vulnerable plaques are plaques prone, in the presence of an appropriate trigger, to events such as ulceration, rupture, erosion, or thrombus that can lead to an acute syndrome. A Nd:YAG-pumped KTP/OPO tunable near-IR laser system is used as a light source for the fiber-optic catheters employed in this research. The BEST algorithm is used to construct chemical-composition images of the intima of the aorta in test subjects in vivo. The long-term goal of these studies is to use near-IR laser spectrometric assays of plaque performed with cardiac catheters in vivo to facilitate assignment of patients to specific drug or surgical interventions selected to match their individual vulnerable plaque characteristics.

KEYWORDS: vulnerable plaque, spectrometric catheter, tunable near-infrared laser, video
INTRODUCTION

The purpose of this research project is to test the hypothesis that near-infrared spectrometric imaging can nondestructively locate and determine Low-Density Lipoprotein (LDL) cholesterol that may serve as an in vivo marker for vulnerable atherosclerotic plaques. Vulnerable plaques are plaques prone, in the presence of an appropriate trigger, to events such as ulceration, rupture, erosion, or thrombus that can lead to an acute syndrome. (A thrombus is a clot of blood formed within a blood vessel that remains attached to its place of origin. A syndrome is a group of signs and symptoms that occur together and characterize a particular abnormality.) Vulnerable plaques are thought to usually contain a sizable lipid pool (which would contain the LDLs) and a thin fibrous cap. Near-infrared imaging spectrometry appears to be ideally suited to measuring the size and chemical composition of the fibrous cap and the lipid pool. Accurate measurement of lipoprotein cholesterol in all its forms is a critical first step in intervention in the diseases of atherosclerosis and ischemic stroke, and is necessary for longitudinal studies of what constitutes a vulnerable plaque and an effective means of stabilizing such plaque. Cholesterol is carried in lipoprotein particles that differ in size and apolipoprotein composition. This variety of lipoproteins can make nondestructive differentiation of lipoprotein composition difficult in the living vessel wall. As a result, most studies focus on in vitro methodologies for determination of plaque lipoprotein composition. However, production of artifacts is common using in vitro methods and has lead to confusion in the literature. At present, there is no good in vivo assay for LDL and forms of oxidized LDL (oxLDL) in solid tissue atherosclerotic plaques. The long-term goal of these studies is to prove that near-IR laser spectrometric assays of plaque performed with cardiac catheters in vivo may facilitate assignment of patients to specific drug or surgical interventions selected to match their individual vulnerable plaque characteristics.

Atherosclerosis without associated thrombosis is often an innocuous disease that is asymptomatic. Many patients with atherosclerosis can be treated surgically or by drugs with high initial success and favorable long-term prognosis. The acute manifestation of atherosclerosis - myocardial infarction - usually arises when a thrombus develops. This potentially life-threatening complication probably develops at the site of plaque fissure or rupture. A number of studies have demonstrated that plaque rupture plays a pivotal role in the pathophysiology (the physiology of abnormal states and the functional changes that accompany a particular syndrome or disease) of acute coronary syndromes. Recent research indicates that it is not the severity of stenosis (a narrowing or constriction of the diameter of an artery by plaque volume) that determines the outcome: it is the type of stenosis (the chemical composition of the plaque) and the extent of collateral growth. The kind of plaque,
determined by composition, consistency, vulnerability and thrombogenicity, varies
greatly from patient to patient, even from plaque to plaque in different locations, and
there is no simple relation among plaque kind, plaque volume or stenosis severity\textsuperscript{3}. Near-IR spectrometry was used in our laboratories to examine lipids \textit{in vitro} and \textit{in vivo} in gerbil brains following experimentally induced stroke and to identify nine
different saturated and unsaturated fatty acids found in the gerbil brain\textsuperscript{4}. Near-IR
spectrometry has also been used in our laboratories to analyze HDL, LDL, and
cholesterol in the blood vessels of rats\textsuperscript{5}. In addition, near-IR imaging has been used in
human stroke patients to analyze atherosclerotic plaque by identifying and locating
oxidized lipoprotein spectral signatures\textsuperscript{6,7}. A major advantage of near-IR spectral
analysis is its chemical imaging ability. Additionally, near-IR spectral imaging
provides information on details of various internal structures including muscle, bone,
and arteries\textsuperscript{8}.

Currently, no accurate nondestructive \textit{in vivo} reference assay exists for LDL or
apolipoproteins immobilized in the walls of living human arteries. Chemical analysis
of lesions \textit{in vivo} will permit the kinetic study of atherogenesis and contribute to the
understanding of lesion formation and growth. Near-IR spectrometry of plaque \textit{in vivo}
may also facilitate assignment of patients to specific new drug interventions that
may affect the course of atherosclerosis (such as TPA, which acts to block thrombi,
Enlimomab, which acts to block granulocyte adhesion to the blood-vessel wall,
Citicoline, which reduces free fatty acids, Lubeluzole, which interferes with the
effects of nitric oxide, Tirilizad, which acts as a free-radical scavenger, or bFGF,
which acts on growth factors) and/or surgical interventions (bypass grafts or
angioplasty). In this research, a Nd:YAG-pumped KTP/OPO laser system has been
used to test experimental spectrometric catheters that are being developed for use in
several projects investigating atherosclerosis and markers of vulnerable plaque. The
purpose of this experiment was to test the hypothesis that a near-IR spectrometric
catheter is capable of early detection of LDL uptake in the arterial wall, even before
the appearance of visible fatty streaks.

**EXPERIMENTAL**

The light source in these experiments is a laser system consisting of a MIRAGE
3000B Mid-Infrared Optical Parametric Generator and a Continuum NY81-10
Nd:YAG Pump Laser. The system provides tunable near-IR light with a wavelength
from 1.4 to 4.1 micrometers and an effective power of 3.3 million watts. The spectra
were scanned in the "water window" between 1650 and 1780 nm, where LDL has two
major peaks.
The catheters were constructed in-house from communications-grade optical fibers (see Fig. 1). The distal reflector tip of the catheter was constructed of gold-plated steel and was 450 micrometers in diameter (Sicon, Berlin, Germany). The diameter of the reflector was selected to match that of the combined transmitting and receiving fiber-optic bundles. One bundle of seven optical fibers was used to transmit light onto the blood vessel wall, while the remaining fibers were used to collect light scattered by the wall and return it to a PbS detector for analysis. The dimensions of the completed catheter assembly were designed to provide a close fit within the artery. The close fit reduced catheter motion with respect to the vessel wall and reduced the optical path length traversed through whole blood.

Spectral data were smoothed using cubic splines, treated with multiplicative scatter correction to reduce baseline variations, and analyzed using the BEST algorithm implemented in Speakeasy (Speakeasy Computing Corp., Chicago, IL, USA). Video clips were prepared in Quicktime format (Apple Computer Corp., Cupertino, CA, USA) or AVI format using Media Studio (Ulead, Torrance, CA, USA).

In this study, rabbits were maintained on a high-cholesterol (2%) diet to create fatty streaks in their arteries. The catheters were inserted into the femoral artery and advanced to the aortic arch. A suture was tied around the catheter to mark the depth of greatest insertion for later correlation between the near-IR images and LDL concentrations determined by a lipoprotein extraction, density-gradient ultracentrifugation, and SDS-PAGE reference procedure. As the catheters were slowly withdrawn, near-IR spectra of the aorta were collected. With all other traditional techniques, vascular tissue must be removed from the animal before cholesterol and ox-LDL can be assayed. The advantage of the near-IR technique is that repeated analyses are possible of the same lesion in the same animal during lesion formation and/or treatment. The technique will make possible tests for plaque vulnerability, tests of theories of lesion formation, and tests of lesion rupture or erosion that are not possible with traditional analytical techniques.

The rabbits were maintained for 6 weeks on high-cholesterol chow. The aorta was scanned in vivo with the tunable near-IR laser, then the heart and aorta of the animal were removed. The excised heart and aorta were photographed and scanned with a conventional spectrometer as a control for the laser procedure. The aorta was cut into 1 cm sections and retained for extraction of lipoproteins in the lesion and analysis of the extract by density-gradient ultracentrifugation and SDS-PAGE. The rabbits were anesthetized with atropine sulfate as a preanesthetic (0.1 mg/kg, i.m.) followed by ketacet (35 mg/kg, i.v.) and xylazine (5 mg/kg, i.v.). With the rabbit anesthetized, an incision near the femoral artery provided access to the aorta. The catheter is a small
near-IR fiber-optic probe that is connected to the tunable laser and a detector, A/D, and computer. The laser is necessary to provide enough light to obtain usable spectra through the intervening whole blood and the arterial tissue. Following the in vivo portion of the experiment, the animals were euthanized by an overdose of anesthetic, and the heart and aorta were removed. The aorta was measured, examined, and all physical aspects recorded. A suture was tied on the catheter at the point of maximum insertion to enable the penetration to the aortic arch to be determined (in cm, see Fig. 2) by measuring the distance between the suture and the femoral artery at each location where spectra were obtained. The catheter was withdrawn in 1 cm increments, and spectra of the vessel wall were obtained at each increment.

RESULTS AND DISCUSSION

Average spectra from each artery segment of a control rabbit and a cholesterol-fed rabbit are depicted in Fig. 3. The spectra were obtained over a wavelength range where LDL and oxLDL cholesterol have absorbance signals. The solid lines represent spectra of the control aorta, while the dot-dashed lines represent spectra of the cholesterol-treated aorta. The cholesterol-fed animals showed a larger signal in the 1700-1800 nm lipid region of the spectra. There is some overlap between the spectra obtained from the cholesterol and control aortas. There are several reasons for the overlap, including:

1. some LDL uptake by the control vessel wall is normal, as LDL cholesterol is needed to synthesize cell membranes for natural growth in the young animal,
2. atherosclerosis is a diffuse disease, and some diseased area of the vessel with LDL uptake will inevitably be near an area of normal vessel wall with relatively little LDL uptake.
3. the uptake of LDL for 6 weeks is not enough to cause visible fatty streaks to appear, which usually requires 12 or more weeks

Nevertheless, the spectra appear to be different in the lipid region upon simple inspection. Statistical analysis of the spectra also reveals differences. The spectral data were analyzed with the BEST algorithm. The calibration set comprised spectra of normal arterial endothelium. The distance in multidimensional BEST SDs of each spectrum of each pixel on the vessel wall was measured to the center of the calibration set of normal (control) arterial endothelium. The distances ranged from 0.57 to 2.35 SDs for the control rabbits, and from 0.45 to 4.88 SDs for the cholesterol-fed rabbits. Quicktime videos were constructed from the image data obtained as the catheters were moved through the arteries. Video clips from one cholesterol subject and one control subject are available online at http://www.pharm.uky.edu/asrg/current/movies.html.
A representative frame from the control video clip is given in Fig. 4, while a representative frame from the cholesterol-fed video clip is shown in Fig. 5. In the video clips online, increasing uptake of LDL is represented as increasing red color (gray areas = little LDL, red areas = increased LDL). In Fig. 4 and Fig. 5, increased LDL uptake is represented as a darker gray color. The uptake of LDL is a natural process in young rabbits, making some pink areas visible even in the control vessel. Although six weeks on high-cholesterol chow is not long enough for the subject to develop actual lesions, analysis of the near-IR results did show significantly increased LDL uptake by the experimental subject as compared to the control, even at this early stage. No oxidation of the LDL cholesterol was noted in the vessel wall of either the cholesterol-fed or control rabbits. This result is not surprising considering the young age of the rabbits and the short duration of the study.

CONCLUSION

Early lesions were detected in vivo using near-IR spectrometric imaging with an intravascular fiber-optic catheter before the lesions were visible on gross examination. The observation of these pre-vulnerable plaques, when combined with similar measurements on grossly visible lesions made in humans, suggest that near-IR spectrometry will be useful in detection of vulnerable plaques at nearly all stages of development. The nondestructive chemical and structural analysis of single lesions over time permits the discovery of markers for vulnerable atherosclerotic plaque. Finding such markers is an important problem because plaque vulnerability will likely turn out to be a complex function of fibrous cap thickness, lipid pool size, the stresses on plaque parts, and the composition of the fibrous cap and lipid pool (which influences factors like thrombogenicity). Near-infrared imaging spectrometry may be ideally suited to measuring these plaque vulnerability parameters.
LITERATURE CITED


Fig. 1. A near-infrared catheter used *in vivo*, shown while illuminated with a HeNe laser. When the catheter is held against the optical table, light can be seen returning down a receiving optical fiber from the catheter tip.

Fig. 2. The catheter was laid beside the heart and aorta and a meter stick. A suture was tied on the catheter at the point of maximum insertion to enable the penetration to the aortic arch to be determined (in cm) by measuring the distance between the suture and the femoral artery at each location where spectra were obtained.
Fig. 3. Average spectra from each 1-cm aorta segment of a control and a cholesterol-fed rabbit. The solid lines represent spectra of the control aorta, while the dot-dashed lines represent spectra of the cholesterol-treated aorta.
Fig. 4. A representative frame from the control video clip. Increased LDL uptake is represented as a darker gray color.

Fig. 5. A representative frame from the cholesterol-fed video clip. Increased LDL uptake is represented as a darker gray color.

CREDIT

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