Intraoperative myocardial ischemia detection with laser-induced fluorescence

Myocardial ischemia can be detected at the mitochondrial level by measuring shifts in nicotinamide adenine dinucleotide and its reduced form. Using a pulsed nitrogen laser and an optical multichannel analyzer, we monitored myocardial metabolism by measuring laser-induced nicotinamide adenine dinucleotide (reduced form) fluorescence in a large animal model of acute ischemia. Eight opened-chest sheep underwent occlusion of branches of the left anterior descending coronary artery, establishing a 15% infarct of the left ventricle. For the simulation of the clinical scenario, after 60 minutes of occlusion, the animals were supported by cardiopulmonary bypass, the aorta was crossclamped, and cold crystalloid cardioplegic solution was administered. The occlusion was removed after 10 minutes, and two additional doses of cardioplegic solution were delivered at 10-minute intervals. The aortic crossclamp was released, and a 30-minute period of reperfusion on bypass ensued. The hearts were then weaned off bypass and allowed to recover. Laser-induced fluorescence was measured inside, outside, and along the border of the infarct. Baseline measurements were made before occlusion, immediately after occlusion, and then at 5, 10, and 20 minutes after occlusion. The results show that immediately after occlusion there is a $200\% \pm 30\%$ (mean $\pm$ standard deviation) increase in laser-induced fluorescence in the infarct zone, a $110\% \pm 30\%$ increase along the border, and no significant change in the area outside the infarct. The fluorescence in the infarct reaches a plateau in 5 minutes at $270\% \pm 30\%$, whereas along the border it reaches a peak near end ischemia of $110\% \pm 40\%$. With the first dose of cardioplegic solution, fluorescence increases outside the infarct and decreases inside the infarct and along the border to $120\% \pm 30\%$, where it remains for all areas until the aortic crossclamp is removed. Fluorescence then drops to $70\% \pm 20\%$ and finally returns to baseline after 5 minutes of recovery. All of these shifts in laser-induced fluorescence were statistically significant ($p < 0.01$). The changes noted with doses of cardioplegic solution reflect the hypothermic and hyperkalemic effects on the myocardium. Laser-induced fluorescence provides a sensitive and specific method of monitoring myocardial ischemia during the operation. It also provides instantaneous feedback of metabolic changes that may be useful in evaluating the effects of different cardioplegic regimens and in monitoring reperfusion injury. (J THORAC CARDIOVASC SURG 1994;107:220-5)

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Received for publication Sept. 2, 1992.

Accepted for publication Mar. 1, 1993.

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0022-5223/94 \$1.00 + .10 12/1/46878

Coronary artery bypass grafting is primarily based on angiographic data that indicate the location of the stenosed or occluded arteries. During the operation, despite the apparent correction of these anatomic abnormalities, it is sometimes difficult to determine whether the revascularization has adequately reversed the ischemia and restored the metabolic function of the myocardium. Additionally, although several methods have been used to monitor the delivery and efficacy of cardioplegia, it has proved troublesome to do this during the operation with sensitivity and specificity. Finally, during operations for acute ischemia, the extent and reversibility of the myocardium at risk is unknown.

As shifts in aerobic and anaerobic metabolism occur, there are concomitant changes in the ratio of the respiratory enzyme nicotinamide adenine dinucleotide (NAD) and its reduced form, NADH. NADH is autofluorescent and therefore can be monitored optically. This study was undertaken to detect and monitor areas of ischemic myocardium with laser-induced NADH fluorescence in a large animal model simulating the clinical scenario of coronary artery bypass grafting after an acute myocardial infarct.

Methods

Eight sheep weighing 25 to 32 kg were anesthetized with intravenous thiopental sodium (Pentothal, 30 mg/kg intravenously) and intubated, and their lungs were ventilated with 100% oxygen. A central venous line was placed in the left jugular vein to monitor the central venous pressure and to deliver fluid. A catheter was placed in the left femoral artery for blood sampling and systemic arterial pressure monitoring. The chest was opened with a median sternotomy, and the heart was suspended in a pericardial cradle. The aortic root was dissected free of the pulmonary artery, and an ultrasonic flow probe (Carolina Medical Electronics, King, N.C.) was placed around the aorta to measure cardiac output. A 5F catheter-tipped micro-manometer (Millar Instruments, Inc., Houston, Tex.) was placed in the left ventricle through the apex and was used to monitor left ventricular pressure. Branches of the left anterior descending artery were encircled with silicone rubber occluders to establish a 15% to 20% infarct of the left ventricle. A pair of ultrasonic dimension crystals (Triton Technology, Inc., San Diego, Calif.) was placed in the subendocardium of the ischemic zone. Analog data were digitized at 200 Hz and stored on a computer. After instrumentation, baseline measurements of the following hemodynamic parameters were recorded: heart rate, mean arterial pressure, aortic flow, left ventricular end-diastolic pressure, and percentage of regional systolic fiber shortening.

Baseline NADH fluorescence was measured with an optical multichannel analyzer. This laser-induced fluorescence system has been previously described¹ and is depicted schematically in Fig. 1. In brief, it uses a pulsed nitrogen laser (VSL-337ND, Laser Science Inc., Cambridge, Mass.) (337 nm, 3 ns pulses at 10 Hz and 200 microjoule/pulse), which delivers the excitation light via a quartz fiber bundle to the myocardium. Fluorescence

for wavelengths of 300 to 800 nm was transmitted between pulses via the same fiber bundle to an intensified diode array, and the spectra were recorded by an optical multichannel analyzer (OMA III, Princeton Applied Research, Princeton, N.J.). The optical multichannel analyzer measured NADH fluorescence by recording all spectra and then decomposing this recording into the intensity contributed by individual fluorophores.¹ This device allows for NADH fluorescence measurements to be made in 3 to 5 seconds. The system was standardized before each experiment by measuring the fluorescence of laser dye of known concentration (DCM, Exciton Inc., Dayton, Ohio).

After baseline measurements, lidocaine 2 mg/kg was given intravenously, and the branches of the left anterior descending coronary artery were occluded. After 60 minutes of regional ischemia, end-ischemia measurements were made, and the sheep were heparinized (300 U/kg intravenously), supported with cardiopulmonary bypass, and systemically cooled to 28° C via cannulas in the right femoral artery and the right atrium. The aorta was crossclamped, and 300 ml of 4° C crystalloid cardioplegic solution (KCl 28 mEq/L and NaHCO₃ 5 mEq/L in 2.5% dextrose in 0.5% normal saline solution, osmolality 330 mOsm/L, pH 7.8) was administered antegradely at 50 mm Hg. After 10 minutes of cardioplegic arrest, the occlusions were released, and two additional 100 ml doses of cardioplegic solution were given at 10-minute intervals. This was done to simulate the intraoperative delivery of cardioplegic solution after performing distal anastomoses. The crossclamp was then removed, and the hearts were reperfused on bypass for 30 minutes and then weaned. During cardiopulmonary bypass and nonworking reperfusion the systemic pressure was maintained at 40 to 60 mm Hg by adjusting the flow rate. Hemodynamic measurements were repeated after 1 hour of recovery. The ischemic area was obvious because it became cyanotic immediately after coronary occlusion. NADH fluorescence was measured inside, outside, and along the border of the ischemic zone. The normal myocardium outside the ischemic zone was used as a reference or control for each animal. In addition to baseline fluorescence, laser-induced fluorescence was recorded immediately after occlusion and then at 5, 10, and 20 minutes after occlusion, after each dose of cardioplegic solution, after removal of the aortic crossclamp, during reperfusion, and during recovery.

Data were analyzed with Student's *t* test with Bonferroni corrections as needed. Differences were considered significant at a *p* value less than 0.05.

All sheep received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Results

The results of the hemodynamic measurements are listed in Table I. All values are expressed as the mean $\pm$ standard error of the mean. There was a significant decrease in the percentage of regional systolic shortening at the end of the ischemic interval when compared with baseline with no significant recovery of systol-

Fig. 1. Laser-induced fluorescence apparatus. The pulsed nitrogen laser is optically coupled to a single 400 μm fiber that transmits the excitation signal to the heart and also returns the fluorescence signal to a polychromator. The fluorescence signal is then recorded with a gated intensified diode array/optical multichannel analyzer.

Fig. 2. NADH fluorescence in relation to the sequence of events after acute myocardial infarction (occlusion), cardioplegic arrest, cardiopulmonary bypass, and recovery. The black line corresponds to fluorescence in the ischemic area, the dark gray line corresponds to outside the ischemic area, and the light gray line corresponds to the border of the ischemic area.

ic shortening during the recovery period. The mean arterial pressure after 1 hour of recovery was significantly lower than at the end of the ischemic interval. Although there was a slight increase in left ventricular end-diastolic pressure after 60 minutes of ischemia and during the recovery period, these differences were not statistically

significant. Similar insignificant trends were seen in the decrease of mean aortic flow and heart rate throughout the experiment.

Fig. 2 depicts the results of the laser-induced fluorescence monitoring. Immediately after occlusion NADH fluorescence in the ischemic zone increased $200\% \pm 30\%$

(mean $\pm$ standard error of the mean). Along the border it increased $110\% \pm 30\%$, and, in the normal, nonischemic area, there was no significant change. Fluorescence in the ischemic zone attained near maximal levels within seconds and reached a stable plateau within 5 minutes of occlusion of the arteries at a level $270\% \pm 30\%$ greater than baseline. The fluorescence increased at a slower rate along the border and reached a peak of $210\% \pm 40\%$ near the end of the ischemic interval. With the first dose of cardioplegic solution, fluorescence increased in the nonischemic area and decreased inside and along the border of the ischemic zone to $120\% \pm 30\%$, where it remained for all areas until the aortic crossclamp was removed. After reperfusion after removal of the crossclamp, the fluorescence dropped to $70\% \pm 10\%$ for all of the areas. The fluorescence returned to baseline in the first 5 minutes of the recovery period. All of these shifts in NADH fluorescence were statistically significant ($p = 0.006$).

Discussion

Reversing ischemia and restoring myocardial metabolism is the objective of coronary artery bypass grafting. At present, there are several methods to monitor ischemia and to detect metabolic changes during the operation. These include electrocardiograms, tissue carbon dioxide tension, and tissue pH.²⁻⁴ Although these techniques may identify ischemia at the tissue level, they do not monitor changes at the intracellular level. The use of NMR spectroscopy is proving beneficial in studying intracellular changes but, at present, is not practical for use in the operating room. One method of measuring these metabolic changes at the mitochondrial level is by measuring NADH fluorescence.⁵⁻¹⁰ NADH is autofluorescent and has an excitation wavelength of 340 nm and an emission wavelength of 480 nm. As the cellular metabolism shifts from aerobic to anaerobic, there is a concomitant change in the NAD/NADH ratio. Therefore, as more NAD is reduced to NADH, there is an increase in fluorescence that can be detected optically. Although NADH is present in the cytosol, as well as the mitochondria, the majority of the fluorescent NADH is mitochondrial.¹¹ Intraoperative NADH fluorescence monitoring is a useful, noninvasive method of detecting changes in myocardial metabolism. It has been shown to be more sensitive than epicardial ST segment mapping and correlates well with electron microscopic analysis of ultrastructural damage.^{13, 14} In addition, NADH fluorescence has demonstrated that the border zone around an infarct, albeit narrow, is composed of heterogeneous cells, which are less likely to be irreversibly damaged.^{15, 16} We have previously investigated the changes in NADH fluorescence with global ischemia in isolated perfused rat hearts and the

Table I. Hemodynamic parameters

Parameter	Baseline	End ischemia	Reperfusion
MAP (mm Hg)	70.3 $\pm$ 8.1	59.7 $\pm$ 8.1	33.4 $\pm$ 8.1
HR (beats/min)	146 $\pm$ 10	126 $\pm$ 10	118 $\pm$ 10
LVEDP (mm Hg)	4.1 $\pm$ 2.0	5.6 $\pm$ 2.0	6.5 $\pm$ 2.0
MAF (L/min)	2.3 $\pm$ 0.3	1.7 $\pm$ 0.3	1.6 $\pm$ 0.3
% Systolic shortening	7.4 $\pm$ 1.8	0.6 $\pm$ 1.7*	1.6 $\pm$ 1.7

MAP, Mean arterial pressure; HR, heart rate; LVEDP, left ventricular end-diastolic pressure; MAF, mean aortic flow.

* $p < 0.05$ versus baseline at end ischemia.

changes in fluorescence seen with reperfusion after the use of different types of cardioplegia.¹² This study was undertaken to evaluate the ability of NADH fluorescence to detect ischemia in a model that parallels the clinical scenario of a patient with an acute myocardial infarct followed by emergency coronary artery bypass operation.

The hemodynamic results indicate that, although the ischemic insult was significant enough to severely diminish regional function, it was not large enough to significantly alter the global function of the heart.

The laser-induced fluorescence data demonstrate dramatic changes with the onset of ischemia. There is a sharp and sudden rise in fluorescence within the ischemic zone after occlusion. In contrast, after occlusion, there is a less dramatic rise in fluorescence along the border and no change in the nonischemic area. This action corresponds to the shift in cellular metabolism from aerobic to anaerobic with ischemia. There is no change in perfusion in the myocardium outside the ischemic zone and therefore no change in the metabolism or fluorescence. The modest yet significant rise in fluorescence along the border reflects the metabolic heterogeneity of the myocardium along the border and the slight increase in the ischemic area with time. The general decrease in fluorescence in and along the ischemic zone after the aorta is crossclamped, the cardioplegic solution is delivered, and cardiopulmonary bypass is commenced, is indicative of the metabolic changes that occur with hypothermia, arrest, and mechanical support.

During these events, there is a significant rise in fluorescence in the nonischemic area. This increase, although not as great as that seen after occlusion, demonstrates the changes in myocardial metabolism induced by hypothermic, hyperkalemic cardioplegia. The fluorescence remained the same in all areas with the subsequent doses of cardioplegic solution. Because the occlusions are released after the first dose of cardioplegic solution, the additional doses reach the ischemic myocardium. There is no change in fluorescence with these doses because the

myocardium in the ischemic zone is also arrested and supported by cardiopulmonary bypass and therefore undergoing no change in metabolism. Fluorescence returned to baseline once the crossclamp was removed and the heart was reperfused with oxygen-rich blood. The fluorescence along the border of the ischemic zone is slightly slower to return to baseline. Although this is merely a trend, it was a constant observation and may reflect the mitochondrial stunning that occurs with reperfusion. Within 5 minutes after the heart is weaned from cardiopulmonary bypass, the laser-induced fluorescence for all areas returns to baseline. This return to baseline correlates with the cellular return to aerobic metabolism. The ensuing functional recovery of the organ may take more time and therefore may not correlate with the fluorescence signal.

The importance of measuring NADH fluorescence lies in the fact that NAD is the primary regulator of mitochondrial respiration and changes in the NAD/NADH ratio are more reflective of shifts in metabolism than other methods, such as measuring high-energy phosphates.¹⁷ The limitations before this study were due to the absorptive and fluorescent effects of hemoglobin. Most of the previous work was accomplished with the use of isolated hearts perfused by hemoglobin-free solutions. Others have demonstrated that NADH fluorescence can be adequately measured in the presence of hemoglobin and, in fact, may be accomplished during coronary catheterization.^{18,19} Although the measurements made in the blood-perfused hearts correlated well with those from crystalloid perfusion experiments, the results were predominantly qualitative. In an effort to improve the precision of these measurements, a laser fluorometer was developed that improved the sensitivity and "real-time" monitoring. A second laser was used to provide a reference wavelength for the measurement of hemoglobin.^{20,21} This optical arrangement was simplified with the use of an optical multichannel analyzer, which permits the measurement of all of the fluorescence in a given spectrum. The wavelengths of interest can then be sorted and quantified by blood-perfused tissue.

After the problem of hemoglobin interference is solved, a limitation of this technique remains: the measurements are made on the epicardial surface and therefore may not reflect subendocardial metabolism. Because the laser is delivered fiberoptically, endocardial measurements are possible. Although this may be useful for more precise measurements of the transmural extent of an ischemic area, it is not necessary for simple, real-time monitoring of metabolic changes that occur before, during, and after interventions.

Laser-induced NADH fluorescence provides a sensitive and specific method of monitoring myocardial metabolism during the operation. It may also be clinically useful in evaluating the delivery and efficacy of different types of myocardial protection, in delineating jeopardized myocardium, and in monitoring reperfusion injury.

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