ELSEVIER

Autofluorescence Spectroscopy in Liver Transplantation: Preliminary Results From a Pilot Clinical Study

O. Castro-e-Silva, A.K. Sankarankutty, R.B. Correa, J. Ferreira, J.D. Vollet Filho, C. Kurachi, and V.S. Bagnato

ABSTRACT

The evaluation of graft function at various stages after transplantation is relevant, particularly at the moment of organ harvest, when a decision must be made whether to use the organ. Autofluorescence spectroscopy is noninvasive technique to monitor the metabolic condition of a liver graft throughout its course, from an initial evaluation in the donor, through cold ischemia transportation, to reperfusion and reoxygenation in the recipient. Preliminary results are presented in six liver transplantations spanning the periods from liver harvest to implant. The laser-induced fluorescence spectrum at 532-nm excitation was investigated before cold perfusion (autofluorescence), during cold ischemia, at the back table procedure, as well as 5 and 60 minutes after reperfusion. The results showed that the fluorescence analysis was sensitive to changes during the transplantation procedure. Fluorescence spectroscopy potentially provides a real-time, noninvasive technique to monitor liver graft function. The information could potentially be valuable for surgical decisions and transplant success.

THE SUCCESS OF liver transplantation depends on a multitude of factors; therefore, the possibility of developing complications is considerable. An important factor is the quality of the donor organ. In certain cases, damage caused by ischemia and reperfusion, superimposed on an already marginal organ, may be a cause of posttransplant liver dysfunction. It is not surprising that the majority of transplanted livers experience a period of compromised function for a few days after the surgery. The possibility to evaluate the metabolic status of the graft would be important for the success of the procedure. To date, potential donor organs have been evaluated by a careful history and physical examination allied to conventional biochemical tests in the donor. Recent experiments[1,2] employing autofluorescence techniques have demonstrated that intrinsic properties of liver tissue strongly correlated with the overall fluorescence spectrum. Such results motivated the development of a real-time, in situ noninvasive technique that allowed observation of the metabolic activity. Several pioneering studies[3–5] have already demonstrated its potential sensitivity for in vivo monitoring of metabolic activity.

Chance and Legallais were the first to discuss autofluorescence emission as a parameter for in vivo monitoring of metabolic activity. The autofluorescence properties of coenzymes such as NAD(P)H and flavins can be extrapolated to provide information on the redox status of cells and tissues.[6]

The contribution of endogenous fluorophores, such as proteins, bound and free NAD(P)H, flavins, vitamin A, and arachidonic acid, to liver autofluorescence has been investigated in tissue homogenates and in isolated hepatocytes by means of spectrofluorometric analysis.[7,8]

According to Croce et al,[1] preservation conditions and cold hypoxia induce an increase in signal amplitude, mainly attributable to NAD(P)H spectral shape modification, ascribable to changes in relative concentrations of NAD(P)H and flavins, the coenzymes involved in energetic metabolism. In an experimental rat model, the same group evaluated the viability of autofluorescence analysis for in vivo monitoring of liver functionality.[1] The ischemia-reperfusion injury was investigated using spectrofluorometric analysis as well as conventional biochemical and histochemical analyses.

Sankarankutty et al[2] investigated the use of laser-induced fluorescence to evaluate rats liver grafts. The mitochondrial respiratory control rate, swelling, and membrane potential

From the Medical School of Ribeirão Preto (O.C.-e.S., A.K.S., R.B.C., J.F.), and Institute of Physics of São Carlos (J.D.V.F., C.K., V.S.B.), University of São Paulo, São Paulo, Brazil.

Financial support: FAPESP–CEPID Program.

Address reprint requests to Orlando de Castro e Silva, Professor, Surgery and Anatomy Department from Ribeirão Preto Medical School, Avenida Bandeirantes, 3.900–CEP 14049-900 Ribeirão Preto–SP, Brasil. E-mail: orlando@fmrp.usp.br

0041-1345/08/$–see front matter
doi:10.1016/j.transproceed.2008.03.005

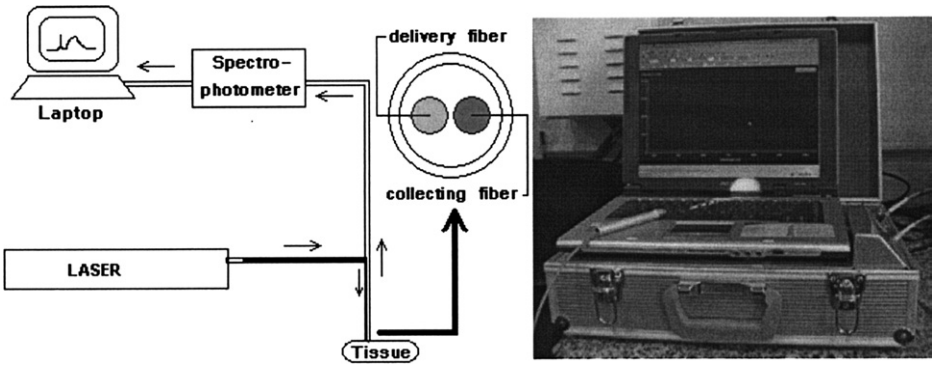

Fig 1. Schematic experimental setup and the homemade fluorescence system.

along with the cellular ATP content were used as functional and metabolic indicators of graft viability. The authors observed that a decrease in mitochondrial function and ATP content occurred progressively after cold perfusion. The results indicated that fluorescence detected metabolic liver changes that were relevant to determine graft function.

In this study we performed, autofluorescence spectroscopy monitoring during six clinical liver transplantations. Herein we have presented the preliminary results. The spectra shape and intensity were correlated with existing experimental work and biochemical analyses performed during the procedure. The results of this pilot clinical study provided enough evidence to further improve this technique to get the desired accuracy and sensitivity. We first presented the experimental procedure, followed by the description of the obtained spectra, and finally a preliminary discussion of the findings.

MATERIALS AND METHODS

Liver Transplantation

Six transplants and seven donor livers were investigated between April 2006 and March 2007. Six patients underwent liver transplantation, two women and four men of overall average age of 44.17 years (range = 15 to 55). One donor liver was discarded as the perfusion was considered inadequate. The University of Wisconsin or the Celsior solution (4°C cold perfusion) was used as the preservation solution. The liver graft was maintained at the same temperature and in the respective solutions until grafting. The grafting was performed using the piggyback technique. Before completing the portal anastomosis, the liver was flushed with approximately 1700 mL of 0.9% NaCl at room temperature to flush out preservation solution and metabolites.

Fluorescence Measurement

A homemade spectroscopy system was assembled with commercial devices. A doubled Nd:YAG laser emitting at 532 nm was used as an excitation source. The system has a Y-type investigation probe (Ocean Optics, USA) with two 600-μm optical fibers, one delivering the excitation laser and the other one collecting the reemitted light from the target tissue. A USB2000 spectrometer (Ocean Optics) allowed fluorescence evaluation in a spectral range between 350 nm and 1000 nm. Three output power options at the end of the fiber optic are available; 4, 6, and 8 mW. A long-pass filter at 550 nm (OGG550, Schott, USA) was used to remove the backscattered light before entering in the spectrometer; only the fluorescence emission was evaluated. A laptop was coupled to the spectrometer and the OBase32.exe software (Ocean Optics) used to acquire and save the spectrum data. The spectroscopy system was housed in a conventional camera recorder case, allowing its portability. Figure 1 shows a schematic design and a picture of the assembled system. Figure 2 shows an example of fluorescence changes at three different transplantation steps in one of the investigated patients.

The investigation probe was protected with a sterilized nonfluorescent plastic film and placed gently in contact with the target tissue. The fluorescence spectrum was collected in a noninvasive measurement; only surface tissue sites were investigated, so no bulk-type effects can be revealed by this method. In experimental models, measured excitation depth was less than 1 mm.

Fluorescence measurements were obtained at specific stages according to the protocol: during the donor procedure, before (considered as liver autofluorescence) and after cold perfusion; at the back table of the explanted liver; as well as in the recipient at 5 minutes and 1 hour after reperfusion. At each stage, at least five measurements were taken from the right lobe, left lateral segment, and segment IV. An attempt was made to perform each measurement in the respective area. The averaged results were used for analysis. A background measurement, with no laser excitation, was

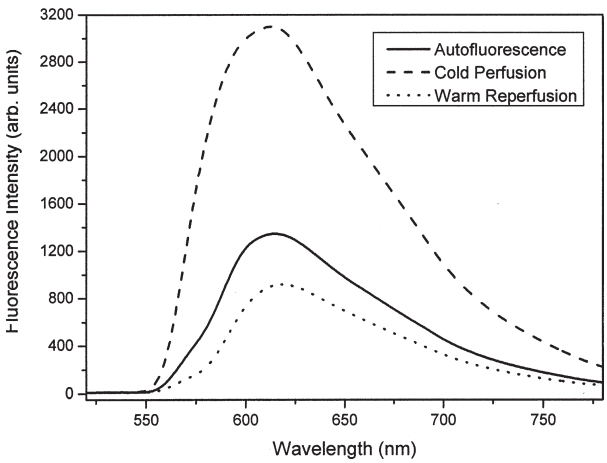

Fig 2. Fluorescence spectra measured at different investigation times—before cold perfusion (autofluorescence), after cold perfusion, and after warm reperfusion—in one of the investigated patients.

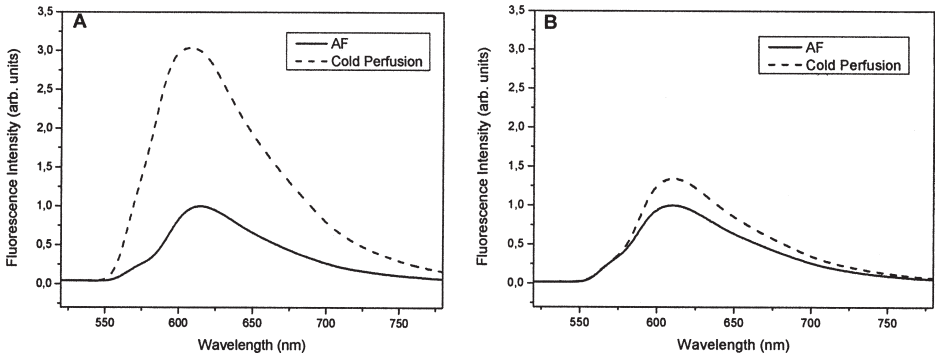

Fig 3. Fluorescence spectra, autofluorescence, and after cold perfusion, measured at the left **(A)** and right **(B)** hepatic lobes in the patient where a nonhomogenous cold perfusion was observed.

performed to ensure that no room lights influenced the fluorescence data.

The experimental protocol was approved by our Ethics Committee.

RESULTS

In one study, an excitation light at 366 nm was used and the authors attributed this behavior to the changes of the relative contributions of NAD(P)H and flavins to the overall fluorescence emission. Even though the same behavior was observed in our results, as the excitation wavelength used in our study was at 532 nm, there was probably no correlation with the same fluorophores.

In one donor in this study, the cold perfusion was not adequate, and clinical differences could be observed between the hepatic lobes. The clinical impression provided by the surgeon was that an adequate cold perfusion had been achieved in the left lobe but not in the right lobe. This situation was shown by distinct fluorescence spectra for the right and left lobes as presented in Fig 3. This evidence was a good indication that fluorescence spectroscopy might be an attractive technique to evaluate perfusion in situ. Such information could be valuable, as inadequately perfused regions could compromise the overall success of the transplant. If proven sensitive enough, a full spatial fluorescence mapping would be an ideal procedure to provide fast information concerning the perfusion quality. This data might be used by the surgeon to improve the decision of whether to proceed with the surgery. In this case, the transplantation team decided not to use this organ based on the clinical impression after cold perfusion.

When the maximum fluorescence amplitude is monitored during the period of investigation, a typical variation can be observed as presented at Fig 4. The fluorescence intensities at 615 nm were normalized by the value from the autofluorescence spectrum. After cold perfusion the liver fluorescence showed increased intensity with rates varying from 30% to 100%. During the back table procedure, a slight increase was observed, but the fluorescence level remained similar. One could speculate that variations in this time interval, such as decreased fluorescence, may be a good indication of liver damage that could later affect the grafting response. Five minutes after warm reperfusion, when rewarming–reoxygenation of the organ occurs, we observed a drop of fluorescence from 0% to 50% of the autofluorescence level, then the fluorescence gradually returned to the autofluorescence level. This observation may probably be an indication of the response to warm reperfusion, involving injury induced by rewarming–reoxygenation, possibly providing relevant information for clinical evaluation and prognosis. Unfortunately, fluorescence monitoring for this purpose is not clinically feasible as yet.

DISCUSSION

Our preliminary results in six liver transplant patients demonstrated the potential utility of fluorescence spectroscopy to monitor graft function. The tissue injury induced by ischemia and reoxygenation may be detected by changes in fluorescence emission when compared with the autofluorescence spectrum. If proven sufficiently sensitive, the technique may be attractive for clinical application due to its ability to noninvasively provide information in real time. Relevant information may help surgeons in decisions gauging the chances of success of the procedure.

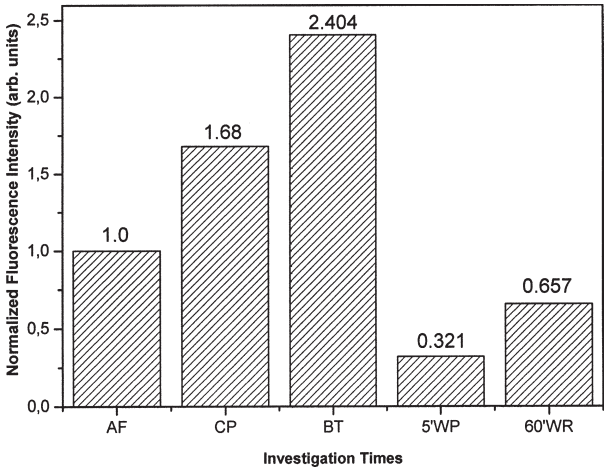

Fig 4. Monitoring of the fluorescence amplitude at 615 nm during the transplantation procedure. The investigation times measured: AF (autofluorescence), CP (cold perfusion), BT (back table), 5′ WR (5 minutes after warm reperfusion), and 60′ WR (60 minutes after warm reperfusion).

REFERENCES

1. Croce AC, Ferrigno A, Vairetti M, et al: Autofluorescence spectroscopy of rat liver during experimental transplantation procedure. An approach for hepatic metabolism assessment. Photochem Photobiol Sci 4:583, 2005

2. Sankarankutty AK, Castro e Silva O, Ferreira J, et al: Use of laser auto-fluorescence for evaluating liver grafts. Las Phys Lett 3:539, 2006

3. Croce A, Bottiroli G: Autofluorescence spectroscopy of cells and tissues as a tool for biomedical diagnosis. In Palumbo G, Pratesi R (eds): Laser and Current Optical Techniques in Biology, Comprehensive Series in Photochemical and Photobiological Sciences. Royal Society of Chemistry, Vol. IV. 2004, p 189

4. Chance B, Legallais V: Differential microfluorimeter for the localization of reduced pyridine nucleotide in living cells. Rev Sci Instrum 30:732, 1959

5. Chance B, Thorell B: Localization and kinetics of reduced pyridine nucleotide in living cells by microfluorometry. J Biol Chem 234:3044, 1959

6. Chance BNS, Warren W, Yurtsever G: Mitochondrial NADH as the bellwether of tissue O2 delivery. Adv Exp Med Biol 566:231, 2005

7. Croce AC, Ferrigno A, Vairetti M, et al: Autofluorescence properties of isolated rat hepatocytes under different metabolic conditions. Photochem Photobiol Sci 3:920, 2004

8. Obi-Tabot ET, Hanrahan LM, Cachecho R, et al: Change in hepatocyte NADH fluorescence during prolonged hypoxia. J Surg Res 6:575, 1993