Published in final edited form as:

J Card Surg. 2008 ; 23(6): 701–708. doi:10.1111/j.1540-8191.2008.00767.x.

Real-time Visualization and Quantification of Retrograde Cardioplegia Delivery using Near Infrared Fluorescent Imaging

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Abstract

Background and Aim of the Study—Homogeneous delivery of cardioplegia is essential for myocardial protection during cardiac surgery. Presently, there exist no established methods to quantitatively assess cardioplegia distribution intraoperatively and determine when retrograde cardioplegia is required. In this study, we evaluate the feasibility of near infrared (NIR) imaging for real-time visualization of cardioplegia distribution in a porcine model.

Methods—A portable, intraoperative, real-time NIR imaging system was utilized. NIR fluorescent cardioplegia solution was developed by incorporating indocyanine green (ICG) into crystalloid cardioplegia solution. Real-time NIR imaging was performed while the fluorescent cardioplegia solution was infused via the retrograde route in 5 ex-vivo normal porcine hearts and in 5 ex-vivo porcine hearts status post left anterior descending (LAD) coronary artery ligation. Horizontal cross-sections of the hearts were obtained at proximal, middle, and distal LAD levels. Videodensitometry was performed to quantify distribution of fluorophore content.

Results—The progressive distribution of cardioplegia was clearly visualized with NIR imaging. Complete visualization of retrograde distribution occurred within 4 minutes of infusion. Videodensitometry revealed that retrograde cardioplegia primarily distributed to the left ventricle and anterior septum. In hearts with LAD ligation, antegrade cardioplegia did not distribute to the anterior left ventricle. This deficiency was compensated for with retrograde cardioplegia supplementation.

Conclusions—Incorporation of ICG into cardioplegia allows real-time visualization of cardioplegia delivery via NIR imaging. This technology may prove useful in guiding intraoperative decisions pertaining to when retrograde cardioplegia is mandated.

Keywords

Cardioplegia; Cardiac Surgery; Myocardial Ischemia; Animal Model

INTRODUCTION

Adequate myocardial protection during the ischemic aortic cross-clamp period is necessary to preserve ventricular function and is critical for achieving good outcomes in cardiac surgery [1]. Homogeneous distribution of cardioplegia to both the right and the left ventricles is essential to achieve adequate myocardial protection[2,3]. Presently, cardioplegia is administered primarily by the antegrade route with or without the supplementation of retrograde cardioplegia. Generally, retrograde cardioplegia is employed when antegrade cardioplegia distribution is perceived to be inadequate. This may be the case when occlusive coronary lesions are present. However, the decision to supplement with retrograde cardioplegia is presently based on the surgeon's judgement, and not on any objective assessment of cardioplegia distribution.

Several techniques, including monitoring intramyocardial pH and temperature and coronary venous blood sampling, have been developed to continuously monitor myocardial protection [4,5]. These methods, however, are invasive and not consistently reliable[6] or not feasible in real-time. For these reasons, they have not been widely adopted. To address these limitations, we developed a near infrared (NIR) fluorescence imaging technique to visualize and quantify cardioplegia delivery in real-time. NIR fluorescence imaging is a high resolution imaging technique that utilizes NIR light to penetrate biological tissues and excite fluorescent dyes[7]. When excited, these dyes emit infrared light which is captured by a NIR camera to visualize vessels and tissues. This technique is appealing because it is portable and non-invasive. This technique is currently utilized to confirm the patency of coronary anastomosis during coronary artery bypass grafting [8].

In this study, we incorporated the NIR fluorescent dye, Indocyanine Green (ICG), into cardioplegia solution. Indocyanine Green is a FDA approved intravascular dye that is used to determine cardiac output and for ophthalmic angiography[9]. We utilized 5 ex-vivo normal porcine hearts and 5 hearts with LAD ligations to evaluate the feasibility of this ICG based NIR imaging system for real-time visualization of cardioplegia distribution.

MATERIALS AND METHODS

Study Design

A portable, intraoperative, real-time NIR imaging system, custom built for use in large animal surgery, was utilized[10]. ICG was incorporated into crystalloid cardioplegia solution to develop a fluorescent cardioplegia solution. This study was performed in two parts. In Part 1, we evaluated the feasibility of NIR imaging to perform real-time visualization of retrograde cardioplegia delivery in 5 ex-vivo normal porcine hearts. In Part 2, we evaluated the feasibility of NIR imaging to determine the adequacy of cardioplegia delivery in 5 ex-vivo porcine hearts with an LAD ligation. In these hearts, we first administered fluorescent cardioplegia by the antegrade route and visualized the defects in the distribution due to the LAD occlusion. We then supplemented the antegrade cardioplegia with retrograde cardioplegia and assessed for improvements in the previously visualized defects. In both parts, we correlated our visual assessment of the images with the fluorescent intensities generated by our imaging system. Cross sections of the hearts were obtained and cardioplegia distribution was quantified with videodensitometry.

Near Infrared Imaging and Fluorescent Dyes

Our NIR fluorescent technology exploits the phenomenon that NIR light, unlike visible light, penetrates biological tissue with minimal scattering. Incident NIR light is therefore able to efficiently penetrate into the blood vessels that have been infused with the fluorescent

cardioplegia solution. The NIR fluorophores present in the cardioplegia solution absorb the incoming NIR light and subsequently emit radiation in the NIR wavelength range, giving rise to fluorescent signals. The fluorescent signals emitted by the fluorophores are captured by a NIR camera to produce an image which can be displayed on a monitor[11]. As NIR light is invisible to the human eye, it is possible to simultaneously irradiate the surgical field with both visible and NIR light.

Three images are simultaneously displayed on our NIR imaging system monitor (Figure 1). The first image corresponds to the surgical field illuminated by visible light (which the surgeon is normally used to seeing). The second image corresponds to the fluorescent signals emitted by the fluorophores in the cardioplegia solution (which are captured by the NIR camera). The third image produced is a merged picture of the visible light images and NIR images. This is an attractive way of displaying to the surgeon where the fluorescent signals are originating from in the actual heart[11] [7,10].

Near Infrared Imaging System

The large animal intra-operative NIR fluorescence imaging system used in this study has been described in detail previously[10]. Briefly, it is composed of two sources of excitation light. One corresponding to white light (wavelength of 400–700 nm) and the other corresponding to near infrared light (725–775 nm) with a power of illumination of 5 mW/cm². The area of field of illumination of the system is 150 cm². All screen images are refreshed 15 times per second. The camera is suspended at a distance of 18" from the operative field.

The NIR fluorescent dye ICG (Akron, Buffalo Grove, IL) used in these experiments is an intravascular dye with a half-life of 30–60 seconds. ICG is approved by the FDA for clinical applications. It is routinely used in ophthalmology angiography[12]. Since ICG is excreted by the liver, it can be used in patients with renal dysfunction[9,12]. The respective excitation and emission wavelengths of ICG are 779 nm and 806 nm [13]. ICG has minimal reported adverse reactions in clinical application [9].

Preparation of Fluorescent Cardioplegia Solution

We developed a fluorescent cardioplegia solution which consisted of standard cold crystalloid cardioplegia solution incorporated with ICG. The standard cold crystalloid cardioplegia solution consisted of 154 mmol/L of sodium, 154 mmol/L of chloride, 4 meq/L of magnesium sulphate, and 40 meq/L of KCL. The ICG stock solution was prepared by mixing 2.5 mg of ICG with 10 ml of saline and allowing the solution to rest at room temperature for one hour. The stock solution was then mixed with 1 L of cold crystalloid cardioplegia solution to achieve a final concentration of 0.025 mg/ml.

Part 1: Cardioplegia Infusion by the Retrograde Route

Five ex-vivo porcine hearts were placed under the field of the large animal NIR imaging system. The coronary sinuses of the porcine hearts were intubated with a 14 French retrograde cardioplegia catheter. The NIR field was checked for background fluorescence to ensure that there were no fluorescent signals detected prior to the onset of cardioplegia infusion. The fluorescent cardioplegia solution was then infused at a rate of 100 ml/min via the coronary sinuses. Near infrared imaging was performed after the initiation of cardioplegia infusion.

Near infrared images were obtained throughout the infusion period. The time interval between the start of the infusion and complete visualization of the coronary venous vasculature was 4 minutes. Fluorescent signals emanating from the anterior surface of the heart were detected and assigned relative values by the imaging system. These relative values were labeled as fluorescent intensity (FI). In general, the values of FI for a particular fluorescent dye (at a

particular wavelength of excitation) are expressed as a relative measurements termed as arbitrary units (AU) and are dependent upon certain intrinsic properties of the imaging system used [14]. In this study, FI was measured at 20 second time intervals after initiating the infusion process and was understood to be indicative of cardioplegia concentration on the surface of the heart. The FI was then plotted against the infusion time. Videodensitometry was used to compare and quantify the values for FI (as described under Videodensitometric Analysis).

Cross sections of the heart were then obtained along the short axis at proximal, middle, and distal LAD levels after the infusion was completed. Videodensitometric analysis was repeated to quantify the distribution of fluorophore content in different segments of the ventricular wall. This distribution of fluorophore content was understood to be indicative of the concentration of cardioplegia in the respective segments of the ventricle.

Part 2: Cardioplegia Infusion After Ligation of the LAD

In another set of 5 ex-vivo porcine hearts, the proximal LAD was occluded with a suture ligature. This was performed to simulate an occlusive lesion in the LAD, which would result in sub-optimal delivery of antegrade cardioplegia. The aorta was clamped and antegrade cardioplegia was infused via the aortic root. NIR imaging was performed as detailed in Part 1. This was followed with the additional infusion of retrograde cardioplegia. NIR imaging was once again performed. Horizontal cross sections of the heart were obtained and analyzed by videodensitometry for distribution of fluorophore content.

Videodensitometric Analysis

Videodensitometry was performed using a custom built software application (Lab View 6, National Instruments, Austin TX). Values for FI were generated electronically using the Labview software application. FI was measured in real-time on the surface and in cross sections of the heart. Cross sections of the heart were demarcated into the anterior left ventricle (LV), posterior LV, lateral LV, septum, and right ventricle (RV). FI was measured in each of these respective segments of the ventricle and was used to quantify the distribution of cardioplegia to these corresponding segments of the ventricle. The mean value of FI was calculated in each segment of the ventricle. This process was repeated 3 times for each specimen in order to eliminate any operator dependent errors. The mean FI in different specimens were compared using the Student's *t* test. A *p* value < 0.05 was considered statistically significant.

RESULTS

Part 1: Retrograde Cardioplegia Distribution

After the initiation of retrograde cardioplegia, NIR images were obtained to document cardioplegia distribution in 5 ex-vivo porcine hearts. These images displayed fluorescent signals originating from the great coronary vein in the anterior LV. As the infusion continued, we were able to appreciate progressive distribution of cardioplegia to the anterior LV, as evidenced by the increasing magnitude of fluorescent signals emanating from the anterior surface of the heart. Near complete visualization of the coronary veins was achieved after infusing 400 cc of cardioplegia. Total time taken for complete visualization of the retrograde vasculature was 4 minutes. The images recorded during the infusion of cardioplegia, during progressive time intervals, are displayed in Figure 1.

Part 2: LAD Occlusion and Cardioplegia Distribution

A separate set of 5 ex-vivo porcine hearts underwent LAD occlusion and antegrade cardioplegia infusion. Figure 2 shows the anterior surface of the heart after infusing 400 cc of antegrade cardioplegia. As expected, the occlusion of the LAD prevented distribution of

antegrade cardioplegia to the anterior wall of the LV. Distribution of cardioplegia to the posterolateral surface of the heart was unaffected, as evidenced by the strong fluorescent signals originating from the circumflex artery and its branches in Figure 3.

Infusion of cardioplegia by the antegrade route was subsequently followed by administering cardioplegia by the retrograde route. Image 4 shows fluorescent signals now originating from the anterior LV despite the LAD occlusion. This is due to the retrograde supplementation. We concluded that these signals originated as a result of cardioplegia distribution via the great cardiac vein. Figure 5 shows the cross sectional images that were obtained in separate hearts after the infusion of antegrade cardioplegia alone and after augmentation with retrograde cardioplegia. After LAD ligation, infusion of antegrade cardioplegia alone displayed a transmural flow defect in cardioplegia distribution to the anterior LV. However, this defect in the anterior LV resolved when retrograde cardioplegia was subsequently infused. This demonstrated that retrograde cardioplegia is essential to achieve global myocardial protection when there is an occlusion of the LAD.

Videodensitometric analysis

Real-time videodensitometric analysis of the images from Part 1 of the experiment revealed that the FI, measured on the surface of the heart, progressively increased with time. This correlated with the volume of cardioplegia infused (Figure 6).

Videodensitometric analysis of the cross sectional images from Part 1 of the experiment revealed that the cardioplegia administered by retrograde route had preferential distribution to the left ventricle and the anterior septum (Figure 7).

Videodensitometric analysis of the cross sectional images from Part 2 of the experiment revealed enhancement in the fluorescent intensities of various segments of the LV wall after supplementation of antegrade cardioplegia with retrograde cardioplegia (Figure 8).

CONCLUSIONS

Achieving optimal protection of the myocardium during the ischemic aortic cross-clamp period is critical in cardiac surgery. Ensuring that the cardioplegia delivered has distributed homogeneously throughout the myocardium is an important means of achieving such optimal levels of myocardial protection. Adequate myocardial protection results in reduced incidence of perioperative myocardial damage and preserves left ventricular function in the postoperative period[15,16]. It also reduces the incidence of postoperative atrial fibrillation and lowers the need for inotropes [16,17]. Presently, no non-invasive methods have been established to visualize the distribution of cardioplegia in real time or direct delivery of cardioplegia intraoperatively. The surgeon thus has no means of objectively assessing cardioplegia distribution during surgery. Routes of cardioplegia administration used (antegrade vs. antegrade + retrograde) to achieve adequate myocardial protection are entirely guided by the surgeon's preference and not by any objective assessment of cardioplegia distribution.

We have demonstrated that the intraoperative visualization of cardioplegia distribution is possible using the NIR fluorescent dye ICG and existing NIR imaging technology. This method is appealing because it is safe, non-invasive, and can be used in real-time[10,11]. Our method takes advantage of the unique properties of NIR fluorescence imaging and provides precise, instantaneous, and high-resolution visualization of cardioplegia distribution. More importantly, the system is not operator dependent or invasive. The entire imaging system is portable, easy to use in a surgical suite, and does not obstruct the operative field (Figure 9). The NIR fluorescent dye ICG is inexpensive and, as stated before, is approved by the FDA for clinical applications [9,12].

We also demonstrated that this NIR imaging technique objectively detects deficiencies in cardioplegia delivery and can guide the decisions required to overcome such deficiencies. Our ICG based NIR imaging system was successful in detecting suboptimal delivery of antegrade cardioplegia due to a simulated LAD occlusion. It allowed recognition that administration of retrograde cardioplegia adequately compensated for this deficit. This technique also corroborates other studies that cardioplegia administration by the retrograde route is critical in situations where distal distribution of antegrade cardioplegia is impaired due to critical coronary lesions[18]. However, this is the first study that demonstrates a visual and intra-operative technique to address this clinical need.

The reason we chose to visualize retrograde cardioplegia delivery over antegrade was because of the fact that antegrade distribution is well characterized and there is little debate about when to use antegrade cardioplegia. On the other hand retrograde vasculature is highly variable and cardioplegia delivered by retrograde route confers variable levels of protection to the myocardium. This is partly due to a higher incidence of anomalies in the venous circulation of the heart as compared to the arterial circulation[19]. Moreover, investigators have found larger number of the basian veins and other venous channels in patients with coronary atherosclerotic obstruction[20]. Also, seventy percent of patients with chronic CAD have been shown to have increased coronary AV shunting[21]. By visualizing and quantifying retrograde cardioplegia delivery, we were able to achieve a better understanding of its mechanics of distribution and identify scenarios where its use would prove to be of added benefit.

An important limitation of the imaging system used in this study is its limited depth of penetration. The depth of penetration of our ICG based NIR imaging system is 1 cm to 1.5 cm for solid tissue and 5 cm for lung tissue [22]. As a result, cardioplegia distribution within the deeper layers of the myocardium, as well as the endocardial distribution of cardioplegia, may not be adequately visualized. Another limitation observed during these experiments was difficulty with repeated visualization of the coronary veins. This was as a result of sustained background fluorescence due to the cumulative accumulation of dye in the myocardium as a result of repetitive administration of dye. During the initial administration of the dye, however, background fluorescence was minimal and visualization was not compromised in any of the ex-vivo hearts. Prior experiments in this lab with in-vivo porcine hearts have also demonstrated the same finding.

Another potential limitation, as mentioned previously, is the variability of retrograde vasculature. For instance, in reviewing Figure 8, it is not clearly understood why there is augmentation of FI in the lateral wall with the use of retrograde cardioplegia (to the same extent as seen in the LAD territory and septum). We attribute this to the fact that venous distribution is relatively variable from specimen to specimen. We believe that this caused different degrees of augmentation of FI in the lateral and posterior segments. The best way to overcome this limitation is to perform a greater number of experiments and perform additional quantitative studies of myocardial perfusion (eg. using microspheres, etc.) in future experiments, to correlate FI with perfusion.

In summary, this study demonstrates the feasibility of the use of an intraoperative imaging system that can be utilized for real-time visualization of cardioplegia delivery. It is our aspiration that this technology will be further developed and refined to optimize techniques of myocardial protection in the field of cardiac surgery.

References

1. Rao V, et al. Predictors of low cardiac output syndrome after coronary artery bypass. *J Thorac Cardiovasc Surg* 1996;112(1):38–51. [PubMed: 8691884]

2. Buckberg GD. Recent advances in myocardial protection using antegrade/retrograde blood cardioplegia. *Eur Heart J* 1989;10(Suppl H):43–8. [PubMed: 2627963]
3. Aldea GS, et al. Inhomogeneous and complementary antegrade and retrograde delivery of cardioplegic solution in the absence of coronary artery obstruction. *J Thorac Cardiovasc Surg* 1994;107(2):499–504. [PubMed: 8302069]
4. Crittenden MD. Intraoperative metabolic monitoring of the heart: I. Clinical assessment of coronary sinus metabolites. *Ann Thorac Surg* 2001;72(6):S2220–6. [PubMed: 11789845]discussion S2267–70
5. Khuri SF, et al. First report of intramyocardial pH in man. II. Assessment of adequacy of myocardial preservation. *J Thorac Cardiovasc Surg* 1983;86(5):667–78. [PubMed: 6632942]
6. Dearani JA, et al. Role of myocardial temperature measurement in monitoring the adequacy of myocardial protection during cardiac surgery. *Ann Thorac Surg* 2001;72(6):S2235–43. [PubMed: 11789847]discussion S2243–4, S2267–70
7. Nakayama A, et al. Functional near-infrared fluorescence imaging for cardiac surgery and targeted gene therapy. *Mol Imaging* 2002;1(4):365–77. [PubMed: 12940233]
8. Reuthebuch O, et al. Novadaq SPY: intraoperative quality assessment in off-pump coronary artery bypass grafting. *Chest* 2004;125(2):418–24. [PubMed: 14769718]
9. Benya R, Quintana J, Brundage B. Adverse reactions to indocyanine green: a case report and a review of the literature. *Cathet Cardiovasc Diagn* 1989;17(4):231–3. [PubMed: 2670244]
10. De Grand AM, Frangioni JV. An operational near-infrared fluorescence imaging system prototype for large animal surgery. *Technol Cancer Res Treat* 2003;2(6):553–62. [PubMed: 14640766]
11. Frangioni JV. In vivo near-infrared fluorescence imaging. *Curr Opin Chem Biol* 2003;7(5):626–34. [PubMed: 14580568]
12. Chen SJ, et al. Indocyanine green angiography of central serous chorioretinopathy. *Zhonghua Yi Xue Za Zhi (Taipei)* 1999;62(9):605–13. [PubMed: 10502851]
13. Sevick-Muraca EM, Houston JP, Gurfinkel M. Fluorescence-enhanced, near infrared diagnostic imaging with contrast agents. *Curr Opin Chem Biol* 2002;6(5):642–50. [PubMed: 12413549]
14. Valeur, B. *Molecular Fluorescence: Principles and Applications*. Vol. 1. Wiley-VCH; 2001.
15. Natsuaki M, et al. Risk factors associated with perioperative myocardial damage in patients with severe aortic stenosis. *J Cardiovasc Surg (Torino)* 2004;45(3):271–7.
16. Onorati F, et al. Determinants and prognosis of myocardial damage after coronary artery bypass grafting. *Ann Thorac Surg* 2005;79(3):837–45. [PubMed: 15734390]
17. Onorati F, et al. Does antegrade blood cardioplegia alone provide adequate myocardial protection in patients with left main stem disease? *J Thorac Cardiovasc Surg* 2003;126(5):1345–51. [PubMed: 14666005]
18. Tian G, et al. Assessment of retrograde cardioplegia with magnetic resonance imaging and localized ³¹P spectroscopy in isolated pig hearts. *J Thorac Cardiovasc Surg* 1997;114(1):109–16. [PubMed: 9240300]
19. Gates RN, et al. Gross and microvascular distribution of retrograde cardioplegia in explanted human hearts. *Ann Thorac Surg* 1993;56(3):410–6. [PubMed: 8379710]discussion 417
20. Lisanti P, Serino W, Petrone M. [Multiple coronary artery-left ventricle fistulas in a patient with apical hypertrophic cardiomyopathy: an unusual cause of angina pectoris]. *G Ital Cardiol* 1988;18(10):858–61. [PubMed: 3246319]
21. Menasche P, et al. Efficacy of coronary sinus cardioplegia in patients with complete coronary artery occlusions. *Ann Thorac Surg* 1991;51(3):418–23. [PubMed: 1998418]
22. Soltesz EG, et al. Intraoperative sentinel lymph node mapping of the lung using near-infrared fluorescent quantum dots. *Ann Thorac Surg* 2005;79(1):269–77. [PubMed: 15620956]discussion 269–77

Figure 1.

Progressive increase in the fluorescent intensity from the anterior surface of the LV was appreciated with increasing time intervals. Complete visualization of the retrograde vasculature was achieved in 4 minutes.

Antegrade Infusion after LAD occlusion

Anterior Surface

Figure 2.

The anterior surface of the heart is devoid of fluorescent signals. This indicates that there is no distribution of antegrade cardioplegia to the anterior LV after LAD occlusion.

Posterolateral Surface

Figure 3.

The posterolateral surface of the heart displaying fluorescent signals after LAD occlusion. These signals are originating from the antegrade cardioplegia distribution in the branches of the circumflex artery.

Antegrade and Retrograde Cardioplegia Infusion after LAD occlusion

Figure 4.
The Anterior LV receiving cardioplegia distribution from the retrograde infusion despite the LAD occlusion

Figure 5.

Comparing the cross-sections of the hearts shows that antegrade cardioplegia augmented with retrograde cardioplegia obliterates the transmural defect produced by the LAD occlusion.

Figure 6. Fluorescent Intensity (FI), measured on the surface of the heart, showed progressive increase with time. This correlated with the volume of cardioplegia infused.

Figure 7. As determined in Part 1 of the study Displays the relative concentration of retrograde cardioplegia in the ventricular segments.(LV corresponds to left ventricle, RV corresponds to the right ventricle.)

Figure 8.

As determined in Part 2 of the study. The Distribution of cardioplegia with antegrade infusion vs. antegrade + retrograde infusion. LV and RV correspond to the left and the right ventricle respectively.

Figure 9.
Large animal NIR imaging system being used in a surgical suite