Myocardial tissue oxygen supply and utilization during coronary artery bypass surgery: evidence of microvascular no-reflow

Mohamed K. AL-OBAIDI*, Philip J. ETHERINGTON†, David J. BARRON*, C. Peter WINLOVE† and John R. PEPPER*

*Department of Cardiothoracic Surgery, National Heart and Lung Institute, Royal Brompton Hospital, Sydney Street, London SW3 6NP, U.K., and †Physiological Flow Study Group, Department of Biological and Medical Systems, Imperial College of Science, Technology and Medicine, London SW7 2AZ, U.K.

ABSTRACT

The supply and utilization of oxygen by the myocardium reflect the dynamic efficiency of the microcirculation. The present study examines these parameters during coronary artery bypass surgery. We used a voltammetric microelectrode technique to assess regional variations in myocardial tissue partial pressure of oxygen ($P_{O_2}$) and myocardial tissue perfusion (MTP) in patients undergoing coronary artery bypass surgery. A total of 29 myocardial regions were studied in 17 patients to assay tissue $P_{O_2}$, and 13 regions in 10 patients to measure MTP. There was an increase in MTP from $53 \pm 9$ m$\text{l min}^{-1} \cdot 100 \text{g}^{-1}$ before cardiopulmonary bypass to $72 \pm 13$ m$\text{l min}^{-1} \cdot 100 \text{g}^{-1}$ after (means ± S.E.M.; $P = 0.05$). Tissue $P_{O_2}$ showed an overall increase from a baseline level of $45 \pm 8$ mmHg to a final level of $88 \pm 10$ mmHg ($P < 0.0001$). Following release of the aortic cross-clamp there was a variable time delay before a change in tissue $P_{O_2}$ was observed. There was an immediate response in five regions, whereas in 20 regions the response was delayed by between 0.5 and 32 min. In the remaining four regions there was no change in tissue $P_{O_2}$. The duration of the delay in response was correlated positively with the cross-clamp time ($r = 0.45$, $P < 0.05$) and negatively with the final tissue $P_{O_2}$ ($r = -0.5$, $P < 0.05$). Voltammetric methods for monitoring changes in oxygen supply and utilization offer new insights into the changes that occur during ischaemia and reperfusion. A delay in the delivery of oxygen to the myocardium occurs in many patients following coronary artery bypass surgery.

INTRODUCTION

In recent years there has been growing interest in the role of the microcirculation in the pathophysiological changes that occur during periods of myocardial ischaemia and during reperfusion [1]. Both structural and functional abnormalities have been demonstrated [2–4].

A particular microvascular problem is the 'no-reflow phenomenon', i.e. the decrease in microcirculatory flow that occurs despite restoration of blood flow after a period of ischaemia [5]. Several associated pathophysiological changes have been described, including distal microvascular leucocyte and platelet plugging, microthrombi, endothelial swelling and distal microvascular spasm [6–8]. Clinically, patients who exhibit no-reflow following regional ischaemia during percutaneous transluminal coronary angioplasty [9] or coronary thrombolysis [10] have increased post-reperfusion mortality and morbidity. Whatever the mode of microvascular injury, the outcome is expected to impair the balance of myocardial tissue oxygen supply and utilization and/or failure of metabolite removal.

Since myocardial oxygen extraction exceeds that of any other organ in the body, increased oxygen demand can
only be met by increasing the coronary flow to improve myocardial perfusion. Patients with coronary artery disease have an impaired coronary reserve and are therefore at increased risk from ischaemic damage. Coronary artery bypass (CABG) surgery improves this balance. However, there is ample evidence to suggest that the surgery itself causes further myocardial damage [11,12].

During CABG surgery involving cardiopulmonary bypass (CPB), a period of global myocardial ischaemia is usually required to allow the surgeon to operate on a motionless, non-beating heart. This period of ischaemia and the ensuing reperfusion may lead to myocardial injury which manifests itself in various ways, from postoperative myocardial infarction, observed in areas supplied by patent grafted vessels [11], to reversible post-ischaemic myocardial dysfunction [13], or ‘stunning’.

There has been extensive work, mainly in animal models, to identify the cellular, biochemical and functional changes that occur, but changes in the microcirculation and their influence on tissue delivery of oxygen, especially in humans, have been more difficult to quantify.

Microelectrode techniques have been employed previously to measure the myocardial partial pressure of oxygen ($P_{O_2}$) in humans [14] in the post-operative period, as well as myocardial perfusion in the operative period [15]. In the present study we used a voltammetric microelectrode technique simultaneously to measure myocardial tissue $P_{O_2}$ and myocardial tissue perfusion (MTP) during CABG surgery.

**METHODS**

**Subjects and surgical procedures**

Patients of either sex selected for elective CABG surgery were studied. Patient consent was obtained according to the rules of the Royal Brompton Ethical Committee, which approved the study. Clinical details were recorded, including the history of the cardiovascular illness, risk factors and any previous cardiac interventions. Preoperative anginal symptoms were graded according to the Canadian Cardiovascular Society grading scale for angina pectoris (CCSS) [16].

A standardized surgical procedure was followed for all operations, which were carried out by a single surgeon. Routine pre-operative coronary angiography was performed for all patients. All patients had three-vessel coronary artery disease, and the left internal mammary artery was grafted to the left anterior descending artery. Saphenous vein grafts were used to bypass coronary lesions of other vessels. Cardioplegic arrest was achieved using antegrade/retrograde cold blood cardioplegia infused at 250–300 ml·min$^{-1}$ for 3 min (15–20 ml·kg$^{-1}$). Further infusions (10 ml·kg$^{-1}$) were administered at 20 min intervals. Infusion pressures of all cardioplegic solutions were closely monitored (antegrade infusion at the patient’s normal diastolic pressure and retrograde infusion up to a maximum of 40 mmHg). $N_2O$ was not used in the induction or maintenance of anaesthesia.

**Tissue $P_{O_2}$ and perfusion measurements**

It is possible to measure selectively both tissue $P_{O_2}$ and partial pressure of $N_2O$ electrochemically using a single silver electrode. This is achieved by an appropriate choice of over-potential ($-0.6$ to $-0.7$ V for $O_2$ and $-1.0$ to $-1.1$ V for $N_2O$) to drive the reduction of the dissolved gas [17]. The potentials are chosen such that the reduction currents generated *in vivo* are diffusion limited and therefore are linearly dependent on the concentration of the analyte of interest.

Determination of the rate of wash-in and wash-out of $N_2O$ introduced via the ventilatory gases, provides a measure of tissue perfusion [18,19]. This measure is most accurately described as the ‘myocardial solute exchange rate’, as it reflects contributions from all the available pathways for the exchange of small solutes to and from the tissue, including vascular pathways and interstitial transport [20]. $N_2O$ current/time curves are constructed during wash-in after switching the ventilatory gases to a mixture containing 30% $N_2O$ (without altering the $O_2$ content) until tissue saturation is reached, and during wash-out after switching back to the previous gas mixture. If the measured $N_2O$ concentration is a mono-exponential function of time, then tissue perfusion can be described by the single-compartment Kety model [21] and the exponent yields a value for the total gas exchange rate per unit volume of tissue. Natural logarithms were calculated for selected current–time data sets from both the wash-in and wash-out portions of the curve, correcting for any baseline drift. Linear regression was then performed to calculate the slope of the natural-log-time plots, yielding the exponent for each portion of the curve. The exponent has units of ml·s$^{-1}$·100 g$^{-1}$.

Microelectrode measurements performed under controlled, non-ischaemic conditions in canine left ventricular myocardium ($n = 10$) revealed good reproducibility over a 45-min period. The coefficients of variation for $P_{O_2}$ and $N_2O$ perfusion measurements made at three time points during this period were 10.3% and 10.8% respectively (P. J. Etherington, unpublished work).

**Myocardial microelectrodes**

Needle microelectrodes were constructed using 125 μm insulated silver wire (Advent Research Materials Ltd., Halesworth, Suffolk, U.K.), stainless steel 23 G hypodermic tubing (epoxy resin) (CY1301; Ciba Geigy plc, Macclesfield, Cheshire, U.K.) and shielded connecting
Myocardial oxygenation during coronary artery bypass surgery

Figure 1 Silver myocardial electrode used for intra-operative measurements of $P_O_2$ and MTP

Instrumentation

A two-channel potentiostat and current-to-voltage converter (EMS Ltd., Oxford, U.K.), incorporating isolation circuitry, was employed. This was connected to a PC (Dell Latitude) via data acquisition hardware (Data Shuttle; Strawberry Tree, Sunnyvale, CA, U.S.A.). Data acquisition software (Workbench PC, v.2.3.1; Strawberry Tree) was used to generate the potential waveforms applied to the microelectrodes and to record the currents. Electrode data were filtered to remove noise resulting from heart motion using a software-controlled 2-s moving average. Data were then stored for later analysis.

Microelectrode distribution and data acquisition

Following sternotomy, the pericardium was incised and the myocardium exposed. Electrodes were sutured securely in regions of the left ventricle that were considered to be ischaemic but viable from examination of pre-operative angiographic and transthoracic echocardiography images. Placement of the electrodes was technically easier on the anterior wall than the inferior wall of the left ventricle. While we attempted to place two electrodes in two ischaemic myocardial regions in each patient, in five out of 17 patients clinical stability before commencing CPB allowed only a single electrode to be placed on the anterior wall of the left ventricle. The length of the microelectrode needle was selected such that the depth of penetration was approx. 6–8 mm from the epicardial surface, taking into account any fat on the surface of the heart. The electrodes were allowed an equilibration period of at least 20 min while the internal thoracic artery was dissected out on a pedicle. The time points at which measurements were recorded are illustrated in Figure 2. A baseline level of tissue $P_O_2$ was recorded for 5 min before changing the applied potential to detect $N_2$O. A pre-CPB wash-in and wash-out curve
was then recorded. The electrodes were re-polarized to measure oxygen for the rest of the operative period, with the exception of an approx. 15 min (range 8–20 min) period post-CPB during which a further N₂O curve was obtained, following routine infusion of protamine sulphate to counteract the effects of heparin. Peak systolic and diastolic pressures, ECG, heart rate and core temperatures were recorded simultaneously with electrode data.

Data analysis
All results are expressed as means ± S.E.M. Since measurements of myocardial PO₂ and MTP could not be presumed to reflect global myocardial values, myocardial regions (rather than individual patients) were used for temporal and spatial comparisons, unless stated otherwise. Oxygen tensions at different times were compared non-parametrically using the Friedman test for related values and Kruskal-Wallis analysis of variance (ANOVA) for non-related values. Otherwise, groups were compared non-parametrically using the Mann Whitney rank sum test. Correlation was tested using the Spearman rank test. The P values quoted are two-tailed, and statistical significance was assumed when P < 0.05.

RESULTS
A total of 17 patients were studied (14 males and three females), with a mean age of 65 ± 1.5 years. All patients were ex-smokers and had ceased smoking for more than 6 months. There were no operative or post-operative complications, and all patients were asymptomatic on 6-week follow up. Eight patients had a previous history of myocardial infarction, of whom three had undergone CABG surgery previously, and four patients had type II diabetes. Table 1 shows the distribution of electrodes in the myocardial regions in the studies performed. Mean cross-clamp time was 81 ± 6 min (range 36–151 min) and 6 min (range 8–20 min) period post-CPB during which a further N₂O curve was obtained, following routine infusion of protamine sulphate to counteract the effects of heparin. Peak systolic and diastolic pressures, ECG, heart rate and core temperatures were recorded simultaneously with electrode data.

Figure 3 Changes in myocardial tissue oxygenation at four points during CABG surgery

Values are expressed as means ± S.E.M. Significant variations in PO₂ among the four time points: P < 0.0001 (ANOVA).

Table 1 Distribution of electrodes in myocardial regions according to the type of measurement performed
A total of 17 patients were studied, 10 of whom provided both PO₂ and MTP measurements.

<table>
<thead>
<tr>
<th>Myocardial regions</th>
<th>PO₂ (n = 17)</th>
<th>PO₂ + MTP (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>Inferior</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Coronary artery supplying</td>
<td></td>
<td></td>
</tr>
<tr>
<td>region studied</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 70% narrowing</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>&gt; 70% narrowing</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>100% narrowing</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

mean CPB time was 112 ± 5 min. Mean blood pressure pre-CPB was 72.6 ± 1.7 mmHg, and that post-CPB was 69.5 ± 1.6 mmHg. Four patients (representing seven myocardial regions) received intra-operative dopamine infusion post-CPB at a dose of 3–4 µg·min⁻¹·kg⁻¹.

PO₂
As shown in Figure 3, the baseline tissue PO₂ of 45 ± 8 mmHg dropped within 5–10 s following application of the aortic cross-clamp to a post-clamp level of 5 ± 2 mmHg. Tissue PO₂ then remained constant throughout the cross-clamp period, other than during infusion of cardioplegic solution, during which tissue PO₂ increased to a maximum of 22 ± 8 mmHg, before returning to the pre-infusion level within 1.2 ± 0.5 min of the end of infusion.

Release of the aortic cross-clamp (after 81 ± 6 min) generally resulted in a transient hyperoxygenation phase lasting 5–10 min. The tissue PO₂ reached a peak value (calculated as the maximum rise in PO₂ following release of the aortic cross-clamp) of 106 ± 14 mmHg, before gradually declining to a final level (calculated as the final PO₂ measurement before removing the electrodes at the end of surgery) of 88 ± 10 mmHg at 52 ± 10 min after release of the cross-clamp. However, the onset of the increase in PO₂ immediately followed release of the cross-clamp in only five regions. In 20 regions a delay occurred, ranging from 0.5 to 32 min (Figures 4a–4c). The delay in onset was inevitably accompanied by a delay in the peak PO₂ reached following release of the aortic cross-clamp. There was a negative correlation between the length of the delay and the final myocardial PO₂ (r = −0.5, P < 0.05). Furthermore, there was a positive correlation between the duration of this delay and the duration of the cross-clamp (r = 0.45, P < 0.05). In four regions there was no change in oxygenation following cross-clamp removal. The delay was noted to be shorter in patients demonstrating more prominent pre-operative anginal symptoms, as assessed by the CCSS score [7.1, 2.0 and
Myocardial oxygenation during coronary artery bypass surgery

Figure 4  Representative examples of changes in myocardial \( P_{O_2} \) following release of the aortic cross-clamp
(a) Immediate response; (b) no response; (c) delayed response.

Table 2  Myocardial \( P_{O_2} \) measured in different left-ventricular myocardial regions
Values are expressed as means ± S.E.M. RCA, right coronary artery. Significant difference compared with baseline: * \( P = 0.007 \) (ANOVA).

<table>
<thead>
<tr>
<th>Region</th>
<th>Baseline</th>
<th>Post cross-clamp on</th>
<th>Post cross-clamp off/RCA clamp off</th>
<th>Final measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inferior (( n = 5 ))</td>
<td>56 ± 27</td>
<td>9 ± 5 *</td>
<td>110 ± 35</td>
<td>95 ± 32</td>
</tr>
<tr>
<td>Proximal anterior (( n = 14 ))</td>
<td>45 ± 8</td>
<td>6 ± 1 *</td>
<td>105 ± 18</td>
<td>85 ± 14</td>
</tr>
<tr>
<td>Distal anterior (( n = 10 ))</td>
<td>41 ± 16</td>
<td>1 ± 1 *</td>
<td>148 ± 20</td>
<td>90 ± 16</td>
</tr>
</tbody>
</table>

0.3 min for CCSS 2 (\( n = 8 \)), CCSS 3 (\( n = 6 \)) and CCSS 4 (\( n = 3 \)) respectively; \( P < 0.01 \).

The mean systemic arterial pressure was higher post-CPB compared with pre-CPB values (by 10.7 ± 2.9 %) in eight patients, and lower than pre-CPB levels (by 6.7 ± 2.1 %) in nine patients (\( P = 0.2 \)). The delay in the increase in myocardial \( P_{O_2} \) was significantly less in the first group compared with the second group (1.2 ± 1.7 and 6.3 ± 3.0 min respectively; \( P < 0.01 \)).

ANOVA did not show any spatial difference in tissue \( P_{O_2} \) (\( n = 29 \)) between sites, categorized by the degree of coronary stenosis, at the pre-CPB or post-CPB time points. Table 2 shows the changes in myocardial \( P_{O_2} \) measured in different myocardial regions. There was no spatial difference in tissue \( P_{O_2} \) before or after CPB. However, there was significant spatial variation in tissue \( P_{O_2} \) during the cross-clamp period (Table 2) (ANOVA: \( P = 0.007 \)).

Changes in MTP
Figure 5 summarizes the changes in MTP and tissue \( P_{O_2} \) before and after CPB (10/17 patients; \( n = 13 \) regions).

MTP rose from a pre-CPB level of 53 ± 9 ml \( \text{min}^{-1} \cdot 100 \text{g}^{-1} \) to a post-CPB level of 72 ± 13 ml \( \text{min}^{-1} \cdot 100 \text{g}^{-1} \) (\( P < 0.05, n = 13 \)). We observed no
significant correlation between MTP and the degree of coronary artery stenosis either before or after CPB. There was no correlation between MTP and myocardial \( \text{PO}_2 \) either before or after CPB. However, in myocardial regions where the operation increased perfusion by more than 50\%, there was only a modest rise in myocardial \( \text{PO}_2 \) (19 ± 35\%). In contrast, in myocardial regions where perfusion increased by less than 50\%, myocardial \( \text{PO}_2 \) rose almost 8-fold (800 ± 352\%) \((P < 0.05)\). Correlation analysis demonstrated a significant inverse relationship between the change in \( \text{PO}_2 \) and the change in MTP \((r = -0.7, P < 0.01, n = 13)\).

There were no correlations between systemic blood pressure and either myocardial \( \text{PO}_2 \) or MTP before or after CPB. Similarly, there were no differences observed post-CPB related to whether the patient received a catecholamine infusion or not. The latter result was probably due to the small number of patients who received a catecholamine infusion intra-operatively, and no firm conclusion can therefore be drawn.

**DISCUSSION**

This study is, to our knowledge, the first to describe the changes in the rate of delivery of oxygen to the myocardium and in its utilization during coronary artery surgery.

The use of a voltammetric microelectrode technique has the advantages of simplicity and good spatial resolution. Measurements are averaged over a tissue volume of approx. 1 mm\(^3\). This high spatial resolution raises questions about the homogeneity of the tissue and how representative the measurement is. This issue is currently being addressed in models of ischaemia and reperfusion using multiple electrode arrays (P. J. Etherington, unpublished work). Such a study in surgical patients is not at present feasible. However, the great advantage of the current technique is that perfusion and \( \text{PO}_2 \) are measured at the same point in the myocardium.

There were some technical limitations in using the microelectrodes in the clinical setting. Movement artifacts resulting from heart motion were overcome by using specially designed electrodes with suture holes to secure them to the surface of the heart. The absence of noise, which correlated with heart motion, provided some evidence that this procedure was effective. The use of diathermy during surgery interfered with the electrode current signal, and so currents recorded during periods of diathermy were not used in the final analysis.

Our baseline myocardial \( \text{PO}_2 \) values were comparable with those obtained in the post-operative period by Wiener et al. \([14]\) using a similar technique in similar patient groups. Given that our baseline myocardial \( \text{PO}_2 \) levels represent the balance between utilization and supply, it seems reasonable to argue that oxygen utilization in ischaemic myocardial tissue is geared down to match the reduced supply. On the other hand, the high final myocardial \( \text{PO}_2 \) measurements may reflect poor oxygen utilization in the immediate post-reperfusion period. It will be important in future investigations to establish the time course of ensuing changes in myocardial tissue \( \text{PO}_2 \).

Tissue \( \text{PO}_2 \) increased following surgery by an average of 95\%. There were no significant differences in myocardial \( \text{PO}_2 \) among different myocardial regions before and after CPB. However, significant variations were observed during the cross-clamp period, with \( \text{PO}_2 \) levels being significantly higher in the inferior wall compared with the anterior wall regions of the left ventricle. This suggests variations in collateral supply to various myocardial regions that assume increased significance during the cross-clamp period. Alternatively, this may represent non-uniform delivery of cardioplegic solution and hence non-uniform oxygen utilization.

In a steady state, myocardial tissue \( \text{PO}_2 \) represents a balance between supply and utilization. However, rapid transients in \( \text{PO}_2 \) reflect supply variations that may occur before this steady-state balance is achieved \([26]\). Under normal conditions, increased oxygen demand is met by increased perfusion. High \( \text{PO}_2 \) levels therefore represent increased supply or under-utilization of oxygen, and low values may mean the reverse. To distinguish between these possibilities, it is necessary to simultaneously measure MTP.

MTP had risen by 35\% at the end in comparison with the beginning of surgery. The most notable observation was the much greater increase in oxygenation than in perfusion following surgery: in fact, there was an inverse relationship between the changes in MTP and those in myocardial \( \text{PO}_2 \). This apparent paradox may arise because of changes in oxygen utilization. There are clearly a number of patients who display a large rise in \( \text{PO}_2 \) presumably because, at least in the immediate post-operative period, the myocardium consumes only a fraction of the oxygen supplied. There was no correlation between MTP and myocardial \( \text{PO}_2 \) before or after CPB, but this may reflect the fact that large variations in tissue perfusion are required to alter the oxygen content in tissue.

Perfusion alone was measured previously by Kawasuji et al. \([15]\) during CABG surgery, using local electrolytic generation of hydrogen. These authors demonstrated that perfusion fell only in myocardial regions supplied by vessels with greater than 90\% stenosis. We did not study patients without coronary artery disease, and within our restricted group we could not establish such a relationship. The main purpose of our measurements was to explore the relationship between perfusion and oxygenation.

A striking observation was the delay in the increase in myocardial \( \text{PO}_2 \) following release of the aortic cross-
clamp. It is possible that the observed delay was a result of impaired microvascular flow or no-reflow. Because we were unable to administer a tracer gas during CPB, we were unable to test this hypothesis directly. This delay in O$_2$ delivery could result in hypoxic myocardial injury at a critical period when myocardial protective measures have stopped, and may explain the high incidence of post-operative myocardial dysfunction that commonly follows coronary artery surgery [13]. The delay is unlikely to be influenced by systemic arterial pressure, since the majority of the delay occurred while patients were still on CPB support with standardized control of systemic blood flow and pressure conditions. However, the delay in the increase in systemic blood flow and pressure conditions. However, were still on CPB support with standardized control of since the majority of the delay occurred while patients were unlikely to be influenced by systemic arterial pressure, post-operative myocardial dysfunction that commonly have stopped, and may explain the high incidence of a critical period when myocardial protective measures rise in tissue symptoms had a significantly shorter delay before the necessary time resolution.

Graphy [28] and positron emission tomography [29], lack microcirculatory dynamics, such as contrast echocardiography large epicardial vessels. Other methods used to determine cine angiography films only determine the flow of radio-opaque dye in delayed coronary flow [27]. However, cine angiography films were used to define the period of cine angiography only to determine the flow of radio-opaque dye in large epicardial vessels. Other methods used to determine microcirculatory dynamics, such as contrast echocardiography [28] and positron emission tomography [29], lack the necessary time resolution.

Patients with more prominent pre-operative anginal symptoms had a significantly shorter delay before the rise in tissue PO$_2$ following release of the aortic cross-clamp. Although it is difficult to explain the mechanism of this association, Komamura et al. [30] have made a similar observation in patients treated with thrombolyis following acute myocardial infarction. They demonstrated that patients who suffered from pre-infarction anginal symptoms had shorter no-reflow duration and a better myocardial functional outcome.

By monitoring myocardial tissue PO$_2$ and perfusion, it is possible to observe the efficacy of various interventions that are aimed at improving myocardial oxygen supply and nutrient and metabolite exchange. Simultaneous assessment of functional myocardial changes may lead to a clearer understanding of changes in myocardial performance during the period that follows reperfusion procedures.

ACKNOWLEDGMENTS

This work was supported by the British Heart Foundation (PG/96160) and by the Garfield-Weston Trust.

REFERENCES

[List of references from the document]