

Ischaemia/reperfusion in the posthypoxaemic re-oxygenated myocardium: haemodynamic study in the isolated perfused rat heart

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In order to study the haemodynamics of reperfusion injury in the post-hypoxaemic heart, we exposed buffer-perfused isolated rat hearts to either: (1) 20-minute low-flow ischaemia or (2) 20-minute hypoxaemia followed by re-oxygenation and further ischaemia/reperfusion. In group 2, the myocardial contractility recovered less ($p < 0.002$) than in group 1. This model therefore represents with sufficient reliability the clinical situation where hypoxaemic hearts are re-oxygenated before ischaemia/reperfusion and receive more severe injury than hearts exposed to ischaemia/reperfusion only. To locate the major site of the injury, further data were obtained (1) with infusion of superoxide dismutase and catalase during hypoxaemia and in the first five minutes of re-oxygenation, and (2) by eliminating re-oxygenation. It appears that the major determinant of reperfusion injury in hypoxaemic hearts is to be looked for in the events underlying hypoxaemia or re-oxygenation, and is mediated by oxygen-derived free radicals.

Introduction

The application of modern techniques to protect the myocardium during surgical repair of cyanotic congenital heart disease has considerably decreased the mortality rate, but this rate still remains high (5%) with respect to elective adult cardiac surgery.^{1–3} Several factors, normally ruled out in adults, may be responsible for this feature, including the age of the patient, ventricular

hypertrophy, the type of the surgical repair and the extent of cyanosis before cardiopulmonary bypass (CPB).⁴ At present, few experimental data are available to assess the importance of these factors. Nevertheless, several indications point to the cyanotic condition existing before the surgical repair as a candidate risk factor,^{1,5–7} but flexible and accessible *in vitro* or *ex vivo* experimental models to test this hypothesis are not available. Indeed, distinguishing hypoxaemia

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from ischaemia with full control of cardiac haemodynamics is difficult with *in vivo* models, and evidence of the critical role of cyanosis in such a clinical setting is therefore only indirect.

In this work, we describe a novel approach to the problem of the reperfusion injury in the posthypoxaemic myocardium. We exposed isolated perfused rat heart preparations to ischaemia/reperfusion (I/R), with and without previous exposure of the heart to hypoxaemia/re-oxygenation (H/R). The myocardial performance was continuously monitored during the various protocols with full control of the composition of the perfusate, the coronary perfusion rate, the loading conditions and the O₂ supply. The purposes of this work were: (1) reproducing the *in vivo* situation under which a posthypoxaemic, cyanotic, re-oxygenated heart exposed to I/R is injured with more severity than a normal heart exposed to I/R only; and (2) locating the major site of the injury. We will show that the oxygen derived free radicals (ODFR) generated during the re-oxygenation of hypoxaemic hearts and before I/R play a pivotal role in determining the injury of cyanotic hearts exposed to I/R.

Materials and methods

The experimental model used in this work is the isolated rat heart perfused through the aorta (Langendorff's model) with a crystalloid oxygenated buffer. The retrograde flow, regulated by an external pump, closes the aortic valve, displacing the perfusate through the coronary circulation. An inflatable balloon in the left ventricle keeps the loading conditions constant and allows measurement of myocardial performance.

Normothermic ischaemia or hypoxaemia were applied for the same time (20 minutes) reducing either the flow or the pO₂ of the perfusate to 10% of baseline. At the end of hypoxaemia or ischaemia, the hearts were returned to baseline conditions to study the effects of the functional derangements which had occurred during the stress on myocardial performance.

The perfusing buffer was composed of 115.5 mmol NaCl, 4.7 mmol KCl, 1.2 mmol KH₂PO₄, 0.5 mmol EDTA, 1.2 mmol Na₂SO₄, 28.5 mmol NaHCO₃, 3 mmol CaCl₂, 1.2 mmol MgCl₂, 16.6 mmol glucose, pH 7.4 and 37 °C. The buffer was

equilibrated in two bubble oxygenators (Shiley S-100 A, Irvine) with either 94% O₂ + 6% CO₂, or 9.4% O₂ + 6% CO₂ (balance N₂). A roller pump (Watson Marlow 503-S, Falmouth) forced the buffer through 8 µm pore size polycarbonate filters to the aortic cannula at flow rates of 15 ml/min or 1.5 ml/min.

We used 33 hearts from male Sprague Dawley rats (weight 250–280 g) anaesthetized by intraperitoneal heparinized sodium thio-pental (10 mg/100 g bodyweight). The hearts were excised and mounted on the aortic cannula and retrograde perfusion was started immediately. An inflatable, saline-filled latex balloon was introduced into the left ventricle. Coronary pressure (CP) was monitored by a pressure transducer (Harvard apparatus 52-9966, Natick), connected to the aortic cannula. An additional transducer connected to the balloon provided the end-diastolic pressure (EDP), the left-ventricle developed pressure (LVDP) and the heart rate (HR). The integral of LVDP versus time (∫LVDP) was evaluated recording the pressure wave of a single contraction by a digital storage oscilloscope (Nicolet Scientific Instruments 3091, Madison) and a chart recorder. The value for ∫LVDP was then obtained by cutting off the area under the wave and weighing it with appropriate controls. The product ∫LVDP × HR is here considered an integrated index of the myocardial contractility since the hearts were not paced.

Data are expressed as mean ± SE. Student's *t* test, one-way ANOVA and the Bonferroni's tests were used as appropriate. The level of significance was set at *p* = 0.05.

This investigation conformed with the *Guide for the care and use of laboratory animals* published by the US National Institutes of Health. (NIH publication No 85-23, revised 1985).

Results

For the purposes of this work, we performed two studies. Study 1 addressed the question as to whether the described experimental model is valid to represent with reasonable accuracy the situation occurring when a cyanotic heart is re-oxygenated and exposed to I/R. Hearts were stabilized for 30 minutes and then divided into two groups: I/R and H/R + I/R (Table 1).

Table 1 Scheme of the experimental protocols of this study. The O₂ supply during baseline and recovery was 14.1 μmol/min (pO₂ = 670 mmHg and coronary flow rate = 15 ml/min). In hypoxaemia, pO₂ was reduced to 67 mmHg at constant coronary flow. In ischaemia, coronary flow was reduced to 1.5 ml/min at constant pO₂. Under both conditions, therefore, the O₂ supply was 1.41 μmol/min.

Group (n)	Time (min)					
	-30	0	20	40	60	80
I/R (7)	Baseline	Hypoxaemia	Recovery			
H/R + I/R (9)	Baseline	Ischaemia	Recovery	Hypoxaemia	Recovery	
			←SOD/CAT*→			
SOD/CAT (7)	Baseline	Hypoxaemia	Recovery	Ischaemia	Recovery	
H/I/R (10)	Baseline	Hypoxaemia	Ischaemia	Recovery		

* Addition of 40 IU/ml superoxide dismutase and 104 IU/ml catalase starting at the fifth minute of hypoxaemia and ending at the fifth minute of recovery

Myocardial performance was compared at the end of the protocols to evaluate the effects of previous exposure to H/R on I/R. Figure 1 shows that both diastolic and systolic performances were significantly depressed in the hearts of the latter group.

Study 2 was designed to investigate the role of re-oxygenation after hypoxaemia. For this purpose we devised three groups: H/R + I/R (as in Study 1); SOD/CAT (hearts exposed to H/R + I/R as in Study 1, but with infusion of 40 IU/ml superoxide dismutase and 104 IU/ml catalase starting at the fifth minute of hypoxaemia and ending at the fifth minute of recovery from hypoxaemia); H/I/R (hearts made ischaemic after hypoxaemia, eliminating the re-oxygenation step).

Figure 2 shows the diastolic and systolic performances of these hearts. One-way, three-group ANOVA revealed highly significant differences among the various groups ($p < 0.007$). Invariably, the recovery of the hearts of the group H/R + I/R was always worse than that of the other two groups. In addition, no significant differences were observed between the recovery of hearts treated with superoxide dismutase and catalase and that of hearts which were not re-oxygenated before I/R.

Discussion

Using the experimental model described here, we monitored myocardial contractility under carefully controlled conditions of coronary

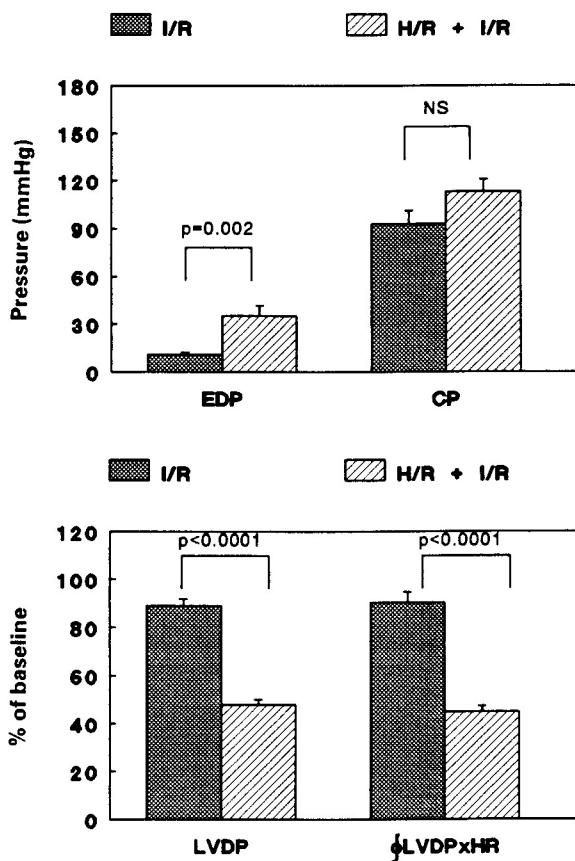


Figure 1 Myocardial performance (mean ± SE) at the recovery from I/R (n = 7) or H/R + I/R (n = 9). The significance of the differences was evaluated using Student's *t* test for unpaired data. EDP = end-diastolic pressure, CP = coronary pressure, LVDP = left ventricle developed pressure, ΔLVDPxHR = (time-pressure integral)·(heart rate).

flow rate, O_2 supply and composition of the perfusate. The fixed perfusate delivery rate prevented the autoregulation of the coronary flow by the hearts. The constant volume of the intraventricular balloon simulated constant loading conditions. The hearts were reperfused or re-oxygenated under the same conditions of baseline to study the effects of the metabolic and functional derangements driven by ischaemia and hypoxaemia on the myocardial recovery. The hearts were not paced and could therefore adjust their O_2 and substrate demand, but the use of

the product (time-pressure integral) \cdot (heart rate) (ϕ LVDP \cdot HR) provided a reliable integrated measure of myocardial function.⁸ The use of blood was discarded at the expense of a departure from physiological conditions, with the advantage of being able to correlate the applied stimuli to the heart responses with fewer interfering factors.

The protocol of the H/R + I/R hearts is closely related to the sequence of events to which cyanotic, hypoxaemic hearts are exposed during most surgical procedures for repair of congenital heart disease: the hypoxaemic heart is first re-oxygenated at the beginning of the CPB, then undergoes ischaemia with aortic cross-clamping for intracardiac repair, and is finally reperfused at the end of the procedure. This surgical protocol is often complicated by elevated hospital mortality regardless of the anatomical success of the intervention^{1,4,7} and the claimed increased tolerance of the neonatal heart to ischaemia with respect to the adult heart.^{9,10}

In our experimental model the hearts were exposed to acute hypoxaemia instead of the clinically more relevant situation of chronic hypoxaemia, limiting the meaning of the comparison between the two situations. Nevertheless, we believe that the present results may provide a partial explanation for the higher degree of severity found in paediatric, compared to adult, cardiac surgery.

Study 2 shows that the exposure of the H/R + I/R hearts to two stresses, compared to only one stress in I/R hearts, may not be the only explanation for their more severe injury. In fact, two interventions that did not decrease the total time of hypoxaemia and ischaemia, i.e. treating the hearts with ODFR scavengers and eliminating re-oxygenation, allowed hearts to recover as in the I/R group. The re-oxygenation before ischaemia appeared the major site of oxidative injury in the hypoxaemic reperfused hearts.^{1,7} There is strong evidence for the involvement of ODFRs in this injury: (1) the final recovery was better in the presence of free radical scavengers; and (2) eliminating re-oxygenation allowed hearts to recover as with the scavengers. In the rat heart, ODFRs are mainly generated from xanthine oxidase and hypoxanthine.¹¹⁻¹³ Although these radicals are generated for up to three hours after reperfusion,¹⁴ they peak at two minutes after reflow consistently with the prevention of

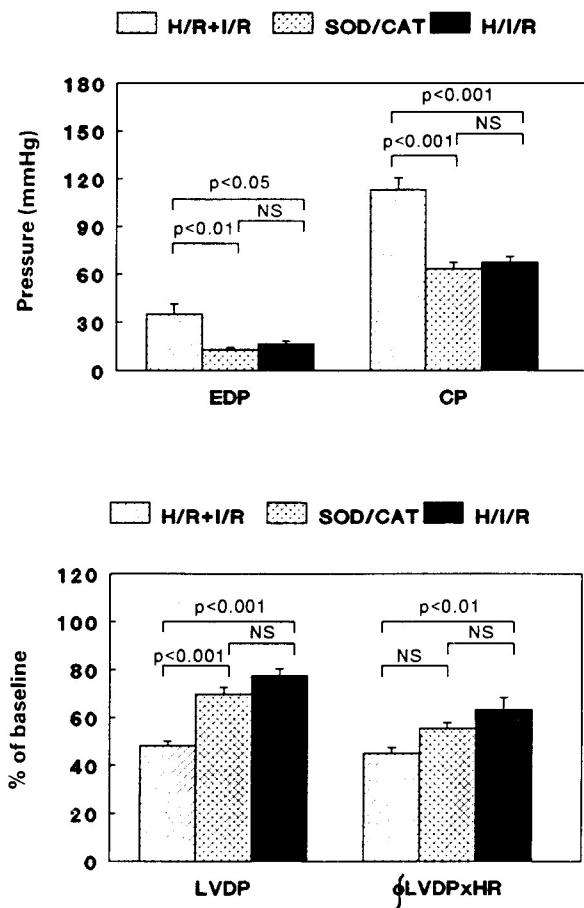


Figure 2 Same data as in Figure 1 in hearts from groups H/R + I/R, SOD/CAT and H/I/R. One-way ANOVA analysis of these data yielded $p < 0.007$. Bonferroni's test was used to define the significance of the differences (NS = not significant).

the oxidative injury by superoxide dismutase and catalase in the first five minutes of reoxygenation. The combined effects of superoxide dismutase and catalase on the prevention of ODFR-induced injury is known.¹⁵ The enzymatic activity of superoxide dismutase and catalase selected for this study approached the optimal.^{16,17}

The hypothesis underlying this work is therefore that the intracellular generation of ODFRs during re-oxygenation is highly involved in determining the size of the reperfusion injury in posthypoxaemic re-oxygenated hearts and is consistent with the occurrence of the injury in the corresponding re-oxygenation of hypoxaemic tissues.^{18,19} Our observations are in agreement with the finding that during elective repair of tetralogy of Fallot, ODRF injury was observed before aortic cross-clamping, i.e. after re-oxygenation and before ischaemia.⁷ The reduced mitochondrial superoxide dismutase and glutathione peroxidase activities in hypoxaemia may be involved.²⁰ However, the possibility that it is not hypoxaemia *per se* but hypoxaemia-derived abnormal loading characteristics which is the real factor leading to functional derangements in ischaemic hearts⁶ cannot be ruled out.

Conclusions

With this model, we could reproduce with reasonable accuracy the higher injury to which posthypoxaemic re-oxygenated hearts are exposed with respect to an ischaemic episode. Although it is difficult to transfer results gathered in an isolated heart preparation to an *in vivo* situation, it appears that the prevention of the oxidative portion of the injury appears feasible using adequate free radicals scavengers, but full protection of the heart is still to be worked out. The deleterious effect of low arterial pO₂ in infants undergoing extracorporeal membrane oxygenation on the development of cardiac stun²¹ emphasizes the importance of this issue. However, our knowledge of the mechanisms involved in ischaemia and hypoxaemia is still limited and important questions have yet to be answered, including the metabolic-functional interactions in the myocardium and the individual roles of the various factors in determining the size of the re-oxygenation or reperfusion injuries. The

effect of chronic or sustained hypoxaemia must also be evaluated in order to offer more reliable explanations to the phenomena observed here.

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