Avoidance of reperfusion injury after cardioplegia

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ABSTRACT   Myocardial damage incurred by ischaemia appears during and seems to be accelerated by reperfusion, which restores recoverable cells and disrupts badly damaged ones. Vicious cycles of oedema, calcium accumulation, acidosis, oxygen toxicity, fibrillation and air and platelet emboli contribute to the reperfusion injury. The philosophy of cool low-pressure reperfusion gradually restoring temperature and pressure to normal is here contrasted experimentally with that of immediate normothermic, normotensive perfusion after 90 minutes of ischaemic cool, cardioplegic arrest. The preparation was a canine heart which was treated according to the usual clinical protocol except that one group was reperfused at normal temperature and pressure, and the other group started reperfusion cool and at a low pressure and over the next 10 minutes pressure and temperature were restored to normal. Isovolumic ventricular function studies were done before ischaemia and after recovery and showed statistically significant differences between the groups in favour of the immediate restoration of normal temperature and pressure of perfusion. Contractile velocity and systolic pressure showed very highly significant ($p = <0.005$) differences, wall stress significant ($p = <0.025$) and compliance not significant differences between the groups. Reperfusion with optimal conditions may prevent "vicious cycle" changes in ischaemically damaged but recoverable myocardium. We have shown that a step in this direction is reperfusion with blood at normal temperature and pressure rather than initially at lowered temperature and pressure.

Myocardial damage appearing during reperfusion has been well-known from the earliest experience of cardioplegia as now used,[1][2] and is acknowledged to be more dramatic than changes found in the ischaemic phase.[3] Reperfusion accentuates the difference between recoverable and dubiously recoverable myocardium, making more obvious the damage previously only detectable by electron microscopy. The characteristic appearances of reperfusion injury result,[2][4-6] with typical deterioration of function[7-9] epitomised by "stiffness" of contraction and relaxation. Not only ischaemia but any withdrawal of metabolites or essential ions is followed by reperfusion injury on restoration of blood supply.

The events which follow reperfusion are of the "vicious cycle" type. *Oedema* impairs the distribution and adequacy of perfusion by increasing the intramyocardial pressure and widening diffusion distances. The restoration of energy supply is further delayed by depressed mitochondrial function. High reperfusion pressures worsen the oedema but

the oedema makes a higher perfusion pressure necessary to avoid the "no reflow phenomenon".[10] Excessive *calcium accumulation* in the cells is characteristic of reperfusion injury and impairs myofibrillar relaxation with resultant reduction of healthy perfusion. Restriction of calcium worsens this reperfusion effect—the calcium paradox.[11] *Acidosis* provoked by ischaemia is increased by the proton efflux of excessive calcium uptake.[12] It impairs membrane efficiency which worsens electrolyte imbalance[13] and oedema (by disturbance of the colloid osmotic pressure from pH effects on the protein buffer system). The acidosis is actually greater than realised at the temperatures at which reperfusion occurs, because the equivalent "alkalinity" to pH 7·4 at 37°C is 7·67 at 20°C.[14]

Reperfusion without *oxygen* worsens the reperfusion syndrome, yet the damage from superoxides is worse in post-ischaemic cells which have not yet restored their defences against this insult. *Ventricular fibrillation* frequently follows reperfusion and impairs the flow and distribution of perfusion. Restoration of blood flow to the heart may also distend it with the same effects, little resistance to this being encountered in the flaccid cardioplegic

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heart. The restored blood flow through the coronary arteries may carry slugs of *air* from the empty large vessels into the small ones where they constitute an impassable barrier to perfusion. *Platelets*, which are programmed to self-destruct on damaged endothelial surfaces will also enter with the restored blood flow and will find little undamaged endothelium throughout most of the coronary tree.

Reperfusion is thus typical of economic debt repaying time—uncomfortable and dangerous for the myocardium but inescapable if recovery is ever to occur. We must thus direct our attention to minimising the dangers. While nearing an optimal solution for cardioplegia we do not know the optimum perfusate for reperfusion, but after multi-dose cardioplegia a pH of 7·8 in the reperfusate has been shown to maintain good contraction.[15]

There is some evidence[16] that the sodium/potassium ratio and myocardial function are better maintained after prolonged preservation if a high potassium solution is used for reperfusion. The effect of the inhibition of electron transport with amylobarbitone to prevent calcium injury has been demonstrated in rat hearts.[17] It is possible that calcium channel blockers may similarly ameliorate calcium injury.

Our investigation is directed to show optimal conditions of temperature and pressure reperfusate, as cardiac surgeons may be roughly divided into those recommencing perfusion with normal temperature and pressure and those starting gently at a low pressure and temperature and gradually restoring conditions to normal.[18–20]

We have recently been seeking an answer to which of these two policies causes the least reperfusion injury. A preparation was used which duplicated the standard clinical situation. Two groups of hearts were managed in exactly the same way until the moment of reperfusion, when one group was reperfused immediately at normal temperature and pressure (100 mm Hg), the other group being reperfused at 50 mm Hg and 28°C initially and these values brought up to normal over the next five to seven minutes. The percentage of the initially established isovolumic ventricular function found after full recovery by reperfusion was used to compare the groups.

Experimental method

Dogs weighing 20-30 kg, premedicated with drop-eridol 1-4 mg and fentanyl 25-100 μg, were anaesthetised with 30-40 mg/kg of pentobarbitone, intubated, and ventilated with a 60/40 N_2O/O_2 mixture. The chest was opened by sternotomy and the cavae and ascending aorta cannulated for bypass, after giving

3 mg/kg of heparin. The femoral artery was cannulated for pressure monitoring, as was the low ascending aorta. A further cannula was placed in the low ascending aorta for root perfusion. Cardiopulmonary bypass was established with a Temptrol Q 100 oxygenator and Pemco Pumps, and the heart fibrillated for a few minutes while the left atrium was opened and a flanged latex balloon fixed in the left ventricle by a purse-string suture in the mitral valve. The balloon was connected to a graduated syringe of saline and to a pressure transducer and the intraventricular pressure raised to 0·65 kPa (5 mm Hg) before defibrillating the heart. With the aortic root perfused at 13 kPa (100 mg Hg) and the heart rate at 100 per minute with pacing if necessary, isovolumic ventricular function tests were made by raising the end-diastolic pressure from 0-2 kPa (0-15 mm Hg) with 2·5 ml increments of saline into the balloon, measuring the systolic pressure on a 0-250 mm Hg scale, and the end-diastolic pressure on a 0-25 mm Hg scale, and the rate of rise of pressure with a differentiator on a 0-2500 mm Hg/s scale. The electrocardiograph, perfusion pressures of the heart and the rest of the animal, temperature of the heart (with a septal needle) and the temperature of the rest of the animal were all recorded on a Devices "M 19" eight-channel recorder. The plan of the preparation is seen in the figure.

The aorta was then clamped if this had not been necessary to maintain a satisfactory cardiac perfusion pressure with differential perfusion. The aortic root pump was stopped and the aortic root filled with St Thomas' solution at 4°C and 7-10 kPa (50-70 mm Hg) of pressure until the myocardial temperature was 15°C. A bag of saline at 4°C was placed on either side of the heart and one upon it to substitute for the cold saline irrigation used in the clinical situation. Ninety minutes of this cold cardioplegic arrest then elapsed, during which time the animal's temperature was allowed to fall to 28°C. If the heart were to be reperfused at normal temperature and pressure, rewarming was started 10 minutes before the end of this ischaemic period. In this group of hearts (group 1, eight dogs) the root perfusion was recommenced at normal temperature and at 13 kPa pressure (100 mm Hg); in the other group (group 2, eight dogs) the initial perfusion pressure was 6·5 kPa (50 mm Hg) and was raised over the next 5-10 minutes while the temperature was being brought from 28°C to 36°C. In all subjects the ventricle was defibrillated if necessary as soon as possible and the end-diastolic pressure was kept below 1·3 kPa (10 mm Hg) by changes in the volume in the balloon; blood gases and chemistry were kept normal throughout. When the recovery of the ventricle was as complete as possible and the
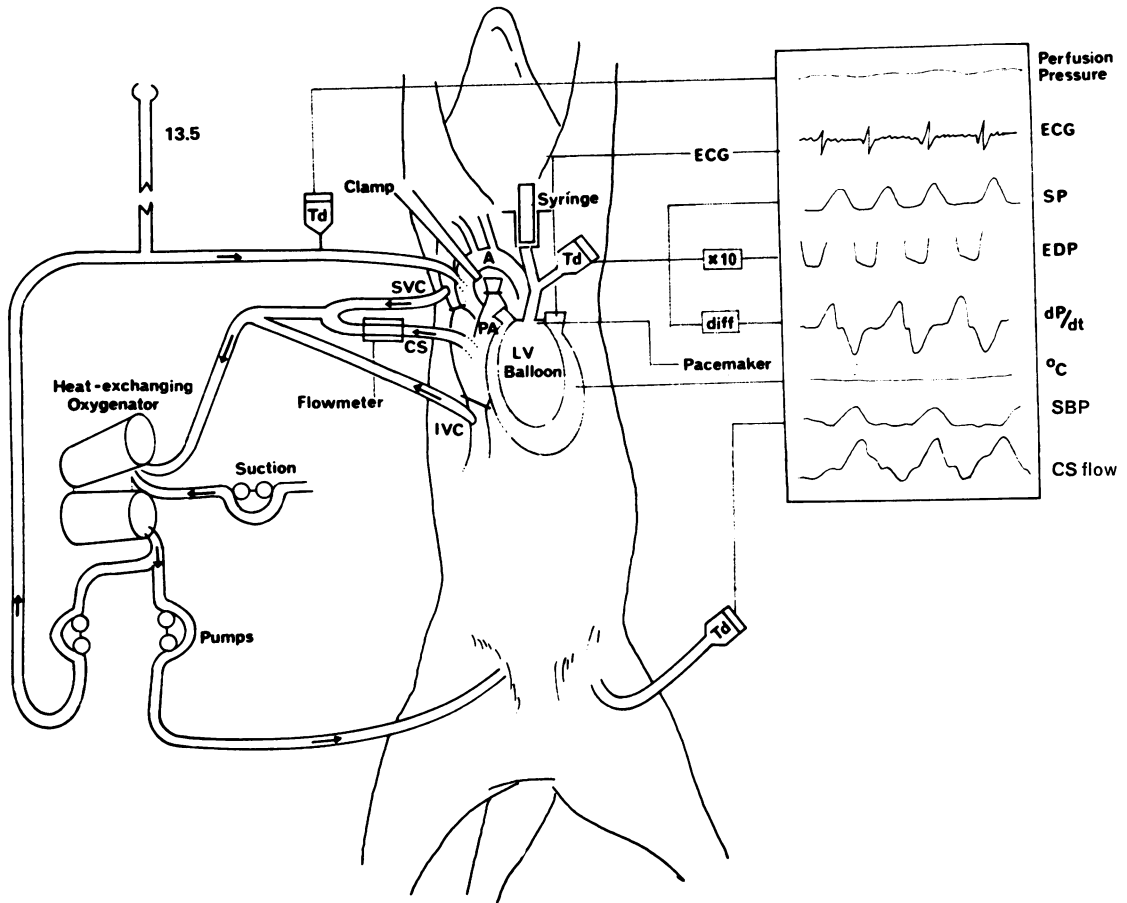
Figure *In-situ heart circuit. A = aorta, CS = coronary sinus, diff = differentiator, EDP = end-diastolic pressure, IVC = inferior vena cava, LV = left ventricle, PA = pulmonary artery, SBP = systemic blood pressure, SP = systolic pressure, SVC = superior vena cava, Td = transducer.*

contractile force and velocity had reached a plateau at a steady state, ventricular function tests were done as before. The left ventricle was then cut from the rest of the heart and its weight and displacement volume measured.

The calculations involved finding the wall thickness at each intraventricular volume (from that volume and volume of the ventricle) which allowed the wall stress at that time to be calculated with the formula s = pr/2h, p being the peak systolic pressure, r the internal radius of the ventricle at that volume, and h the wall thickness at that volume. These values and the intraventricular volume, the peak systolic pressure, and the rate of rise of pressure, were plotted against end-diastolic pressure, and the value for each at an end-diastolic pressure of 10 mm Hg were calculated from the regression formula. The final values for these were expressed as percentages

of the initial values. Mean and standard error for each group could then be found for volume (compliance), peak systolic pressure (force), wall stress (contractile force) and rate of rise of pressure (dp/dt—expressing the velocity of contraction) and the significance of differences between the groups found using Student's *t*-test.

Results

COMPLIANCE (table a)
Both groups showed some reduction in the volume producing an end-diastolic pressure of 10 mm Hg, but in neither case was this significant and the groups did not differ significantly.

PEAK SYSTOLIC PRESSURE (table b)
There was 17% loss of this index of contractile force

in the hearts of group 1, which was not significantly different from the initial levels. Group 2, gradually rewarmed and reperfused, lost a very significant amount (60%) of the initial value and this was significantly worse than group 1.

PEAK SYSTOLIC WALL STRESS (table c)
There was a similar pattern to that of pressure. Immediately reperfused hearts lost insignificant amounts of their original systolic wall stress whereas the gradually reperfused hearts lost very significant amounts and the groups were significantly different in this respect.

CONTRACTILE VELOCITY (table d)
In this respect also there was only significant loss of original dp/dt in the gradually reperfused and rewarmed hearts and the groups were significantly different.

Discussion

It is always difficult to get something for nothing, so our realistic ambition must be to maximise the benefits of cardioplegia and reperfusion and minimise the costs of ischaemia and reperfusion injury. The cardioplegic benefit of lowering the calcium was offset by the paradoxical reperfusion calcium injury. Frequent reperfusion, while seeming beneficial, means frequent reperfusion injury,[21] and high perfusion pressures are more likely to worsen oedema and contusion.[22] More oxygen delivered to the depleted cell exposes it to the risks of super-oxide toxicity and oxygen-dependent myocardial stiffness.[23] Restoring contractility to the myocardium may make

it fibrillate, producing inadequate and ill-distributed perfusion. Left ventricular venting to prevent over-distension of the heart during reperfusion gives access to air with all its risks, but no venting may permit distension to discourage healthy perfusion.

The attraction of reperfusing with cool blood is that at a vulnerable time the minimum metabolic demands would be made upon the myocardial cells. This possible advantage is however theoretically offset by the increased likelihood of oxygen toxicity and of adverse shifts in pH and other ions. Our experiments seem to bear this out and would support the policy of normothermic reperfusion. Similarly the theoretical advantages of gentle low pressure reperfusion in preventing injury to the damaged and therefore "leaky" endothelium of the myocardial blood vessels and oedema of the vulnerable myocardial cells, are offset by the resultant delay in recovery of these tissues and possibly encouragement of the "no-reperfusion syndrome". The philosophy of normal pressure, normal temperature reperfusion is amply supported by our experiments.

We have now shown that generous reperfusion with blood at normal temperature is optimal and the explanation for this fact could be that the technique rapidly flushes and buffers the myocardial acidity, at a temperature at which pH 7·4 is appropriate and the efficiency of a cell and mitochondrial membranes is best assured.

Table *Comparison of results in group 1 animals (immediate reperfusion and rewarming) and group 2 animals (gradual reperfusion and rewarming)*

	Final initial %	Significance of change from initial values	Significance of difference between groups
Compliance (vol) (a)			
Group 1 (n = 8)	80·43 ± 8·67	p = <0·0125	
Group 2 (n = 8)	85·21 ± 16·15	p = <0·15	p = <0·4
Force (PSP) (b)			
Group 1	83·63 ± 12·56	p = <0·1	
Group 2	38·99 ± 6·74	p = <0·0025	p = <0·005
Wall Stress (Pr/2h) (c)			
Group 1	74·2 ± 10·66	p = <0·1	
Group 2	28·23 ± 11·98	p = <0·0025	p = <0·025
Velocity (dp/dt) (d)			
Group 1	76·78 ± 15·355	p = <0·1	
Group 2	27·32 ± 5·36	p = <0·0005	p = 0·005

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