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Iloprost (ZK 36374) enhances recovery of regional myocardial function during reperfusion after coronary artery occlusion in the pig

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1 Ligation of the left anterior descending coronary artery in open-chest pigs for 20 min caused a complete loss of regional myocardial function, which did not recover during the first two hours of reperfusion.

2 Infusion of the stable prostacyclin analogue Iloprost (100 ng kg^{-1} min^{-1}) did not prevent the loss of systolic wall function during ischaemia.

3 Recovery of regional myocardial function during the first two hours of reperfusion was enhanced to 40% of baseline by Iloprost.

4 This effect of Iloprost cannot be explained by a decreased O_2-demand during ischaemia or an enhanced recovery of myocardial ATP content.

Introduction

In the experimental animal, prostacyclin exerts a beneficial effect on acutely ischaemic myocardium (Lefer *et al.*, 1978; Melin & Becker, 1983). However, its chemical instability limits its use. Iloprost ZK 36374; 5-{(E)-(1S, 5S, 6R, 7R)-7-hydroxy-6-|(E)-(3S, 4RS)-3-hydroxy-4-methyl-oct-1-en-6-yn-yl | -bicyclo- | 3.3.0-octan-3-yliden}-pentanoic acid) a more stable analogue of prostacyclin, has been shown to reduce ST-segment elevation and myocardial creatine kinase depletion after coronary artery occlusion in the cat (Schrör *et al.*, 1981) and to possess a pronounced antithrombotic effect in the pig (van der Giessen *et al.*, 1984). We now describe the effect of Iloprost on regional myocardial function in a model of coronary artery occlusion followed by reperfusion.

Methods

General

Studies were performed in young Yorkshire pigs (22–30 kg). The animals were sedated with azaperone (120 mg i.m.) and anaesthesia was induced with 150 mg metomidate, administered via a dorsal ear vein. Subsequently the animals were intubated and connected to a respirator for artifical ventilation with a mixture of O_2 and N_2O (1:2). Pentobarbitone (6–12 mg kg^{-1} h^{-1}) was administered intravenously for maintenance of anaesthesia. Iloprost or its solvent was infused via a catheter in the superior caval vein. Central aortic pressure was obtained via a 7F Cournand catheter. Left ventricular pressure was measured via an 8 F Millar catheter. A standard lead ECG was recorded continuously.

After exposure of the heart, an electromagnetic flow probe (Skalar, Delft) was placed around the ascending aorta for the measurement of aortic blood flow. Stroke volume was computed from the integral of this flow signal.

Myocardial wall thickness was monitored with a 5 MHz ultrasonic transducer (Krautkramer-Branson, Lewistown, Pa, USA) sutured onto the epicardial surface of that part of the left ventricle perfused by the left anterior descending coronary artery (LAD). From the tracings, the local wall thickness was measured at end-diastole (EDT) and end-systole (EST), while systolic wall thickening (SWT) was calculated as:

$SWT = (EST - EDT)/EDT$ (Verdouw *et al.*, 1980)

Transmural myocardial biopsies were taken from control and ischaemic areas with a Trucut Travenol needle and immediately placed and stored in liquid nitrogen until analysis. The biopsies were homogen-

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Table 1 Systemic haemodynamic values before and after a 10 min infusion of Iloprost and vehicle; during occlusion and after reperfusion

		Baseline	*Start occlusion*	*End of occlusion*	*Reperfusion*	
		− 10 min	*0 min*	*15 min*	*30 in*	*120 min*
Heart rate (min^{-1})	Iloprost	90 ± 3	93 ± 4	94 ± 4	102 ± 6[f]	99 ± 6[f]
	Solvent	84 ± 4	82 ± 4	85 ± 4	83 ± 4	84 ± 4
LV-syst.pr (mm Hg)	Iloprost	106 ± 3	97 ± 4$*	87 ± 4*	84 ± 4†	78 ± 3
	Solvent	102 ± 4	101 ± 3	90 ± 3*	86 ± 5	83 ± 5
Mean art.pr (mm Hg)	Iloprost	82 ± 3	82 ± 3$*	73 ± 3*	68 ± 4†	62 ± 4
	Solvent	85 ± 3	85 ± 3	76 ± 3*	73 ± 4	70 ± 4
Aortic flow ($l\ min^{-1}$)	Iloprost	2.5 ± 0.1	2.3 ± 0.1†	2.1 ± 0.1*	2.1 ± 0.1	2.2 ± 0.2
	Solvent	2.4 ± 0.2	2.4 ± 0.2	1.9 ± 0.1*	1.9 ± 0.2	1.7 ± 0.2
Systemic vasc. resist. ($mm\ Hg\ l^{-1}\ min^{-1}$)	Iloprost	37.8 ± 2	36.3 ± 2	35.6 ± 2	33.7 ± 3	29.6 ± 3
	Solvent	36.8 ± 3	37.3 ± 2	40.6 ± 2	41.0 ± 3	46.2 ± 6

Data shown are means ± s.e.mean.
[f] $P < 0.05$ vs solvent; [$] $P < 0.01$ vs solvent (change 0 vs − 10); * $P < 0.01$ vs previous data point; † $P < 0.05$ vs previous data point.

ized at liquid nitrogen temperatures in 4% perchloric acid. After neutralization the high energy phosphates of adenosine, adenosine 5'-triphosphate, ATP), adenosine 5'-diphosphate (ADP) and adenosine 5'-phosphate (AMP)) and creatine phosphate (CP) were measured using high-pressure liquid chromatrography as described by Harmsen *et al.* (1982). Protein content was estimated according to the method of Bradford (1976) using a Bio-Rad protein assay (Bio-Rad, München, FGR).

Experimental procedure

Baseline measurements were recorded after the preparation had been stable for at least 30 min. Two groups of animals were studied. One group of 16 animals received 100 $ng\ kg^{-1}\ min^{-1}$ Iloprost (supplied by Schering Chemicals, Ltd, UK) during the entire course of the experiment, while 12 other animals received the solvent. This dose of Iloprost was chosen because it caused an acceptable decrease in blood pressure (15–20%) and the haemodynamic changes would be comparable with other data on the compound (Coker & Parratt, 1983). Furthermore, this specific dose has proved to be effective against experimental coronary thrombosis (van der Giessen *et al.*, 1984). Ten minutes after the start of the infusions, the LAD was completely occluded distal to its first diagonal branch using a microsurgical clamp. Twenty minutes later the clamp was released and the ischaemic myocardium abruptly reperfused for two hours.

In the solvent group one animal was withdrawn from the protocol due to sustained ventricular tachyarrhythmias in the early reperfusion phase. In the Iloprost group, 6 animals did not complete the entire protocol as 4 of these had persistent ventricular fibrillation and two others were withdrawn because their mean arterial blood pressure had declined below 50 mmHg. Of the four pigs with ventricular fibrillation, in three this occurred during collection of a biopsy. This procedure may have triggered the arrhythmia. Data obtained from these animals were included until the time of withdrawal.

Statistical analysis

Student's *t* test was employed to determine whether the Iloprost-induced haemodynamic changes were statistically significant ($P < 0.05$). The same test was used to assess changes in ATP or CP. Since repeated testing occurred for the assessment of changes in SWT, only $P < 0.01$ was considered statistically significant. All data are expressed as means ± s.e.mean.

Results

Infusion of the solvent did not cause changes in cardiovascular performance (Table 1). Iloprost did not affect heart rate, but produced a 9% decrease in left ventricular systolic pressure (from 106 ± 3 to 97 ± 3 mmHg, $P < 0.01$) and mean arterial pressure (from 92 ± 3 to 82 ± 3 mmHg, $P < 0.01$), mainly due to systemic vasodilatation as cardiac output (from 2.5 ± 0.1 to 2.3 ± 0.1 $l\ min^{-1}$) only slightly decreased. Occlusion of the LAD resulted in similar decreases in blood pressure in both groups: 11 ± 1% in the Iloprost group and 10 ± 2% in the solvent group (Table 1).

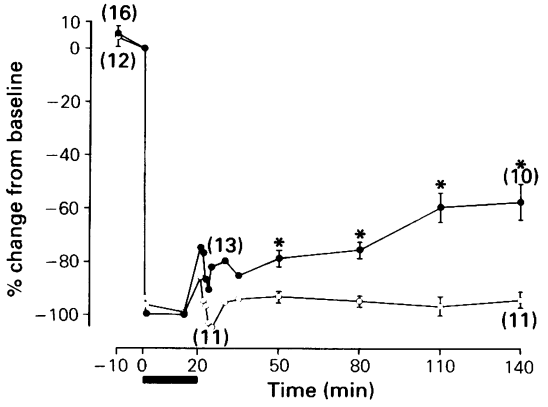

Figure 1 Recovery of systolic wall thickening after 20 min of left anterior descending coronary artery occlusion (solid bar) in (●) Iloprost (100 ng kg^{-1} min^{-1}) and (○) solvent-treated pigs. Data are expressed as percentage change from baseline (0.40 ± 0.02 for the Iloprost and 0.35 ± 0.03 for the solvent group). Each point represents the mean, and vertical lines s.e.mean, of the number of animals in parentheses. *$P < 0.01$.

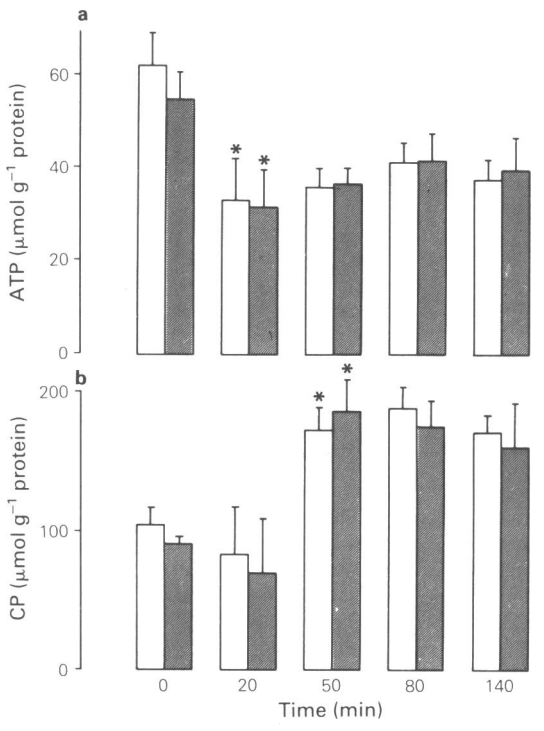

Figure 2 The amount of (a) ATP and (b) creatine phosphate (CP) in the area of the left ventricle perfused by the left anterior descending coronary artery (LAD) in Iloprost (shaded columns)- and solvent (open columns)-treated pigs. Biopsies were obtained before (0) and after (20) occlusion, and after 30 (50), 60 (80) and 120 (140) min of reperfusion. *$P < 0.02$ vs previous data point.

This decline in blood pressure was caused by a decrease in stroke volume (17 ± 3% for the solvent-group versus 10 ± 2% for the Iloprost group), while heart rate remained unchanged. After removing the obstruction, heart rate increased slightly (from 94 ± 4 to 102 ± 6 beats min^{-1}, $P < 0.05$) in the Iloprost group, while other haemodynamic parameters remained relatively similar between the groups.

Systolic wall thickening of the area perfused by the LAD (0.40 ± 0.02) was not affected by either Iloprost or the solvent (Figure 1). Immediately following LAD occlusion, SWT was completely abolished in both groups of animals. During the first two hours of reperfusion, no recovery of function of the ischaemic zone was observed in the solvent group. Immediately after reperfusion there was a slight recovery in SWT in the Iloprost-treated group which was maintained for the first hour. Subsequently, there was a further increase and after two hours of reperfusion SWT had returned to about 40% of its pre-occlusion value.

Animals not completing the protocol had a slightly lower SWT before occlusion (0.37 ± 0.02, compared to 0.42 ± 0.02 for the survivors), but shortly before the start of the fatal arrhythmia SWT in these animals was at least as good (0.11 ± 0.04 compared to 0.04 ± 0.01 in the survivors).

During occlusion ATP levels in the ischaemic area declined from 62 ± 7 to 33 ± 9 μmol g^{-1} protein and from 55 ± 6 to 32 ± 8 μmol g^{-1} protein in the vehicle- and Iloprost-treated groups, respectively (Figure 2). After reperfusion ATP recovered slightly, but did not reach pre-ischaemic values in either group (Figure 2). In the non-ischaemic tissue ATP remained constant (60 ± 5 μmol g^{-1} protein) during the experiments in both groups. The energy charge decreased slightly but not significantly during ischaemia and returned to pre-ischaemic values after reperfusion (Table 2). In the ischaemic area CP decreased during and just after occlusion from 104 ± 12 to 84 ± 33 and from 90 ± 6 to 70 ± 38 μmol g^{-1} protein in the solvent and drug-treated groups, respectively (Figure 2). After the ischaemic period CP had increased to a significantly higher level than that in the pre-ischaemic period ($P < 0.02$; Figure 2) in both groups. However, there were no significant differences in the biochemical changes between the groups.

Discussion

No recovery in regional function was observed in the solvent-treated animals during the first two hours of

Table 2 Adenylate energy charge (ATP + 0.5 ADP/ATP + ADP + AMP) in transmural biopsies obtained from the occluded segment

	Baseline	*End of occlusion*	*Reperfusion*	
	− 10 min	15 min	30 min	120 min
Treatment				
Solvent	0.85 ± 0.01	0.78 ± 0.03	0.83 ± 0.01	0.85 ± 0.01
Iloprost	0.86 ± 0.01	0.79 ± 0.02	0.85 ± 0.01	0.85 ± 0.01

Data shown are means ± s.e.mean.

reperfusion following 20 min of coronary artery occlusion. This finding is consistent with that reported by Murphy *et al.* (1982) in the same species. In the Iloprost-treated animals, regional function showed a partial recovery. The mechanism by which Iloprost improved regional function during reperfusion is unclear, although a number of factors can be eliminated. A lowered myocardial O_2 demand at the time of occlusion is an unlikely factor as the double product of heart rate x left ventricular systolic pressure was very similar for both groups. The biochemical parameters ATP, energy charge and CP were also not significantly affected by the Iloprost treatment. Our data also showed that inhibition of ATP depletion during ischaemia or an enhanced recovery during reperfusion by Iloprost was not an important factor. This mechanism of action is possibly operative in experimental hypothermic cardiac arrest in the rat (van Gilst *et al.*, 1983). The CP level overshoot and the lower level of ATP, compared to pre-ischaemic values, occurring during reperfusion is consistent with results obtained by others, as summarized by Ichihara & Abiko (1984).

Schrör *et al.* (1982) have suggested that prostaglandin I_2 may exert a beneficial action via structural preservation of myocardial membrances or adrenergic nerve endings. We cannot exclude the possibility that such a mechanism is also effective for Iloprost.

The favourable effect of Iloprost demonstrated in this study may be of value to certain patients, but the greater vulnerability of the heart to reperfusion arrhythmias, an observation previously made in the dog (Coker & Parratt, 1983), might pose a serious problem. We observed ventricular tachyarrhythmias (VT,VF) in 42% of the solvent-treated and in 56% of the Iloprost-treated animals during the first 15 min after reperfusion. Most of the ventricular fibrillations occurred during collection of the biopsies and mechanical irritation of vulnerable myocardium may have triggered those fatal arrhythmias. The higher incidence of ventricular fibrillation in the Iloprost-treated animals could indicate that the hearts of these animals were electrically more unstable, but the design of the protocol (reperfusion combined with needle biopsies) is not really suited to draw any definite conclusions.

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