Defibrotide reduces infarct size in a rabbit model of experimental myocardial ischaemia and reperfusion

¹Christoph Thiernemann, G. Roger Thomas & John R. Vane

The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ

1 Defibrotide, a single-stranded polydeoxyribonucleotide obtained from bovine lungs, has significant anti-thrombotic, pro-fibrinolytic and prostacyclin-stimulating properties.

2 The present study was designed to evaluate the effects of defibrotide on infarct size and regional myocardial blood flow in a rabbit model of myocardial ischaemia and reperfusion.

3 Defibrotide (32 mg kg^{-1} bolus + $32 \text{ mg kg}^{-1} \text{ h}^{-1}$, i.v.) either with or without co-administration of indomethacin ($5 \text{ mg kg}^{-1} \times 2$, i.v.) was administered 5 min after occlusion of the left anterior-lateral coronary artery and continued during the 60 min occlusion and subsequent 3 h reperfusion periods.

4 Defibrotide significantly attenuated the ischaemia-induced ST-segment elevation and abolished the reperfusion-related changes (R-wave reduction and Q-wave development) in the electrocardiogram. In addition, defibrotide significantly improved myocardial blood flow in normal and in ischaemic, but not in infarcted sections of the heart. The improvement in blood flow in normal perfused myocardium, but not in the ischaemic area was prevented by indomethacin.

5 Although the area at risk was similar in all animal groups studied, defibrotide treatment resulted in a 51% reduction of infarct size. Indomethacin treatment abolished the reduction of infarct size seen with defibrotide alone.

6 The data demonstrate a considerable cardioprotective effect of defibrotide in the reperfused ischaemic rabbit myocardium. This effect may be related, at least in part, to a stimulation of endogenous prostaglandin formation. Other possible mechanisms are discussed.

Introduction

Defibrotide is the sodium salt of polydeoxyribonucleotide obtained by controlled depolymerization of deoxyribonucleic acid of bovine lungs. The active fraction, corresponding to about 60 nucleotides with a defined ratio of purine and pyrimidine bases, has a molecular weight of approximately 20 kDa. However, the precise chemical structure of the compound is not yet known. Defibrotide has significant profibrinolytic (Pescador *et al.*, 1983) and plasminogen-activating properties (Mussoni *et al.*, 1979), eventually resulting in the dissolution of pre-formed arterial and venous platelet thrombi *in vivo* (Niada *et al.*, 1981; Grodzinska *et al.*, 1987). Most notably, Löbel & Schrör (1985) have demonstrated an increase of coronary vascular prostacyclin forma-

tion in the presence of platelets and defibrotide. Furthermore, in cat (Niada *et al.*, 1985; Thiernemann *et al.*, 1985) and rabbit (Berti *et al.*, 1987) models of acute myocardial ischaemia and reperfusion, defibrotide exerts considerable beneficial effects on biochemical, electrocardiographic and functional indices of ischaemia- and reperfusion-induced tissue injury. However, an improvement of biochemical indicators of ischaemic tissue injury, such as the loss of cytosolic marker enzymes from the ischaemic myocardium is not necessarily associated with a reduction of infarct size (Smith *et al.*, 1984a).

The present study was designed to evaluate the effects of defibrotide on infarct size in an open-chest rabbit model of acute myocardial ischaemia and reperfusion. A further aim was to determine if the cardioprotective effects of defibrotide are related to

¹ Author for correspondence.

an increased formation of endogenous prostaglandins and/or associated with changes in myocardial collateral blood flow.

An account of some of these results was presented to the British Pharmacological Society in Dublin, (Thiemermann *et al.*, 1988).

Methods

Animal selection and exclusion criteria

The study was carried out on male rabbits (New Zealand White SPF Rabbits, Regal, Great Bookham, U.K.) weighing 2.0 to 2.8 kg receiving a standard diet and water *ad libitum*. All data obtained from rabbits which died during the course of the experiment due to either ventricular fibrillation or cardiac failure were excluded from the study.

Surgical procedures

Ten min before surgery, all animals were premedicated with 0.1 ml kg^{-1} Hypnorm i.m. (containing 0.315 mg ml^{-1} fentanyl citrate and 10 mg ml^{-1} flunitrazepam; Janssen Pharmaceutical Ltd.). General anaesthesia was then induced with sodium pentobarbitone (30 mg kg^{-1} , i.v.; Sagatal, May & Baker) and anaesthesia was maintained with supplementary doses of sodium pentobarbitone as required. The rabbits were intubated and ventilated with air from a Harvard ventilator at a rate of 36–40 strokes per min and a tidal volume of 18–20 ml. Arterial blood gases and pH were checked periodically on a Corning 168 pH blood gas analyzer. When necessary, the ventilation rate and/or oxygen flow rate were adjusted to maintain physiological blood gas values (P_{O_2} : 90–130 mmHg, P_{CO_2} : 30–35 mmHg, and pH 7.38–7.42). Body temperature was maintained at $38 \pm 1^\circ\text{C}$ by means of a rectal probe thermometer attached to a homeothermic blanket control unit (CFP 8185). The left femoral vein was cannulated for drug administration. The right femoral artery was catheterized for phasic aortic blood pressure recordings made from a Bell & Howell 40-422-0001 pressure transducer. The left femoral artery was cannulated for the withdrawal of a reference blood sample during the microsphere injection. Another catheter was placed in the left ventricle via the right common carotid artery for measurement of left ventricular systolic pressure (LVP) and subsequent injection of microspheres and Evans blue dye solution. A 2–3 cm left intercostal thoracotomy (4th intercostal space) was performed and the heart was suspended in a temporary pericardial cradle. A snare occluder was placed around the

first antero-lateral branch of the left coronary artery (LAL) 1 cm distal from its origin. In contrast to other species, the rabbit LAL supplies most of the left ventricle and apex of the left ventricular myocardium (Flores *et al.*, 1984). Care was taken not to include any veins draining blood from this area. Upon completion of the surgical procedure, fluid loss was replaced with 3 ml of physiological saline i.v. The animals were then allowed to stabilize for 30 min before LAL ligation.

The coronary artery was occluded at time 0 by a two-stage tightening of the occluder. The first stage was a tightening of the snare-occluder until a blue hue appeared on the epicardium. One min later this procedure was followed by a complete occlusion of the coronary artery, which was associated with the typical electrocardiographic and haemodynamic changes of myocardial ischaemia. After 60 min of acute myocardial ischaemia (MI), the occluder was re-opened to allow a 3 h reperfusion period. The presence of reperfusion was confirmed by the appearance of an 'epicardial blush'.

Three experimental groups were studied: controls (vehicle, $n = 10$); defibrotide ($n = 8$) and indomethacin-defibrotide ($n = 11$).

Drug regimens

Defibrotide (Procyclide; loading dose of 32 mg kg^{-1} ; followed by a continuous infusion of $32 \text{ mg kg}^{-1} \text{ h}^{-1}$) or saline (vehicle) were infused i.v. at a rate of 0.6 ml h^{-1} starting 5 min after LAL occlusion and maintained for the remainder of the experiment. In indomethacin-defibrotide-treated animals, indomethacin (5 mg kg^{-1} ; Sigma, Poole, Dorset) was given as an i.v. bolus injection 10 min before coronary artery occlusion and 1 h after the start of reperfusion. Bult *et al.* (1980) have shown that even lower doses of indomethacin (2.5 mg kg^{-1}) are sufficient to inhibit prostacyclin formation (measured as 6-keto-PGF_{1 α} from extracted plasma samples by radioimmunoassay) in the rabbit. Even when the rabbits were subjected to endotoxin (a potent stimulus of endogenous prostacyclin formation), this low dose of indomethacin abolished the endotoxin-induced rise in plasma 6-keto-PGF_{1 α} levels (as demonstrated in control animals) for at least 3 h. In the present study, indomethacin-treated animals were subjected to the same defibrotide protocol as that described for the defibrotide alone group. Defibrotide was a generous gift of Crinos Research Laboratories, Villa Guardia, Como, Italy.

Haemodynamic measurements and electrocardiogram

Haemodynamic parameters, including heart rate (HR), systolic and diastolic pressure and left ven-

tricular systolic pressure (LVP) were continuously recorded on a 4-channel Grass 7D polygraph recorder (Quincy, Mass., U.S.A.). However, detailed data analysis was only performed at time 0 (baseline measurement), 20, 40, 60 min (occlusion period) and every hour during the subsequent reperfusion period. Arterial blood gasses were measured 10 min before and at 30 min intervals after occlusion. Lead II electrocardiograms (ECGs) were recorded from subdermal platinum electrodes on a 7P4H Grass ECG-amplifier attached to Grass 4-channel recorder (Grass, Mass., U.S.A.) R-wave and Q-wave amplitude together with ST-segment changes were calculated as absolute differences with respect to the J-point of the QRS complex (of at least 10 cardiac cycles) and expressed as delta mV. The heart rate was automatically calculated from left ventricular systolic pulse curves by means of a Grass 7P4H tachograph. The pressure-rate index, a relative indicator of myocardial oxygen consumption (Baller *et al.*, 1981) was calculated as the product of mean arterial blood pressure and heart rate.

Measurement of regional myocardial blood flow

^{113}Sn -labelled microspheres (New England Nuclear, Boston, Mass., U.S.A.) were used to determine regional myocardial blood flow (RMBF) at the end of the 3 h reperfusion period. Details of this procedure have been previously described (Heymann *et al.*, 1977) and validated for the rabbit (Maxwell *et al.*, 1987). Microspheres $15 \pm 3 \mu\text{m}$ diameter were suspended in physiological saline solution containing 0.01% Tween 80. To ensure adequate dispersion, the microsphere suspension, prewarmed to $38 \pm 1^\circ\text{C}$, was sonicated for 10 s immediately before use. The LAL was then re-occluded and 150,000 to 200,000 microspheres (0.6 ml total volume) were injected into the left ventricle over a period of 30 s. This was followed by 1 ml of physiological saline solution over 15 s. Concurrently, blood was withdrawn from the left femoral artery at a rate of 2 ml min^{-1} (Braun, Melsungen, F.R.G.) and continued for 90 s after the saline flush.

Measurements of area at risk and infarct size

Subsequent to microsphere injection, Evans blue dye solution (4 ml of 1% w/v; Sigma, Dorset) was injected into the left ventricle to distinguish between perfused and non-perfused (myocardium at risk) sections of the heart. The Evans blue solution stains the perfused myocardium while the occluded vascular bed remains uncoloured. The dose of Evans blue dye was well within the range reported for nearly exclusive binding to plasma albumin or other proteins in

the rabbit (Lindner & Heinle, 1982). The rabbits were killed with an overdose of pentobarbitone sodium. The heart was excised and left ventricular weight determined after removing the right ventricular wall, valves, papillary muscle and epicardial fat deposits. Area at risk and non-ischaemic myocardium were separated by following the line of demarcation between blue stained and unstained tissue. To distinguish between ischaemic and infarcted tissue, the area at risk was sectioned into 2–3 mm thick slices, which were incubated in 0.5 mg ml^{-1} *p*-nitroblue tetrazolium (NBT) (Sigma, Poole, Dorset) for 20 min at $37 \pm 1^\circ\text{C}$. In the presence of intact dehydrogenase enzyme systems (normal myocardium), NBT forms blue coloured precipitates, while areas of necrosis lack dehydrogenase activity and therefore fail to stain (Nachlas & Shnitka, 1963).

Regional myocardial blood flow

Non-ischaemic left ventricular myocardium, area at risk and infarcted tissue were blotted dry and placed in separate vials for weighing and isotope counting. Additionally, the reference blood sample and any radioactive material remaining in the injection syringe or cannula were counted. Furthermore, both kidneys were removed, cleared of adhering connective tissue, blotted dry, weighed and counted. Subsequently, renal flow values were used as a control for adequate microsphere dispersion and mixing. All tissue samples and blood probes were counted in a 1282 LKB gamma counter (Wallak Oy, Turku, Finland) with a $2'' \times 2''$ NaI(Tl) well type detector. Regional myocardial blood flow (RMBF) was calculated by using the reference blood flow method (Heymann *et al.*, 1977) according to the formula,

$$Q_m = \frac{Q_r \times C_m}{C_r}$$

where Q_m = myocardial blood flow (ml min^{-1}); Q_r = reference blood flow (ml min^{-1}); C_m = counts min^{-1} in myocardial sample; C_r = counts min^{-1} in reference sample. RMBF was expressed as $\text{ml min}^{-1} 100 \text{ g}^{-1}$ wet weight. Additionally, the NBT solution was checked for the presence of microspheres lost from the heart tissue as a result of the staining technique. Radioactivity in the NBT solution did not exceed background activity ($n = 3$).

Statistical comparison

All values in the text, figures and tables are expressed as the mean $\pm$ s.e. mean of n observations. Where repeated measures were taken over the course of the experimental period the results were analysed by a

Table 1 Haemodynamic data for left ventricular pressure (LVP), heart rate (HR), pressure-rate-index (PRI) in vehicle (Veh), defibrotide (Def) and indomethacin-defibrotide (Indo-Def)-treated rabbits subjected to LAL-occlusion and reperfusion

Time (min)	LVP (mmHg)			HR (beats min ⁻¹)			PRI (mmHg min ⁻¹ 10 ²)		
	Veh	Def	Indo-Def	Veh	Def	Indo-Def	Veh	Def	Indo-Def
0	99 ± 3	102 ± 5	93 ± 4	248 ± 5	254 ± 5	248 ± 7	197 ± 11	200 ± 9	196 ± 5
20	91 ± 5	91 ± 6	85 ± 4	227 ± 5	240 ± 7	239 ± 9	170 ± 11	171 ± 9	171 ± 11
40	96 ± 3	94 ± 4	89 ± 3	243 ± 5	254 ± 8	240 ± 7	186 ± 10	192 ± 6	180 ± 5
60	98 ± 3	98 ± 3	91 ± 5	241 ± 6	255 ± 7	241 ± 6	197 ± 8	199 ± 5	189 ± 8
120	99 ± 6	100 ± 3	85 ± 4*	251 ± 6	251 ± 7	238 ± 13	209 ± 18	205 ± 9	176 ± 10*
180	107 ± 6	104 ± 3	84 ± 3*	250 ± 4	254 ± 5	248 ± 7	228 ± 15	214 ± 9	177 ± 10*
240	107 ± 3	106 ± 5	85 ± 3*	250 ± 5	254 ± 4	246 ± 9	236 ± 11	220 ± 11	181 ± 9*

Values are given as mean ± s.e.mean of 5–7 observations for each group. * $P < 0.05$, when compared to vehicle at the same time-point.

two-way analysis of variance, while endpoint experiments were analysed by a one-way analysis of variance. Analyses of variance were followed by a least significance difference procedure (LSD) to determine the nature of the response (SPSS Inc., Chicago, IL, U.S.A.). A P value of less than 0.05 was considered statistically significant.

Results

Haemodynamic data

Values for LVP, HR and PRI measured during the course of the experiment are given in Table 1. Baseline (time 0) haemodynamic data were similar in all 3 animal-groups studied. Coronary artery occlusion resulted in a consistent but not significant 8–11 mmHg decrease in LVP. In comparison to the vehicle-control group, defibrotide infusion, starting 5 min after LAL ligation, did not influence LVP or HR. Furthermore, the PRI, an index of myocardial oxygen consumption (Baller *et al.*, 1981), was similar in vehicle- and defibrotide-treated groups. In the indomethacin-defibrotide-treated animal group, LVP and PRI were lower ($P < 0.05$), but HR was not significantly different from vehicle-treated animals.

Reduction of myocardial infarct size

A total of 29 rabbits were used to determine the effects of defibrotide on myocardial infarct size resulting from ligation of the LAL for 60 min followed by subsequent reperfusion. Two rabbits of the control group, 1 rabbit of the defibrotide-treated group and 6 rabbits of the indomethacin-defibrotide-treated group were excluded from the study because of ventricular fibrillation. Furthermore, one rabbit of the control group having an area at risk and infarct

size of 65 and 48% of the left ventricle, was excluded due to cardiac failure during the reperfusion period (3 h).

Area at risk expressed as a percentage of left ventricle and myocardial infarct size expressed as a percentage of the area at risk are given in Figure 1. The percentage of the left ventricle subjected to ischaemia and constituted the area at risk was similar in all 3 groups studied ($P > 0.05$). Defibrotide-treatment resulted in a 51% reduction of the amount of infarcted myocardium within the area at risk when compared with the vehicle-treated control group ($P < 0.05$, Figure 1). Furthermore, infarct expressed as a percentage of the total left ventricle (IN/LV) was similarly decreased by defibrotide-treatment: control ($n = 7$), IN/LV $26 \pm 3\%$; defibrotide IN/LV $13 \pm 2\%$; $P < 0.05$. In contrast, infarct size of the indomethacin-defibrotide-treated animal group was not different from the vehicle-treated control group (Figure 1). Thus, indomethacin treatment ablated the infarct size reduction seen with defibrotide.

Electrocardiogram alterations

In vehicle-treated control animals, LAL occlusion was associated with a rapid increase in the ST-segment amounting to 0.28 ± 0.02 mV after 60 min of permanent coronary artery ligation. Defibrotide infusion significantly attenuated this ischaemia-induced ST-segment elevation to 0.12 ± 0.01 mV at 60 min ($P < 0.05$). In comparison to control, defibrotide-indomethacin treatment did not influence the ischaemia-induced rise in the ST-segment (0.24 ± 0.02 mV at 60 min), indicating that the beneficial effects of defibrotide on ST-segment elevation were largely prevented by indomethacin (Figure 2).

Upon reperfusion, a rapid and continuous decrease of the ST-segment was observed in all animal groups studied, reaching control levels at the

Figure 1 Area at risk expressed as percentage of left ventricle (LV) (a) and infarct size expressed as percentage of area at risk (b) of vehicle- ($n = 7$, solid columns), defibrotide- ($n = 7$, hatched columns) and indomethacin-defibrotide- ($n = 5$, stippled columns) treated rabbits after 1 h of LAL-occlusion, followed by 3 h of reperfusion. Values are mean of n observations, vertical lines show s.e.mean. * $P < 0.05$, when compared to vehicle.

end of the 4 h experimental period (Figure 2). In vehicle-treated controls, reperfusion was associated with a change of the QRS-complex, namely a reduction of R-wave amplitude and the concomitant development of a pathological Q-wave. The R-wave amplitude was reduced by more than 40% from 0.64 ± 0.08 mV at 1 h to 0.36 ± 0.08 mV at 4 h ($P < 0.05$; Table 2), while the Q-wave magnitude increased from 0.02 ± 0.01 mV (1 h) to 0.25 ± 0.05 mV at the end of the reperfusion period ($P < 0.05$; Figure 2). These reperfusion-related

Figure 2 Changes in ST-segment (a) and Q-wave amplitude (b) in vehicle- (■), defibrotide- (●) and indomethacin-defibrotide- (▲) treated rabbits, subjected to occlusion (hatched bar) and reperfusion (open bar) of the LAL-coronary artery. Values are mean of 5–7 observations, vertical lines show s.e.mean. * $P < 0.05$, when compared to vehicle at the same time point.

changes of the QRS-complex were abolished by defibrotide-infusion. In comparison to vehicle-treated controls, indomethacin-defibrotide treatment did not affect the loss in R-wave amplitude, but partly reduced the development of a pathological Q-wave during reperfusion ($P < 0.05$). Thus, indomethacin reversed the beneficial effects of defibrotide on ST-segment elevation, R-wave and partially on Q-wave development.

Microsphere studies

The data on changes in regional myocardial blood flow are given in Figure 3. In vehicle-treated controls, coronary artery occlusion was associated with a reduction of RMBF from 214 ± 34 ml min⁻¹ 100 g⁻¹ (in the non-ischaemic myocardium) to 35 ± 6 ml min⁻¹ 100 g⁻¹ in the ischaemic and 7 ± 2 ml min⁻¹ 100 g⁻¹ in the infarcted sections of the area at risk. In comparison, defibrotide treatment

Table 2 Changes of R-wave amplitude after LAL-occlusion and reperfusion in vehicle-(Veh), defibrotide-(Def) and indomethacin-defibrotide-(Indo-Def) treated rabbits

Time (min)	R-wave-amplitude (mV)		
	Veh	Def	Indo-Def
0 (CO)	0.54 ± 0.07	0.47 ± 0.05	0.59 ± 0.08
20 (CO)	0.74 ± 0.12	0.68 ± 0.05	0.82 ± 0.06
40 (CO)	0.69 ± 0.12	0.58 ± 0.04	0.71 ± 0.05
60 (CO)	0.64 ± 0.08	0.57 ± 0.03	0.61 ± 0.07
120 (REP)	0.40 ± 0.08	0.56 ± 0.02*	0.44 ± 0.06
180 (REP)	0.41 ± 0.07	0.56 ± 0.03*	0.37 ± 0.07
240 (REP)	0.36 ± 0.08	0.53 ± 0.03*	0.38 ± 0.07

CO: LAL-occlusion period; REP: LAL-reperfusion period

Values are expressed as mean ± s.e.mean of 5–7 observations for each group. Statistical significance from vehicle-controls was determined by two-way analysis of variance followed by a least square difference analysis. * $P < 0.05$ when compared with vehicle at the same timepoint.

significantly increased RMBF to the non-ischaemic myocardium by 64% and to the ischaemic part of the area at risk by 300% ($P < 0.05$). However, RMBF to the actual infarcted myocardium was not affected by defibrotide treatment ($7 \pm 2 \text{ ml min}^{-1} 100 \text{ g}^{-1}$). Indomethacin abolished the defibrotide-induced increase in RMBF in the normal perfused myocardium, but did not affect the defibrotide-induced increase in RMBF in the ischaemic sections of the area at risk. Furthermore, indomethacin-defibrotide treatment did not influence blood flow in the infarcted myocardium, when compared to either vehicle or defibrotide-treated animal groups.

Figure 3 Regional myocardial blood flow (RMBF) distribution in non-ischaemic (NMI), ischaemic (MI) and infarcted (IN) sections of the myocardium after coronary artery occlusion in rabbits treated with vehicle (solid columns), defibrotide (hatched columns) or indomethacin-defibrotide (stippled columns). Values are mean of 5–7 observations, vertical lines show s.e.mean * $P < 0.05$, when compared to vehicle.

Discussion

Defibrotide reduced infarct size in an occlusion-reperfusion model of myocardial ischaemia in the rabbit. Other indications of the anti-ischaemic effect of defibrotide were attenuation of ST-segment elevation, a widely accepted indicator of ischaemic injury (Kjekshus *et al.*, 1972; Ross, 1976) and abolition of the pathological changes in the R- and Q-waves during reperfusion. Such changes in the QRS-complex have also been related to myocardial tissue injury (Hinohara *et al.*, 1984).

The mechanism by which defibrotide protects the ischaemic reperfused myocardium is still largely unknown. Maroko *et al.* (1971) suggested that a reduction in blood pressure would protect the ischaemic heart by reducing oxygen demand. However, defibrotide did not affect left ventricular pressure, mean arterial blood pressure or heart rate. Additionally, defibrotide did not alter the pressure-rate-index, so that a reduction in myocardial oxygen consumption is unlikely to play a role in the mechanism of action of the compound.

Alternatively, an improvement of myocardial oxygen supply, i.e. by enhancement of collateral perfusion, should also protect against ischaemia (Maroko *et al.*, 1971). Our results clearly show an improvement in blood flow to the ischaemic myocardium in defibrotide treated rabbits, which was associated with a reduction in infarct size. However, blood flow to the infarcted area was not improved, but this was to be expected because blood flow was greatly reduced (by 96%), as also found by Maxwell *et al.* (1987) and Chiariello *et al.* (1988) in the rabbit.

What mechanisms, then, can account for the limitation in infarct size and the increase in myocardial blood flow seen with defibrotide? Niada *et al.* (1981) have suggested that an increase of prostacyclin formation contributes to the anti-ischaemic effects of defibrotide. Interestingly, Löbel & Schrör (1985) have shown that defibrotide released prostacyclin in the isolated, platelet-perfused guinea-pig heart in sufficient quantities to cause an increase in platelet cyclic AMP as well as a reduction in coronary perfusion pressure. Surprisingly, in our experiments, defibrotide increased blood flow to the normal myocardium and this effect was abolished by indomethacin. Thus, a cyclo-oxygenase product, possibly prostacyclin, may well bring about this effect. Also in agreement with a prostacyclin-dependent mechanism is our result in which indomethacin abrogated the beneficial effects of defibrotide on infarct size, on ST-segment elevation and partly on Q-wave development. In support of a prostacyclin-dependent mechanism, Berti *et al.* (1987) showed that the ability of defibrotide to release prostacyclin and to prevent ischaemia-induced myocardial contracture in the

ischaemic rabbit heart *in vitro* were both completely abolished by indomethacin.

Indomethacin pretreatment appears to be associated with a higher incidence of fatal ventricular fibrillation. An aggravation of ischaemic myocardial tissue injury by indomethacin has been shown in experimental myocardial ischaemia in dogs (Kirmser *et al.*, 1976; Jugdutt *et al.*, 1979) and rabbits (Berti *et al.*, 1988) as well as in patients suffering from ischaemic heart disease (Friedman *et al.*, 1981). Berti *et al.* (1988) concluded that the deleterious effects of indomethacin in ischaemic Langendorff-hearts of the rabbit were related to the ablation of vasodilator prostaglandins, such as prostacyclin.

The one result which is difficult to attribute to a cyclo-oxygenase dependent mechanism is the improvement in blood flow brought about by defibrotide in the ischaemic border zone, for this was insensitive to indomethacin. This result is in accord with the lack of effect of prostacyclin in improving collateral blood flow in the infarcted centre and in the ischaemic border zone of the occluded vascular bed as demonstrated in cat (Smith *et al.*, 1984a), dog (Simpson *et al.*, 1987) and rabbit (Chiariello *et al.*, 1988) hearts *in vivo*.

The improvement in blood flow brought about by defibrotide in the ischaemic border zone could also be related to an effect of the compound on the microvasculature rather than a direct action on collateral blood supply. Reperfusion of the ischaemic microvasculature is often associated with a 'no-reflow' phenomenon (Kloner *et al.*, 1974) mainly due to plugging of the capillaries with neutrophils (Engler *et al.*, 1983) and/or platelets (Vic-Mo, 1978). Since defibrotide prevents platelet aggregation *in vivo* (Thiemermann *et al.*, 1985) and inhibits neutrophil activation *in vitro* (Di Perri *et al.*, 1987), defibrotide may well interfere with some of the initial events leading to an impaired perfusion of the microvasculature.

References

- ARAKI, H. & LEFER, A.M. (1980). Cytoprotective actions of prostacyclin during hypoxia in the isolated perfused cat liver. *Am. J. Physiol.*, **238**, 176-181.
- BALLER, D., BRETSCHNEIDER, H.J. & HELIGE, G. (1981). A critical look at currently used indices of myocardial oxygen consumption. *Basic Res. Cardiol.*, **76**, 163-181.
- BERTI, F., ROSSINI, G., OMINI, C., FOLCO, G., DAFIONCHI, L., VIGANO, T. & TONDO, C. (1987). Defibrotide, an anti-thrombotic substance which prevents myocardial contracture in ischaemic rabbit heart. *Eur. J. Pharmacol.*, **135**, 375-382.
- BERTI, F., ROSSINI, G., MAGNI, F., CARUSO, D., OMINI, C., PUGLISI, L., & GALLI, G. (1988). Nonsteroidal anti-inflammatory drugs aggravate acute myocardial ischaemia in the perfused rabbit heart: A role for prostacyclin. *J. Cardiovasc. Pharmacol.*, **12**, 438-444.
- BULT, H., BEETENS, J., HERMAN, A.G. (1980). Blood levels of 6-oxo-prostaglandin F₁ during endotoxin-induced hypotension in rabbits. *Eur. J. Pharmacol.*, **63**, 47-56.
- CHIARIELLO, M., GOLLINO, P., CAPELLI-BIGAZZI, M., AMBROSIO, G., TRITTO, I. & SALVATORE, M. (1988). Reduction of infarct size by the prostacyclin analogue iloprost (ZK 36374) after experimental coronary artery occlusion-reperfusion. *Am. Heart J.*, **115**, 499-504.
- DEMBINSKA-KIEĆ, A., KOSTKA TRABKA, E. & GRYGLEWSKI, R.J. (1982). Effect of prostacyclin on fibrinolytic activity in patients with arteriosclerosis obliterans. *Thromb. Haemost.*, **47**, 190.
- DI PERRI, T., LAGHI PASINI, F., CAPECCHI, P.L., CECCATELLI, L., PASQUI, A.L. & ORRICO, A. (1987). Defibrotide *in vitro* inhibits neutrophil activation by a calcium-involving mechanism. *Int. J. Tiss. React.*, **9**, 399-406.
- ENGLER, R.L., SCHMID-SCHONBEIN, R.W. & PAYCLEC, C.T. is a recipient of a research grant from the Deutsche Forschungsgemeinschaft (DFG), Bonn, F.R.G. The William Harvey Research Institute is supported by a grant from Glaxo Group Research Limited.

- R.S. (1983). Leukocyte capillary plugging in myocardial ischemia and reperfusion in the dog. *Am. J. Pathol.*, **111**, 98–111.
- FLORES, N.A., DAVIES, R. L., PENNY, W.J. & SHERIDAN, D.J. (1984). Coronary microangiography in the guinea-pig, rabbit and ferret. *Int. J. Cardiol.*, **6**, 459–471.
- FRIEDMAN, P.L., BROWN, E.J., GUNTHER, S., ALEXANDER, R.W., BARRY, W.H., MUDGE, G.H. & GROSSMAN, W. (1981). Coronary vasoconstrictor effect of indomethacin in patients with coronary artery disease. *N. Eng. J. Med.*, **305**, 1171–1172.
- GRODZINSKA, L., KÖNIG, E. & SCHRÖR, K. (1987). Defibrotide is equipotent in stimulating arterial and venous thrombolysis. *Pharmac. Res. Comm.*, **19**, 609–615.
- HEYMANN, M.A., PAYNE, B.D., HOFFMANN, J.I.E. & RUDOLPH, A.M. (1977). Blood flow measurements with nucleotide-labelled particles. *Prog. Cardiovasc. Dis.*, **20**, 55–79.
- HINOHARA, T., HINDMAN, N.B., WHITE, R.D., IDEKER, R.E. & WAGNER, G.S. (1984). Quantitative QRS-criteria for diagnosing and sizing myocardial infarcts. *Am. J. Cardiol.*, **53**, 875–878.
- JUGDUTT, B.I., HUTCHINS, G.M., BULKLEY, B.H., PITT, B., BECKER, L.C. (1979). Effect of indomethacin on collateral blood flow and infarct size in the conscious dog. *Circulation*, **4**, 734–743.
- KIRMSE, R., BERGER, H.J., COHEN, L.S., WOLFSON, S. (1976). Effect of indomethacin, a prostaglandin inhibitor, on epicardial ST-segment elevation and myocardial blood flow after coronary artery occlusion. *Circulation*, **54**, II-194.
- KJEKSHUS, J.K., MAROKO, P.R. & SOBEL, B.E. (1972). Distribution of myocardial injury and its relation to epicardial ST-segment changes after coronary artery occlusions in dogs. *Cardiovasc. Res.*, **6**, 490–499.
- KLONER, R.A., GANOTE, C.E. & JENNINGS, R.B. (1974). The "No-reflow" phenomenon after temporary coronary artery occlusion in the dog. *J. Clin. Invest.*, **54**, 1496–1508.
- LINDNER, V. & HEINLE, H. (1982). Binding properties of circulating Evans blue dye in rabbits as determined by disc electrophoresis. *Atherosclerosis*, **43**, 417–422.
- LÖBEL, P. & SCHRÖR, K. (1985). Selective stimulation of coronary vascular PGI₂ but not of thromboxane formation by defibrotide in the platelet perfused heart. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **331**, 265–275.
- MAROKO, P.R., KJEKSHUS, J.K., SOBEL, B.E., WATANABE, T., COVELL, J.W., ROSE, J. & BRAUNWALD, E. (1971). Factors influencing infarct size following experimental coronary artery occlusions. *Circulation*, **43**, 67–82.
- MAXWELL, M.P., HEARSE, D.J. & YELLON, D.M. (1987). Species variation in the coronary collateral circulation during regional myocardial ischaemia: a critical determinant of the rate of evolution and extent of myocardial infarction. *Cardiovasc. Res.*, **21**, 737–746.
- MULLANE, K.M., READ, N., SALMON, J.A., MONCADA, S. (1984). Role of leukocytes in acute myocardial infarction in anaesthetized dogs: Relation to myocardial salvage by anti-inflammatory drugs. *J. Pharmacol. Exp. Ther.*, **228**, 510–522.
- MUSSONI, L., EVOLVI, C. & DONATI, M.B. (1979). Activation of plasma and vascular fibrinolytic activity by a polydeoxyribonucleotide substance, fraction P, in rats and rabbits. *Thromb. Haemost.*, **42**, 388.
- NACHLAS, M.M. & SHNITKA, T.K. (1963). Macroscopic identification of early myocardial infarct by alterations in dehydrogenase system. *Am. J. Pathol.*, **43**, 379–405.
- NIADA, R., MANTOVANI, M., PRINO, G., PESCADOR, R., BERTI, F., FOLCO, G.C. & OMINI, C. (1981). Anti-thrombotic activity of a polydeoxyribonucleotide substance extracted from mammalian organs: a possible link with prostacyclin. *Thromb. Res.*, **23**, 233–246.
- NIADA, R., PORTA, R., PESCADOR, R., MANTOVANI, M. & PRINO, G. (1985). Cardioprotective effects of defibrotide in acute myocardial ischaemia in the cat. *Thromb. Res.*, **38**, 71–81.
- OGLETREE, M.L., LEFER, A.M., SMITH, J.B. & NICOLAOU, K.C. (1979). Studies on the effect of prostacyclin in acute myocardial ischaemia. *Eur. J. Pharmacol.*, **56**, 95–103.
- PESCADOR, R., MANTOVANI, M., PRINO, G. & MADONNA, M. (1983). Pharmacokinetics of defibrotide and its fibrinolytic activity in the rabbit. *Thromb. Res.*, **30**, 1–11.
- ROBERT, A. (1984). On the mechanism of cytoprotection by prostaglandins. *Ann. Clin. Res.*, **16**, 335–338.
- ROMSON, J.L., HOOK, B.G., KUNKEL, S.L., ABRAMS, G.D., SCHORK, M.A. & LUCCHESI, B.R. (1983). Reduction of the extent of ischaemic myocardial injury by neutrophil depletion in the dog. *Circulation*, **67**, 1016–1023.
- ROSS, J. (1976). Electrocardiographic characterisation of myocardial ischaemic injury and infarction. *Circulation*, **53**, 73–86.
- SCHRÖR, K., OHLENDORF, R. & DARIUS, H. (1981). Beneficial effects of a new carbacyclin derivative, ZK 36374, in acute myocardial ischaemia. *J. Pharmacol. Exp. Ther.*, **219**, 243–249.
- SIMPSON, P.J., MITSOS, S.E., VENTURA, A., GALLAGHER, K.P., FANTONE, J.C., ABRAMS, G.D., SCHORK, M.A. & LUCCHESI, B.R. (1987). Prostacyclin protects the ischaemic reperfused myocardium in the dog by inhibition of neutrophil activation. *Am. Heart J.*, **113**, 129–137.
- SMITH III, E.F., GALLENKÄMPER, W., BECKMANN, R., THOMSEN, T., MANNESMANN, G. & SCHRÖR, K. (1984a). Early and late administration of a PGI₂-analogue, ZK 36374 (iloprost): Effects on myocardial preservation, collateral blood flow and infarct size. *Cardiovasc. Res.*, **18**, 163–173.
- SMITH III, E.F., KLOSTER, G., STÖCKLIN, G. & SCHRÖR, K. (1984b). Effect of iloprost (ZK 36 374) on membrane integrity in ischaemic rabbit hearts. *Biomed. Biochem. Acta*, **43**, S155–S158.
- THIEMERMANN, C., LÖBEL, P. & SCHRÖR, K. (1985). Usefulness of defibrotide in protecting ischaemic myocardium from early reperfusion damage. *Am. J. Cardiol.*, **56**, 978–982.
- THIEMERMANN, C., THOMAS, G.R., WALDER, C. & VANE, J.R. (1988). Defibrotide reduces infarct size in a rabbit model of acute myocardial ischaemia and reperfusion. *Br. J. Pharmacol.*, **95**, 497P.
- UTSUNOMIYA, T., KRANSZ, M.M., VALERI, G.R., SHEPRO, D. & HECHTMAN, H.B. (1980). Treatment of pulmonary embolism with prostacyclin. *Surgery*, **88**, 25–30.
- VIK-MO, H. (1978). Platelet accumulation in the myocardium during non-thrombotic coronary artery occlusion in dogs. *Scand. J. Haematol.*, **21**, 225–232.

(Received September 26, 1988

Revised January 30, 1989

Accepted February 15, 1989)