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Early captopril prevents myocardial infarction-induced hypertrophy but not angiogenesis

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Abstract

Delayed captopril, started after the healing phase of myocardial infarction, improves perfusion by reducing tissue weight without affecting the vascular capacity of the heart. Early captopril, during the healing phase, prevents reactive hypertrophy, but the effects on angiogenesis are unknown. Therefore, the effects of early captopril (2 g/l drinking water, from 1 day until 3 weeks after myocardial infarction) on regional coronary flow related to tissue mass, were studied in isolated perfused hearts from rats, subjected to coronary artery ligation. Regional maximal vascular capacity was measured during nitroprusside-induced vasodilation, using radioactive microspheres. Maximal vascular capacity was not changed by captopril. Reactive hypertrophy in infarcted hearts only reached statistical significance in the left ventricular free wall. Since captopril prevented hypertrophy but did not affect regional capacity, peak tissue perfusion was improved. Indicating effects on metabolism, captopril restored the increased lactate/purine ratio in infarcted hearts. Thus, early captopril treatment prevented post-myocardial infarction hypertrophy but did not suppress angiogenesis, thus beneficially influencing the vascularization/tissue mass ratio, probably reflected by preservation of aerobic metabolism.

Keywords: Myocardial infarction; Hypertrophy; Captopril; Coronary flow; Cardiac metabolism

1. Introduction

Post myocardial infarction treatment with angiotensin I converting enzyme inhibitors has been shown to decrease reactive hypertrophy and attenuate progressive ventricular dilatation in experimental animals as well as in patients (Sharpe et al., 1990; Pfeffer, 1991; Pfeffer et al., 1992; Galcera-Tomas et al., 1993; Ray et al., 1993; Jugdutt, 1995). Moreover, captopril treatment has been proved to reduce morbidity and mortality (Pfeffer et al., 1987; Rutherford et al., 1994; ISIS-4, 1995).

We have previously reported that delayed captopril treatment (started after completion of scar formation, at 3 weeks) in the rat myocardial infarction model restored cardiac function (Schoemaker et al., 1991). This beneficial effect of captopril could be due to its ability to induce regression of myocardial hypertrophy without affecting flow capacity of the coronary vascular bed, consequently improving cardiac perfusion and aerobic metabolism (Kalkman et al., 1996b). On the other hand, early treatment with captopril (started 1 day after infarction) failed to improve pump capacity of the heart (Gay, 1990; Schoemaker et al., 1991). Since in addition to hypertrophy and remodelling, angiotensin II is an important growth factor in angiogenesis (Le Noble et al., 1993; Munzenmaier and Greene, 1996), we hypothesized that the failure of early captopril treatment to improve cardiac function in infarcted rats is caused by the prevention of reactive hypertrophy as well as the adaptive vascular growth. In order to test this hypothesis, regional cardiac mass and regional capacity of the coronary vasculature were studied in isolated perfused hearts from rats that had been treated with captopril from 1 day to 3 weeks after myocardial infarction. To evaluate the consequences, cardiac metabolism was studied in a separate group of rats, and to support functional findings, the structure of resistance arteries was examined by light microscopy in viable myocardium as well as scar tissue of infarcted hearts.

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2. Materials and methods

Male Wistar rats (270–320 g, Harlan Zeist, The Netherlands) were used in this study. The rats were housed under a 12-h light/dark cycle with standard rat chow and water available ad libitum. Seventy four animals were subjected to either coronary artery ligation ($n = 54$) or sham operation ($n = 20$). Infarcted rats were randomised to receive either normal drinking water ($n = 25$) or captopril ($n = 29$) treatment (2 g/l of drinking water; (Pfeffer et al., 1987; Gay, 1990; Kalkman et al., 1996b; Schoemaker et al., 1996). Captopril was a generous gift of Squibb, Princeton, NJ, USA. Captopril treatment was started 24 h after infarction and captopril-containing drinking water was supplied continuously until the end of the experiment at 3 weeks after surgery. Since previous studies had shown no effects of long-term captopril treatment on hypertrophy or remodelling parameters in sham rats (Van Krimpen et al., 1991), we only studied effects of treatment on rats with myocardial infarction. The experiments were approved by the University Ethics Committee for the use of experimental animals.

2.1. Coronary artery ligation

Under pentobarbital (60 mg/kg, i.p.) anaesthesia, the left anterior descending coronary artery was ligated as described in detail elsewhere (Fishbein et al., 1978; Schoemaker et al., 1991). Briefly, after the trachea was intubated, an incision was made in the skin overlying the 4th intercostal space. The overlying muscles were separated and kept aside. The animals were put on positive pressure ventilation (frequency 65/min, tidal volume 3 ml), and the thoracic cavity was opened by cutting the intercostal muscles. The heart was left in situ and a 6-0 silk suture was looped under the left coronary artery near the origin of the pulmonary artery. The suture was tied except in the sham operation. The ribs were pulled together with 3-0 silk. Subsequently, the muscles were returned to their normal position, and the skin was sutured. Post-myocardial infarction mortality is approximately 30%, and occurs mainly in the first 24 h.

This procedure leads to a major and transmural infarction in the left ventricular free wall, resulting in heart failure, as indicated by a significantly reduced cardiac output and stroke volume (Schoemaker et al., 1991, 1998). The anatomy of the rat coronary vasculature is such that accidental occlusion of only a side branch leads to a substantially smaller infarct (< 20%). Data from rats with these small infarctions were excluded from the analysis, since small infarctions are known to be hemodynamically fully compensated (Schoemaker et al., 1991).

2.2. Maximal coronary flow and regional distribution

Under pentobarbital anaesthesia, the heart was rapidly excised and mounted for perfusion with an oxygenated Krebs Henseleit buffer (composition in mmol/l: NaCl 125, KCl 4.7, $CaCl_2$ 1.35, $NaHCO_3$ 20, NaH_2PO_4 0.4, $MgCl_2$ 1.0, D-glucose 10; pH = 7.4; 37°C) at a constant pressure of 85 mm Hg, using the Langendorff technique. Heart rate was kept constant at 350 beats/min by ventricular pacing (4 V, 2 ms) with a Grass stimulator (Grass Medical Instruments, Quincy, MA, USA). A water-filled, latex balloon was inserted into the left ventricle via the left atrium, and connected to a pressure transducer (Viggo-Spectramed, Oxnard, USA). The pressure signal was fed into a 68B09-based pre-processor and microcomputer for on-line recording of left ventricular pressure, its first derivative (dP/dt) and heart rate. Left ventricular end-diastolic pressure was set to 5 mm Hg for all hearts by adjusting the balloon volume. Coronary flow was measured by an in-line flow-probe (Transonic Systems, Ithaca, NY, USA) placed in the tubing to monitor the flow of buffer passing through the probe just before the buffer entered the coronary arteries. The distribution of coronary flow was determined with 15 ± 1 (S.D.) μm diameter microspheres labelled with either ^{113}Sn, ^{95}Nb, ^{103}Ru or ^{46}Sc (NEN Dupont, Boston, USA). After a stabilisation period of 20 min, microspheres were injected to obtain baseline values for regional coronary flow. Subsequently, maximal coronary flow was determined using a 0.1 ml bolus injection of a 10^{-2} mol/l sodium nitroprusside solution (Dijkzigt University Hospital pharmacy, Rotterdam, The Netherlands). The dose of sodium nitroprusside was based upon complete dose–response curves obtained in pilot experiments. Microspheres were injected when maximal coronary flow was reached. For each measurement a suspension of 0.1 ml containing about 8000 microspheres, labelled with one of the isotopes, was mixed and injected into the perfusing buffer just before it entered the coronary arteries. In pilot experiments, coronary flow after injection of about 25,000 microspheres (10.5 ± 2.4 ml/min, $n = 5$), did not differ from the baseline coronary flow (11.4 ± 2.7 ml/min, $n = 5$). Coronary effluent was collected during the first minutes after injection of the microspheres in order to quantify microspheres by-passing the capillary bed through leakage or arteriovenous anastomotic shunting. After the experiment, the ventricles were separated from the atria and large vessels and subsequently divided into right ventricle, interventricular septum and left ventricle free wall. Left ventricular free walls of infarcted hearts were further divided into viable (= red) tissue and scar (= white) tissue, based on macroscopic appearance (Kalkman et al., 1996a). The demarkation between infarcted and viable tissue was clear since the transmural infarcted area is completely replaced by scar tissue. Tissues were weighed and their radioactivity was counted for 10 min in a γ-scintillation counter (Packard, Miniaxi autogamma 5000), using suitable windows for discriminating the different isotopes. All data were processed with specially designed computer programs (Saxena et al., 1980). Coronary flow was expressed both as absolute values in milliliter/minute, used as an index of the flow capacity of

the coronary vascular bed, and values corrected for tissue weight (mainly myocytes), representing cardiac perfusion.

2.3. Cardiac metabolism

In order to evaluate the metabolic consequences of improved perfusion, cardiac metabolism was studied from the release of purines and lactate into the coronary effluent in a separate group of rats. After stabilisation, coronary effluent was sampled and stored at −80°C until assayed for purines and lactate. The release of purines into the coronary effluent, calculated as concentration times flow per heart weight, was used to evaluate the loss of ATP catabolites from the myocytes (Achterberg et al., 1984). The loss of ATP catabolites from the heart correlates well with myocardial ATP breakdown, as measured with [^{31}P]-nuclear magnetic resonance (Harmsen and Seymour, 1988). The concentration of purines was determined as described in detail by Smolenski et al. (1990). Briefly, the ATP catabolites, uric acid, adenosine, inosine, hypoxanthine, and xanthine were measured by high-performance liquid chromatography on a C_{18}-μBondapak column (Millipore Waters, Milford, MA, USA). Coronary effluent (100 μl) was injected directly into the system, eluted with a 15% (v/v) solution of acetonitrile in 150 mM potassium dihydrogen orthophosphate, containing 150 mM potassium chloride adjusted to pH 6.0 with potassium hydroxide. Peaks were monitored by absorption at 254 nm. Although analyzed separately, the catabolites were grouped together as purines and, since measured in the coronary effluent, can be regarded as lost for the heart to re-form ATP.

The release of lactate into the coronary effluent in relation with purine release was used as an indicator of anaerobic ATP formation through glycolysis in the cardiomyocyte (Vrobel et al., 1982). Lactate concentration in coronary effluent was determined as described in detail by Marbach and Weil (1967) (reagents, Sigma Diagnostics, Deisenhofen, Germany). Briefly, lactic acid was converted by lactate oxidase to pyruvate and H_2O_2. In the presence of the H_2O_2 formed, peroxidase catalyzed the oxidative condensation of chromogen precursors to produce a coloured dye with an absorption maximum at 540 nm. Lactate concentration could then be determined, being directly proportional to the increase of absorption at 540 nm.

2.4. Histological analysis of resistance arteries

Hearts were fixed by perfusion with 3.6% phosphate-buffered formaldehyde. The ventricles were cut into four slices from apex to base, after removal of atria and large vessels. The slices were dehydrated and paraffin embedded. Deparaffinized 5 μm thick sections were incubated for 90 min with a resorcin–fuchsin solution at 60°C, and subsequently for 2 min with a Van Gieson solution, flushed with alcohol, dehydrated, and mounted with Entellan (Merck, Darmstadt, Germany). Resistance sized arteries (< 250 μm) were studied in scar tissue as well as in the spared left ventricular free wall and septum of infarcted hearts; the comparison between vessels in sham and infarcted hearts has been extensively studied in our previous investigations (Kalkman et al., 1996a, 1997). Vascular wall thickness was indexed by the ratio of wall to lumen area in at least 20 vessels in each heart. To avoid measurements of components of the venous side of the coronary vascular bed, only vessels with an internal elastic membrane were examined (Simionescu and Simionescu, 1988).

2.5. Data analysis

Data are expressed as group means 'S.E.M.', unless indicated otherwise. One rat of the non-treated infarcted group was excluded because of too small infarction. The data were analyzed using one-way analysis of variance (ANOVA), followed by post-hoc analysis according to Bonferroni (Wallenstein et al., 1980). Differences in structural parameters of vessels in captopril-treated and untreated infarcted hearts were analyzed with Student's *t*-test for independent groups. Differences were considered statistically significant if $P < 0.05$.

3. Results

3.1. Regional tissue perfusion

MI hearts weighed significantly more than sham-operated control hearts. Since body weight did not differ

Table 1
Body weight and regional myocardial tissue weight

	SHAM	MI	MI + CAP
n	16	16	20
BW (g)	368 ± 5	359 ± 4	334 ± 4[ab]
Total ventricular			
Weight (mg)	1071 ± 21	1232 ± 56[a]	1033 ± 41[b]
Weight/BW (mg/g)	2.92 ± 0.07	3.45 ± 0.18[a]	3.11 ± 0.14
n	9	9	13
LV free wall			
Weight (mg)	572 ± 18	638 ± 17[a]	516 ± 14[ab]
Weight/BW (mg/g)	1.54 ± 0.04	1.74 ± 0.05	1.52 ± 0.04[b]
Interventricular septum			
Weight (mg)	237 ± 20	257 ± 17	209 ± 10[b]
Weight/BW (mg/g)	0.64 ± 0.05	0.70 ± 0.05	0.62 ± 0.03
Right ventricle			
Weight (mg)	220 ± 6	253 ± 28	190 ± 8[b]
Weight/BW (mg/g)	0.60 ± 0.02	0.69 ± 0.08	0.56 ± 0.02

SHAM, hearts from sham-operated rats; MI, myocardial infarction; CAP, captopril; BW, body weight; LV free wall, left ventricular free wall (including scar tissue in infarcted hearts).
In the upper part of the table, data from metabolic studies and regional flow studies are combined. In the lower part of the table, only data from the regional flow studies (in which regional tissue weight was determined) are presented.
[a] $P < 0.05$ vs. sham values; [b] $P < 0.05$ vs. values for untreated infarcted hearts.

Table 2
Distribution of coronary flow (ml/min) and local tissue perfusion (ml/min per g)

	Tissue flow			Tissue perfusion		
	SHAM	MI	MI + CAP	SHAM	MI	MI + CAP
Baseline						
Viable LV free wall	6.4 ± 0.5	4.5 ± 0.3	5.1 ± 0.3	11.1 ± 0.6	9.8 ± 0.8	12.2 ± 0.7[a]
Scar tissue	–	0.7 ± 0.1	0.6 ± 0.1	–	4.3 ± 0.5	5.9 ± 0.7
Interventricular septum	2.9 ± 0.3	2.9 ± 0.2	2.8 ± 0.2	12.4 ± 0.7	11.4 ± 0.9	13.4 ± 0.7
Right ventricle	1.8 ± 0.1	2.3 ± 0.2	2.1 ± 0.2	8.2 ± 0.5	9.4 ± 0.8	11.3 ± 1.0
Atria and large vessels	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	1.1 ± 0.4	1.5 ± 0.4	1.9 ± 0.4
By-pass flow	0.6 ± 0.4	0.4 ± 0.2	0.4 ± 0.1			
Nitroprusside						
Viable LV free wall	11.1 ± 0.7	7.5 ± 0.5	9.0 ± 0.7	19.3 ± 0.9	16.9 ± 1.1	21.4 ± 1.0[a]
Scar tissue	–	1.3 ± 0.3	1.0 ± 0.2	–	6.6 ± 1.2	9.7 ± 1.3
Interventricular septum	5.3 ± 0.4	5.3 ± 0.3	5.8 ± 0.5	22.8 ± 1.7	20.5 ± 1.6	27.3 ± 1.7[a]
Right ventricle	4.4 ± 0.2	5.8 ± 0.6	4.4 ± 0.5	19.9 ± 1.0	22.2 ± 1.3	22.8 ± 2.0
Atria and large vessels	0.8 ± 0.2	1.0 ± 0.2	1.3 ± 0.5	2.5 ± 0.7	2.8 ± 0.4	4.9 ± 1.5
By-pass flow	0.6 ± 0.3	0.7 ± 0.2	0.6 ± 0.1			

Distribution of coronary flow and local tissue perfusion at baseline, and during nitroprusside-induced maximal vasodilation over the different regions of hearts from sham-operated rats (SHAM), untreated infarcted rats (MI), and infarcted rats with captopril treatment (MI + CAP).
[a] $P < 0.05$ vs. untreated infarcted hearts.

between myocardial infarction and sham-operated rats, the heart weight to body weight ratio was also increased (Table 1). Locally, only the tissue weight of the left ventricular free wall was significantly increased in in-

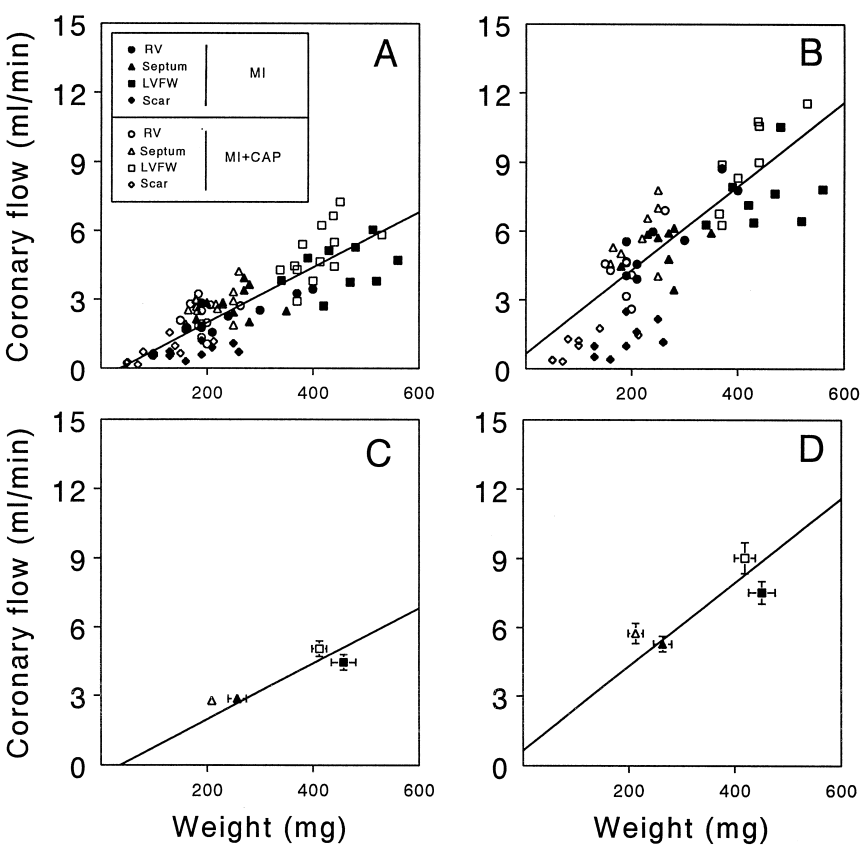

Fig. 1. Relationship between regional coronary flow and tissue weight of different parts of hearts from untreated infarcted rats (black symbols) and captopril-treated infarcted rats (MI + CAP, open symbols). Panel A: During baseline perfusion. Panel B: During nitroprusside-induced maximal vasodilation. The line represents the relationship between coronary flow and weight of different parts of hearts from sham-operated hearts, at baseline: Coronary flow (ml/min) = 0.012 * Weight (mg) − 0.446; During maximal vasodilation: Coronary flow (ml/min) = 0.018 * Weight (mg) + 0.648. Panels C and D show the relationship of mean coronary flow and mean tissue weight of interventricular septa (triangles) and left ventricular free walls (squares) of infarcted hearts to the regression line of normal hearts.

farcted hearts (+12%), despite replacement of most of the myocyte mass by relatively light scar tissue (180 ± 19 mg). However, if scar weight was subtracted from the total weight of the left ventricular free wall, the remaining viable myocardial tissue weight was not significantly different from that of sham hearts. Captopril treatment caused a reduction in body weight of infarcted rats, as well as prevention of reactive hypertrophy. The reduction of cardiac mass by captopril treatment was not restricted to the most hypertrophied region, the left ventricular free wall, but resulted from a reduced weight of all parts of infarcted hearts, including the infarct scar (105 ± 13 mg).

Baseline coronary flow to the ventricles was the same in the experimental groups (12.4 ± 0.9, 11.4 ± 0.5 and 11.8 ± 0.5 ml/min in sham, untreated infarcted and captopril-treated infarcted hearts, respectively). Similarly, coronary flow during nitroprusside-induced maximal vasodilation, indicating total coronary vascular capacity, was not different in sham and infarcted hearts (20.7 ± 0.7 and 19.9 ± 0.9 ml/min, respectively), and also not in hearts from captopril-treated rats (20.1 ± 0.8 ml/min). Baseline as well as maximal coronary flow to the different regions of infarcted hearts was not influenced by captopril treatment (Table 2).

In hearts from sham-operated animals, there was a highly significant correlation between regional coronary flow and tissue weight (at baseline: $r = 0.941$, $P < 0.0001$; during maximal vasodilation: $r = 0.954$, $P < 0.0001$; Fig. 1, Panels A and B), indicating a rather steady myocardial perfusion, independent of its location in the heart. Since captopril could affect both local tissue weight and local flow, an effect which may then be masked in the perfusion data, the results are presented in relation to the regression line for control hearts. Right ventricles and interventricular septa of infarcted hearts showed a weight–flow relationship similar to that of sham-operated rats, but left ventricular free walls deviated from the weight–flow relationship of control hearts. However, in infarcted hearts from captopril-treated rats, all contractile parts, including the left ventricular free walls, showed a weight–flow relationship similar to that of tissues from sham hearts. Global ventricular myocardial perfusion during peak vasodilation was significantly decreased in infarcted hearts compared to that in shams (17.1 ± 0.8 vs. 20.2 ± 0.8 ml/min g), which was

Table 3
Concentrations (μM) of purines and lactate in coronary effluent

	SHAM	MI	MI+CAP
Purines	1.7±0.2	1.5±0.2	1.2±0.2
Lactate	50±6	72±11	32±3[b]
Lactate/purines	29.8±2.9	48.6±4.8[a]	26.9±2.6[b]

Concentrations of purines and lactate, and the lactate/purines ratio in coronary effluent, collected during baseline perfusion.
MI, untreated infarcted hearts; MI+CAP, captopril-treated infarcted hearts.
[a] $P < 0.05$ vs. sham hearts; [b] $P < 0.05$ vs. untreated infarcted hearts.

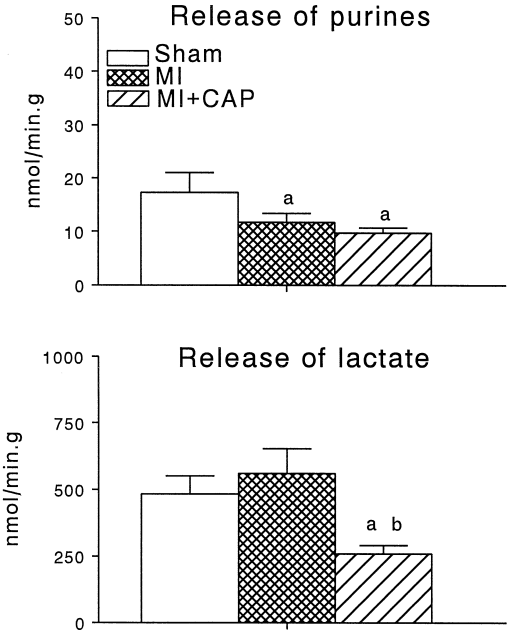

Fig. 2. Myocardial release of purines (panel A) and lactate (panel B) into the coronary circulation during baseline perfusion in sham, non-treated infarcted (MI) and captopril treated infarcted (MI+CAP) rats. [a] $P < 0.05$ vs. sham values; [b] $P < 0.05$ vs. untreated infarcted hearts.

significantly restored after captopril treatment (21.9 ± 1.0 ml/min g). This restored peak perfusion was explained by an improved maximal perfusion of left ventricular free walls and interventricular septa of infarcted hearts with captopril treatment (Table 2). Whereas the improvement in perfusion of interventricular septa was mainly due to the reduction of septal weight, the increased perfusion of left ventricular free walls was instead caused by a combination of weight reduction and increased vascular capacity in this region, as indicated by an upward and leftward shift shown in Fig. 1, Panels C and D. In these two panels only the major findings with regard to cardiac function are summarised since right ventricular values were on the control line, whereas scar perfusion is supposed not to contribute to contractile function. Scar tissue of infarcted hearts was hypoperfused compared to contractile parts, both in untreated and captopril-treated infarcted hearts, especially during maximal vasodilation of the coronary vasculature (Fig. 1, Panels A and B).

3.2. Cardiac function and metabolism

Left ventricular developed pressure was similar in untreated infarcted hearts and shams (78 ± 7 vs. 84 ± 5 mm Hg, respectively), but tended to be lower in infarcted hearts after captopril (65 ± 7 mm Hg). Peak velocity of contraction and relaxation was lower in infarcted hearts, being significantly depressed in the infarcted group with captopril ($+dP/dt_{max}$: 2.7 ± 0.2; 2.3 ± 0.2 and 1.8 ± 0.2 10^3 mm Hg/s, $-dP/dt_{max}$: 2.0 ± 0.2; 1.8 ± 0.2 and 1.3 ± 0.2 10^3 mm Hg/s, in sham, non-treated infarcted and captopril treated infarcted rats, respectively).

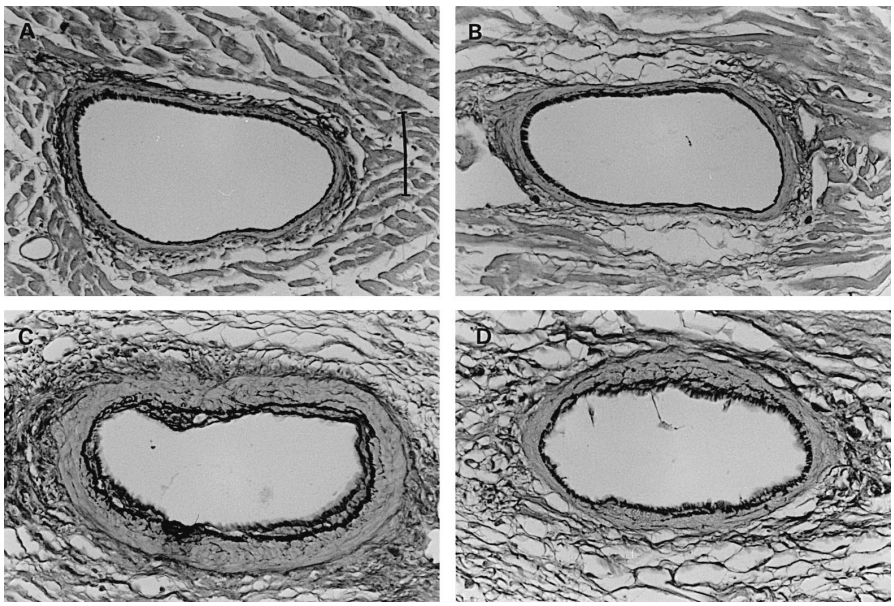

Fig. 3. Photomicrographs of resorcine–fuchsin-stained sections, showing resistance sized arteries in non-treated (A and C) and captopril-treated (B and D) infarcted hearts. Panels A and B present arteries in viable myocardium, whereas panels C and D show vessels in the infarct scar. The bar in panel A represents 100 μm.

The concentration of purines in coronary effluent was similar in all experimental groups (Table 3). Since coronary flow differed between experimental groups, purine release was calculated as concentration times flow per heart weight (Fig. 2). Release of purines was lower in infarcted hearts than in sham hearts, irrespective of treatment.

Concentrations of lactate in coronary effluent did not differ between infarcted hearts and normal hearts, but were significantly reduced in captopril-treated infarcted hearts (Table 3). Corrected for flow and tissue mass, the cardiac release of lactate into the coronary circulation did not differ between normal control hearts and infarcted hearts. However, lactate release of captopril-treated infarcted

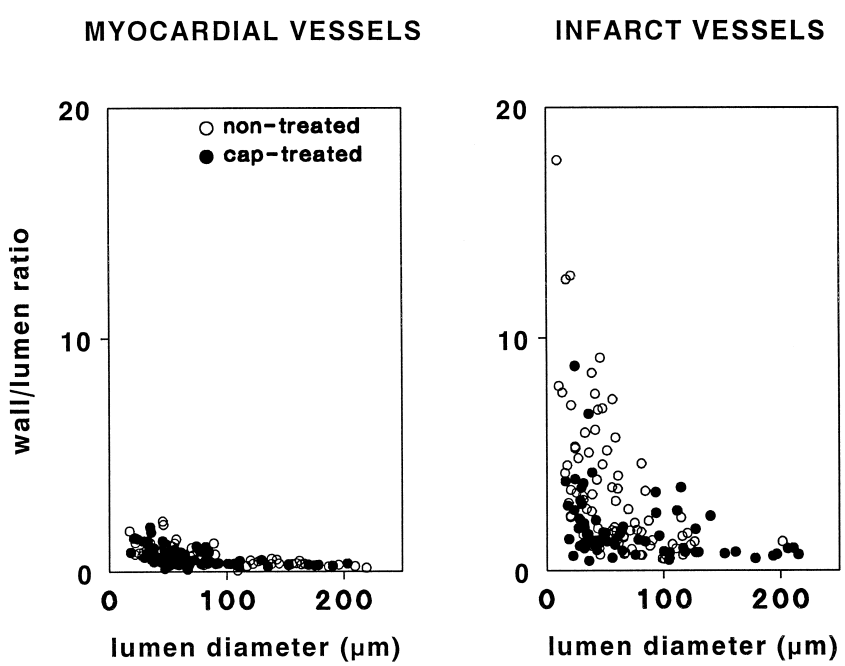

Fig. 4. Wall-to-lumen ratios of resistance-sized arteries in vital myocardium (left panel) and in the scar (right panel), plotted against the actual vessel diameter for captopril-treated and non-treated infarcted hearts.

hearts was reduced compared to that of both sham-operated hearts and untreated infarcted hearts (Fig. 2). The lactate/purine ratio, as an estimate of the relative contribution of anaerobic metabolism, was significantly increased in infarcted compared to that in sham hearts and was normalized by captopril (Table 3).

3.3. Structure of resistance arteries

Examples of resistance arteries in different areas from non-treated and captopril-treated hearts are presented in Fig. 3. Resistance arteries in viable myocardium of infarcted hearts appeared similar to arteries in sham hearts, whereas resistance arteries in the infarcted area appeared strikingly different with regard to the vessel wall. The latter wall abnormalities were less pronounced in captopril-treated hearts. These observations are supported by the actual measurements performed on the vessels. The wall-to-lumen ratios of vessels in viable myocardium did not differ for non-treated and captopril-treated hearts (0.68 ± 0.08 and 0.62 ± 0.09, respectively), at similar lumen diameters (78 ± 5 and 72 ± 5 μm). Moreover, no differences were found between arteries in the spared left ventricular free wall, close to the infarct area, and vessels in the septum, remote from the infarct. In contrast, vessels in the infarcted area displayed an increased wall thickness (wall/lumen ratio: 3.36 ± 0.41). Although this effect concerns the media, the volume of which consists mainly of smooth muscle cells, a small contribution of collagen and elastin cannot be excluded. Besides thickening of the vessel wall, the frequent appearance of a (partially) second internal elastic membrane (see Fig. 3) may indicate growth outside its initial boundaries, resulting in significant encroachment of the vessel lumen (diameter: 59 ± 3 μm). The wall-to-lumen ratio of non-treated infarct vessels was significantly reduced by captopril (1.90 ± 0.34) but remained significantly above normal. Lumen diameter was normalized by captopril (77 ± 6 μm). The wall-to-lumen ratio was related to the actual vessel diameter (Fig. 4). The smaller the diameter, the more pronounced the effects on the wall-to-lumen ratio in the infarct, and the more pronounced the effects of captopril. However, after captopril, even the largest infarct vessels remained at values that were about twice the values of normal viable myocardial vessels.

4. Discussion

The aim of the present study was to investigate the effects of early captopril treatment of infarcted rats on the regional capacity of the coronary vascular bed in relation to regional myocardial mass, as well as to find the consequences of these structural adaptations for cardiac metabolism. The main findings were: (i) significant MI-induced reactive hypertrophy could be shown only in the left ventricular free wall and was prevented by captopril; (ii) maximal coronary flow during vasodilation, representing vascular capacity, was not affected by captopril; (iii) early captopril treatment significantly improved peak perfusion of both the viable part of the left ventricular free wall and the interventricular septum of infarcted hearts; (iv) microscopic studies showed that resistance vessels in viable myocardium were not affected, whereas the aberrant structure of infarct vessels was less pronounced after captopril; and (v) captopril-treated infarcted hearts showed restored lactate/purine ratios, by releasing less lactate, suggesting preservation of aerobic metabolism.

4.1. Angiotensin converting enzyme inhibition, tissue weight and cardiac function

The increase in heart weight and heart weight/body weight ratio after 3 weeks of infarction indicates reactive hypertrophy and is in agreement with our previous findings (Schoemaker et al., 1991; Van Krimpen et al., 1991; Kalkman et al., 1997). Regionally, the reactive hypertrophy reached statistical significance in the left ventricular free wall, an effect which was prevented by captopril. Although the weight of right ventricles and interventricular septa of infarcted hearts was not significantly increased compared to that in control hearts, captopril treatment still reduced the mass of these regions. However, since captopril also inhibited the increase in body weight, the tissue/body weight ratio remained normal. The exact reason for the reduced gain in body weight after captopril, as seen earlier (Schoemaker et al., 1991), is still unclear, but may be related to loss of retained body fluid, due to reduced angiotensin II and aldosterone levels after captopril. Whereas the changes in mass of viable myocardium are associated with effects on hypertrophy, changes in scar mass may be attributable to a different mechanism. The collagen content of the scar was not reduced after captopril, while whole tissue density tended to be lower ($P < 0.07$; non-published data).

In previous studies we had not observed any effect of early captopril treatment on either heart weight or collagen content and associated increased DNA synthesis (Van Krimpen et al., 1991). Therefore, we did not study effects in sham rats further. Moreover, the present results indicated that improved tissue perfusion due to captopril treatment only occurred in the viable left ventricular free wall; the area closest to the infarction, shown in previous studies (Kalkman et al., 1997) to be the only area with significant hypertrophy and underperfusion due to MI. Therefore, we do not expect any effects of captopril on tissue perfusion in sham rats.

Left ventricular developed pressure as well as peak velocity of contraction and relaxation were not significantly lower in infarcted than in sham hearts. This could be related to the observation that total viable tissue was normalized at 3 weeks after infarction. In captopril-treated

infarcted hearts, this compensatory growth was prevented, which could have resulted in the decreased peak velocity of contraction and relaxation.

4.2. Angiotensin converting enzyme inhibition and vascular growth

Baseline coronary flow as well as baseline perfusion were not significantly different for the experimental groups. However, because of similar cardiac loading conditions, the fixed heart rate due to pacing, and the lack of circulating neurohumoral factors, which are supposed to be different in vivo due to infarction and treatment, the effects on baseline coronary flow in the isolated hearts did not necessarily represent in vivo effects on baseline coronary flow. Therefore, effects on baseline coronary flow will not be discussed in detail. Maximal coronary flow is less influenced by the above parameters.

The maximal vascular capacity of infarcted hearts was similar to that of control hearts, despite permanent occlusion of one of the three coronary arteries, which acutely reduces coronary flow by approximately 30% (unpublished data). Whereas the restoration of baseline flow could be attributed to a lower resting vascular tone, the restored maximal capacity at 3 weeks after coronary artery ligation implies angiogenesis in the vascular beds perfused by the two remaining coronary arteries. This is in agreement with previous findings (Nelissen-Vrancken et al., 1996; Kalkman et al., 1996a, 1997). However, in contrast to the latter study, hypoperfusion of the viable left ventricular free wall in infarcted heart in the present study did not reach statistical significance. Captopril treatment did not reduce vascular capacity but, instead, maximal coronary flow in the spared part of the left ventricular free wall tended to be even higher. These findings indicate that early captopril treatment does not inhibit vascular growth during the early phase of post myocardial infarction cardiac remodelling and scar formation. With regard to the viable myocardium, the above findings are supported by the lack of effect of captopril on vessel structure; vessel diameter and wall/lumen ratio. The lack of interference with vascular growth by captopril may be interpreted as a paradoxical finding because angiotensin II is a recognized angiogenic factor (Le Noble et al., 1993; Munzenmaier and Greene, 1996). However, stimulation of vascular growth during angiotensin converting enzyme inhibition has been reported as well (Unger et al., 1992; Olivetti et al., 1993). Unger et al. (1992) hypothesized that potentiation of kinins, through an angiotensin converting enzyme inhibitor-related decrease in their breakdown, would increase vascularization. Another potential mechanism for angiotensin converting enzyme inhibitor-induced stimulation of vascular growth may be a reduction of angiotensin AT_2 receptor-mediated inhibition of endothelial cell proliferation (Stoll et al., 1995).

In contrast to the effects on viable cardiac tissue, the effects of captopril on infarct resistance arteries could not be associated with effects on baseline or maximal flow capacity. An explanation could be that although average vessel diameter was increased after captopril, the total number of vessels may be lower, resulting in similar total cross-sectional area; the determinant of vascular capacity (Prewitt et al., 1982). In this regard it is interesting that the vessels in the infarct area grow in an environment with a relatively high renin angiotensin system activation (Sun and Oberley, 1994; Sun et al., 1994).

4.3. Captopril and cardiac metabolism

Treatment with angiotensin converting enzyme inhibitors has been reported to restore biochemical parameters in hearts of stroke-prone spontaneously hypertensive rats, even at a dose that neither lowered blood pressure nor attenuated left ventricular hypertrophy (Gohlke et al., 1994). Biochemical abnormalities in hearts from SHRs included increased cardiac release of lactate dehydrogenase, creatine kinase, and lactate into the coronary circulation, and decreased myocardial tissue levels of the energy-rich phosphates, ATP and creatine phosphate. The effects of chronic angiotensin converting enzyme inhibitor treatment on these biochemical parameters, but not the effects on hypertrophy, could be prevented by co-treatment with a bradykinin receptor blocker, suggesting a role for bradykinin in the biochemical but not in the structural changes induced by angiotensin converting enzyme inhibition.

Similarly to previous findings with the same experimental protocol (Kalkman et al., 1996b), hypertrophied infarcted hearts did not have an increased release of ATP catabolites. In fact, release of purines from infarcted hearts was reduced compared to that from sham hearts. Also, lactate release appeared to be even below sham values. Tissue perfusion in all parts of the hearts of captopril-treated infarcted rats was slightly higher than that in sham hearts, while left ventricular developed pressure was lower. Thus, the lower purine and lactate release in captopril-treated infarcted hearts compared that in to sham hearts could be attributable to a combination of a slightly better oxygen supply and lower oxygen demand. However, the increased lactate/purine ratio may indicate a shift to a relatively higher anaerobic metabolism, resulting in lower oxygen expenditure, as reflected by lower velocity of contraction and relaxation in infarcted hearts. A favourable vascularization/myocyte mass ratio, as reflected by the improved tissue perfusion in captopril-treated infarcted hearts, would decrease oxygen diffusion distance and therefore contribute to better preservation of aerobic metabolism, a possibility which is supported by the reduced lactate release and normalized lactate/purines ratio.

4.4. Implications for clinical outcome

Prevention of reactive hypertrophy with early captopril treatment or regression of established hypertrophy with

delayed captopril treatment (Kalkman et al., 1996b) led to a similar reduction of ventricular weight. The vascular capacity of infarcted hearts was not affected by either early or delayed captopril treatment. Consequently, the impaired peak ventricular perfusion was restored by both early and delayed captopril therapy to its values in hearts from sham-operated rats. This suggests that the more favourable vascularization/myocyte mass ratio improved cardiomyocyte oxygenation, as indicated by the preserved aerobic metabolism (lactate/purines ratio). This is in agreement with the finding that treatment of infarcted rats with angiotensin converting enzyme inhibitors reversed the reduction in mitochondrial oxygen consumption rate (Sanbe et al., 1995). However, while delayed angiotensin converting enzyme inhibitor treatment is generally associated with improved prognosis, this is still a matter of debate in the case of early treatment.

Clinical trials evaluating early intervention with angiotensin converting enzyme inhibitors in myocardial infarction patients have not yielded uniform results. Whereas decreased mortality has been reported from some trials (Ambrosioni et al., 1995; ISIS-4, 1995; Sanbe et al., 1995; GISSI-3, 1996), others did not find improved survival (Sharpe et al., 1991; Swedberg et al., 1992; Ray et al., 1993; CCS-1, 1995). In the rat myocardial infarction model, improved heart function was found with delayed captopril treatment, whereas early intervention failed in this respect (Schoemaker et al., 1991). In fact, myocardial infarction rats that received early captopril therapy, had the same cardiac output at rest, resulting from a lower stroke volume and a higher heart rate. Similar results were obtained in anaesthetized rats by Gay (1990), where they coincided with attenuation of left ventricular dilation and decreased left ventricular filling pressures. Whereas the former effects are considered unfavourable, the latter effects are regarded as beneficial. Results from the present study indicated that, independently of effects on hemodynamics, survival of myocardial infarction patients could be improved after early intervention with captopril through improved cardiac perfusion and aerobic metabolism. The subgroup of patients with generalized coronary atherosclerosis would benefit specially, since this group is most at risk of additional ischemic episodes. Although delayed captopril treatment was found to reduce the number of further ischemic events (Søgaard et al., 1993, 1994; Rutherford et al., 1994), to our knowledge, similar studies have not yet been performed for immediate post-myocardial infarction captopril therapy.

5. Conclusion

Early captopril treatment of myocardial infarction rats prevents reactive hypertrophy but not adaptive vascular growth, hence improving peak perfusion of the viable part of the left ventricular free wall. The absence of hemodynamic improvement after early captopril treatment is thus not explainable by prevention of myocardial infarction-induced angiogenesis. The more beneficial vascularization/tissue mass ratio is reflected in better preservation of aerobic metabolism. This may explain why the clinical outcome could improve, without improvement of hemodynamics, in some of the clinical trials with early angiotensin converting enzyme inhibitor treatment.

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