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Enhanced reduction of myocardial infarct size by combined ACE inhibition and AT_1-receptor antagonism

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1 The effects of the angiotensin-converting-enzyme inhibitor (ACEI) ramiprilat, the angiotensin II type 1 receptor antagonist (AT_1A) candesartan, and the combination of both drugs on infarct size (IS) resulting from regional myocardial ischaemia were studied in pigs.

2 Both ACEI and AT_1A reduce myocardial IS by a bradykinin-mediated process. It is unclear, however, whether the combination of ACEI and AT_1A produces a more pronounced IS reduction than each of these drugs alone.

3 Forty-six enflurane-anaesthetized pigs underwent 90 min low-flow ischaemia and 120 min reperfusion. Systemic haemodynamics (micromanometer), subendocardial blood flow (ENDO, microspheres) and IS (TTC-staining) were determined. The decreases in left ventricular peak pressure by ACEI (by 9 ± 2 (s.e.mean) mmHg), AT_1A (by 11 ± 2 mmHg) or their combination (by 18 ± 3 mmHg, $P<0.05$ *vs* ACEI and AT_1A, respectively) were readjusted by aortic constriction prior to ischaemia. With placebo ($n=10$), IS averaged $20.0\pm3.3\%$ of the area at risk. IS was reduced to $9.8\pm2.6\%$ with ramiprilat ($n=10$) and $10.6\pm3.1\%$ with candesartan ($n=10$). Combined ramiprilat and candesartan ($n=10$) reduced IS to $6.7\pm2.1\%$. Blockade of the bradykinin-B_2-receptor with icatibant prior to ACEI and AT_1A completely abolished the reduction of IS ($n=6$, $22.8\pm6.1\%$). The relationship between IS and ischaemic ENDO with placebo was shifted downwards by each ACEI and AT_1A and further shifted downwards with their combination ($P<0.05$ *vs* all groups); icatibant again abolished such downward shift.

4 The combination of ACEI and AT_1A enhances the reduction of IS following ischaemia/reperfusion compared to a monotherapy by either drug alone; this effect is mediated by bradykinin. *British Journal of Pharmacology* (2000) **131,** 138–144

Keywords: Ramiprilat; candesartan; myocardial ischaemia; reperfusion; bradykinin; swine

Abbreviations: ACE, angiotensin-converting-enzyme; AT_1, angiotensin II type 1 receptor; AT_2 angiotensin II type 2 receptor; LAD, left anterior descending coronary artery; LV, left ventricular; LV_pP, left ventricular peak pressure; RAS, renin-angiotensin-system; s.e.mean, standard error of the mean; TTC, triphenyl tetrazolium chloride; WT, systolic wall thickening

Introduction

The renin-angiotensin-system (RAS) is activated during acute myocardial ischaemia. Its final mediator, angiotensin II, exerts a number of effects that exacerbate the consequences of myocardial ischaemia *via* activation of the angiotensin II type 1 receptor (AT_1), including arrhythmias, epinephrine release, and vasoconstriction (for review, see Zughaib *et al*., 1993; Jalowy *et al*., 1999). The predominant pathway for cardiac angiotensin II formation in humans is through the angiotensin-conversion-enzyme (ACE) (Zisman *et al*., 1995), but chymase and other enzymes also contribute (for review see Jalowy *et al*., 1999).

The ACE is also responsible for the degradation of kinins, and following ACE inhibition, the concentration of bradykinin is increased (Baumgarten *et al*., 1993). ACE inhibition attenuates ischaemia/reperfusion injury, i.e. attenuates stunning (Ehring *et al*., 1994) and reduces infarct size (Hartman *et al*., 1993), and bradykinin mediates these protective effects (Hartman *et al*., 1993; Ehring *et al*., 1994).

Also, the AT_1 antagonists EXP3174 (Schwarz *et al*., 1997) and candesartan (Jalowy *et al*., 1998a; Shimizu *et al*., 1998; 1999) reduce infarct size in pigs. AT_1 antagonism increases plasma angiotensin II (Campbell *et al*., 1995), which subsequently activates the angiotensin II type 2 receptor (AT_2), resulting in enhanced kininogen activity (Tsutsumi *et al*., 1999) and bradykinin formation (Liu *et al*., 1997). Indeed, the infarct size reduction by candesartan is abolished by blockade of the AT_2-(PD123319) or the bradykinin B_2-receptor (icatibant) (Jalowy *et al*., 1998a).

ACE inhibitors and AT_1 antagonists alone each reduce morbidity and mortality in patients with heart failure post myocardial infarction (Pfeffer *et al*., 1992; The Acute Infarction Ramipril Efficacy (AIRE) Investigators, 1993; GISSI 3, 1994; ISIS-4 Collaborative Group, 1995; Pitt *et al*., 1997); their combination reduces blood pressure (Di Pasquale *et al*., 1999; McKelvie *et al*., 1999), plasma aldosterone (Baruch *et al*., 1999) and end systolic LV volume (McKelvie *et al*., 1999) in an additive manner.

Experimental data on the combined use of ACE inhibitors and AT_1 antagonists during acute myocardial ischaemia/reperfusion are lacking. Therefore, in the present study, the effect of the ACE inhibitor ramiprilat, the AT_1 antagonist candesartan and their combination on myocardial infarct size was assessed in pigs. Since both ACE inhibitors and AT_1 antagonists act through bradykinin (Ehring *et al*., 1994; Jalowy *et al*., 1998a), the importance of bradykinin in mediating the infarct size reduction by combined treatment was also determined.

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Methods

The experimental protocols employed in this study were approved by the Bioethical Committee of the district of Düsseldorf, and they adhere to the guiding principles of the American Physiological Society.

Experimental preparation

The experimental model has been described previously (Jalowy *et al*., 1998a). In brief: in 46 enflurane-anaesthetized Göttinger minipigs, a micromanometer (P7, Konigsberg Instruments, Pasadena, California, U.S.A.) was placed into the left ventricle (LV) through the apex. A silicon tube was passed around the descending thoracic aorta to permit adjustment of left ventricular peak pressure (LVpP) by controlled aortic constriction.

Ultrasonic crystals were then implanted in the anterior wall within the perfusion bed of the cannulated left anterior descending coronary artery (LAD, distal to its first branch) to measure wall thickness using standard techniques (Theroux *et al*., 1974). To verify the stability of the preparation a set of ultrasonic crystals was implanted in the lateral wall (control zone) within the perfusion bed of the left circumflex coronary artery. Systolic wall thickening (WT) was calculated as per cent of end-diastolic wall thickness.

The LAD was dissected free from surrounding tissue for a distance of approximately 2 cm just prior to its first diagonal branch, but distal to the branching of the left circumflex coronary artery. After heparinizing the swine (20,000 IU, initial dose, followed by 10,000 IU h^{-1}), the LAD was ligated and rapidly cannulated. Perfusion pressure was measured through a distal side arm of the cannula. Pressure drop from the cannula tip to the side arm was measured *in vitro* using heparinized blood at 36.8°C over a flow range from 0–100 ml min^{-1}; the maximum difference that occurred was 1.2 mmHg at a flow rate of 100 ml min^{-1}. Since the maximum flow rate for perfusing the LAD under control condition was less than 70 ml min^{-1}, the maximum error introduced by measuring coronary artery pressure through the side arm of the cannula was less then 1 mmHg. Blood was supplied by an extracorporeal circuit which included an occlusive roller pump (Masterflex, Cole & Parmer Instrument Co., Chicago, IL, U.S.A.), windkessel as well as two side ports: one for regional infusion of drugs and one for microspheres injection. The microsphere injection port was proximal (just distal to pump and windkessel) in the extracorporeal circuit and spheres were injected in the opposite direction of flow to facilitate their mixing with the blood (Schulz *et al*., 1989).

Heart rate was maintained constant by left atrial pacing (Hugo Sachs Elektronik Type 215/T, Hugstetten, Germany). Pacing rate was set to about 10 beats · min^{-1} above the spontaneous heart rate.

Under control conditions the perfusion pump was adjusted so that minimum coronary arterial pressure did not fall below 70 mm Hg to avoid any initial hypoperfusion. Radiolabelled microspheres (15 μm diameter, ^{141}Ce, ^{114}In, ^{103}Ru, ^{95}Nb or ^{46}Sc; NEN, Du Pont, Boston, MA, U.S.A.) were injected into the coronary perfusion circuit ($1-2\times10^5$ suspended in 1 ml saline) to determine regional myocardial blood flow and its distribution throughout the LAD perfusion bed (Schulz *et al*., 1989). Blood flow to the tissue at the site of the ultrasonic crystals is reported (this piece of tissue was divided into transmural thirds). The averaged subendocardial blood flow of the entire LAD-perfused territory was related to infarct size.

At the end of each experiment the heart was excised, sectioned into five to six slices parallel to the atrio-ventricular groove, and incubated in a 1% triphenyl tetrazolium chloride (TTC)-solution (25 min, 37°C) to demarcate infarcted areas. The LV area at risk was assessed using the microspheres technique (Vatner *et al*., 1988; Post *et al*., 1998). Reduction of blood flow during ischaemia by more than 85% was taken to indicate myocardium at risk (Vatner *et al*., 1988). Infarct size is expressed as a percentage of the LV area at risk.

Protocols

Group 1: Placebo + 90-min severe ischaemia (n = 10) Following control measurements of systemic haemodynamics, regional myocardial function and blood flow, 40 ml saline was infused intravenously over 30 min. Coronary inflow was then decreased to reduce anterior WT by >95%. This level of hypoperfusion was maintained for 90 min. Measurements were repeated at 5 and 85 min after the commencement of myocardial ischaemia. Following 90 min ischaemia, the myocardium was reperfused for 120 min to facilitate the identification of necrotic tissue. During reperfusion, coronary inflow was again set to maintain minimum coronary arterial pressure above 70 mmHg.

Group 2: Ramiprilat + 90-min severe ischaemia (n = 10) Following control measurements, the ACE inhibitor ramiprilat (50 μg kg^{-1} i.v. in 40 ml saline) was infused over 30 min; this dose has previously been demonstrated to reduce infarct size in anaesthetized rabbits (Hartman *et al*., 1993). The decrease in LVpP with ramiprilat was adjusted to predrug values by aortic constriction. Measurements were repeated at matched LVpP, before coronary inflow was reduced to match the coronary arterial pressure measured in group 1. Thereafter, the protocol of group 2 was identical to that of group 1.

Group 3: Candesartan + 90-min severe ischaemia (n = 10) Following control measurements, the AT_1 antagonist candesartan (1 mg kg^{-1} i.v. in 40 ml saline) was infused over 30 min; this dose has previously been demonstrated to abolish the coronary vasoconstriction induced by infusion of angiotensin II (15 μg min^{-1} i.c.) and to reduce infarct size in pigs (Jalowy *et al*., 1998a) and stunning in dogs (Dörge *et al*., 1999). Thereafter, the protocol of group 3 was identical to that of group 2.

Group 4: Ramiprilat + Candesartan + 90-min severe ischaemia (n = 10) After control measurements, pigs received both ramiprilat (50 μg kg^{-1} i.v.) and candesartan (1 mg kg^{-1} i.v.). Thereafter, the protocol of group 4 was identical to that of group 2.

Group 5: Icatibant + Ramiprilat + Candesartan + 90 min severe ischaemia (n = 6) In a second step, the importance of bradykinin in mediating the infarct size reduction by combined ramiprilat and candesartan was assessed.

Fifteen minutes prior to the ramiprilat and candesartan infusion, the icatibant infusion was started. Icatibant was given through the distal side port directly into the perfusion system at an infusion rate of 0.5 ml min^{-1}, resulting in a final concentration of 0.1 μg $kg^{-1}min^{-1}$, until the end of ischaemia. Icatibant *per se* at this dose did not increase infarct size in three additional pigs, as previously shown for a lower dose (Jalowy *et al*., 1998a). With ongoing icatibant infusion, ramiprilat (50 μg kg^{-1} i.v.) and candesartan (1 mg kg^{-1} i.v.) were then infused, and the subsequent protocol of group 5 was identical to that of group 4.

Data analysis and statistics

Haemodynamic and functional parameters were digitized and recorded over a 20 s period during each microspheres injection using CORDAT II software (Skyschally *et al*., 1993). Calculation of all haemodynamic parameters was done on a beat-to-beat basis, and data were then averaged.

Statistical analysis was performed using SYSTAT software (Urbana, IL, U.S.A.). Data on haemodynamics, WT and myocardial blood flow were compared using a two-way ANOVA, accounting for the different time points throughout the protocols and the five groups of pigs. When significant differences were detected, individual mean values were compared using LSD *post-hoc* tests. Area at risk and infarct size were compared by one-way ANOVA. Data are reported as mean values ± standard error of the mean (s.e.mean), and a P value less than 0.05 was accepted as indicating a significant difference. Linear regression analyses between subendocardial blood flow at 5 min and 85 min ischaemia and infarct size were performed in all groups and compared by ANCOVA.

Results

Haemodynamics, regional myocardial function and blood flow

There were no significant differences in any parameter between the five groups under control conditions (Table 1). Heart rate was held constant by atrial pacing, and WT of the posterior control wall remained stable throughout the experimental protocol.

LVpP was decreased by 9 ± 2 mmHg ($P<0.05$ *vs* Control) with ramiprilat and then readjusted by aortic constriction. At readjusted LVpP, ramiprilat had no effect on $LVdP/dt_{max}$, anterior WT and blood flow. Also, with candesartan LVpP was decreased by 11 ± 2 mmHg ($P<0.05$ *vs* Control) and readjusted by aortic constriction. At readjusted LVpP, also candesartan had no effect on $LVdP/dt_{max}$, anterior WT and blood flow. Combined ramiprilat and candesartan decreased LVpP more than either drug alone (18 ± 3 mmHg, $P<0.05$ *vs* Control, Ramiprilat and Candesartan groups). At readjusted

Table 1 Systemic haemodynamics, regional myocardial function and blood flow

	Group	*Control*	*Drug*	*Drug + pressure match*	*5 min ischaemia*	*85 min ischaemia*
HR	1	101 ± 2			103 ± 3	106 ± 3
(min^{-1})	2	102 ± 2	102 ± 2	102 ± 2	104 ± 3	103 ± 3
	3	99 ± 3	99 ± 3	100 ± 3	103 ± 3	105 ± 5
	4	102 ± 2	102 ± 2	102 ± 2	102 ± 3	106 ± 4
	5	99 ± 3	97 ± 3	97 ± 3	99 ± 3	98 ± 3
LVedP	1	5 ± 1			12 ± 1*	9 ± 1
(mmHg)	2	5 ± 1	5 ± 1	5 ± 1	9 ± 1	11 ± 3*
	3	5 ± 1	5 ± 1	5 ± 1	10 ± 2*	10 ± 1
	4	6 ± 2	4 ± 1	5 ± 1	9 ± 1	9 ± 1
	5	4 ± 2	4 ± 1	4 ± 1	11 ± 2*	11 ± 3*
LVpP	1	87 ± 3			76 ± 3*	79 ± 3*
(mmHg)	2	86 ± 4	77 ± 4*	87 ± 3#	75 ± 3*#	72 ± 3*
	3	93 ± 3	82 ± 3*	91 ± 3#	79 ± 3*#	80 ± 5*
	4	91 ± 3	72 ± 4*	91 ± 4#	77 ± 4*#	74 ± 2*
	5	95 ± 3	91 ± 4	96 ± 4	82 ± 4*#	79 ± 3*
$LVdP/dt_{max}$	1	1143 ± 66			885 ± 39*	1046 ± 160
($mmHg\ s^{-1}$)	2	1256 ± 82	1033 ± 136*	1159 ± 82	874 ± 41*#	843 ± 44*
	3	1273 ± 54	1057 ± 58*	1174 ± 44	862 ± 28*#	1013 ± 71*
	4	1390 ± 97	997 ± 87*	1193 ± 62	905 ± 58*#	1017 ± 70*
	5	1281 ± 72	1126 ± 102	1168 ± 87	935 ± 70*	991 ± 54*
CAP	1	115 ± 2			30 ± 2*	28 ± 1*
(mmHg)	2	113 ± 3	110 ± 3	116 ± 3	31 ± 1*#	30 ± 1*
	3	117 ± 2	107 ± 11	119 ± 2	29 ± 1*#	28 ± 1*
	4	115 ± 3	118 ± 4	123 ± 6	33 ± 2*#	30 ± 1*
	5	114 ± 2	109 ± 3	115 ± 5	27 ± 1*#	28 ± 3*
CI	1	36 ± 3			6 ± 1*	6 ± 1*
($ml\ min^{-1}$)	2	40 ± 3	48 ± 4	51 ± 6	7 ± 1*#	7 ± 1*
	3	33 ± 5	33 ± 5	35 ± 5	5 ± 1*#	5 ± 1*
	4	33 ± 4	40 ± 3	44 ± 4*	6 ± 1*#	6 ± 1*
	5	27 ± 3	29 ± 4	29 ± 4	5 ± 1*#	5 ± 1*
WT	1	39.7 ± 4.9			−1.2 ± 0.8*	−0.3 ± 0.6*
(%)	2	38.2 ± 2.3	36.1 ± 2.4	34.9 ± 2.3	5.1 ± 1.3*#	6.2 ± 1.4*
	3	36.2 ± 4.6	33.3 ± 6.5	33.1 ± 6.0	1.7 ± 1.5*#	2.4 ± 1.0*
	4	42.3 ± 3.0	33.9 ± 2.8	34.2 ± 3.0	2.1 ± 1.5*#	2.5 ± 1.7*
	5	47.0 ± 6.9	45.2 ± 7.5	44.5 ± 7.3	−0.3 ± 2.6*#	2.5 ± 2.2*
TMF	1	0.82 ± 0.06			0.11 ± 0.01*	0.12 ± 0.02*
($ml\ min^{-1}\ g^{-1}$)	2	0.94 ± 0.06	1.05 ± 0.09	1.02 ± 0.09	0.13 ± 0.02*#	0.14 ± 0.02*
	3	0.80 ± 0.06	0.92 ± 0.09	0.93 ± 0.08	0.13 ± 0.02*#	0.12 ± 0.02*
	4	0.88 ± 0.07	1.11 ± 0.12*	1.25 ± 0.14*	0.12 ± 0.02*#	0.13 ± 0.02*
	5	0.72 ± 0.04	0.85 ± 0.06	0.85 ± 0.08	0.14 ± 0.02*#	0.16 ± 0.02*

HR, heart rate; LVedP, left ventricular end-diastolic pressure; LVpP, left ventricular peak pressure; $LVdP/dt_{max}$, maximum of the first derivative of left ventricular pressure; CAP, mean coronary arterial pressure; CI, coronary inflow; WT, anterior systolic wall thickening in per cent of end-diastolic wall thickness; TMF, mean transmural blood flow; *$P<0.05$ *vs* control; #$P<0.05$ *vs* preceding value; group 1, placebo; group 2, ramiprilat; group 3, candesartan; group 4, ramiprilat + candesartan; group 5, icatibant + ramiprilat + candesartan.

LVpP, again, the combined drugs had no effect on $LVdP/dt_{max}$ and anterior WT. Transmural myocardial blood flow, however, was increased with the combined drugs. Icatibant abolished the decreases in LVpP and in transmural myocardial blood flow with ramiprilat and candesartan.

During ischaemia, LVpP, $LVdP/dt_{max}$ and transmural myocardial blood flow were decreased to a similar extent in all groups. Ischaemic WT tended to be somewhat increased with ramiprilat over placebo (NS).

Infarct size

Body weights averaged (in kg): 41 ± 2, 38 ± 3, 34 ± 3, 33 ± 3 and 35 ± 4 for groups 1 to 5, respectively. Area at risk was comparable among groups (group 1: $47 \pm 2\%$; group 2: $47 \pm 4\%$; group 3: $50 \pm 2\%$; group 4: $47 \pm 3\%$; group 5: $39 \pm 2\%$ of LV mass, Figure 1). Infarct size with placebo was $20.0 \pm 3.3\%$ of the area at risk (group 1, Figure 1) and reduced to $9.8 \pm 2.6\%$ with ramiprilat (group 2). Also, infarct size for any given subendocardial blood flow at 5 min ischaemia was reduced with ramiprilat over placebo (Figure 2). Candesartan reduced infarct size to $10.6 \pm 3.1\%$. Infarct size for any given subendocardial blood flow at 5 min ischaemia was also reduced with candesartan over placebo (Figure 2). Combined ramiprilat and candesartan reduced infarct size to $6.7 \pm 2.1\%$. Infarct size for any given subendocardial blood flow at 5 min ischaemia was reduced over that in all other groups (Figure 2). Combined ramiprilat and candesartan with icatibant no longer reduced infarct size. Also, infarct size for any given subendocardial blood flow at 5 min ischaemia was no longer different from that with placebo (Figure 3). Similar results were obtained when subendocardial blood flows at 85 min ischaemia were related to infarct size.

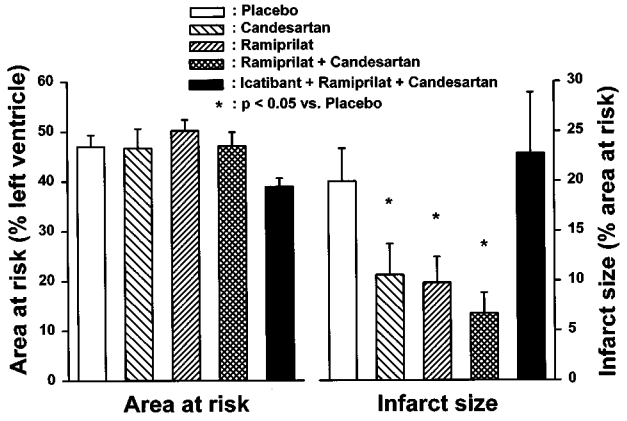

Figure 1 Area at risk (AAR, left bars) was comparable between groups. Infarct size was reduced with ramiprilat, candesartan and both drugs combined. The infarct size reduction by combined drugs tended to be greater than that by either drug alone. Infarct size reduction by combined drugs was abolished by the bradykinin-B_2-receptor antagonist icatibant.

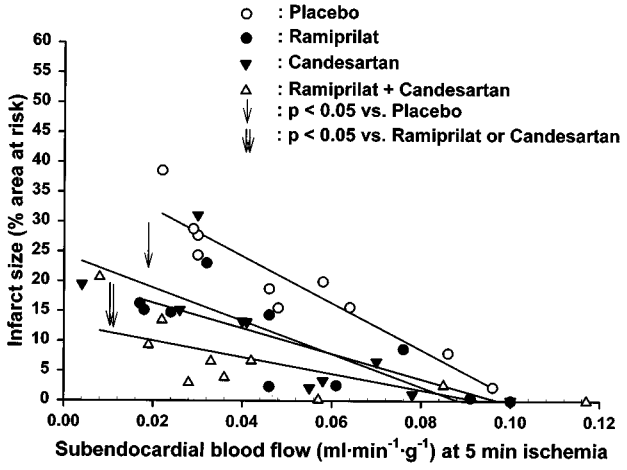

Figure 2 Relationships between subendocardial blood flow at 5 min ischaemia and infarct size. Subendocardial blood flow correlated inversely to infarct size in all groups of pigs. Infarct size for any given subendocardial blood flow was significantly reduced in pigs receiving ramiprilat ($y = -212.6 \times +20.7$, $n = 10$, $r = -0.79$) and candesartan ($y = -276.9 \times +24.5$, $n = 10$, $r = -0.79$) compared to placebo ($y = -392.4 \times +40.0$, $n = 10$, $r = -0.94$, $p < 0.05$). The relationship between subendocardial blood flow and infarct size with combined drugs ($y = -137.4 \times +12.8$, $n = 10$, $r = -0.71$) was further shifted downwards and different from the relationships of all other groups ($P \leqslant 0.05$).

Discussion

Critique of methods

Infarct size as determined by TTC-staining after 90 min ischaemia and 120 min reperfusion was one major end point of the present study. Although the validity of TTC-staining to identify myocardial necrosis within the time frame of 90 min ischaemia and 120 min reperfusion has not been rigorously confirmed by electron microscopy, numerous studies from different laboratories have used it within such time frame of ischaemia/reperfusion (Klein *et al*., 1981; Go *et al*., 1988).

Pigs were used in the present study because their coronary anatomy (Weaver *et al*., 1986), extent of collateral flow (White & Bloor, 1981) and time course of infarct development most closely resemble that observed in humans (Schaper *et al*., 1988). In pigs, complete coronary occlusion frequently results in ventricular fibrillation (45% within 20 min) (Hill & Gettes, 1980) and extensive infarction of the LV with subsequent pump failure. Therefore, in the present study, the LAD perfusion territory was hypoperfused at low but maintained flow, resulting in a large area at risk (48% of LV mass on the average), but a small infarct size when expressed as per cent of the area at risk ($20 \pm 3\%$ with placebo). However, infarct size as per cent of total LV mass in the present study averaged $9 \pm 1\%$ with placebo and was similar to that in a study with total occlusion of one distal LAD branch in pigs (7%) (Schott *et al*., 1990).

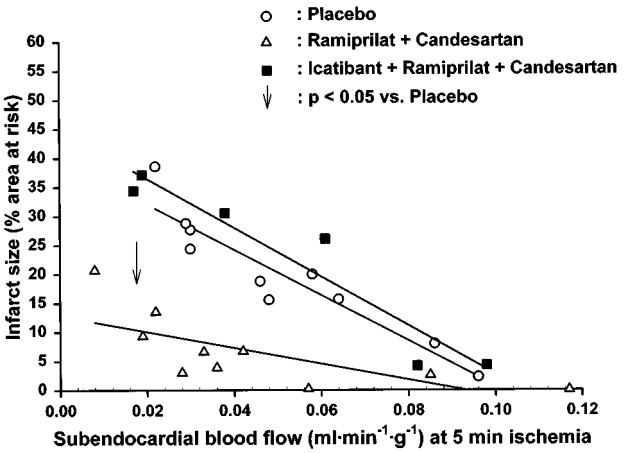

Figure 3 Relationships between subendocardial blood flow at 5 min ischaemia and infarct size. The relationships for placebo and combined ramiprilat and candesartan in the presence of icatibant ($y = -422.2 \times +44.9$, $n = 6$, $r = -0.95$) were superimposable.

Many patients suffering from coronary artery disease over prolonged periods of time develop an extensive collateral circulation (McFalls *et al*., 1993; Sasayama, 1994). In this scenario, an acute total occlusion of one coronary artery will result in low-flow rather than no-flow ischaemia in the dependent myocardium. Technically, perfusion at low flow permits the delivery of drugs throughout the ischaemic period, and to perform the bradykinin-B_2-receptor blocking experiments using icatibant without inducing systemic effects. Using low-flow ischaemia, infarct development is not as homogeneous as seen with a total coronary artery occlusion model in other species. Accordingly, our analysis is based on the relationships between residual ischaemic blood flow and infarct size, as previously described in a variety of experimental models from different laboratories (Lefkowitz *et al*., 1988; Kloner *et al*., 1992; Black *et al*., 1994; Yao & Gross, 1994), rather than on mean infarct size data, as used in experiments with collateral-deficient species using total coronary artery occlusion.

Only one dose of candesartan and ramiprilat was used in the present study. Therefore, increased doses of each drug alone could have possibly decreased infarct size further. However, in prior studies infarct size reduction by candesartan was identical at doses of 2 or 20 $\mu g\ kg^{-1}$ (Shimizu *et al*., 1998) or 50 $\mu g\ kg^{-1}$ (Shimizu *et al*., 1999) in anaesthetized pigs. Similarly, blood pressure reduction was comparable in patients receiving either 16 or 32 mg candesartan once daily (Reif *et al*., 1998), while the addition of an ACE inhibitor on top of 16 mg candesartan had an additive effect (McKelvie *et al*., 1999).

Systemic haemodynamics

In the present study, combined ACE inhibition and AT_1 antagonism reduced LVpP in an additive manner, consistent with prior results in animals (Ménard *et al*., 1997) and humans (Azizi *et al*., 1995; McKelvie *et al*., 1999). At maintained coronary arterial pressure, combined ACE inhibitor and AT_1 antagonist also increased transmural myocardial blood flow in an additive manner. This increase in transmural blood flow under baseline conditions was, however, completely lost during ischaemia. In dogs, the increase in coronary blood flow with ACE inhibitors has been related to bradykinin (Sudhir *et al*., 1993; Kitakaze *et al*., 1995). Also in the present study, this increase was obviously mediated by bradykinin and therefore prevented by icatibant.

Infarct size

Ramiprilat, candesartan and both drugs combined reduced LVpP. Apart from ischaemic blood flow and the size of the area at risk, the rate-pressure product is a determinant of myocardial infarct size in anaesthetized and conscious dogs (Reimer *et al*., 1985). However, the main determinant of the increase in infarct size when the rate-pressure product is increased relates to the enhanced heart rate rather than the enhanced left ventricular pressure, since an increase in left ventricular pressure by more than 25 mmHg does not increase infarct size in pigs (Post *et al*., 2000). Thus, the reduction in left ventricular pressure following treatment with ramiprilat, candesartan or the combination of both drugs is expected to have little impact on infarct size – if anything, it could result in a slight reduction of infarct size—as long as heart rate remains unaltered.

Ramiprilat, candesartan and both drugs combined reduced infarct size, and this beneficial effect was not related to favourable changes in heart rate, LVpP and blood flow during ischaemia. Infarct size for a given ischaemic blood flow was less with combined drugs than with either drug alone. The reduction of infarct size by ACE inhibitors (Hartman *et al*., 1993) and AT_1 antagonists (Seyedi *et al*., 1995; Jalowy *et al*., 1998a,b) is mediated through bradykinin (for review see Jalowy *et al*., 1999). AT_1 antagonists increase the formation of bradykinin and ACE inhibitors reduce bradykinin breakdown; as expected, the more pronounced decrease in infarct size with combined ACE inhibition and AT_1 antagonism was also mediated by bradykinin and prevented by icatibant. Interestingly, these drugs, by increasing bradykinin, share an important mediator with ischaemic preconditioning (Vegh *et al*., 1994; Wall *et al*., 1994; Goto *et al*., 1995; Schulz *et al*., 1998), the most powerful procedure to reduce infarct size known so far (Yellon *et al*., 1998).

Activation of bradykinin-B_2 receptors increases the concentration of cytosolic calcium (Busse & Lamontagne, 1991), resulting in a stimulation of cyclo-oxygenase with a subsequently enhanced synthesis of prostacyclin (Revtyak *et al*., 1990). Indeed, inhibition of cyclo-oxygenase blocked the protective effects of ACE inhibition on myocardial stunning in dogs (Ehring *et al*., 1994) and the reduction of infarct size by candesartan in pigs (Jalowy *et al*., 1998a). The involvement of prostaglandins in the enhanced reduction of infarct size by ACE inhibition and AT_1 antagonism in the present study was not elucidated. Activation of bradykinin-B_2 receptors might also increase formation of nitric oxide (Linz *et al*., 1999); however, endogenous nitric oxide appears to be of no importance for infarct development during 90 min ischaemia in pigs (Post *et al*., 2000).

Clinical implications

In pacing-induced heart failure in pigs, combined ACE inhibitors and AT_1 antagonists improve ventricular function and myocardial blood flow and decrease neurohormonal activation (Spinale *et al*., 1997; Krombach *et al*., 1998). In patients with heart failure, ACE inhibitors (Pfeffer *et al*., 1992; The Acute Infarction Ramipril Efficacy (AIRE) Investigators, 1993) and AT_1 antagonists (Pitt *et al*., 1997) reduce morbidity and mortality. Again, the combined use of ACE inhibitors and AT_1 antagonists appeared to be more beneficial in preventing left ventricular dilatation and suppressing neurohormonal activation than that of ACE inhibitors alone (McKelvie *et al*., 1999).

When extrapolating the results of the present study, patients under treatment with ACE inhibitors, AT_1 antagonists and particularly under combined treatment for indications such as hypertension and ventricular remodelling after myocardial infarction may also have improved outcome when suffering from an acute myocardial infarction.

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