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Na^+/H^+-exchange inhibition and aprotinin administration: promising tools for myocardial protection during minimally invasive CABG ☆

M. Hendrikx[a,b,*], F. Rega[b], L. Jamaer[c], T. Valkenborgh[c], H. Gutermann[b], U. Mees[b]

[a]*Limburgs Universitair Centrum, Universitaire Campus, 3590 Diepenbeek, Belgium*
[b]*Department of Cardiothoracic and Vascular Surgery, Virga Jesse Hospital, B-3500 Hasselt, Belgium*
[c]*Department of Cardiac Anaesthesiology, Virga Jesse Hospital, Stadsomvaart 11, B-3500 Hasselt, Belgium*

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Abstract

Objective: Minimally invasive coronary artery bypass grafting (CABG), carried out on the warm beating heart, does not allow conventional myocardial protection. The objective was to investigate the possibility of enhancing tolerance to ischemia during short episodes of coronary artery occlusion, based on a pharmacological approach using a selective Na^+/H^+-exchange inhibitor (cariporide) or a serine protease inhibitor (aprotinin). **Methods**: Four groups ($n = 6$ in each group) of sheep were subjected to 20 min of normothermic regional ischemia (first lateral branch of the circumflex artery occlusion) followed by 1 h of reperfusion. Regional wall thickening was measured using sonomicrometry, and expressed as the percentage of thickening fraction compared with baseline. Group I was the control with no treatment, group II received cariporide (1 mg/kg administered over 2 min prior to ischemia), group III was treated with aprotinin (2.10^6 kallikrein inactivation units (KIU) load followed by 500.000 KIU/h). Group IV was treated with a combination of cariporide and aprotinin at the same concentrations as in groups II and III, respectively. **Results**: Wall thickening measurements showed that, compared with control, cariporide was largely able to suppress secondary loss of wall thickening after initial recovery during early reperfusion. Wall thickening in the ischemic/reperfused myocardial area improved from 10 ± 31 to 51 ± 17% at 1 h of reperfusion ($P = 0.002$). Aprotinin improved wall thickening at the end of 1 h of reperfusion to 70 ± 13% ($P = 0.0001$). However, in this group, there was a transient loss of regional contractility similar in amplitude and time course to the one observed in the control group. A combination of cariporide and aprotinin suppressed transient contractile loss and resulted in improved wall thickening at the end of 1 h of reperfusion (65 ± 22%, $P = 0.0002$ vs. control). This value was not significantly different from the cariporide ($P = 0.263$) or aprotinin ($P = 0.704$) group. **Conclusion**: These data indicate that both Na^+/H^+-exchange inhibition and aprotinin administration are promising tools for cardioprotection during minimally invasive CABG. A combination of both treatments is able to adequately suppress loss of contractility during early reperfusion as a consequence of reperfusion injury, and results in significantly improved wall thickening at the end of 1 h of reperfusion.

Keywords: Beating heart surgery; Myocardial protection; Na^+/H^+-exchange inhibition; Aprotinin

1. Introduction

Increasing numbers of coronary artery bypass grafting (CABG) cases are carried out as off-pump (OPCAB) procedures. In the STS database, the percentage of OPCAB surgery for coronary surgery in 1999 was 8%. A recent Belgian national survey showed that during 1999, 10% of all coronary surgery was carried out as off-pump procedures, and there was a projected increase of another 5% for the year 2000. A lot of effort has been spent on the development of devices facilitating exposure during beating heart procedures. This has undoubtedly lead to the more widespread acceptance of OPCAB as a valid technique in the surgical community. Much less attention has been paid to means of myocardial protection during those procedures. Minimally invasive coronary surgery, carried out on the beating heart, does not allow conventional myocardial protection, such as cardioplegic arrest, fibrillatory arrest or intermittent aortic cross-clamping.

Temporary occlusion of the coronary artery at the anastomotic site, using either snares or occluding devices, is a commonly used technique during those interventions. Short episodes of coronary occlusion, although not causing irreversible myocardial damage, may result in myocardial

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* Corresponding author. Department of Cardiothoracic and Vascular Surgery, Virga Jesse Hospital, B-3500 Hasselt, Belgium. Tel.: +32-11-309060; fax: +32-11-309068.

E-mail address: marc.hendrikx@virgajesse.be (M. Hendrikx).

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stunning, which may last for a considerable time during reperfusion.

The aim of this study was to investigate the possibility of enhancing the tolerance to ischemia during short episodes of coronary artery occlusion, based on a pharmacological approach.

Calcium-overload during ischemia and reperfusion has been incriminated as one of the major factors causing post-ischemic dysfunction. However, the factors contributing to this calcium-overload and their relative importance remain largely unknown. Increasing evidence points towards substantial calcium-overload secondary to activation of the Na^+/H^+-exchanger during ischemia, and especially, early reperfusion. Inhibition of this exchanger is able to reduce infarct size, both in experimental models and in humans. However, the role of Na^+/H^+-exchange inhibition in reducing stunning remains unclear, and data available to date are scarce. Therefore, we wanted to investigate its role in reducing the negative effects of short-term regional ischemia in a setting mimicking the conditions of minimally invasive CABG surgery. We have used cariporide (HOE642), a selective Na^+/H^+-exchanger subtype I (the prevailing cardiovascular subtype) to inhibit sodium–proton exchange during ischemia and reperfusion.

Aprotinin has well-known antifibrinolytic properties, as well as inhibitory action on the kallikrein–bradykinin system. Furthermore, recent reports have suggested a reduction of myocardial damage following full dose administration of aprotinin prior to aortic cross-clamping in the context of conventional CABG using extracorporeal circulation [1,2]. A possible effect on reversible contractile dysfunction following regional ischemia during OPCAB procedures remains to be elucidated.

2. Materials and methods

The animals used in this study received humane care in compliance with the European Convention on Animal Care. The study was approved by the institutional ethics committee. Twenty-six Suffolk sheep, weighing between 60 and 90 kg were fasted overnight and premedicated with an intramuscular combination of ketamin (10 mg/kg) and midazolam (0.1 mg/kg). They were intubated and ventilated with a mixture of room air and oxygen (6 l), to maintain the arterial pCO_2 between 35 and 40 mmHg and PO_2 between 150 and 200 mmHg. Anesthesia was maintained using fluothane (2–3%). Additional pain relief prior to thoracotomy was achieved using a bolus injection of sufentanil (100 μg). Muscle relaxation was obtained by administering cisatracurium (10 mg bolus). Body temperature was monitored and normothermia was maintained using heating blankets.

Continuous electrocardiogram (EKG) monitoring was carried out throughout the experiments. Precordial leads were monitored using an EKG transducer connected to a Hewlett–Packard console. The peripheral arterial pressure was monitored via a Tygon catheter inserted in the ear artery. A Swan–Ganz catheter (Edwards Laboratories, USA) was inserted via the left jugular vein and directed into the pulmonary artery for pulmonary artery pressure measurements and the measurement of cardiac output. Cardiac output was determined with the thermodilution technique as an average of three successive measurements. Arterial pressure was monitored through a fluid-filled catheter connected to a Statham transducer. The heart rate was measured from the EKG tracings.

A left thoracotomy was performed, the pericardium was opened and the heart was suspended in a pericardial cradle. The first lateral branch of the circumflex artery was identified and encircled proximally using a Prolene 4/0 stitch and a tourniquet.

Ventricular pressure and its first derivative, dP/dt, were recorded by inserting an 18 gauge needle (Terumo) into the left ventricular cavity via the apex. This needle was connected to a pressure transducer via a fluid-filled line.

Changes in systolic wall thickening during coronary occlusion and reperfusion in the ischemic-reperfused circumflex area and the non-ischemic left anterior descending (LAD) region were recorded, using a pulsed Doppler displacement epicardial transducer (Triton Technologies, San Diego, CA), implanted on the myocardial surface, in the perfusion territories of the circumflex artery and the LAD coronary artery. The range gate was set to sample echoes returning from a fixed point in the mid-myocardium set at 10 mm from the epicardial surface. The ultrasonic frequency of the transducer was factory set at 20 MHz with a pulse repetition frequency of 31 250 Hz and a pulse width of 0.4 μs. The echoes are shifted in frequency by an amount proportional to the velocity of the structures passing through the sample window. The reflected velocity signals are integrated in the displacement module to form a displacement signal output. The regional wall thickening was expressed as percent of preocclusion (baseline) values. The recordings of five beats were averaged for each measurement.

Coronary flow in the first lateral branch of the circumflex artery was measured using a small vessel transit time flow probe of appropriate size. Complete occlusion of the vessel was confirmed by the absence of flow recorded by the flow probe and the almost immediate occurrence of paradoxical motion in the ischemic area.

The time constant of isovolumic relaxation, T, was calculated, assuming that the isovolumic pressure decrease between maximum dP/dt− and the time of mitral valve opening approximates a single exponential with a non-zero asymptote. Since the time of mitral valve opening cannot be confidently identified from the left ventricular pressure signal, we used the period from the point of maximum dP/dt− to the time at which pressure decreased to the level of end-diastolic pressure of the previous beat. The exponential method was used since a previous study has

shown this model to closely approximate the measured pressure [3].

Data collection was performed by analog to digital conversion at 500 Hz with 16-bit resolution using the Labview (Labview 4.0, National Instruments, Austin, TX) software system.

After a stabilization period of 15 min, baseline hemodynamic measurements were recorded. The animals were then randomized to one of four groups ($n = 6$ in each group). Group I was the control group. Animals received no treatment, but an equivalent volume of 0.9% sodium chloride as in the other groups. In group II, cariporide at a dose of 1 mg/kg was infused over 2 min prior to ischemia. In group III, aprotinin was administered according to the Hammersmith scheme. A loading dose of 2 million kallikrein inactivation units (KIU) was given, followed by an infusion of 0.5×10^6 KIU/h until the end of the reperfusion phase. Group IV was treated with a combination of cariporide and aprotinin at the same concentrations as in groups II and III, respectively. Hemodynamic and myocardial segment measurements were recorded 15 min after the cariporide bolus injection or the administration of aprotinin loading dose to investigate possible drug effects on baseline parameters. All animals were subjected to a 20 min interruption of circumflex coronary artery blood flow, followed by 1 h of reperfusion. If ventricular fibrillation occurred during ischemia or at the onset of reperfusion, the animals were defibrillated using DC countershock (20 J). No antiarrhythmic treatment was given to any of the animals. Global hemodynamic and regional wall motion variables were recorded throughout ischemia and reperfusion.

Two animals had to be excluded from this study protocol: one in the control group due to incomplete occlusion of the coronary artery and one in the cariporide group due to a technical failure of the Doppler displacement transducer during the reperfusion period.

3. Statistical analysis

Statistical analyses were performed using SAS. The data are reported as means ± SD.

At each time point, the treatment groups were compared using one-way analysis of variance of the regional wall thickening, expressed as a percentage of preocclusion values. All hypothesis tests were performed using a two-sided 5% level of significance. In case a significant treatment effect was noted, pairwise treatment comparisons were performed. In this paper, no adjustments to P values to allow for the multiplicity of statistical testing are reported. At 4 min of reperfusion, one missing measurement in the control group was imputed by the mean.

Besides the classical comparison tests, longitudinal analyses were undertaken in order to study the profiles of the regional wall thickening over time. Our mixed regression model assumed an unstructured variance–covariance matrix and a random intercept. The model included period-specific (ischemic and reperfusion) treatment effects, and period-specific linear and quadratic effects of time. Type III F-statistics were used to evaluate the statistical significance of these fixed effects. Pairwise comparisons between the active treatments and the control group were adjusted for multiple comparison by means of the Dunnett's test.

4. Results

Left ventricular developed pressure, heart rate, end-diastolic pressure, as well as cardiac output and the time constant of isovolumic relaxation (T) during occlusion of the circumflex artery and reperfusion are shown in Table 1. There were no intra- or inter-group differences during ischemia or following reperfusion. Electrocardiographic changes included ST-segment elevation during coronary occlusion, which rapidly reversed upon reperfusion.

The effect of administration of cariporide or bolus injection of 2.10^6 KIU aprotinin on baseline hemodynamic measurements is also shown in Table 1. Neither of the treatments (or the combination of both) had a significant effect on baseline hemodynamic parameters.

During coronary occlusion, the active wall thickening was replaced by paradoxical systolic bulging. There was no treatment effect on this systolic bulging.

During ischemia, the following rhythm disturbances were observed: in the control group, one animal developed a run of ventricular tachycardia at the onset of ischemia, and one animal went into ventricular fibrillation 5 min into ischemia. It was converted to sinus rhythm, using DC countershock (20 J). In the cariporide group, one animal developed bradycardia with multifocal ventricular extrasystoles (VES) at the onset of ischemia. The animals receiving aprotinin only, showed no arrhythmias at the onset of ischemia. One animal had an episode of multifocal VES at 10 min ischemia. In the aprotinin + cariporide group, there were two animals suffering from VES at the onset of ischemia. Generally speaking, the incidence of arrhythmias after occlusion of the coronary artery was limited.

On the other hand, at the onset of reperfusion, arrhythmias were frequent: ventricular fibrillation occurred in two animals in the control group, one animal in the cariporide group, two animals in the aprotinin group and three animals in the aprotinin + cariporide group. All animals were successfully converted to sinus rhythm using DC countershock at 20 J. No antiarrhythmic drugs were administered in any experiment. One animal in the cariporide group developed an episode of ventricular tachycardia, which spontaneously reverted to sinus rhythm.

In the control group, reperfusion was accompanied by an acute trend towards the disappearance of paradoxical movement. However, active wall thickening during systole in the early reperfusion phase could not be observed (percentage

Table 1
Hemodynamic parameters and myocardial function during ischemia and reperfusion[a,b]

		Baseline	Drug	Ischemia (20 min)	Reperfusion (60 min)
LVDP (mmHg)					
	Control	90 ± 15		88 ± 17	91 ± 24
	Cariporide	88 ± 10	86 ± 17	89 ± 12	84 ± 7
	Aprotinin	83 ± 17	84 ± 7	76 ± 10	83 ± 12
	Cari + Apro	82 ± 10	80 ± 15	78 ± 7	78 ± 22
HR (bpm)					
	Control	105 ± 22		111 ± 24	105 ± 20
	Cariporide	105 ± 15	96 ± 15	93 ± 15	97 ± 17
	Aprotinin	108 ± 5	95 ± 20	90 ± 12	92 ± 10
	Cari + Apro	111 ± 17	101 ± 10	100 ± 15	94 ± 15
LVEDP (mmHg)					
	Control	4.5 ± 2.2		3.8 ± 7.8	5.7 ± 11.0
	Cariporide	4.6 ± 5.9	4.8 ± 5.4	6.7 ± 6.4	5.2 ± 4.7
	Aprotinin	9.4 ± 7.8	6.0 ± 2.0	7.7 ± 6.4	8.1 ± 4.9
	Cari + Apro	5.6 ± 2.9	5.6 ± 5.1	4.4 ± 5.9	6.2 ± 4.9
LVdP/dt+ (mmHg/s)					
	Control	1240 ± 314		1084 ± 274	1098 ± 328
	Cariporide	1351 ± 872	1064 ± 652	1130 ± 473	1200 ± 443
	Aprotinin	1252 ± 169	1266 ± 282	1096 ± 164	1026 ± 365
	Cari + Apro	1177 ± 208	1011 ± 142	937 ± 216	1067 ± 240
LVdP/dt− (mmHg/s)					
	Control	1146 ± 314		1023 ± 304	1018 ± 255
	Cariporide	1336 ± 573	1258 ± 534	1097 ± 389	1112 ± 326
	Aprotinin	1146 ± 122	1210 ± 267	960 ± 164	855 ± 277
	Cari + Apro	1043 ± 336	1068 ± 240	974 ± 191	970 ± 262
CO (l/min)					
	Control	5.0 ± 1.0		4.8 ± 1.0	5.2 ± 0.5
	Cariporide	5.1 ± 0.2	5.0 ± 0.2	5.1 ± 0.7	4.9 ± 0.2
	Aprotinin	4.9 ± 0.7	4.9 ± 1.2	5.0 ± 0.5	5.0 ± 0.7
	Cari + Apro	5.2 ± 0.7	5.3 ± 1.0	5.0 ± 0.7	5.1 ± 0.5
T (ms)					
	Control	47 ± 15		41 ± 17	43 ± 20
	Cariporide	44 ± 10	50 ± 10	49 ± 12	46 ± 12
	Aprotinin	44 ± 7	46 ± 24	44 ± 12	50 ± 10
	Cari + Apro	43 ± 7	44 ± 7	41 ± 5	46 ± 12

[a] Data are reported as means ± SD.
[b] LVDP, left ventricular developed pressure; HR, heart rate; LVEDP, left ventricular end diastolic pressure; LVdP/dt, peak rate of change in ventricular pressure; CO, cardiac output; *T*, exponential time constant of isovolumic relaxation; Cari, cariporide; Apro, aprotinin.

wall thickening at 3 min, 2 ± 16%). However, wall thickening then rapidly deteriorated again. The lowest value was observed at 15 min reperfusion (−50 ± 65%). Thereafter, wall thickening in the ischemic area gradually recovered and at the end of 1 h of reperfusion; wall thickening in the ischemic area was 10 ± 31% of the preischemic control.

In the cariporide treated group, paradoxical wall motion also disappeared at the onset of reperfusion. Active wall thickening, however, was not observed. As opposed to the control group, there was no secondary deterioration of wall thickening during reperfusion. When the overall curve during reperfusion was compared with the control group, a trend towards better recovery was observed, which, however, did not reach statistical significance ($P = 0.114$). At the end of 1 h of reperfusion, wall thickening in the ischemic/reperfused myocardial area was 51 ± 17% (unadjusted $P = 0.002$ vs. control; Fig. 1).

The administration of aprotinin resulted in improved recovery of function in the reperfused segment throughout reperfusion ($P = 0.0009$). Active wall thickening was recovered during the first minutes of reperfusion, which resulted in significantly higher wall thickening compared with controls. The percentage wall thickening was 53 ± 15% of the preischemic value at 1 min (vs. −12 ± 48% in control, $P = 0.002$) and 49 ± 22% at 2 min (vs. −16 ± 38% in control, $P = 0.002$). However, also in this group, the regional contractility deteriorated during the course of reperfusion. The nadir was obtained at the same point in time as in the control group: at 15 min reperfusion, active wall thickening had greatly diminished to 16 ± 12%. At the end of 1 h of reperfusion, regional wall thickening recovered to 70 ± 13 vs. 10 ± 31% in controls ($P = 0.0001$; Fig. 2).

When cariporide and aprotinin treatments were combined, wall thickening recovered to 50 ± 26% of the preischemic control at the first minute of reperfusion. This

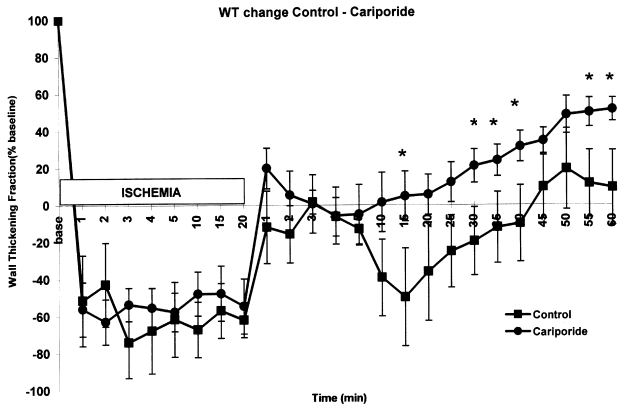

Fig. 1. Wall thickening changes during 20 min ischemia and 1 h reperfusion in the ischemic/reperfused circumflex area in the control group and the group treated with cariporide. Wall thickening in the preischemic phase is taken as 100%. Results are shown as means ± SEM. Asterisks represent significant differences at $P < 0.05$. P values are only descriptive. Note that cariporide was able to improve recovery of wall thickening in the ischemic/reperfused circumflex area. Secondary decline during reperfusion remains largely absent.

value was significantly higher than the recovery in the control group ($P = 0.003$). A decline of wall thickening during reperfusion, as could be observed in the control and aprotinin groups, was absent. Wall thickening throughout reperfusion was significantly improved by the combined treatment as compared with the control group ($P = 0.0004$). At the end of 1 h of reperfusion, the recovery of wall thickening was 65 ± 22% of the preischemic base ($P = 0.0002$ vs. control). This value was not significantly different from either the cariporide (51 ± 17%, $P = 0.263$) or the aprotinin group (70 ± 13%, $P = 0.704$; Fig. 3).

There were no differences in wall thickening changes among or within groups in the non-ischemic LAD perfusion area during coronary occlusion or after re-establishment of coronary blood flow in the circumflex area.

5. Discussion

In this study, we observed important differences in the postischemic regional systolic myocardial performance after the administration of cariporide or aprotinin in clinically relevant doses. The loss of regional contractility is marked after a single episode of ischemia. Preischemic values were not obtained after 1 h of reperfusion. Data on the effect of ischemia on myocardial stunning in the sheep model are scarce. Appleyard and Cohn have published data on pharmacological interventions reducing stunning in the sheep model following coronary occlusion and cardioplegic arrest on bypass [4]. To our knowledge, this is the first paper describing a model of stunning only in the sheep model in an off-pump situation and discussing the effect of pharmacological interventions. The sheep is specific in its coronary anatomy by the complete absence of collateral circulation. Therefore, coronary occlusion immediately leads to severe contractile dysfunction [5]. Since more marked transmural myocardial ischemia occurs after coronary occlusion in species with underdeveloped collateral circulation, it can be expected that severe and lasting myocardial stunning will be observed. However, even in the dog, which is known to have a better developed collateral circulation, severe myocardial stunning is observed after a single coronary occlusion. It is interesting to note that in a subgroup of dogs with collateral flows of <10% of the non-ischemic zone flow, the recovery of thickening fraction at 1 h of reperfusion was similar to the recovery, observed in our study [6].

The duration of ischemia was chosen to be relevant in the

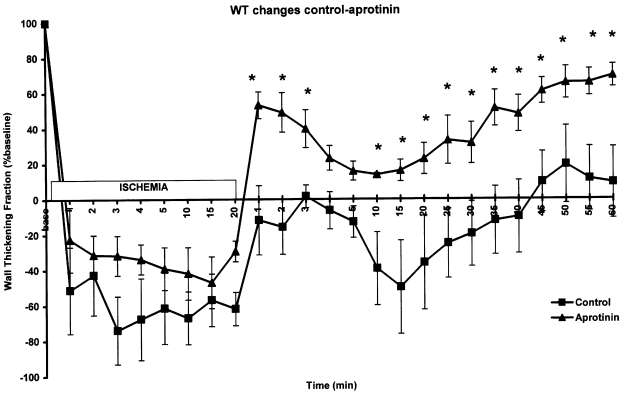

Fig. 2. Wall thickening changes during 20 min ischemia and 1 h reperfusion in the ischemic/reperfused circumflex area in the control group and the group treated with aprotinin. Wall thickening in the preischemic phase is taken as 100%. Results are shown as means ± SEM. Asterisks represent significant differences at $P < 0.05$. P values are only descriptive. Note that aprotinin also improved wall thickening throughout reperfusion. A decline during reperfusion, similar in amplitude and time course as in the control group can be observed.

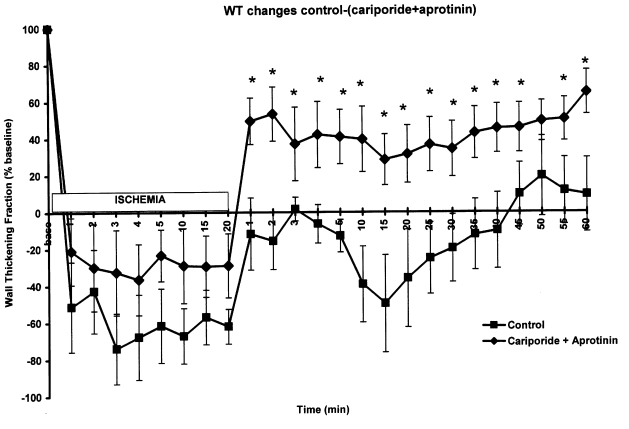

Fig. 3. Wall thickening changes during 20 min ischemia and 1 h reperfusion in the ischemic/reperfused circumflex area in the control group and the group treated with cariporide and aprotinin. Wall thickening in the preischemic phase is taken as 100%. Results are shown as means ± SEM. Asterisks represent significant differences at $P < 0.05$. P values are only descriptive. A combination of both treatments suppresses loss of regional contractile function during reperfusion and results in improved functional recovery.

context of minimally invasive CABG surgery. A recent report indicated that, when robotic telesurgery was used, even after a learning curve, the duration of a coronary anastomosis was still around 20 min [7].

There is substantial evidence showing that Na^+/H^+-exchange inhibition is protective after an extended normothermic ischemia [8]. However, its effect on myocardial stunning is not that well documented. Two previous reports have shown improved functional recovery after repetitive episodes of short-term ischemia in the pig model [9,10]. This study describes the effect of pretreatment with a selective Na^+/H^+-exchange inhibitor on functional recovery after a single episode of regional ischemia in the sheep model. Cariporide (HOE642; 4-isopropyl-3-methylsulfonylbenzoyl-guanidine methanesulphonate) was used as a potent and specific Na^+/H^+-exchange inhibitor. The Na^+/H^+-exchanger has been proposed as an important source of Na^+ entry during ischemia and reperfusion. Suppression of Na^+/H^+-exchange at the moment of reperfusion has been shown to be protective. Several investigators have reported that staged [11] or acidotic [12] reperfusion lessens myocardial stunning. Crucial to the argument for a role for the Na^+/H^+-exchanger in ischemic/reperfusion injury is the acidic intracellular environment that occurs during ischemia. Studies in isolated heart preparations have shown a rapid decline of the intracellular pH during ischemia, which can be as low as pH 6.0 [13,14]. Reperfusion washes out the extracellular space and creates a H^+ gradient across the sarcolemmal membrane. These conditions stimulate the Na^+/H^+-exchanger to remove H^+ from the cell in exchange for extracellular Na^+. This process will, in turn, stimulate the Na^+/Ca^{2+}-exchange pathway to remove Na^+ for extracellular Ca^{2+}. This ultimately leads to cellular Ca^{2+}-overload. Therefore, it can be expected that Na^+/H^+-exchange plays an important role in the occurrence of cardiac dysfunction during ischemia/reperfusion.

It should be noted however, that cariporide also partially inhibits the veratridine and lysophosphatidylcholine induced slowly inactivating component of the sodium current in isolated ventricular myocytes. Therefore, part of its protective action may originate from this non-selective action [15].

Improved systolic function in the cariporide group could, in part, be explained by the fact that, as opposed to the control group, there was no secondary deterioration of systolic function during reperfusion. The initial overshoot of systolic function in the control group may be explained by the fact that contractile function is dependent not only on free cytosolic Ca^{2+}, but also on Ca^{2+} sensitivity of contractile proteins. Intracellular alkalinization has a potent sensitizing effect on tension development [16]. Studies using ^{31}P-NMR spectroscopy in buffer perfused heart have established that Na^+/H^+-exchange blockers delay intracellular alkalinization during reperfusion [17]. An alkaline overshoot during early reperfusion was previously described by our group in isolated blood perfused rabbit hearts [14], and could account for the fact that in the control group after initial recovery, systolic function progressively deteriorated again with normalization of intracellular pH. Furthermore, several studies have shown a significant delay of realkalinization in hearts treated with selective Na^+/H^+-exchange blockers [13,14]. Intracellular acidosis during the early phase of reperfusion can protect the myofilaments against reperfusion injury [18] and may explain why a secondary decline of systolic function was not observed in cariporide pretreated animals.

The protective effect of aprotinin on the myocardium has previously been documented in the context of cardiopulmonary bypass (CPB) when the major endpoint was cardiac troponin release as a marker of myocardial damage [1,2]. Its protective action has also been demonstrated as infarct size limitation, both in the isolated globally ischemic rat heart [19] and in the 'in vivo' model of regional ischemia in the dog [20]. A reduction of postischemic myocardial stunning after short-term ischemia (15 min) was reported in the dog model [21].

In the group treated with aprotinin, contractility in the reperfusion period was better preserved than in the control group, although a secondary deterioration of contractile function persisted. This finding is consistent with the fact that aprotinin is unable to suppress an alkaline overshoot in the early reperfusion phase, as cariporide does. Hallett and colleagues [22] have shown that aprotinin can inhibit the production and release of free radicals from activated neutrophils. Activated neutrophils produce the superoxide radical via the NADPH oxidase system and H_2O_2 via dismutation of superoxide. Since hydrogen peroxide formation has been implicated in the development of stunning, its inhibition by aprotinin administration may result in improved postischemic function.

Furthermore, there is experimental evidence in cardiac sarcolemmal vesicles that Na^+/Ca^{2+}-exchange activity is stimulated in the presence of superoxide radicals and H_2O_2 [23]. Since aprotinin is able to reduce the production of oxygen free radicals, Ca^{2+}-overload may be suppressed via this pathway.

In the group treated with cariporide and aprotinin, the beneficial effects of both treatments were combined: secondary deterioration of contractile function was negligible and contractility was higher than in the control group throughout the reperfusion period. There are data available that indicate that the decreased Ca^{2+} responsiveness of stunned myocardium is due to intrinsic alterations of the myofilaments. This could account for the fact that even in the buffer perfused isolated rat heart model, aprotinin was able to reduce the extent of myocardial damage [24]. Ca^{2+} activated protease activity, such as calpain I, decreases the Ca^{2+} responsiveness of the cardiac myofilaments by reperfusion induced Ca^{2+}-overload [25]. This may be a site where the protective action of cariporide and aprotinin converge, since cariporide is able to reduce Ca^{2+}-overload via Na^+/H^+-exchange and Na^+/Ca^{2+}-exchange inhibition, hence inhibiting the Ca^{2+} activated protease activity,

whereas aprotinin, as a non-specific protease inhibitor, may directly interfere with Ca^{2+} activated proteases. This, together with a common inhibition of the Na^+/Ca^{2+}-exchanger, may explain why at the end of the reperfusion period, functional recovery in the group treated with cariporide and aprotinin is not significantly different from the groups in which cariporide or aprotinin only was administered.

The fact that regional contractility did not recover to preischemic values despite the administration of cardioprotective drugs warrants further investigation in this field.

6. Conclusion

Our study shows that functional recovery from myocardial ischemia–reperfusion injury after short-term ischemia at normothermia can be improved both by pretreatment with a Na^+/H^+-exchange blocker and the administration of aprotinin. Therefore, both treatments may be a useful strategy for pharmacological myocardial protection in the context of off-pump beating heart surgery. Combining administration of cariporide and aprotinin will result in suppressed loss of contractility during early reperfusion and better preserved wall thickening at the end of 1 h of reperfusion in the ischemic/reperfused area.

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