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European Journal of Pharmacology 528 (2005) 124–131
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Calpain inhibition reduces infarct size and improves global hemodynamics and left ventricular contractility in a porcine myocardial ischemia/reperfusion model

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Received 1 August 2005; received in revised form 27 September 2005; accepted 7 October 2005

Abstract

Calpains, a family of Ca^{2+}-dependent cysteine proteases, are activated during myocardial ischemia and reperfusion. This study investigates the cardioprotective effects of calpain inhibition on infarct size and global hemodynamics in an ischemia/reperfusion model in pigs, using the calpain inhibitor A-705253.

The left anterior descending coronary artery was occluded for 45 min and reperfused for 6 h. A bolus of 1.0 mg/kg A-705253 or distilled water was given intravenously 15 min prior to induction of ischemia and a constant plasma level of A-705253 was maintained by continuous infusion of 1.0 mg/kg A-705253 during reperfusion. Infarct size was assessed histochemically using triphenyltetrazolium chloride staining. Macromorphometric findings were verified by light microscopy on hematoxylin–eosin- and *Tunel*-stained serial sections. Global hemodynamics, including the first derivate of the left ventricular pressure (dP/dt_{max}), were measured continuously throughout the experiment.

A-705253 reduced the infarct size by 35% compared to controls ($P<0.05$). Hemodynamic alterations, including heart rate, aortic blood pressure, central venous pressure and left atrial pressure, were attenuated mainly during ischemia and the first 2 h during reperfusion by A-705253. Cardiac function improved, as determined by dP/dt_{max}, after 6 h of reperfusion ($P<0.003$).

Our results demonstrate that myocardial protection can be achieved by calpain inhibition, which decreases infarct size and improves left ventricular contractility and global hemodynamic function. Hence, the calpain–calpastatin system might play an important pathophysiological role in porcine myocardial ischemia and reperfusion damage and A-705253 could be a promising cardioprotective agent.

Keywords: Cardiovascular; Heart; Ischemia and reperfusion; Myocardial infarction; Cysteine protease; Calpain inhibitor

1. Introduction

Intracellular acidosis caused by myocardial ischemia results in a cytosolic increase in Na^+ and subsequent exchange of Na^+ by extracellular Ca^{2+}—a possible key event in myocardial cell damage (Tany and Neely, 1989). Evidence has been provided that cellular Ca^{2+}-overload leads to an overactivation of calpains, initiated by autoproteolysis of a N-terminal peptide (Tolanai and Korecky, 1986; Steenbergen et al., 1990; Mellgren, 1980). Calpains, i.e. Ca^{2+}-activated neutral cysteine proteases, are found in most mammalian tissues (Goll et al., 1999), and their activation is required for many cellular processes of a unidirectional nature, such as mitosis, cell differentiation and protein turnover (Wang and Yuen, 1999). However, uncontrolled activation is presumed to be involved in the pathogenesis of cerebral, cardiac and hepatocellular ischemia (Wang and Yuen, 1994; Liebertau et al., 1990). A growing body of literature has demonstrated various morphological alterations related to the proteolytic activation of calpain following

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doi:10.1016/j.ejphar.2005.10.032

ischemia and reperfusion. Calpains are presumed to be involved in myocardial reperfusion injury, cardiac hypertrophy and myocardial stunning (Iizuka et al., 1993; Yoshida et al., 1995; Arthur and Belcastro, 1997). Several cytoskeletal proteins are degraded, and protein kinases and phosphatases cleaved by calpain (Goll et al., 1999, 2003). Trumbeckaite et al. (2003) recently demonstrated, in isolated rabbit hearts, that calpain inhibition attenuated mitochondrial injury secondary to ischemia and reperfusion. Different synthetic and endogenous calpain inhibitors have been investigated for their therapeutic potential in various ischemia-related diseases (Wang and Yuen, 1994; Urthaler et al., 1997; Iwamoto et al., 1999). However, problems of low calpain specificity, limited cell membrane permeability, cell toxicity and difficulties in administration were encountered with several inhibitors. More recently, a new water-soluble, selective, membrane-permeable calpain inhibitor A-705253 (recently renamed by the manufacturer and formerly CAL 9961) has been demonstrated to reduce both calpain isoforms significantly in a rodent myocardial infarction model (Sandmann et al., 2002; Lubisch et al., 2003). We previously demonstrated, in an isolated heart model, that this inhibitor protects myocardial and mitochondrial function in post-ischemic reperfusion (Neuhof et al., 2003). However, its relevance, in terms of functional and morphological organ protection in an intact large animal model, needs to be clarified. The present study addresses these issues, using the calpain inhibitor A-705253 (Abbott, Ludwigshafen, Germany; Lubisch et al., 2003), in a randomised, vehicle-controlled porcine ischemia and reperfusion model.

2. Material and methods

2.1. Study design

Domestic farm pigs (18–22 kg) of the same breed were obtained from the Teaching and Research Farm of the University of Munich (Germany). The experimental protocol was approved by the Animal Care and Use Committee of the Government of Upper Bavaria in Munich and was in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Six animals were used for validation of the experimental protocol and subjected to the described procedures. However, they did not undergo regional myocardial ischemia (sham procedure, $n=3$) or reperfusion ($n=3$) to evaluate the impact of the protocol on hemodynamics and resulting myocardial infarction. Twenty-one animals were used for the prospective randomised vehicle-controlled study.

2.2. Anesthesia and experimental protocol

The pigs were premedicated with a combination of azaperone (1.8–2.2 mg/kg), ketamine (14.0–17.0 mg/kg) and atropine (0.01 mg/kg) by intramuscular injection. A bolus of fentanyl (6.0 μg/ kg) and midazolam (0.4 mg/kg) was given into a lateral ear vein for induction of anesthesia followed by continuous infusion of 25.0 μg/kg/h fentanyl and 1.0 mg/kg/h midazolam. Pancuronium bromide (0.2 mg/kg/h) was used for neuromuscular blockade. Animals were ventilated mechanically using a 900C respirator (Siemens Elma AB, Sweden) after orotracheal intubation. Body temperature was kept constant using heating blankets and monitored by a rectal temperature probe. For anticoagulation, heparin (100 IU/kg/h) was administered intravenously and monitored by repeated measurements of the activated clotting time. A standard lead of ECG (electrocardiogram) was monitored throughout. During early reperfusion, drops of lidocaine 1.0% were intermittently applied topically, as a prophylaxis for vasospasm, at the level where the left anterior descending coronary artery manipulation occurred. Otherwise, no antiarrhythmic agents were used. Direct electric countershocks (CodeMaster 100; Hewlett Packard, USA) were used to terminate ventricular fibrillation. The pediatric paddles were placed carefully outside the area at risk.

Experiments were performed on the animal in a supine position. Multi-purpose catheters were inserted into the groin and neck vessels for blood sampling and cardiovascular monitoring (Statham® Pressure Transducer, Viggo-Spectramed, Ohmeda, Germany; Sirecust monitor unit, Siemens, Germany). A 5-French Mikro-Tip®-Catheter (Millar Inc., USA) was inserted via the carotid artery into the left ventricle for measuring dP/dt_{max}, using a RS transducer and differentiator (Gould-Statham Instruments, USA), and the jugular vein for central venous pressure monitoring. A midline sternotomy was performed: the pericardium opened in longitudinal fashion and a catheter inserted through the left atrial appendage to monitor the left atrial pressure. A resting period of 30–60 min was allowed after the end of surgery and baseline physiological variables determined. The left anterior descending coronary artery was occluded by a 3-0 suture (Vicryl®; Ethicon, Germany) and a silicon tourniquet. The left anterior descending coronary artery was occluded, after 40–50% of its total length measured from its origin, for 45 min followed by a reperfusion period of 6 h. For reperfusion, the tourniquet was opened but left in place. Finally the animal was sacrificed and the heart excised.

Criteria for sufficient ischemia were: (i) no residual blood flow distal to the occlusion, (ii) no visible collateral flow into the risk region, (iii) myocardial fading, (iv) prestenotic left anterior descending coronary artery dilatation in terms of flow redistribution, and (v) typical ECG changes; and for sufficient reperfusion (i) refilling and reflow of the left anterior descending coronary artery distal to the former occlusion, (ii) recoloration of the myocardium in the risk region, (iii) no prestenotic dilatation, (iv) resolution of ECG changes.

2.3. Slicing and staining of the hearts

Excised hearts were perfused with 0.9% saline first. Thereafter, the tourniquet around the left anterior descending coronary artery was tightened again and the non-risk region stained, pressure- and volume-controlled, with 0.5% Evans blue dye (Sigma-Aldrich Chemie, Germany). Evans blue stains the perfused myocardium blue while the occluded vascular bed remains uncoloured, thus allowing the differentiation between non-risk region and area at risk. The hearts were frozen at −20

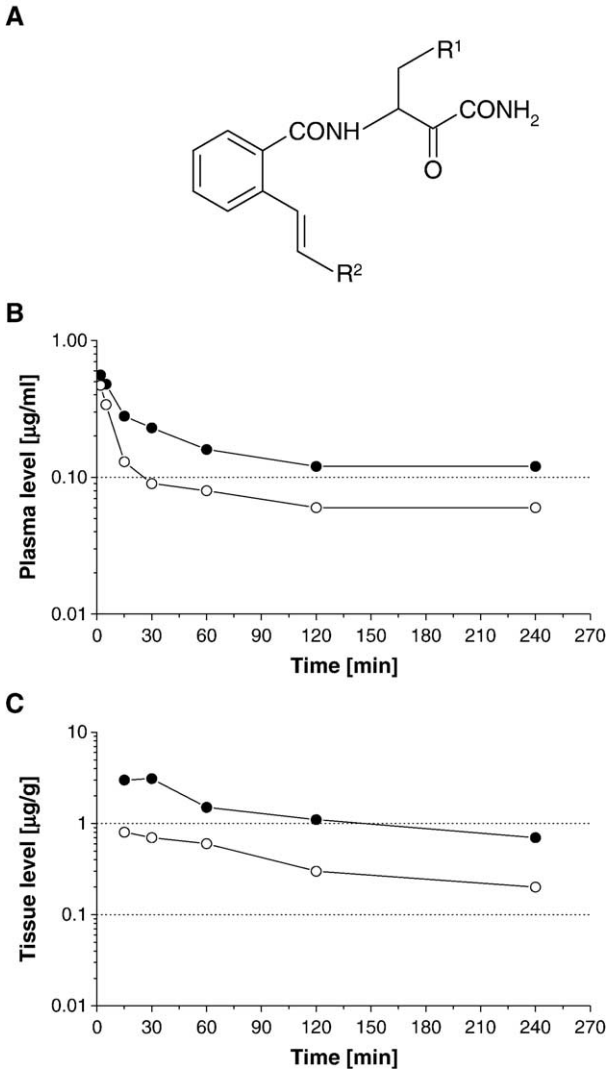

Fig. 1. Structural formula of the calpain inhibitor A-705253 and kinetics. Compound with ketoamide as reactive moiety. R^1, *phenyl*; R^2, *4-(C_6H_4)-$CH_2N(CH_2CH_3)_2$* (A). Inhibitor plasma (B) and tissue (C) levels after i.v. administration of either 1.0 (●) or 0.3 mg/kg (○), (n=6).

°C for 24 h and thereafter cut into 5-mm thick slices from the apex towards the atrioventricular groove. All slices were incubated in phosphate-buffered (pH 7.40) 0.1 M 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich Chemie) solution at 37 °C for 20 min. Tetrazolium stained the myocardium red due to intact dehydrogenase activity, while infarcted myocardium lacking dehydrogenase activity remained unstained. Slices were fixed with 4.0% paraformaldehyde (Merck, Germany) for 30 min to bleach the tissue and extravasated blood. Apical and basal view of all slices was photographed (Coolpix 950 digital camera; Nikon, Japan) for planimetric evaluation.

2.4. Micromorphometric correlation of histochemical results

Slices of six experiments (four from calpain inhibitor- and two from vehicle-treated animals) were evaluated. From each slice, transition regions of infarct area/area at risk and area at risk/no-risk regions from the left ventricular free wall and septum were cut out, embedded in paraffin, serially sectioned and stained with hematoxylin–eosin and *Tunel*.

2.5. Experimental drug protocol

A-705253 is a selective benzoylalanine-derived ketoamide (M_r ~1725 Da) calpain inhibitor (Fig. 1A) with a K_i value for calpain I of 27.0 nM (Lubisch et al., 2003). The inhibitor was dissolved in $NaHCO_3$-buffered distilled water. A-705253 porcine plasma (Fig. 1B) and myocardial tissue levels from the left ventricular free wall (Fig. 1C) were assessed in pilot kinetic experiments by the manufacturer of the compound in a high-performance liquid chromatographic (HPLC)-based assay. Inhibitor concentration in the plasma was 0.16 µg/ml at 1 h and 0.12 µg/ml at 2 and 4 h, respectively, after i.v. administration of 1.0 mg/kg. The A-705253 content in the infarcted area of the left ventricular free wall after intravenous administration of 1.0 mg/kg was 1.5 µg/g at 1 h, 1.1 µg/g at 2 h and 0.7 µg/g at 4 h, which corresponds to tissue concentrations of 2.7, 1.9 and 1.2 µM, respectively. A calpain inhibitor tissue concentration of 1.0 µg/g reduced cardiac calpain, significantly (Moeller, Abbott, Ludwigshafen, Germany, personal communication).

Animals assigned to the calpain inhibitor group were given a bolus of 1.0 mg/kg A-705253 i.v. 15 min prior to ischemia and 1.0 mg/kg/h during reperfusion, based on these kinetic experiments, while controls received the vehicle solution.

2.6. Statistics analysis

One investigator, blind to the experimental drug protocol, traced all slices for infarct size measurement using Image Tool 2.0 software package (University of Texas, San Antonio, TX, USA). Apical and basal views of all slices containing areas at risk were traced, divided by two and multiplied by the slice thickness for left ventricular infarct volume calculation. Measurements were expressed as mean±S.E.M. Data were proved for normal distribution and, if appropriate, a two-tailed t-test was used for comparison of means between groups. Planimetric data were evaluated using covariance analysis. Infarct size, as well as the area at risk of the left ventricle, was

Table 1
Survival and ventricular fibrillation

Variable	A-705253	Vehicle
Animals prepared for the experiment	11	10
Fatal	1	0
Death from ventricular fibrillation	0	0
Animals finally evaluated	10	10
Animals with non-fatal ventricular fibrillation	3	2
Ventricular fibrillation episodes	3	2
During ischemia	2[a]	1[b]
During reperfusion	1[c]	1[d]
Number of attempts at defibrillation	7[e]	3[e]
Energy (J)	10, 20, 30	10, 20
Death from ventricular fibrillation	0	0

Absolute numbers of survivors are given. [a]30, 45 min, [b]30 and 45 min (two episodes), [c]1 min, [d]2 min, [e]all defibrillations were eventually successful.

plotted against the area at risk and entire left ventricular area, respectively, and risk size or left ventricular size was used as a covariate when infarct size or area at risk was analysed by ANOVA (*Analysis of Variance*) among groups. For comparison of dP/dt_{max} values, we also used ANOVA. A Bonferroni post hoc test for multiple testing was also be performed, when appropriate. A two-sided test at an α-level of 0.05 rejected alternative hypotheses and was considered statistically significant.

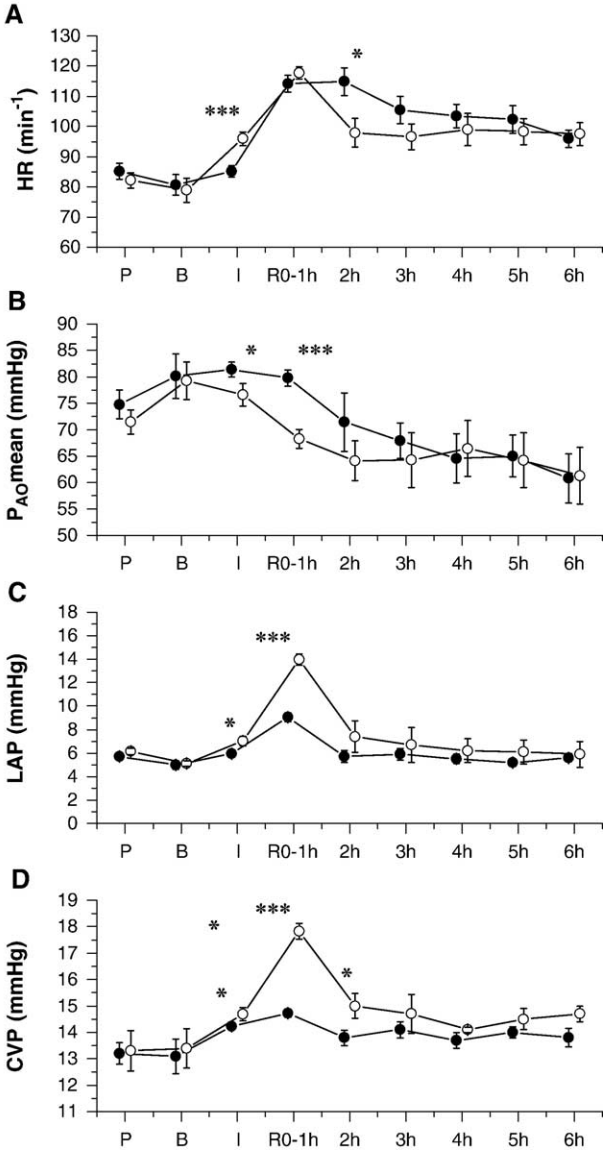

Fig. 2. Effect of calpain inhibition on global hemodynamic changes during ischemia and reperfusion in pigs subjected to 45 min ischemia and 6 h reperfusion, treated either with the calpain inhibitor A-705253 (●) or vehicle solution (○) ($n=10$). Values are expressed as mean±S.E.M.; $*P<0.05$, $***P<0.001$. HR, heart rate (A); P_{AOmean}, mean aortic pressure (B); LAP, left atrial pressure (C), and CVP, central venous pressure (D). Time axis: P indicates *during preparation*; B, *at baseline*; I, *during ischemia*, and R, *reperfusion*. During preparation (2 measurements within 5 min each) measurements were acquired (i) after insertion of the arterial catheter (except for CVP and LAP), (ii) 5 min before sternotomy (except for LAP), (iii) 10 min after sternotomy. Baseline values were measured at the end of the rest period (at 25 and 30 min). During ischemia and 1 h of reperfusion variables were measured twice in intervals of 5 min. During 2–6 h of reperfusion means of two measurements within 5 min were recorded.

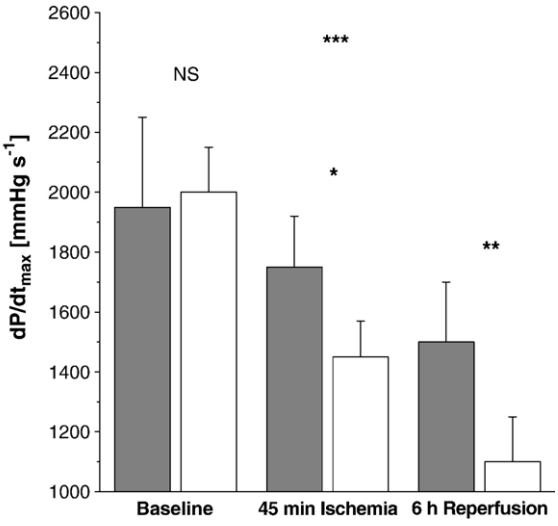

Fig. 3. Effect of calpain inhibition on left ventricular contractility after 45 min of ischemia and 6 h of reperfusion ($n=10$). Grey bars (■) indicate the calpain inhibitor A-705253 and white bars (□) the vehicle-treated group. Baseline values were recorded during the resting period prior to induction of regional myocardial ischemia. dP/dt_{max} indicates first derivate of left ventricular pressure. Values are given as mean±S.D.; $***P<0.001$; $**P<0.003$, $*P<0.05$; NS, not significant.

3. Results

3.1. Validation of the experimental protocol

Surgical manipulation did not lead to arrhythmias or hemodynamic depression over time, as measured by dP/dt_{max} and global hemodynamics, in animals that did not undergo ischemia. The infarct creation procedure, by placing a suture around the left anterior descending coronary artery, did not result in detectable myocardial infarction, if the tourniquet was not tightened, as verified histochemically. However, ischemia without reperfusion resulted in an almost complete infarction of the incorporated area and severe hemodynamic depression, as verified by dP/dt_{max} and global hemodynamics.

3.2. Explorative study with the calpain inhibitor A-705253

One of 21 animals died before creation of ischemia due to technical problems. The remaining 20 pigs survived to the end of the experiment and were used for evaluation.

3.3. Effect of calpain inhibition on arrhythmias

Rhythm disturbances were characterized by a sudden increase in heart rate during late ischemia and early reperfusion. In most experiments, reperfusion induced tachyarrhythmias. Two of the 10 control animals had three episodes of ventricular fibrillation compared to three episodes in three of the 10 animals

treated with the calpain inhibitor ($P>0.05$). Sinus rhythm could be restored immediately in all cases using direct electric countershocks (Table 1).

3.4. Effect of calpain inhibition on global hemodynamics and left ventricular contractility

Hemodynamic changes, including heart rate, mean aortic pressure, central venous pressure and left atrial pressure, are summarised in Fig. 2A–D. At baseline, no differences were observed between groups. Regional myocardial ischemia resulted in depressed cardiac function in both groups. In comparison to baseline, heart rate remained elevated ($P<0.001$) and mean aortic pressure depressed ($P<0.05$), whereas central venous pressure and left atrial pressure ($P>0.1$) reached almost baseline values within both groups. Differences between groups were seen during ischemia and after 2 h of reperfusion. The increase in heart rate during ischemia was less pronounced in the calpain inhibitor-treated group compared to control group ($P<0.001$ and $P<0.001$, respectively). The mean aortic pressure was found to be higher during ischemia and after 1 h of reperfusion in the treatment group ($P<0.05$ and $P<0.001$, respectively). Similar results, except the time frame, were found for the left atrial pressure ($P<0.001$ and $P<0.05$, respectively). At baseline, dP/dt_{max} did not differ significantly between groups. The left ventricular contractility was depressed after 45 min of regional ischemia in both groups with a significant lower mean in the control group ($P<0.05$). After 6 h of reperfusion, dP/dt_{max} was depressed in both groups ($P<0.001$). However, calpain inhibition attenuated the loss of contractility compared to controls, significantly ($P<0.003$) (Fig. 3).

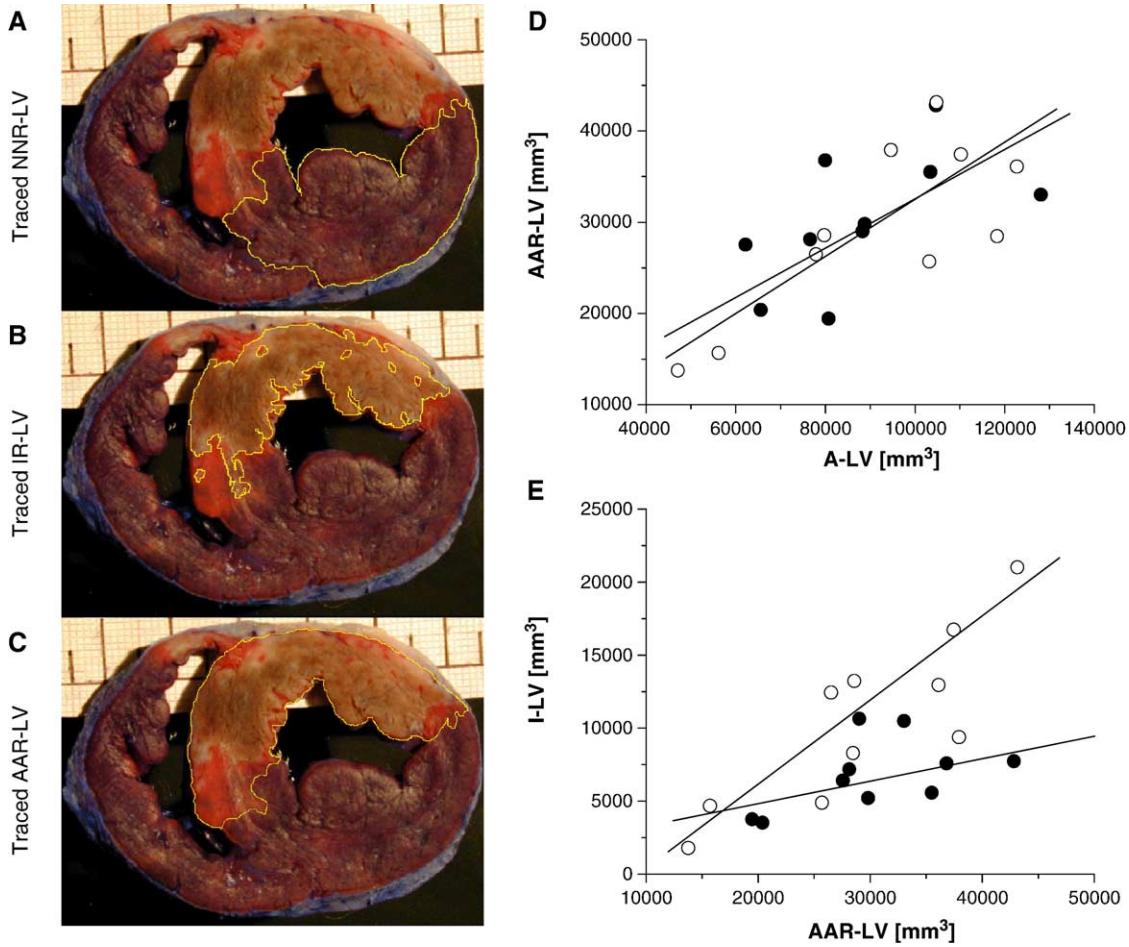

Fig. 4. Representative heart slices after histochemical staining for planimetric left ventricular infarct size measurement (A–C). Apical view of heart slices (5 mm thick). In general apical and basal view of 9–12 slices were evaluated. Evans blue stained the tissue blue thus indicating the non-risk region and, therefore, marks the border to the area at risk. The area at risk comprises the entire heart muscle area which underwent 45 min of ischemia and 6 h of reperfusion. Within this area the red coloured region represents myocardial tissue covered by reperfusion based on an intact dehydrogenase activity (triphenyltetrazolium stain). Myocardium lacking dehydrogenase activity remains unstained (white/brown), indicating infarcted myocardium. The brown colour is due to formaldehyde. The yellow lines of demarcation indicate the border for plane calculation of the different areas. Regression plot of planimetric infarct size measurement. Animals were treated with calpain inhibitor A-705253 (●) or received vehicle solution (○). Area at risk plotted against the entire left ventricular area (D): $y=0.21x+2366.8$, $R^2=0.34$; $y=0.30x+509.1$, $R^2=0.61$ for A-705253 and vehicle, respectively ($n=10$, $P>0.8$). AAR-LV indicates area at risk of the left ventricle and A-LV the entire left ventricular area. Left ventricular infarct size plotted against area at risk (E): $y=0.52x-969.81$, $R^2=0.73$; $y=0.18x+292.73$, $R^2=0.27$ for vehicle and A-705253, respectively ($n=10$, $P<0.009$). I-LV indicates left ventricular infarct size, AAR-LV left ventricular area at risk. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

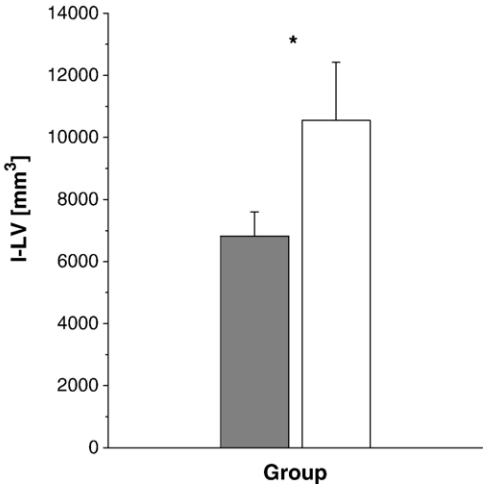

Fig. 5. Effect of calpain inhibition on absolute left ventricular infarct volume. The grey bar (■) indicates the calpain inhibitor-treated and the white bar (□) the vehicle-treated group. Data are presented as mean±S.E.M. (n=10, *P<0.05).

3.5. Effect of calpain inhibition on infarct size

Heart weights did not differ between groups (113.3±3.7 vs. 119.5±2.6 g, P>0.1). All slices containing left ventricular tissue were traced for left ventricular size measurement—in general, 9–12 slices contained the area at risk. Representative stained heart slices, including yellow lines of demarcation for plane calculation of the different areas, are shown in Fig. 4A–C. Electric countershocks for ventricular fibrillation did not affect later infarct size measurement if the paddles were placed carefully outside the area at risk. Total left ventricular volume (87,813±6,268 vs. 91,485±8,100 mm^3, P>0.7) and area at risk (30,254±2,269 vs. 29,330±3,033 mm^3, P>0.8), distal to the ligation, did not differ between groups, as well as risk size, when plotted against the left ventricle (P>0.8) (Fig. 3D). Calpain inhibition reduced the percentage of the infarct volume at the area at risk (33%, P=0.009) and the total infarct volume (35%, 6,820 vs. 10,549 mm^3, P<0.05), as shown in Figs. 4E and 5.

3.6. Micromorphometric correlation of histochemical results and detection of myocardial apoptotic cell death

Hematoxylin–eosin- and *Tunel*-stained serial sections of transition regions of infarct area/area at risk and the area at risk/no-risk regions confirmed histochemical findings, in terms of a distinct separation between the different areas.

4. Discussion

We have investigated the potential effects of A-705253, a novel, water-soluble, cell membrane-permeable calpain inhibitor, on infarct size, left ventricular contractility and hemodynamic function in ischemic reperfused porcine hearts. This compound has recently been demonstrated to reduce calpain I and II activity significantly in chronically infarcted rat myocardium (Sandmann et al., 2002). The infarct size in experimental regional myocardial ischemia/reperfusion is known to be influenced by various factors, including the model itself, duration of ischemia/reperfusion, myocardial and core temperature, size of the ischemic area and loading condition of the heart (Pich et al., 1988; Chien et al., 1994; Duncker et al., 1996; Birnbaum et al., 1997; Schwartz et al., 1997; Rappaport, 2000). The advantage of using a porcine model, in comparison to others, is the very low residual blood flow via collateral vessels into the area at risk (Sjörquist et al., 1984). Infarct size-influencing factors were kept constant in all experiments. Triphenyltetrazolium staining, a well described and widely utilised method, was used for detection of myocardial infarction (Doerr, 1950; Nachlas and Shnitka, 1963; Klein et al., 1981). The applied inhibitor did not influence the staining procedure, as confirmed by histology. The infarct size, created in the present study is large compared to the literature on porcine ischemia models with no residual blood flow. Therefore, we were able to trace a larger number of heart slices (9–12 slices) in comparison to literature reports (4–6 slices), which is especially remarkable with regard to reliable results (Klein et al., 1995; Rohmann et al., 1995). According to Klein et al. (1991), electric countershocks do not affect infarct size when applied outside the ischemic area. Therefore, ventricular fibrillation was not an exclusion criterion.

According to Rappaport (2000), calpain appears to be a relevant candidate for myofilament proteolysis following ischemia and reperfusion. Electron microscopic studies have shown similar structural alterations in the ischemic myocardium when compared to those caused by calpain digestion of myofibrils. Consequently, it has been concluded that a major portion of tissue damage in the infarcted myocardium may be due to overactivation of calpain (Goll et al., 1999). Indeed, a growing body of evidence has demonstrated numerous effects of calpains in various in vivo and vitro models (Iizuka et al., 1993; Goll et al., 1999; Iwamoto et al., 1999; Trumbeckaite et al., 2003). Using cardiomyocytes, Papp et al. (2000) recently demonstrated a major reduction in maximal isometric force and degradation of desmin caused by calpain. Other studies showed that calpain inhibition prevented troponin I degradation in ischemic rats (Gao et al., 1997). Gao et al. (1996) demonstrated, in skinned cardiac muscle, that the maximal Ca^{2+}-activated force, as well as Ca^{2+}-sensitivity, decreased after administration of calpain I. In contrast, Atsma et al. (1995) claimed that the calpain I inhibitor does not attenuate cell death of cardiomyocytes during metabolic inhibition and, therefore, does not play a major role in apoptosis.

Myocardial ischemia is characterized by an intracellular increase in Ca^{2+}, and it has long been recognized that prophylactic administration of calcium antagonists reduces infarct size and improves functional recovery (Lefer et al., 1979), as well as preventing left ventricular hypertrophy (Linz et al., 1990) in myocardial ischemia/reperfusion and hypertension, respectively. Moreover, evidence has been provided that the blockade of Ca^{2+} channels decreases myocardial necrotic and apoptotic cell death, and improves contractile function. (Gao et al., 2001; Liu et al., 2004). Calpains are activated by intracellular Ca^{2+} and evidence suggests their involvement in

myocardial ischemia and reperfusion injury (Iizuka et al., 1993; Yoshida et al., 1995; Arthur and Belcastro, 1997). In recent years, various calpain inhibitors have been considered for their therapeutic potential, but none of them proved to be a suitable candidate. In ischemic reperfused rat hearts, calpain inhibitor I reduced infarct size when given prior to ischemia, whereas post-ischemic myocardial function did not improve (Wang and Yuen, 1994; Perrin et al., 2003). Urthaler et al. (1997) demonstrated that calpain inhibition, using another compound (MDL-28170), attenuates the effects of mechanical stunning in reperfused ferret hearts. To our knowledge, the present study provides the first direct evidence of the effectiveness of calpain inhibition on infarct size, using an intact large animal model. Moreover, in our study, calpain inhibition by A-705253 was shown not only to reduce infarct size, but also to improve hemodynamic and contractile function, compared to previous reports (Wang and Yuen, 1994; Perrin et al., 2003). The infarct size was significantly reduced and the decrease in dP/dt_{max} at 6 h of reperfusion was significantly attenuated by calpain inhibition compared to vehicle. However, tachyarrhythmias and ventricular fibrillation were not influenced by this calpain inhibitor.

In spinal cord injury, Banik et al. (1997) found significantly elevated calpain levels 1 h (70%) and a maximum of 4 h (90%) after injury compared to controls. In chronically infarcted rat myocardium, Sandmann et al. (2002) measured the highest calpain I values and calpain II activity 14 and 3 days post-myocardial infarction, respectively. Studies on cerebral ischemia have suggested a 6-h window of therapeutic opportunity following the onset of ischemia during which calpain inhibition prevented tissue damage (Markgraf et al., 1998). This potential therapeutic window may reflect the major difference and advantage of calpain inhibition in attenuating myocardial ischemia and reperfusion-induced cell damage compared to other recently used potential compounds, which demonstrate effectiveness predominately when administered before or during ischemia (Wang and Yuen, 1994; Klein et al., 1995). However, in the present experiments, the calpain inhibitor A-705253 was administered before ischemia and during reperfusion to investigate whether myocardial protection could be achieved in an intact large animal model. Whether A-705253 acts cardioprotectively when administered during ischemia or at the onset of reperfusion remains to be investigated.

Acknowledgements

This study was supported in part and the calpain inhibitor A-705253 supplied by Abbott GmbH and Co. KG, Ludwigshafen, Germany. We thank Drs. A. Moeller and W. Lubisch from Neuroscience Discovery Research, Abbott GmbH and Co. KG, Ludwigshafen for helpful discussion.

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