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European Journal of Pharmacology 494 (2004) 205–212

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Sustained protective effects of 7-monohydroxyethylrutoside in an in vivo model of cardiac ischemia–reperfusion

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Received 26 February 2004; received in revised form 6 May 2004; accepted 11 May 2004

Abstract

Earlier studies have shown that 7-monohydroxyethylrutoside (monoHER), an antioxidant flavonoid, protects against doxorubicin-induced cardiotoxicity. In this study, we investigated potential sustained cardioprotective effects of monoHER in a model of ischemia–reperfusion (I/R) in mice. Ischemia was induced for 30 min by ligating the left anterior descending coronary artery. Afterwards, the ligature was removed and reperfusion was allowed for 6 or 24 h or 2 weeks. MonoHER (500 mg/kg) was given intraperitoneally (i.p.) one hour before ischemia. Treatment with monoHER significantly attenuated myocardial neutrophil influx both at 6 and 24 h after reperfusion by 77% and 76%, respectively. Infarct size was also significantly reduced, 24 h and 2 weeks after reperfusion by 58% and 49%, respectively. Whereas ischemia–reperfusion had no influence on basal levels of cardiac contractility (+dp/dt), responses to dobutamine were blunted 24 h and 2 weeks after reperfusion. In mice treated with monoHER, cardiac contractility response was significantly restored. These results indicate that monoHER exerts a sustained cardioprotective effect on ischemia–reperfusion injury and prevents deterioration of cardiac contractility.

Keywords: MonoHER; Reperfusion; Oxidative stress; Necrosis; Neutrophil; Cardiac function

1. Introduction

Myocardial reperfusion through thrombolysis, percutaneous transluminal coronary angioplasty or coronary artery bypass grafting is standard treatment in acute myocardial infarction. However, these therapies initiate a second phase of myocardial injury either by acceleration of detrimental processes initiated during ischemia or by inducing additional pathological processes following reperfusion (Dhalla et al., 2000; Jolly et al., 1984). Many studies support a role of "reactive oxygen species" in myocardial ischemia–reperfusion (I/R) injury (Jolly et al., 1984; Lefer and Granger, 2000).

The mechanism implicates the sequential reduction of molecular oxygen into these "reactive oxygen species", including superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\cdot}$), in an amount that overwhelms the scavenging capacity of endogenous antioxidants in the heart. The interaction of these "reactive oxygen species" with cell membrane lipids and essential proteins contributes to myocardial cell damage, leading to inflammatory reactions, irreversible tissue injury and, consequently, to impaired cardiac function (Kaminski et al., 2002). Reperfusion injury triggers an acute inflammatory response in which polymorphonuclear neutrophils infiltrate the myocardium, under the influence of chemotactic attraction and activation of the complement cascade (Frangogiannis et al., 2002; Jordan et al., 1999). Although essential in wound healing, these neutrophils may have detrimental effects by producing additional "reactive oxygen species" and proteolytic enzymes (Duilio et al., 2001; Jordan et al., 1999; Kaminski et al., 2002).

Flavonoids are a group of naturally occurring polyphenolic compounds with excellent iron chelating as well as radical scavenging properties (Rekka and Kourounakis, 1991). The semisynthetic flavonoid, 7-monohydroxyethyl-

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doi:10.1016/j.ejphar.2004.05.017

rutoside (monoHER), has been shown to protect in vivo against chronic doxorubicin-induced cardiotoxicity in mice (van Acker et al., 2000b; van Acker et al., 1995) and is now being tested in a phase II clinical trial.

Potential protective effects of exogenously administered antioxidants on reperfusion injury have already been described in literature, but the results are controversial (Lefer and Granger, 2000). Moreover, most of these studies only investigated possible short-term protective effects after initiation of reperfusion (range 1–24 h) and did not prove sustained protective effects.

In the present study, we investigated potential short- (6 and 24 h) and long-term (2 weeks) protective effects of monoHER after cardiac ischemia and reperfusion in mice. We did this by examining neutrophil tissue infiltration, infarct size and cardiac contractility. Possible dose-dependent effects of monoHER on mean arterial pressure and heart rate were examined in a separate set of experiments.

2. Materials and methods

2.1. Animals

Outbred Swiss male mice, 8–11 weeks old, weighing 35–40g were used. The mice were purchased from Charles River (The Netherlands). Experiments were performed according to the guidelines of the Universiteit Maastricht and were approved by the Institutional Animal Ethics Committee. The animals were kept on a 12:12-h light–dark cycle in a temperature-controlled (21±2 °C) room. After surgery, animals were housed individually with ad libitum access to standard food pellets (type Ssniff, Soest; Germany) and water.

2.2. Measurement of blood pressure and heart rate in conscious animals

A catheter was implanted in the femoral artery and in the jugular vein under isoflurane anesthesia (1.5–2.5%), as described in detail (Janssen et al., 2000). The arterial catheter was connected to a pressure transducer (microswitch, model 156PC 156WL, Honeywell; Amsterdam, The Netherlands) and to an amplifier that delivered a high-voltage signal to an analog-to-digital converter board (model 2814, Data Translation; CN Rood, Rijswijk, the Netherlands) mounted in an IBM 486-compatible computer. The blood pressure signal was sampled at 2000 Hz (~200 data samples/beat). Beat-to-beat values of mean arterial pressure were calculated as the area under the curve of each pressure wave using the end diastolic value to determine the heart rate. Animals were allowed to recover from surgery for 2 days and on the third day, blood pressure and heart rate were measured continuously in the conscious state after intravenous (i.v.) administration of monoHER (pH:7.9–8.1) in a dose range from 1 to 100 mg/kg with an interval of 15 min between each dose.

2.3. Ischemia-reperfusion of the murine heart in vivo

Mice were anaesthetised with ketamine [100 mg/kg intramuscularly (i.m.)] and xylazine [5 mg/kg subcutaneously (s.c.)]. Body temperature was monitored with a rectal probe and maintained at 37 °C using a warm pad and a heating lamp.

The trachea of each mouse was intubated perorally with a stainless steel tube connected to a respirator (Hugo Sachs Elektronic rodent ventilator type 845; March-Hugstetten, Germany), set at a stroke volume of 250 μl and a rate of 210 strokes/min. After left thoracotomy and exposure of the heart, the left anterior descending coronary artery was ligated with 6–0 polypropylene (Surgipro; Connecticut, USA) just proximal to its main branching point. The suture was tied over a 3-mm long polyethylene tube (PE-10) that was left in place for 30 min. Blood flow was then reestablished by removal of the tube. The occurrence of reperfusion was assessed by the observation of blood flow in the epicardial coronary arteries through a surgical microscope. Sham procedures were identical, with the exception of the actual tying of the polypropylene suture. The chest was closed with 5–0 silk sutures. The animals were then weaned from the respirator, and the intratracheal tube was removed once they were breathing spontaneously. Afterwards, 0.2-mg/kg buprenorphine was given s.c. as an analgesic.

2.4. Treatment

MonoHER (7-monohydroxyethylrutoside, mol. wt. 654.6) was kindly donated by Zyma (Nyon, Switzerland). Before injection, monoHER was dissolved in 36 mM NaOH in sterile water, in a final concentration of 20 mg/ml (pH=7.9–8.1). Based upon its proven efficacy in earlier studies, monoHER was given i.p. in a dose of 500 mg/kg, 1 h before induction of ischemia (van Acker et al., 1995). Control mice were given 25 μl/g 0.9% NaCl solution (same pH) i.p., 1 h before ischemia. In our study, four groups were compared: sham saline, sham monoHER, ischemia–reperfusion saline and ischemia–reperfusion monoHER.

2.5. Evaluation of ischemic area at risk (AAR) and infarct size

Depending on the time point of reperfusion (24 h or 2 weeks), infarct size was measured on triphenyltetrazolium chloride (TTC)-stained (Nachlas and Shnitka, 1963) or AZAN (Azocarmine Anilineblue)-stained tissue sections with the use of a computerised morphometry system (Quantimet 570, Leica; Cambridge, UK). Briefly we describe these methods.

In the 24-h reperfusion group, mice were anesthesised with pentobarbital (120 mg/kgm, i.p.), the jugular vein was

canulated and the thorax was reopened, the left anterior descending coronary reoccluded and 500 μl of 2.5% trypan blue was injected into the jugular vein to delineate the nonischemic tissue and to quantify the area at risk. The heart was then excised, briefly washed with isotonic saline and cut into two parts in the frontal plane, central through the ventricles. These parts were then incubated for 20 min at 37 °C with 5 ml of 1% 2,3,5-triphenyltetrazolium chloride solution (TTC, Sigma; St. Louis, MO, USA). Viable myocardium is stained red by TTC and the necrotic, infarcted area remains unstained (Vivaldi et al., 1985). The surface of the left ventricle, the area at risk (AAR) and the infarcted area was measured on both parts of the heart. The percentage AAR/left ventricle, infarct/AAR and infarct/left ventricle was calculated and expressed as the mean of both parts.

For the 2 week reperfusion group, mice were also anesthesised under pentobarbital (120 mg/kg, i.p.), hearts were excised, washed with isotonic saline and cut into 2 parts in the frontal plane, central through the ventricles and were embedded in paraffin. From each part, one slide (5 μm) was taken and stained with AZAN. The surface of the infarct and the left ventricle were measured on both slides and the percentage infarct/left ventricle was calculated and expressed as the mean of both slides.

2.6. Evaluation of ventricular function

Mice were anesthesised with urethane (2.5 mg/g body weight, i.p.; Sigma). Body temperature and respiration were controlled as described before.

A 1.4 French high-fidelity catheter tip micromanometer (SPR-671, Millar Instruments; Houston, TX) was inserted through the right carotid artery into the left ventricular cavity. Ventricular pressure was measured and was sampled at a rate of 2 kHz. Maximal positive pressure development (+dp/dt) and heart rate were determined on a beat to beat basis and 1-s averages were stored on disk. The heart was then stimulated by a ramp-infusion of i.v. dobutamine (0.5 to 5 ng/g/min) using a microinjection pump (Model 200 Series, KdScientific; Boston, MA). Each dose was infused for 2 min.

In the 2 week reperfusion group, the hearts were additionally stressed by loading the circulation with an infusion of 2.5 ml Ringer's solution (warmed to 37 °C) for 1 min. Maximal values for +dp/dt were recorded and the difference between the maximal value and the value at baseline was calculated. At the end of the experiment, hearts were excised, washed with isotonic saline and ventricular weights were determined.

2.7. Immunohistochemistry

To determine the number of infiltrating polymorphonuclear neutrophils, heart specimens were immediately frozen and stored at −80 °C. Frozen sections (6 μm) were stained for neutrophils with a rat antimouse neutrophil-specific antibody, NIMP-R14 (McLaren et al., 1987), using peroxidase-labeled rabbit antirat immunoglobulin (Ig) as the secondary mAb and 3-amino-9-ethylcarbazole as a chromogen followed by a hematoxylin counterstain. The numbers of polymorphonuclear neutrophils per grid (0.25 mm^2) were counted under a high-power microscope field (×200) in a blinded fashion. Average numbers of polymorphonuclear neutrophils were obtained by counting nine grids per slide in eight apical slides per heart and were compared between saline and monoHER-treated animals at both 6 and 24 h reperfusion.

2.8. Statistical analysis

All parameters are expressed as mean±S.E.M. Numbers of neutrophils, infarct size and increase in +dp/dt by dobutamine and volume loading were evaluated using Student's *t*-test. Dose response curves for dobutamine were compared using a two-way analysis of variance (ANOVA) and a posthoc Bonferonni test. P values<0.05 were regarded as statistically significant.

3. Results

3.1. General

A total of 50 animals were subjected to sham surgery or ischemia–reperfusion and were studied for ventricular contractility after 24 h reperfusion. Six of these animals died prematurely because of complications due to anesthesia, arrhythmia or bleeding. In five animals, we could not measure contractility because of difficulties in passing the cardiac valves with the catheter tip.

For the 2 week reperfusion group, 43 animals were subjected to surgery. Two animals died during or shortly after surgery, two animals died between 3 and 7 days after reperfusion and one animal died after 12 days for an unknown reason. In three animals, we could not measure contractility due to technical complications as mentioned above.

Table 1 summarises age and body weights of mice in the different groups used for evaluation of cardiac contractility. There were no significant differences between groups in any of the parameters. The ventricular weight/body weight ratio was slightly increased in the 2-week saline reperfusion group compared to the sham groups and the monoHER-treated reperfusion group at that time point.

3.2. Blood pressure and heart rate

Fig. 1 shows mean arterial pressure and heart rate in conscious mice (n=4) measured under control conditions (marker C) and after i.v. administration of cumulative

Table 1
Biometric data of mice subjected to sham surgery and ischemia–reperfusion (I/R)

Time	Group		*n*	Age (weeks)	Body weight (g)	Ventricular weight/body weight (%)
24 h	Sham	Saline	10	11.0±0.5	37.3±1.0	0.44±0.01
		MonoHER	10	10.7±0.5	38.0±1.1	0.45±0.01
	I/R	Saline	10	10.3±0.5	37.1±0.9	0.44±0.02
		MonoHER	9	10.4±0.4	37.7±0.7	0.40±0.05
2 weeks	Sham	Saline	9	10.1±0.3	39.2±0.8	0.45±0.01
		MonoHER	7	11.0±0.3	38.3±0.7	0.44±0.01
	I/R	Saline	10	10.7±0.3	40.0±1.6	0.49±0.02
		MonoHER	9	11.1±0.2	39.1±0.9	0.44±0.05

Number of mice (*n*), age, body weight and ventricular weight/body weight are presented.

amounts of monoHER. MonoHER was found to have no acute influence on blood pressure and heart rate.

3.3. Neutrophil infiltration

Fig. 2 illustrates the inflammatory response following ischemia–reperfusion as evidenced by neutrophil infiltration within the injured tissue. In the saline group, the amount of neutrophils in cardiac tissue was more than doubled from 6 to 24 h after reperfusion. The number of neutrophils was significantly lower in the monoHER-treated group 6 h after reperfusion (saline, 22±5; monoHER, 5±1; P=0.006) and 24 h after reperfusion (saline, 51±11; monoHER, 12±3; P=0.007). In the sham groups, no neutrophils were detected in cardiac tissue.

3.4. Myocardial infarct size

Twenty-four hours after reperfusion, the area at risk (AAR) of the left ventricle was comparable in the saline group and the monoHER-treated group (47±2% and 50±7%, respectively), indicating that the ligation was placed reproducibly (Fig. 3B). However, the infarct/AAR ratio was significantly smaller in the monoHER-treated group than in the saline group (saline, 51±8%; monoHER, 21±6%; P=0.009). The infarct/left ventricle ratio was also significantly smaller in the monoHER-treated group than in the saline group (saline, 21±4%; monoHER, 9±4%; P=0.005).

Fig. 3A represents two sections of a heart from the saline group versus two sections of the heart of a monoHER-treated animal, 2 weeks after reperfusion. Infarct size 2 weeks after reperfusion was significantly smaller in the monoHER-treated group than in the saline group (Fig. 3B; saline, 13±4%; monoHER, 7±2%; P=0.014).

3.5. Ventricular contractility

Cardiac function was evaluated 24 h and 2 weeks after reperfusion by measuring heart rate and the rate of left ventricular pressure development (+dp/dt). Under basal conditions, +dp/dt and heart rate did not differ between sham and ischemia–reperfusion groups. Basal heart rate was significantly higher in the saline ischemia–reperfusion group compared to the saline sham group (P<0.01) after 24-h reperfusion. When the heart was stimulated with an increasing dose of dobutamine (i.v.), +dp/dt levels increased dose-dependently in the sham animals, whereas the increase was significantly blunted in the saline ischemia–reperfusion animals (Fig. 4A). The increase of +dp/dt was significantly restored in the monoHER-treated ischemia–reperfusion group at both time points (24 h and 2 weeks after reperfusion). Dose response curves for heart rate were comparable between groups (Fig. 4B). In the 24 h ischemia–reperfusion

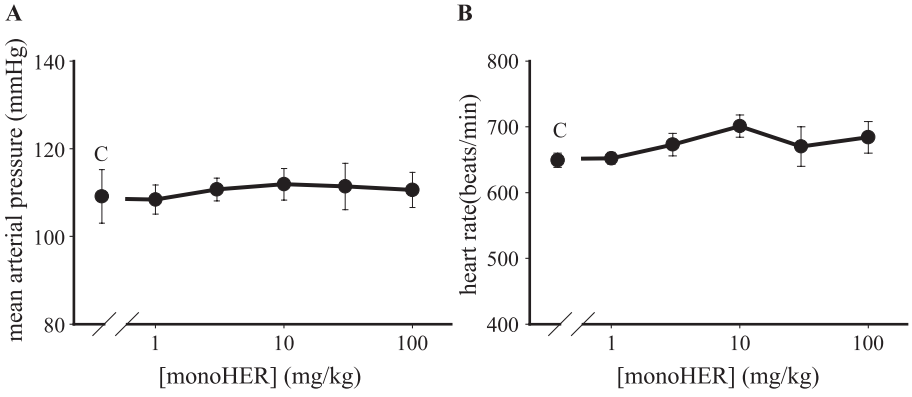

Fig. 1. Mean arterial pressure and heart rate measured in conscious mice (n=4) under control conditions (marker C) and after i.v. administration of monoHER in a cumulative fashion from 1–100 mg/kg. Data points represent the mean values obtained from 5–15 min after injection.

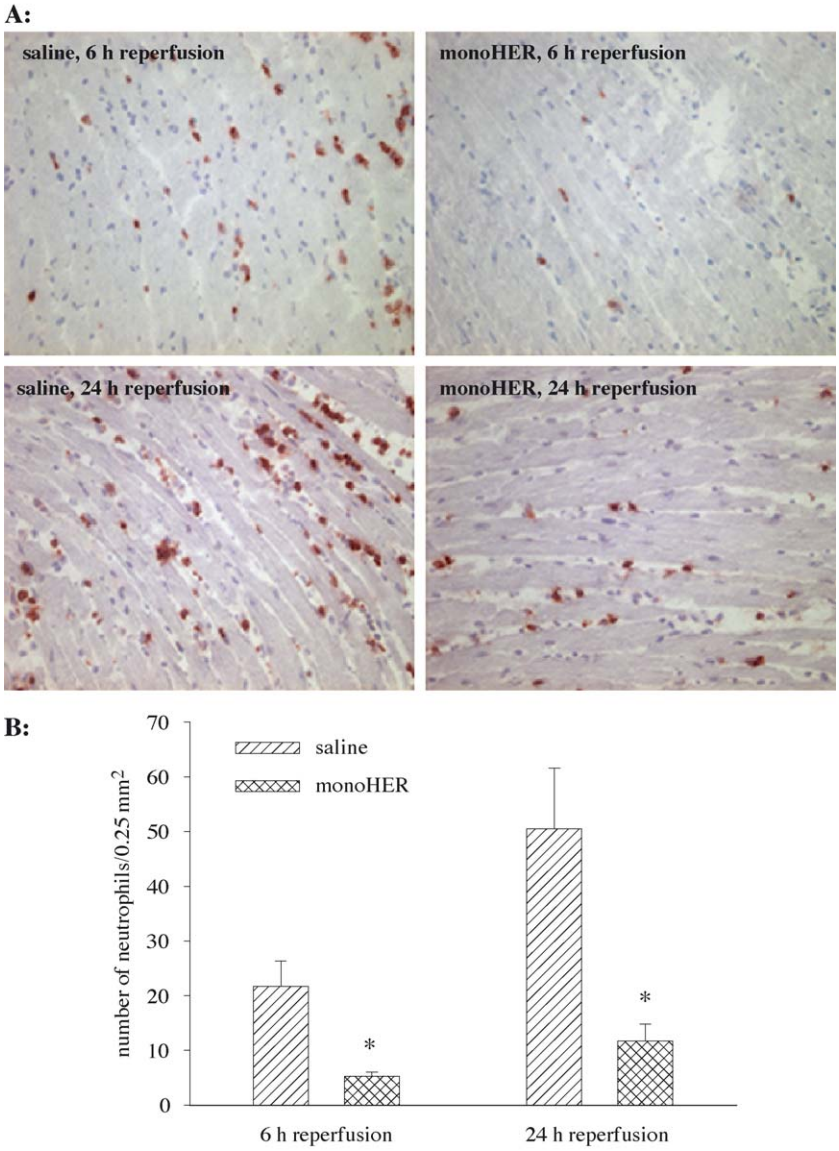

Fig. 2. Neutrophil infiltration in ischemic heart tissue of saline and monoHER-treated mice after 6 h (n=10/group) and 24 h of reperfusion (n=10/group). (A) Cardiac tissue sections with NIMP-R14 positive cells (brown), representing neutrophils. (B) Average number of infiltrating neutrophils per grid (magnification ×200). At both 6 and 24 h after reperfusion, neutrophil numbers were significantly lower (*P<0.01) in monoHER-treated mice than in time-matched saline groups.

saline group, increase in +dp/dt was significantly smaller (+2456±475 mmHg/s) than in the sham saline group (+5939±928 mmHg/s; P=0.004) and the ischemia–reperfusion monoHER-treated group (+5515±928 mmHg/s; P=0.0003). Similar effects were seen in the 2-week ischemia–reperfusion saline group where the increase in +dp/dt (+3174±1066 mmHg/s) was significantly lower than in the sham saline group (+7487±1209 mmHg/s; P=0.01) and the monoHER-treated ischemia–reperfusion group (+7274±687 mmHg/s; P=0.005). Comparable results were obtained following volume loading (Fig. 4C). The increase of +dp/dt in the 2-week ischemia–reperfusion saline group (+1758±991 mmHg/s) was significantly smaller than in the sham saline group (+6129±1247 mmHg/s; P=0.02) and the monoHER-treated ischemia–reperfusion group (+5723±575 mmHg/s; P=0.006).

4. Discussion

The results of this study show that 7-monohydroxyethylrutoside (monoHER) exerts a significant cardioprotective effect after ischemia and reperfusion in mouse hearts and that this effect is sustained.

MonoHER was chosen for this study because it has strong radical scavenging and iron chelating properties (van Acker et al., 2000a). This capacity makes it a potential drug for the prevention of oxidative stress-related

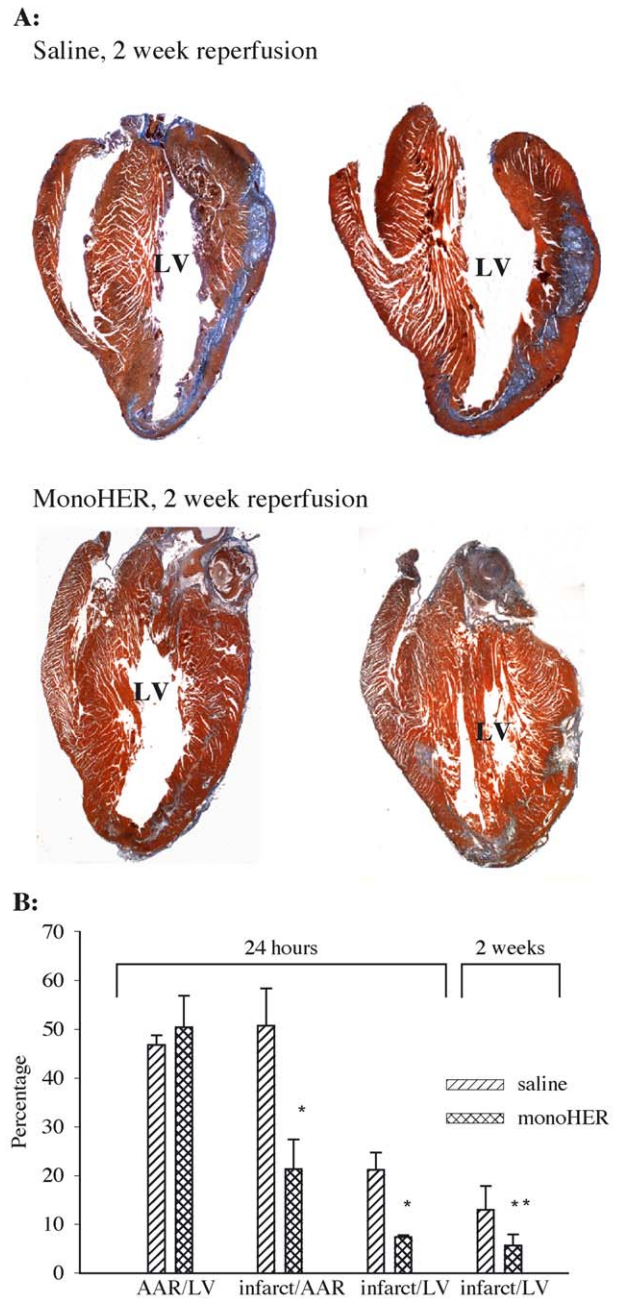

Fig. 3. Evaluation of area at risk (AAR) and infarct size from monoHER-treated mice and mice from the saline group, 24 h (n=13/group) and 2 weeks after reperfusion (n=10/group). (A) AZAN-stained heart tissue sections of 2 week reperfusion animals (one from the saline group and one from the monoHER-treated group). Infarcted area (blue) in the wall of the left ventricle (indicated as LV) versus noninfarcted area (red). Note the reduced infarcted area in the monoHER-treated animal. (B) There was no significant difference in myocardial AAR/left ventricle (indicated as AAR/LV) ratios in monoHER-treated mice and mice from the saline group at 24 h of reperfusion. Infarct/AAR and infarct/left ventricle (indicated as infarct/LV) ratios were significantly smaller in monoHER-treated mice than in mice from the saline group (*P<0.01) after 24 h and 2 weeks of reperfusion (**P=0.014).

reperfusion injury. In addition, monoHER protects against doxorubicin-induced cardiotoxicity in vivo (van Acker et al., 2000b; van Acker et al., 1995). Flavonoids have effects on a variety of inflammatory responses and immune function (Manthey, 2000). An in vitro study showed that monoHER has antiinflammatory effects by reducing neutrophil adhesion through inhibition of doxorubicin-induced expression of vascular cell adhesion molecule (VCAM) and E-selectin (Abou El Hassan et al., 2003). In the present study, we showed that monoHER also reduces the inflammatory response in vivo by reducing influx of neutrophils in the infarcted area of the heart. The reason for this reduction in the number of neutrophils by monoHER in our model is probably dual. First, due to a reduction in damage and therefore a reduced stimulus for an inflammatory response, and second, due to a direct antiinflammatory effect of monoHER. However, the role of the reduction in neutrophils itself by monoHER treatment on the reduced infarct size 2 weeks after reperfusion is unknown. A direct causal association between the number of neutrophils and myocardial cell death is still under debate (Arai et al., 1996; Baxter, 2002; Birnbaum et al., 1997; Briaud et al., 2001; Hoffmeyer et al., 2000; Metzler et al., 2001). To our knowledge, there is only one in vivo study that has examined the effects of neutrophil depletion after a prolonged time (in terms of weeks) of reperfusion. Metzler et al. (2001) demonstrated that in mice deficient in intercellular adhesion molecule-1 (ICAM-1), protection occurred in the early phase after reperfusion, but that at a later stage, 1 and 3 weeks after reperfusion, scar size in the ventricles was comparable between knockout and wild-type mice. This suggests that neutrophil infiltration into the infarcted area may help promote repairing cardiac tissue, and consequently, the enhancement of leukocyte recruitment might be beneficial.

However, following monoHER treatment, infarct size remained significantly smaller 2 weeks after reperfusion, indicating that monoHER prevents, rather than delays myocardial ischemia–reperfusion injury. In contrast to the study of Metzler et al. (2001), monoHER treatment was applied to prevent the initial damage before the influx of neutrophils occurs. This means that the reduction in myocardial neutrophil influx and infarct size and the prevention of deterioration of cardiac contractility by monoHER was effective and that no significant additional damage occurred during the subsequent period of reperfusion. The present data therefore support the view that ischemia–reperfusion injury due to free radicals occurs in the initial phase after initiation of reperfusion (Bolli et al., 1989; Shao et al., 2002; Wang and Zweier, 1996; Zweier et al., 1987) and effective antioxidant treatment must therefore be directed to this phase.

In the saline groups, infarct size/left ventricle ratio was higher at 24 h than at 2 weeks after reperfusion, most likely due to the different techniques that were required to measure infarct size. TTC staining is used to detect infarct size shortly after reperfusion whereas AZAN staining is used after longer

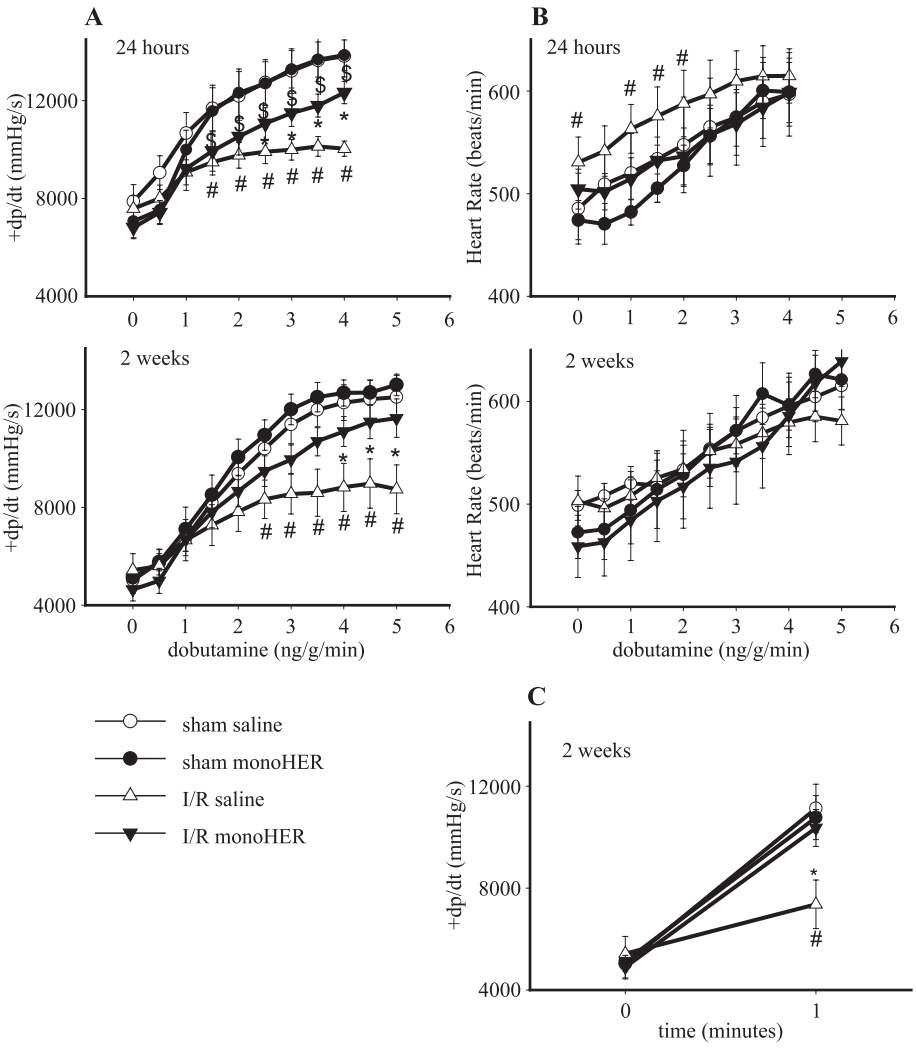

Fig. 4. In vivo assessment of left ventricular contractility. (A) Cardiac contractility during dobutamine stimulation at 24 h (top) and 2 weeks (bottom) after reperfusion. $^{\#}P<0.01$: significantly different between nontreated ischemia–reperfusion (I/R saline) and sham-operated controls (sham saline). $^{*}P<0.01$: significantly different between nontreated ischemia–reperfusion (I/R saline) and monoHER-treated ischemia–reperfusion (I/R monoHER). $^{\$}P<0.01$: significantly different between monoHER-treated ischemia–reperfusion (I/R monoHER) and monoHER-treated sham (sham monoHER). (B) Increase in heart rate after dobutamine stimulation. At both time points, 24 h (top) and 2 weeks (bottom) after reperfusion, there was no significant difference between the groups. Basal heart rate in the saline ischemia–reperfusion group was significantly higher than in the saline sham group ($^{\#}P<0.01$). (C) Contractility (+dp/dt) measured before and after volume loading at 2 weeks after reperfusion. $^{*}P=0.006$: significantly different between nontreated ischemia–reperfusion (I/R saline) and monoHER-treated ischemia–reperfusion group (I/R monoHER). $^{\#}P\leq0.02$: significantly different between nontreated ischemia–reperfusion (I/R saline) and sham-operated controls (sham saline).

periods of reperfusion to detect the amount of granulation tissue. There is, however, a clear reduction in infarct size in the monoHER-treated reperfusion group compared to the saline reperfusion group at both time points.

MonoHER did not influence baseline levels of heart rate and mean arterial pressure in conscious mice. This finding excludes the notion that protective effects of monoHER are due to possible hemodynamic changes which can have preconditioning and therefore protective effects (Thornton et al., 1992).

Baseline +dp/dt levels were not different between the ischemia–reperfusion and sham-operated mice at any time point. This is probably due to the fact that the infarct size is relatively small after cardiac ischemia–reperfusion in mice. Earlier experiments from our group showed a significantly reduced cardiac contractility at baseline in mice with permanent myocardial infarction and related larger infarcts of around 45% of the left ventricle (Lutgens et al., 1999). The significantly higher heart rate at basal level, 24 h after reperfusion in the saline group is probably due to neurohumoral compensation to maintain cardiac output after cardiac damage. This phenomenon was not observed 2 weeks after reperfusion, suggesting a normalisation of this response. Stressing the heart by an infusion of dobutamine did reveal differences in cardiac contractility between groups. Ischemia–reperfusion resulted in a blunted response to dobutamine. Treatment with monoHER improved the increase of cardiac contractility, both 24 h and 2 weeks after reperfu-

sion. To examine if the blunted response to dobutamine was primarily due an impaired β-adrenergic effect, we also stressed the heart by volume loading. Because the increase in contractility was comparable to the effects obtained by dobutamine stimulation, we can conclude that the improved cardiac response is due to a reduced infarct size rather than a change in β-adrenergic effectiveness.

Timing, route and amount of monoHER administration in this study were based on the design of the earlier in vivo studies in which monoHER was proven to act protective in a model of doxorubicin cardiotoxicity. For later clinical application of monoHER in the reduction of ischemia–reperfusion injury, further studies have to be done in which different time-points of administration (as postischemical) and lower amounts of monoHER should be tested.

In conclusion, the present study shows that the antioxidant 7-monohydroxyethylrutoside strongly reduced myocardial neutrophil influx, infarct size and prevented deterioration of cardiac contractility in an in vivo mouse model of cardiac ischemia–reperfusion. To our knowledge, this is the first time that sustained protective effects (in terms of weeks) of a flavonoid are demonstrated in a model of ischemia–reperfusion.

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