ELSEVIER

Resuscitation 56 (2003) 199–206

RESUSCITATION

www.elsevier.com/locate/resuscitation

Magnesium reduces free radical concentration and preserves left ventricular function after direct current shocks

Yi Zhang, Loyd R. Davies, Sean M. Martin, Imran M. Bawaney, Garry R. Buettner, Richard E. Kerber *

Department of Internal Medicine, The Cardiovascular Center, College of Medicine, University of Iowa Hospital, 200 Hawkins Drive, Iowa City, IA 52242, USA

Received 26 June 2002; received in revised form 20 September 2002; accepted 20 September 2002

Abstract

Objective: Our objective was to determine if magnesium reduces free radicals generated by direct current countershock and preserves left ventricular contractile function. *Background:* We have previously shown that magnesium reduces free radicals in a coronary occlusion-reperfusion model, and therefore also might reduce free radical generation by direct current shocks. *Methods:* In eight swine weighing 18–27 kg (mean: 22 kg), using electron paramagnetic resonance, we monitored continuously the coronary sinus concentration of ascorbate free radical, a measure of free radical generation (total oxidative flux). Epicardial shocks (30 J) using a truncated exponential biphasic waveform (5/5 ms) were administered. Each animal received two shocks, one without and one with magnesium, 80 mg/min IV, beginning 10 min before the shock and continuing to 15 min after the shock. Percent fractional area shortening of the left ventricular cavity was determined by 2-dimensional echocardiography. *Results:* Magnesium shocks resulted in a significantly lower increase in the ascorbate free radical concentration ($0.6 \pm 4.6\%$) than no-magnesium shocks ($16 \pm 3.3\%$, $P < 0.05$) at 12 min after the shock. Total radical flux was reduced 72% ($P < 0.05$), and left ventricular fractional area shortening was preserved: baseline: $69 \pm 2.6\%$, no-magnesium shocks: $41 \pm 2.8\%$ ($P < 0.05$, versus baseline) and magnesium shocks $61 \pm 3.7\%$. *Conclusions:* Magnesium pre-treatment reduced oxygen free radicals generated by direct current shocks; post-shock left ventricular contractile function was not impaired. Magnesium may be cardioprotective during epicardial ('surgical') defibrillation.

Keywords: Defibrillation; Free radicals; Ascorbate; Magnesium

Resumo

Objectivo: O nosso objectivo foi determinar se o magnésio reduz os radicais livres gerados por uma corrente de choques directa e se preserva a função contráctil do ventriculo esquerdo. *Contexto:* Evidenciamos préviamente que o magnésio reduz os radicais livres num modelo coronário de oclusão-reperfusão, e portanto também pode diminuir a produção de radicais livres por choques de corrente directa. *Método:* Monitorou-se de forma contínua, utilizando a ressonância electrónica paramagnética, a concentração de radicais livres de ascorbato no seio coronário, uma medição da produção de radicais livres (fluxo oxidativo total) em oito porcos pesando 18–27 kg (média = 22 kg). Foram administrados choques epicárdicos (30 J) utilizando 'ondas bifásicas exponenciais e truncadas' (5/5 ms). Cada animal recebeu dois choques, um sem e outro com magnésio, 80 mg/min, iniciado 10 min antes do choque e continuado até 15 min. depois do choque. Foi determinada, por ecocardiograma bidimensional, a fracção de encurtamento do ventrículo esquerdo. *Resultados:* Os choques com magnésio resultaram num aumento significativamente menor da concentração de radicais livre ascorbato ($0.6 \pm 4.6\%$) do que os choques sem magnésio ($16 \pm 3.3\%$; $P < 0.05$) aos 12 min após o choque. O fluxo total de radicais foi reduzido a 72% ($P < 0.05$), e a fracção de encurtamento do ventrículo esquerdo foi preservada: linha de base: $69 \pm 2.6\%$, choques sem magnésio: $41 \pm 2.8\%$ ($P < 0.05$, versus linha de base) e choques com magnésio $61 \pm 3.7\%$. *Conclusões:* O pré-tratamento com magnésio reduz a produção de radicais livres de oxigénio produzidos por choques de corrente directa; a função

* Corresponding author. Tel.: +1-319-356-2739; fax: +1-319-356-4552.
E-mail address: richard-kerber@uiowa.edu (R.E. Kerber).

0300-9572/02/$ - see front matter
PII: S0300-9572(02)00353-2

ventricular esquerdo após o choque não foi alterada. O magnésio pode ser cardioprotector durante a desfibrilhação epicárdica ('cirúrgica').

Palavras chave: Desfibrilhação; Radicais livres; Ascorbato; Magnésio

Resumen

Objetivo: Nuestro objetivo fue determinar si acaso el magnesio reduce los radicales libres generados por la descarga de corriente directa y preserva la función contráctil del ventrículo izquierdo. Antecedentes: Hemos mostrado previamente que el magnesio reduce los radicales libres en un modelo oclusión coronaria-reperfusión, y por lo tanto podría también reducir la generación de radicales libres por descarga de corriente directa. *Métodos*: Por medio de resonancia paramagnética en 8 cerdos que pesaban 18–27 kg (promedio 22 kg), hicimos monitoreo contínuo de concentración de radicales libres de ascorbato en el seno coronario, una medida de la generación de radicales libres (flujo oxidativo total). Se administraron descargas epicárdicas (30 J) usando corriente en una onda bifásica exponencial truncada (5/5 ms). Cada animal recibió dos descargas, una sin y una con magnesio, 80 mg/min ev, empezando 10 minutos antes de la descarga y continuado hasta 15 min después de la descarga. Se determinó el acortamiento porcentual del área de la cavidad del ventrículo izquierdo con ecografía bidimensional. *Resultados*: Los pacientes con magnesio y descarga presentaron un aumento en la concentración de los radicales libres de ascorbato ($0.6 \pm 4.6\%$) significativamente menor que los pacientes que no recibieron magnesio($16 \pm 3.3\%$, $P < 0.05$) al medirlo 12 minutos después de la descarga. El flujo total de radicales se redujo 72% ($P < 0.05$), y el acortamiento fraccional de área de ventriculo izquierdo se mantuvo: linea basal: $69 \pm 2.6\%$, sin bolos de MG, $41 \pm 2.8\%$ ($P < 0.05$ versus basal), con magnesio $61 \pm 3.7\%$. *Conclusiones:* El pretratamiento con magnesio reduce los radicales libres generados por descargas de corriente directa; la función contráctil ventricular izquierda no se daño. El magnesio puede ser cardioprotector durante desfibrilación epicárdica (quirurgica).

Palabras clave: Radicales libres; Ascorbato; Magnesio

1. Introduction

Direct current (DC) countershocks have been the standard treatment for cardiac arrest patients with ventricular fibrillation for decades. In spite of the effectiveness of DC shocks for the termination of ventricular fibrillation (VF), anatomic and functional myocardial damage after transthoracic and epicardial shocks has been observed [1–3]. The mechanism of this defibrillation-induced myocardial damage has not been fully elucidated.

Oxygen free radicals are generated when molecular oxygen is reintroduced into ischemic myocardium on reperfusion [4,5]. Those free radicals play a central role in the pathogenesis of myocardial ischemia-reperfusion injury (IRI) [6,7], resulting in lipid peroxidation, cellular dysfunction and myocardial stunning [8].

Increasing evidence has suggested that DC countershocks can generate free radicals. Jackson et al. [9] demonstrated that an electrical current passing through a physiologic buffer solution could generate free radicals. Lecour et al. [10] confirmed this source of free radical production and showed that the quantity of free radicals induced by electrolysis was directly proportional to the intensity of the current. These observations support the proposal of free radicals generated by DC shocks as a mechanism of post-defibrillation myocardial damage. Our laboratory has reported previously that direct current epicardial shocks generate free radicals [11]. Furthermore, the angiotensin converting enzyme inhibitor captopril, with free radical scavenging properties, lowered coronary venous free radical concentration after direct current cardiac shocks [12].

Magnesium has been used clinically in patients with acute myocardial infarction [13] Magnesium may act as a 'physiological calcium channel blocker', which preserves left ventricular function, limits infarct size and reduces mortality [14,15]. Afanas'ev et al. [14] demonstrated that magnesium could inhibit reduced nicotinamide-adenine dinucleotide phosphate oxidase, an enzyme that produces superoxide radical. Garcia et al. [15] reported that magnesium reduced free radical concentration after coronary occlusion-reperfusion sequences.

The purpose of this study was to examine the effect of magnesium on the production of free radicals and left ventricular function after DC shocks. Electron paramagnetic resonance (EPR) was used to measure real-time production of ascorbate free radical ($Asc^{\bullet -}$), a marker of total oxidative stress.

2. Methods

2.1. Animal preparation

The study was done on deeply anesthetized, intubated swine weighing 18–27 kg (mean: 22 kg). Swine of either sex were fasted overnight and anesthetized with a mixture of Ketamine (20 mg/kg) and Acepromazine

(0.2 mg/kg). Sodium pentobarbital at a concentration of 50 mg/ml was used for supplemental intravenous anesthesia as necessary. The animals then underwent tracheal intubation, and ventilation was begun with a volume-cycled respirator. The arterial blood gas was maintained in the physiologic range of pH (7.35–7.45) and $pO_2 > 100$ Torr by adjusting the tidal volume, respiratory rate and fraction of inspired oxygen content, and serum bicarbonate as necessary.

Under fluoroscopic guidance, a 6Fr USCI Gensini catheter was placed via the right jugular vein into the right atrium and advanced into the coronary sinus. The right carotid artery and right external jugular vein were cannulated for passing blood through the EPR spectrometer. The EKG (lead II) and arterial pressure were monitored continuously.

The heart was exposed through a midline sternotomy. Heparin was administered to prevent thrombosis in cannulae and tubing carrying blood to and from the EPR spectrometer.

2.2. Electron paramagnetic resonance

The ascorbate free radical ($Asc^{\bullet -}$) is a resonance-stabilized tricarbonyl species that is readily formed upon the one-electron oxidation of ascorbate, $AscH^-$. Because of the low reduction potential of the $Asc^{\bullet -}/AscH^-$ couple, ascorbate is considered to be the terminal small molecule antioxidant [16–18]. Nearly all oxidants that could be present in a biological system will bring about the one-electron oxidation of ascorbate [16]. Thus, the concentration of ascorbate free radical, as monitored by EPR, is an excellent measure of the degree of free radical stress (total oxidative flux) in chemical, biochemical, and biological systems [19].

For this study we used a method we have previously described in detail [20]. A brief description is given here. A Varian E-4 spectrometer with a TM_{110} cavity and an aqueous flat cell was used to monitor $Asc^{\bullet -}$. The lower end of the flat cell was connected to Teflon tubing (OD 0.5 mm), which was connected to a manifold. The upper end of the flat cell was connected to the femoral vein with a variable speed infusion pump. The coronary sinus catheter and the femoral artery catheter were connected to different ports of the manifold. Thus coronary venous blood was circulated continuously through the spectrometer at a determined rate. In order to scan the arterial blood the manifold was switched from coronary sinus to femoral artery. The blood sample was scanned within 5 s of leaving the vein or artery [11].

The EPR instrument settings used to acquire the $Asc^{\bullet -}$ spectra were: nominal power = 40 mW; modulation amplitude = 1 Gauss; time constant 1 s; scan rate = 1 Gauss per 24 s. The spectrum of $Asc^{\bullet -}$ ($a^H = 1.8$ Gauss) is typically a doublet. The concentration of $Asc^{\bullet -}$ was determined from the peak-to-peak height (in mm) of the $Asc^{\bullet -}$ EPR spectrum as previously described, using 3-carboxy-proxyl as a standard and accounting for saturation effects [21]. For our experimental conditions, 1 mm of normalized $Asc^{\bullet -}$ signal height corresponds to 0.073 nM $Asc^{\bullet -}$ in the blood being sampled since the volume of blood in the EPR flat cell remains constant.

Without ascorbic acid supplementation, a weak $Asc^{\bullet -}$ signal (about 1 nM) can often be detected in the arterial blood, but not in the coronary sinus blood. In order to obtain an adequate EPR signal, 1 g of L-ascorbic acid was infused intravenously as a bolus, followed by a slow continuous infusion. The $Asc^{\bullet -}$ signal could then be obtained from arterial and coronary venous blood. The $Asc^{\bullet -}$ concentration varied from animal to animal; the arterial $Asc^{\bullet -}$ signal was 14 ± 4 nM; the venous $Asc^{\bullet -}$ signal was 8 ± 3 nM. The negative arterial-venous gradient results from the repair of the ascorbate radical by the nicotinamide adenine dinucleotide (NAD) system as red blood cells pass through the myocardium [11]. We maintained a steady-state level of $Asc^{\bullet -}$ by adjusting the rate of ascorbic acid infusion (the rate ranged from 15.3 to 76.4 mg/min, after the initial loading dose of 1 g); the arterial $Asc^{\bullet -}$ level was rechecked frequently. Arterial pH was maintained between 7.35 and 7.45 by respirator adjustments.

2.3. Left ventricular function by echocardiography

Two-dimensional echocardiograms were obtained to determine the functional injury to the myocardium as a result of DC countershock and to correlate this injury with the ascorbate radical generation. Left ventricular systolic contraction was measured by transthoracic echocardiography by a Hewlett Packard (Hewlett Packard 77020A Ultrasound System, Andover, MA) Ultrasound Imaging System. The animal was lying on its right side with the transducer placed below the animal on the chest wall, not into the thoracotomy incision. This allows the chest wall to serve as a 'standoff'. Two-dimensional left ventricular cavity images in the parasternal short axis view were obtained at the papillary muscle level at baseline, and again 15 min after no-magnesium shock or magnesium shock. Left ventricular end-diastolic and end-systolic areas were determined by planimetry by an observer blinded as to treatment group, and the percent functional area shortening was determined using the following formula: end-diastolic area minus end-systolic area divided by end-diastolic area.

2.4. Protocol

The animals were prepared as described earlier in this section. As discussed above, the concentration of

ascorbate free radical in swine coronary venous blood is too low for detection by EPR spectroscopy. Therefore, an intravenous infusion of ascorbate was given (1 g bolus followed by 15.3 to 76.4 mg/min infusion) to amplify the endogenous ascorbate free radical signal. This infusion was continued throughout the experiment.

The technique of ascorbate loading was developed during our preliminary studies with intact animal preparations. Consecutive ascorbate free-radical EPR scans were obtained from the femoral artery until the signal amplitude of the spectrum was stable. The ascorbate free-radical concentration was then monitored from the coronary sinus. The arterial ascorbate free-radical concentration was rechecked periodically throughout the study to ensure the stability of the system. Ascorbate infusion adjustments were not made during the post-shock monitoring period.

Once stable $Asc^{\bullet -}$ signals were obtained, a baseline echocardiogram was done. After saline infusion for 10 min, a single truncated biphasic waveform (5/5 ms) 30 J synchronized shock was delivered from our custom-made defibrillator (115 μF capacitor, total tilt: 98% and each individual phase tilt: 87% with 30 J stored energy and 21 Ω discharge impedance.). The shocks were delivered from the hand-held paddle electrodes (44.2 cm^2, Agilent Technologies, model number: M1741A), placed on the epicardial surface, on opposite lateral aspects, to 'cradle' the heart. The $Asc^{\bullet -}$ concentration was measured with consecutive EPR scans. The coronary sinus $Asc^{\bullet -}$ signal was monitored continuously for 15 min after the shock. Another echocardiogram was performed at 15 min after the shock. The femoral artery $Asc^{\bullet -}$ signal was rechecked to verify $Asc^{\bullet -}$ stability at baseline and at approximately 15 min after the shock. Arterial blood gases were obtained at the time before the shock and at 15 min after the shock.

The pigs were allowed to stabilize for 30–60 min before the second shock. At 10 min before delivering the second shock, magnesium infusion (80 mg/min) was initiated. Then we repeated the shock, echo and blood gas measurements; the magnesium infusion continued for 15 min after the shock. Our previous study has shown that repeated DC countershocks resulted in similar $Asc^{\bullet -}$ increase after each shock [11]; thus differences in $Asc^{\bullet -}$ rise after the second shock can be attributed to the intervention, magnesium, and not simply to a repeated shock. Euthanasia was achieved by massive intravenous barbiturate overdose at the end of the experiment.

2.5. Statistical analysis

A repeated measures analysis was used to compare % change in $Asc^{\bullet -}$ concentration between no-magnesium and magnesium groups. The repeated measures factor for this variable was time (1.5–15 min at 1.5 min increments). The same analysis was used to compare the percent area shortening by echocardiography among two groups (no-magnesium vs. magnesium pretreatment). Bonferroni's method was applied to the P-values to adjust for the number of tests performed within each set. A Bonferroni adjusted P-value < 0.05 was considered to be statistically significant. The statistical analyses for this study were performed using the SAS/STAT procedure MIXED [22].

3. Results

There were no differences between the two groups with respect to baseline systolic and diastolic arterial pressure. The dose of magnesium used in the present study did not markedly reduce arterial pressure ($P =$ NS), comparing no-magnesium condition and magnesium-treated condition. There was also no difference in heart rate between the two conditions (Table 1).

The changes in coronary venous $Asc^{\bullet -}$ concentration in no-magnesium and magnesium conditions are summarized in Fig. 1. After the no-magnesium shock, the $Asc^{\bullet -}$ concentration increased by 18% at 9 min, and then gradually decreased. In comparison, shocks given after magnesium pretreatment caused only a 5% peak $Asc^{\bullet -}$ increase. Statistical comparison of the two $Asc^{\bullet -}$ curves shows that there is a significant difference overall between the two curves. Further, a significant decrease ($P < 0.05$) in the $Asc^{\bullet -}$ concentration was observed at the specific time point of 12 min post-shock, comparing the no-magnesium versus magnesium curves. The integrated area under the entire no-magnesium shock curve was 182 (arbitrary units) versus 51 (arbitrary units) in the magnesium curve ($P < 0.05$), indicating an overall reduction in total free radical flux of 72%.

Echocardiographic measured fractional area shortening is shown in Fig. 2. At baseline before shock, the fractional area shortening was $69 \pm 2.6\%$. The no-magnesium shock resulted in a significantly lower fractional area shortening ($41 \pm 2.8\%$) compared to baseline ($P < 0.001$). However, the magnesium shock caused no significant fall in percent fractional area shortening, indicating the preservation of left ventricular function by magnesium infusion after biphasic waveform shocks.

4. Discussion

The major findings of the present study are: (1) magnesium attenuates $Asc^{\bullet -}$ production after DC countershock; (2) the decrease in free radical concentration is accompanied by less post-shock myocardial dysfunction.

Table 1
Hemodynamics before and after magnesium

	No-magnesium		Magnesium	
	Baseline	Post-shock	Baseline	Post-shock
Heart rate (beat/min)	179±9	182±11	182±9	184±10
Systolic arterial pressure (mmHg)	119±5	112±5	118±6	109±6
Diastolic arterial pressure (mmHg)	95±4	93±4	93±5	88±5

Mean±S.E. None of these changes are statistically significant.

Jackson et al. [9] were the first to demonstrate that an electrical current passing through a physiologic buffer solution resulted in the production of free radicals. Subsequent studies have applied the technique of electrolysis to generate free radicals and investigated the adverse effects of free radicals on myocardial and left ventricular function [10,23–25]. Lecour et al. [10] identified the free radicals generated during the electrolysis of the solution used to perfuse isolated rat heart Langendorff preparations; the quantity of free radicals induced was directly proportional to the intensity of the current. Paolocci et al. [23] demonstrated that oxygen free radicals generated by electrolysis could impair NO-mediated coronary vasorelaxation severely affecting both basal and agonist-evoked NO release.

There is increasing evidence that free radicals are also produced by DC countershocks directly. Trouton et al. [26] reported that DC countershock alone, delivered to normal beating hearts, could generate free radicals and those countershock-induced free radicals contributed to cellular injury. Using an EPR resonance method to monitor in real-time the production of free radicals, Caterine et al. [11] demonstrated previously that free radicals were generated when epicardial DC countershocks were delivered to heart; there was a significant linear relation between the shock energy and percent free radical increase. The free radicals generated were similar whether the heart was in ventricular fibrillation or in sinus rhythm, and repeated shocks generated similar $Asc^{\bullet -}$ rise after each shock [11], thus supporting our conclusion in this study that the blunting of the $Asc^{\bullet -}$ rise after the magnesium pretreatment may be attributed to the magnesium, not simply to a repeated shock.

The evidence of free radical generation by DC countershocks provides a possible mechanism for shock-induced myocardial damage. This shock-induced injury occurs commonly, resulting from both epicardial and transthoracic shocks [1–3]. However, its mechanism is still uncertain. Several studies have shown that DC countershocks resulted in metabolic and cellular changes in the myocardium. Trouton et al. [26] suggested that DC countershocks lead to mitochondrial dysfunction, which generated superoxide and hydrogen production via leakage from the electron transport. In another study, Trouton et al. [27] found that electrical

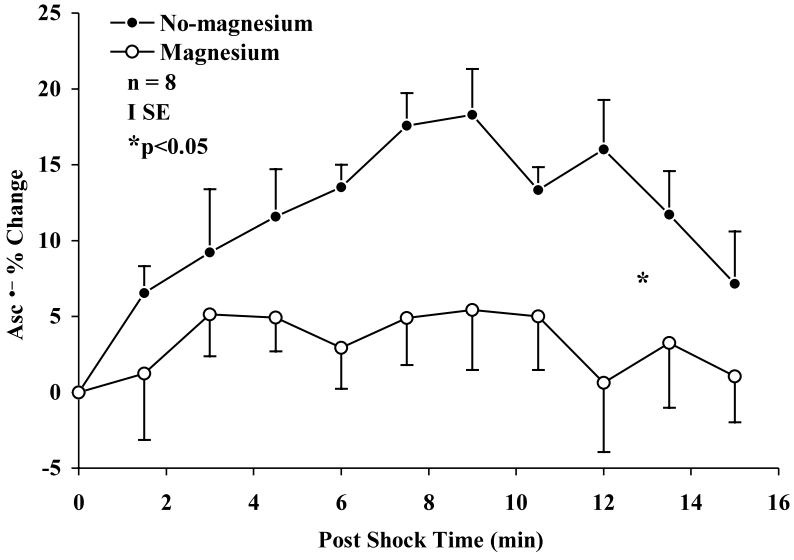

Fig. 1. Changes in coronary sinus concentration of ascorbate free radicals ($Asc^{\bullet -}$) after a biphasic shock. The $Asc^{\bullet -}$ concentration was significantly lower for the entire curve ($P < 0.05$) and at the specific time point 12 min after the shock ($P < 0.05$) for the magnesium shocks compared to the no-magnesium shocks.

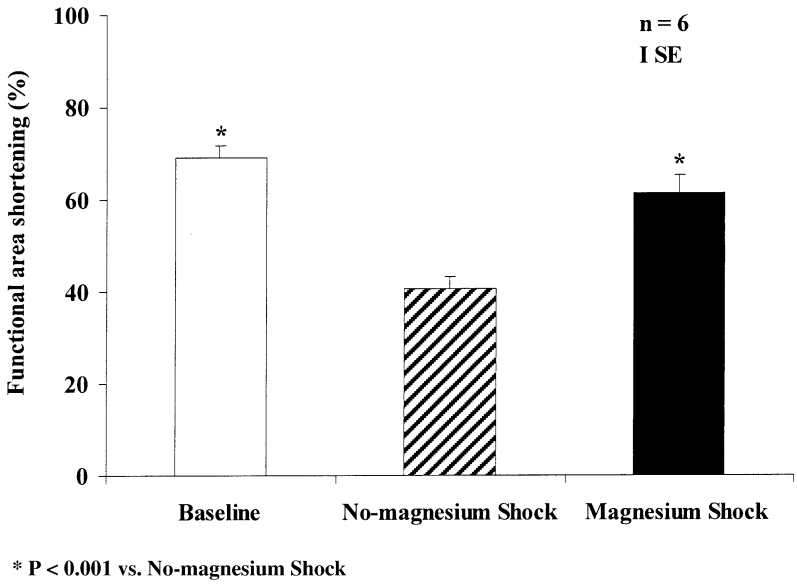

Fig. 2. Fractional area shortening by echocardiography after the no-magnesium shock was significantly lower than the baseline (no-magnesium

discharges did not directly depress mitochondrial function and postulated that free radical formation might be related to the reduction in mitochondrial oxygen consumption after transthoracic shocks. Such free radicals may cause lipid peroxidation, cellular dysfunction and myocardial stunning [8].

Pharmacological interventions have been tested to reduce free radical production by DC countershocks. Caterine et al. [11] found that the antioxidant enzymes superoxide dismutase (SOD) and catalase reduced free radical generation by DC countershocks. However, SOD and catalase are not used clinically. Pagan-Carlo et al. [12] reported that the angiotensin converting enzyme inhibitor captopril, which is known to possess free radical scavenging properties, lowered coronary venous free radical concentration after high-energy epicardial DC shocks. In the present study magnesium pretreatment infusion attenuated the generation of free radicals by epicardial DC shocks, which was accompanied by preserved left ventricular function. This finding may have clinical implications since it is common for cardiac surgeons to deliver an epicardial DC shock to terminate VF during cardiac surgery. If our experimental findings are clinically applicable, magnesium administration several minutes prior to anticipated epicardial defibrillation might be cardioprotective.

Magnesium has been used clinically in patients with acute myocardial infarction. Its role of preserving left ventricular ejection fraction and limiting infarct size may be due to its actions as a 'physiologic calcium channel blocker' [13]. We previously showed that magnesium pre-treatment reduced free radical concentration in coronary sinus blood after coronary occlusion–reperfusion sequences in dogs [15]. The results of our present study indicate the beneficial effect of magnesium may extend to other cardioprotective effects, such as reducing free radicals generated by DC shocks. Magnesium lowers free radical concentration by one or more of the following possible mechanisms:

(1) Inhibition of free radical production: magnesium has been shown to inhibit nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, which in turn lowers production of the superoxide radical [14]. A high level of magnesium was found equivalent to other excellent inhibitors of NADPH oxidase, such as copper [14].

(2) Magnesium deficiency causing myocardial oxidative stress [28–32]: Kharb et al. [28] found that magnesium deficiency could potentiate free radical production associated with myocardial infarction and worsen oxidative injury to post ischemic myocardium. Magnesium deficiency has also been shown to contribute to prolonged myocardial stunning [29], to enhance oxidative injury [30] and myocardial function loss [31], and to cause oxidative neuronal death in murine cortical cultures [3].

(3) Scavenging free radicals: Matkovics et al. [33] reported that magnesium treatment resulted in a significant increase (10–50%) in the activation of the enzymes superoxide dismutase and catalase as well as in the concentration of the non-enzymatic antioxidant glutathione.

(4) Magnesium also appears to play a role in the release of endothelium-derived relaxing factor, EDRF or NO. Calcium causes endothelium-dependent arterial relaxation, attributed to endothelium-derived NO. Gold et al. [34] showed that extracellular magnesium and calcium elicit mutually antagonistic or reciprocal actions at the level of the formation or release of endothelium-derived NO. In a setting of defibrillation, the calcium-

antagonizing action of magnesium may result in less endothelial-derived NO release. NO combines with superoxide to form peroxynitrite, a cardiotoxic, highly unstable and reactive radical that causes lipid peroxidation. If magnesium results in less NO release, less peroxynitrite should be formed and less myocardial injury will result. We have previously shown that the NO synthase inhibitor N^G-nitro-L-arginine (L-NNA) reduces the concentration of free radicals after direct current shocks [35]. Thus, the action of magnesium to reduce free radicals may in part be due to its effects on the NO–peroxynitrite system.

4.1. Limitations

There are several limitations of the present study. We studied normal animal hearts without coexisting hypoxia, acidosis or other anomalies that often accompany or precipitate cardiac surgery and epicardial defibrillation. In addition, we cannot exclude entirely a role of magnesium in preserving myocardial contractility by vasodilation [36], although arterial pressure in our study was not lowered by magnesium.

A 30 J epicardial shock was used to generate free radicals in the present study, somewhat higher than the usual energies delivered during cardiac surgery (5–20 J) [37]. The increase in free radical concentration (18%) after 30 J biphasic shocks in this study is higher than the observed increase after 40J monophasic shocks in our previous study [11] (5.8%). There are two obvious differences between these two studies: species and shock waveform. Whether other factors may contribute is unknown.

The deleterious effect of the 30 J shock on ventricular function may be attributed in part to the experimental design (open-chest model) and the duration of the experiments (up to 1 h may be required to obtain a stable free radical signal).

Another limitation of our study is the confounding effects of repeated shocks. Although our previous study [11] showed that free radical generation remained constant with repeated same-energy shocks, left ventricular function may be adversely affected.

The dose of magnesium in this study was arbitrary. The optimal dose of magnesium remains to be established.

We did not repeat the echo between the two shocks. Thus there is no pre-magnesium baseline echo data.

5. Conclusions

Magnesium pre-treatment reduced oxygen free radicals generated by epicardial shocks and preserved post-shock left ventricular contractile function. Magnesium may be cardioprotective during epicardial ('surgical') defibrillation.

Acknowledgements

Supported by American Heart Assoc. #0020656Z (YZ), National Heart Lung and Blood Institute grant HL53284 (REK), and CA84462 (GRB, SMM).

References

[1] Warner ED, Dahl C, Ewy GA. Myocardial injury from transthoracic defibrillator countershock. Arch Pathol 1975;99:55–9.
[2] Kerber RE, Martins JB, Gascho JA, Marcus ML, Grayzel J. Effect of direct-current countershocks on regional myocardial contractility and perfusion. Experimental studies. Circulation 1981;63:323–32.
[3] Tang W, Weil MH, Sun S, et al. The effects of biphasic and conventional monophasic defibrillation on postresuscitation myocardial function. J Am Coll Cardiol 1999;34:815–22.
[4] Zweier JL, Flaherty JT, Weisfeldt ML. Direct measurement of free radical generation following reperfusion of ischemic myocardium. Proc Nat Acad Sci USA 1987;84:1404–7.
[5] Bolli R, Patel BS, Jeroudi MO, Lai EK, McCay PB. Demonstration of free radical generation in 'stunned' myocardium of intact dogs with the use of the spin trap alpha-phenyl N-tert-butyl nitrone. J Clin Invest 1988;82:476–85.
[6] Ambrosio G, Tritto I. Reperfusion injury: experimental evidence and clinical implications. Am Heart J 1999;138:S69–75.
[7] Bolli R, Marban E. Molecular and cellular mechanisms of myocardial stunning. Physiol Rev 1999;79:609–34.
[8] Kramer JH, Misik V, Weglicki WB. Lipid peroxidation-derived free radical production and postischemic myocardial reperfusion injury. Ann N Y Acad Sci 1994;723:180–96.
[9] Jackson CV, Mickelson JK, Stringer K, Rao PS, Lucchesi BR. Electrolysis-induced myocardial dysfunction. A novel method for the study of free radical mediated tissue injury. J Pharmacol Methods 1986;15:305–20.
[10] Lecour S, Baouali AB, Maupoil V, Chahine R, Abadie C, Javouhey-Donzel A, Rochette L, Nadeau R. Demonstration of the production of oxygen-centered free radicals during electrolysis using E.S.R. spin-trapping techniques: effects on cardiac function in the isolated rat heart. Free Radic Biol Med 1998;24:573–9.
[11] Caterine MR, Spencer KT, Pagan-Carlo LA, Smith RS, Buettner GR, Kerber RE. Direct current shocks to the heart generate free radicals: an electron paramagnetic resonance study. J Am Coll Cardiol 1996;28:1598–609.
[12] Pagan-Carlo LA, Garcia LA, Hutchison JL, Buettner GR, Kerber RE. Captopril lowers coronary venous free radical concentration after direct current cardiac shocks. Chest 1999;116:484–7.
[13] Steurer G, Yang P, Rao V, Mohl W, Glogar D, Smetana R. Acute myocardial infarction, reperfusion injury, and intravenous magnesium therapy: basic concepts and clinical implications. Am Heart J 1996;132:478–82.
[14] Afanas'ev IB, Suslova TB, Cheremisina ZP, Abramova NE, Korkina LG. Study of antioxidant properties of metal aspartates. Analyst 1995;120:859–62.
[15] Garcia LA, Dejong SC, Martin SM, Smith RS, Buettner GR, Kerber RE. Magnesium reduces free radicals in an in vivo coronary occlusion-reperfusion model. J Am Coll Cardiol 1998;32:536–9.

[16] Buettner GR. The pecking order of free radicals and antioxidants: lipid peroxidation, (-tocopherol, and ascorbate. Arch Biochem Biophys 1993;300:535–43.
[17] Frei B, Stocker R, Ames BN. Antioxidant defenses and lipid peroxidation in human blood plasma. Proc Natl Acad Sci USA 1988;85:9748–52.
[18] Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci USA 1989;86:6377–81.
[19] Buettner GR, Jurkiewicz BA. Ascorbate free radical as a marker of oxidative stress: an EPR study. Free Radic Biol Med 1993;14:49–55.
[20] Sharma MK, Buettner GR, Spencer KT, Kerber RE. Ascorbyl free radical as a real-time marker of free radical generation in briefly ischemia and reperfusion hearts. An electron paramagnetic resonance. Circ Res 1994;74:650–8.
[21] Buettner GR, Kiminyo KP. Optimal EPR detection of weak nitroxide spin adduct and ascorbate free radical signals. J Biochem Biophys Meth 1992;24:147–51.
[22] SAS Version 8, SAS Institute Inc., 1999
[23] Paolocci N, Biondi R, Bettini M, Lee CI, Berlowitz CO, Rossi R, Xia Y, Ambrosio G, L'Abbate A, Kass DA, Zweier JL. Oxygen radical-mediated reduction in basal and agonist-evoked NO release in isolated rat heart. J Mol Cell Cardiol 2001;33:671–9.
[24] Chahine R, Chen X, Yamaguchi N, de Champlain J, Nadeau R. Myocardial dysfunction and norepinephrine release in the isolated rat heart injured by electrolysis-induced oxygen free radicals. J Mol Cell Cardiol 1991;23:279–86.
[25] Jackson CV, Mickelson JK, Pope TK, Rao PS, Lucchesi BR. O_2 free radical-mediated myocardial and vascular dysfunction. Am J Physiol 1986;251:H1225–31.
[26] Trouton TG, Allen JD, Yong LK, Rooney JJ, Adgey AA. Metabolic changes and mitochondrial dysfunction early following transthoracic countershock in dogs. Pacing Clin Electrophysiol 1989;12:1827–34.
[27] Trouton TG, Barry JJ, Allen JD, Adgey AA. Failure of counter-shock-type pulses in vitro to adversely alter mitochondrial oxidative phosphorylation. Ann Emerg Med 1992;21:132–6.
[28] Kharb S, Singh V. Magnesium deficiency potentiates free radical production associated with myocardial infarction. J Assoc Phys India 2000;48:484–5.
[29] Herzog WR, Atar D, Mak IT, Alyono D, MacCord C, Weglicki WB. Magnesium deficiency prolongs myocardial stunning in an open-chest swine model. Int J Cardiol 1994;47:105–15.
[30] Freedman AM, Cassidy MM, Weglicki WB. Magnesium-deficient myocardium demonstrates an increased susceptibility to an in vivo oxidative stress. Magnes Res 1991;4:185–9.
[31] Kramer JH, Misik V, Weglicki WB. Magnesium-deficiency potentiates free radical production associated with postischemic injury to rat hearts: vitamin E affords protection. Free Radic Biol Med 1994;16:713–23.
[32] Regan RF, Guo Y. Magnesium deprivation decreases cellular reduced glutathione and causes oxidative neuronal death in murine cortical cultures. Brain Res 2001;890:177–83.
[33] Matkovics B, Kiss I, Kiss SA. The activation by magnesium treatment of anti-oxidants eliminating the oxygen free radicals in Drosophila melanogaster in vivo. Magnes Res 1997;10:33–8.
[34] Gold ME, Buga GM, Wood KS, Byros RE, Chaudhuri G, Iganaro LJ. Antagonistic modulatory roles of magnesium and calcium on release of endothelial derived relaxing factor and smooth muscle tone. Circ Res 1990;66:355–66.
[35] Clark CB, Bissing JW, Davies LR, Martin SM, Buettner GR, Kerber RE. The NO synthase inhibitor L-NNA decreases defibrillation-induced free radical generation. J Am Coll Cardiol 1999;33:369A.
[36] Altura BT, Altura BM. Endothelium-dependent relaxation in coronary arteries requires magnesium ions. Br J Pharmacol 1987;91:449–51.
[37] Kerber RE, Carter J, Klein S, Grayzel J, Kennedy J. Open chest defibrillation during cardiac surgery. Energy and current requirements. Am J Cardiol 1980;46:393–6.