Captopril Lowers Coronary Venous Free Radical Concentration After Direct Current Cardiac Shocks*

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Study objectives: **Direct current (DC) shocks to the heart cause morphologic and functional myocardial damage. Previous studies have suggested that acute DC shock injury is free radical mediated and that the administration of antioxidant enzymes superoxide dismutase and catalase can reduce the level of DC shock-induced free radicals. Angiotensin-converting enzyme (ACE) inhibitors are clinically used drugs that may scavenge free radicals or reduce free radical generation. The objective of our study was to determine whether the ACE inhibitor captopril lowers free radicals after DC shocks.**

Design: **In six open-chest dogs, we administered 100-J DC shocks to the epicardium, before and after administration of captopril, 3 mg/kg. We used electron paramagnetic resonance measurements of arterial and coronary venous ascorbate free radical (AFR) as a real-time marker of free radical generation (total oxidative flux).**

Measurements and results: **Captopril resulted in a significant lowering of coronary venous AFR concentration: the peak rise in AFR after 100-J shocks was 17.3 ± 3.4% (mean ± SEM before captopril vs 3.2 ± 4.0% after captopril; $p < 0.05$).**

Conclusions: **Captopril lowers coronary venous AFR concentration after high-energy epicardial shocks.** ***(CHEST 1999; 116:484–487)***

Key words: angiotensin-converting enzyme inhibitors; captopril; cardioversion; defibrillation; free radicals

Abbreviations: ACE = angiotensin-converting enzyme; AFR = ascorbate free radical; DC = direct current; EPR = electron paramagnetic spin resonance

Both transthoracic and epicardial direct current (DC) countershocks have been demonstrated to cause transient and permanent myocardial damage.[1,2] However, the mechanism of this DC shock injury has been uncertain. There is considerable evidence that free radicals play a major role in DC shock-induced myocardial injury. The passage of a current through a physiologic buffer solution has been shown to result in the generation of free radicals.[3] DC countershocks to the heart have been shown to result in the production of free radicals.[4] Caterine et al[5] have shown that free radical generation is primarily dependent on the peak energy delivered and that it is not dependent on the presence or absence of ventricular fibrillation.

In models of myocardial ischemia and reperfusion, *in vivo* protection against free radical injury is obtained from free radical scavengers such as superoxide dismutase and catalase. However, these endogenous antioxidant enzymes are not used clinically. In contrast, angiotensin-converting enzyme (ACE) inhibitors are a class of drugs that are widely used in the treatment of hypertension and congestive heart failure. Some studies have suggested that ACE inhibitors can act as free radical scavengers.[6–9] For example, Przyklenk and Kloner[8] have shown that administration of ACE inhibitors improved recovery of contractile function in "stunned" canine myocardium, a phenomenon associated with the production of oxygen radicals. Because free radicals are generated by DC shocks to the heart, ACE inhibitors might scavenge such radicals or inhibit their production; this may be a clinically applicable method of reducing defibrillation injury.

Recently we described an electron paramagnetic spin resonance (EPR) method that allows real-time,

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ex vivo quantification of free radical generation.[10] The method uses the ascorbate free radical (AFR) concentration in coronary venous blood as a real-time marker of myocardial free radical generation. The AFR has a low reduction potential, thereby facilitating the oxidation of ascorbate by almost all oxidizing radicals generated by biological systems.[11–15] For example, free radicals such as lipid peroxyl radicals can be eliminated by vitamin E in the lipid membrane, but this results in the formation of the vitamin E radical. This radical in turn can be repaired by vitamin C in the aqueous phase resulting in AFR production.[11] The AFR is a resonance-stabilized radical that is easily detectable by EPR.

In this study, we used this new method in a series of experiments undertaken to test the hypothesis that the ACE inhibitor captopril can act as a free radical inhibitor or scavenger after epicardial DC shocks, thereby lowering the postshock coronary venous concentration of AFR.

Materials and Methods

Animal Preparation

An open-chest model previously described was used for this study.[10] Adult mongrel dogs of either gender were used. Body weights ranged from 20 to 30 kg. Sedation and anesthesia were induced with IV fentanyl/droperidol, 1.2 mg/60 mg, and sodium pentobarbital, 400 mg. The animals underwent endotracheal intubation and were placed on a volume-cycled respirator. Routine monitoring of blood gases was performed to maintain physiologic conditions. Intravascular access was obtained bilaterally via the internal jugular veins, femoral arteries, and femoral veins. Both arterial BPs and ECGs were monitored throughout the experiments. A left lateral thoracotomy was performed in the fifth intercostal space, and the heart was suspended in a pericardial cradle. The left atrial appendage was retracted to allow for adequate exposure of the coronary sinus. A catheter was advanced from the left internal jugular vein into the coronary sinus and secured with a ligature in the distal great cardiac vein. A venous-venous shunt was fashioned between the great cardiac vein and the right femoral vein. Heparin was administered in the IV fluid and the indwelling cannulas and tubing to prevent clotting.

EPR Methods

The measurement of AFRs has been previously described and validated as a measure of total oxidative stress.[15] Our method of AFR measurement has previously been described in detail.[10] Briefly, the EPR spectra of the AFR were obtained using a spectrometer (Varian E4 spectrometer; Varian Associates; Palo Alto, CA) with a transverse mode110 cavity and an aqueous flat cell. Scans were collected serially on blood being continuously withdrawn from either the great cardiac vein or the femoral artery. An infusion pump was used to circulate the sample through the spectrometer and return it to the animal. In this fashion, blood could be scanned for AFRs within 7 s of leaving the animal. The following instrument settings were used for all studies because they provide the largest AFR signal: nominal power, 40 mW; modulation amplitude, 0.63 gauss; time constant, 1 s; and scan rate, 1 G/24 s. To amplify the endogenous AFR signal, which normally is too low in canine blood for detection by EPR, a 1-g bolus of ascorbate was given followed by an IV infusion of ascorbate (8 to 30 mg/min). The AFR EPR signal is partially saturated at 40 mW nominal power. Appropriate corrections in ascorbate infusion rates were made to achieve steady-state AFR concentrations in the femoral artery and coronary venous blood. The femoral artery AFR signal is higher than the coronary venous signal and must be stable to allow meaningful interpretation of rises in the coronary venous signal, which indicates increased myocardial radical generation.[10]

The concentration of the AFR was determined from the signal height of the AFR EPR spectrum after calibration using double-integration techniques and 3-carboxy peroxyl as the standard. All signal heights were normalized to the full gain of the instrument, 10^5. In our experimental conditions, after accounting for saturation effects, 1 mm of signal height corresponds to 0.0734 nmol/L AFR.[16]

Protocol

In six dogs, the effect of DC shocks on the generation of AFR was studied. After obtaining baseline (steady-state) AFR measurements from the femoral artery and great cardiac vein, an initial 100-J DC shock was delivered to the epicardium of the beating heart. All shocks were administered using a damped sine waveform defibrillator (Datascope MD2-J; Datascope Corp; Paramus, NJ). All shocks were administered via hand-held electrode paddles cradling the heart.

After the shock, we monitored AFR signals for 10 min. Typically, the coronary venous AFR signal briefly fell during the first 1 to 2 min after shocks, probably reflecting the expected transient postshock hypotension and bradycardia.[17] Because this coronary venous AFR fall is artifactual, we have omitted AFR data from the first 2 min after each shock.

After the postshock monitoring was completed, we prepared for the second shock. We first verified that the arterial AFR signal was unchanged. If necessary, we adjusted the ascorbate infusion rate and monitored the arterial AFR signal further until a steady-state arterial signal was regained before administering the second shock.

Once the AFR monitoring was completed after the first 100-J shock and a steady-state was reestablished, an IV infusion of the ACE inhibitor captopril, 3 mg/kg, was started 15 min before delivering the second 100-J DC shock. The second shock was administered after one half of the total captopril dose had been infused, and the infusion was continued through the 10-min postshock monitoring period. The ACE inhibitor dose was based on studies of the cardioprotective effects of ACE inhibitors by other investigators, in settings of postischemic stunned myocardium.[18–21] After the second 100-J shock, the AFR level was again measured.

Statistical Analysis

Analysis of the AFR concentration generated by the shocks in the absence or presence of captopril was done by repeated measures of the analysis of variance with both the intervention and time as repeated factors. *Post hoc* comparisons of intervention (captopril) vs no intervention (no captopril) at each point were done by using Bonferroni's method ($p < 0.05$). BP comparisons before and after captopril were done by using the Student's paired *t* test. All results are reported as mean ± SEM.

Results

After the initial 100-J shock, the mean AFR concentration in coronary venous blood rose from 7.8 ± 0.2 nmol/L at baseline to a peak of 9.1 ± 0.3 nmol/L (17.3 ± 3.4% rise). After the administration of captopril and a second 100-J shock, the AFR concentration rose minimally, from 7.6 ± 0.4 nmol/L to 7.8 ± 0.4 nmol/L (3.2 ± 4.0% rise; $p < 0.05$ compared with the AFR rise induced by the initial precaptopril shock). The rise in AFR generation was significantly higher after the first 100-J shock compared with the second 100-J shock that followed captopril (Fig 1).

After the first 100-J shock (given before the administration of captopril), mean arterial pressure fell from 90 ± 10 mm Hg to 76 ± 10 mm Hg ($p < 0.05$). After the administration of captopril and the second shock, the arterial pressure fell from 69 ± 8 mm Hg to 66 ± 8 mm Hg (not significant).

Discussion

In this study, we have shown that the shock-induced increase in coronary venous AFR concentration was virtually abolished by the administration of captopril before delivering high-energy epicardial shocks.

Myocardial injury from DC shocks (defibrillation injury) is a well-established phenomenon, resulting from both transthoracic and epicardial shocks.[1,2] The nature of the morphologic injury is patchy and seems to be related to the amount of current that traverses the myocardium.[22] Functional injury (contraction abnormalities, atrioventricular block) has been reported.[1,23] Transesophageal echocardiographic studies show atrial dysfunction ("atrial stunning") after elective cardioversion for atrial fibrillation.[24]

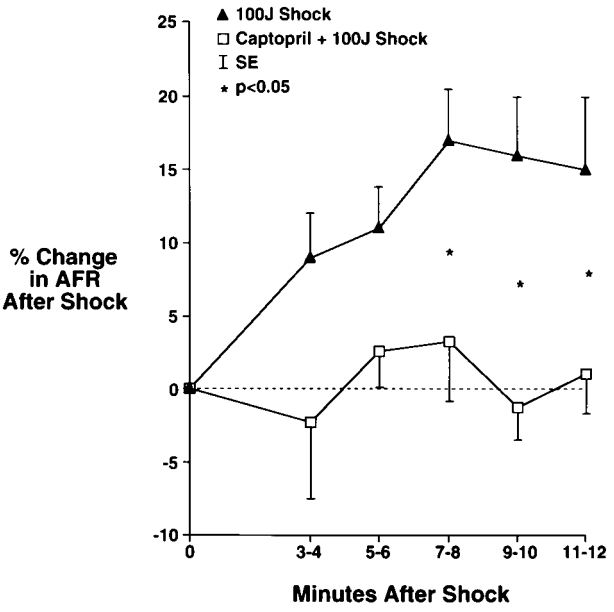

Figure 1. Percent change in AFR vs time after 100-J shocks before and after captopril. The peak percent AFR rise after an initial 100-J shock was 17.3 ± 3.4%. After the administration of captopril, the peak AFR rise after a second 100-J shock was lower, 3.2 ± 4.0% ($p < 0.05$ vs initial shock-induced AFR rise).

Trouton et al[4] have postulated that this damage may be related to the observed mitochondrial dysfunction. Mitochondria are sources of oxygen radicals via leakage from the electron transport chain. Any damage to mitochondria may result in additional seepage of electrons from the chain, resulting in superoxide and hydrogen peroxide production.[25]

Previous work in our laboratory has shown that the increase of AFR after epicardial shocks is energy dependent, and it can be attenuated by the administration of superoxide dismutase and catalase.[5] Superoxide dismutase and catalase are not used clinically, whereas ACE inhibitors are used clinically and may also have free radical scavenging or inhibitory effects. Przyklenk and Kloner[8] noted that ACE inhibitors improved contractile function in stunned myocardium, a phenomenon in which free radicals have been implicated,[26–29] by as much as five to seven times. Studies by Westlin and Mullane[21] have demonstrated, both *in vitro* and *in vivo*, that captopril scavenges superoxide radicals and improves myocardial dysfunction. Chopra et al[7] found that the ACE inhibitors captopril, epicaptopril, and zofenopril significantly scavenged free radicals. Although some studies have suggested that the sulfhydryl portion of the ACE inhibitors is required for the optimal free radical scavenging effect,[7,21,30] other studies[6,9,31] have found that ACE inhibitors both with and without the sulfhydryl moiety act as effective free radical scavengers. The ACE inhibitor we used, captopril, contains a sulfhydryl group.

We have previously shown that in the absence of antioxidant enzymes, repeated shocks at the same energy generate equivalent amounts of AFRs.[26] Thus, the fall in ascorbate generation we observed after administration of captopril and a second 100-J shock cannot simply be ascribed to a nonspecific effect of repeated shocks, but must be caused by the scavenging or inhibiting effects of captopril.

The dose of captopril we chose was based on the work of previous investigators who have studied the cardioprotective effects of ACE inhibitors.[8,18–21] The captopril dose we used, 3 mg/kg IV, is much higher than doses typically used in patients. Whether or not lower doses more analogous to clinical usage would have the same radical-lowering effect is not established by this study.

One limitation of this study is that we did not attempt to measure the functional consequences of free radical generation; we did not demonstrate that

the reduction of the shock-induced rise in free radicals, which occurred because of the administration of captopril, was accompanied by a preservation of, for example, myocardial contractility. Whether or not captopril is functionally cardioprotective in the setting of DC shocks remains to be established.

ACE inhibitors are already shown to be useful drugs in the treatment of congestive heart failure and hypertension. The present results cannot be directly extrapolated to the clinical setting, but they do suggest that lowering of free radical concentration may already be clinically possible by use of captopril. Further studies are needed to determine whether captopril may be used preemptively in the clinical setting of defibrillation or cardioversion to prevent free radical damage.

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