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Neuroprotective antioxidant STAZN protects against myocardial ischemia/reperfusion injury

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ABSTRACT

Background: Protecting the myocardium from ischemia-reperfusion injury has significant potential to reduce the complications of myocardial infarction and interventional revascularization procedures. Reperfusion damage is thought to result, in part, from oxidative stress. Here we use a novel method of percutaneous coronary occlusion to show that the potent antioxidant and neuroprotective free-radical scavenger, stilbazulenyl nitrone (STAZN), confers marked cardioprotection when given immediately prior to reperfusion.

Methods and results: Physiologically controlled male Sprague-Dawley rats were anesthetized with isoflurane, paralyzed with pancuronium and mechanically ventilated. A guide wire was introduced via the femoral artery and advanced retrogradely via the aorta into the left coronary artery under fluoroscopic guidance. Rats with established coronary ischemia (85 min after occlusion) were given STAZN 3.5 mg/kg or its vehicle 5 min before and 2 h after reperfusion, and were subjected to functional and histopathologic studies at 3 days. Ischemia-associated Q wave amplitude was reduced by 73% in STAZN-treated rats ($P = 0.01$), while infarct-related ejection fraction, fractional shortening and severe regional wall-motion impairments were improved by 48%, 54% and 37%, respectively, relative to vehicle-treated controls ($P = 0.05$). Total myocardial infarct volume in STAZN-treated rats was correspondingly reduced by 43% ($P < 0.05$), representing a sparing of 14% of the total left ventricular myocardium.

Conclusions: STAZN, a second-generation azulenyl nitrone with potent neuroprotective efficacy in brain ischemia, is also a rapidly acting and highly effective *cardio*protective agent in acute coronary ischemia. Our results suggest the potential for clinical benefit in the setting of acute coronary syndromes.

1. Introduction

Thrombolytic therapy and percutaneous coronary interventions reduce mortality from acute myocardial infarction (AMI), and it has become standard practice to administer these treatments as early as possible [1]. However, adjunctive therapeutic strategies are needed because significant mortality and disability ensue despite the timely administration of these therapies, which are intended to restore blood flow following ischemia. Elevated levels of oxidizing free radicals

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following reperfusion provide a rationale for the testing of antioxidants in models of myocardial ischemia/reperfusion injury [2]. Previous animal studies have shown therapeutic cardioprotective effects for various antioxidants. The lack of successful translation of these strategies to the clinic to-date [3] may relate, not only to possible inter-species differences in drug efficacy, but also to the fact that many previous experimental studies have involved treatments administered only at the onset of ischemia and have failed to substantiate a beneficial effect at longer survival periods [4].

Controversy as to the role of reactive oxygen species (ROS) in cardioprotection also stems from observations of others that the cardioprotective effects of acute preconditioning [5] and of preconditioning-mimetics such as opioids [6] or volatile anesthetic agents [7] are blocked by ROS scavengers, suggesting that ROS are actually necessary for cardioprotective signaling. It is possible that complete neutralization of oxidative stress might reduce both its salutary and damaging influences.

Stilbazulenyl nitrone (STAZN) is a novel, second-generation azulenyl-nitrone free-radical scavenger that confers enduring neuroprotection in models of transient focal cerebral ischemia [8]. In pharmacokinetic and biodistribution studies, STAZN exhibited a long circulating half-life and attained significant myocardial tissue levels. Given a common role of free radicals in cerebral and myocardial reperfusion injury, we hypothesized that STAZN might be cardioprotective in a model of AMI in rats.

2. Materials and methods

2.1. Chemical synthesis of STAZN

STAZN was synthesized from guaiazulene in an efficient five-step procedure that has been published in detail, and its purity was confirmed via reference to archived spectroscopic data for this compound [9].

2.2. Animal preparation

All studies were approved by the University of Miami's Animal Care and Use Committee. Twelve male Sprague-Dawley rats (492 ± 34 g (S.D.), age ~5 months) were allowed rat chow and water ad libitum before surgery. Anesthesia was induced with 4% isoflurane, 70% nitrous oxide and a balance of oxygen delivered into a closed jar. To permit controlled ventilation, rats were orotracheally intubated (2.1 mm o.d. × 45 mm B&D Insyte catheter tubing; Becton Dickinson Infusion Therapy Systems Inc., Sandy UT), and ventilated with a rodent respirator (Stoelting Co., Wood Dale, IL) on a mixture of 70% nitrous oxide, 1.0–2.0% isoflurane and a balance of oxygen passed through a humidifier containing 1 ml of Mucomyst-10 (acetylcysteine) in water. Animals were immobilized with pancuronium bromide (0.75 mg/kg i.v.). The left femoral arteries and veins were cannulated with PE-50 polyethlene tubing. Arterial blood pressure was continuously monitored (model RS3400 polygraph; Gould Instrument Systems Inc., Cleveland, OH). Arterial blood gases (pO_2, pCO_2, pH) were measured (model ABL 330; Radiometer America Inc., Westlake, OH) and maintained in the normal range by ventilatory adjustments. With rats in a supine position, ECG needle electrodes were placed subcutaneously in all four limbs. The precordial (chest) lead was placed (under fluoroscopic guidance) to the left of the sternum between the fourth and fifth ribs, approximately at the mid-clavicular line. Three limb leads, the corresponding unipolar leads and the precordial lead were continuously monitored (ECG switch box, Bio Amp, and Chart 4 software, ADInstruments Inc., Colorado Springs, CO). Rectal temperature was monitored by a thermistor and maintained at 36.5 ± 0.5 °C by a heating pad or heating lamp (CMA/150 Temperature Controller; CMA/Microdialysis AB, Stockholm, Sweden).

2.3. Left coronary artery occlusion (LCAo)

The left coronary artery was reversibly occluded for 90 min by a novel percutaneous method employing the intraluminal insertion of a guidewire. A custom-designed, coated microcatheter (O.D. 940 μm, I.D. 760 μm, VasCon LLC, Miami FL) with a smooth bullet-shaped head and loaded with a 360 μm guidewire (Choice PT-Plus, Boston Scientific, Maple Grove, MN) was introduced via the right femoral artery by inguinal cut-down. Under X-ray fluoroscopic guidance, the microcatheter was advanced retrogradely into the descending aorta; the wire was then used to navigate the bend of the aortic arch while the catheter was advanced over it and thence into the ascending aorta until it abutted against the aortic valve. The microcatheter was then retracted slightly and served as a guide for the wire. The wire was advanced outside the microcatheter until it reached the left aortic sinus.

To reduce the incidence of fatal ventricular tachycardia and fibrillation subsequent to arterial occlusion, two preventive strategies were employed: moderate hypotension and prophylactic lidocaine. By briefly increasing the inspired isoflurane from 2.0% to 4.0%, it was possible to induce moderate hypotension (77 ± 5 mmHg, range: 71–89 mmHg) at the time of the onset of ischemia. The rats received a prophylactic dose of lidocaine (10 mg/kg, i.v., 1 mg/kg/min) starting 5 min before insertion of the wire and occlusion of the artery, and continuing 5 min thereafter.

Under fluoroscopic guidance, the wire was introduced into the left coronary artery and was advanced until changes were observed on the ECG, indicating successful coronary occlusion. The duration of occlusion was 90 min. Blood pressure and ECG were continuously monitored. Blood gases were measured 15 and 85 min after insertion of the wire and were maintained in the normal range by ventilatory adjustments. Rats were randomized to receive two doses of either STAZN (3.5 mg/kg i.v. in 30% Solutol HS 15 and 70% isotonic saline at 2 ml/h, $n = 6$) or vehicle (0.37 ml/kg 30% Solutol HS 15 and 70% isotonic saline at 2 ml/h, $n = 6$). These agents were infused i.v. over 5 min, beginning at 85 min of arterial occlusion and again at 2 h of reperfusion, respectively. After 90 min of occlusion, the wire was withdrawn, the catheters removed, the incisions closed, and rats returned to their cages. Weight, temperature and ECG were recorded 1, 2 and 3 days afterwards by an investigator blinded to the treatment.