CARDIOVASCULAR

Dopamine Induces Postischemic Cardiomyocyte Apoptosis In Vivo: An Effect Ameliorated by Propofol

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Background. **Dopamine is commonly used to improve postischemic myocardial contractile function. However, there is evidence that dopamine augments apoptosis after ischemia through increased intracellular calcium and opening of the mitochondrial permeability transition pore. Propofol (2,6-diisopropylphenol) is an anesthetic that has been shown to prevent mitochondrial permeability transition pore opening. We evaluated the effects of propofol given during reperfusion on dopamine-mediated apoptosis.**

Methods. **Hearts from 8-week-old inbred New Zealand White rabbit siblings were subjected to 2 hours of cold cardioplegic ischemia and 6 hours of reperfusion in a heterotopic transplant model. Controls consisted of the recipient rabbit's nonischemic heart. The ischemia-reperfusion (IR) group consisted of postischemic hearts reperfused with no drugs; the IR plus dopamine (IR+D) group received dopamine (20 $\mu g \cdot kg^{-1} \cdot min^{-1}$) continuously; the IR+D plus propofol (IR+D+P) group received dopamine (20 $\mu g \cdot kg^{-1} \cdot min^{-1}$) plus propofol (500 to 600 $\mu g \cdot kg^{-1} \cdot min^{-1}$); and the IR plus propofol (IR+P) group received propofol only (500 to 600 $\mu g \cdot kg^{-1} \cdot min^{-1}$) throughout reperfusion (n = 7 to 9 in each group). Myocardial function was measured using a left ventricular balloon; terminal nick-end labeling (TUNEL) staining, DNA electrophoresis, and immunoblotting for caspase-3 cleavage were performed at the end of reperfusion.**

Results. **Dopamine increased the number of TUNEL-positive nuclei significantly (14.0 ± 2.0/1,000 for IR+D versus 6.7 ± 2.0/1,000 for IR, $p = 0.01$). Propofol (IR+D+P) reduced the total number of apoptotic cells in hearts receiving dopamine (7.1 ± 1.8/1,000, $p = 0.01$ versus IR+D) to the extent seen in IR alone. DNA laddering and caspase-3 cleavage were observed at greater frequency in the IR+D group compared with the IR and IR+D+P groups. Propofol had no effect on dopamine-mediated increased systolic function, but improved diastolic function after ischemia.**

Conclusions. **Dopamine infusion has a positive inotropic effect on the postischemic heart at the expense of increased cardiomyocyte apoptosis. The addition of propofol prevents dopamine-induced apoptosis after ischemia while maintaining positive inotropy.**

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Postoperative myocardial dysfunction is highly prevalent after complex cardiac procedures and can lead to low cardiac output with a potential for multiorgan dysfunction. Inotropic agents improve myocardial contractility and systemic perfusion. For the postischemic heart, however, concern exists as to whether use of catecholamines can further augment myocardial injury by mechanisms such as intracellular calcium overload, or damage from production of reactive oxygen species. Inotropic agents, through adrenergic stimulation, further increase postischemic cytosolic calcium by promoting calcium release from sarcoplasmic reticulum and by causing cyclic adenosine monophosphate activation and phosphorylation of L-type calcium channels by protein kinase A, leading to opening of the membrane channels with increased entry of calcium into the cell [1, 2]. In turn, increased cytosolic calcium after ischemia has been shown to affect mitochondria by promoting opening of the mitochondrial permeability transition pore (mPTP) [3], which can lead to apoptosis.

In previous studies using an isolated rabbit heart model of global ischemia and reperfusion, we demonstrated that mean cytosolic calcium levels are elevated after ischemia owing to impaired calcium cycling, and that catecholamine-type inotropic agents such as dopamine further augmented calcium levels. That was associated with an increase in apoptosis and a deterioration of ventricular diastolic function during reperfusion, despite improved systolic function [4]. However, the question remains whether preventing dopamine-induced apoptosis during reperfusion would maintain diastolic function and result in significant preservation of cardiomyocyte viability. This phenomenon is especially relevant to the population affected by congenital heart de-

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fects, for whom the need for multiple surgeries and prolonged use of inotropic agents can lead to myocyte loss at critical stages and contribute to late ventricular dysfunction.

Propofol (2,6-diisopropylphenol) is a lipid-soluble anesthetic agent commonly used in cardiac surgery and for postoperative sedation that has been reported to have protective effects against ischemic injury in excitable cells [5–9]. Propofol has been reported to reduce oxidative stress [10] and intracellular calcium levels in isolated cardiomyocytes [11, 12], and in isolated heart experiments, it maintained mitochondrial respiration and prevented mPTP opening after ischemia [8]. The purpose of this work was to study the effects of propofol in an in vivo model of global myocardial ischemia and reperfusion in the presence of dopamine. We hypothesized that a continuous propofol infusion during reperfusion would prevent dopamine-induced apoptosis after ischemia and preserve diastolic ventricular compliance.

Material and Methods

Animal Care

All animals used for these experiments received care according to the "Principles of Laboratory Animal Care" from the National Society of Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No 85-23, revised 1996). The current protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Children's Hospital Boston.

Animal Model

Inbred 8-week-old New Zealand White (NZW) male rabbit siblings were used in a heterotopic heart transplant model. Under general anesthesia induced with an intramuscular injection of ketamine (100 mg/kg) and xylazine (5 mg/kg) and maintained with isofluorane inhalation (1% to 3%), the donor heart was harvested after cold crystalloid cardioplegic arrest (modified Krebs-Henseleit solution supplemented with KCl 22.5 mmol/L). The heart was maintained in an isotonic solution at 4°C for 2 hours, transplanted in a nonworking fashion to the recipient's abdominal aorta and inferior vena cava, and then reperfused in vivo for 6 hours. The animals were maintained under mild general anesthesia with isofluorane (0% to 1%) throughout reperfusion, and the hearts were collected at end of reperfusion.

Absence of Rejection

Preliminary survival studies using inbred siblings were conducted to confirm the absence of acute and subacute rejection. The donor hearts were explanted after 1 and 7 days, and the tissue was stained with hematoxylin-eosin or Masson's trichrome stain, and bright-field microscopy was used to determine rejection according to the International Society of Heart and Lung Transplant (ISHLT) criteria [13, 14]. One day after transplantation, there was no histologic evidence of rejection (n = 2) in this model. By 7 days, histologic examination showed only mild perivascular lymphocytic infiltrate without myocyte destruction or fibrosis (n = 1). Conversely, nonrelated animals showed severe rejection by day 7 (n = 3). We concluded that an inbred sibling transplant model was suitable for studies in which the reperfusion period did not exceed 1 day. In the subsequent experiments, a 6-hour reperfusion period was used, and histologic examination was performed (n = 34).

Experimental Groups

Five groups of hearts were studied after ischemia and reperfusion (IR), and nonischemic controls (C) consisted of the recipient animal's native heart (n = 34). In postischemic groups, the transplanted heart was subjected to 2 hours of cold ischemia (4°C) after cardioplegic arrest and reperfused for 6 hours in vivo. Group IR (n = 7) received no drugs during reperfusion. For the remaining four groups, the drugs were started at the onset of, and continued throughout, reperfusion. Group IR+D (n = 8) received a continuous dopamine infusion (20 $\mu g \cdot kg^{-1} \cdot min^{-1}$; Bristol-Myers Squibb, New York, New York). The dose was determined during a preliminary dose-response study (n = 3), and 20 $\mu g \cdot kg^{-1} \cdot min^{-1}$ resulted in an increase in heart rate by 10% without affecting the mean arterial blood pressure. Group IR+D+P (n = 9) received a continuous infusion of dopamine (20 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and propofol (AstraZeneca, Wilmington, Delaware) during reperfusion. Propofol was started (100 $\mu g \cdot kg^{-1} \cdot min^{-1}$) at the onset of reperfusion and increased as tolerated to 500 to 600 $\mu g \cdot kg^{-1} \cdot min^{-1}$ within the first hour, then maintained at that rate throughout reperfusion. Based on a dose-response study, 500 to 600 $\mu g \cdot kg^{-1} \cdot min^{-1}$ propofol was required to maintain light anesthesia and did not result in systemic hypotension. Group IR+P (n = 7) was reperfused with an infusion of propofol only, following the same protocol. Group IR+D+L (n = 3) received an infusion of dopamine (20 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and 20% soy-based lipid solution similar to the vehicle for propofol, initiated and maintained at the same volume/rate as the propofol infusion.

Myocardial Function Measurements

A separate set of animals was used to measure myocardial function (n = 7 in each group). The left ventricle (LV) intracavitary balloon was progressively inflated to keep the diastolic pressure at 5 mm Hg during the first 45 minutes of reperfusion and maintained at that volume from 60 minutes to 6 hours of reperfusion. The postischemic heart's left ventricular systolic and diastolic pressures, heart rate, intraventricular balloon volume, as well as the recipient's native heart rate and blood pressure were monitored every 30 minutes. Left ventricular developed pressure (LVDP) was calculated by subtracting the diastolic from the systolic pressure for each time point. Pressure-volume relationships were determined at the end of the reperfusion period by filling the LV balloon in stepwise increments of 0.1 mL and recording the diastolic and systolic pressures.