A Novel Peroxynitrite Decomposer Catalyst (FP-15) Reduces Myocardial Infarct Size in an In Vivo Peroxynitrite Decomposer and Acute Ischemia-Reperfusion in Pigs

Cesario Bianchi, MD, PhD, Hidetaka Wakiyama, MD, Renato Faro, PhD, Tanveer Khan, MD, James D. McCully, PhD, Sidney Levitsky, MD, Csaba Szabó, MD, and Frank W. Sellke, MD

Division of Cardiothoracic Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, and Inotek Corporation, Beverly, Massachusetts

Background. **Reactive oxygen and nitrogen species generated after reperfusion injury result in organ dysfunction. Peroxynitrite, a reactive nitrogen molecule produced from the reaction of superoxide anions and nitric oxide, is thought to be a causative agent in oxidative reperfusion injury. The aim of this study was to investigate the effects of a novel peroxynitrite decomposition catalyst (FP-15) in an acute myocardial ischemia/reperfusion model.**

Methods. **Pigs were subjected to 60 minutes of regional ischemia by reversibly ligating the left anterior descending coronary artery followed by 180 minutes of reperfusion. In the treatment group (n = 6), an FP-15 (1 mg/kg) bolus was infused through the jugular vein after 30 minutes of ischemia followed by a continuous infusion ($1\ mg \cdot kg^{-1} \cdot h^{-1}$) during reperfusion. Vehicle was infused in the control group (n = 6). Coronary flow was recorded by an ultrasonic flow probe and infarct size determined by tetrazolium staining. Arterial and left ventricular pressures were monitored continuously and regional myocardial function determined by sonomicrometry.**

Results. **No significant differences were observed in either hemodynamics or ischemic area at risk. However, the infarct size was significantly reduced (35.3% ± 3.5% versus 21.6% ± 2.6% of the ischemic area, control versus FP-15-treated groups, respectively, $p < 0.05$). +dP/dt was transiently improved in the FP-15-treated groups while during most of the reperfusion period coronary flow, and was significantly lower in the FP-15-treated group as compared to the control group ($p < 0.01$).**

Conclusions. **FP-15 administration reduces myocardial infarct size and reactive hyperemia. These data support the pathogenic role of endogenously produced peroxynitrite and that FP-15 is effective in preventing myocardial reperfusion injury.**

(Ann Thorac Surg 2002;74:1201–7)

The pathophysiology of ischemia/reperfusion has been studied extensively; however, the biochemical agents mediating tissue injury during the reperfusion period are not completely known. Among biochemical mediators, reactive oxygen radicals and more recently, reactive nitrogen radicals appear to play an important role in preconditioning and reperfusion injury [1, 2].

Under physiologic conditions, nitric oxide (NO) production appears to be salutary to cardiac function as inferred from experiments using NO synthase knockout mice and NO inhibitors [1, 2]. Conversely, higher concentrations of NO (mimicking ischemia/reperfusion conditions), either pharmacologically delivered or overexpressed in cardiac myocytes [3], have a clear detrimental effect.

It has been well documented that cardiac ischemia/reperfusion is invariably accompanied by cardiac dysfunction during the reperfusion period. It is early during reperfusion that superoxide anions (O_2^-) and NO are produced at elevated levels [2]. The high levels of O_2^- and NO lead to the formation of the highly reactive oxidant peroxynitrite ($ONOO^-$), implicated as an important mediator of cardiac dysfunction and cell death in various forms of shock, inflammation, and reperfusion injury [4, 5].

Because $ONOO^-$ is likely instrumental in ischemia/reperfusion injury encountered clinically during coronary artery bypass grafting, heart transplant, and thrombolytic therapy, agents that neutralize $ONOO^-$ may offer a therapeutic approach to mitigate cardiac necrosis after cardiac ischemia/reperfusion.

In experimental models, $ONOO^-$ contributes to myo-

Accepted for publication Jun 26, 2002.

Address reprint requests to Dr Sellke, Beth Israel Deaconess Medical Center, 110 Francis St, LMOB Suite 2A, Boston, MA 02215; e-mail: fsellke@caregroup.harvard.edu.

Dr Szabó discloses that he has a financial relationship with Inotek Corporation.

Published by Elsevier Science Inc

0003-4975/02/$22.00
PII S0003-4975(02)03953-X

cardial stunning in ischemia/reperfusion injury [6], and loss of cardiac function in isolated working hearts [7–9]. Accordingly, we investigated the acute effects of a novel $ONOO^-$ decomposition catalyst (FP-15) [10] in our in vivo pig model of myocardial ischemia–reperfusion injury.

Material and Methods

Animals

Pigs were used because they lack significant collateral blood flow, similar to humans, making a more comparative experimental model. Animals were housed individually and provided with laboratory chow and water ad libitum. All experiments were approved by the Beth Israel Deaconess Medical Center Animal Care and Use Committee and the Harvard Medical Area Standing Committee on Animals (Institutional Animal Care and Use Committee) and conformed to the U. S. National Institutes of Health guidelines regulating the care and use of laboratory animals (NIH publication 5377-3, 1996).

Surgical Preparation

Yorkshire pigs of either sex (32 to 42 kg, n = 13) were sedated with ketamine hydrochloride (20 mg/kg, intramuscularly, Abbott Laboratories, North Chicago, IL), and anesthetized with a bolus infusion of thiopental sodium (Baxter Healthcare Corporation, Inc, Deerfield, IL; 5.0 to 7.0 mg/kg intravenously). A tracheotomy was performed through a midline cervical incision, and ventilation begun with a volume-cycled ventilator (model Narkomed II-A; North American Drager, Telford, PA; oxygen, 40%; tidal volume, 1,000 mL; ventilation rate, 12 breaths/min; positive end-expiratory pressure, 3 cm H_2O; inspiratory to expiratory time, 1:2). The right internal jugular vein was cannulated for intravenous access and the right common carotid artery was cannulated for arterial blood sampling and intra-arterial blood pressure monitoring (Millar Instruments, Houston, TX). General anesthesia was established with 3.0% sevoflurane (Ultane; Abbott Laboratories) at the beginning of the surgical preparation, and then maintained with 1.0% throughout the experiment. Heparin sodium (Elkins-Sinn, Inc, Cherry Hill, NJ; 5,000 IU intravenously) and 1% lidocaine (Elkins-Sinn; 5 mL intravenously) were given before sternotomy. Heparin was administered at the same dose every 30 minutes to the end of the experiment. The pericardial sac was exposed through a median sternotomy and was opened to form a pericardial cradle. A catheter-tipped manometer (Millar Instruments) was introduced through the apex of the left ventricle to record left ventricular (LV) pressure. The distal third of the left anterior descending coronary artery or its large second diagonal branch was reversibly occluded with a 0 silk tie (0 silk, Ethicon, Somerville, NJ) using a Ramel tourniquet. Subepicardial coronary blood flow, distal to the ligation site, was continually monitored using a transit time ultrasonic flow probe (3 mm RS-Series, Transonic Systems Inc, Ithaca, NY) with a T206 flowmeter (Transonic Systems). Myocardial ischemia was confirmed visually by regional cyanosis of the myocardial surface [11, 12].

Experimental Protocol

Thirteen pigs were randomly divided into two groups. After 60 minutes of equilibrium, hearts were subjected to 60 minutes of regional ischemia followed by 180 minutes of reperfusion. Thirty minutes after the initiation of regional ischemia, control pigs (n = 7) received a (phosphate buffer saline) infusion through the right internal jugular vein, which was continued throughout the experiment. Treatment animals (n = 6) received a bolus of FP-15 (1.0 mg/kg intravenously; Inotek Corp., Beverly, MA) through the right internal jugular vein 30 minutes after the initiation of ischemia, followed by a maintenance infusion of FP-15 ($1.0\ mg \cdot kg^{-1} \cdot h^{-1}$ intravenously) continued until the termination of 180 minutes of reperfusion. FP-15, a novel peroxynitrite decomposition catalyst, was synthesized as previously described [10]. Hemodynamic variables were continuously acquired throughout the experiment using a PO-NE-MAH digital data acquisition system (Gould, Valley View, OH), with an Acquire Plus processor board, LV pressure analysis software, and a Gould ECG/Biotach [11, 12].

Regional Myocardial Function

Regional myocardial function was assessed by sonomicrometry (Sonometrics Digital Ultrasonic Measurement System, Sonometrics Corp., London, CA) using five digital piezoelectric ultrasonic probes (2.0 mm) implanted in the subepicardial layer approximately 10 mm apart within the ischemic area, with two pairs placed parallel to the minor axis of the heart and secured to the epicardium using polypropylene stitches (5-0 Prolene, Ethicon). The probes were left in place until the end of the experiment. Digital data were inspected for correct identification of end-diastolic and end-systolic points using post-processing software (Sono View, Sonometrics). Measurements were made during at least three cardiac cycles in normal sinus rhythm and then averaged. The ventilator was stopped during data acquisition to eliminate the effects of respiration. End-diastolic segment length was measured at the onset of the positive first derivative of pressure over time (+dP/dt), and the end-systolic segment length at peak negative dP/dt (−dP/dt). Regional contractility was assessed by segment shortening. Wall motion abnormalities were assessed as systolic bulging defined as the bulging of the myocardium after the end of diastole. Time course changes in percent segment shortening were calculated from the mean ± standard error of the mean of four to five unique horizontal or longitudinal distances and expressed as a percent of baseline to minimize variability among individual animals.

Coronary Blood Flow, Arterial Blood Gases, and Hematocrit

Coronary blood flow was continuously monitored distal to the site of ligation using a transit time ultrasonic flow probe (3 mm RS-Series, Transonic) placed around the left anterior descending coronary artery connected to a T206

flowmeter (Transonic). Arterial blood gases and hematocrit were monitored every 10 to 15 minutes using a Corning 238 pH/blood gas analyzer and a Corning 270 CO-oximeter (Chiron Diagnostics, Emeryville, CA). Blood gases and acid–base parameters were maintained at partial pressure of oxygen $>$ 100 mm Hg, partial pressure of carbon dioxide $<$ 45 mm Hg, and pH 7.3 $\pm$ 0.3.

Core Temperature

Core temperature was continuously monitored by rectal thermometer using a YSI model 44-TD tele-thermometer (Yellow Springs Instrument Co. Inc., Yellow Springs, OH) and kept at 37°C.

Measurement of Infarct Size

The measurements of ischemic area at risk (AAR) and infarct size were performed according to standard methods as described previously [11]. After 180 minutes of reperfusion, AAR was delineated by religation of the left anterior descending coronary artery and injection of a 1:5 dilution in PBS of monastryl blue pigment (aka phthalocyanine blue, Engelhard Corp., Louisville, KY) into the aorta. The AAR was assessed by the weight of unstained, ischemic myocardium as a percentage of the total LV mass weight. Immediately after the evaluation of AAR, the heart slices were immersed into 1% triphenyl tetrazolium chloride (TTC, Sigma Chemical Co, St. Louis, MO) in phosphate buffer (pH 7.4) at 38°C for 20 minutes to determine myocardial infarct size. The AAR and infarct size were measured by computerized planimetry (Scion Image, Scion Corp., Frederick, MD).

Immunohistochemical Detection of Nitrotyrosine in the Reperfused Myocardium

Heart samples were harvested at the conclusion of the experiments, frozen in liquid nitrogen and stored at −80°C until used. Nitrotyrosine immunohistochemistry was performed as previously described [13]. Briefly, cryomicrotome frozen (10 μm) sections were incubated for 10 minutes in PBS (pH 7.4) and then treated with 0.3% hydrogen peroxide for 15 minutes to eliminate endogenous peroxidase activity, and rinsed in 10 mmol/L of PBS. Nonspecific binding was blocked by incubating the slides for 1 hour in PBS containing 2% horse serum. Rabbit polyclonal antibody against nitrotyrosine (Upstate Biotech, Lake Placid, NY) was applied to the sections and isotype-matched control antibody (negative control) was applied in a dilution of 1:100 for 2 hours at room temperature. After extensive washing (5 $\times$ 5 minutes) with PBS, immunoreactivity was detected with a biotinylated goat antirabbit secondary antibody and the avidin-biotin-peroxidase complex both supplied in the Vector Elite kit (Vector Laboratories, Burlingame, CA). Color was developed using Ni-DAB substrate (95 mg of diaminobenzidine, 1.6 g of NaCl, 2 g of $NiSO_4$ in 200 mL of 0.1 mmol/L acetate buffer). Sections were then counterstained with nuclear fast red, dehydrated and mounted in 50% glycerol in H_2O. Photomicrographs were taken with a Zeiss Axiolab microscope (Carl Zeiss Inc, Thornwood, NY) equipped with a Fuji HC-300C digital camera (Fuji Photo Film Corp, Ltd, Tokyo, Japan).

Statistical Analysis

Data are expressed as mean $\pm$ standard error of the mean. Statistical significance was determined by analysis of variance following a Bonferroni correction to adjust for the multiplicity of tests. Statistical significance is defined as $p < 0.05$.

Results

Ventricular Fibrillation and Ventricular Arrhythmia

Of the 13 pigs that underwent coronary artery occlusion, 1 animal from the control group was excluded because of unsuccessful defibrillation during the ischemia period, after which 12 pigs remained (6 control and 6 FP-15-treated animals). The incidence of premature ventricular contractions was 77% in both groups (five animals/group). Premature ventricular contractions started between 20 and 25 minutes after coronary artery occlusion and continued throughout the ischemia period (60 minutes) in both groups. We experienced one ventricular fibrillation in the control group just after reperfusion (1 minute of reperfusion), and the heart was resuscitated by electrical cardioversion (75 J) with concomitant bolus injection of 1% lidocaine (2 mg/kg). No significant differences were observed in hemodynamic parameters between animals that had arrythmias and those that did not experience premature ventricular contractions (data not shown).

Blood Gases, Hematocrit, and Core Temperature

The partial pressure of carbon dioxide (35.2 $\pm$ 3.8 mm Hg) and partial pressure of oxygen (381 $\pm$ 17 mm Hg) in control group did not significantly differ from the FP-15-treated animals (37.3 $\pm$ 5.1 mm Hg and 397 $\pm$ 15 mm Hg, respectively). Core temperature, hemoglobin concentration, and blood electrolyte analysis also showed no significant differences between groups (data not shown).

Hemodynamic Parameters

There was a significant difference in +dP/dt between the groups at 210 and 240 min ($p < 0.05$) (Fig 1), which paralleled the changes in segment shortening. No significant differences in mean arterial pressure, LV developed pressure, or heart rate (Table 1) were observed between control and FP-15-treated animals during the equilibrium period and during ischemia. In the control group, coronary flow increased immediately with reperfusion, with maximal flow observed 30 minutes after the occluder was released. At all time points after the first 10 minutes of reperfusion, the reflow values were significantly reduced in the FP-15-treated group (Fig 2; $p < 0.01$). In the treated animals, the coronary flow returned to baseline, whereas in the control group it remained high throughout the reperfusion period (Fig 2).

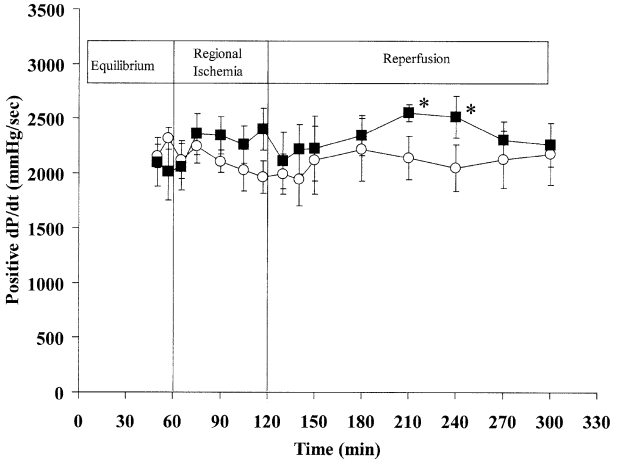

*Fig 1. Evolution of positive first derivative of left ventricular pressure over time (+dP/dt) in control (n = 6) and FP-15-treated animals (n = 6) throughout the experiment. *p < 0.05. (–○– = control; –■– = FP-15.)*

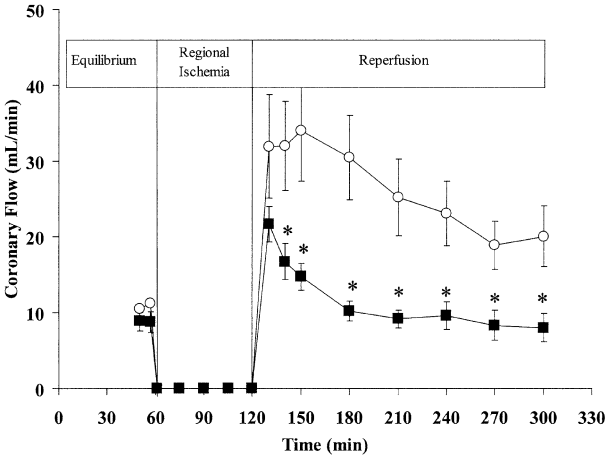

*Fig 2. Evolution of coronary blood flow in control (n = 6) and FP-15 (n = 6)-treated pigs. Coronary blood flow was continuously monitored using a transit time ultrasonic flow probe (3-mm RS-Series) placed around the left anterior descending coronary artery connected to a T206 flowmeter. *p < 0.01. (–○– = control; –■– = FP-15.)*

Area at Risk and Infarct Size

No significant differences were observed in the ischemic area at risk. However, the infarct size was significantly reduced (35.3% ± 3.5% versus 21.6% ± 2.6% of the area at risk, control versus treatment group, respectively; $p < 0.05$) (Fig 3).

Regional Myocardial Function

There was a suppression in segment shortening in the control group during the period of ischemia, which did not recover over time during the period of reperfusion. In contrast, in the FP-15-treated animals, there was a partial improvement of the segment shortening during the reperfusion period (210 min, $p = 0.08$), although it did not reach statistical significance (Fig 4). Using sonomicrometry to record regional myocardial function, no significant differences in systolic bulging were observed between groups during the periods of ischemia and reperfusion (not shown).

Peroxynitrite Staining in Heart Tissues

Myocardial sections from the ischemia area representative animals in both the control and treated groups were stained for nitrotyrosine (a marker for $ONOO^-$ production). Nitrotyrosine levels were readily detectable in the ischemic area of control pigs but were undetectable in FP-15-treated animals (Fig 5).

Comment

We show in this study that an intravenous infusion of FP-15, a $ONOO^-$ decomposer catalyst, reduces myocardial infarct size in a porcine model of ischemia/reperfusion injury. The infusion of FP-15 has no effect on arterial blood pressure, heart rate, or LV pressure. Segment shortening and +dP/dt are slightly improved in FP-15-treated compared to control groups during part of the reperfusion period. On the other hand, coronary reactive hyperemia is greatly decreased during most of the reperfusion period in the myocardium of FP-15-treated animals as compared to untreated ones, probably reflecting the reduced extent of myocardial necrosis. However, it is more likely that endothelial or vascular dysfunction due to FP-15 may have limited the degree of vascular relaxation and hyperemia.

Table 1. Hemodynamic Parameters

Parameters	Groups	Time Intervals (min)								
		S [59]	I [5]	I [30]	I [60]	R [5]	R [30]	R [60]	R [120]	R [180]
LVP (mm Hg)	Control	69.6 ± 2.7	64.0 ± 2.1	66.7 ± 7.0	67.2 ± 3.9	67.8 ± 4.4	67.2 ± 4.6	68.2 ± 3.7	64.0 ± 5.8	67.8 ± 5.3
	FP-15	69.4 ± 2.5	68.3 ± 5.4	70.8 ± 1.7	71.5 ± 3.4	67.0 ± 5.7	68.1 ± 4.1	69.8 ± 3.0	72.3 ± 3.1	68.2 ± 3.4
MAP (mm Hg)	Control	72.3 ± 1.2	68.5 ± 2.3	73.3 ± 6.7	71.3 ± 3.8	72.8 ± 3.2	67.5 ± 4.6	71.8 ± 3.9	69.5 ± 5.9	71.5 ± 5.3
	FP-15	73.6 ± 1.2	72.7 ± 5.0	76.2 ± 4.0	69.8 ± 5.8	67.8 ± 5.0	69.8 ± 5.2	69.2 ± 3.1	70.8 ± 2.5	69.9 ± 3.0
Heart rate (beats/min)	Control	102.9 ± 7.3	105.2 ± 9.0	99.8 ± 6.5	98.8 ± 9.0	108.8 ± 7.2	105.5 ± 6.8	109.5 ± 5.0	102.7 ± 8.7	101.0 ± 6.7
	FP-15	91.2 ± 5.4	92.3 ± 5.4	98.0 ± 8.6	94.0 ± 8.6	96.2 ± 10.6	90.5 ± 6.0	93.8 ± 7.8	95.8 ± 7.3	99.7 ± 7.5

Values are presented as mean ± standard error of the mean.

I = ischemia period; LVP = left ventricular developed pressure; MAP = mean arterial pressure; R = reperfusion period; S = stabilization period.

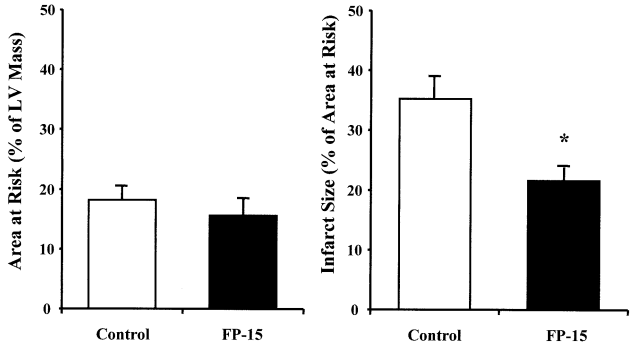

*Fig 3. Histograms showing the area at risk (left) and infarct size (right) in the left ventricle (LV) at the end of the experimental protocol in control (n = 6) and FP-15-treated pigs (n = 6). Values are expressed as mean ± standard error of the mean. *p < 0.05.*

Because $ONOO^-$ has a very short half-life, the measurement of the more stable bioproduct, nitrotyrosine, using specific antibodies has became an acceptable and reliable biomarker for the production of $ONOO^-$ in an in vivo model of ischemia/reperfusion [14]. We must note, nevertheless, that additional in vivo pathways may also lead to tyrosine nitration, and therefore this assay must be used with caution [15]. We also show immunohistochemically that after ischemia/reperfusion, an important accumulation of nitrotyrosine is present in the injured myocardium of control animals but not in the FP-15-treated group. Our results demonstrate the production of $ONOO^-$ during ischemia/reperfusion and that infusion of FP-15 decomposes $ONOO^-$, thereby limiting myocardial reperfusion injury. These results point to a deleterious effect of $ONOO^-$ produced during ischemia/reperfusion of the porcine heart leading to myocardial cell death. Indeed, Hayashi and colleagues [16] also detected the presence of nitrotyrosine in the coronary sinus effluent 5 minutes into the cardiopulmonary bypass reperfusion period of patients undergoing mitral valve replacement, also observed by Zhang and colleagues [6] in an in vivo dog model of ischemia/reperfusion.

The deleterious effects of $ONOO^-$ in isolated hearts, cell cultures, and in vivo ischemia/reperfusion models were well documented in previous works. Ishida and associates [17] showed that a continuous infusion of $ONOO^-$ into isolated hearts impaired cardiac contractility of papillary muscle and contractile function of cardiac myocytes. In isolated rat hearts, cytokine-induced depression of myocardial contractility was associated with increased levels of nitrotyrosine that were blocked by the $ONOO^-$ decomposer catalyst FeTPPS [18]. Given that $ONOO^-$ is formed from the reaction of NO and O_2^-, inhibition of NO formation with N^G-nitro-L-arginine (L-NNA) and O_2^- formation with the superoxide scavanger Tion, nitrotyrosine formation is decreased [18]. Accordingly, Zhang and colleagues [6] in a dog model of ischemia/reperfusion showed that pretreatment with a NO synthase inhibitor L-NNA ameliorated myocardial stunning after ischemia reperfusion and decreased nitrotyrosine formation. In a human cell line and primary neuron cultures treated with $ONOO^-$ and LPS, respectively, an inverse correlation between nitrotyrosine staining and cell viability was observed. As expected, the presence of FeTTPS decreased nitrotyrosine accumulation and increased cell viability [19].

However, considering that NO has cardioprotective effects [1], blocking the formation of NO may be detrimental to organ function. Accordingly, inducible NO synthase 2 (NOS_2) knock-out ($NOS_2^{-/-}$) mice subjected to ischemia/reperfusion in vivo had enhanced myocardial damage and increased mortality rate as compared to wild-type littermates [20]. In isolated mice hearts, Flogel and colleagues [21] showed that acute recovery from ischemia/reperfusion was less pronounced in the wild type as compared to the $NOS_3^{-/-}$, whereas Sumeray and colleagues [22] observed a similar recovery in both wild type and $eNOS_3^{-/-}$, but significant larger myocardial infarction in the knock-out animals.

The vasoactive effects of $ONOO^-$ seem to be complex. A continuous infusion of $ONOO^-$ in isolated rat hearts caused initial vasodilation that converted to vasoconstriction at a higher concentration [23, 24]. At lower concentrations, only vasodilation was observed [24]. However, as we also observed less reactive hyperemia and infarct size during reperfusion in animals treated with a poly ADP ribose synthase inhibitor PJ-34 [25], the mechanism leading to less hyperemic reaction may be explained by metabolic factors [26] produced in proportion to the area of infarcted myocardium, rather than solely by $ONOO^-$ concentration. A more likely explanation is that the agent caused endothelial and vascular dysfunction resulting in diminished vascular relaxation and hyperemia.

This study addressed the effects of a $ONOO^-$ decomposer catalyst in an acute model of ischemia/reperfusion in vivo. The previously published cardiac effects of $ONOO^-$ seem clearly to point to a cardiotoxic effect in

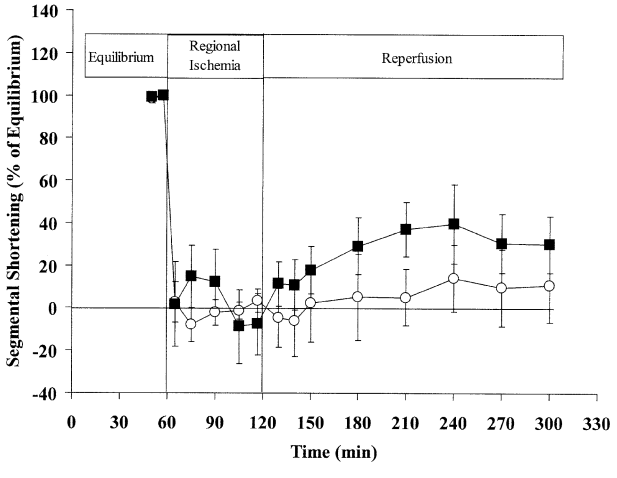

Fig 4. Evolution of segment shortening in the ischemic area (n = 6) of control and FP-15 (n = 6)-treated pigs. Regional myocardial function was obtained by sonomicrometry using piezoelectric ultrasonic probes (2.0 mm) implanted in the subendocardial layer. (–○– = control; –■– = FP-15.)

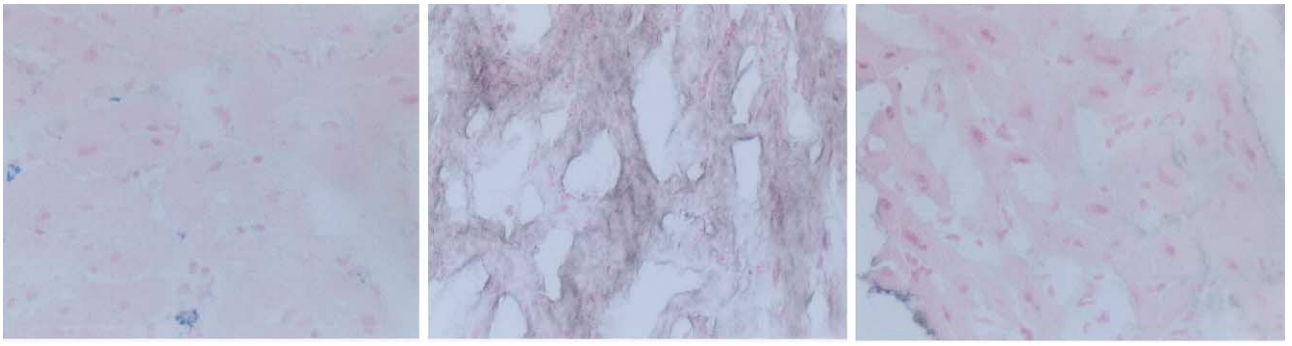

*Fig 5. Immunohistochemical localization of nitrotyrosine in left ventricle sections from the ischemic area of (left) a control animal with unrelated isotype-matched first antibody (negative control); (middle) a control animal with anti-nitrotyrosine antibody; and (right) an FP-15-treated animal with anti-nitrotyrosine antibody. Left ventricular samples were harvested and frozen sections (10 μm) were processed for immunohistochemistry. Note the presence of nitrotyrosine (*brown staining*) in the tissue (middle) that is not present in the negative control (left) or in the myocardium of pigs treated with FP-15 (right). (*MI/R = *myocardial ischemia/reperfusion.)*

culture [19] and ex vivo [27], and the current data from our in vivo experimental model appear to confirm this notion.

The development of compounds that neutralize and decompose $ONOO^-$ may be a potential clinically useful approach to myocardial infarction, cardiopulmonary bypass, and possibly other disease states involving ischemia/reperfusion injury where $ONOO^-$ is being produced at cytotoxic rates.

This work was supported in part by the National Heart, Lung, and Blood Institute R43 HL-65863 and RO1 HL-46716. Dr Renato Faro was a postdoctoral fellow supported by "Fundação de Amparo à Pesquisa do Estado de São Paulo"—Brazil (FAPESP - #99/07234-8).

References

1. Bolli R. Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: an overview of a decade of research. J Mol Cell Cardiol 2001;33:1897–918.
2. Shah AM, MacCarthy PA. Paracrine and autocrine effects of nitric oxide on myocardial function. Pharmacol Ther 2000;86:49–86.
3. Brunner F, Andrew P, Wolkart G, Zechner R, Mayer B. Myocardial contractile function and heart rate in mice with myocyte-specific overexpression of endothelial nitric oxide synthase. Circulation 2001;104:3097–102.
4. Szabo C. The pathophysiological role of peroxynitrite in shock, inflammation, and ischemia-reperfusion injury. Shock 1996;6:79–88.
5. Liaudet L, Soriano FG, Szabo C. Biology of nitric oxide signaling. Crit Care Med 2000;28:N37–52.
6. Zhang Y, Bissing JW, Xu L, et al. Nitric oxide synthase inhibitors decrease coronary sinus-free radical concentration and ameliorate myocardial stunning in an ischemia-reperfusion model. J Am Coll Cardiol 2001;38:546–54.
7. Wang P, Zweier JL. Measurement of nitric oxide and peroxynitrite generation in the postischemic heart. Evidence for peroxynitrite-mediated reperfusion injury. J Biol Chem 1996;271:29223–30.
8. Yasmin W, Strynadka KD, Schulz R. Generation of peroxynitrite contributes to ischemia-reperfusion injury in isolated rat hearts. Cardiovasc Res 1997;33:422–32.
9. Gupte SA, Okada T. Prostaglandins and nitric oxide mediate superoxide-induced myocardial contractile dysfunction in isolated rat hearts. J Mol Cell Cardiol 2001;33:1107–17.
10. Szabo C, Mabley JG, Moeller SM, et al. FP 15, a novel, potent peroxynitrite decomposer catalyst: in vitro cytoprotective actions and protection against diabetes mellitus and diabetic cardiovascular complications. FASEB J 2002;16:A1166.
11. Uematsu M, Gaudette GR, Laurikka JO, Levitsky S, McCully JD. Adenosine-enhanced ischemic preconditioning decreases infarct in the regional ischemic sheep heart. Ann Thorac Surg 1998;66:382–7.
12. Toyoda Y, Di Gregorio V, Parker RA, Levitsky S, McCully JD. Anti-stunning and anti-infarct effects of adenosine-enhanced ischemic preconditioning. Circulation 2000;102:III326–31.
13. Liaudet L, Soriano FG, Szabo E, et al. Protection against hemorrhagic shock in mice genetically deficient in poly-(ADP-ribose)polymerase. Proc Natl Acad Sci USA 2000;97:10203–8.
14. Liu P, Hock CE, Nagele R, Wong PY. Formation of nitric oxide, superoxide, and peroxynitrite in myocardial ischemia-reperfusion injury in rats. Am J Physiol 1997;272:H2327–36.
15. Brennan M-L, Wu W, Fu X, et al. A tale of two controversies: defining the role of peroxidases in nitrotyrosine formation in vivo using eosinophil peroxidases and myeloperoxidase deficient mice; and defining the nature of peroxidase-generated reactive netrogen species. J Biol Chem 2002;277:17415–27.
16. Hayashi Y, Sawa Y, Ohtake S, Fukuyama N, Nakazawa H, Matsuda H. Peroxynitrite formation from human myocardium after ischemia–reperfusion during open heart operation. Ann Thorac Surg 2001;72:571–6.
17. Ishida H, Ichimori K, Hirota Y, Fukahori M, Nakazawa H. Peroxynitrite-induced cardiac myocyte injury. Free Radic Biol Med 1996;20:343–50.
18. Ferdinandy P, Danial H, Ambrus I, Rothery RA, Schulz R. Peroxynitrite is a major contributor to cytokine-induced myocardial contractile failure. Circ Res 2000;87:241–7.

19. Misko TP, Highkin MK, Veenhuizen AW, et al. Characterization of the cytoprotective action of peroxynitrite decomposition catalysts. J Biol Chem 1998;273:15646–53.
20. Zingarelli B, Hake PW, Yang Z, O'Connor M, Denenberg A, Wong HR. Absence of inducible nitric oxide synthase modulates early reperfusion-induced NF-kappaB and AP-1 activation and enhances myocardial damage. FASEB J 2002;16: 327–42.
21. Flogel U, Decking UK, Godecke A, Schrader J. Contribution of NO to ischemia–reperfusion injury in the saline-perfused heart: a study in endothelial NO synthase knockout mice. J Mol Cell Cardiol 1999;31:827–36.
22. Sumeray MS, Rees DD, Yellon DM. Infarct size and nitric oxide synthase in murine myocardium. J Mol Cell Cardiol 2000;32:35–42.
23. Wang W, Sawicki G, Schulz R. Peroxynitrite-induced myocardial injury is mediated through matrix metalloproteinase-2. Cardiovasc Res 2002;53:165–74.
24. Villa LM, Salas E, Darley-Usmar VM, Radomski MW, Moncada S. Peroxynitrite induces both vasodilatation and impaired vascular relaxation in the isolated perfused rat heart. Proc Natl Acad Sci USA 1994;91:12383–7.
25. Faro R, Toyoda Y, McCully JD, et al. Myocardial protection by PJ34, a novel potent poly (ADP-ribose) synthetase inhibitor. Ann Thorac Surg 2002;73:575–81.
26. Berne RM. Physiology. In: MW B, ed. Human physiology. St Louis: Mosby, 1998, 478–501.
27. Zweier JL, Fertmann J, Wei G. Nitric oxide and peroxynitrite in postischemic myocardium. Antioxid Redox Signal 2001;3: 11–22.