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Myocardial Function After Gut Ischemia/Reperfusion: Does NFκB Play a Role?

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Background. **Mesenteric ischemia/reperfusion (I/R) is a common problem in critically ill patients and is frequently associated with myocardial dysfunction. Several potential mechanisms have been proposed to be involved in the myocardial dysfunction associated with mesenteric I/R, including nuclear factor kappa B (NFκB)-mediated tumor necrosis factor alpha (TNF-α) release leading to cardiodepression. Thus, we sought to investigate the effect of NFκB inhibition on mesenteric I/R-associated myocardial dysfunction in a large animal model (dog).**

Materials and methods. **A total of 21 mongrel dogs were anesthetized and mechanically ventilated. Animals were instrumented with a Swan–Ganz Catheter, left ventricle (LV) pressure manometer, and ultrasonic crystals. Mesenteric I/R consisted of 60 min of ischemia followed by 180 min of reperfusion. Seven animals received pyrrolidine dithiocarbamate (PDTC, 100 mg/kg) prior to mesenteric I/R (I/R PDTC). Another group of animals ($n = 7$) without mesenteric ischemia received PDTC following baseline measurements and served as control for the effect of PDTC alone (PDTC). Preload recruitable stroke work, ±dp/dt(max), isovolumic relaxation (tau), and cardiac output were measured. Myocardial tissue was analyzed for NFκB activity, TNF-α production, and myocardial apoptosis.**

Results. **Mesenteric I/R impaired both LV systolic and diastolic function. Administration of PDTC worsened LV function impairment following I/R. In addition, PDTC resulted in decreased LV function even without mesenteric I/R. NFκB, TNF-α, and myocardial apoptosis were not different among the groups.**

Conclusions. **Mesenteric I/R affects LV function independent of NFκB and TNF-α pathways. PDTC acts as a cardiac depressant through a thus far unknown mechanism. Therefore, evalutation of cardiac and hemodynamic function in experimental setups using PDTC has to be carefully interpreted.**

Key Words: **mesenteric ischemia/reperfusion; cardiac function; NFκB; TNF-α.**

INTRODUCTION

Mesenteric ischemia/reperfusion (I/R) is a common problem in critically ill patients and is frequently associated with myocardial dysfunction, which further increases mortality in these patients [1–3]. Several potential mechanisms have been proposed to be involved in the myocardial dysfunction associated with mesenteric I/R. Some of these studies point to the primed and subsequently activated polymorphonuclear leukocytes (PMNs) as the link between mesenteric I/R and myocardial dysfunction. Data suggesting activated PMNs as the source of cardiac injury after mesenteric injury are found in studies from Horton's group, who found that both plasma and heart tissue malonyldialdehyde concentrations increased after mesenteric I/R, and reversal of lipid peroxidation returned myocardial function to control levels [4]. Using a similar model, Shahani showed that mesenteric I/R increased myocardial myeloperoxidase and tumor necrosis factor alpha (TNF-α) [5]. Furthermore, myocardial TNF-α synthesis has been shown to increase after shock and resuscitation (associated with mesenteric I/R) [6]. Although TNF-α is a direct myocardial depressant, it also promotes leukosequestration [7]. Taken together, these data suggest a pathophysiologic sequence of increased myocardial TNF-α production fol-

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lowed by proinflammatory mesenteric lymph entering the myocardial circulation. PMNs primed by mesenteric lymph could be activated by myocardial TNF-α, resulting in lipid peroxidation and myocardial edema (microvascular barrier injury).

TNF-α expression is in part regulated by nuclear factor kappa B (NFκB) [7]. NFκB is a transcriptional regulator that is activated by oxidant stress in the myocardium. When activated, NFκB dissociates from it inhibitory subunit (IκB), translocates to the nucleus, and binds to the consensus sequence for its multiple promoter/enhancer regions on the DNA, resulting in TNF-α mRNA transcription.

Our hypothesis for the presented study is based on a sequence of events, including priming and activation of PMNs during mesenteric I/R with subsequent oxidant stress generation by PMNs to the myocardium, thus activating NFκB followed by TNF-α increase, leading to cardiodepression. Thus, we sought to ameliorate mesenteric I/R-associated myocardial dysfunction by NFκB inhibition using pyrrolidine dithiocarbamate (PDTC) in a large animal model (dog) of mesenteric I/R.

MATERIALS AND METHODS

Animal Preparation

All procedures were approved by the University of Texas Animal Welfare Committee and were consistent with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" (NIH publication 85-23, revised 1985). Conditioned mongrel dogs ($n = 21$) of either sex were anesthetized by intravenous administration of 25 mg/kg thiopental sodium (Pentothal; Abbott Laboratories, North Chicago, IL) and underwent tracheal intubation and mechanical ventilation with 100% oxygen using a volume-cycled respirator (Siemens–Elema AB, Syndbyberg, Sweden). Anesthesia was maintained with intravenous infusion of 1% thiopental sodium in Ringer's solution. Temperature was maintained by using a heating blanket.

Fluid-filled catheters were placed into the right femoral artery and vein and connected to pressure transducers for arterial pressure monitoring, arterial blood sampling, and fluid administration, respectively. A 7-French Swan–Ganz thermodilution catheter was inserted into the pulmonary artery via the right jugular vein for pressure and cardiac output measurements. Following a median sternotomy and pericardiotomy, we placed sonomicrometry crystals (10 MHz; Sonometrics, London, ON, Canada) in the subendocardium at midventricular level across the septum/free wall axis (short axis) and also at the apex and base (long axis) of the left ventricle (LV). A micromanometer tipped pressure transducer (Millar Instruments, Houston, TX) was introduced in the LV cavity through the apex. We placed a snare around the inferior vena cava for cardiac preload manipulation.

Hemodynamics and Preload Recruitable Stroke Work

Systolic function was determined by using proload recruitable stroke work (PRSW) and the maximum rate of change of LV pressure dP/dt_{max}. The time constant of isovolumic relaxation tau (τ) and $-dP/dt_{max}$ were used as measures of diastolic function. Hemodynamic data were simultaneously logged into a Macintosh Quadra 700 computer via an analogue-to-digital data acquisition device (MacLab; World Precision Instruments, New Haven, CT). Cardiac output was measured continuously by using the Edwards Lifesciences (Irvine, CA) monitor system. Mean arterial pressure (MAP) and pulmonary artery pressure (PAP)/pulmonary artery occlusion pressure (PCWP) were measured with arterial catheters interfaced to pressure transducers. LV pressure was measured with a micromanometer, and the LV long- and short-axis diameters were obtained with a sonomicromanometer. These data were recorded at a frequency of 200 Hz during 10 s of inferior vena cava occlusion (Sonolab/Sonoview; Sonometrics).

PRSW was calculated as the slope of the relation between LV end-diastolic volume and LV stroke work (SW) from pressure–volume loops obtained using inflow occlusion of the inferior vena cava as previously described [8]. All measures were taken in triplicate.

Experimental Protocol/Mesenteric I/R

A midline laparotomy was used to gain access to the peritoneal cavity. The mesenteric arteries were encircled with cotton tape for subsequent occlusion. Following baseline measurements, atraumatic vascular clamps were used to occlude the mesenteric vessels, and with application, the distal mesenteric vessels lost all pulsation and the

TABLE 1

Hemodynamics in I/R and I/R-PDTC

	Baseline	60'I	60'Rep	120'Rep	180'Rep
MAP, (mmHg)					
I/R	106 ± 5	77 ± 5#	91 ± 5	103 ± 6	92 ± 6
I/R-PDTC	107 ± 8	65 ± 8#	85 ± 8	99 ± 4	86 ± 7
PAP, (mmHg)					
I/R	16.8 ± 1.2	15.4 ± 1.1	16.8 ± 1.0	18.1 ± 1.5	17.4 ± 1.6
I/R-PDTC	18.8 ± 2.0	15.9 ± 2.2	17.4 ± 1.8	21.0 ± 2.4	23.2 ± 3.0
PCWP, (mmHg)					
I/R	9.8 ± 1.2	8.1 ± 1.0	8.8 ± 1.2	9.0 ± 1.1	8.1 ± 1.4
I/R-PDTC	12.8 ± 1.9	10.9 ± 2.2	9.6 ± 2.3	11.4 ± 1.9	13.2 ± 2.6
CVP, (mmHg)					
I/R	6.9 ± 1.0	7.4 ± 1.2	8.0 ± 1.2	8.4 ± 1.3	9.4 ± 1.6
I/R-PDTC	9.9 ± 1.1	10.4 ± 1.2	11.3 ± 1.6	12.6 ± 1.4	13.3 ± 1.4

Note. Values are mean ± standard error.

MAP = mean arterial pressure; PAP = pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; CVP = central venous pressure.

# $P < 0.05$ for differences from baseline period.

TABLE 2

Hemodynamics in PDTC Control

	Baseline	60 min	120 min	180 min	240 min
MAP, (mmHg)	120 ± 7	100 ± 4#	106 ± 6	104 ± 6	104 ± 4
PAP, (mmHg)	21 ± 1	17 ± 1#	18 ± 1	19 ± 1	20 ± 2
PCWP, (mmHg)	14 ± 1	12 ± 1	12 ± 1	13 ± 1	12 ± 1
CVP, (mmHg)	8 ± 1	7 ± 1	7 ± 1	7 ± 1	7 ± 1

Note. Values are mean ± standard error.

MAP = mean arterial pressure; PAP = pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; CVP = central venous pressure.

# $P < 0.05$ for differences from baseline period.

bowel serosa became a characteristic pale white color. Mesenteric ischemia was maintained for 60 min followed by reperfusion for 3 h ($n = 14$). In seven animals, PDTC was administered 20 min prior to mesenteric ischemia (I/R PDTC; 100 mg/kg), whereas control animals ($n = 7$) received vehicle only (I/R control). Another group of animals ($n = 7$) without mesenteric ischemia received PDTC following baseline measurements and served as control for the effect of PDTC alone (PDTC).

Preparation of Cytoplasmic and Nuclear Extracts

All tissue samples were frozen in liquid nitrogen immediately after collection. Frozen tissue was ground with mortar and pestle over liquid nitrogen. Nuclear and cytoplasmic extracts were prepared by using a nuclear extract kit (Active Motif, Carlsbad, CA). Briefly, cold hypotonic buffer containing protease inhibitors (Halt Kit Protease Inhibitor Cocktail; Pierce Biotechnology, Rockford, IL) and phosphatase inhibitors (2 mM orthovanadate and 2 mM sodium fluoride) was added to the ground tissue, and the samples were homogenized. After 15 min of incubation at 4°C, the samples were centrifuged at 850*g* at 4°C for 10 min. The supernatant was saved. The pellet was resuspended in hypotonic buffer containing protease inhibitors and phosphatase inhibitors and incubated on ice for 15 min. After addition of 0.05% detergent, the samples were vortexed then centrifuged for 10 min at 14,000 RPM at 4°C. The supernatant was saved and combined with the first supernatant and called the cytoplasmic extract. The pellet was resuspended in lysis buffer, vortexed, and incubated on ice for 30 min. The samples were then centrifuged, and the supernatant was called the nuclear extract. All samples were aliquotted and frozen until use.

Detection of NFκB p65 Subunit

Translocation of the NFκB p65 to the nucleus was analyzed in myocardial nuclear extraxts by using the TransAM NF-κB p65 kit (Active Motif North America, Carlsbad, CA).

Determination of TNF-α Concentrations

TNF-α concentrations were measured by using a canine TNF-α Immunoassay (R&D Systems, Minneapolis, MN).

Cell Death Detection Enzyme-Linked Immunosorbent Assay

Myocardial cytoplasmatic fractions (lysates) were analyzed for mono- and oligonucleosomes as indicators of apoptosis-associated DNA fragmentation.

Statistical Analysis

All data presented are mean ± standard error. To examine our data for changes over time, we used an analysis of variance for repeated measures. Post hoc comparisons were performed by using Tukey's Test. For analysis between groups, we used Student's *t*-test. A value of $P < 0.05$ was considered significant.

RESULTS

Hemodynamic Measurements

Hemodynamic results (MAP, PAP, PCWP, CVP) are shown in Tables 1 and 2. Cardiac output (shown in Fig. 1) decreased significantly after 60 min of mesenteric ischemia in both I/R and I/R-PDTC. However, cardiac output reduction was even more pronounced in ani-

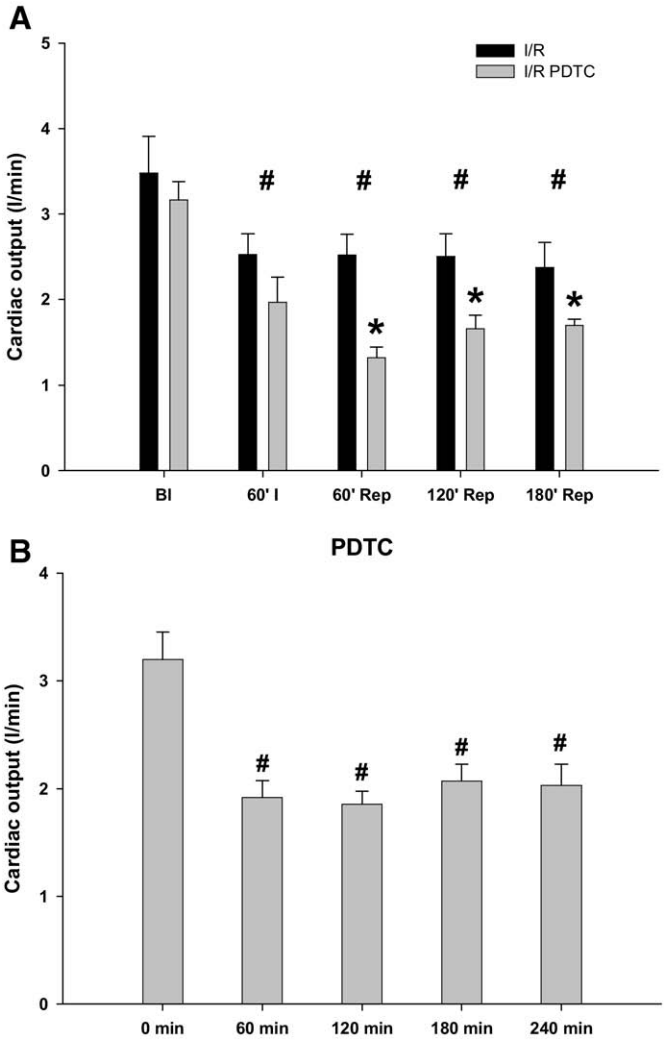

FIG. 1. Cardiac output decreased significantly following 60 min of mesenteric ischemia in both I/R and I/R-PDTC. However, cardiac output reduction was even more pronounced in animals treated with PDTC (A). In animals treated with PDTC in the absence of mesenteric ischemia, cardiac output was also significantly reduced (B). #, $P < 0.05$ *versus* baseline; *, $P < 0.05$ *versus* I/R.

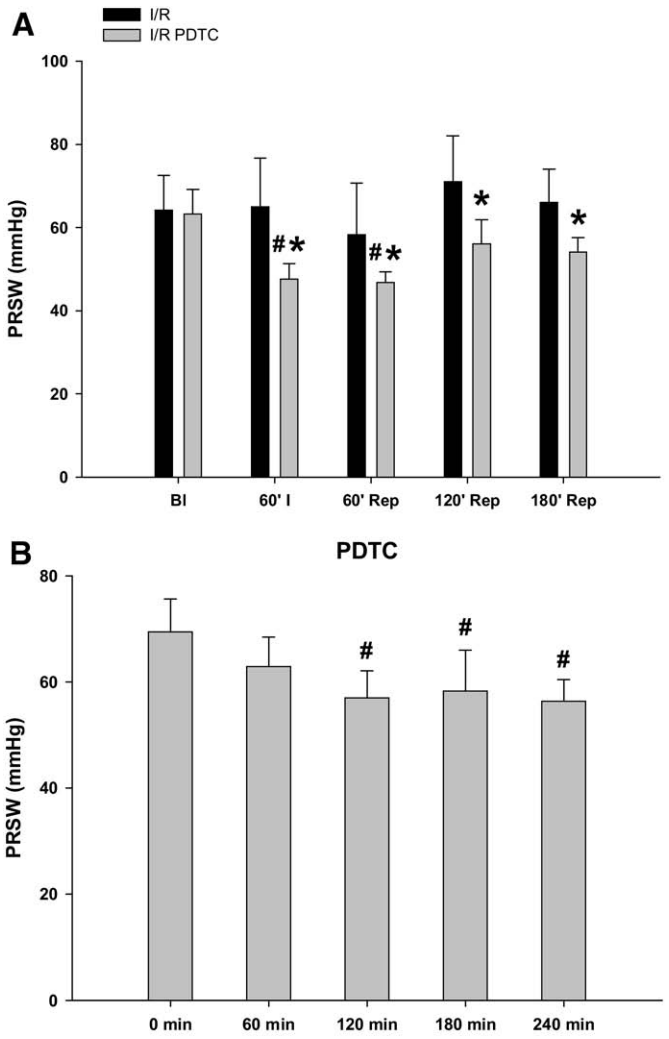

FIG. 2. Although PRSW remained unchanged in I/R animals, treatment with PDTC resulted in significant PRSW reduction following mesenteric ischemia (A). In PDTC control animals, PRSW values decreased over time and were significantly reduced at 120 min and throughout the remaining time points (B). #, $P < 0.05$ *versus* baseline; *, $P < 0.05$ *versus* I/R.

mals treated with PDTC (I/R PDTC, Fig. 1A). In animals treated with PDTC in the absence of mesenteric ischemia, cardiac output was also significantly reduced (Fig. 1B). LV function as measured by PRSW remained unchanged in I/R animals. Treatment with PDTC (I/R-PDTC), however, resulted in significant PRSW reduction following mesenteric ischemia (Fig. 2A). In PDTC control animals (PDTC), PRSW values decreased over time and were significantly reduced at 120 min and throughout the remaining time points (Fig. 2B). After 60 min mesenteric I/R, dp/dt_{max} was significantly reduced in both I/R and I/R-PDTC. However, whereas dp/dt_{max} recovered in I/R animals, I/R-PDTC showed markedly reduced dp/dt_{max} throughout the experiment (Fig. 3A). In animals treated with PDTC alone, dp/dt_{max} decreased significantly 60 min following PDTC administration and remained reduced thereafter (Fig. 3B). Diastolic function as measured by tau and $-dp/dt_{max}$ is depicted in Figs. 4 and 5. Whereas tau remained unchanged in I/R animals, values were significantly increased in I/R-PDTC at 60 min of reperfusion (Fig. 4A). In PDTC animals, tau increased significantly 60 min following PDTC administration and remained elevated throughout the experiment (Fig. 4B). After 60 min, mesenteric I/R $-dp/dt_{max}$ was significantly reduced in both I/R and I/R-PDTC and remained decreased in both groups except for 120 min reperfusion (Fig. 5A). In animals treated with PDTC alone, $-dp/dt_{max}$ values were significantly reduced at 60, 120, and 180 min post PDTC administration (Fig. 5B).

Tissue Analyses

Neither NFκB p65 nuclear translocation (Fig. 6A) nor TNF-α (Fig. 6B) was different among experimental

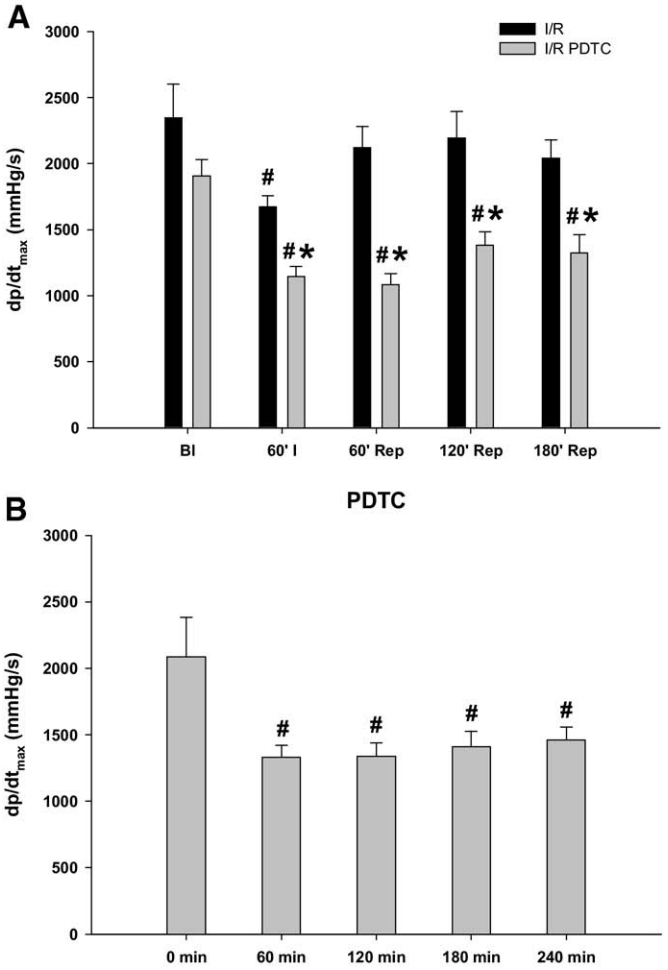

FIG. 3. After 60 min, mesenteric I/R dp/dt_{max} was significantly reduced in both I/R and I/R-PDTC. However, whereas dp/dt_{max} recovered in I/R animals, I/R-PDTC showed markedly reduced dp/dt_{max} throughout the experiment (A). In animals treated with PDTC alone, dp/dt_{max} decreased significantly 60 min following PDTC administration and remained reduced thereafter (B). #, $P < 0.05$ *versus* baseline; *, $P < 0.05$ *versus* I/R.

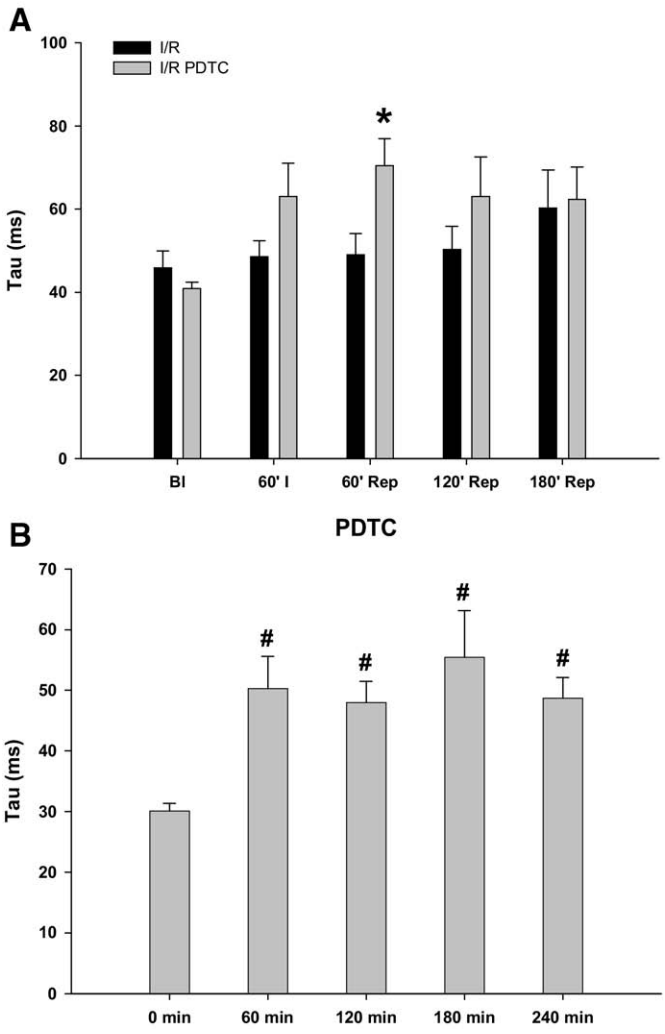

FIG. 4. Whereas tau remained unchanged in I/R animals, values were significantly increased in I/R-PDTC at 60 min of reperfusion (A). In PDTC animals, tau increased significantly 60 min following PDTC administration and remained elevated throughout the experiment (B). #, $P < 0.05$ *versus* baseline; *, $P < 0.05$ *versus* I/R.

groups. Apoptosis/DNA fragmentation assays also did not reveal any differences between the groups (Fig. 6C).

DISCUSSION

Our data show significant decrease in hemodynamic and LV function following 60 min of mesenteric I/R. Both systolic and diastolic LV function were affected. Administration of PDTC aggravated mesenteric I/R-associated cardiac dysfunction. Moreover, in control animals, PDTC administration in the absence of mesenteric I/R caused significant LV and hemodynamic reduction. Tissue analysis did not show significant changes in NFκB activity, TNF-α production, or myocardial apoptosis in either group.

In previous studies, we and others have shown that mesenteric I/R is associated with LV dysfunction and myocardial edema, which could be prevented by mesenteric lymphatic diversion [9]. Furthermore, we could show a PMN-mediated mechanism for mesenteric I/R-induced myocardial dysfunction since post I/R mesenteric lymph primed PMNs and lymph diversion prevented myocardial PMN leukosequestration [9]. Many studies point to the primed and subsequently activated PMN as the link between mesenteric I/R and myocardial dysfunction [5, 6]. In addition, Shahani and coworkers showed that mesenteric I/R increases myocardial myeloperoxidase and TNF-α [5]. Myocardial TNF-α synthesis has also been demonstrated to increase after shock and resuscitation (associated with mesenteric I/R) [6, 10]. TNF-α in turn promotes leukosequestration and also acts as a direct myocardial depressant [5, 7, 9].

The NFκB is an integral component of the cascade leading to TNF-α production in the heart [7]. Therefore, we hypothesized that mesenteric I/R results in myocardial NFκB production, leading to increased

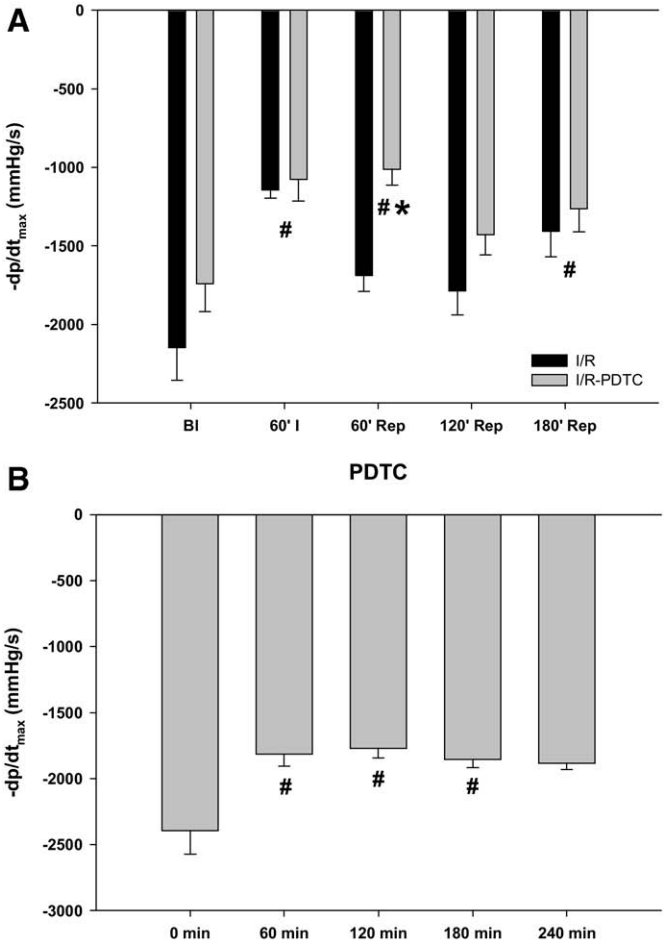

FIG. 5. After 60 min, mesenteric I/R $-dp/dt_{max}$ was significantly reduced in both I/R and I/R-PDTC and remained decreased in both groups except for 120 min reperfusion (A). In animals treated with PDTC alone, $-dp/dt_{max}$ values were significantly reduced at 60, 120, and 180 min post PDTC administration (B). #, $P < 0.05$ *versus* baseline; *, $P < 0.05$ *versus* I/R.

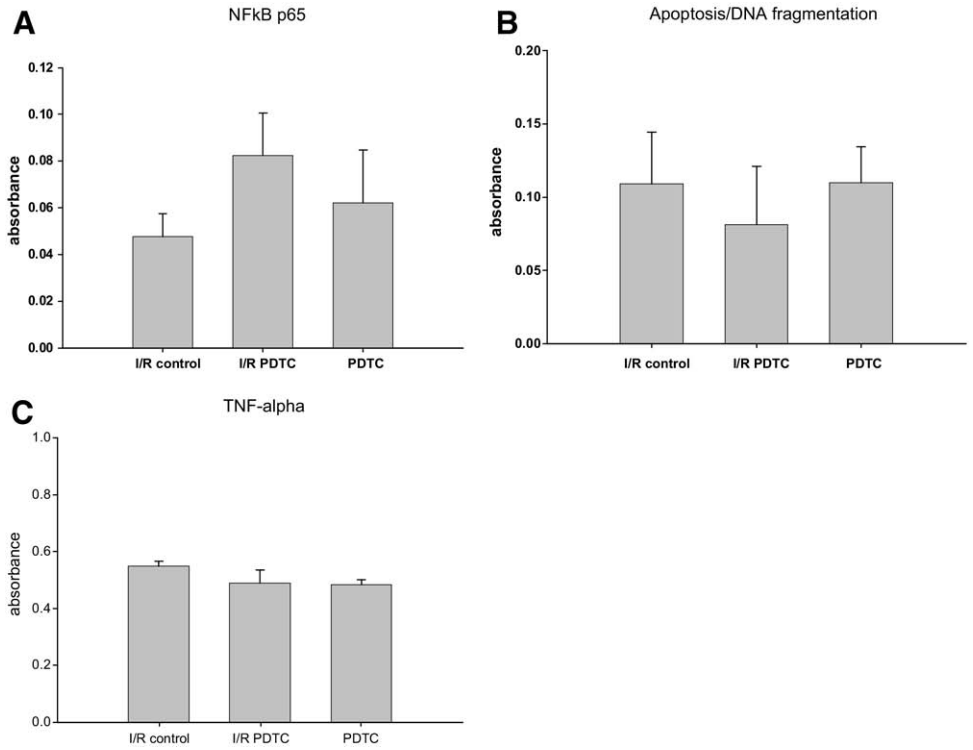

FIG. 6. Neither NFκB p65 nuclear translocation (A) nor TNF-α (B) was different among experimental groups. Apoptosis/DNA fragmentation assays also did not reveal any differences between the groups (C).

TNF-α generation. Consequently, inhibition of NFκB would reduce myocardial TNF-α generation and thus preserve myocardial function following mesenteric I/R. However, we did not find significant changes in myocardial NFκB production following mesenteric I/R. Furthermore, administration of the NFκB inhibitor PDTC exacerbated LV dysfunction without altering NFκB status.

To investigate for mesenteric I/R-independent effects of PDTC on LV function, we added another experimental control group in which animals received PDTC only. In these animals, we found a significant reduction in LV function in the absence of mesenteric I/R. NFκB in those animals was not different from the other experimental groups. Thus, pharmacological properties other than NFκB inhibition must be involved in the PDTC-associated cardiodepression. In addition to its properties as NFκB inhibitior, PDTC also acts as an antioxidant and has been shown to induce apoptosis in vascular smooth muscle cells [11–13]. Myocardial apoptosis induction during ischemia/reperfusion has been associated with LV dysfunction. Consequently, inhibition of the apoptosis signal-cascade improves LV function. Therefore, we also analyzed tissue samples from our experiments for apoptosis/DNA fragmentation. However, we did not find significant differences between the groups.

In summary, we found significant LV dysfunction associated with mesenteric I/R that was not correlated with pathways involving NFκB. In addition to our initial hypothesis and experimental approach, we found a thus far unknown pronounced cardiodepressant effect of the NFκB inhibitor PDTC, which is not related to NFκB pathways. We also did not find a pathophysiological correlate to PDTC-mediated cardiodepression, which could be attributed to its known pharmacological actions. Therefore, evalutation of cardiac and hemodynamic function in experimental set-ups using PDTC have to be carefully interpreted. Future studies are required to elucidate the effects of PDTC on myocardial function.

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