Sulforaphane pretreatment prevents systemic inflammation and renal injury in response to cardiopulmonary bypass

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Objectives: Systemic inflammatory responses are a major cause of morbidity and mortality in patients undergoing cardiac surgery with cardiopulmonary bypass. However, the underlying molecular mechanisms for systemic inflammation in response to cardiopulmonary bypass are poorly understood.

Methods: A porcine model was established to study the signaling pathways that promote systemic inflammation in response to cardiac surgery with cardiopulmonary bypass under well-controlled experimental conditions. The influence of sulforaphane, an anti-inflammatory compound derived from green vegetables, on inflammation and injury in response to cardiopulmonary bypass was also studied. Intracellular staining and flow cytometry were performed to measure phosphorylation of p38 mitogen-activated protein kinase and the transcription factor nuclear factor-κB in granulocytes and mononuclear cells.

Results: Surgery with cardiopulmonary bypass for 1 to 2 hours enhanced phosphorylation of p38 (2.5-fold) and nuclear factor-κB (1.6-fold) in circulating mononuclear cells. Cardiopulmonary bypass also modified granulocytes by activating nuclear factor-κB (1.6-fold), whereas p38 was not altered. Histologic analyses revealed that cardiopulmonary bypass promoted acute tubular necrosis. Pretreatment of animals with sulforaphane reduced p38 (90% reduction) and nuclear factor-κB (50% reduction) phosphorylation in leukocytes and protected kidneys from injury.

Conclusions: Systemic inflammatory responses after cardiopulmonary bypass were associated with activation of p38 and nuclear factor-κB pathways in circulating leukocytes. Inflammatory responses to cardiopulmonary bypass can be reduced by sulforaphane, which reduced leukocyte activation and protected against renal injury. (J Thorac Cardiovasc Surg 2014;148:690-7)

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Cardiac surgery with cardiopulmonary bypass (CPB) can lead to a spectrum of postoperative end-organ complications.[1-4] Systemic inflammation after CPB is believed to contribute to various pathophysiologic outcomes, including renal, pulmonary, and myocardial damage.[5,6] The underlying mechanism is likely to be multifactorial, involving operative trauma, hemodilution and endothelial damage by edema, ischemia/reperfusion of organs, and contact-activation of blood components during CPB.[1-8] Despite these insights, the mechanisms that control systemic inflammatory responses to cardiac surgery remain poorly characterized at the molecular level. To illuminate this subject, we performed CPB under highly controlled experimental conditions using a porcine model. Pigs were chosen because they have a circulation that is anatomically and physiologically similar to that of humans. In addition, the pig is becoming an increasingly attractive species for cardiovascular research, with sequencing of the porcine genome completed and an expanding reagent base.[9,10] The activities of proinflammatory p38 mitogen-activated protein (MAP) kinase and RelA (a member of the nuclear factor-κB [NF-κB] family of transcription factors)[11-17] were monitored in leukocytes and in renal, lung, and myocardial tissues of pigs exposed to CPB to assess

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Abbreviations and Acronyms

ATN = acute tubular necrosis
CPB = cardiopulmonary bypass
IL = interleukin
IV = intravenous
NF-κB = nuclear factor-κB
MAP = mitogen-activated protein
PE = phycoerythrin
RT-PCR = real-time polymerase chain reaction
TNFα = tumor necrosis factor-α

the potential role of these molecules in systemic inflammatory responses.

Given that inflammation in response to cardiac surgery with CPB is induced by a predictable stimulus, it may be possible to prevent or reduce inflammation by pretreatment with an anti-inflammatory compound. We focused on the potential anti-inflammatory effects of sulforaphane. This compound is an isothiocyanate that acts as a potent inducer of antioxidants via the transcription factor Nrf2.[18-22] Sulforaphane is produced during dietary consumption of the precursor glucoraphanin, which is found in green vegetables and is particularly abundant in broccoli sprouts.[23,24] Preclinical studies from our group and others have demonstrated that sulforaphane suppresses inflammation by inhibiting activation of p38 and NF-κB.[21,22,25-27] We examined whether pretreatment using sulforaphane can reduce inflammation and tissue injury in response to CPB.

MATERIALS AND METHODS

Materials

Fluorescent (phycoerythrin [PE]-Cy7-conjugated) antibodies that recognize Thr180/Tyr182 phosphorylated p38 and PE-conjugated antibodies that recognize Ser529 phosphorylated RelA (NF-κB) were purchased from BD Biosciences (San Jose, Calif). Buffers for intracellular staining were obtained from BD Biosciences. Primary antibodies that recognize Thr180/Tyr182 phosphorylated p38 or Ser536 phosphorylated RelA (NF-κB) were purchased from New England Biolabs (Ipswich, Mass). All other reagents were obtained from Sigma-Aldrich (St Louis, Mo).

Animal Model

Female Landrace pigs (50-60 kg) were used according to UK Home Office regulations and Directive 2010/63/EU of the European Parliament and in compliance with the *Guide for the Care and Use of Laboratory Animals* (http://www.nap.edu/catalog.php?record_id=12910). They were acclimatized for 1 week before surgery, and their health and absence of infection were verified by veterinary inspection. Animals were sedated using intramuscular ketamine (20 mg/kg)/xylazine (2 mg/kg) before the induction of anesthesia with 5% isoflurane gas and oxygen flow rates of 8 to 10 L/min. A 21-gauge cannula was inserted in an ear vein to facilitate intravenous (IV) administration of substances. After intubation, anesthesia was maintained with an isoflurane/O_2 mixture. Immediately after the induction of anesthesia, pigs were treated with a single dose of sulforaphane (2 mg/kg, IV injection) or an equivalent volume of the vehicle (saline, IV injection) as a control (5 animals per group). Continuous arterial blood pressure monitoring was instituted via an indwelling arterial line in the external carotid artery after surgical neck dissection. After median sternotomy using a gigli saw, IV heparin was administered (300 IU/kg). The aorta and right atrium were then cannulated, and normothermic (38°C-39°C) nonpulsatile CPB was maintained for 2 hours using a Stöckert multiflow roller pump (Sorin Group GmbH, Munich, Germany) generating a forward flow of 2 to 4 L/min with line pressures less than 300 mm Hg. Gas exchange was achieved via a hollow fiber-membrane oxygenator apparatus (Dideco Biomedical, Mirandola, Italy). Mean arterial pressures between 50 and 65 mm Hg were achieved with incremental doses of metaraminol, as required. The adequacy of CPB was checked by blood gas analysis at 30-minute intervals. Lung ventilation was discontinued during this period. Arterial blood samples were taken before surgery, after sternotomy and cannulation but before CPB, and at varying times after the commencement of CPB. On completion of the CPB period, the experimental animal was terminated using IV phenobarbital. Immediately after termination, renal, pulmonary, and myocardial tissues were removed. Portions were snap-frozen using liquid nitrogen and stored at −80°C (before analysis by Western blotting or real-time polymerase chain reaction [RT-PCR]). Portions were fixed using formalin and embedded in paraffin before the preparation of histologic sections.

Intracellular Staining for Phosphorylated p38 Mitogen-Activated Protein Kinase and Nuclear Factor-κB

Whole blood samples were incubated with Becton Dickinson (Franklin Lakes, NJ) lyse/fix buffer (containing phosphatase inhibitors) for 10 minutes at 37°C. Leukocytes were then isolated by centrifugation (300*g* for 5 minutes) and washed once with phosphate-buffered saline. Cells were resuspended in 0.5 mL prechilled Becton Dickinson Perm Buffer III, vortexed, and incubated on ice for 30 minutes. After this, cells were washed twice with phosphate-buffered saline and resuspended in 0.5 mL Becton Dickinson Stain Buffer (fetal bovine serum) before incubation for 30 minutes (at room temperature) with PE-Cy7– or PE-conjugated antibodies that recognized Ser529 phosphorylated RelA (p65; NF-κB) or Thr180/Tyr182 phosphorylated p38 or isotype-matched controls, with subsequent washing and analysis by flow cytometry (Becton Dickinson). Fluorescence of PE-conjugated p65 and PE-Cy7–conjugated p38 MAP kinase antibodies was quantified in granulocytes or mononuclear cells (identified by forward and side scatter plots) using Summit 4.3 software (Dako, Glostrup, Denmark).

Comparative Real-Time Polymerase Chain Reaction

RNA was extracted using the EZNA Total RNA Kit I (Omega Bio-Tek, Norcross, Ga) and reverse transcribed into cDNA using qScript cDNA Supermix (Quanta BioSciences Inc, Gaithersburg, Md). Transcript levels were quantified by comparative RT-PCR using gene-specific primers for porcine tumor necrosis factor-α (TNFα) (sense, 5′-GACAGATGGGCTGTACCTCA-3′; antisense, 5′-GAGGTTGACCTTGGTCTGGT-3′), interleukin (IL)-8 (sense, 5′-GACCAGAGCCAGGAAGAGAC-3′; antisense, 5′-ACAGAGAGCTGCAGAAAGCA-3′), and IL-6 (sense, 5′-GCTTCCAATCTGGGTTCAAT-3′; antisense, 5′-CTAATCTGCACAGCCTCGAC-3′) using PerfeCTa SYBR Green Supermix (Quanta BioSciences Inc) and the CFX96 RT-PCR Detection System (Bio-Rad Laboratories, Inc, Hercules, Calif). Reactions were performed in triplicate. Relative gene expression was calculated by comparing the number of thermal cycles that were necessary to generate threshold amounts of product. Data were pooled from 3 independent experiments, and mean values were calculated with standard deviations.

Cell Culture

Porcine aortas were obtained from a local abattoir. Porcine aortic endothelial cells were collected and cultured as described.[28]

Western Blotting

Levels of particular proteins were measured in cytosolic or nuclear lysates prepared using the Nuclear Extraction Kit (Active Motif, Carlsbad,