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Original Article

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Effects of Preoperative Curcumin on the Inflammatory Response During Mechanical Circulatory Support: A Porcine Model

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Abstract

Background: Curcumin is a polyphenol extracted from the turmeric plant which may have anti-inflammatory properties. We hypothesized that curcumin pretreatment would result in a reduction in inflammatory markers in a large animal model of extracorporeal support.

Methods: A total of seven samples were obtained from three swine treated with curcumin and 16 samples were obtained from six swine in the control group (procedure terminated in two swine before last sample could be obtained).

Results: Samples for interleukin (IL)-8 and IL-1b had concentrations below the limit of detection at all points and were discarded from further analysis. IL-6, tumor necrosis factor (TNF)-α, and intercellular adhesion molecule (ICAM)-1 concentrations were lower in curcumin pretreated animals when compared to control animals. This decrease was statistically significant for TNF-α, and ICAM-1.

Conclusions: This project may provide information for the development of a translational study in humans as we noted that curcumin pretreatment in a large animal model of cardiopulmonary bypass (CPB) and extracorporeal support resulted in a decrease in TNF-α and ICAM-1 expression compared to control animals.

Keywords: Curcumin; Circulatory support; Inflammation

Introduction

Curcumin is a polyphenol extracted from the rhizomes of *Curcuma Longa*, the turmeric plant. Recent publications suggest its potential as an agent with anti-inflammatory, anti-oxidant, and anti-bacterial capabilities, as well as therapeutic properties in cancer and for preservation of myocardial function [1, 2]. Curcumin down-regulates the production of various inflammatory cytokines including tumor necrosis factor (TNF-α), interleukin (IL)-1, IL-6, IL-8, and IL-12, most likely through nuclear transcription factor (NF)-κB [3]. It also possesses strong anti-oxidant activities, as both a potent scavenger of reactive oxygen species and by an increase in the transcription of enzymes protecting cells from oxidative stress [4].

Recent studies have shown that curcumin pretreatment is effective in ameliorating ischemia/reperfusion injury in rat cardiac tissue and related mitochondria with attenuation of maladaptive cardiac repair [5, 6]. Curcumin pretreated animals have shown improvements in oxidative stress enzyme levels, improved left ventricular end diastolic volume, reduction in scar tissue, and attenuation of mitochondrial injury induced by reactive oxygen species [5, 6]. It has a long safety profile as it has been consumed for centuries, even at high doses, with a lack of serious short- or long-term adverse effects. The main reported adverse effect has been diarrhea [7, 8]. Given the clinical effects of inflammation during cardiopulmonary bypass (CPB) and the potential role of curcumin in reducing inflammation, we performed a blinded, prospective pilot study evaluating the inflammatory markers TNF-α, IL-6, IL-1b, and intercellular adhesion molecule (ICAM)-1 in a swine model of mechanical circulatory support. We hypothesized that curcumin pretreatment would result in a reduction in inflammatory markers in a large animal model of CPB and extracorporeal support.

Methods

All animals used for this study received care according to the "Guide for the Care and Use of Laboratory Animals (2011)" prepared by the United States Department of Health and Human Services and published by National Institutes of Health. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Nationwide Children's Hospital with strict adherence to the IACUC guidelines regarding humane use of animals.

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Common crossbred swine between 12 - 20 kg were divided into two groups: curcumin (n = 3) and no curcumin (n = 6). The curcumin group was fed 130 mg/kg of curcumin orally for 3 days prior to surgery. The curcumin extract was hidden in their food. The animals were anesthetized with a combination of telazol or ketamine and xylazine with an anti-cholinergic agent administered intramuscularly. Once sedated, the animals had the larynx sprayed with a topical lidocaine spray followed by endotracheal intubation and mechanical ventilation. Mechanical ventilation was accomplished using a pressure-limited mode via an AVEA ventilator (Carefusion, Yorba Linda, CA). The animals were placed on a warming blanket to maintain normothermia. Central femoral venous and arterial access was obtained to facilitate drug delivery as well as arterial blood gas, laboratory, electrolyte, and hemodynamic monitoring. Anesthesia was maintained by continuous infusions of fentanyl and midazolam. This was supplemented with either ketamine, telazol, or pentobarbital, as needed. After ensuring a surgical level of anesthesia was present, rocuronium was administered as needed to maintain neuromuscular blockade throughout the procedure.

Heparin guided by activated clotting time (ACT measurements) provided anticoagulation during extracorporeal membrane oxygenation (ECMO) support. Cannulation for ECMO was via external jugular vein or the right atrial appendage and the carotid artery, femoral artery and aorta. The external jugular and right atrial appendage catheter utilized was the BioMedicus 14 French (Medtronic, Minneapolis, MN, USA) venous wire-wound with a multi-end hole. The BioMedicus 12 French arterial wire-wound cannulas were used for arterial access. The ECMO circuit consisted of a 1/4" × 1/4" internal diameter polyvinyl chloride tubing circuit with Cortiva Bio-Active surface coating (Medtronic, Minneapolis, MN, USA) and a 3/8" step-up and step-down connections using a ROTAFLOW centrifugal pump (Maquet USA, Wayne, NJ, USA) and Quadrox ID pediatric oxygenator (Maquet USA, Wayne, NJ, USA). A small chest tube was placed for suction to facilitate fluid and air removal. The chest cavity was closed in layers using standard surgical approaches. This surgical period required approximately 90 min.

Connections were made to the ECMO circuit and VA-ECMO was initiated with a circuit primed with Normosol-R®. The total ECMO circuit prime volume was approximately 685 mL. Anticoagulation was maintained with an hourly administration of heparin. VA-ECMO flow was increased to 100 mL/kg/min (dependent on cannula size, size of animal and bladder pressure recordings) to simulate full VA-ECMO support. After stable ECMO flows were reached, cardiorespiratory support parameters were titrated to provide normal pH, $PaCO_2$, and PaO_2. Ionized calcium and hemoglobin was monitored using point-of-care testing.

Whole blood samples (10 mL) were collected at three time points for inflammatory mediator analysis: pre cannulation following anesthetic induction, after 60 min on ECMO, and pre-termination of ECMO (180 min). The animals were then euthanized with pentobarbitol and euthasol followed by exsanguination via the common carotid ECMO cannula. Assays measuring IL6, IL8, IL1-b and ICAM-1 levels were performed for each sample using commercially available ELISA kits and were analyzed using a multiplexed electro chemiluminescent platform (MesoScale Discovery) and associated protocols.

Statistical analysis

IL-6, TNF-α, and ICAM-1 concentrations were compared between samples obtained from swine receiving curcumin and samples obtained from the control swine. Data were summarized as medians with interquartile ranges, and non-parametric Mann-Whitney U-tests were used to account for a non-normal distribution of study outcomes. The Hodges-Lehmann method was used to calculate 95% confidence intervals (CIs) of differences in median concentration, expressing concentrations below the limit of detection as 0. As this was a pilot study, tests of statistical significance were not adjusted for multiple comparisons. Data analysis was performed in Stata/IC 14.2 (College Station, TX: StataCorp, LP), and two-tailed $P < 0.05$ was considered statistically significant.

Results

A total of seven samples were obtained from three swine treated with curcumin (one swine expired before the second and third samples could be obtained) and 16 samples were obtained from six swine in the control group (procedure terminated in two swine before last sample could be obtained). Samples for IL-8 and IL-1b had concentrations below the limit of detection and were discarded from further analysis. IL-6, TNF-α, and ICAM-1 concentrations are compared between samples from each group in Table 1. On rank-sum tests, concentrations of TNF-α were significantly elevated among control animals compared to animals receiving curcumin (difference in medians: 110; 95% CI: 20, 388; P = 0.045). Concentrations of ICAM-1 were also elevated among control animals, compared to animals receiving curcumin (difference in medians: 17; 95% CI: 7, 22; P = 0.019).

Discussion

Cardiac surgery with CPB and techniques of extracorporeal oxygenation results in a significant systemic inflammatory response through several mechanisms. These include blood contact with extracorporeal and CPB circuit components, ischemia-reperfusion injury, heparin-protamine interactions, and surgical trauma [9]. This inflammatory response and the resultant mediators can have systemic effects ranging from mild edema to severe multi-organ dysfunction. The biochemistry of inflammation involves many mediators that are only beginning to be understood. It is known that IL-1, IL-6, IL-8, and TNF-α are all potent activators of neutrophils and it has been demonstrated that postoperative inflammatory maker levels for interleukin IL-6 and IL-8 have been correlated with intensive care unit (ICU) length of stay [10]. Furthermore, the endothelial ICAM-1 demonstrates increased expression in the

Table 1. Inflammatory Marker Concentration by Experimental Group Assignment (n = 23 samples)

Inflammatory marker	Curcumin (seven samples) Median (IQR)	Control (16 samples) Median (IQR)	Difference in medians (95% CI)	P value
IL-1b	0 (0, 0)*	0 (0, 52)	0 (0, 0)	0.105
IL-6	251 (201, 315)	324 (177, 815)	73 (-68, 482)	0.349
IL-8	0 (0, 0)	0 (0, 11)	0 (0, 0)	0.517
TNF-α	303 (144, 387)	413 (307, 697)	110 (20, 388)	0.045
ICAM-1	23 (20, 31)	40 (29, 45)	17 (7, 22)	0.019

*Concentrations below limits of detection rendered as 0. Sample concentrations are compared using non-parametric methods to account for unquantifiable low concentrations in some samples. CI: confidence interval; ICAM: intercellular adhesion molecule; IL: interleukin; IQR: interquartile range; TNF: tumor necrosis factor.

face of increased IL-1 and TNF-α levels.

Our preliminary data demonstrate that the inflammatory mediators, TNF-α and ICAM-1, were reduced in animals who received curcumin preoperatively compared to those who did not (Table 1). These results suggest the potential utility of this novel agent in mediating the inflammatory response that is initiated during CPB and extracorporeal support. These data suggest that additional study is warranted, particularly when considered in conjunction with other studies demonstrating the properties of curcumin including interruption of inflammatory pathways. Given the roles of IL-1 and TNF-α in activation of ICAM-1, the significant reduction of ICAM-1 expression in the curcumin treated group suggests a role for curcumin in mediating inflammatory responses in neutrophil activation pathways. Further studies in a larger cohort may demonstrate a more robust effect of curcumin in reducing of IL-6 and TNF-α levels as the numerical differences within these samples were suggestive of a potentially significant effect.

The current study was meant as a preliminary study to provide data and information on which to build future studies with a larger study cohort. We saw no change in the inflammatory markers, IL1b and IL8. In fact, the levels were below the detectable level in all samples suggesting no role for these mediators. Despite the low cohort size, these data suggest a significant effect of curcumin in cytokine burdens in the animals studied. This reduction in cytokine burden correlates well with prior work suggesting an anti-inflammatory role for curcumin, which could extend to cardio-protective and anti-inflammatory effects during CPB and extracorporeal support. Future studies should also attempt to evaluate cytokine burden not only early (2 h), but also later in the course at 18 - 24 h following CPB, as these time points have been shown to be the times for peak TNF-α expression [11]. This project may provide information for the development of a translational study in humans as we noted that curcumin pretreatment in a large animal model of CPB and extracorporeal support resulted in a decrease in TNF-α and ICAM-1 expression compared to control animals.

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