Attenuation of Lipopolysaccharide Induced Cardiac Toxicity and the Role of Curcumin

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Abstract

Lipopolysaccharides (LPS) are the major virulence factors of Gram-negative bacteria and the principal agents of bacteria induced cardiac toxicity. On the other hand, curcumin is an extract from the rhizome of the plant Curcuma Longa (a.k.a spice turmeric; an age old medicinal remedy and cooking ingredient in ancient India and Asia). This study demonstrates the effects of this curcumin in lipopolysaccharide induced cardiac toxicity and began by assigning mice into 4 groups (3 males & 3 females in each group). The groups can be specified as: (A) Control (B) Curcumin: 100μg/kg of body weight by intraperitoneal route, (C) LPS: 60mg/kg by intraperitoneal route, (D) LPS + curcumin; both were given at previously stated concentrations by intraperitoneal route. At first, curcumin and curcumin + LPS group received intraperitoneal (IP) injections of curcumin (100μg/kg of body weight) to see the preventive effects of curcumin. 24hrs after that, LPS and Curcumin + LPS group received LPS: 60mg/kg IP injection. All mice were sacrificed after the next 24 hrs. The mice hearts were collected and Hematoxylin and Eosin (H&E) staining was done with histologic sections of mice myocardial tissue. Mice from the LPS treated group showed: a local area of mineralization, cell death and inflammatory cell infiltrates. Mice from the curcumin + LPS treated group showed: fewer areas of mineralization and reduced cell death and inflammatory cell infiltrates, indicating that curcumin either substantially minimized or prevented cell damage. To assess the type of mineralization, i.e. the deposition of calcium versus another metal; the sections were subjected to Von Kossa staining. LPS treated groups showed brown-black mineral deposition (mainly calcium) and inflamed areas in the cardiac muscle. The curcumin treated mice did not demonstrate the level of mineral deposition and inflammation seen with the LPS induced myocardial injury.

Introduction

Gram negative bacteria can cause a wide variety of infections and can spread among humans in many ways. One instance of the significance of the threat they pose is that, in The United States food borne pathogens cause nearly 76 million illnesses annually and in most cases they are a few gram-negative bacteria such as Salmonella, Escherichia and Campylobacter together with viruses and protozoa (CDC, 2007).

Among the most dreaded consequences of gram-negative bacterial infection is the grave situation called endotoxemia. Here endotoxin or lipopolysaccharide from the outer membrane of gram negative bacteria enters patient’s blood stream and ultimately leads to significant cardiovascular damage and septic shock. This lipopolysaccharide (LPS), is a complex glycolipid that is made up of two distinct regions: polysaccharide and lipid A (Matsuguchi et al., 2000). Infact most of the LPS-induced biological effects are caused by its lipid A portion. Being the innermost component of LPS, lipid A is generally released (and therefore toxic) only after the death of bacterial cell (Todar, 2009). The molecular study of pathogenesis claims that, interaction of LPS with its cellular receptors leads to stimulation of transcription of some pro-inflammatory cytokines like tumor necrosis factor α (TNF-α), interleukin (IL-1), prostaglandins and nitric oxide (NO). This unregulated cytokine production leads to the immunopathological features of endotoxemia or sepsis presented clinically as vasodilatory shock, circulatory collapse and fatal myocardial toxicity (Ramana et al., 2006). While attempts to interrupt this uncontrolled progression of inflammation is frequently sought after, potentials of various natural agents (like curcumin) in attenuating LPS induced pathology are yet to be explored thoroughly and can be a far cost-effective one.

Curcumin is derived from turmeric (a.k.a Curcuma longa plant), a tropical plant that grows in southern and southeastern tropical Asia (Chowdhury et al., 2013). This perennial (living for more than two years) herb of the ginger family is famous for its brilliant yellow-orange color and for numerous therapeutic applications (Chowdhury et al., 2013). The spice turmeric has about 2-5% of curcumin as an ingredient (Chowdhury et al., 2013). This study was designed to determine the potentials of this natural agent curcumin in attenuating LPS induced toxicity in cardiac myocytes. Additionally...
Figure 1: The section from the control group was within normal histological limits. The section from the Curcumin treated group contained multiple sarcoplasmic vacuolation of the cardiocytes. The section from the LPS treated group showed foci of cardiocyte death with subsequent mineralization and inflammatory cell infiltration. The section from the Curcumin + LPS treated group evidenced a lesser degree of cardiocyte death, mineralization, and inflammatory cell infiltration.

H&E Stain:
Figure: 1a(Control) 600X  Figure: 1b(Curcumin)

Figure: 1c(LPS)  Figure: 1d(LPS+Curcumin)

the mechanism of action for this attenuation was investigated.

Materials and Method
Preparation of curcumin stocks
Curcumin C3 complex (R) was collected from Sabinsa Corporation, Hyderabad India.

LPS source and uses
We collected LPS sc-3535 from Santa Cruz biotechnology and LPS was used as 60mg/kg body weight dose (dissolved in distilled water).

Animal Models
An experiment protocol (protocol#R0804-12-1) was approved by Animal Care and Use Committee of Tuskegee University. The adult 129SvEv mice (6–8 weeks old) was used in this study. This study began by assigning mice into 4 groups (3 males & 3 females in each group), to demonstrate the effects of curcumin in lipopolysaccharide induced cardiac toxicity. The groups can be specified as: (A) Control (B) Curcumin: 100ìg/kg of body weight by intraperitoneal route, (C) LPS: 60mg/kg by intraperitoneal route, (D) LPS + curcumin; both were given at previously stated concentrations by intraperitoneal route. At first, Curcumin and Curcumin + LPS group received intraperitoneal (IP) injections of curcumin (100ìg/kg of body weight) to see the preventive effects of curcumin. 24hrs after that, LPS and Curcumin + LPS group received LPS: 60mg/kg IP injection. All mice were sacrificed after the next 24 hrs.

We performed all necropsies and subsequent analyses in the necropsy room of Williams Bowie Hall. The hearts were collected and preserved in 10% regular formalin in 50 ml tube.

Hematoxylin and Eosin (H&E) Staining
To see whether curcumin could attenuate LPS induced cardiotoxicity, Hematoxylin and Eosin (H&E) staining was done on histologic sections of murine myocardial tissue. Following histological processing, the tissues were embedded in paraffin (Product# Leica EG 1120 and Company-Leica) using a Leica embedding center (Product# Leica EG 1160 and Company-Leica). The paraffin embedded blocks were then sectioned at 4 microns using a Leica Microtome (product# Leica RM 2155 and company-Leica) and placed on microscope slides (product# Leica CV5030 and company-Leica). These sections were stained by routine H&E staining procedures.

Von Kossa Silver Test for Calcium
The Von Kossa Silver Test is a metal substitution technique for demonstration of calcium salts. It depends on the anionic part of the calcium salt and hence is not specific for the calcium ion itself. In this technique, sections are treated with a silver nitrate solution and the silver deposited, presumably by replacing the calcium, and thereby visualized in the tissue section as metallic silver. Alcohol were used for fixation and frozen and paraffin sections were used for embedding. For the staining procedure, 5% aqueous silver nitrate solution, 5% sodium thiosulfate and nuclear fast red solutions were used. The sections were hydrated in double-distilled water (ddH₂O) and immersed in the 5% silver nitrate solution. The
Figure 2: The sections from the control and Curcumin treatment groups showed no calcium deposition. The section from the LPS treatment group showed marked calcification as indicated by the intense brown black staining. The section from the Curcumin + LPS treatment group showed a lesser degree of calcification.

immersed slides were exposed to bright light or ultraviolet light for 10 - 20 minutes. Following this exposure, the slides were washed several times in ddH₂O. The unreacted silver was removed with 5% sodium thiosulfate for 2 minutes after which the slides were counterstained for 3-5 minutes with nuclear fast red. The slides were rinsed again in several changes of ddH₂O. The slides were dehydrated, cleared in xylene, and cover slipped using a synthetic mounting medium. The calcium salts stain black to brown black, the nuclei stain red, and the cytoplasm stains pink.

Results and Discussion
Curcumin Attenuates Cardiac Toxicity
To test whether curcumin could attenuate LPS induced cardiotoxicity, Hematoxylin and Eosin (H&E) staining was done with histologic sections of murine myocardial tissue. Figure 1 illustrates representative H&E stained sections of cardiac tissue from each treatment group. The section from the control group (Figure 1a) was within normal histologic limits. In the Curcumin treated group (Figure 1b), the cardiomyocytes contained multiple cytoplasmic vacuoles which are consistent with reversible injury. In the LPS treated group (Figure 1c) there were areas of cell death characterized by deposits of mineral and inflammatory cell infiltrates. The arrow in the photomicrograph depicts a dead cardiocyte undergoing mineralization. In the Curcumin + LPS treated group (Figure 1d) there was a lesser degree of cell death and mineralization, indicating that curcumin decreased the extent of cell damage induced by LPS.

To determine the types of mineralization present in experiments, the histologic sections were stained with von kossa stain. Figure 2 illustrates representative von kossa stained sections of cardiac tissue from each treatment group. The sections from the Control (Figure 2a) and Curcumin treated (Figure 2b) groups showed little to no mineralization. The LPS treated group (Figure 2c) showed marked brown to black staining indicative of calcium deposition. The section from the Curcumin + LPS treated group (Figure 2d) showed a lesser degree of brown to black staining than was seen in Figure 2c. This indicates that there was less calcification of the cardiocytes in this treatment group.

Conclusion
The present study showed that Curcumin attenuated LPS induced cardiac toxicity in Rodents. Cardiac toxicity can be evidenced by increased mineral deposition and cell death. Curcumin showed its anti-cardiotoxicity role by preventing mineral deposition and cell death.

References

