Effect of Curcumin in Experimental Peritonitis

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Abstract Despite medical advancements, the inflammatory cascade and oxidative stress worsen the prognosis in most cases of peritonitis. Curcumin has emerged as a potential antioxidant and anti-inflammatory agent in few of the acute inflammatory and infective conditions. We examined the effect of intraperitoneal injection of curcumin in endotoxin-induced peritonitis in rats. The blood and peritoneal fluid samples were collected at 3 and 24 h following the induction of peritonitis. Animals were sacrificed at 24 h and the organs preserved. The histopathological report of lung, liver, and intestines in the curcumin-treated rats showed maintenance of tissue architecture to a large extent compared to the control group which showed massive congestion, hemorrhage, and necrosis. The blood and peritoneal fluid total count and differential neutrophil counts were significantly higher at 24 h of induction of peritonitis. Serum amyloid assay and lipid peroxidation were significantly lower, and myeloperoxidase assay was higher in the curcumin-treated group at the end of 24 h; thus, curcumin probably demonstrated

a neutrophil-mediated immunopotential and anti-inflammatory action thereby protecting the animal from endotoxemia-induced multi-organ damage.

Keywords Peritonitis · Curcumin · Immunopotential · Anti-inflammatory

Introduction

Despite sophisticated surgical techniques, good antibiotic therapy, and modern intensive care, peritonitis is associated with very high morbidity and mortality rates [1, 2]. In most cases which go into complications, uncontrolled inflammatory cascade and oxidative stress seem to be the culprit [3, 4]. Although lavage using several antioxidants and anti-inflammatory agents had shown therapeutic benefits in animal models and septic patients [5–7], the ability of any lavage to remove the source of infection is questionable. Even if the source of infection decreases immediately, they return to prelavage levels in 24 h [8, 9]. Curcumin (diferuloylmethane), a component of turmeric, a spice obtained from rhizomes of the plant *Curcuma longa* and a member of the family Zingiberaceae, has several anti-inflammatory and antioxidant properties [10, 11]. In vitro studies have shown curcumin to inhibit lipopolysaccharide (LPS)-induced production of nitric oxide in macrophages and nitrite in peritoneal cells. Oral and intragastric administration of curcumin could suppress inflammation through multiple pathways by decreasing inflammatory markers and reactive oxygen species in several chronic inflammations [12]. Acute infections involving oxidative stress have also been shown to respond to curcumin [13, 14]. The present study proposes to evaluate the effect of curcumin as intraperitoneal injection following the induction of peritonitis.

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Methods

Animals Male Wistar rats ($n=14$) 6–8 weeks old and weighing 180–200 g (inbred at Animal house of St. John's Research Institute, Bangalore, India) were kept under routine laboratory conditions at the Central Animal Laboratory of the St. John's Research Institute, SJAHs. The rats received standard laboratory chow and had free access to food and water. The study was approved by the Animal Ethics Committee (CPCSEA) for Care and Use of Laboratory Animals.

Experimental Design Experimental peritonitis was induced in all the animals by giving intraperitoneal injection of 5 mg/kg of LPS (lipopolysaccharide endotoxin) from *Salmonella enteritidis* (Sigma Chemical Co., St. Louis, MO) to each rat [8, 10]. The animals were divided into two groups. The test group ($n=7$) received intraperitoneal injection of 2 ml

curcumin solution (30 mg/kg body weight in DMSO) [9] 30 min after LPS injection while the control group received normal saline. Blood samples were collected at the third hour after the toxin administration. Animals were monitored for survival and other clinical signs of peritonitis (ruffled fur, lethargy, and appearance of diarrhea). The two groups were monitored for signs of peritonitis and sacrificed at the end of 24 h. Blood, peritoneal fluid, tissue specimens of the intestine, lung, and liver were collected for analysis [8].

Total leucocyte count (TLC) and differential count (DC) of blood and peritoneal fluid samples were performed using the Improved Neubauer chamber. Leucocyte infiltration of the intestine, lung, and liver specimens were looked for with H and E staining. Myeloperoxidase activity and total antioxidant capacity of plasma were assessed by spectrophotometry, lipid peroxidation was assessed using TBARS assay, and levels of serum amyloid A were measured using ELISA.

Results

Total and Differential Count Total count in the peripheral blood sample was not significantly different at 3 h in the two groups, whereas the counts were significantly higher in the experiment group as compared to the control group at 24 h.

Neutrophil count was significantly higher, and lymphocyte count was significantly lower in the experimental group compared to the control group both at 3 and 24 h (Table 1).

Peritoneal Fluid Differential Count Neutrophil count in the peritoneal fluid was significantly higher, and lymphocyte

Table 1 Blood total and differential leucocyte counts

Parameter	Control		Experimental		<i>p</i> value
	3rd hour	24th hour	3rd hour	24th hour	
TLC	3257±487	7600±502	4375±599	10,675±1017	0.049
Neutrophil	55±4	73±5	72±5	86±4	0.001
Lymphocyte	42±7	24±8	26±7	13±6	0.001

p<0.05 was considered to be significant

count was significantly lower in the experimental group compared to the control group both at 3 and 24 h (Table 2).

Serum Amyloid A, Lipid Peroxidation, and Total Antioxidant Levels in Plasma Curcumin treatment resulted in a significant decrease in the levels of serum amyloid A (SAA) both at 3 and 24 h. Lipid peroxidation was lower at both time points in the curcumin-treated group; however, the values were not statistically significant. There was no difference in the total antioxidant levels between the two groups and between the two time points (Table 3).

Myeloperoxidase Activity Myeloperoxidase (MPO) activity was significantly lower at 3 h, whereas it increased significantly at 24 h in the experimental group as compared to the control group (Table 3).

Leucocyte Infiltration of the Intestine, Lung, and Liver Specimens On histopathological examination, those animals, where endotoxemia was induced by intraperitoneal injection of lipopolysaccharide (5 mg/kg body weight), showed features of disseminated intravascular coagulation as evidenced by severe congestion, hemorrhage, and marked neutrophilic infiltrate in the liver, lung, and intestines. In addition, liver showed hepatocyte necrosis at multiple foci (Fig. 1a), and features suggestive of acute alveolar injury was noted in the lung (Fig. 1b). The microscopy of intestines showed transmural necrosis along with inflammation and congestion of the serosa (Fig. 1c). In contrast, the animals treated with curcumin did not show the histopathological features of septic shock as described above, although minimal inflammatory infiltrate and congestion was noted in the liver, lung, and intestines (Fig. 1d–f).

Discussion

Turmeric has been used for centuries as a treatment of inflammatory diseases, pain, and trauma [15]. Various preclinical cell culture and animal studies suggest that curcumin has potential as a therapeutic agent in wound healing [16]. Curcumin exhibits anti-inflammatory [10], antioxidant [17], anticarcinogenic [18], antiviral [19], and antimicrobial activity [20]. Besides these, curcumin has a variety of potentially therapeutic

properties, such as antineoplastic, antiapoptotic, antiangiogenic, cytotoxic, immunomodulatory [12], antithrombotic, wound healing, antidiabetogenic, antistressor, and antilithogenic actions [10].

Extensive research within the last half a decade has confirmed that curcumin mediates anti-inflammatory effects through the downregulation of transcription factor nuclear factor- κ B (NF- κ B) [21], tumor necrosis factor (TNF) [22], interleukin-6 (IL-6) [23], interleukin-8 (IL-8) [24], adhesion molecules [25], inducible nitric oxide synthase (iNOS) [26], matrix metalloproteinase-9 (MMP-9) [27], cyclooxygenase-2 (COX-2) [28], and 5-lipoxygenase (5-LOX). Curcumin has both prooxidant and antioxidant properties. While the prooxidant mechanism mediates apoptotic effects, the antioxidant mechanism mediates NF- κ B-suppressive effects [29].

The present study demonstrates the effect of intraperitoneal injection of curcumin in LPS-induced experimental peritonitis. Curcumin was injected 30 min following the injection of LPS. The ability of delayed administration of curcumin to curtail the progress of the disease fulfills an essential prerequisite for anti-endotoxemic agent, because treatment is started after the onset of sepsis in patients.

Curcumin prevented peritonitis-associated multiple organ damage observed at the end of 24 h of induction of peritonitis as demonstrated by the histopathological specimens of the lung, liver, and intestines. Curcumin-treated group showed minimal inflammatory infiltration, and the architecture of the organs was largely retained while the control group showed marked neutrophil infiltration, hemorrhage, and multiple organ injury. The therapeutic effect of curcumin seems to be mediated through an immune stimulatory effect which is shown by an increase in the neutrophil count in the peripheral blood. Increased peritoneal fluid neutrophil count suggests an increase in neutrophil recruitment into the peritoneal cavity. In agreement with our results, in systemic sepsis due to any

Table 2 Peritoneal fluid differential leucocyte count

Peritoneal fluid DC	Control	Experimental	<i>p</i> value
Neutrophils	25	48	0.01
Lymphocytes	53	44	0.03

p<0.05 was considered to be significant

Table 3 Myeloperoxidase assay, total antioxidants, and serum amyloid assay

Parameters	Control		Experimental		<i>p</i> value
	3rd hour	24th hour	3rd hour	24th hour	
MPO activity (U/ml)	14.9±4.2 ^a	10.8±3.7 ^b	5.9±3.2 ^a	29.1±8 ^b	^a 0.01 ^b 0.04
TAO levels (nmol/ml)	3477.2±884 ^a	4326±456 ^b	3104.5±867 ^a	3913.5±586 ^b	^a 0.49 ^b 0.2
SAA assay (ng/ml)	0.53±0.07 ^a	0.53±0.02 ^b	0.16±0.09 ^a	0.13±0.01 ^b	^a 0.04 ^b 0.04

p<0.05 was considered to be significant

^a Comparison at the 3rd hour between the groups

^b Comparison at the 24th hour between the groups

cause, neutrophil count in the peripheral blood and its influx into the peritoneal cavity would increase and reach a maximum level by 24 h of the insult followed by a gradual decrease in the counts [30]. The key to improved survival is an optimal immune stimulation such that the increased neutrophil count is sufficient to combat the insult and not damage the host tissue. In the present study, the tissue architecture was preserved suggesting that probably curcumin had produced an optimal immune stimulatory response that protected the organs from systemic effects of sepsis. Earlier studies have shown that even if the circulating levels of neutrophils increased with the treatment, it would not necessarily relate to organ injury [31]. Our study proposes that curcumin had a more targeted approach to neutrophil manipulation in sepsis.

The serum MPO activity was significantly higher in the experimental group correlating with the increased circulating pool of neutrophils. Although increased MPO activity indicates an increased level of lipid peroxidation and, thus, increased levels of proinflammatory mediators, studies have shown that better survival occurred without suppression of the typical proinflammatory mediators, suggesting that the deaths were not mediated by excessive cytokine-driven inflammation [32]. However, the levels of lipid peroxides in the current study were lower in the curcumin-treated group, suggesting an antioxidant effect of curcumin.

Serum amyloid assay is a hepatic acute-phase protein which responds rapidly to inflammation. SAA levels increase during sepsis and contribute to the disseminated intravascular

Fig. 1 Histopathology of lung, liver, and intestines.

Photomicrograph of liver, lung, and intestines in animals with endotoxemia (**a**, **b**, **c**, left panel) and those treated with curcumin (**d**, **e**, **f**, right panel). **a** Liver showing marked neutrophilic infiltration and hepatocyte necrosis (×200, original magnification, H&E, inset showing necrosis, ×400). **b** Lung showing features of acute alveolar injury (×40, original magnification, H&E). **c** Intestine showing transmural necrosis and serosal congestion (×40, original magnification, H&E, inset showing necrosis of mucosal glands, ×400). **d**, **e**, **f** Liver, lung, and intestine with mild inflammation and congestion (×400, ×40, and ×40; original magnification, H&E, respectively)

coagulation and microthrombosis observed in sepsis [32]. Similar to C reactive protein (CRP), levels of acute-phase SAA increase within hours after inflammatory stimulus, and the magnitude of increase may be greater than that of CRP. Treatment with curcumin significantly reduced the levels of SAA both at 3 and 24 h suggesting that curcumin had an anti-inflammatory action.

The antioxidant levels were not significantly different in the two groups showing that the antioxidant effect of curcumin did not play a significant role in protecting against inflammatory damage; rather, it was proinflammatory and immune stimulant in the first 24 h which minimized the tissue damage. This is supported by previous studies which showed that curcumin has both pro- and antioxidant effects [33].

Several recent studies have potentiated the anti-inflammatory, antioxidant, and analgesic effect of curcumin. A randomized controlled study regarding the use of dietary spices, of which curcumin was one of the major constituent, in postoperative stapled hemorrhoidopexy patients, showed that there was reduced usage of analgesics in the spice intake group demonstrating the analgesic effect of the compound [34].

Another recent double-blind, randomized placebo-controlled study demonstrated significant benefits of curcumin in respect of postoperative pain and fatigue-related outcomes after laparoscopic cholecystectomy [35].

In summary, this work identifies curcumin as a potent immune stimulator with the capacity to protect the organs from LPS-induced peritonitis as observed at the end of 24 h of induction of peritonitis. Further studies are warranted to see the long-term effects such as survival benefits following peritonitis and also in frank bacterial peritonitis.

The present work proposes yet another mechanism of action of curcumin based on neutrophil-mediated immunopotentialization and thereby protecting the organs from LPS-induced lethality.

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Ethical Approval All applicable institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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Conflict of Interest The authors declare that they have no competing interests.

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