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Original Research Article

Curcumin protects hepatocytes from sepsis by regulating inflammatory response and hepatocyte apoptosis

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Abstract

Purpose: *To investigate the effect of curcumin on sepsis in rat hepatocytes, and the mechanism involved.*
Methods: *60 Wistar rats were used: 45 rats in experimental group, and 15 rats in sham operated group. The expression levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), percentage apoptosis, and protein expression levels of peroxisome proliferator activated receptor (PPAR-γ) and nuclear factor-κ B (NF - κ b) were determined.*
Results: *The levels TNF-α and IL-6 were significantly lower in the curcumin-treated rats than in septic rats, and lower in high-dose curcumin group than in low-dose curcumin group ($p < 0.05$). The protein expression levels of PPAR-γ in liver tissue of curcumin-treated rats were significantly up-regulated, relative to that in septic rats, but the expression of NF-κ B protein was down-regulated, when compared to that in septic rats ($p < 0.05$). The protein expression level of PPAR-γ increased in liver tissue of high-dose curcumin-exposed rats when compared to the liver tissue of low-dose curcumin rats, while NF-κ B protein was down-regulated in rats given a higher dose of curcumin.*
Conclusion: *Curcumin reduces inflammatory reaction and suppresses apoptosis of liver cells by up-regulating PPAR-γ and down-regulating the expression of NF-κ β, thereby protecting rat hepatocytes from sepsis-induced injury.*

Keywords: *Curcumin, Inflammatory response, Sepsis, Hepatocyte injury*

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INTRODUCTION

Sepsis is a common complication of severe trauma and shock, and it may lead to multiple organ dysfunction [1]. Usually, sepsis is of sudden onset, and it is associated with high mortality. The liver is the site of energy metabolism. Acute liver injury can occur in sepsis at any time. Therefore, abnormal liver function often occurs at the onset of sepsis due to a combination of several factors [2,3]. Sepsis may lead to different degrees of pathological changes in liver cells, and in severe cases, it may result in necrosis and fibrosis. The disease may deteriorate to liver dysfunction or liver damage, leading ultimately to liver failure [4]. The

pathogenesis of sepsis is complicated, and it is strongly associated with the pathophysiological abnormalities of infection and inflammatory response in multiple systems and organs [5]. In the early clinical stage of sepsis, a large number of antibiotics, organ support therapy and other interventions are usually given, but the clinical efficacy of these treatment strategies are not significant [6]. Therefore, there is need for development new and more effective treatment for sepsis-induced liver injury so as to mitigate liver cell injury and block the expression of inflammatory factors. Pharmacological studies have shown that curcumin exerts anti-inflammatory and anti-tumor effects, with low toxic and side effects and great potential for clinical application [7]. It has been reported that curcumin effectively mitigated the degrees of hepatic and kidney degeneration and necrosis in rats with sepsis [8]. The present study was aimed at investigating the effect of curcumin on hepatocytes in septic rats, and the mechanism involved.

EXPERIMENTAL

Materials

Sixty male rats of Wistar strain weighing 230 – 270 g were maintained in SPF animal house and fed *ad libitum* for 7 days before commencement of the study. Ethical approval for the animal study was given by the institutional ethical committee, and the study followed international guidelines for animal studies.

Main reagents and equipment

Kits for assay of TNF-α and IL-6 were purchased from Jiangxi Eboyin Biotechnology Co. Ltd, while BCA protein quantitation kit was bought from Fuzhou Feijing Biotechnology Co. Ltd. Primers for PPAR-γ and NF-κb were products of Wuxi Yuncui Biotechnology Co. Ltd. Curcumin was supplied by Shanghai Baoman Biotech. Co. Ltd, while ELISA kit was obtained from Nanjing Shanben Biotechnology Co. Ltd. Xylene was purchased from Shanghai Jingke Chemical Technology Co. Ltd. Anhydrous ethanol was bought from Shenzhen Zhenqiang Biotechnology Co. Ltd. Enzyme tag analyzer was purchased from Shanghai Jingkang Bioengineering Co. Ltd. Ultra-clean workbench was bought from Nanjing Beiden Medical Co. Ltd. Table centrifuge was purchased from Sichuan Shuke Instrument Co. Ltd. Optical microscope was product of Jiaozuo Yunzhiyu Biotechnology Co. Ltd, while automatic slicing machine was bought from Dongguan Spectral standard Experimental Equipment Technology Co. Ltd.

Study design

Sixty (60) Wistar rats were used: 45 rats in experimental group, and 15 rats in sham operated group in which only the distal cecum and mesocolon were separated via laparotomy without ligation or perforation. The 45 rats in the experimental group were used to establish sepsis model by cecal ligation and perforation. Thereafter, the model rats were randomly divided into 3 groups: sepsis, and two curcumin groups (50 and 100 mg/kg). The two curcumin doses were administered intraperitoneally. Animals in sham and sepsis groups were injected equivalent volumes of normal saline in place of curcumin.

Determination of severity of sepsis

The improved sepsis severity scoring system was used to evaluate the severity of sepsis in rats in each group. The higher the score, the more severe the disease. Rats in each group were sacrificed via decapitation, after which the liver tissues were excised. Pathological changes in the liver tissues in each group were determined using hematoxylin and eosin (H & E) staining. Liver tissues were processed into sections after fixing in glutaraldehyde solution. The fixed tissues were dehydrated in alcohol gradient, embedded in wax, and sectioned using a microtome. The sections were subjected to H & E staining, cleared and sealed. Then, histological analysis was carried out on hepatic tissue in each group.

Determination of TNF-α and IL-6 levels

The concentrations of these factors in hepatic tissue homogenate in each group were measured using enzyme linked immunosorbent assay (ELISA) kits strictly in line with the kit instructions.

TUNEL assay

The percentage apoptosis of liver tissue cells in each group was analyzed using TUNEL assay. The total numbers of normal and apoptotic cells were determined under the microscope in ten randomly selected high magnification fields. The data were used to calculate percentage apoptosis in each group.

Western blot assay

Total hepatic protein extraction and quantification were done. The protein expression levels of PPAR-γ and NF-κ B in liver tissues of rats in each group were assayed with Western blotting. The protein was resolved using SDS-

polyacrylamide gel electrophoresis, followed by electro-transfer to PVDF membrane which was subsequently sealed with 5 % fat-free milk. Thereafter, the membrane was incubated overnight at 4 °C with 1° antibodies, i.e., *β*-actin, PPARγ, IκB*α,* followed by rinsing and incubation with 2° antibody conjugated with horse radish peroxidase for 60 min at laboratory temperature. Then, the blots were subjected to ECL, while Image J was used for quantitation of relative protein expressions.

Statistical analysis

Data were analyzed using the Statistical Package for The Social Sciences (SPSS) version 22.0 software. Results for IL-6 and TNF-α contents were consistent with normal distribution, and are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for multi-group comparisons, while SNK-q test was used for two-group comparisons. Values of $p < 0.05$ were considered as indicative of statistically significant differences.

RESULTS

Sepsis severity score

The score on severity of sepsis was significantly higher in septic rats than in sham-operated rats, but it was significantly lower in curcumin-treated rats than in septic rats. There was no significant difference in sepsis severity score between the two curcumin dose groups ($p > 0.05$). These results are presented in Table 1.

Table 1: Comparison of sepsis severity score amongst the groups (mean ± SD, n = 15)

Group	Sepsis severity score
Sham	4.56±0.22
Sepsis	19.99±1.23[a]
Low-dose curcumin	16.01±0.39[b]
High-dose curcumin	15.23±0.41[b]
F	1387.570
P-value	< 0.001

[a]$P < 0.05$, vs sham; [b]$p < 0.05$, vs sepsis rats

Pathological and morphological changes in liver tissues

There were no marked changes in the morphologies of hepatic lobule, hepatic sinuses and hepatocytes in the sham group. In the sepsis group, there was congestion in the central vein and hepatic sinusoid; the liver cells were disordered and necrotic, and a large number of inflammatory cells infiltrated the portal area. In contrast, the degrees of congestion in central vein and hepatic sinus in the two curcumin groups were mild, and there was lower level of inflammatory cell infiltration in portal area. Moreover, the degree of liver tissue injury was markedly reduced in the curcumin groups, relative to the sepsis group. These data are presented in Figure 1.

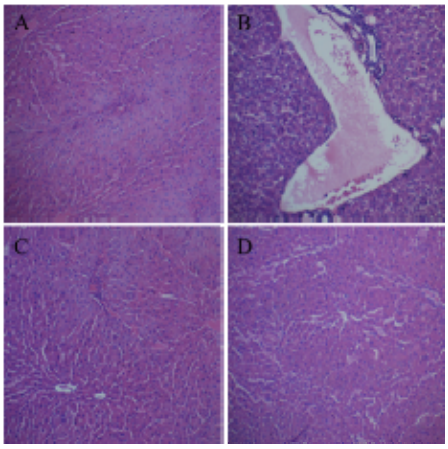

Figure 1: Lesions in liver tissues. A: Sham, B: Sepsis, C: Curcumin (low); D: Curcumin (high). (H & E staining, ×200)

Hepatocyte concentrations of TNF-α and IL-6

Hepatocyte levels of these cytokines were up-regulated in the septic rats, relative to the sham-operated rats, but they were markedly down-regulated in the two curcumin-treated rats than in septic rats. However, there were markedly lower IL-6 and TNF-α levels in rats given high-dose curcumin than in those that received low-dose curcumin. These results are presented in Table 2.

Table 2: Comparison of some cytokine levels in hepatic tissue (mean ± SD, n = 15)

Group	IL-6 (ng/L)	TNF-α (ng/L)
Sham	123.23±3.08	90.08±5.44
Sepsis	988.83±6.61[a]	760.24±12.42[a]
Low-dose group	603.55±16.82[b]	428.99±16.83[b]
High-dose group	410.80±20.42[bc]	311.16±14.39[bc]
F	10506.23	6957.690
P-value	< 0.001	< 0.001

[a]$P < 0.05$, vs sham; [b]$p < 0.05$, vs septic rats; [c]$p < 0.05$, vs curcumin low-dose group

Apoptosis of liver tissue cells

The percentage hepatocyte apoptosis in sepsis group was significantly higher than that in sham group, but it was markedly reduced in the two curcumin groups, relative to septic rats. Moreover, the percentage apoptosis of hepatocytes was markedly milder in high-dose

curcumin rats than in low-dose curcumin rats (Table 2).

Table 3: Apoptosis of hepatocytes (mean ± SD, n = 15)

Group	Apoptosis (%)
Sham	19.49±2.14
Sepsis	53.97±8.41
Low-dose curcumin	28.47±6.48
High-dose curcumin	16.57±2.47
F	210.040
P-value	< 0.001

Expression levels of PPAR-γ and NF-κB in liver tissues

The PPAR-γ protein was markedly lower in sepsis group than in sham group, but there was markedly higher NF-κB protein expression level in septic rats than in sham-operated rats. Protein expression levels of PPAR-γ were markedly up-regulated in curcumin dose groups, relative to sepsis group, while protein expression level of NF-κb was markedly decreased, relative to that in sepsis group. Moreover, PPAR-γ protein in high-dose curcumin group was markedly up-regulated, relative to the corresponding level in curcumin low-dose group, but it was significantly reduced, when compared with that in low-dose curcumin group ($p < 0.05$; Figure 2).

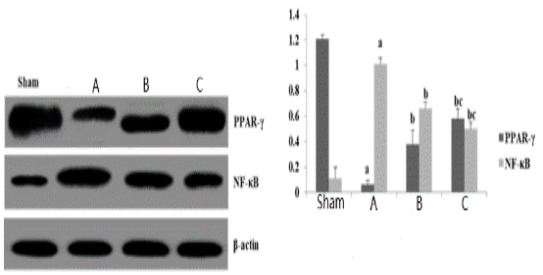

Figure 2: Protein levels of PPAR-γ and NF-κB in liver tissues of each group. A: Sepsis; B: Curcumin (low); C: Curcumin (high). [a]$P < 0.05$, vs sham; [b]$p < 0.05$, vs sepsis group; [c]$p < 0.05$, vs curcumin (low)

DISCUSSION

Curcumin is extracted from curcumin rhizome. Due to its low toxicity and good clinical application potential, it has become the focus of research in China and other countries. Curcumin exerts pharmacological effects on sepsis through several mechanisms, and its use in sepsis research can provide a scientific basis for its application in the treatment of sepsis [9, 10]. It has since been revealed that curcumin is a highly potent NF-κB blocker, and it mediates the pathogenesis of many types of acute and chronic inflammatory diseases by blocking NF-κB [11].

In the present study, it has been demonstrated that curcumin markedly reduced sepsis severity score and mitigated sepsis-induced liver damage in septic rats. These results indicate that curcumin exerts hepatoprotective effects. The systemic response to sepsis due to uncontrolled infection is manifested in increased inflammatory response in which the levels of pro-inflammatory factors are elevated. When stimulated with lipopolysaccharide (LPS), tissue myocytes synthesize and release inflammation-inducing proteins, which then induce secretion of a large amount of pro-inflammatory factors and inflammatory mediators, and eventually cause multi-organ failure [12]. Sepsis induces substantial apoptosis in hepatocytes and other tissues and organs, thereby aggravating the disease [13]. It is known that NF-κB is associated with the release of inflammatory factors, thereby participating in the onset of sepsis.

It has been reported that LPS induced NF-κB synthesis and secretion [14]. With time, NF-κB activity increases, leading to intensified inflammatory cascade which has significantly higher regulatory effects than anti-inflammatory factors, eventually resulting in uncontrolled sepsis-induced inflammation. Moreover, NF-κB acts as a central factor that potentiates the release of various cytokines. Thus, the blocking of NF-κB activity is of great significance in the treatment of sepsis. It is known that PPAR-γ is expressed in fat, liver and other tissues. The activation of PPAR-γ blocks the transcription of NF-κB gene, thereby blocking the secretion and activation of NF-κB and inflammatory factors, resulting in mitigation of tissue damage [15].

The levels of PPAR-γ protein in curcumin-treated rats were markedly up-regulated, relative to the corresponding expression level in sepsis group. Moreover, cytokine levels, percentage apoptosis and NF-κB protein were markedly reduced in curcumin groups, relative to the sepsis group.

These findings indicate that curcumin exerts a hepatoprotective effect in sepsis, most likely through mechanisms involving inhibition of the expression of NF-κB and up-regulation of the expression of PPAR-γ, reduction of secretion of pro-inflammatory factors, and suppression of hepatocyte apoptosis. Thus, curcumin attenuated inflammatory response and suppressed apoptosis of hepatocytes by up-regulating PPAR-γ and down-regulating the expression of NF-κ β, thereby protecting rat hepatocytes from sepsis-induced lesions.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Minjie Zhou designed the study, supervised the data collection, and analyzed the data. Guoyong Hu interpreted the data and prepared the manuscript for publication. Donglian Wang, Lihui Jiang, Lina Xu and Lidong Zhao supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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