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Effect of curcumin on *TNFR2* and *TRAF2* in unilateral ureteral obstruction in rats

Reem M. Hashem Ph.D. [a,b,*], Rasha H. Mohamed Ph.D. [c], Dina M. Abo-El-matty Ph.D. [d]

[a] *Centre for Skin Science, Faculty of Life Sciences, Bradford, Yorkshire, United Kingdom*
[b] *Biochemistry Department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt*
[c] *Biochemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt*
[d] *Biochemistry Department, Faculty of Pharmacy, Suez-Canal University, Ismailia, Egypt*

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ABSTRACT

Objectives: Tumor necrosis factor α (TNF-α) is implicated in the pathophysiology of renal obstruction through its interactions with two TNF-α receptors: *TNFR1* and *TNFR2*. Activation of *TNFR1* leads to the recruitment of the adaptor TNFR-associated death domain protein (TRADD), which binds the Ser/Thr kinase receptor-interacting protein (RIP) and TNFR-associated factors 2 (*TRAF2*). This *TRADD-RIP-TRAF* complex causes activation of the antiapoptotic pathway and inhibits caspase 8 activation. Meanwhile, activation of TNFR2 leads to depletion of TRAF2 and enhancement of the apoptotic pathway. Curcumin, the major component found in turmeric spice, has been reported to possess a protective role against renal injury elicited by unilateral ureteral obstruction (UUO). The present study aimed mainly to address the cytoprotective role of curcumin-rich diet (5% w/w) on the apoptotic pathway induced by UUO in rats after 30 d of ligation.
Methods: The levels of mRNA for *TNFR1*, *TNFR2*, *RIP*, *TRAF2*, and *caspase 8* were measured by reverse transcription-polymerase chain reaction. The levels of TNF-α was determined by ELISA. Kidney sections were exposed to histologic and morphometric studies.
Results: Administration of curcumin decreased TNF-α, *TNFR2*, and *caspase 8* without affecting *TNFR1* levels. The gene expression levels of the antiapoptotic molecules *RIP* and *TRAF2* were increased.
Conclusions: The cytoprotective role of curcumin relies on its ability to decrease the *TNFR2* mRNA and enhance the antiapoptotic molecules *RIP* and *TRAF2* to decrease the apoptotic pathway via decreasing the *caspase 8*.

Introduction

Upper urinary tract obstruction is an important cause of renal failure in both children and adults. Progressive tubulointerstitial fibrosis and apoptotic renal tubular cell death are manifestations of obstructive renal injury. Tumor necrosis factor α (TNF-α) is a multifunctional cytokine that promotes inflammation [1]; it is implicated in the pathophysiology of renal obstruction; it exerts its biological functions by interactions with two members of the TNF receptor superfamily, a 55- to 60-kDa type 1 receptor (TNF-R1; p55/60; CD120 a) and a 75- to 80-kDa type 2 receptor (TNF-R2; p75/80; CD120 b) [2,3].

TNFR2 expression is known to be highly regulated and confined to cells of the immune system, whereas TNFR1 is expressed by virtually all tissues [4]. Activation of TNFR1 leads to the recruitment of the adaptor TNFR-associated death domain protein (TRADD), which serves as a platform to recruit additional signaling adaptors [5,6]. These include the Fas-associated death domain (FADD) protein, TNFR1-associated factor 2 (TRAF2), and the receptor interacting protein (RIP) [6].

The association of FADD with TRADD initiates the apoptosis program by activation of caspase 8, whereas the TRADD-RIP-TRAF complex causes activation of the antiapoptotic pathway [7–10].

The TRAF family of proteins consists of six members that are characterized by a highly conserved TRAF domain at the protein C-terminus. With the exception of TRAF1, the TRAFs contain an N-terminal RING domain followed by five or seven zinc-finger

* Corresponding author. Tel.: +44 (0)1274 235 917; fax: +44 (0)1274 232225.
E-mail address: r.ahmed29@bradford.ac.uk (R. M. Hashem).

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motifs [11,12]. These TRAFs serve as scaffold proteins and regulate signal transduction by most members of the TNFR superfamily, resulting in expression of genes that suppress death-receptor- and stress-induced apoptosis [11,13].

Curcumin is a yellow curry spice that is derived from turmeric. It is widely used to treat various disorders and pathologies. The organic extract of turmeric, a ground powder from the root of the *Curcuma* plant, contains 79–85% curcumin as the major component according to the National Cancer Institute and the Food and Drug Administration [14].

A large body of evidence from in vitro and in vivo studies of both animals and humans has indicated that curcumin exhibits a variety of biological effects, such as antioxidant, anti-inflammatory, antitumor, and wound-healing properties [15]. The curcumin effectively attenuates oxidative stress, inflammation, and renal fibrosis, which suggests that curcumin hold promising potential for safe treatment of chronic kidney disease [16].

This study has been undertaken to further address the cytoprotective effect in UUO in attempting to answer the following questions: 1) Does curcumin affect the expression of TNF receptors *TNF-R1* and *TNF-R2*? 2) Is the cytoprotective effect of curcumin attributed to the enhanced expression of antiapoptotic proteins TRAF2 and RIP?

Materials and methods

Experimental animal

Thirty male Wistar rats weighing 260 ± 45 g were supplied by the Egyptian Organization for Biological Products and Vaccines. All studies were conducted in accordance with the guidelines of the Animal Care and Use Committee of the Biochemistry Department, Faculty of Pharmacy, Beni-Suef University. Rats had free access to water *ad libitum* and a standard laboratory diet; all animals were subjected to controlled conditions of temperature and illumination.

Experimental design

Before surgery, the rat was kept without food for 12 h, but allowed access to water. After the rat was anesthetized with thiopental (100 mg/kg i.p.) the abdomen was shaved and a low-midline abdominal incision was done. UUO was performed as described previously [17]. Briefly, the left ureter was ligated with 3 to 0 silk suture at two points. After the abdominal wound was closed in two layers, the rat was returned to the cage. The diet was supplemented with turmeric powder (Sigma-Aldrich, St. Louis, MO, USA) in a dose of 5% w/w (n = 10), whereas the remaining UUO rats were kept on the standard chow diet (n = 10). Sham-operated rats without ureteral ligation (n = 10) were used as a control. After 30 d of the study, blood samples were collected via retro-orbital bleeding for serum separation and stored at −20°C for further analysis. Rats were then sacrificed; the obstructed kidney was harvested for mRNA extraction and histologic examination.

Creatinine and blood urea nitrogen

Concentrations of creatinine and blood urea nitrogen (BUN) were determined enzymatically using commercially available kits (Spinreact, Gerona, Spain) and were expressed as mg/dL.

Determination of renal content of TNF-α

Serum TNF-α concentration was estimated using enzyme-linked immunosorbent assay kits provided by Biosource (Camarillo, CA, USA).

Detection of TNFR1, TNFR2, TRAF2, RIP, *and* caspase 8 *genes expression by reverse transcription-polymerase chain reaction*

RNA extraction

Total RNA was extracted from 50 mg kidney tissue by the acid guanidinium thiocyanate-phenol-chloroform method [18], and RNA content and purity was measured by a UV spectrophotometer. The A260/A280 ratio should be 1.8 to 2.0. The RNA was of high integrality, as detected by agarose gel electrophoresis.

Reverse transcription-polymerase chain reaction experiments

Reverse transcription-polymerase chain reaction (RT-PCR) was done using the extracted RNA for detection of *TNFR1*, *TNFR2*, *TRAF2*, *RIP*, and *caspase 8* genes.

For amplification of the target genes, reverse transcription and PCR were run in two separate steps. Briefly, equal amounts of total RNA (6 μg) were heat denatured and reverse transcribed by incubation at 42°C for 90 min with 12.5 U avian myeloblastosis virus reverse transcriptase (Promega Corp., Madison, WI, USA), 20 U ribonuclease inhibitor RNase (Promega Corp.), 200 nM deoxynucleoside 5′-triphosphate (dNTP) mixture, and 1 nM oligo-dT primer in a final volume of 30 μL of 1× avian myeloblastosis virus reverse transcriptase buffer were added. The reactions were terminated by heating at 97°C for 5 min and cooling on ice. the cDNA samples were amplified in 50 μL of 1× PCR buffer in the presence of 2.5 U Taq DNA polymerase (Promega Corp.), 200 nM dNTP mixture, and the appropriate primer pairs (1 nM of each primer). These sets of primers are shown in Table 1.

PCR consisted of a first denaturing cycle at 97°C for 5 min, followed by 35 to 40 cycles of amplification defined by denaturation at 96°C for 1.5 min, annealing for 1.5 min, and extension at 72°C for 3 min. A final extension cycle at 72°C for 15 min was included, and PCR detection of β-actin was run with each sample as a control.

Agarose gel electrophoresis

All PCR products were electrophoresed on 2% agarose stained with ethidium bromide and observed by UV transilluminator.

Quantitation of the PCR product

The PCR products were then quantitated by using a quantitation kit (from Promega Corp.). This method depends on purification of the PCR using Promega Wizard PCR preps DNA purification kit (Promega Corp.). The mixture for quantitation consisted of DNA quantitation buffer, sodium pyrophosphate, NDPK enzyme solution, T4 DNA polymerase, and DNA. All these contents were incubated at 37°C for 10 min. Then, 100 μL of Enliten L/L reagent was added. Immediately, the reaction was read using a luminometer. The same steps were done on DNA of known concentrations provided by the kit, and a standard curve was drawn by plotting the readings of the luminometer against the concentrations. Then, the readings of the amplified PCR products of the five different genes after using the luminometer were obtained from the standard curve. The results were expressed as μg/mg wet tissue [19].

Table 1
Oligonucleotide primer sequences of studied genes

Gene	Primer sequence	Annealing temperature, °C	Product size, bp
TNFR1	Forward primer: 5′-ACCAAGTGCCACAAAGGAAC-3′ Reverse primer: 5′-CTGCAATTGAAGCACTGGAA-3′	55	441
TNFR2	Forward primer: 5′-GTT GGACTG ATTGTG GGTGTG A-3′ Reverse primer: 5′-AGG GGCTGGAATCTGTGTCTC-3′	50	413
TRAF2	Forward primer: 5′-ACCAGCCCAGTCCTCAGATTTCAGA-3′ Reverse primer: 5′-CTAGGAATGCTCCCTTCTCTCTCCAG-3′	62	226
RIP	Forward primer: 5′-TGGGAAAGCACTGGAAAAC-3′ Reverse primer: 5′-GTCGATCCTGGAACACTGGT-3′	51	251
Caspase 8	Forward primer: 5′-GTTCACACCATTCTCCTGCC-3′ Reverse primer: 5′-GAAACC CCGTCTCTACTAAA-3′	55	156
β-actin	Forward primer: 5′-TGTTGTCCCTGTATGCCTCT-3′ Reverse primer: 5′-TAATGTCACGCACGATTTCC-3′	55	306