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Research Article

EFFECT OF NANOCURCUMIN ON CISPLATIN-INDUCED ACUTE KIDNEY INJURY: A FOCUS ON NRF2 AND KEAP1

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ABSTRACT

Objective: This study aimed to investigate the effect of nanocurcumin (NC) against cisplatin-induced acute kidney injury, focusing on Nrf2 and Keap1.

Methods: Male Sprague-Dawley rats (n=23) were divided into five groups (Control, CP, CP+Cur, CP+50 NC, and CP+100 NC) and sacrificed 7 days after treatment. Whole kidneys were collected for reverse transcription polymerase chain reaction (RT-PCR) analysis of Nrf2 and Keap1.

Results: There were no statistically significant differences in the RT-PCR results among the groups ($p>0.05$). However, Keap1 expression was upregulated in rats treated with 100 mg of NC, which may have been caused by increased Nrf2 activation and activation of a negative feedback loop, which upregulated transcription of Keap1.

Conclusion: NC increased Keap1 levels, but not significantly.

Keywords: Cisplatin, Keap1, Nanocurcumin, Nephrotoxicity, Nrf2.

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INTRODUCTION

Cisplatin (CDPP) is a platinum-based drug for the treatment of cancer. Despite its effectiveness, CDPP induces acute kidney injury (AKI). A quarter to one-third of patients receiving CDPP develop signs of acute nephrotoxicity such as reduced glomerular filtration and decreased serum magnesium and potassium [1].

Uptake of CDPP by the kidney is very high, which can result in nephrotoxicity. The known mechanism of CDPP-induced nephrotoxicity is through activation of the tubular intracellular signaling pathway, which includes the activation of apoptotic mechanisms and increased production of oxygen radicals [2]. In addition, CDPP can react with glutathione and inhibit enzymes involved in the antioxidant system such as glutathione peroxidase, glutathione S-transferase, and superoxide dismutase. Moreover, CDPP can bind to cytochrome P450 in microsomes, thereby increasing the production of radical oxygen species. All of these mechanisms lead to oxidative stress, which is characterized by a shift in intracellular oxidative balance and activation of the apoptotic pathway, resulting in cell death [3,4].

The Nrf2 gene plays a role in the antioxidant system and has been shown to be protective against various diseases including AKI [5]. In its normal state, Nrf2 is suppressed by association with Keap1, resulting in the intracellular pooling of inactive Nrf2. Dissociation of Keap1 activates Nrf2, which functions to protect against oxidative stress and protease damage [6].

Curcumin is an active compound of the turmeric plant (*Curcuma longa*) rhizome that has been shown to ameliorate organ damage due to oxidative stress by increasing Nrf2 activation and reducing the cytoplasmic abundance of Keap1, thus allowing Nrf2 activation [7-9].

METHODS

Animals

A total of 23 male Sprague-Dawley rats were obtained from the Agency for Health Research and Development (Jakarta, Indonesia) and housed at 22±2°C and relative humidity of 65±10% with *ad libitum* access to standard rat chow and water. All experimental protocols were approved by the Animal Ethics Committee of Universitas Indonesia.

Chemicals

Nanocurcumin (NC) and curcumin were purchased from Plamed Green Science Group (Xi'an, China). Molecular analysis showed that the average size of NC was 240.7±53.5 nm, while that of curcumin was 331.7±91.0 nm. Curcumin and NC were suspended in 0.5% carboxymethylcellulose (CMC).

CDPP was obtained from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in normal saline (0.9% NaCl).

All reagents for reverse transcription polymerase chain reaction (RT-PCR) analysis were purchased from Genetika Science (Jakarta, Indonesia), while disposable materials (gloves, pipette tip, etc.) were a generous gift from the Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Indonesia.

Experimental treatment

The rats were acclimatized for 7 days with *ad libitum* access to food and water, and administered 0.5% Na-CMC. Before experimentation, the rats were randomly allocated to one of five groups: Control (four rats), CDPP (four rats), CDPP+100 mg curcumin (CDPP+Cur) (five rats), CDPP+50 mg NC (CDPP+50 NC) (five rats), and CDPP+100 mg NC (CDPP+100 NC) (five rats). Rats in the control group were given 0.5% CMC-Na once daily p.o. (orally) for 9 days, with a single intraperitoneal injection of 0.9% normal saline on day 7. Rats in the CDPP group were given 0.5% CMC-Na once daily p.o. for 9 days, with a single intraperitoneal injection of CDPP (7 mg/kg BW) on day 7. Rats in the CDPP+Cur group were given curcumin (100 mg/kg BW) once daily p.o. for 9 days, with a single intraperitoneal injection of CDPP (7 mg/kg BW) on day 7. Rats in the CDPP+50 NC group were given NC (50 mg/kg BW) once daily p.o. for 9 days, with a single intraperitoneal injection of CDPP (7 mg/kg BW) on day 7. Rats in the CDPP+100 NC group were given NC (100 mg/kg BW) once daily p.o. for 9 days, with a single intraperitoneal

injection of CDPP (7 mg/kg BW) on day 7. After treatment, the rats were anesthetized and sacrificed by decapitation. Whole kidneys were collected and stored at −80°C for further analysis.

Kidney homogenization

The kidneys were homogenized in ice-cold saline (0.9% NaCl) using a rotor-stator and then centrifuged at 3000 g for 10 min at 4°C. The supernatant was then stored at −80°C for RT-PCR analysis.

Nrf2 and Keap1 gene expression analysis

cDNA was synthesized using a cDNA synthesis kit (Roche Life Science, Penzberg, Germany). A Nanodrop 2000 spectrophotometer (NanoDrop Technologies, LLC, Wilmington, DE, USA) was used to assess cDNA purity and concentration. RT-PCR analysis of Nrf2 and Keap1 was performed.

After RT-PCR, gene expression was examined by electrophoresis (Bio-Rad Laboratories, Hercules, CA, USA). Samples were mixed with loading dye (SYBR Green) and then electrophoresed for 10 min at 220 V in a 2% agarose gel in 2% Tris-acetate-ethylenediaminetetraacetic acid buffer. Results were interpreted using the Gel Doc™ EZ Gel Documentation System (Bio-Rad Laboratories).

RESULTS

The RT-PCR results of the Nrf2 gene showed that the average relative expression levels in the CDPP, CDPP+Curcumin, CDPP+50 NC, and CDPP+100 NC groups were 1.40, 1.08, 0.85, and 0.80, respectively (Fig. 1, Table 1). However, since the data distribution was not normal, the median values were used for analysis.

The average relative Keap1 gene expression levels of the CDPP, CDPP+Curcumin, CDPP+50 NC, and CDPP+100 NC groups were 3.85, 3.80, 2.34, and 2.92, respectively (Fig. 2, Table 1).

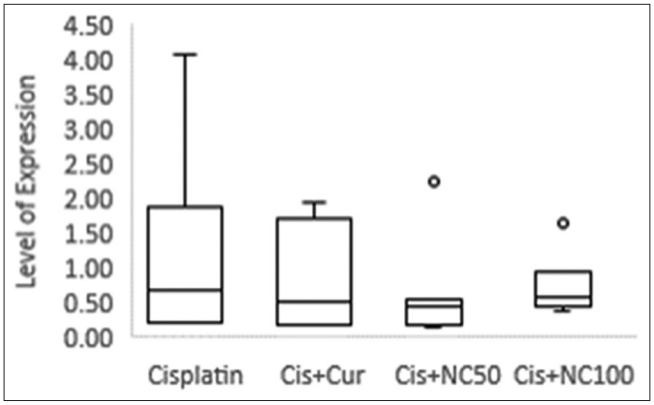

Fig. 1: Nrf2 expression levels

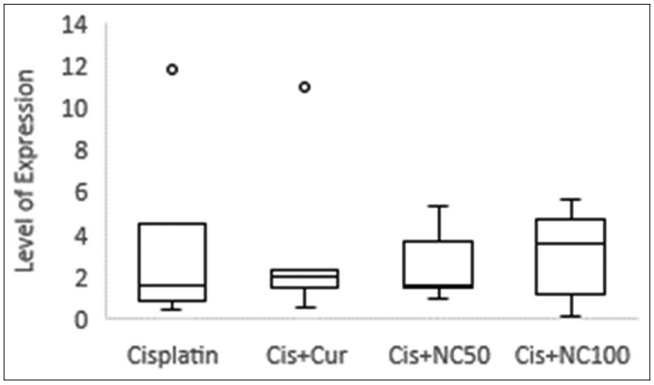

Fig. 2: Keap1 expression levels

The statistical analysis results showed that there was no significant difference in Nrf2 and Keap1 expression levels among the groups ($p>0.05$).

After RT-PCR analysis, electrophoresis was performed, but the results were inconclusive due to the very small differences between the genes. However, several variations within groups were observed (Figs. 3 and 4).

DISCUSSION

A previous study by Waly *et al*. reported an association between curcumin administration and CDPP-induced kidney cell damage, through the activation of antioxidant enzymes [10].

An animal study also found that curcumin conveyed a nephroprotective effect against CDPP-induced nephrotoxicity. However, that study focused on antioxidant enzymes rather than gene expression profiles [11].

Our research suggests that there is no association between NC administration and Keap1 and Nrf2 gene expression. A possible explanation for this discrepancy is the failure of this study to differentiate between nuclear and cytoplasmic levels, as Nrf2 might be translocated to the nucleus.

A previous study reported that curcumin administration significantly increased the level of internalized Nrf2 [12]. However, no study has yet investigated the effect of curcumin on Nrf2 at the transcript level. In addition, studies that described the effect of curcumin on Nrf2 related the findings to chronic kidney failure [6,12]. In the present study, it

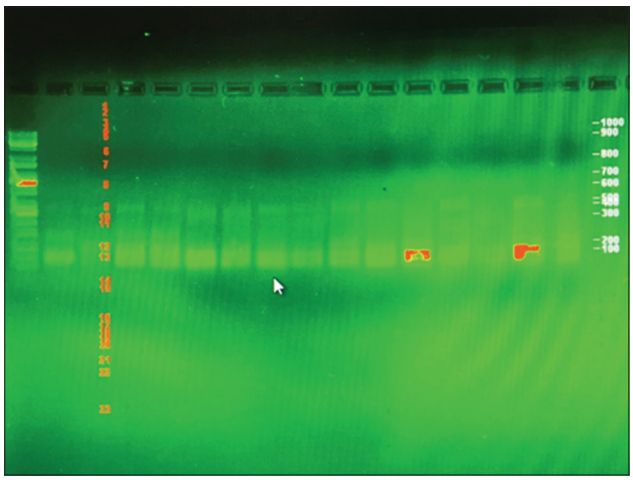

Fig. 3: Electrophoresis result for Keap1

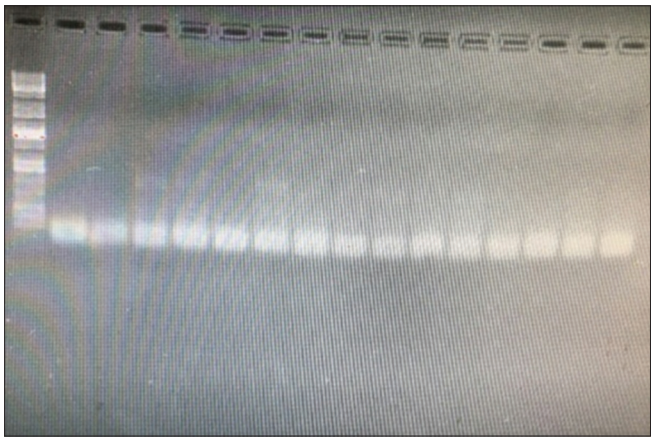

Fig. 4: Electrophoresis result for Nrf2

Table 1: Median gene expression levels based on the Livak method

Treatment Groups	Control	CDPP	CDPP+Cur	CDPP+50 NC	CDPP+100 NC
Gene					
Nrf2	1	0.66984992	0.51405691	0.44751254	0.69015868
Keap1	1	1.56920297	1.99308053	1.58556827	3.53081199

CDPP: Cisplatin, NC: Nanocurcumin

is possible that Nrf2 levels did not significantly increase because the intracellular pool of Nrf2 had not yet been depleted.

A literature search retrieved only one study addressing the effect of curcumin on Keap1 expression, which found that curcumin can relieve Keap1 inhibition of Nrf2 [13]. However, in the present study, curcumin had no effect on Keap1 at the transcript level.

The results of this study showed that Keap1 transcription was upregulated in rats treated with 100 mg of NC. Thus, it is possible that NC increased the level of unbound Nrf2, which then activated a negative feedback mechanism that caused the cell to increase Keap1 transcription.

NC tended to upregulate, but not significantly, Keap1 gene expression, suggesting that the bioavailability of NC is greater than that of curcumin. In fact, it has been reported that the bioavailability of NC is 5 times greater than that of curcumin [14].

There were several limitations to this study. First, the data were not normally distributed, thus the possibility of result bias was high. Second, since two rats were excluded, the number of rats varied among the groups, which may have affected the results.

CONCLUSION

NC was found to upregulate the transcription of Keap1, though not significantly. Otherwise, NC had no effect on Nrf2 in regard to acute CDPP-induced nephrotoxicity.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

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