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EFFECTS OF CURCUMIN AND NANOCURCUMIN ON CISPLATIN-INDUCED NEPHROTOXICITY IN RAT: COPPER TRANSPORTER 1 AND ORGANIC CATION TRANSPORTER 2 AS DRUG TRANSPORTERS

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ABSTRACT

Objective: This study aimed to investigate the efficacy of curcumin (CMN) and nanocurcumin (NC) at preventing cisplatin (CDPP)-induced nephrotoxicity.

Methods: Two membrane transporters, copper transporter 1 (CTR1) and organic cation transporter 2 (OCT2), have been identified involved in active accumulation of CDPP into renal tubular cells. We analyzed OCT2 transcription levels in rat kidney tissue and determined whether renoprotective mechanism of CMN involves CTR1. Rats were randomly divided into five groups: (1) Control, (2) CDPP (7 mg/kg as single dose (i.p.), (3) CDPP+CMN (7 mg/kg CDPP as a single dose, i.p.+100 mg/kg/day of CMN), (4) CDPP+50 mg NC (7 mg/kg CDPP as single dose, i.p.+50 mg/kg/day NC), and (5) CDPP+100 mg NC (7 mg/kg CDPP as single dose, i.p.+100 mg/kg/day NC). Quantitative reverse transcription-polymerase chain reaction was performed to calculate relative expression of CTR1 and OCT2 genes in rat kidney.

Results: Expression of CTR1 was unassociated with administration of CMN or NC, indicating CTR1 is uninvolved in renoprotective mechanism of CMN. The administration of 100 mg/kg/day NC increased expression of OCT2; this increase was higher compared with normal expression levels. This may be due to another regulatory mechanism from the CMN itself.

Conclusion: NC has a better renoprotective effect compared with curcumin, suggested by the increased OCT2 expression on its administration in CDPP-treated rats.

Keywords: Cisplatin nephrotoxicity, Copper transporter 1, Curcumin, nanocurcumin, Organic cation transporter.

INTRODUCTION

Cisplatin (CDPP) (cis-diamminedichloroplatinum [II]) is a potent antineoplastic drug used for the treatment of solid tumors. It has been extensively used for a wide variety of neoplasms including those of the lung, head and neck, breast, ovary, blood, brain, kidney, and testes [1]. However, the dose of CDPP is ultimately limited due to its adverse effects, which reduces its overall effectiveness. The clinical usefulness of this drug is limited due to the presence of platinum in the kidney and liver [2]. The disproportionate accumulation of CDPP in kidney tissue contributes to CDPP-induced nephrotoxicity [3]. It clinically manifests as a lower glomerular filtration rate, higher serum creatinine, and reduced serum magnesium and potassium levels occurring in about one-third of patients undergoing CDPP treatment [4]. This adverse effect typically evolves slowly and predictably after initial and repeated exposure [5].

The major pathological basis of CDPP nephrotoxicity is renal tubular cell death on exposure [4]. CDPP is taken up by tubular cells to be eliminated from the body. In recent years, it has become evident that the cellular uptake of CDPP is mediated, at least in part, by specific transport proteins. Transport proteins that have been reported to be associated with CDPP uptake into renal tubular cells and consequent nephrotoxicity include copper transporter 1 (CTR1) and organic cation transporter 2 (OCT2) [6-8].

Curcumin (CMN) is an active compound extracted from the rhizome of the plant *Curcuma longa* and has been reported to have renoprotective effects [9]. CMN has been known for its numerous pharmacological activities including antioxidant, anti-inflammatory and antimicrobial properties, and chemopreventive and chemotherapeutic activity [10]. In addition, CMN appears to reverse the effects of CDPP, which includes a decrease in the expression of OCT2. This decreases in OCT2 expression in response to CDPP is believed to be a natural renoprotective mechanism of the kidney to prevent further toxicity from CDPP, the uptake of which is mediated by this transporter. Hence, a decrease in the reduction level of OCT2 in the kidneys of animals treated with CDPP and CMN indicates less renal damage compared with kidneys treated with CDPP alone. This is consistent with histopathological findings of CDPP-induced changes (particularly interstitial edema, tubular vacuolation, necrosis, and atrophy). These histopathological changes are found significantly less in CMN+CDPP-treated groups. Various studies have also demonstrated that CMN is extremely safe even at very high doses [11-16].

Unfortunately, pharmacokinetic studies of CMN have revealed poor absorption, rapid metabolism, and elimination, resulting in poor bioavailability [17]. Another factor that contributes to the poor bioavailability of CMN is its low solubility in water, and soluble CMN molecules are unstable at physiological pH [18]. Efforts have been made to enhance the bioavailability of CMN including the use of adjuvants, liposomes, micelles, phospholipid complexes, and nanoparticles [17]. In this study, nanocurcumin (NC) was used to increase the bioavailability of CMN. With enhanced bioavailability, the improved renoprotective abilities of CMN are expected to better attenuate CDPP-induced nephrotoxicity. Therefore, the aim of this study was to investigate the differences between the effects of CMN and NC in preventing CDPP-induced nephrotoxicity. To this end, we analyzed OCT2 transcription levels in rat kidney tissue and examined the effects of CMN and NC on CTR1.

METHODS

Ethics statement and experimental animals

Male Sprague-Dawley rats weighing 150–300 g were obtained from LITBANGKES (Jakarta, Indonesia). Rats were acclimatized for a week before the study. Rats were housed under standard laboratory conditions at a temperature of 22±2°C with a relative humidity of 65±10% and were provided a standard pellet rodent diet and water *ad libitum* during the study. Experiments were approved by the Institutional Animal Ethics Committee of Universitas Indonesia.

Drugs and chemicals

Cisplatin (CDPP) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and was suspended in normal saline (0.9% NaCl). CMN and NC were purchased from PT Plamed Green Science Limited (China). Molecular size analysis was performed, and it was determined that the average molecular size of NC was 240.7 nm (standard deviation [SD]=53.5), whereas the average molecular size of CMN was 331.7 nm (SD=91.0). CMN and NC were suspended in 0.5% carboxymethylcellulose (CMC).

Experimental design

A total of 23 rats were divided into five groups at random: (1) Control rats (n=4), which received daily administrations of 0.5% CMC-Na orally for 9 consecutive days and a single intraperitoneal (i.p.) injection of 0.9% normal saline on the 7th day; (2) the CDPP group (n=4), which received daily administrations of 0.5% CMC-Na orally for 9 consecutive days and a single i.p. injection of CDPP (7 mg/kg dissolved in 0.9% normal saline) on the 7th day; (3) the CDPP+CMN group (n=5), which received daily administrations of CMN (100 mg/kg/day dispersed in 0.5% CMC-Na) orally for 9 consecutive days and a single i.p. injection of CDPP (7 mg/kg dissolved in 0.9% normal saline) on the 7th day; (4) the CDPP+50 mg NC group (n=5), which received daily administrations of NC (50 mg/kg/day dispersed in 0.5% CMC-Na) orally for 9 consecutive days and a single i.p. injection of CDPP (7 mg/kg dissolved in 0.9% normal saline) on the 7th day; and (5) the CDPP + 100 mg NC group (n=5), which received daily administrations of NC (100 mg/kg/day dispersed in 0.5% CMC-Na) orally for 9 consecutive days and a single i.p. injection of CDPP (7 mg/kg dissolved in 0.9% normal saline) on the 7th day. Rats were weighed regularly during the experiment. 24 h after CDPP injection, all rats were placed individually in metabolic cages for urine collection. At the end of the experiment (i.e., on the 10th day), rats were anesthetized with ether and sacrificed by cervical decapitation. Kidneys were isolated and stored at −80°C for gene expression analysis by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and electrophoresis.

Data collection and analysis

Kidney tissue was homogenized with ice-cold saline (0.9% sodium chloride) using a rotor-stator homogenizer. The mixture was centrifuged for 10 min at 3000×*g* at 4°C. The supernatant was then collected, and RNA was isolated using the Tripure Isolation Reagent. Next, cDNA was synthesized using a Transcriptor First Strand cDNA Synthesis Kit (Roche) before two-step qRT-PCR was performed. The purity and concentration of the cDNA in the samples was assessed using a Nanodrop 2000 spectrophotometer. qRT-PCR for CTR1, OCT2, as well as beta-actin as a reference gene was then carried out. The relative expression of CTR1 and OCT2 was calculated using the Livak method by comparing the quantitation cycle values of the treated group with those of the control group. Analysis of the data was carried out using the Statistical Package for the Social Sciences software, version 20. After qRT-PCR, visualization of gene expression was performed by electrophoresis (Bio-Rad). Gels were 2% agarose immersed in 2% tris-acetate-ethylenediaminetetraacetic acid buffer. Samples were mixed with SYBR Green loading dye and electrophoresed for 10 min at 220 V.

RESULTS

The results of qRT-PCR for CTR1 indicated no association between the administration of CMN and NC and respective gene expression as shown in Fig. 1. In the analysis, the median was used instead of the average due to the abnormal spread of the data.

The median of OCT2 qRT-PCR results showed an increased expression in rats treated with CDPP+100 mg NC compared with the other groups, although this finding was not significant (Fig. 2).

Statistical analysis showed no statistically significant result for either CTR1 or OCT2 (p>0.05). Electrophoresis of the CTR1 qRT-PCR product was inconclusive due to only a very slight difference between the expressions (Fig. 3).

Electrophoresis of the OCT2 qRT-PCR product showed an increased expression in the CDPP+100 mg NC group (Fig. 4, the two rightmost wells).
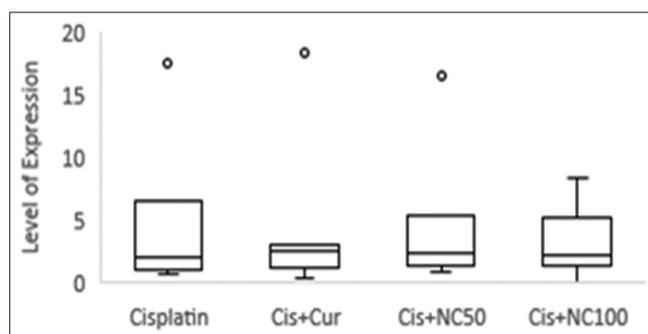
Fig. 1: Expression level of copper transporter 1
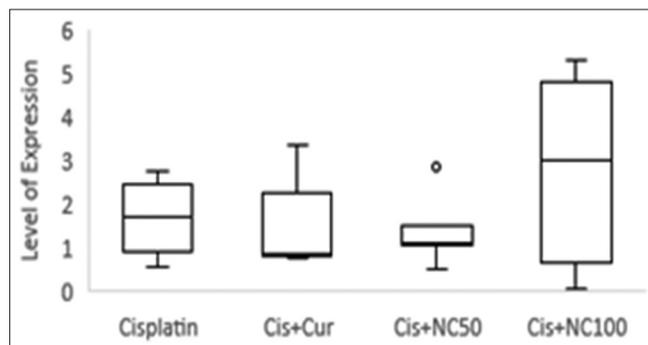
Fig. 2: Expression level of organic cation transporter 2
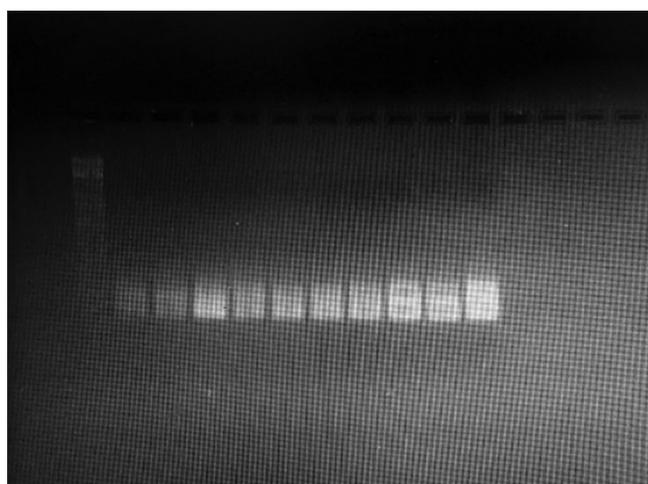
Fig. 3: Electrophoresis of copper transporter 1
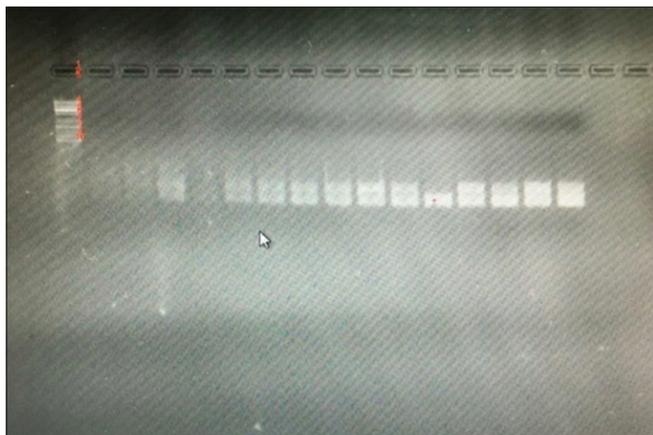
Fig. 4: Electrophoresis of organic cation transporter 2

DISCUSSION

An important process mediating the cellular accumulation of CDPP inside renal tubular cells is transporter-mediated uptake [6]. Two identified membrane transporters involved in the active accumulation of CDPP into renal tubular cells are CTR1 and OCT2 [19]. However, no study has yet elucidated the effects of CDPP toward CTR1 gene expression or the effect of CMN toward CTR1 gene expression in CDPP-induced nephrotoxicity. As for OCT2, several studies have elucidated that OCT2 expression is suppressed on CDPP treatment, which is presumed to be a natural renoprotective mechanism of the kidney to prevent further toxicity [20]. Therefore, CTR1 and OCT2 expression levels were used in this study to determine whether the renoprotective mechanism of CMN involves CTR1 and whether NC has better renoprotective effects compared with CMN.

Collectively, our research suggested no association between the administration of CMN or NC with CTR1 expression level. Thus, the renoprotective mechanism of CMN does not appear to involve CTR1. However, in accordance with previous studies, OCT2 gene expression was increased in the CDPP+100 mg/kg/day NC group. Although the expression was higher than normal levels, this indicates a better renoprotective effect of NC compared with CMN. These results are expected as the molecular size of NC would make it more readily dispersed in aqueous solutions, with subsequent better physical-chemical properties compared with CMN. As for the overexpression, this may be caused by another regulatory mechanism from the CMN itself.

CONCLUSION

The administration of 100 mg/kg/day NC increased OCT2 expression in rats treated with CDPP; however, this increase was higher compared with normal expression levels. In contrast, CTR1 expression was not associated with the administration of CMN or NC; thus, it does not appear to be involved in the renoprotective mechanism of CMN.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

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