

Comparative Effects of Curcumin and Nanocurcumin on Cisplatin-Induced Acute Kidney Injury

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Abstract

Cisplatin is a highly efficacious chemotherapeutic drug; however, it can trigger nephrotoxicity. Previous studies have suggested that curcumin can protect kidneys from cisplatin-induced toxicity. However, its low bioavailability hampers its usage. This study aimed to evaluate the effect of different nanocurcumin concentrations on cisplatin-induced acute kidney injury in rats.

Sprague Dawley rats were randomly divided into five groups, each receiving a different treatment for 9 days: normal, cisplatin, cisplatin + curcumin, cisplatin+nanocurcumin50, and cisplatin+nanocurcumin100. Kidney and plasma samples were analyzed.

A larger concentration of curcumin was detected in the kidneys of the curcumin-treated group than in the nanocurcumin-treated groups, however no statistically significant differences between groups. The concentration of nanocurcumin in the kidneys of the group treated with cisplatin + nanocurcumin 50 mg/kgBW group was higher than in those treated with cisplatin + nanocurcumin 100 mg/kgBW group. These results are consistent with the expression levels of kidney injury molecule (KIM)-1 and neutrophil gelatinase-associated lipocalin (NGAL) in kidney, as both these genes were expressed at lower levels in the nanocurcumin 50 mg/kgBW.

We conclude that nanocurcumin at a dose of 50 mg/kgBW might protect the kidney against cisplatin-induced damage through decreased levels of KIM1 and NGAL.

Experimental article (J Int Dent Med Res 2019; 12(2): 803-808)

Keywords: Cisplatin, bioavailability, oxidative stress

Received date: 11 February 2019

Accept date: 17 March 2019

Introduction

Cisplatin is one of the most effective chemotherapeutic drugs and is commonly used in solid organ cancer. However, cisplatin is a nephrotoxic agent, and may induce renal damage.¹ Over one-third of patients experience cisplatin-induced nephrotoxicity despite prevention procedures.² However, there is still no treatment that can prevent the occurrence of acute kidney injury (AKI). The main preventative treatment that is currently used consists of mannitol administration together with hydration, which only slows the progress of nephrotoxicity.³ The mechanisms underlying cisplatin-induced nephrotoxicity are complex and involve different

processes. Two of the main mechanisms studied in cisplatin-induced nephrotoxicity involve the inflammatory and oxidative stress signaling pathways. Inflammatory reactions occur as cisplatin triggers the production of $TNF\alpha$, a major cytokine in the inflammation process. The end result of cisplatin's inflammatory effect is kidney dysfunction and fibrosis. Oxidative stress can simultaneously contribute to cisplatin-induced nephrotoxicity by reducing antioxidant levels, inducing ROS production through cytochrome p450 induction and causing mitochondrial damage.⁴ As the damage occurs, the kidney tries to recover and protect its function by producing several substances, namely creatinine, cystatin C, monocyte chemotactic peptide (MCP-1), and Netrin-1.⁵ Two of them are kidney injury molecule (KIM)-1 and neutrophil gelatinase-associated lipocalin (NGAL), which can be detected as early as within 2 and 12 h and are better than other traditional biomarkers such as blood urea nitrogen, and serum creatinine (Scr).⁶⁻⁸

Curcumin is known to have antioxidant and anti-inflammatory effects, which may prevent the inflammatory effects and oxidative stress

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caused by cisplatin. However, the use of curcumin is hampered due to its low bioavailability. Many methods have been tested to increase the bioavailability of curcumin. One of them consists of generating curcumin nanoparticles.⁹ A previous study has shown that nanocurcumin has a better saturation solubility and dilution rate than free curcumin.⁹ Furthermore, curcumin nanoparticles have been found to increase its bioavailability up to 5-fold.¹⁰ This research was conducted to determine whether particle size might affect the uptake of curcumin into the kidney in cisplatin-treated rats and investigate its protective effect against cisplatin-induced nephrotoxicity in rat kidneys, as measured by KIM-1 and NGAL, biomarkers for kidney injury.

Materials and methods

Chemicals and drugs

Cisplatin and carboxymethylcellulose sodium (CMC-Na) were purchased from Sigma Aldrich (St. Louis, MO, USA). Curcumin was purchased from Plamed green Science Limited, China. The particle size of curcumin was 339,8 nm (SD = 91.0). Nanocurcumin was bought from Xi'an Pincredit Bio-Tech Co., Ltd, China and the particle size was 240.7 nm (SD = 53.5).

Animals

Male Sprague Dawley rats (150–300 g) were obtained from LITBANGKES (Jakarta, Indonesia) and kept in standardized temperature (26–28°C) and humidity conditions, as well as adequate lighting. The rats were acclimatized for 7 days before treatment and were given food and drink ad libitum. All experimental protocols were ratified by the Animal Ethics Committee, University of Indonesia (Protocol no. 536/UN2.F1/ETIK/2016).

Experimental design

The rats were divided into 5 groups at random order (n = 5 rats/group) and treated for 9 consecutive days. Group 1 (Normal) was given 0.5% CMC-Na orally using gavage for 9 days and a single dose of normal saline through intraperitoneal (i.p) injection on day 7. Group 2 (Cisplatin) was given 0.5% CMC-Na orally

through gavage for 9 days and a single dose of cisplatin (Cis) (7 mg/kgBW) by i.p injection on day 7. Group 3 (Cis+Cur) was administered with Curcumin (100 mg/kgBW) through oral gavage for 9 days and a single dose of cisplatin (7 mg/kgBW) through i.p injection on day 7. Group 4 (Cis+NC 100 mg/kgBW) was administered with nanocurcumin (NC) (100 mg/kgBW) for 9 days through oral gavage and a single dose of cisplatin (7 mg/kgBW) injected intraperitoneally on day 7. Group 5 (Cis+ NC 50 mg/kgBW) was given nanocurcumin (50 mg/kgBW) for 9 consecutive days through oral gavage and a single dose of cisplatin (7 mg/kgBW) through i.p injection on day 7. The curcumin and cisplatin doses were selected based on previous literature searches.¹¹⁻¹⁴ On 10th day, all rats were anesthetized using ether and sacrificed by using the cardiac puncture method. Kidney tissue was collected, cleaned, and weighed. Left kidney tissue from groups 3, 4, and 5 were taken and homogenized using 1 ml NaCl 0.9% for LC-MS/MS detection, while small part of the kidneys from groups 1, 2, 4, and 5 were stored in RNA later solution for qRT-PCR. The homogenized kidney tissue was then stored at -80°C before being analyzed.

Curcumin concentration measurement in kidney tissue homogenate

Curcumin concentration was measured by LC-MS/MS. The LC-MS/MS system that was used comprised UPLC (Waters) and tandem mass spectroscopy with positive electrospray ionization and monitored with m/z 367.25→177.06 for curcumin and 260.06→183.17 for internal standard. Analytes in the sample were separated using liquid chromatography with Acquity UPLC® BEH C18 column (2.1 × 50 mm, 1.7 μm). The mobile phase used formic acid 0.1% and an acetonitrile mixture with the ratio of 72:28. The analyte rate was 0.3 mL/minute and the volume of injection was 3 μL.

Nanocurcumin concentration analysis

Samples were prepared by adding 20 μL of internal standard and 2 mL MTBE as an extraction solvent to 200 μL of kidney homogenate sample. The mixture was then vortexed for 1 minute and centrifuged for 10 min at 3000 rpm. The supernatant of the centrifuged

samples was moved to test tubes and evaporated with nitrogen at 60°C for 2 min in an evaporator. The residue was reconstituted with 100 µL mobile phase and vortexed for 30 s. The solution was then centrifuged for 5 min at 12000 rpm at 20°C. It was then analyzed using LC-MS/MS.

RNA isolation and cDNA synthesis

Total RNA was isolated from the whole kidney samples using Trizol (Takara, Japan) following the manufacturer's instruction. Total RNA was then reverse using commercial kit (Roche). Real-time PCR amplifications were implemented using ABI 7500 system (Thermo Electron Corporation, USA). The primers for RT-PCR are displayed in Table 1.

Gene	Forward	Reverse
KIM1	GCA-GCG-GTC-GAT-ACA-ACA-ATA	CTC-CAC-TCG-GCA-ACA-ATA-CA
NGAL	CGA-ATG-CGG-TCC-AGA-AAG-A	GAG-GAT-GGA-AGT-GAC-GTT-GTA-G

Table 1. The primers used for real-time PCR analysis.

KIM-1 and NGAL qRT-PCR and electrophoresis

Isolated cDNA was measured using a spectrophotometer (NanoDrop 2000) to check sample purity and concentration. The samples were then combined with PCR mix. After the samples underwent PCR, gene expression was assessed using electrophoresis to verify the gene and check for contamination. The electrophoresis gel was prepared by mixing 2% agarose gel powder and 1% TAE buffer solution. 5 µl GelRed stain was then added. The samples were mixed with loading dye and electrophoresis was carried out for 20 min at 120 V and 400 A. The results were analyzed using BioRad's GelDoc Electrophoresis Reading System.

Data analysis

The results for nanocurcumin concentrations are presented as mean ± SEM, whereas KIM-1 and NGAL expression are shown as the median. Statistical analysis was performed using ANOVA with p<0.05 indicating statistical significance. If the data were not normally distributed, Kruskal-Wallis was used to analyze the data. SPSS was used.

Results

Concentration of nanocurcumin in kidney tissues as assessed by LC-MS/MS

Nanocurcumin particle size was 240.7 nm, which is 29% lower than that of curcumin particle size (339.8 nm); however, it is not as low as the usual classification of nanoparticle (<100 nm). The concentration of curcumin in rat kidney homogenate was slightly higher compared to that of nanocurcumin (Figure 1). Surprisingly, administration of 50 mg/kgBW nanocurcumin resulted in a slightly higher concentration the kidney compared to administration of nanocurcumin 100 mg/kgBW, although the difference between the three treatments was not statistically significant.

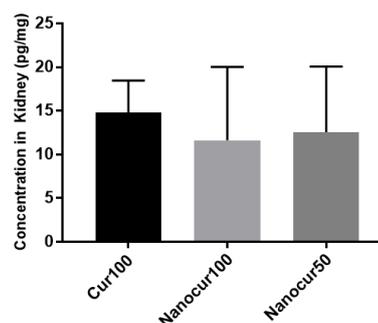


Figure 1. Concentration of Curcumin and Nanocurcumin in Rat Kidney Homogenate. There are no significantly different among the groups treated with curcumin or nanocurcumin.

Expression of KIM-1 and NGAL in kidney tissue assessed by qRT-PCR

qRT-PCR results showed that KIM-1 and NGAL were expressed at different levels (Table 2 & Figure 2). As the data distribution was not normal, median and boxplot were used to present the data.

Table 2. Median gene expression of NGAL and KIM-1 (Livak Method).

Treatment	Control	Cisplatin	Cis + Cur	Cis+NC50	Cis+NC100
Gene					
KIM-1	1	2.21 (0.353-16.737)	2.13 (0.524-15.834)	1.52 (0.837-10.777)	1.87 (0.134-3.329)
NGAL	1	1.54 (0.469-13.594)	1.69 (0.490-16.680)	1.48 (0.850-9.127)	1.50 (0.114-3.599)

The median expression of NGAL in Normal, Cisplatin, Cisplatin+Nanocurcumin 50 mg/kgBW, and Cisplatin+Nanocurcumin 100 mg/kgBW were 1, 1.70, 1.48, and 1.50,

respectively. Meanwhile, the median expression of KIM-1 in Control, Cisplatin, Cisplatin+Nanocurcumin 50 mg/kgBW, and Cisplatin+Nanocurcumin 100 mg/kgBW were 1, 2.13, 1.52, 1.87, respectively.

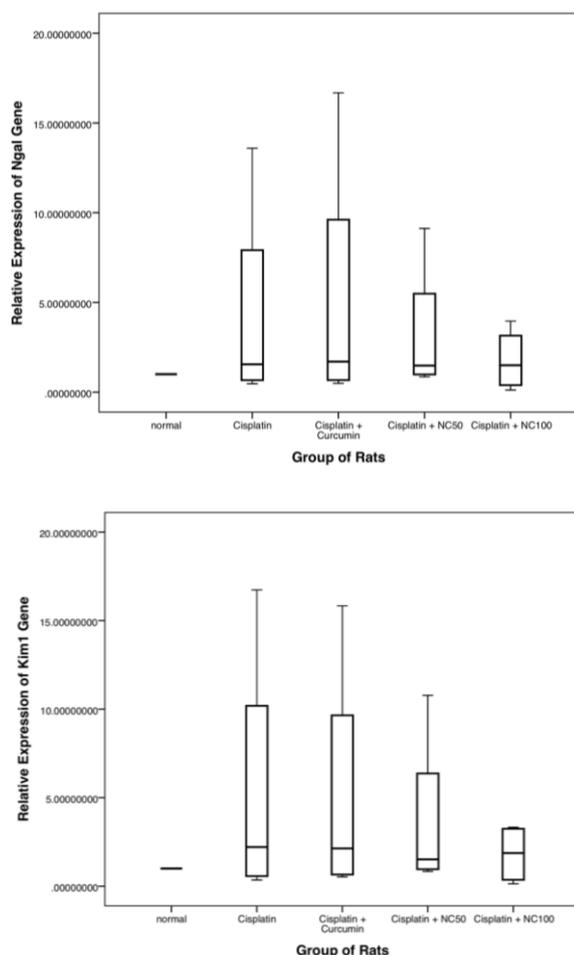


Figure 2. Relative Gene Expression of KIM-1 and NGAL. There are no significantly different among the groups treated with curcumin or nanocurcumin, though cisplatin group showed increased gene expression of KIM-1 and NGAL compared to that of normal group.

The data was analyzed using the Kruskal Wallis test. The results were not significant for KIM-1 or NGAL ($p > 0.05$). However, the median expression in groups administered with nanocurcumin (at both concentrations) showed lower expression of both KIM-1 and NGAL in comparison with both control and cisplatin group.

Discussion

Curcumin is known to have antioxidant and anti-inflammatory effects, which may ameliorate

cisplatin-induced nephrotoxicity. However, its benefits are hampered by its low solubility, physicochemical stability, rapid metabolism, and poor pharmacokinetics. According to research by Yallapu et al. nanoparticles of 100–200 nm in size are good to be used for medical purposes.¹⁵ Because the particle size of nanocurcumin is 29% smaller than that of curcumin, we predicted that it would be better absorbed in kidney tissue. From the results, we can see that the administration of curcumin 100 mg/kgBW leads to a slightly higher concentration in kidney than nanocurcumin (at both doses of 50 mg/kgBW and 100 mg/kgBW). Comparing the groups that were given nanocurcumin, the group that was administered 50 mg/kgBW of nanocurcumin had a slightly higher concentration of nanocurcumin in kidney than the group treated with 100 mg/kgBW nanocurcumin. However, the differences were not significant ($p > 0.05$). Therefore, it can be concluded that there is no significant correlation between a decrease in particle size and the concentration of curcumin in kidney. This result might be due to the size of the nanocurcumin that was used, which was bigger than the curcumin nanoparticles used in another study,¹⁵ and might hamper the uptake of nanocurcumin in kidney. Besides the particle size, the low concentration of nanocurcumin might also be due to the fast metabolism of curcumin. In the liver, kidney, and body fat, only 0.015% of administered curcumin was detected after 3 hours.¹⁶ In this study, the kidney was harvested 24 hours after the last administration of curcumin and nanocurcumin, thus it shows very low concentration of curcumin or nanocurcumin inside the cytoplasm. Cisplatin-induced renal damage may also affect the uptake of curcumin into kidney tissue. To the best of our knowledge, there is no reported correlation between cisplatin concentration and curcumin concentration in kidney.

KIM-1 and NGAL are two compounds that are produced in the kidney during injury. KIM-1 is important for eliminating dying cells and cell debris in damaged tubules, whereas NGAL plays a role in reducing apoptosis, and maintaining the function and increasing the proliferation of new cells.^{6, 17-19} Both KIM-1 and NGAL have been proposed to be promising early biomarkers of AKI as they can be detected 2 and 12 hours, respectively, after injury occur. In the present study, we demonstrated that cisplatin increases

the gene expression levels of KIM-1 and NGAL by 2.21-fold and 1.55-fold, respectively, compared to the normal group (Figure 2). These results were similar to a previous study by Oh et al. which showed a 1.7-fold increase of both KIM-1 and NGAL expression after 24 hours using ELISA.²⁰ These findings indicate that the kidney injury has indeed occurred. Although the data is not statistically significant, in comparison with the cisplatin group, the nanocurcumin-treated groups had lower expression levels of KIM-1 and NGAL, suggesting that nanocurcumin had the same effect as curcumin.¹⁰ Curcumin has previously been shown to counteract the main mechanisms of cisplatin-induced nephrotoxicity, namely oxidative stress and inflammatory reaction.^{1,21} The lower expression levels of KIM-1 and NGAL in the nanocurcumin-treated groups indicate that nanocurcumin can also protect against cisplatin nephrotoxicity. We found a lower expression of KIM-1 and NGAL in the sample group administered with nanocurcumin 50 mg/kgBW, compared with the group treated with nanocurcumin 100 mg/kgBW. This is in agreement with the higher concentration of nanocurcumin detected in kidney in the nanocurcumin 50 mg/kgBW than in the nanocurcumin 100 mg/kgBW-treated group. There are no previous reports of a correlation between nanocurcumin concentration and KIM-1 or NGAL expression.

Conclusions

In this study, we have shown that the data is not statistically significant and there are differences between the effects of the curcumin and nanocurcumin concentrations that were tested, therefore to use nanocurcumin to be able to protect the kidney against cisplatin-induced damage needs a further research.

Acknowledgements

We thank Department of Pharmacology and Therapeutics for the support to finish this research. We also thank Dr Paramitha, M. Biomed and Bantari Wisnu M. for their support during this research. This research is funded by Universitas Indonesia through PITTA grant. The publication of this manuscript is supported by Universitas Indonesia.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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