Cisplatin-Induced Nephrotoxicity in Mouse Kidney Cortical Collection Duct Cells: Protective Role of Curcumin

Bilal Çiğ

1 Ahi Evran University Medicine Faculty, Department of Physiology, Kirşehir, Turkey

INTRODUCTION

Platinum-based cancer drugs provide effective treatment opportunities in malignancies. Cisplatin cis-diamminedichloroplatinum II (Cisp), a platinum-based primary care antineoplastic agent, discovered approximately 60 years ago, is used in the treatment of cancers of the head, neck, lung, ovaries and testicles. In cancer treatment, cisplatin, which shows its antineoplastic effect by binding to the DNA double helix crosswise, turns into its metabolites by rapid enzymatic differentiation. The use of platinum-based chemotherapeutics is limited to many severe negative effects. As these chemotherapy agents have to be used systemically in the treatment, they potentiate a risk of systemic complications. This is the platinum accumulation in the renal system for cisplatin usage and this dose-limiting side effect appears as nephrotoxicity. The molecular mechanism underlying these side effects remains uncertain. In Cisp-induced nephrotoxicity, the role of reactive oxygen species (ROS), mainly occurring in kidney tubular cells, is an undeniable reality. There are studies suggesting the clearance of ROS in the environment with antioxidative agents to prevent renal toxicity.

Glutathione peroxidase (GSH-Px) is one of the leading natural antioxidant enzymes that eliminate oxidative damages in the cells. In this study, GSH, GSH-Px and LP levels were discussed in case of Cisp-induced oxidative stress. Thus, we have evaluated how the strongest mammalian antioxidant enzyme GSH-Px contributes to normalization by eliminating lipid hyperoxides. The second aim of this study is to investigate how much the levels of oxidative stress parameters (ROS, Mitochondrial membrane depolarization, caspase3, caspase-9 and apoptosis) will change with CurC. In addition, the contribution of Curcumin (CurC) to this situation was investigated. Curcumin is an important phenolic compound obtained from Rhiza curcuma longa which has spread all over the world from Southeast Asia. Curcumin, which reacts to reactive oxygen species by reacting directly to reactive nitrogen species, has been shown to exhibit a very strong anti-oxidant and anti-inflammatory effects. In this study, the protective effect of CurC was investigated, on oxidative stress in Cisp-induced nephropathy.

MATERIAL and METHODS

Curcumin, JC-1, Dihydro-rhodamine 123 (DHR 123) and Cell viability dye were obtained from Sigma Aldrich USA. Mouse kidney cortical collection duct cells (mpkCCDc14) were provided by Prof Dr Franziska Theilig at the University of Friborg in Germany. The mpkCCDc14 cells were preserved as...
described previously. The cells were passaged in 25 cm² culture flasks at 37 °C 5% CO₂, 95% air incubator (HF90, Heal Force Bio-meditech Holdings Limited, Shanghai, China).

**The groups were designed as follows**

**Control:** These cells were kept in an incubator for 24 hours without incubation

**CurC:** These cells were treatment with Curcumin (10 µM) for 24 hours.

**Cisp:** These cells were treated for 24 hours with Cisp (80 µM).

**Cisp+CurC:** These cells were treated for 24 hours with Cisp (80 µM), CurC (10 µM). Incubator conditions were: (5% CO₂, 95% air, 37 °C).

**Apoptosis and Caspase-3, Caspase-9 analysis**

Detection of Cisp-induced apoptotic cells in mouse kidney cortical collection duct cells (mpkCCDcl4, 1x10⁶ cell/ml) was performed as described in another study with the ApoPercentage kit (Biocolor Ltd., Northern Ireland). Asymmetry loss occurs in the membrane of cells that undergo apoptosis. The membranes of these cells, which have lost their asymmetry, are painted in red by APOPercentage kit and transported into the cell. In this way, quantitative apoptosis can be determined with the help of spectrophotometer. The determination of the activities of these caspases, which are compared to the stair steps on the road to apoptosis, was made on the basis of a previously reported method. Caspase-3 and -9 were analysed with multiple well reader (plate reader, Tecan Austria) at 360, 460 nm excitation and emission wavelengths, respectively. Data were evaluated by calculating fluorescent units/mg protein compared to control.

**Mitochondrial membrane depolarization**

The cells (mpkCCDcl4, 1x10⁶ cells/ml) were loaded with JC-1 (1µM, 15 minutes at 37 °C) in a shaking water bath as done before in other studies. After loading with dye, fresh Na-HEPES was added on the cells and resuspended and centrifuged. JC-1 are cationic dyes that show mitochondrial potential dependent deposition. Determining the level of mitochondrial membrane depolarization is determined by a shift rate from green (525 nm) to red (590) fluorescent aggregation. The green/red conversion ratio depends on the mitochondrial membrane depolarisation that can affect single-component fluorescent signals.

**Intracellular ROS production**

Intracellular ROS production was analyzed with multiple well reader (Pleate reader, infinite pro200 brand, Austria). This process was determined by measuring the fluorescence intensity of rhodamine-123 (Rh-123). Briefly, cells (1x10⁶ cells/ml) were loaded by incubating with 2µM dihydrodoramide-123 (DHR123) in a shaking water bath at 37 °C for 30 minutes. DHR123 marker, treated with these cells for 30 minutes, is converted into rhodamine 123 with fluorescent properties and used to determine the amount of reactive oxygen species. The excitation is 488 nm and the emission wavelengths are determined as 543 nm for this rhodamine.

**Cell viability (MTT) analysis**

The cell viability activities were evaluated using the MTT assay, as described before. In the cell viability analysis, the diphenyltetrazolium bromide substance, which is yellow in color, is transformed into a purple colored formocant crystal by mitochondrial respiratory tract by living cells. If the cells are not alive, this reaction does not take place. Color formation was also evaluated quantitatively on a colorimetric basis by spectrophotometric method. MTT dye (0,5 mg/ml), cells (1x10⁶ cells/ml) were incubated in the dark for 60 minutes in a shaking water bath at 37°C. Optical density was performed at the same absorbance values (490, 650 nm) for each well using a plate reader.

**Analysis of lipid peroxidation, GSH and GSH-Px levels**

The determination of lipid peroxidation (LP) as the release of malondialdehyde (MDA) in Cisp-induced nephrotoxicity in kidney collection duct cells was evaluated by measuring the thiobarbituric acid (TBA) reaction as described in a previous study. While GSH levels were determined with the help of Ellman reagent (412 nm wavelength), GSH-Px activity were analysed in the light of the literature.

**Statistical analysis**

SPSS statistics program (version 17.0, software, SPSS, Chicago, IL) was used to analyze the data. Expressing the results was mean ± standard deviation (SD). Unpaired Mann-Whitney U-test and Variance analysis (ANOVA) were used and p values were accepted as less than 0.05 and equal. Values with statistical significance were evaluated with the lowest significance difference test.
RESULTS

Cisplatin-induced oxidative stress-related apoptosis and caspase findings in mpkCCD

The increase in oxidative stress parameters in the environment causes disruptions in cellular energy production, disruption of mitochondrial membrane integrity and mitochondrial dysfunction leading to apoptosis. Here the researchers wanted to examine the stages that started from this mitochondrial dysfunction and dragged the cell to apoptosis. In this study, where the protective role of CurC in Cisp-induced nephrotoxicity was investigated (Pict.1), although there was a significant increase in reactive oxygen species production levels (ROS) and apoptosis values, these values approached to normal by CurC treatment (Fig.1 a, b, (ap ≤ 0.05, bp ≤ 0.001 vs control, cp ≤ 0.01 vs Cisp)). The caspase-3, -9 levels, also known as pro-apoptotic cascades, also increased in the Cisp group. CurC treatment significantly reduced this toxic effect (Fig. 2 a, b, (p ≤ 0.01 vs control, p ≤ 0.05 vs Cisp)). The most important effect of apoptotic pathways is the decrease of mitochondrial membrane potential, namely membrane depolarization. Therefore, in this study, also mitochondrial membrane depolarization was evaluated. Cisp significantly increased mitochondrial membrane depolarization and CurC improved these values (Fig.3e, (ap ≤ 0.001, bp ≤ 0.01 vs control; cp ≤ 0.01 vs Cisp)). Also, Cisp treatment caused a decrease in cell viability values and CurC reversed this situation (Fig.3 f, (ap ≤ 0.05, bp ≤ 0.01 vs control, cp ≤ 0.01 vs Cisp)). Our data show that the use of CurC to eliminate oxidative damage in Cisp-induced nephrotoxicity will be beneficial, and the use of CurC can be beneficial in renal failure problems in cancer patients undergoing Cisp treatment.

Effect of Curcumin on GSH, GSH-Px and LP values in mpkCCD cells treated with Cisplatin.

Lipid peroxidation levels were higher in Cisplatin-treated mpkCCD mouse kidney cortical collection duct cells compared to the control group (p <0.001), with a decrease in GSH and GSH-Px values (ap <0.01 and cp <0.001). Although in the curcumin treated mpkCCD cells, GSH-Px levels significantly increased when compared to the control group, LP levels decreased significantly (p <0.01). The LP levels of cells in Cisp+CurC groups showed a significant decrease compared

Pict.1 Images of cells belonging to all groups under Sterio microscope
to Cisplatin (p < 0.001), but there was a significant increase in GSH (p < 0.01) and GSH-px levels (p < 0.001) (Table 1).

**Table 1** Glutathione peroxidase (GSH-Px), reduced glutathione (GSH) and Lipid Peroxidation (LP) levels in mpkCCDCl4 mouse kidney cortical collection channel cells (Control, Curcumin, Cisplatin and Cisp + CurC) (mean ± SD) (*p* < 0.01 and *p* < 0.001 compared to control; *p* < 0.01 and *p* < 0.001 compared to Cisplatin).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Curcumin (CurC)</th>
<th>Cisplatin (Cisp)</th>
<th>Cisp+CurC</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (µg/g protein)</td>
<td>11.67 ± 0.61</td>
<td>12.63 ± 0.45</td>
<td>7.14 ± 0.20*</td>
<td>10.50 ± 0.76*</td>
</tr>
<tr>
<td>GSH-Px (IU/g protein)</td>
<td>24.11 ± 2.94</td>
<td>28.55 ± 1.25*</td>
<td>14.10 ± 2.50</td>
<td>20.77 ± 1.41*</td>
</tr>
<tr>
<td>LP (µmol/g protein)</td>
<td>16.98 ± 0.71</td>
<td>13.34 ± 1.76*</td>
<td>24.24 ± 1.29*</td>
<td>17.58 ± 0.94*</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In this study, we investigated the caspase cascades and apoptosis responses by using molecular techniques to investigate the Cisp-induced nephrotoxicity model in vitro using mouse kidney collection channel cells (mpkCCDCl4). Previous studies on cisplatin-induced nephrotoxicity have been targeted to form DNA lesions as a source of apoptosis caused by this toxicity. According to studies, platinum damage was found in tubular and glomerular kidney cells in Cisp-mediated nephrotoxicity. This resulted in the division of procaspase-3 and procaspase-7, and ultimately with apoptosis. Here, CurC prevented Cisp-induced apoptosis, which forms DNA-platinum adducts by entering the cell nucleus (Fig.1a, bp ≤ 0.001 vs control, cp ≤ 0.01). Our findings are consistent with the histopathological findings obtained from previous Cisp-induced nephrotoxicities. In addition, Curcumin significantly suppressed the ROS, Mitochondrial membrane depolarization levels and caspase-3, caspase-9 activities, which increased with the Cisp effect. Curcumin's contribution to cell vitality is by maintaining cell proliferation with DNA damage prevention by fighting against DNA-platinum additions. As seen in Fig.3f, CurC contributed significantly to cell viability when given to the cell with Cisp (p ≤ 0.01). The protective role of curcumin against Cisp-induced nephrotoxicity has not been previously investigated in renal cortical collection duct cells. We have shown here that Curcumin's protection against this toxicity develops by removing reactive oxidative species from the cellular environment.

Cisplatin-induced nephrotoxicity is the source of ROS, and this reactive oxidative stress in cells is expected to depolarize the mitochondria. Here we wanted to investigate the events that will occur after this mitochondria membrane depolarization after Cisp treatment. It is released into cytosol due to cytochrome C membrane depolarization, which is normally located between the two membranes of the mitochondria and has a very important role in the electron transport chain. The release of cytochrome C into cytosol triggers caspase cascades. Protecting the membrane integrity of mitochondria, BCL-2, BCL-X proteins act anti-apoptotic by preventing the release of any protein from mitochondria to cytosol. When damage occurs in a cell DNA, P53 triggers protein production. This protein leads to the transformation of many proteins. Bim, Bid and bad proteins, which are triggered for reasons such as toxic effects occurring in the cells, prevent the activities of BCL-2 and Bcl-x proteins, and help the membrane integrity of the mitochondria. In this case, Bax and bak proteins form channels in the mitochondria membrane and accelerate this situation. In this way, the cytochrome C flowing into the cytosol combines with Apaf-1 to form the apoptosome. Apoptosome catalyzes the conversion of caspase-9 from procaspase-9. Active caspase-9, on the other hand, triggers the active caspase-3 transformation from procaspase-3 and eventually takes the cell to apoptosis. From these stages, we performed mitochondrial membrane depolarization, caspase-3, caspase-9, apoptosis and cell viability analysis, especially ROS.

To summarize, we observed that in the in vitro Cisp-induced nephrotoxicity model in kidney collection duct cells, the increase in ROS amount, depolarized the mitochondrial membranes, increased caspase-3, caspase-9 and apoptosis. CurC played an important role in the inhibition of apoptotic pathways by reversing these effects. While Cisp increased LP levels, it decreased GSH and GSH-Px levels. Curcumin reversed this situation and exhibited a complete antioxidant property and exhibited a nephroprotective property. Consequently, to prevent kidney complications, researchers should consider curcumin supplementation in cancer treatment protocols. More research on the benefits of using CurC in cancer treatment will be valuable for the topic under discussion.

**Conflicts of Interest**

The authors declare that they have no conflict of interests.
REFERENCES


