SIRT1 activation by resveratrol ameliorates cisplatin-induced renal injury through deacetylation of p53

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Departments of 1Internal Medicine, 2Microbiology, and 3Diagnostic Radiology, and 4Institute for Medical Sciences, Chonbuk National University Medical School, Jeonju; and 5Food Function Research Division, Korea Food Research Institute, Song Nam, South Korea

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Ki-m DH, Jung YJ, Lee JE, Lee AS, Kang KP, Lee S, Park SK, Han MK, Lee SY, Ramkumar KM, Sung MJ, Kim W. SIRT1 activation by resveratrol ameliorates cisplatin-induced renal injury through deacetylation of p53. Am J Physiol Renal Physiol 301: F427–F435, 2011. First published May 18, 2011; doi:10.1152/ajprenal.00258.2010.—Nephrotoxicity is one of the important dose-limiting factors during cisplatin treatment. There is a growing body of evidence that activation of p53 has a critical role in cisplatin-induced renal apoptotic injury. The nicotinamide adenine dinucleotide-dependent protein deacetylase SIRT1 decreases apoptosis through deacetylation of p53, and resveratrol is known as an activator of SIRT1. To study the role of SIRT1 in cisplatin-induced renal injury through interaction with p53, mouse proximal tubular cells (MPT) were treated with cisplatin and examined the expression level of SIRT1, acetylation of p53, PUMA-α, Bax, the cytosolic/mitochondrial cytochrome c ratio, and active caspase-3. The expression of SIRT1 was decreased by cisplatin. Resveratrol, a SIRT1 activator, ameliorated cisplatin-induced renal injury through interaction with p53, mouse proximal tubular cells (MPT) were treated with cisplatin and examined the expression level of SIRT1, acetylation of p53, PUMA-α, Bax, the cytosolic/mitochondrial cytochrome c ratio, and active caspase-3. The expression of SIRT1 was decreased by cisplatin. Resveratrol, a SIRT1 activator, ameliorated cisplatin-induced acetylation of p53, apoptosis, and cytotoxicity in MPT cells. In addition, resveratrol remarkably blocked cisplatin-induced decrease of Bcl-xL in MPT cells. Further specific SIRT1 inhibition with EX 527 or small interference RNA specific to SIRT1 reversed the effect of resveratrol on cisplatin-induced toxicity. Inhibition of p53 by pifithrin-α reversed the effect of EX527 in protein expression of PUMA-α, Bcl-xL, and caspase-3 and cytotoxicity in MPT cells. SIRT1 protein expression after cisplatin treatment was significantly decreased in the kidney. SIRT1 activation by resveratrol decreased cisplatin-induced apoptosis while improving the glomerular filtration rate. Taken together, our findings suggest that the modulation of p53 by SIRT1 could be a possible target to attenuate cisplatin-induced kidney injury.

apoptosis; cisplatin nephrotoxicity

CISPLATIN IS A CHEMOTHERAPEUTIC agent widely used for the treatment of malignant tumors in solid organs. One of the important dose-limiting factors of cisplatin treatment is nephrotoxicity. Direct DNA damage, inflammatory injury, and oxidative stress have been recognized as the mechanism of cisplatin-induced renal injury. Especially, cisplatin-induced apoptotic cell death after DNA damage is the major mechanism in cytotoxicity in renal tubule cells. In response to DNA damage, p53 can induce cell cycle arrest and apoptosis. p53-induced apoptosis affects its transcriptional activity and Bcl2 family members in mitochondria (24). In kidney disease, p53 is involved in the apoptotic injury in ischemic injury and aristolochic acid-induced nephrotoxicity (11, 30, 33). There is also a growing body of evidence that p53 plays a critical role in cisplatin-induced renal injury (2, 9, 30). Furthermore, it has been demonstrated that downregulation of p53 by small interference (si) RNA is an effective way of preventing or treating cisplatin-induced nephrotoxicity (25). Activation of p53 is regulated by posttranslational modification of p53 such as ubiquitination, phosphorylation, or acetylation (1). Especially, acetylation of p53 affects its DNA binding affinity (18). These findings have suggested that modulation of deacetylation or acetylation of p53 can be an important avenue to prevent or treat cisplatin-induced nephrotoxicity.

Among the four major classes of 18-histone deacetylase (HDAC), SIRT1 belongs to HDAC class III, being different from class I and II enzymes in that their activity depends on NAD+ (7). Recently, considerable importance has been given to SIRT1 due to its role in multiple diverse processes, including metabolism, development, stress response, neurogenesis, hormone responses, and apoptosis (12, 23). SIRT1 deacetylates histone and other substrates such as p53, forkhead box O (FOXO), and NF-κB (19, 26, 29, 31). Actually, it has been demonstrated that deacetylation of p53 by SIRT1 decreases the apoptosis mediated by oxidative stress (29). A very recent study has reported that cisplatin induces p53 acetylation at lysine-379 (3). It has also been demonstrated that kidney-specific overexpression of SIRT1 mitigates the cisplatin-induced decrease in renal function and apoptosis in the kidney (5). However, a role of SIRT1 in p53 deacetylation in cisplatin-induced nephropathy remains to be examined.

Since cisplatin increases acetylation of p53 and SIRT1 deacetylates p53, we have hypothesized that activation of SIRT1 decreases cisplatin-induced apoptotic renal injury through p53 deacetylation. In the present study, we have demonstrated that SIRT1 protein expression was decreased by cisplatin in mouse proximal tubular (MPT) and that resveratrol, a SIRT1 activator, reduced cisplatin-induced p53 acetylation and apoptosis. In addition, in vivo study also revealed that SIRT1 activation by resveratrol decreased the cisplatin-induced apoptosis in the kidney.

MATERIALS AND METHODS

Animal experiments. Male C57BL/6 mice (7 wk old, weighing 20–22 g) were purchased from Orient Bio (Seoul, Korea) and maintained under a standard laboratory diet with water and libitum. The animal experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Chonbuk National

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University. The dose of cisplatin and the time of treatment were selected based on our previous study (10). Resveratrol (10 mg/kg) was administered orally once a day for 7 days, followed by intraperitoneal injection of cisplatin (6, 15). EX527 (10 mg/kg) was administered intraperitoneally once a day for 7 days at the same time with resveratrol. Maximal renal injury, as assessed by functional and histological measurements, was observed at 72 h after intraperitoneal injection of 20 mg/kg cisplatin.

Cell culture. MPT cells (generously provided by Dr. Lloyd G. Cantley, Yale University School of Medicine, New Haven CT), an

Fig. 1. SIRT1 enzyme activity assay (A) and immunoblot analyses (B) of SIRT1 protein expression in cisplatin-treated mouse proximal tubule (MPT) cells. MPT cells were harvested at indicated time after cisplatin treatment. Blots were probed with an anti-SIRT1 antibody. The membrane was stripped and reprobed with an anti-β-actin (Actin) to control for protein loading in each lane. Bars are presented as the relative ratio of SIRT1 to actin. The relative ratio measured in MPT cells treated with control buffer is arbitrarily presented as 1. Results from 3 independent experiments were similar. *P < 0.05, **P < 0.01 vs. control buffer treatment.

Fig. 2. Immunoblot analyses of acetyl-p53 after treatment of MPT cells with SIRT1 inhibitor EX527, small interference (si) RNA SIRT1, or p53 inhibitor pifithrin-α. A: MPT cells were treated with resveratrol (Res; 100 µmol/l) and/or EX527 (10 µmol/l) for 8 h in the presence or absence of cisplatin (Cis). B: MPT cells were incubated with Res and/or siRNA SIRT1 for 8 h with or without Cis. C: MPT cells were treated with Res plus EX527 and/or pifithrin-α (20 µmol/l) for 8 h in the presence or absence of cisplatin. Total cell lysate was collected for immunoblot analyses of acetyl-p53 (lysine-379) and β-actin (Actin). Densitometric analyses are presented as the relative ratio of acetyl-p53 to actin. The relative ratio measured in MPT cells treated with control buffer is arbitrarily presented as 1. Results from 3 independent experiments were similar. *P < 0.05, **P < 0.01 vs. control buffer treatment. ‡P < 0.05 vs. Cis alone. #P < 0.05 vs. Cis plus Res. ##P < 0.01 vs. Cis plus Res. $P < 0.05 vs. Cis plus Res plus EX527.
immortalized proximal tubular epithelial cell line from mice, were grown in α-MEM media supplemented with 10% fetal calf serum and antibiotics (100 U/ml penicillin G, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin). Cells were seeded in a six-well tissue culture plate and allowed to adhere for 16 h in an incubator at 37°C with 5% CO₂ in 95% air and were subsequently serum starved. To evaluate the effect of SIRT1 inhibition, cells were preincubated with EX527 (10 μmol/l, Tocris, Bristol, UK) for 30 min and then treated with/resveratrol (100 μmol/l) for 30 min (28). Thereafter, cells were incubated with cisplatin for 8 h. To examine the effect of p53 inhibition in MPT cells, cells were preincubated with pifithrin-α (Calbiochem, San Diego, CA) for 30 min and then treated with/resveratrol (100 μmol/l) for 30 min.

**Cytosolic and nuclear protein extraction.** Cytosolic and nuclear protein extractions from MPT cells were performed with a Nuclear and Cytoplasmic Extraction Kit (Pierce, Rockford, IL) as described previously (16). The nuclear lysate (20 μg) was immunoblotted with anti-acetyl p53 antibody.

**Mitochondrial protein extraction.** Mitochondrial and cytosolic fractions were extracted using a Mitochondria Isolation kit (Pierce), and the proteins were subjected to immunoblotting with anti-Bax and anti-cytochrome c antibody.

**RNA interference.** The siRNA to mouse ON-TARGETplus SMARTpool SIRT1 and negative control ON-TARGETplus siCONTROL Nontargeting siRNA were purchased from Thermo Scientific (Fremont, CA). MPT cells on a six-well plate were transfected with SIRT1 siRNA using the Nucleofector Kits (Lonza, Basel, Switzerland). After 24 h, the cells were coexposed to cisplatin (20 μg/ml) and resveratrol (100 μmol/l) for 8 h, and the levels of SIRT1 expression were determined by immunoblot analysis. **Immunoblot analysis.** Immunoblot analysis was performed as described previously (13). Kidney tissues were homogenized in PBS with a protease inhibitor cocktail (Calbiochem). The samples from kidney or MPT cells were incubated overnight at 4°C with rabbit anti-SIRT1 antibody (Millipore, Billerica, MA), rabbit anti-mouse acetyl p53 antibody (lysine 379; Cell Signaling Technology, Danvers, MA), goat anti-mouse PUMA-α antibody (Novus Biological, Littleton, CO), rabbit anti-mouse Bcl-xL (Cell Signaling Technology), rabbit anti-mouse cytochrome c (Cell Signaling Technology), mouse anti-Bax (Thermo Scientific), or rabbit anti-mouse caspase-3 (Cell Signaling Technology). Signals were visualized with a Chemiluminescent Detection Kit according to the manufacturer’s protocol (Amersham Pharmacia Biotech, London, UK). The membranes were reprobed with anti-actin antibody. All signals were analyzed by densitometric scanning (LAS-3000; FujiFilm, Tokyo, Japan).

**SIRT1 activity assay.** The activity of SIRT1 was measured using a SIRT1 Fluorescent Activity Assay Kit (Enzo Life Science, Plymouth Meeting, PA) according to the manufacturer’s instructions using a fluorescent emission at 460 nm with excitation at 360 nm.

**Determination of apoptosis and cell viability.** Apoptosis in the kidney was assessed by terminal deoxynucleotidyl transferase-mediated uridine triphosphate (dUTP) nick-end labeling (TUNEL) assay.
using an ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Millipore) according to the manufacturer’s protocol, and the number of apoptotic cells in each section was calculated by counting the number of TUNEL-positive apoptotic cells in 10 random, nonoverlapping fields/slide at a ×400 magnification (10). Cell viability was determined by CCK-8 assay as described previously (14).

**ELISA of kidney injury molecule-1.** The urine concentrations of kidney injury molecule-1 (KIM-1) were determined using an ELISA kit (COSMO BIO, Tokyo, Japan). For measurements, 100 μl of 24-h urine were analyzed in duplicate.

**Measurement of glomerular filtration rate.** Determination of glomerular filtration rate was performed using FITC-inulin (Sigma-Aldrich) as described previously (13).

**Statistical analysis.** Data are expressed as means ± SD. Multiple comparisons were examined for significant differences using Student’s unpaired t-test or one-way ANOVA followed by individual comparison with the Tukey post hoc test. A statistical value of $P < 0.05$ was considered significant.

**RESULTS**

Cisplatin decreases activity and protein expression of SIRT1 in MPT cells. To evaluate effects of cisplatin on SIRT1 activity, MPT cells were incubated with cisplatin (20 μg/ml) for 4, 8, and 24 h. Cisplatin treatment significantly decreased SIRT1 deacetylase activity (Fig. 1A). The maximum decrease was observed at 24 h after cisplatin treatment. Western blot analysis revealed that cisplatin treatment significantly reduced the level of SIRT1 expression in a dose-dependent manner (Fig. 1B). At dose of 20 μg/ml, the decrease in the SIRT1 protein expression was ~40%.

Resveratrol ameliorates cisplatin-induced acetylation of p53 through SIRT1 signal-dependent pathway in MPT cells. It has been shown that p53 is associated with cisplatin-induced nephrotoxicity and cisplatin exposure leads to p53 acetylation at the site of lysine-379 (3). Thus we evaluated whether resveratrol, a SIRT1 activator, decreased cisplatin-induced acetylation of p53 in MPT cells. Immunoblot analyses showed that treatment of MPT cells with cisplatin significantly increased acetylation of p53 (lysine-379) whereas resveratrol significantly suppressed the acetylation (Fig. 2A). To confirm that the effect of resveratrol is associated with an SIRT1-dependent mechanism, we used an inhibitor of SIRT1, EX527, in this experiment.

Treatment with EX527 significantly reversed the effect of resveratrol on cisplatin-induced acetylation of p53 (Fig. 2A). Knocking down SIRT1 by siRNA blunted the suppressive effect of resveratrol on the acetylation of p53 induced by cisplatin. These data suggest that the effect of resveratrol on p53 deacetylation is mediated by SIRT1 activation (Fig. 2B).

**SIRT1 signaling pathway is involved in cisplatin-induced increase in PUMA-α, Bax, and suppression of Bcl-xL protein expression.** PUMA-α, a BH3-only member of the Bcl-2 family, is a p53-responsive proapoptotic factor and neutralizes the antiapoptotic members including Bcl-xL, resulting in activation of Bax and Bak (8). Therefore, we evaluated the effect of resveratrol on cisplatin-induced PUMA-α expression in MPT cells. Cisplatin treatment significantly increased PUMA-α protein expression compared with that in the control buffer-treated cells (Fig. 3A). Treatment with resveratrol effectively decreased the cisplatin-induced PUMA-α expression whereas inhibition of SIRT1 with EX527 abolished the suppressive effect of resveratrol on cisplatin-induced PUMA-α expression. Resveratrol or EX527 alone did not induce PUMA-α expression.

Bax is present in the cytosol in a monomeric form while enforced dimerization leads to its mitochondrial translocation (27). Previous studies have shown that Bax undergoes conformational changes in response to death signals from cytotoxic insults, resulting in Bax homodimerization and mitochondrial translocation.

![Graph A](image)

**Graph A:** Immunoblot analyses of active caspase-3 expression from the MPT cells after treatment with Cis, Res, and/or SIRT1 inhibitors. MPT cells were treated with Res and/or EX527 (SIRT1 inhibitor) for 8 h in the presence or absence of Cis. Total cell lysate was collected for immunoblot analyses of active caspase-3 and β-actin (Actin). Densitometric analyses are presented as the relative ratio of active caspase-3 to actin. The relative ratio measured in MPT cells treated with control buffer is arbitrarily presented as 1. Results from 3 independent experiments were similar. B: cell viability was determined by CCK-8 assay. MPT cells were treated with Res and/or EX527 for 8 h in the presence or absence of Cis. Ten microliters of kit reagent was added to the cells and incubated for 3 h. Cell viability was expressed as percentage of controls. **$P < 0.01$ vs. control buffer treatment. $^\ddagger P < 0.05$ vs. Cis alone. $^\ddagger P < 0.01$ vs. Cis alone. $^# P < 0.05$ vs. Cis plus Res. $^{##P} < 0.01$ vs. Cis plus Res.

![Graph B](image)

**Graph B:** Effect of resveratrol and EX527 on cell viability.
membrane insertion (4). To determine Bax translocation, we prepared cytosolic and mitochondrial fractions from the control and experimental cells. Cisplatin treatment increased the level of Bax in the mitochondria fraction (Fig. 3B). Treatment with resveratrol reduced the cisplatin-induced Bax translocation. Inhibition of SIRT1 with EX527 abolished the suppressive effect of resveratrol on cisplatin-induced Bax translocation. No change in the Bax level was observed by the treatment of resveratrol or EX527 alone. The cytosolic Bax levels were not altered under any conditions applied.

We also investigated the expression of antiapoptotic protein Bcl-xL after treatment with cisplatin and resveratrol in the presence and absence of EX527 in MPT cells. Cisplatin significantly suppressed Bcl-xL protein expression, which was ameliorated by resveratrol treatment (Fig. 3C). EX527 treatment reversed the effect of resveratrol on cisplatin-induced Bcl-xL protein expression. Knockdown of SIRT1 using siRNA also decreased the effect of resveratrol on the cisplatin-induced decrease in Bcl-xL protein expression (Supplementary Fig. S1; all supplementary material for this article is available online at the journal web site).

To determine whether Bax translocation was accompanied by the release of cytochrome c, MPT cells were treated with cisplatin for 8 h, and levels of cytochrome c in the mitochondrial and cytosolic fractions were analyzed by immunoblotting. The levels of cytochrome c in cytosolic fractions were significantly increased after cisplatin treatment; accordingly, mitochondrial levels of cytochrome c were reduced (Fig. 3D). The cisplatin-induced release of cytochrome c was blocked by resveratrol treatment. EX527 reversed the blocking effect of resveratrol on cisplatin-induced release of cytochrome c. Resveratrol or EX527 alone did not change the release of cytochrome c compared with the control.

Inhibition of SIRT1 abrogates the effect of resveratrol on the expression of cisplatin-induced active caspase-3. To determine the role of SIRT1 in expression of active caspase-3, MPT cells were treated with cisplatin and resveratrol in the presence and absence of EX527. The expression of active cleaved caspase-3

![Fig. 5. Immunoblot analyses of PUMA-α (A), Bcl-xL (B), and active caspase-3 (C) from MPT cells after treatment with Cis, Res, EX527, and/or pifithrin-α. MPT cells were treated with EX527 and/or pifithrin-α for 8 h in the presence or absence of Res (100 μmol/l). Total cell lysate was collected for immunoblot analyses of PUMA-α, Bcl-xL, and caspase-3. Densitometric analyses are presented as the relative ratio of PUMA-α, Bcl-xL, or caspase-3 to β-actin (Actin). The relative ratio measured in MPT cells treated with control buffer is arbitrarily presented as 1. Results from 3 independent experiments were similar. D: cell viability was determined by CCK-8 assay. Cell viability was expressed as percentage of controls. *P < 0.05 vs. control buffer treatment. **P < 0.01 vs. control buffer treatment. ‡P < 0.01 vs. Cis alone. #P < 0.05 vs. Cis plus Res. ###P < 0.01 vs. Cis plus Res. $P < 0.05 vs. Cis plus Res plus EX527. $$$P < 0.01 vs. Cis plus Res plus EX527.](http://ajprenal.physiology.org/)

AJP-Renal Physiol • VOL 301 • AUGUST 2011 • www.ajprenal.org
was increased ~26-fold after cisplatin treatment compared with the expression level in control buffer-treated cells (Fig. 4A). Resveratrol treatment decreased the cisplatin-induced active caspase-3 expression by ~54.6%. EX527 significantly reversed the effect of resveratrol on the cisplatin-induced expression of active caspase-3. Supporting the observations, the number of apoptotic cells measured by annexin-V assay was increased ~4.5-fold in MPT cells after cisplatin treatment, and resveratrol treatment reduced the number of apoptotic cells induced by cisplatin (Supplementary Fig. S2). EX527 reversed the reducing effect of resveratrol on the number of apoptotic cells.

**SIRT1** inhibition reverses the protective effect of resveratrol in cisplatin-induced cytotoxicity in MPT cells. To determine the role of SIRT1 in cisplatin-induced cytotoxicity, MPT cells were treated with cisplatin and resveratrol in the presence and absence of EX527 and a CKK-8 assay was performed. CKK-8 assay revealed that treatment with resveratrol significantly decreased cisplatin-induced cytotoxicity in MPT cells (Fig. 4B). Pretreatment with EX527 significantly reversed the protective effect of resveratrol in cisplatin-induced cytotoxicity.

Inhibition of p53 reverses the effect of EX527 on protein expression of PUMA-α, Bcl-xL, and caspase-3 and cytotoxicity in MPT cells. The date above indicated that inhibition of SIRT1 by EX527 reversed the effect of resveratrol on PUMA-α, Bcl-xL, and caspase-3 protein levels and cytotoxicity in cisplatin-treated cells. We also found that inhibition of p53 abolished the effect of EX527 on the acetyl-p53 protein level in resveratrol- and cisplatin-treated MPT cells. Therefore, we evaluated whether inhibition of p53 by pifithrin-α changed...
the effect of EX527 on PUMA-α, Bcl-xL, and caspase-3 protein levels in resveratrol- and cisplatin-treated MPT cells. Our immunoblot data revealed that treatment with pifithrin-α reversed the effect of EX527 on PUMA-α, Bcl-xL, and caspase-3 protein levels in resveratrol- and cisplatin-treated MPT cells (Fig. 5, A–C). We also examined whether inhibition of p53 by pifithrin-α alters the effect of EX527 on cell viability in resveratrol- and cisplatin-treated MPT cells. Treatment with pifithrin-α significantly reversed the effect of EX527 on cytotoxicity in resveratrol- and cisplatin-treated MPT cells (Fig. 5D).

**Activation of SIRT1 inhibits cisplatin-induced apoptosis in the kidney and cisplatin-induced increase in KIM-1 urine level.** We evaluated SIRT1 protein expression and SIRT1 activity in the kidney after administration of cisplatin. Immunoblotting analyses showed that cisplatin treatment significantly decreased SIRT1 protein expression in the kidney 3 days after cisplatin injection compared with that for control buffer (Fig. 6A). The cisplatin-induced decrease in the SIRT1 protein expression was ~52%. The SIRT1 deacetylase activity was also decreased ~31% (Fig. 6B). To evaluate whether administration of resveratrol increases enzyme activity of SIRT1 in the kidney, we measured the activity of SIRT1 in kidney tissues after administration of resveratrol. The treatment of cisplatin-treated mice with resveratrol increased the SIRT1 activity by ~1.2-fold 3 days after cisplatin treatment compared with that in the kidneys from cisplatin-treated mice. Resveratrol alone did not increase the SIRT1 protein expression in the kidney (data not shown). We also evaluated the effect of resveratrol on cisplatin-induced apoptotic renal injury. The number of apoptotic cells in the kidney detected by TUNEL assay was increased at 72 h after cisplatin treatment compared with that in the kidneys of control mice (Fig. 7, C and D). Resveratrol decreased the cisplatin-induced increase in the number of apoptotic cells in the kidney. We also measured urinary KIM-1 levels after treatment with cisplatin and resveratrol in the presence and absence of EX527 by ELISA. Cisplatin significantly increased the urinary KIM-1 level, which was decreased by resveratrol treatment (Fig. 6E). EX527 treatment reversed the effect of resveratrol on the cisplatin-induced increase in urinary KIM-1 level. Taken together, these data suggest that activation of SIRT1 by resveratrol inhibits cisplatin-induced apoptosis and renal injury.

Resveratrol ameliorates cisplatin-induced decrease in renal function through SIRT1. To evaluate the change in renal function, we measured the glomerular filtration rate by inulin clearance. The inulin clearance of mice treated with cisplatin was significantly lower than that in mice treated with vehicle (Fig. 7). Treatment with resveratrol partially ameliorated the cisplatin-induced decrease in inulin clearance. SIRT1 inhibition by EX527 significantly blocked the protective effect of resveratrol on the cisplatin-induced decrease in inulin clearance.

**DISCUSSION**

In this study, we have demonstrated that activation of SIRT1 by resveratrol reduces cisplatin-mediated p53 acetylation and ameliorates cisplatin-induced kidney injury through inhibition of the apoptotic pathway. Cisplatin increased expression of PUMA-α, Bax, and active caspase-3 in MPT cells, and resveratrol reduced the expression of these apoptotic proteins. In addition, resveratrol remarkably increased the cisplatin-induced decrease in Bcl-xL in MPT cells. Inhibition of SIRT1 with EX527 reversed the effect of resveratrol on cisplatin-induced apoptotic renal cell injury. Furthermore, in vivo studies with mice revealed that administration of resveratrol increased the SIRT1 activity decreased by cisplatin treatment and reduced cisplatin-induced apoptosis in the kidney. These findings have suggested that activation of SIRT1 by resveratrol inhibits cisplatin-induced apoptosis in the kidney.

Numerous studies have evidenced that resveratrol activates SIRT1. As SIRT1 activation leads to protection from apoptosis, it is reasonable to suspect that SIRT1 may be associated with an antia apoptotic effect. Supporting the notion, our data showed that resveratrol decreased cisplatin-induced apoptosis in MPT cells and in the kidneys of mice. Moreover, specific inhibition of SIRT1 with EX527 reversed the protective effect of resveratrol on cisplatin-induced apoptosis and cytotoxicity. These data have suggested that the effect of resveratrol on cisplatin-induced apoptosis and cytotoxicity is mediated through an SIRT1-dependent mechanism. In contrast, resveratrol has been reported to enhance the TNF-related apoptosis-inducing ligand (TRAUL)- or CD95-induced apoptosis of human preadipocytes (21). Thus the effect of resveratrol on apoptosis may differ depending on the cell type and proapoptotic stimuli.

p53 has become an important pathogenic mechanism in cisplatin-induced nephrotoxicity (9, 17). Recently, it has been demonstrated that siRNA to p53 ameliorated cisplatin-induced apoptosis and histological injury in the kidney (25). Activation of p53 is regulated by posttranslational modification of p53 such as ubiquitination, phosphorylation, or acetylation (1). Especially, acetylation of p53 is increased after DNA damage and regulated by histone acetyl transferase and HDAC. There are several acetylation sites at lysine residues (Lys 370, Lys 371, Lys 372, Lys 381, Lys 382 Lys320) in the p53 carboxy-terminal regulatory domain (1). Very recently, it has been reported that cisplatin treatment increased acetylation of p53 at lysine 379 in rat kidney proximal tubular cells (3). Supporting the findings, we also found that cisplatin increased acetylation of p53 in MPT cells. Taken together, these findings suggest that inhibition of p53 activation can be a therapeutic target in cisplatin-induced nephrotoxicity.

Fig. 7. Glomerular filtration rate was measured by inulin clearance. Inulin clearance was measured from mice treated with Res (10 mg/kg body wt) or Res plus EX527 with/without Cis (n = 8 mice/group). **P < 0.01 vs. control. †P < 0.05 vs. Cis alone. ‡P < 0.05 vs. Cis plus Res.
In addition, the acetylation status of p53 has been reported to be modulated by HDAC (20). Recently, Dong et al. (3) have demonstrated that inhibition of HDAC with suberoylanilide hydroxamic acid and trichostatin A decreases cisplatin-induced apoptosis in renal tubular cells, suppressing cisplatin-induced p53 activation. However, it is well known that HDAC inhibition increases acetylation of histone and nonhistone substrates such as p53 (32). In this study, we demonstrated that cisplatin-induced acetylation of p53 was suppressed by resveratrol and that inhibition of SIRT1 with a specific inhibitor, EX527, blocked the suppressive effect of resveratrol on cisplatin-induced acetylation of p53 in MPT cells. Therefore, the mechanism of the HDAC inhibitor on cisplatin-induced p53 acetylation from the data from Dong et al. (3) may be different from our experiment.

As an increase in SIRT1 protein level has a protective role in apoptosis, the cisplatin-induced decrease in SIRT1 protein expression may be associated with apoptotic renal injury. Matsushita et al. (22) have reported that cisplatin increased proapoptotic injury in SIRT1- and SIRT2-deficient cells. Recently, Hasegawa et al. (5) have also demonstrated that kidney-specific overexpression of SIRT1 mitigates the cisplatin-induced decrease in renal function and apoptosis in the kidney. Our data demonstrated that the deacetylation activity of SIRT1 activation by resveratrol significantly decreases cisplatin-induced apoptosis in MPT cells and the kidney. Therefore, it can be suggested that SIRT1 activation may be a new therapeutic candidate for prevention or treatment of cisplatin-induced renal injury.

PUMA is a p53-responsive proapoptotic gene in cisplatin-induced nephrotoxicity (8). Cisplatin treatment increases PUMA-α in cultured renal tubular cells and the mouse kidney (3). Increased PUMA-α in mitochondria interacts with Bcl-xL and releases cytochrome c into the cytosol. In this study, our data showed that resveratrol treatment decreased cisplatin-induced PUMA-α expression and release of cytochrome c. We also found that resveratrol ameliorated Bcl-xL expression that was suppressed by cisplatin treatment and decreased cisplatin-induced active caspase-3.

In summary, our findings suggest that SIRT1 plays a crucial role in the apoptosis induced by cisplatin through the deacetylation of p53 and that activation of SIRT1 may prevent or treat cisplatin nephrotoxicity.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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