Mcl-1 is downregulated in cisplatin-induced apoptosis, and proteasome inhibitors restore Mcl-1 and promote survival in renal tubular epithelial cells

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Yang C, Kaushal V, Shah SV, Kaushal GP. Mcl-1 is downregulated in cisplatin-induced apoptosis, and proteasome inhibitors restore Mcl-1 and promote survival in renal tubular epithelial cells. Am J Physiol Renal Physiol 292: F1710–F1717, 2007. First published February 20, 2007; doi:10.1152/ajprenal.00505.2006.—Mcl-1 is an antiapoptotic member of the Bcl-2 family that plays an important role in cell survival. We demonstrate that proteasome-dependent regulation of Mcl-1 plays a critical role in renal tubular epithelial cell injury from cisplatin. Protein levels of Mcl-1 rapidly declined in a time-dependent manner following cisplatin treatment of LLC-PK1 cells. However, mRNA levels of Mcl-1 were not altered following cisplatin treatment. Expression of other antiapoptotic members of the Bcl-2 family such as Bcl-2 and BclxL, was not affected by cisplatin treatment. Cisplatin-induced loss of Mcl-1 occurs at the same time as the mitochondrial release of cytochrome c, activation of caspase-3, and initiation of apoptosis. Treatment of cells with cycloheximide, a protein synthesis inhibitor, revealed rapid turnover of Mcl-1. In addition, treatment with cycloheximide in the presence or absence of cisplatin demonstrated that cisplatin-induced loss of Mcl-1 results from posttranslational degradation rather than transcriptional inhibition. Overexpression of Mcl-1 protected cells from cisplatin-induced caspase-3 activation and apoptosis. Preincubating cells with the proteasome inhibitor MG-132 or lactacystin not only restored cisplatin-induced loss of Mcl-1 but also resulted in an accumulation of Mcl-1 that exceeded basal levels; however, Bcl-2 and BclxL levels did not change in response to MG-132 or lactacystin. The proteasome inhibitors effectively blocked cisplatin-induced mitochondrial release of cytochrome c, caspase-3 activation, and apoptosis. These studies suggest that proteasome regulation of Mcl-1 is crucial in the cisplatin-induced apoptosis via the mitochondrial apoptotic pathway and that Mcl-1 is an important therapeutic target in cisplatin injury to renal tubular epithelial cells.

acute kidney injury; Bcl-2 family

MITOCHONDRIAL DYSFUNCTION is an important component of cisplatin-induced nephrotoxicity (5, 13, 18, 33, 43, 48, 57) and other genotoxic stresses (12, 31) that initiate the intrinsic pathway of caspase activation and result in cell death. Cisplatin-induced apoptosis is associated with permeabilization of the mitochondrial outer membrane, which leads to release of proapoptotic mitochondrial intermembrane proteins, including cytochrome c (3, 37, 38, 40, 44), Omi/HtrA2 (8), Smac/Diablo (29), and AIF (45). Permeabilization of the mitochondrial outer membrane is regulated by interplay between the pro- and antiapoptotic Bcl-2 family of proteins (9, 12).

Bcl-2 family members are characterized by the presence of one of four relatively conserved Bcl-2 homology (BH) domains. Antiapoptotic Bcl-2 and BclXL each have four BH domains, whereas antiapoptotic Mcl-1 (myeloid cell leukemia sequence 1) has three putative BH domains, suggesting that this protein may interact with a different set of proteins than those interacting with Bcl-2 and BclXL. Proapoptotic Bcl-2 family members have either three BH domains or a single BH3-only domain. In response to apoptotic stimuli, proapoptotic members such as Bak and Bad are activated by conformational changes and oligomerization that trigger mitochondrial membrane permeabilization (2, 9). Proteins such as Bid and Bim that carry the BH3-only domain stimulate the proapoptotic function of Bak and Bak by inducing their oligomerization, which subsequently results in mitochondrial membrane permeabilization. In addition, most BH3-only proteins promote apoptosis by binding and inhibiting antiapoptotic Bcl-2, BclXL (7), and Mcl-1 (19, 54). Thus antiapoptotic Bcl-2 family members antagonize the effects of proapoptotic members and protect mitochondrial integrity.

Mcl-1, an antiapoptotic member of the Bcl-2 family, has not been studied as extensively as other antiapoptotic members of the Bcl-2 family (12, 55). Mcl-1 was originally shown to play a significant role in the survival of leukemia and other hematopoietic cells (10, 59); however, recent studies have shown that Mcl-1 also plays a pivotal role in the survival of non-hematopoietic cells responding to a wide variety of apoptotic stimuli (39, 55). The role of Mcl-1 has not been examined previously in renal tubular epithelial cell injury. Tubular epithelial cells are primary targets of cisplatin-induced acute kidney injury, so we investigated the expression and role of Mcl-1 in these cells in response to cisplatin injury. We examined the effects of overexpression of Mcl-1 on cell survival following cisplatin injury, the regulatory mechanisms of Mcl-1 expression by the proteasome system, and the role of proteasome inhibitors in cisplatin-induced apoptosis in renal tubular epithelial cells.

MATERIALS AND METHODS

Cell culture and reagents. The renal tubular epithelial cell line used in the study was porcine kidney proximal tubule epithelial cells (LLC-PK1) from American Type Culture Collection (ATCC; Manassas, VA); these were cultured as described in our previous studies (30). HEK-293 human embryonic kidney cells from ATCC were also used and were cultured under conditions recommended by their depositors. Cultures were maintained in a humidified incubator with 5% CO2-95% air at 37°C and were provided fresh medium at 48- to 72-h intervals. Cells were plated at a density of 5x104 cells/cm² in six-well plates 18 h before experimental treatments. When cells were...
~75% confluent, cisplatin was added to cultures to a final concentration of 25 μM.

Caspase substrates were purchased from Peptide International (Louisville, KY), and antibodies to caspase-3, α-tubulin, and β-actin were from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody to active caspase-3 was obtained from Cell Signaling Technology (Beverly, MA). Proteasome inhibitors MG-132 and lactacystin were from Calbiochem (San Diego, CA). Amino-4-methylcoumarin (AMC)-tagged caspase substrates and pancaspase inhibitor, benzoxycarbonyl-Val-Ala-Asp-fluoromethylketone (Z-VAD-fmk), were obtained from Enzyme System Products (Livermore, CA). Cell transfection reagents, primers, and one-step RT-PCR kit were from Invitrogen (Carlsbad, CA). Plasmid preparation kit, nucleotide purification kit, and RNeasy RNA purification kit were from Qiagen (Valencia, CA). All other chemicals were from Sigma (St. Louis, MO) or Fisher Scientific (Hampton, NH).

**Immunofluorescence localization of caspase-3.** Cells were grown on sterile glass coverslips and treated with 25 μM cisplatin for 12 h in the presence or absence of inhibitors. Following treatments, cells were washed in PBS and fixed in 2% paraformaldehyde in PBS for 15 min. After being washed twice in PBS, cells were permeabilized for 1 h in blocking buffer containing 1% BSA, 1% goat serum, 0.1% saponin, 1 mM CaCl2, 1 mM MgCl2, and 2 mM Na2VO4 in PBS. The cells were then incubated with rabbit anti-caspase-3 (active) antibody (1:200) for 1 h in a 37°C humidified incubator. After three washes in washing buffer containing 1% BSA and 0.1% saponin in PBS, cells were incubated at 37°C in a humidified incubator for 1 h with 1:500 of Alexa fluor-conjugated secondary antibody (goat anti-rabbit Alexa fluor 594) in blocking solution and again washed with washing buffer. Nuclei were stained with 0.5 μg/ml of DAPI for 5 min, and the cells were washed twice in washing buffer. Coverslips were then mounted on slides using antiadhesive mounting medium (Molecular Probes, Eugene, OR). Localization of active caspase-3 and changes in nuclear morphology were analyzed using a Zeiss deconvolution microscope.

**Overexpression of Mcl-1.** Transfection was carried out with Lipofectamine 2000 (Invitrogen) as per manufacturer's recommendation with plasmid DNA (3xFLAGCMVMcl-1, kindly provided by Dr. X. Wang); plasmid DNA without the insert was used as a control. LLC-PK1 cells growing at 80–90% confluence were transfected with Mcl-1 plasmid DNA or empty vector for 36 h and then treated with 25 μM cisplatin.

**Caspase activity assay.** LLC-PK1 cells were harvested by centrifugation, and the pellets were washed twice in cold PBS. The washed cell pellets were lysed with 20 mM HEPES (pH 7.5) containing 10% sucrose, 0.1% CHAPS, 2 mM DTT, 1 mM EDTA, 1 mM PMSF, 1 μg/ml leupeptin, and 1 μg/ml pepstatin A at 4°C. After centrifugation, supernatants were used to determine caspase-3/7 activity by fluorometric assay using DEVD-AMC substrate as described previously (30). The supernatants (each containing 50 μg protein) were incubated with 100 mM HEPES (pH 7.4) containing 10% sucrose, 0.1% CHAPS, 10 mM DTT, and 50 μM caspase substrate in a total reaction volume of 0.2 ml at 37°C for 60 min. The liberated fluorescent group, AMC, was detected using a spectrofluorometer (Perkin Elmer) with an excitation wavelength of 380 nm and an emission wavelength of 460 nm.

**Western blot analysis.** Cell lysates were prepared as described above for caspase assay, and equal amounts of protein samples were resolved by SDS-polyacrylamide gel electrophoresis using 8% polyacrylamide gels as previously described (30). The proteins were electrophoretically transferred to a Transblot membrane (Bio-Rad, Scientific (Hampton, NH)).

![Fig. 1. Time course of Mcl-1 loss, cytochrome c (Cyto-C) release, and caspase-3 activation in cisplatin (CP)-treated LLC-PK1 cells. A: time course expression of antiapoptotic Mcl-1, Bcl-2, and Bcl-xL proteins in CP-treated LLC-PK1 cells. LLC-PK1 cells were treated with 25 μM CP for varying lengths of time as indicated. Cell lysates (50 μg protein in each lane) were analyzed by Western blot using antibodies to Mcl-1, Bcl-2, and Bcl-xL. Graph shows quantitative analysis by densitometry of protein levels of Mcl-1, Bcl-2, and Bcl-xL from 3 different experiments (3 Western blots). Values shown are means ± SD, n = 3 (*P < 0.05 and **P < 0.025 compared with control). B: time course of Cyto-C release in response to CP treatment. Cells were treated with 25 μM CP for varying lengths of time as indicated. Mitochondrial and cytosolic fractions were isolated, and 10 μg of mitochondrial and 50 μg of cytosolic fractions were electrophoresed and immunoblotted for Cyto-C. C: time course of proteolytic processing of procaspase-3 to active caspase-3 in response to CP treatment. Cells were treated with 25 μM CP for varying lengths of time as indicated. Cell lysates (50 μg protein in each lane) were analyzed by Western blot with antibodies specific for caspase-3 (both proform and active form).
RT-PCR. Total RNA from the cultured cells was obtained by using the RNeasy Mini Kit (Qiagen). Approximately 1 μg of total RNA was used for reverse transcription. Expression levels of the Mcl-1 gene were determined by RT-PCR. The primer pair used for pig Mcl-1 (LLC-PK₁ cells) was 5'-TGGGTTTGAGGAGCTTCTCC (forward), 5'-GCCAGCTTCAAGTCCACCTTC (reverse); the primer pair for human Mcl-1 (HEK 293 cells) was 5'-TGCAAGTGTGTGAGGATG (forward), 5'-CCTCTGACCACCTTTGTCTTC (reverse); and the primer pair for β-actin internal control was 5'-AGCCATGTAGTAGCCATCG (forward), 5'-TCTCGCTGTGGTGTGAAG (reverse). To ensure that the PCR reaction had not reached a saturation point that would skew the quantitation, aliquots were removed after certain cycle numbers and analyzed in series on an agarose gel. The minimum number of cycles that initially gave optimal product was used in the experiment.

Isolation of cytosolic and mitochondrial fractions. Cytosolic and mitochondrial fractions from LLC-PK₁ cell were prepared as previously described (45).

Cell viability and apoptosis analysis. Cells (5,000/well) were plated in 96-well dishes and treated with 5 μM proteasome inhibitors (MG-132, or lactacystin) 1 h before treatment with 25 μM cisplatin. Inhibition of cell proliferation was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Roche Diagnostics, Indianapolis, IN) according to the manufacturer’s protocol. To detect apoptosis, cells were grown on glass coverslips in six-well plates. The cells were treated with 5 μM proteasome inhibitors for 1 h before treatment with 25 μM cisplatin for 12 h. Apoptosis was detected on the basis of nuclear morphology. Cells were fixed with 4% paraformaldehyde and stained with DAPI for 5 min to reveal fragmented and condensed nuclei. Cells were mounted on glass slides, covered, and analyzed using fluorescence microscopy. For statistical analysis of each experiment, 10–20 fields (×400 magnification) were counted.

RESULTS

Expression of Mcl-1 in LLC-PK₁ cells. We first examined expression of antiapoptotic Bcl-2 family members (Mcl-1, Bcl-2, and BclXL) in response to cisplatin treatment in renal tubular epithelial cell line LLC-PK₁. Mcl-1 protein levels rapidly declined in a time-dependent manner after cells were treated with cisplatin; Mcl-1 levels began decreasing 8 h after cisplatin treatment and were almost completely eliminated 24 h after the treatment (Fig. 1A). Levels of Bcl-2 and BclXL did not change in response to cisplatin treatment (Fig. 1A). Interestingly, cisplatin-induced loss of Mcl-1 began at the same time as mitochondrial release of cytochrome c (Fig. 1B) and activation of caspase-3 (Fig. 1C). Because cisplatin-induced initiation of apoptosis in renal tubular epithelial cells begins at the same time as cytochrome c release and caspase-3 activation (40, 45), our studies suggest that Mcl-1 loss is associated with cisplatin-induced caspase-3 activation and apoptosis.

Cisplatin-induced reductions in Mcl-1 levels result from protein destabilization rather than decreased mRNA levels. We tested whether rapid loss of Mcl-1 protein in LLC-PK₁ cells caused by cisplatin treatment is due either to downregulation at the transcriptional level or to posttranslational degradation at the protein level. To distinguish between these possibilities, we first examined Mcl-1 mRNA levels by semiquantitative RT-PCR. The results indicated that Mcl-1 mRNA levels were not altered in response to cisplatin treatment (Fig. 2A). Further...
more, when cells from another kidney cell line, HEK 293, were treated with cisplatin, Mcl-1 mRNA levels were not altered (data not shown).

To examine the effects of cisplatin on downregulation of Mcl-1 protein, we blocked de novo protein synthesis in LLC-PK₁ cells by using cycloheximide and then determined the Mcl-1 protein levels in the presence or absence of cisplatin treatment for various times (Fig. 2B). Cycloheximide treatment alone resulted in a considerable loss of Mcl-1 suggesting fast turnover of Mcl-1. However, elimination of Mcl-1 occurred more rapidly when cells were treated with both cycloheximide and cisplatin together than with cycloheximide alone, suggesting that exposure to cisplatin results in posttranslational degradation of Mcl-1 (Fig. 2B). Quantitative analysis of Mcl-1 protein levels from a representative Western blot by densitometry indicated significant decrease in Mcl-1 protein at 12 and 16 h following treatment with cycloheximide and cisplatin together compared with that of cycloheximide alone. These results indicate that cisplatin-induced loss of Mcl-1 is a posttranslational event triggered by protein destabilization.

Overexpression of Mcl-1 inhibits cisplatin-induced apoptosis. To determine whether cisplatin-induced loss of Mcl-1 is involved in the induction of apoptosis, we overexpressed full-length Mcl-1 by transient transfection of LLC-PK₁ cells and examined cisplatin-induced caspase-3 activation and apoptosis. Transient transfection with MCL1 expression plasmid (p3xFLAGCMV/MCL1) resulted in high expression levels of Mcl-1 in LLC-PK₁ cells (Fig. 3A). Cells carrying the control plasmid without the Mcl-1 insert showed Mcl-1 expression at the same level as untransfected cells (Fig. 3A). Overexpression of Mcl-1 provided marked protection from cisplatin-induced caspase-3 activation and cell death following cisplatin treatment (Fig. 3B). We further examined cisplatin-induced apoptosis by assessing the morphology of DAPI-stained cells following either transfection with Mcl-1 expression plasmid or control plasmid; overexpression of antiapoptotic Mcl-1 blocked cisplatin-induced apoptosis in LLC-PK₁ cells (Fig. 3C).

Proteasome inhibitors restore cisplatin-induced elimination of Mcl-1 and rescue apoptosis. Unlike other members of the Bcl-2 family, Mcl-1 has a short half-life and can be subjected to proteasomal degradation (36), so we investigated whether cisplatin-mediated loss of Mcl-1 results from proteasomal degradation. Treatment of LLC-PK₁ cells with cisplatin resulted in gradual decrease of Mcl-1 in a time-dependent manner (Fig. 4). As shown in the figure, at 24 h after cisplatin treatment most of the Mcl-1 was eliminated in the cells.
Incubating the cells with proteasome inhibitor MG-132 prevented the time-dependent loss of Mcl-1 (Fig. 4). In fact, in the presence of MG-132 accumulation of Mcl-1 was more than the basal level even at 24 h after cisplatin treatment. MG-132 had no effect on levels of Bcl-2 or BclxL proteins. Other proteasome inhibitors (i.e., lactacystin and bortezomib) similarly restored cisplatin-induced elimination of Mcl-1 (data not shown).

Because proteasome inhibitors caused accumulation of Mcl-1 protein, we examined whether treating cells with proteasome inhibitors provides protection from cisplatin-induced injury. Proteasome inhibitors MG-132 and lactacystin were very effective in blocking cisplatin-induced mitochondrial release of cytochrome c (Fig. 5A). In addition, cisplatin-induced caspase-3 activation was blocked by the proteasome inhibitors in a time-dependent manner (Fig. 5B). In a separate experiment, the effect of proteasome inhibitors alone was also examined on caspase-3 activation in a time course manner. Proteasome inhibitors alone did not alter basal caspase-3 activity (data not shown). Since activation of executioner caspase-3 results from proteolytic processing of procaspase-3, we examined whether cisplatin-induced procaspase-3 cleavage is inhibited by the proteasome inhibitors. Preincubating cells with MG-132 or lactacystin 1 h before cisplatin treatment dose dependently decreased cleavage of procaspase-3 to form active caspase-3 (Fig. 5C). Immunohistochemical analysis also revealed that proteasome inhibitors protected cells against cisplatin-induced caspase-3 activation (Fig. 5D). Nuclear staining with propidium iodide revealed that proteasome inhibitors provided protection to cells from cisplatin-induced nuclear fragmentation and condensation (Fig. 5E). Proteasome inhibitors (tested up to 20 μM) by themselves had no effect on renal tubular epithelial cell apoptosis or caspase-3 activation (data not shown).

**DISCUSSION**

Our results demonstrate that the antiapoptotic Bcl-2 family member Mcl-1 is an important target of cisplatin in renal tubular epithelial cells. Cisplatin triggered a rapid decline in Mcl-1 but not in Bcl-2 or BclxL, suggesting that, among these survival proteins of the Bcl-2 family, Mcl-1 downregulation is crucial for cisplatin-induced renal tubular epithelial cell injury. In the presence of cisplatin, cells overexpressing Mcl-1 demonstrated statistically significant increases in cell survival over control cells.

Previous studies have documented loss of Mcl-1 protein in nonrenal cells, including hematopoietic and tumor cells, in response to growth factor withdrawal (14), UV irradiation (36, 60), adenoviral infection (11), cytotoxic agents (15, 32, 41, 60), oxidative stress (27), staurosporine (26, 56), and tumor necrosis factor-α-related apoptosis-inducing ligand (TRAIL)-induced apoptosis (52). In most of these studies, overexpression of Mcl-1 provided protection from cell death or prolonged survival. Our studies in LLC-PK1 cells show that overexpression of Mcl-1 provided significant protection from cisplatin injury. These findings suggest that a decrease in Mcl-1 level is a common event in renal and nonrenal cells in response to apoptotic stimuli and that Mcl-1 mediates cisplatin-induced injury.

Mcl-1 mRNA levels did not change following treatment with cisplatin, suggesting that the cisplatin-induced rapid loss of Mcl-1 protein is not controlled at the transcriptional level. Caspases, particularly caspase-3 and caspase-8, are required to cleave Mcl-1 in some cell lines undergoing apoptosis triggered by agents such as sodium salicylate, staurosporine, Fas, actinomycin D, etoposide, and TRAIL (15, 21, 23, 35, 52). Caspase-8 and caspase-3 cleave Mcl-1 at aspartic acid residues 127 and 157, resulting in cleaved products. Although caspase-3 is highly activated in response to cisplatin injury in renal tubular epithelial cells (30), we did not detect cisplatin-induced, caspase-mediated cleaved products of Mcl-1 (data not shown). These data suggest that cisplatin-induced caspase-3 activation does not play significant role in regulation of Mcl-1 turnover. Conversely, we demonstrated that cisplatin-induced rapid elimination of Mcl-1 is dependent on the proteasome system; proteasome inhibitors MG-132 and lactacystin prevented cisplatin-induced degradation of Mcl-1 and promoted...
its accumulation to levels far above basal levels. Proteasome inhibitor MG-132 was previously shown to block degradation of Mcl-1 in apoptosis mediated by UV irradiation (36) and staurosporine (56). Thus the ability of proteasome inhibitors to restore Mcl-1 levels in renal tubular epithelial cells treated with cisplatin suggests that the ubiquitin proteasome pathway of Mcl-1 degradation plays a key role in cisplatin-induced apoptosis. In further support of these data, Mcl-1 was recently shown to be ubiquitinated by a ubiquitin ligase, Mule/ARF-BPI (51, 58), and such ubiquitination is required for the proteasome to recognize proteins specifically targeted for proteasome degradation (17, 24).

Fig. 5. Proteasome inhibitors prevent CP-induced Cyto-C release, caspase-3 activation, and apoptosis in LLC-PK1 cells. A: proteasome inhibitor MG-132 prevents CP-induced Cyto-C release. Cells were treated with 25 μM CP overnight in the presence or absence of 5 μM MG-132. Cytosolic fractions were analyzed by Western blot using antibody to Cyto-C. Controls were untreated cells. CP + MG-132 in lanes 3 and 4 are duplicate run. B: proteasome inhibitors MG-132 and lactacystin prevent CP-induced caspase-3 activation. Cells were treated with 5 μM MG-132 or 5 μM lactacystin for 1 h before treatment with 25 μM CP for various times as indicated. Cell lysates (50 μg protein) were assayed for caspase-3 activity using DEVD-AMC substrate. Control (C) are untreated cells. Results are presented as means ± SE (n = 5, independent samples); *P < 0.01 for CP-treated cells vs. CP plus MG-132 or lactacystin. C: dose-dependent effect of proteasome inhibitors on CP-induced processing of procaspase-3 to active caspase-3. Cells were treated with indicated doses of MG-132 or lactacystin 1 h before 12-h treatment with 25 μM CP. Cell lysates were subjected to Western blot with antibody for the active form of caspase-3 to reveal cleavage of procaspase-3. D: immunohistochemical analysis of active caspase-3 and cell apoptosis in the presence of proteasome inhibitor MG-132. LLC-PK1 cells were grown on glass coverslips treated with 25 μM CP overnight in the presence or absence of proteasome inhibitor MG-132. Fixed cells were processed with caspase-3 antibody followed by Alexa fluor-conjugated secondary antibody (red). Cell nuclei (blue) were stained with propidium iodide to examine nuclear fragmentation and condensation. Fluorescence for active caspase-3 nuclear apoptosis was visualized using a deconvolution fluorescence microscope. E: effect of proteasome inhibitors on CP-induced apoptosis. Cells grown in 6-well plates were treated with 25 μM CP for varying lengths of time in the presence or absence of 5 μM MG-132 or lactacystin. Apoptotic nuclei (fragmented and condensed nuclei) were visualized by DAPI staining and scored after CP treatment. Percentages of apoptotic nuclei averaged from 20 fields are shown. Results are presented as means ± SE (n = 4, independent samples); **P < 0.005 for CP-treated cells vs. CP plus MG-132 or lactacystin.
Recent studies provide evidence that Mcl-1 is capable of sequestering proapoptotic proteins of the Bcl-2 family including Bak, Bim, and tBid. It is expressed in many cell types, and whether they play a role in the proteasome-mediated degradation of Mcl-1 is not determined whether in renal cells Mcl-1 sequesters Bak or Bim and whether they play a role in the proteasome-mediated degradation of Mcl-1.

Our studies also demonstrated that proteasome inhibitors MG-132 and lactacystin provided protection from cisplatin-induced cytochrome c release, caspase activation, and cell death. The proteasome system is known to control levels of the Bcl-2 family of proteins (6, 16, 36, 50), so part of this protective mechanism in LLC-PK1 cells might be mediated by Mcl-1 accumulation due to proteasome inhibition. Evidence suggests that proteasome inhibitors prevent apoptosis in various settings (4, 20, 25, 46, 47, 50), while other data indicate that proteasome inhibitors, either alone or in combination with death-inducing stimuli, cause apoptosis (1, 22, 42, 50). Specifically, proteasome inhibitors are effective in killing cancer cells while sparing normal cells (1, 47, 50). Our results demonstrate that in renal cells proteasome inhibitors protect antiapoptotic proteins from proteasome-mediated degradation, which leads to cell survival. In support of our conclusions, proteasome inhibitors were previously shown to protect renal tubular epithelial cells from acute kidney injury in response to ischemia (28, 49).

In summary, our studies provide the first demonstration that 1) among the antiapoptotic members of the Bcl-2 family, Mcl-1 expression is rapidly declined at the posttranslational level in renal tubular epithelial cells in response to cisplatin; 2) overexpression of Mcl-1 provides protection from cisplatin-induced apoptosis; 3) proteasome inhibitors restore cisplatin-induced rapid loss of Mcl-1; and 4) proteasome inhibitors block cisplatin-induced cytochrome c release, caspase-3 activation, and apoptosis in renal tubular epithelial cells. These studies demonstrate that Mcl-1 plays a key role in cisplatin-induced renal tubular epithelial cell apoptosis. Thus, proteasome inhibitors may provide a therapeutic benefit in response to cisplatin-induced acute kidney injury.

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Mcl-1 IN RENAL TUBULAR EPITHELIAL CELL INJURY


