Cisplatin Induces Apoptosis in LLC-PK1 Cells via Activation of Mitochondrial Pathways

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Abstract. Cisplatin, a commonly used chemotherapeutic agent, has a major limitation because of its nephrotoxicity. Recent studies have shown that cisplatin causes apoptotic cell death in renal tubule cells, but the underlying molecular mechanisms remain to be elucidated. In this study, cisplatin was found to induce apoptosis in a dose- and duration-dependent manner in cultured proximal tubule (LLC-PK1) cells, as evidenced by DNA laddering and TdT-mediated dUTP nick end-labeling assay. Pretreatment with the specific caspase 9 inhibitor LEHD-CHO completely prevented the apoptosis, whereas the caspase 8 inhibitor IETD-fmk had no effect. Furthermore, the activity of caspase 9 was upregulated about sixfold by cisplatin in a dose-dependent manner. These results implicated the activity of caspase 9 was upregulated about sixfold by cisplatin and DNA laddering and Western blots. Cisplatin treatment also resulted in the duration-dependent activation and mitochondrial translocation of the pro-apoptotic molecule Bax, by immunofluorescence. Finally, cisplatin induced a duration-dependent onset of the mitochondrial permeability transition. Our results indicate that cisplatin induces apoptosis in LLC-PK1 cells via activation of mitochondrial signaling pathways. The sequence of events may be summarized as follows: activation of Bax induces mitochondrial permeability transition, leading to release of cytochrome c, activation of caspase 9, and entry into the execution phase of apoptosis. Inhibition of this specific pathway may provide a strategy to minimize cisplatin-induced nephrotoxicity.

Cisplatin is one of the most widely used chemotherapeutic agents for the treatment of several human malignancies. The efficacy of cisplatin is dose dependent, but the risk of nephrotoxicity frequently hinders the use of higher doses to maximize its antineoplastic effects. Nephrotoxicity after cisplatin treatment is common and may manifest after a single dose with acute renal failure or may present with a chronic syndrome of renal electrolyte wasting. Cisplatin accumulates in cells from all nephron segments but is preferentially taken up by the highly susceptible proximal tubule cells within the S3 segment, which bear the brunt of the damage (1). The cytotoxic effects of cisplatin are postulated to occur via several mechanisms, including inhibition of protein synthesis, mitochondrial injury, and DNA damage (2,3), leading ultimately to activation of programmed cell death pathways in tumor cells (4) as well as renal tubule cells (5–15).

Apoptosis or programmed cell death is characterized by distinct morphologic changes consisting of cell shrinkage, nuclear condensation, and internucleosomal DNA fragmentation. Renal tubule cell apoptosis has recently been observed in an increasing array of renal disorders (12) and is emerging as a final common pathway in response to a wide variety of cellular stresses applied at an intensity below the threshold for necrosis. This observation also holds true for cisplatin nephrotoxicity, in which necrotic cell death is encountered with higher doses whereas lower concentrations induce apoptosis (5,11). Although cisplatin-induced renal tubule cell apoptosis has been well documented, the intracellular pathways involved in the stimulus recognition, signal transduction, and execution phases of apoptosis after cisplatin exposure remain under active investigation.

In recent years, specific intracellular proteases belonging to the caspase family have emerged as crucial effectors of apoptosis (16,17). Members of this family (now totaling at least 14) are expressed as pro-enzymes and require activation by upstream stimuli to commit a cell into the execution phase of apoptosis. It is convenient to classify the major intracellular apoptotic pathways according to the type of pro-caspase that is activated (16,17). Activation of the initiator pro-caspase 8 results predominantly from signaling via integral membrane death receptors such as Fas and TNFRI (18). On the other hand, activation of the initiator pro-caspase 9 is dependent primarily on mitochondrial signaling pathways regulated by members of the Bcl-2 family (19). Activation of pro-apoptotic Bcl-2 family members such as Bax can trigger a sequence of events that leads to alterations in mitochondrial permeability transition, release of mitochondrial cytochrome c into the cytosol, and activation of pro-caspase 9 (20–22). Once activated,
both caspases 8 and 9 participate in a cascade that culminates in the activation of caspase 3, which cleaves several substrates, resulting in chromosomal DNA fragmentation and cellular morphologic changes characteristic of apoptosis (17). The anti-apoptotic Bcl-2 family members such as Bcl-2 itself play a pivotal and overriding protective role by preserving mitochondrial structure and function, preventing onset of mitochondrial permeability transition, and inhibiting the release of cytochrome c into the cytosol (20–22).

In this study, cisplatin was found to induce apoptosis in a dose- and duration-dependent manner in cultured proximal tubule (LLC-PK1) cells. Pretreatment with the specific caspase 9 inhibitor LEHD-CHO completely prevented the apoptosis, whereas the caspase 8 inhibitor IETD-fmk had no effect. Furthermore, the activity of caspase 9 was upregulated about sixfold by cisplatin in a dose-dependent manner, whereas caspase 8 activity was not significantly altered. Cisplatin also resulted in a duration-dependent translocation of cytochrome c from the mitochondria to the cytosol, activation and mitochondrial translocation of Bax, and induction of the mitochondrial permeability transition. Our results indicate that cisplatin induces apoptosis in cultured proximal tubule cells via activation of a mitochondrial signaling pathway.

Materials and Methods

Cell Culture and Cisplatin Treatment

LLC-PK1 cells (porcine proximal tubule cells, American Type Culture Collection, Rockville, MD) were cultured in plastic six-well tissue culture plates (Costar, Cambridge, MA) in minimal essential medium alpha supplemented with 10% fetal bovine serum (Life Technologies BRL, Gaithersburg, MD). Confluent cells were incubated with varying concentrations (0, 25, 50, or 100 μM) of cisplatin (Sigma, St. Louis, MO) for different time periods. For the caspase inhibitor studies, cells were pretreated with either the caspase 8 inhibitor IETD-fmk or the caspase 9 inhibitor LEHD-CHO (both from Clontech, La Jolla, CA) 1 h before cisplatin exposure.

Apoptosis Assays

Internucleosomal DNA fragmentation was detected primarily by DNA laddering assay (23). In brief, equal number of cells were resuspended in 500 μl of lysis buffer (1% sodium dodecyl sulfate, 25 mM ethylenediaminetetraacetic acid, and 1 mg/ml proteinase K [pH 8]) and incubated overnight at 50°C. Ribonuclease A (10 mg/ml) was then added for an additional 2-h incubation at 37°C. The chromosomal DNA was extracted with phenol/chloroform, precipitated with ethanol, and analyzed by agarose gel electrophoresis followed by staining with ethidium bromide to reveal the fragmentation pattern. DNA fragmentation was confirmed in situ by use of the TdT-mediated dUTP nick end-labeling (TUNEL) assay (ApoAlert DNA Fragmentation Assay Kit, Clontech), by which fluorescein-dUTP incorporation at the free ends of fragmented DNA is visualized by use of fluorescence microscopy (23). Cells grown on cover slips and subjected to control conditions or cisplatin were washed with phosphate-buffered saline (PBS) and fixed with 4% formaldehyde/PBS for 30 min at 4°C. After permeabilization with 0.2% Triton X-100/PBS for 5 min at 4°C, cells were incubated with a mixture of nucleotides and TdT enzyme for 60 min at 37°C in a dark, humidified incubator. The reaction was terminated with 2 X SSC, the cells washed with PBS, and the cover slips mounted on glass slides with Crystal/mount (Biomedia, Foster City, CA). Fluorescent nuclei were detected by visualization with a microscope equipped with fluorescein filters (IX70, Olympus).

Caspase Assays

Caspase 8 and caspase 9 activity assays were performed by use of the ApoAlert Caspase 8 Colorimetric Assay and the Caspase 9 Fluorescence assay kits (both from Clontech), respectively. Equal numbers of control or cisplatin-treated cells were incubated in cell lysis buffer for 10 min, centrifuged, the supernatants incubated in reaction buffer that contained IETD-AFC (specific substrate for caspase 8) or LEHD-AMC (specific substrate for caspase 9) at 37°C for 1 h. The activity was assayed by use of a spectrophotometer for caspase 8 and a fluorometer for caspase 9. The specificity of changes in caspase 9 activity was confirmed by the addition of the caspase 9 inhibitor LEHD-CHO before incubation in caspase 9 substrate, in parallel experiments.

Western Blot Analyses

For the detection of released cytochrome c, cytosolic extracts were prepared as described elsewhere (22). In brief, cells were washed with PBS, incubated for 30 min on ice in 300 μl mitochondrial buffer (68 mM sucrose, 200 mM mannitol, 50 mM KCl, 1 mM ethyleneglycol-bis[β-aminoethyl ether]-N,N’tetraacetic acid, 1 mM dithiothreitol, and 1 X Complete [Roche Molecular Biochemicals, Indianapolis, IN] protease inhibitor) homogenized by six passages through a 25-gauge needle, and centrifuged at 4°C at 800 x g for 10 min and the resultant supernatant (cytosol) subjected to Western blotting. Protein concentrations were determined by the Bradford assay (Bio-Rad, Hercules, CA), and equal amounts of protein were loaded (30 μg in each lane). Monoclonal antibody to cytochrome c (clone C-7, Upstate Biotechnology, Lake Placid, NY) was used at 1:1000 dilution. Monoclonal antibody to α-tubulin (Sigma) was used at 1:10,000 dilution for confirmation of equal protein loading. Immunodetection of transferred proteins was performed by use of enhanced chemiluminescence (Amersham, Arlington Heights, IL).

Immunocytochemical Analyses

The subcellular distribution of cytochrome c and Bax in LLC-PK1 cells was determined by double labeling as described elsewhere (24). Briefly, cells grown on cover slips to confluence were incubated with 50 μM cisplatin for varying time periods, washed twice with cold 1X PBS, fixed in 4% formaldehyde on ice for 25 min, permeabilized in 0.2% Triton X-100 in PBS on ice, and blocked with goat serum at room temperature for 60 min. After a 60-min incubation simultaneously with both primary antibodies at room temperature, the cells were washed extensively with PBS and exposed simultaneously to rhodamine-conjugated goat anti-mouse (GAM-Cy3, Amersham) and fluorescein-conjugated goat anti-rabbit (GAR-Cy2, Amersham) secondary antibodies at 1:1000 dilution for 30 min in the dark. After being washed, the cover slips were mounted on glass slides with crystal mount and visualized with a microscope equipped with rhodamine and fluorescein filters. Monoclonal antibody to cytochrome c (clone C-7, Upstate Biotechnology) was used at 1:400 dilution in 2% bovine serum albumin/10% goat serum. The polyclonal antibody to the N-terminal of Bax (Bax-NT, Upstate Biotechnology) that recognizes only the activated form of Bax was used at 1:250 dilution. In a separate set of experiments, cells were double stained with polyclonal anti-Bax-NT as above, and with a monoclonal antibody against the mitochondrial voltage-dependent anion channel (Calbiochem, La
Jolla, CA) at a 1:250 dilution, to confirm the mitochondrial localization of activated Bax.

**Mitochondrial Permeability Transition Detection**

Mitochondrial permeability transition was assessed by use of the ApoAlert Mitochondrial Membrane Sensor kit (Clontech), which contains a cationic dye (MitoSensor) that freely enters the mitochondria of normal cells and aggregates into polymers that are detected by red fluorescence. Alterations in mitochondrial membrane potential result in a cytosolic accumulation of monomeric dye, which emits a green fluorescence. Cells grown on cover slips to confluence were incubated with 50 μM cisplatin for varying time periods, rinsed with serum-free medium, and incubated for 15 min at 37°C in 1 ml of incubation buffer that contained 1 μl MitoSensor dye. After a gentle rinse with incubation buffer, the cells were examined under a fluorescence microscope.

**Results**

**Cisplatin Induces a Dose- and Duration-Dependent Apoptosis in LLC-PK1 Cells**

Cultured LLC-PK1 cells were incubated with varying concentrations of cisplatin for 24 h and subjected to two distinct apoptosis assays. In multiple experiments, internucleosomal DNA fragmentation was clearly evidenced by the characteristic 180-bp laddering pattern in cisplatin-treated cells. The DNA laddering was just detectable in 25 μM cisplatin but was clearly evident in the 50 and 100 μM cisplatin-treated cells (Figure 1A). These results indicate that the pro-apoptotic effects of cisplatin are dose-dependent, with the maximum DNA laddering seen in the 50 μM cisplatin-treated cells. We then examined the effect of varying the duration of cisplatin treatment, using the 50 μM dose. The DNA laddering was evident only after 12 h of incubation (Figure 1B), which indicates that the pro-apoptotic effects of cisplatin are also duration-dependent. The presence of apoptosis was confirmed by TUNEL assay, which revealed intense TUNEL-positive nuclei with nuclear fragmentation in the cells treated with 50 μM cisplatin for 12 or 24 h (Figure 2).

**Cisplatin-Induced Apoptosis Is Caspase 9–Dependent**

Because it was of interest to identify the intracellular apoptotic pathways induced by cisplatin, we sought to first determine the type of pro-caspase that was activated. Pretreatment with the specific caspase 8 inhibitor IETD-fmk had no effect on cisplatin-induced DNA laddering, whereas pretreatment with the specific caspase 9 inhibitor LEHD-CHO dramatically abrogated the apoptosis (Figure 1C). Furthermore, the activity of caspase 8 was essentially unchanged in cisplatin-treated cells (Figure 3), whereas caspase 9 activity was significantly upregulated by cisplatin in a dose-dependent manner (about sixfold with the 50 μM dose), as shown in Figure 4. This increase in activity was completely prevented by addition of caspase 9 inhibitor to the assay mixture, which attests to the specificity of the activation.

**Cisplatin Induces a Duration-Dependent Translocation of Cytochrome c**

The above results implicated the caspase 9–dependent mitochondrial apoptotic pathways, which we investigated first by determining the distribution of cytochrome c. Subcellular fractionation followed by Western blots showed that cytochrome c was released from the mitochondria into the cytosol in a duration-dependent fashion (Figure 5). The release of cytochrome c was evident at the 9-h incubation point with 50 μM cisplatin and appeared to be complete, with no evidence for enhanced release at further time points. The duration-dependent escape of cytochrome c into the cytosol was confirmed by immunofluorescence. Cytochrome c was detected in a punctate mitochondrial distribution in control cells and cells treated with 50 μM cisplatin for 6 h (Figure 6). However, cytochrome c assumed a diffuse cytosolic localization at the 9-h incubation point and remained cytosolic at 12 h (Figure 6).

**Cisplatin Induces a Duration-Dependent Activation and Translocation of Bax**

Because cytochrome c release can occur after activation and mitochondrial insertion of Bax, it was next of significant interest to assess the status of Bax after cisplatin exposure. We used an antibody directed to the N-terminal of Bax. The N-terminal is normally inaccessible in the inactive conformation and is exposed for antibody recognition only when activated. Accordingly, activated Bax could not be detected in control cells and cells treated with 50 μM cisplatin for 6 h (Figure 6), which indicates absence of activation. However, activated Bax was clearly evident in a punctate mitochondrial
distribution at the 9- and 12-h incubation points (Figure 6). Indeed, in multiple experiments, double labeling of cells at the 9-h treatment period with antibodies to cytochrome c and Bax-NT consistently showed that every cell that was positive for cytosolic cytochrome c was also decorated by activated mitochondrial Bax (Figure 6). The mitochondrial distribution of activated Bax at the 9-h incubation point was confirmed by colocalization with voltage-dependent anion channel (not shown).

**Cisplatin Induces a Duration-Dependent Mitochondrial Permeability Transition**

Because one of the postulated mechanisms by which activation and mitochondrial insertion of Bax causes cytochrome c release involves changes in mitochondrial membrane potential, we assayed for mitochondrial permeability transition after cisplatin exposure using the MitoSensor probe. In control cells and cells treated with 50 μM cisplatin for 6 h, the cationic dye entered the mitochondria, aggregated into polymers, and displayed a punctate mitochondrial pattern of intense red fluorescence by use of rhodamine filters and no green fluorescence (Figure 7). In marked contrast, the majority of cells at the 9-h incubation point (and all the cells at the 12-h incubation period) were devoid of punctate red fluorescence and instead displayed a diffuse cytosolic pattern of green fluorescence when fluorescein filters were used (Figure 7). This is indicative of an inability of the cationic dye to enter the mitochondria secondarily to a duration-dependent alteration in mitochondrial permeability transition, with the resultant persistence of monomeric dye in the cytoplasm.

**Discussion**

Cisplatin is known to cause apoptotic cell death in renal tubule cells, but the underlying mechanisms remain to be
elucidated. In this study, cisplatin was found to induce apoptosis in a dose- and duration-dependent manner in cultured proximal tubule (LLC-PK1) cells. Pretreatment with the specific caspase 9 inhibitor LEHD-CHO completely prevented the apoptosis, whereas the caspase 8 inhibitor IETD-fmk had no effect. Furthermore, the activity of caspase 9 was upregulated about sixfold by cisplatin in a dose-dependent manner, whereas caspase 8 activity was not significantly altered. Cisplatin exposure resulted in a duration-dependent release of cytochrome c into the cytosol, activation and mitochondrial translocation of Bax, and onset of the mitochondrial permeability transition. Our results indicate that cisplatin induces apoptosis in LLC-PK1 cells via activation of mitochondrial pathways.

Cisplatin is one of the most widely used chemotherapeutic agents for the treatment of human malignancies. The efficacy of cisplatin is dose dependent, but nephrotoxicity frequently precludes the use of higher doses to maximize its antineoplastic effects. The renal cytotoxic effects of cisplatin may occur via the formation of DNA adducts, which can inhibit DNA replication and transcription, leading to inhibition of protein synthesis, mitochondrial injury, DNA damage, and, ultimately, to activation of programmed cell death pathways (2–4). Renal tubule cell apoptosis after cisplatin exposure has been well documented both in vivo (9,13) and in vitro (5–15). However, the intracellular pathways involved in the stimulus recognition, signal transduction, and effector phases of apoptosis after cisplatin exposure have not been fully elucidated.

In this study, cisplatin reproducibly induced apoptosis in LLC-PK1 cells in a dose- and duration-dependent manner. Apoptosis was barely detectable after a 24 h exposure to 25 μM cisplatin but was clearly evident by two distinct assays (DNA laddering and TUNEL assay) in the cells treated with the 50 μM dose. These results are in agreement with studies elsewhere that have shown that lower doses of cisplatin (8 to 100 μM range) result in apoptosis of LLC-PK1 (11,14), mouse proximal tubule (5), and human proximal tubule cells (13,25), whereas higher doses (300 to 1000 μM range) cause necrotic cell death. Also, we detected the internucleosomal DNA fragmentation only after 12 h of exposure to 50 μM cisplatin, similar to results of a recent report (11). Collectively, these results lend support to the concept that apoptosis can result from stimuli that are applied at an intensity and duration below the threshold for necrosis.

Specific proteases belonging to the caspase family are the major effectors of apoptosis, and the type of intracellular apoptotic pathway involved may be deduced from the class of pro-caspase activated, i.e., pro-caspase 9 versus 8 (16,17). Our studies demonstrate for the first time that caspase 9-dependent pathways predominate in cisplatin-induced renal cell apoptosis in vitro, because (1) caspase 9 activity was upregulated by cisplatin in a dose-dependent manner (about threefold with the 25 μM dose and sixfold with the 50 μM dose), whereas caspase 8 activity was essentially unchanged; and (2) pretreatment with the specific caspase 9 inhibitor LEHD-CHO abrogated the cisplatin-induced apoptosis, whereas caspase 8 inhibitor had no effect. It is well known that activated caspase 9 triggers a cascade that culminates in the activation of caspase 3, which cleaves several substrates, resulting in chromosomal DNA fragmentation and cellular morphologic changes characteristic of apoptosis (17). Thus our results are in agreement with studies elsewhere that have demonstrated the activation of caspase 3, the final common mediator of apoptosis in general, after cisplatin exposure (7,11,15).

Activation of caspase 9 usually occurs downstream of cytochrome c release from mitochondria (19–22). Although mitochondrial alterations in the form of dysfunction and calcium accumulation have been previously elucidated in cisplatin nephrotoxicity (2), our results demonstrate for the first time the activation of specific mitochondrial apoptotic pathways. We documented a duration-dependent activation and mitochondrial translocation of Bax, onset of mitochondrial permeability transition, and release of cytochrome c into the cytosol. All these events were noted to occur coordinately at the 9-h incubation point with 50 μM cisplatin and appeared to be complete, with no evidence for significantly enhanced occurrence at further time points. This is in full concordance with a recent study that demonstrated that the coordinate release of cytochrome c during apoptosis induced by a variety of stimuli in HeLa cells is rapid, complete, and kinetically invariant (22). Similarly, other studies have shown that cytochrome c release occurs within minutes of Bax activation (26). Our detection of DNA laddering first at the 12-h incubation point is also consistent with the induction of mitochondrial pathways at the 9-h point, because some additional time requirement would be anticipated for the activation of caspase cascades and endonucleases with resultant internucleosomal DNA cleavage. Of interest, a similar mitochondrial signaling pathway involving translocation of Bax and release of cytochrome c also results in apoptosis of cultured proximal tubule cells after an unrelated stimulus, namely hypoxia/reoxygenation injury (20). Furthermore, our result implicating activation of pro-apoptotic Bax fully complements studies elsewhere that have shown that induction or overexpression of the pivotal anti-apoptotic mol-
ecule Bcl-2 attenuates cisplatin-induced renal cell apoptosis both in vitro (6) and in vivo (10). Collectively, these results suggest that the integration of diverse pro- and anti-apoptotic signals may occur at the mitochondria and that the release of cytochrome c may decide cell fate (27).

It has been reported that cisplatin-induced apoptosis in cultured human proximal tubule cells is temporally correlated with an upregulation of the Fas/Fas ligand system (25). However, no direct cause-and-effect association was defined. Also, induction of Fas-dependent pathways would be expected to result in activation of pro-caspase 8 (18), and we found no evidence for its significant activation in our study. Thus it is likely that Fas-dependent pathways may play a major role in cisplatin-induced renal tubule cell apoptosis in LLC-PK1 cells. However, the scenario may be quite different in a complex in vivo system such as the kidney, in which previous studies have shown activation of Fas-dependent apoptosis after ischemia (28), allograft rejection (29), and chronic renal disease (30). Indeed, activation of the Fas pathway is a common mechanism by which cytotoxic drugs such as cisplatin induce apoptosis in cell lines derived from various tumors (31). Examples include hepatoma (32), neuroblastoma (33), and colon carcinoma (34) cells. On the other hand, cisplatin-induced apoptosis has been shown to be Fas-independent in leukemic (35,36) and lung cancer (37,38) cells, and several recent studies have implicated activation of mitochondrial apoptotic pathways after cisplatin exposure of ovarian cancer (39), squamous cell carcinoma (40), melanoma (41,42), and HeLa (43) cells. Thus, it is likely that the mechanism of cisplatin-induced apoptosis may not be similar in all cells but rather may be specific to the cell type.

The mechanisms by which cisplatin activates mitochondrial apoptotic pathways remain unknown. However, a role for oxidative stress may provide an attractive hypothesis (44). Several studies have documented the importance of reactive oxygen metabolites in cisplatin-induced renal cell apoptosis (12). A variety of antioxidants attenuate cisplatin-induced apoptosis in cultured mouse (5) or human (13) proximal tubule cells, in a rat model of nephrotoxicity (10), and in a mouse model of heme oxygenase-1 gene ablation (13), resulting in improved renal function (13). Cisplatin may directly lead to the generation of reactive oxygen species (5) or may induce (via mitochondrial damage) the release of reactive oxygen molecules normally sequestered within that organelle (12). Such molecules may then trigger several of the apoptotic mechanisms identified in this study. For example, first, reactive oxygen species may have been shown to promote the onset of the mitochondrial permeability transition (45). Second, oxidant stress can activate p53 (46), which in turn is a direct activator of Bax (47). Third, reactive oxidant molecules can trigger the release of cytochrome c (48) and even activate caspases (49).

In summary, we have shown that cisplatin induces a dose- and duration-dependent apoptosis in LLC-PK1 cells, via activation of mitochondrial signaling pathways. The sequence of events is as follows: activation and mitochondrial translocation of Bax leads to onset of mitochondrial permeability transition, release of cyto-

![Figure 6. Cisplatin induces a duration-dependent translocation of Bax. Control cells or cells treated with 50 μM cisplatin for various periods were double-labeled with antibodies to cytochrome c and Bax-NT and visualized by immunofluorescence microscopy with rhodamine filter (to detect cytochrome c) or fluorescein filter (for activated Bax). Control cells and cells at the 6-h incubation period showed a punctate mitochondrial distribution for cytochrome c and absence of Bax activation. Treated cells at the 9- and 12-h incubation points revealed a diffuse cytosolic pattern of cytochrome c staining and a distinct punctate mitochondrial staining with Bax-NT antibody, which indicates activation and mitochondrial translocation of Bax. Cells after 9 h of cisplatin also showed a colocalization of Bax and the voltage-dependent anion channel, which confirms the mitochondrial distribution of activated Bax. Representative of three experiments. Bar, 5 μm.

chrome c into the cytosol, activation of caspase 9, and entry into the execution phase of apoptosis. Selective inhibition of this pathway may provide a strategy to minimize cisplatin-induced nephrotoxicity. However, in clinical practice, any attempts at altering the apoptotic response of renal cells to cisplatin must necessarily be tempered by the potential of such maneuvers to interfere with the chemotherapy of the primary tumor.

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References


