Role of an Endoplasmic Reticulum Ca$^{2+}$-Independent Phospholipase A$_2$ in Cisplatin-Induced Renal Cell Apoptosis

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ABSTRACT

It has been demonstrated recently that rabbit renal proximal tubule cells (RPTC) express a novel Ca$^{2+}$-independent phospholipase A$_2$ (iPLA$_2$) whose activity localizes to the endoplasmic reticulum (ER-iPLA$_2$) and is similar to group VIB PLA$_2$. In this study, the expression of group VIB PLA$_2$ was examined and the role of ER-iPLA$_2$ in cisplatin-induced apoptosis was determined. Cisplatin induced both time- and concentration-dependent RPTC apoptosis as determined by p53 nuclear localization, annexin V staining, caspase 3 activity, and chromatin condensation. Inhibition of ER-iPLA$_2$ with bromoelactone (5 MuM) reduced cisplatin-induced annexin V binding 40%, chromatin condensation 55%, and caspase 3 activity 42%, but had no effect on p53 nuclear localization. Treatment of RPTC with the protein kinase C stimulator phorbol 12-myristate 13-acetate increased the activity of ER-iPLA$_2$ 2-fold and increased cisplatin-induced RPTC apoptosis. These studies demonstrate that group VIB PLA$_2$ is expressed in RPTC and suggest that RPTC ER-iPLA$_2$ is the rabbit homolog of group VIB PLA$_2$. These data also demonstrate that ER-iPLA$_2$ acts downstream of p53 and upstream of caspase 3 to mediate cisplatin-induced RPTC apoptosis. Finally, ER-iPLA$_2$ seems to be regulated by protein kinase C.

Phospholipase A$_2$ (PLA$_2$) are esterases that hydrolyze glycerophospholipids at the sn-2 position of the glycerol backbone, releasing a fatty acid and a lysophospholipid (Six and Dennis, 2000). PLA$_2$ are classified according to their nucleotide and amino acid sequence into 19 separate groups (Balsinde et al., 2002). Historically, PLA$_2$ have been classified into three groups: secretory PLA$_2$ (sPLA$_2$), cytosolic (cPLA$_2$), and Ca$^{2+}$-independent PLA$_2$ (iPLA$_2$). sPLA$_2$ are approximately 15 kDa, use a histidine amino acid to facilitate hydrolysis, and, with one exception (group XII), require Ca$^{2+}$ for activity (Six and Dennis, 2000; Balsinde et al., 2002). In contrast, sPLA$_2$, cPLA$_2$ (group IVA, B, and C) use a serine at their catalytic site and are larger than sPLA$_2$, ranging in size from 61 to 110 kDa (Six and Dennis, 2000). Groups IVA and B PLA$_2$ require Ca$^{2+}$ for translocation to membranes upon activation (Balsinde et al., 2002), whereas Group IVC is membrane associated and Ca$^{2+}$-independent (Underwood et al., 1998). Group VI members are typically referred to as iPLA$_2$ because they do not require Ca$^{2+}$ for either their activity or translocation to the membrane upon activation (Balsinde et al., 2002) and use a serine to catalyze hydrolysis (Six and Dennis, 2000).

Group VI PLA$_2$ represent some of the newest PLA$_2$ to be characterized and include group VIA-1, VIA-2, and VIB PLA$_2$. Group VIA and VIA-2 PLA$_2$ are splice variants of the same gene, are 85-kDa, and reside in the cytosol. Group VIA-1 and VIA-2 PLA$_2$ also are referred to as iPLA$_2$$\gamma$. In contrast, group VIA-1 and VIA-2 PLA$_2$ also are referred to as iPLA$_2$$\gamma$. In contrast, group VIB PLA$_2$ is the rabbit homolog of group VIB PLA$_2$. These data also demonstrate that ER-iPLA$_2$ acts downstream of p53 and upstream of caspase 3 to mediate cisplatin-induced RPTC apoptosis. Finally, ER-iPLA$_2$ seems to be regulated by protein kinase C.
more, the role of ER-iPLA2 in oncosis seems to be specific to renal cells (Cummings et al., 2002). Further, using BEL potentiated oxidant-induced lipid peroxidation. Thus, one role for microsomal group VIB PLA2, such as ER-iPLA2, is the protection of cells from oxidant-induced oncosis. However, several studies have demonstrated that the cytosolic group VIA PLA2 mediates apoptosis (Enari et al., 1996; Sapirstein et al., 1996; Atsumi et al., 1998; De Valck et al., 1998; Atsumi et al., 2000). For example, group VIA PLA2-mediated arachidonic acid release correlates to caspase 3 activation and the progression of apoptosis (Atsumi et al., 1997, 1998, 2000). The goals of this study were to determine whether ER-iPLA2 mediates RPTC apoptosis and to determine where apoptosis signaling ER-iPLA2 acts.

Materials and Methods

Materials. Female New Zealand White rabbits (1.5–2.0 kg) were purchased from Myrtle’s Rabbitry (Thompson Station, TN). L-Ascorbic acid-2-phosphate (magnesium salt) was obtained from Wako Chemicals USA (Richmond, VA). DEVD-afc (caspase 3 substrate), IETD-afc (caspase 8 substrate), and LEHD-afc (caspase 9 substrate) were purchased from BioVision (Palo Alto, CA). The rat kidney cDNA library was purchased from Stratagene (La Jolla, CA). The caspase 3 inhibitor DEVD-fmk, the general pan caspase inhibitor ZVAD-fmk, and annexin-FITC were obtained from R&D Systems (San Diego, CA). The antibody to p53, AOCF3, phospholipase D, and annexin F-12 (without d-glucose, phenol red, and sodium pyruvate) supplemented with 15 mM HEPES buffer, 2.5 mM L-glutamine, 15 mM sodium bicarbonate, and 6 mM lactate. Hydrocortisone (50 nM), selenium (5 ng/ml), human transferrin (5 μg/ml), bovine insulin (10 nM), and L-ascorbic acid-2-phosphate (50 μM) were added to fresh culture medium immediately before daily media change. In general, confluent RPTC were treated with inhibitors or diluent control [DMSO at <0.1% (v/v)] for 30 min before treatment with cisplatin.

Reverse-Transcriptase-Polymerase Chain Reaction. RTPCR using total RNA isolated from primary cultures of RPTC or PCR using a rat kidney cDNA library (Stratagene) was performed using primers designed against the sequences of group VIA PLA2 and group VIA PLA2 reported in Mancuso et al. (2000) and Ma et al. (1998), respectively. The RT product was performed at 50°C for 30 min followed by 2 min at 92°C to inactivate the RT. The PCR was then performed with 35 cycles of 30 s at 72°C, 90 s at 55°C, and 30 s at 92°C followed by a final extension step of 2 min at 72°C. For group VIA PLA2, the conditions were exactly the same as reported by Ma et al. (1998). RT-PCR products were analyzed by agarose gel electrophoresis. Candidate bands were excised and extracted from the gel using Millipore Ultrafree-DA extraction columns (Bedford, MA), ethanol precipitated, and subjected to automated fluorescence based sequencing at the Biotechnology Resource Laboratory at the Medical University of South Carolina. Candidate sequences were compared with those previously entered into GenBank.

Measurement of iPLA2 Activity. PLA2 activity was determined under linear conditions in microsomes and cytosol as described previously (McHowat et al., 1998). Activity was measured using synthetic (16:0, [3H]18:1) plasmenylcholine and diacylcholine substrates (100 μM) in the absence of Ca2+ (presence of 4 mM EGTA). For PLA2 activity inhibition studies, confluent RPTC were exposed to either a solvent control [DMSO] or 5 μM BEL for 30 min (Cummings et al., 2002).

Measurement of Annexin V and PI Staining. Annexin V and PI staining were determined using flow cytometry as described previously (Schutte et al., 1998; Goldberg et al., 1999; Meijerman et al., 1999) with modifications (Cummings and Schnellmann, 2002). Briefly, media were removed, RPTC were washed twice with PBS, and incubated in binding buffer (10 mM HEPES, 140 mM NaCl, 5 mM KCl, 1 mM MgCl2, 0.8 mM CaCl2, pH 7.4) containing annexin V-FITC (25 μg/ml) and PI (25 μg/ml) for 10 min. Cells were washed three times in binding buffer, released from the monolayers using a rubber policeman, and stained quantified using a BD Biosciences FACS Calibur flow cytometer. For each measurement, 10,000 events were counted.

### Table 1

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence</th>
<th>Product Sizes</th>
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<tbody>
<tr>
<td>Group VIB sense</td>
<td>5′-ATTGATGGTGGAGGACAAAGG-3′</td>
<td>475 bp</td>
</tr>
<tr>
<td>Group VIB antisense</td>
<td>5′-ATGGCCTGCGCACTTTTTATAC-3′</td>
<td></td>
</tr>
<tr>
<td>Group VIA sense</td>
<td>5′-GATGCAGTTCTTGGAGGCCCTGG-3′</td>
<td>1.5 and 1.6 kb</td>
</tr>
<tr>
<td>Group VIA antisense</td>
<td>5′-AATGCCCAGGGCCAGATGC-3′</td>
<td></td>
</tr>
</tbody>
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kb, kilobase.
**Determination of Caspase Activities.** Caspase 3, 8, and 9 activities were determined using the fluorometric substrates DEVD-afc (caspase 3 substrate), IETD-afc (caspase 8 substrate), and LEHD-afc (caspase 9 substrate) following the protocols of the caspase activity assay kit from BioVision (Cummings and Schnellmann, 2002). At 2, 4, 8, 12, and 24 h both attached and detached cells were isolated by scraping the dish with a rubber policeman and centrifugation at 400 g for 10 min. The supernatant was removed, and the pellet suspended in 100 μl of lysis buffer (BioVision), incubated at 4°C for 10 min, followed by centrifugation at 12,000 g for 10 min. Aliquots (50 μl) of the supernatant were removed and placed in a 96-well plate containing reaction buffer (BioVision). Substrate was added and the microplate was incubated at 37°C for 30 min. Activity was monitored as the linear cleavage and release of the afc side chain and compared with a linear standard curve generated on the same microplate.

**Immunocytochemistry.** RPTC were exposed to either solvent control or cisplatin for 4, 8, 12, and 24 h, fixed for 20 min using 10% buffered formalin/4% formaldehyde, and washed with PBS (Cummings and Schnellmann, 2002). RPTC were permeabilized, washed, and nonspecific binding blocked by incubation of RPTC in PBS/8% bovine serum albumin for 30 min. After washing, RPTC were incubated at 4°C overnight with either the primary antibody against p53 (10 μg/ml) or an IgG control, washed three times, and incubated with a secondary antibody conjugated to FITC for 2 h. Samples were washed three times, covered with mounting media, and coverslips applied. Staining was done using a Zeiss 410 confocal laser scanning microscope. p53 Staining was evaluated using a double blind protocol.

**Assessment of Chromat in Condensation.** Chromatin condensation was assessed in both treated and control RPTC using the DNA stain DAPI. After treatment, cells were washed twice with PBS and exposed to 16 μM DAPI at room temperature with gentle shaking. After 2 h, DAPI was removed, cells washed three times with PBS, mounted media was added, and coverslips applied. DAPI staining was assessed using a Nikon TE300 Eclipse microscope at 350/461-nm excitation/emission wavelengths. Apoptotic nuclei were those nuclei that exhibited chromat condensation (margination) at the periphery of the nucleus in the presence or absence of nuclear condensation. Chromatin condensation was evaluated using a double blind protocol.

**Measurement of DNA Hypoploidy and Cell Shrinkage.** DNA hypoploidy and cell shrinkage were assessed using methods described previously (Cummings et al., 2000). Briefly, RPTC were washed twice with sample buffer [PBS plus glucose (1 g/l)], dislodged using Cell Stripper (Mediatech, Herndon, VA), centrifuged at 400 g for 10 min, and suspended in sample buffer. Cells were fixed in ice-cold ethanol [70% (v/v)] and stained with PI (50 μg/ml) or an IgG control, washed twice with sample buffer [PBS plus glucose (1 g/l)], and Schnellmann, 2002). RPTC were permeabilized, washed, and nonspecific binding blocked by incubation of RPTC in PBS/8% bovine serum albumin for 30 min. After washing, RPTC were incubated at 4°C overnight with either the primary antibody against p53 (10 μg/ml) or an IgG control, washed three times, and incubated with a secondary antibody conjugated to FITC for 2 h. Samples were washed three times, covered with mounting media, and coverslips applied. DAPI staining was assessed using a Zeiss 410 confocal laser scanning microscope. p53 Staining was evaluated using a double blind protocol.

**Protein Determination.** Protein determination was determined using the bicinchoninic acid assay method as described by Sigma-Aldrich.

**Statistical Analysis.** RPTC isolated from one rabbit represented one experiment (n = 1). The appropriate analysis of variance was performed for each data set using SigmaStat statistical software. Individual means were compared using Fisher’s protected least significant difference test with P ≤ 0.05 being considered indicative of a statistically significant difference between mean values.

**Results**

**Expression of Group VIB PLA₂ in the Kidney.** Using immunoblot analysis and an antibody directed against cytosolic group VIA PLA₂, an immunoreactive 85-kDa protein was identified in RPTC microsomes. In contrast, no protein was identified in the RPTC cytosol. This study identified and characterized an ER-iPLA₂ activity, and we hypothesized that the ER-iPLA₂ was a rabbit homolog of group VIB PLA₂. RT-PCR using total RPTC RNA and primers against group VIA PLA₂ (Table 1) did not result in any detectable cDNA product (Fig. 1A). As previously demonstrated by Ma et al. (1997, 1998), these same sets of primers did result in multiple 1.5- to 1.7-kb products using a rat kidney cortex cDNA library (Fig. 1A). RT-PCR using total RPTC RNA and primers designed against the sequence of group VIB PLA₂ (Mancuso et al., 2000) (Table 1) resulted in one 475-bp cDNA product (Fig. 1A) whose sequence was 89% identical to the DNA surrounding the active site of group VIB PLA₂ sequence. These data support our previous report that rabbit RPTC express group VIA PLA₂, but not group VIB PLA₂ (Cummings et al., 2002) and support the hypothesis that ER-iPLA₂ is the rabbit homolog of the microsomal group VIB PLA₂.

**Effect of ER-iPLA₂ Inhibition on Cisplatin-Induced Annexin V Staining, Chromatin Condensation, and DNA Hypoploidy.** To address the role of ER-iPLA₂ in RPTC apoptosis, cisplatin-induced apoptosis in the presence and absence of BEL was studied. Similar to previous studies, cisplatin (50 μM), a common chemotherapeutic and nephrotoxicant, induced chromatin condensation and DNA hypoploidy in 40% of the cells, and a 4-fold increase in annexin V staining after 24 h of exposure (Figs. 2 and 3). Treatment of RPTC with BEL (5 μM) (a concentration previously shown to inhibit 90% of ER-iPLA₂ activity in RPTC; Cummings et al., 2002) before cisplatin exposure decreased all apoptosis markers studied 30 to 50% (Figs. 2E and 3). In contrast, treatment of RPTC with the cPLA₂ inhibitor AAOCF₃ had no effect on cisplatin-induced annexin V staining (Fig. 3A). Similar to BEL, treatment of RPTC with the caspase 3 inhibitor DEVD-fmk and the general pan caspase inhibitor ZVAD-fmk dem-

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**Fig. 1.** Expression of ER-iPLA₂ in RPTCs. Total RNA was isolated from primary cultures of RPTC and subjected to RT-PCR. A demonstrates the absence of cDNA products in reactions performed using RPTC total RNA and primers against group VIA PLA₂. Note the presence of multiple 1.5- to 1.8-kb cDNA products in reactions using rat kidney cortex cDNA library. B, demonstrates the presence of a 475-bp cDNA product in reactions using RPTC total RNA and primer against group VIA PLA₂. Results using RT-PCR total RNA are typical of at least three separate RNA isolations from three separate rabbits.
creased cisplatin-induced apoptosis approximately 50%. The
decrease in apoptosis was not the result of a shift to oncosis
because no increases in PI staining were detected at any time
point throughout treatment with BEL (data not shown).
These results suggest that microsomal iPLA₂ mediates apo-
ptosis, acting upstream of events signaling chromatin con-
densation, DNA hypoploidy, and annexin V staining.

**Effect of ER-iPLA₂ Inhibition on p53 Nuclear Local-
ization.** Cisplatin-induced RPTC apoptosis is mediated in
part by p53 nuclear localization (Cummings and Schnell-
mann, 2002), which increases in RPTC within 4 h after
exposure to cisplatin (Fig. 4, A and B). Therefore, the hypoth-
thesis that ER-iPLA₂ inhibition reduces cisplatin-induced
RPTC apoptosis by inhibiting p53 nuclear localization was
tested. Treatment of RPTC with BEL alone had no affect on
p53 nuclear localization. BEL treatment did not affect cispla-
tin-induced p53 nuclear localization (Fig. 4C). These data suggest that ER-iPLA₂ acts down-

![Image](image-url)
stream of p53 nuclear localization in cisplatin-induced RPTC apoptosis.

**Effect of ER-iPLA₂ Inhibition on Caspase Activity.** Approximately 50% of cisplatin-induced RPTC apoptosis is mediated by caspase 3 activation in response to p53 nuclear localization (Cummings and Schnellmann, 2002). Furthermore, inhibition of caspases using the caspase 3 inhibitor DEVD-fmk, or caspases in general using the pan caspase inhibitor ZVAD-fmk, decreased cisplatin-induced RPTC apoptosis 50% (Figs. 2 and 3; Cummings and Schnellmann, 2002). As such, the hypothesis that inhibition of microsomal iPLA₂ decreases cisplatin-induced caspase 3 activity was tested. Treatment of RPTC with cisplatin increased caspase 3 activity as assessed by cleavage of DEVD-afc (Fig. 5). Caspase 3 activation was time-dependent with initial increases being measured at 8 h and increasing at both 12 and 24 h. Treatment of RPTC with BEL before cisplatin exposure totally inhibited caspase 3 activity at 12 h and resulted in a 50% inhibition of activity after 24 h. Neither cisplatin alone, nor cisplatin in combination with BEL, increased caspase 8 or 9 activity compared with control (data not shown and previous observations; Cummings and Schnellmann, 2002). These data suggest that ER-iPLA₂ acts downstream of p53 nuclear localization and upstream of caspase 3 in cisplatin-induced RPTC apoptosis.

**Effect of PMA on ER-iPLA₂ Activity and RPTC Apoptosis.** If inhibition of ER-iPLA₂ decreases cisplatin-induced RPTC apoptosis, then activation may increase apoptosis. To investigate this hypothesis, the effect of PMA on both microsomal iPLA₂ activity and cisplatin-induced apoptosis was determined. Treatment of RPTC with cisplatin increased caspase 3 activity as assessed by cleavage of DEVD-afc (Fig. 5). Caspase 3 activation was time-dependent with initial increases being measured at 8 h and increasing at both 12 and 24 h. Treatment of RPTC with BEL before cisplatin exposure totally inhibited caspase 3 activity at 12 h and resulted in a 50% inhibition of activity after 24 h. Neither cisplatin alone, nor cisplatin in combination with BEL, increased caspase 8 or 9 activity compared with control (data not shown and previous observations; Cummings and Schnellmann, 2002). These data suggest that ER-iPLA₂ acts downstream of p53 nuclear localization and upstream of caspase 3 in cisplatin-induced RPTC apoptosis.

**Discussion**

Recently, several new PLA₂ isoforms have been described (Tang et al., 1997; McHowat and Creer, 1998; Mancuso et al., 2000; Tanaka et al., 2000; Cummings et al., 2002). Our laboratory has demonstrated that RPTC contain an ER-iPLA₂ activity that is plasmalogen-selective, is inhibited by BEL, and is relatively insensitive to AAOCF₃ and MAFP (Schnellmann et al., 1994; Cummings et al., 2002). Using immunoblot analysis and an antibody directed against cytosolic group VIA PLA₂, an immunoreactive 85-kDa protein was identified in RPTC microsomes. In contrast, no protein was identified in RPTC cytosol. The data reported in this study further support the hypothesis that RPTC express group VIB, but not group VIA PLA₂. Sequencing of the 475-bp cDNA product generated by RT-PCR and comparison with the sequence for human group VIB PLA₂ in GenBank resulted in an 89% nucleotide sequence identity. Thus, these data suggest that
rabbit RPTC express the rabbit homolog of human group VIB PLA₂, but not group VI PLA₂.

The discovery of group VIB PLA₂ raises several questions as to the role of a microsomal iPLA₂, such as ER-iPLA₂, in cell physiology and cell injury and death. Although a recent study demonstrated that ER-iPLA₂ plays a protective role during oxidant-induced RPTC oncosis (Cummings et al., 2002), a role for ER-iPLA₂ in RPTC apoptosis was not addressed. In the present study, treatment of RPTC with BEL before cisplatin exposure decreased all apoptosis markers studied with the exception of p53 nuclear translocation. The effect of BEL on annexin V staining, DNA hypoploidy and chromatin condensation mirrored that seen with DEVD-fmk or ZVAD-fmk, and BEL inhibited caspase 3 activation. These results suggest that ER-iPLA₂ acts downstream of p53 and upstream of caspase 3 activation after cisplatin exposure.

Inhibition of ER-iPLA₂ activity in RPTC only inhibited 50% of cisplatin-induced RPTC apoptosis after 24 h. This is the same level of inhibition seen with the caspase 3 inhibitor DEVD-fmk and the general pan caspase inhibitor ZVAD-fmk (Cummings and Schnellmann, 2002). The remaining 50% of RPTC apoptosis proceeds in the absence of caspase 3, 8, or 9 activity and is not affected by inhibition of p53 nuclear translocation. Furthermore, treatment of RPTC with both ZVAD-fmk and BEL does not result in further protection against cisplatin-induced RPTC apoptosis (data not shown). These data suggest that ER-iPLA₂ mediates caspase-dependent RPTC apoptosis and does not play a role in caspase-independent apoptosis.

Cisplatin-induced p53 translocation results in caspase 3 activity and RPTC apoptosis via a mechanism that progresses in the absence of significant mitochondrial dysfunction (Cummings and Schnellmann, 2002). In the absence of mitochondrial dysfunction, we propose that the ER may provide a link between p53 and caspase 3 activation. p53-Mediated alterations of ER function during apoptosis have been reported (Froesch et al., 1999). Furthermore, several studies have reported links between apoptosis, the release of ER-Ca²⁺ (Nakagawa et al., 2000; Niwa et al., 2001), and the activation of caspases (Nakagawa and Yuan, 2000; Nakagawa et al., 2000). Nakagawa et al. (Nakagawa and Yuan, 2000; Nakagawa et al., 2000) reported that ER-Ca²⁺ releasing agents such as tunicamycin and thapsigargin induced caspase 3 activation independently of caspase 8 or 9 or mitochondrial dysfunction. Finally, disruption of ER function by cisplatin may lead to increased ER-iPLA₂ activity. Thus, a number of possibilities exist for stimulation of ER-PLA₂ activity and the release of arachidonic acid. We postulate that arachidonic acid or a metabolite mediates downstream apoptotic events.

Recent studies suggested that group VIA PLA₂ (cytosolic iPLA₂) activity may increase in response to oxidative stress or activation of protein kinase C (Balboa and Balsinde, 2002; Steer et al., 2002). For example, cytosolic group VIA iPLA₂-
mediated cleavage and release of phospholipids from U937 cells is increased after exposure to H$_2$O$_2$ (Balboa and Balsinde, 2002). However, this increase also may be mediated by an increase in phospholipid oxidation as opposed to a direct activation of group VIA PLA$_2$. In contrast, pretreatment of cardiomyocytes with protein kinase C activating concentrations of PMA resulted in increases in group VI PLA$_2$ activity (Steer et al., 2002). In this study, we demonstrate that treatment of RPTC with similar concentrations of PMA increased the activity of ER-iPLA$_2$ 2-fold. Furthermore, increases in ER-iPLA$_2$ activity correlated to increases in cisplatin-induced apoptosis. These results suggest that ER-iPLA$_2$ is regulated by protein kinase C and further support an important role for ER-iPLA$_2$ in apoptosis.

Previously, a 28-kDa cytosolic iPLA$_2$ has been suggested to mediate hypoxia-induced injury in renal proximal tubules (Portilla et al., 1994, 1998; Portilla and Dai, 1996). We also have detected cytosolic iPLA$_2$ activity in RPTC (Cummings et al., 2002) whose activity is comparable with that reported by Portilla and Dai (1996). However, the IC$_{50}$ for BEL is approximately 3-fold higher for 28-kDa iPLA$_2$ than ER-iPLA$_2$ and was not inhibited by 5 $\mu$M BEL (Portilla et al., 1994; Cummings et al., 2002). Thus, although cytosolic 28-kDa iPLA$_2$ may mediate hypoxia-induced injury in renal tubules, it probably does not mediate cisplatin-induced injury. Furthermore, it is unlikely that decreases in cisplatin-induced RPTC are a result of phophatidic acid phosphohydrolase-1 inhibition by BEL because phophatidic acid phosphohydrolase-1 is only inhibited 50% by 50 $\mu$M BEL (Balsinde and Dennis, 1996). In contrast, BEL almost totally inhibits ER-iPLA$_2$ at concentration of 5 $\mu$M (Cummings et al., 2002). BEL does inhibit cytosolic group VIA PLA$_2$ with equal potency as ER-iPLA$_2$. However, data included in this report, and from previous studies (Cummings et al., 2002), demonstrate that group VIA PLA$_2$ is not expressed in RPTC.

We have demonstrated that group VIA PLA$_2$ is expressed in mammalian RPTC and that rabbit RPTC ER-iPLA$_2$ may represent the rabbit homolog of human group VIA PLA$_2$. Data from this study also suggest that ER-iPLA$_2$ mediates apoptosis. These data, combined with our previous studies on ER-iPLA$_2$ in oxidant-induced oncosis (Cummings et al., 2002), demonstrate that ER-iPLA$_2$ plays divergent roles in RPTC oncosis and apoptosis. During oxidant-induced oncosis ER-iPLA$_2$ protects RPTC, whereas ER-iPLA$_2$ seems to facilitate cispllatin-induced apoptosis. We hypothesize that the divergent roles are the result of differences in the initiating insult and the ensuing signaling pathways. For example, the role of ER-iPLA$_2$ in oxidant injury is due to oxidant stress and lipid peroxidation that leads to mitochondrial dysfunction and oncosis. ER-iPLA$_2$ activity may serve to cleave and remove peroxidized phospholipids, preventing their conversion to malondialdehyde, and to prohibit propagation of free radicals. In contrast, the concentration of cisplatin used in this study produced apoptosis exclusively through DNA damage, p53 activation, and activation of caspase-dependent and -independent pathways (Cummings and Schnellmann, 2002). We have no evidence that oxidative stress plays a role in cisplatin-induced apoptosis under these conditions. ER-iPLA$_2$ may facilitate the release of the lipid signaling molecule arachidonic acid. Arachidonic acid may serve to activate caspases (Atsumi et al., 1998) and other downstream signaling apoptotic pathways. In summary, we have demonstrated that ER-iPLA$_2$ mediates cisplatin-induced apoptosis in RPTC. Although we have demonstrated that ER-iPLA$_2$ mediates oncosis induced by diverse oxidants, the role of ER-iPLA$_2$ in apoptosis induced by chemotherapeutics or chemicals other than cisplatin is not known. ER-iPLA$_2$ may represent a novel therapeutic target for either the prevention of chemotherapeutic-induced nephrotoxicity or for the development of novel anticancer agents.

References
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