Pathophysiology of Nephrotoxic Acute Renal Failure

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Humans are exposed intentionally and unintentionally to a variety of diverse chemicals that harm the kidney. As the list of drugs, natural products, industrial chemicals and environmental pollutants that cause nephrotoxicity has increased, it has become clear that chemicals with very diverse chemical structures produce nephrotoxicity. For example, the heavy metal $HgCl_2$, the mycotoxin fumonisin B_1, the immunosuppresant cyclosporin A, and the aminoglycoside antibiotics all produce acute renal failure but are not structurally related. Thus, it is not surprising that the cellular targets within the kidney and the mechanisms of cellular injury vary with different toxicants. Nevertheless, there are similarities between chemical-induced acute tubular injury and ischemia/reperfusion injury.

The tubular cells of the kidney are particularly vulnerable to toxicant-mediated injury due to their disproportionate exposure to circulating chemicals and transport processes that result in high intracellular concentrations. It is generally thought that the parent chemical or a metabolite initiates toxicity through its covalent or noncovalent binding to cellular macromolecules or through their ability to produce reactive oxygen species. In either case the activity of the macromolecule(s) is altered resulting in cell injury. For example, proteins and lipids in the plasma membrane, nucleus, lysosome, mitochondrion and cytosol are all targets of toxicants. If the toxicant causes oxidative stress both lipid peroxidation and protein oxidation have been shown to contribute to cell injury.

In many cases mitochondria are a critical target and the lack of adenosine triphosphate (ATP) leads to cell injury due to the dependence of renal function on aerobic metabolism. The loss of ATP leads

CHAPTER 15

to disruption of cellular ion homeostasis with decreased cellular K^+ content, increased Na^+ content and membrane depolarization. Increased cytosolic free Ca^{2+} concentrations can occur in the early or late phase of cell injury and plays a critical role leading to cell death. The increase in Ca^{2+} can activate calcium activated neutral proteases (calpains) that appear to contribute to the cell injury that occurs by a variety of toxicants. During the late phase of cell injury, there is an increase in Cl^- influx, followed by the influx of increasing larger molecules that leads to cell lysis. Two additional enzymes appear to play an important role in cell injury, particularly oxidative injury. Phospholipase A_2 consists of a family of enzymes in which the activity of the cytosolic form increases during oxidative injury and contributes to cell death. Caspases are a family of cysteine proteases that are activated following oxidative injury and contribute to cell death. Following exposure to a chemical insult those cells sufficiently injured die by one of two mechanisms, apoptosis or oncosis.

Clinically, a vast number of nephrotoxicants can produce a variety of clinical syndromes-acute renal failure, chronic renal failure, nephrotic syndrome, hypertension and renal tubular defects. The evolving understanding of the pathophysiology of toxicant-mediated renal injury has implications for potential therapies and preventive measures. This chapter outlines some of the mechanisms thought to be important in toxicant-mediated renal cell injury and death that leads to the loss of tubular epithelial cells, tubular obstruction, "backleak" of the glomerular filtrate and a decreased glomerular filtration rate. The recovery from the structural and functional damage following chemical exposures is dependent on the repair of sublethally-injured and regeneration of noninjured cells.

Clinical Significance of Toxicant-Mediated Acute Renal Failure

CLINICAL SIGNIFICANCE OF TOXICANT–MEDIATED RENAL FAILURE

- Nephrotoxins may account for approximately 50% of all cases of acute and chronic renal failure.
- Nephrotoxic renal injury often occurs in conjunction with ischemic acute renal failure.
- Acute renal failure may occur in 2% to 5% of hospitalized patients and 10% to 15% of patients in intensive care units.
- The mortality of acute renal failure is approximatley 50% which has not changed significantly in the last 40 years.
- Radiocontrast media and aminoglycosides are the most common agents associated with nephrotoxic injury in hospitalized patients.
- Aminoglycoside nephrotoxicity occurs in 5% to 15% of patients treated with these drugs.

FIGURE 15-1
Clinical significance of toxicant-mediated renal failure.

REASONS FOR THE KIDNEY'S SUSCEPTIBILITY TO TOXICANT INJURY

- Receives 25% of the cardiac output
- Sensitive to vasoactive compounds
- Concentrates toxicants through reabsorptive and secretive processes
- Many transporters result in high intracellular concentrations
- Large luminal membrane surface area
- Large biotransformation capacity
- Baseline medullary hypoxia

FIGURE 15-2
Reasons for the kidney's susceptibility to toxicant injury.

FACTORS THAT PREDISPOSE THE KIDNEY TO TOXICANT INJURY

- Preexisting renal dysfunction
- Dehydration
- Diabetes mellitus
- Exposure to multiple nephrotoxins

FIGURE 15-3
Factors that predispose the kidney to toxicant injury.

EXOGENOUS AND ENDOGENOUS CHEMICALS THAT CAUSE ACUTE RENAL FAILURE

- Antibiotics
  - Aminoglycosides (gentamicin, tobramycin, amikacin, netilmicin)
  - Amphotericin B
  - Cephalosporins
  - Ciprofloxacin
  - Demeclocycline
  - Penicillins
  - Pentamidine
  - Polymixins
  - Rifampin
  - Sulfonamides
  - Tetracycline
  - Vancomycin
- Chemotherapeutic agents
  - Adriamycin
  - Cisplatin
  - Methotraxate
  - Mitomycin C
  - Nitrosoureas (*eg*, streptozotocin, lomustine)
- Radiocontrast media
  - Ionic (*eg*, diatrizoate, iothalamate)
  - Nonionic (*eg*, metrizamide)
- Immunosuppressive agents
  - Cyclosporin A
  - Tacrolimus (FK 506)
- Antiviral agents
  - Acyclovir
  - Cidovir
  - Foscarnet
  - Valacyclovir
- Heavy metals
  - Cadmium
  - Gold
  - Mercury
  - Lead
  - Arsenic
  - Bismuth
  - Uranium
- Organic solvents
  - Ethylene glycol
  - Carbon tetrachloride
  - Unleaded gasoline
- Vasoactive agents
  - Nonsteroidal anti-inflammatory drugs (NSAIDs)
  - Ibuprofen
  - Naproxen
  - Indomethacin
  - Meclofenemate
  - Aspirin
  - Piroxicam
  - Angiotensin-converting enzyme inhibitors
  - Captopril
  - Enalopril
  - Lisinopril
  - Angiotensin receptor antagonists
  - Losartan
- Other drugs
  - Acetaminophen
  - Halothane
  - Methoxyflurane
  - Cimetidine
  - Hydralazine
  - Lithium
  - Lovastatin
  - Mannitol
  - Penicillamine
  - Procainamide
  - Thiazides
  - Lindane
- Endogenous compounds
  - Myoglobin
  - Hemoglobin
  - Calcium
  - Uric acid
  - Oxalate
  - Cystine

FIGURE 15-4
Exogenous and endogenous chemicals that cause acute renal failure.

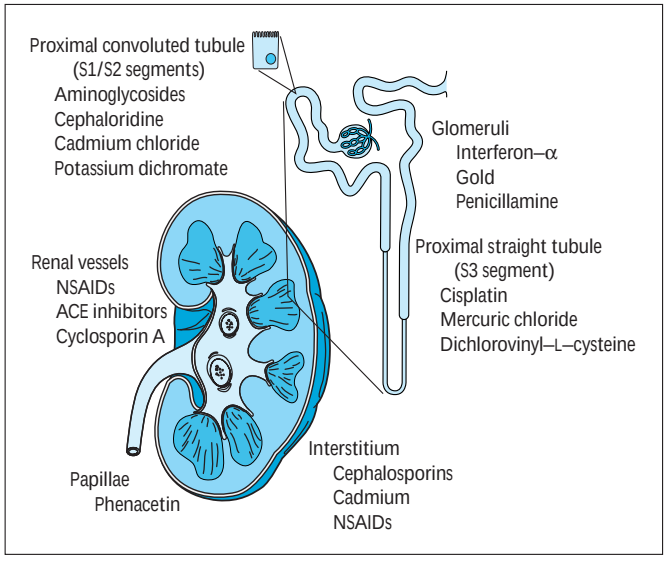

FIGURE 15-5
Nephrotoxicants may act at different sites in the kidney, resulting in altered renal function. The sites of injury by selected nephrotoxicants are shown. Nonsteroidal anti-inflammatory drugs (NSAIDs), angiotensin-converting enzyme (ACE) inhibitors, cyclosporin A, and radiographic contrast media cause vasoconstriction. Gold, interferon-alpha, and penicillamine can alter glomerular function and result in proteinuria and decreased renal function. Many nephrotoxicants damage tubular epithelial cells directly. Aminoglycosides, cephaloridine, cadmium chloride, and potassium dichromate affect the S1 and S2 segments of the proximal tubule, whereas cisplatin, mercuric chloride, and dichlorovinyl-L-cysteine affect the S3 segment of the proximal tubule. Cephalosporins, cadmium chloride, and NSAIDs cause interstitial nephritis whereas phenacetin causes renal papillary necrosis.

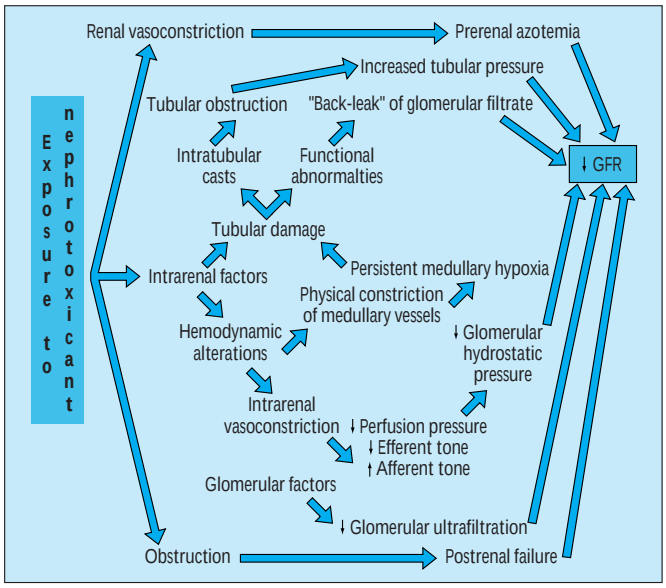

FIGURE 15-6

Mechanisms that contribute to decreased glomerular filtration rate (GFR) in acute renal failure. After exposure to a nephrotoxicant, one or more mechanisms may contribute to a reduction in the GFR. These include renal vasoconstriction resulting in prerenal azotemia (*eg*, cyclosporin A) and obstruction due to precipitation of a drug or endogenous substances within the kidney or collecting ducts (*eg*, methotrexate). Intrarenal factors include direct tubular obstruction and dysfunction resulting in tubular backleak and increased tubular pressure. Alterations in the levels of a variety of vasoactive mediators (*eg*, prostaglandins following treatment with nonsteroidal anti-inflammatory drugs) may result in decreased renal perfusion pressure or efferent arteriolar tone and increased afferent arteriolar tone, resulting in decreased in glomerular hydrostatic pressure. Some nephrotoxicants may decrease glomerular function, leading to proteinuria and decreased renal function.

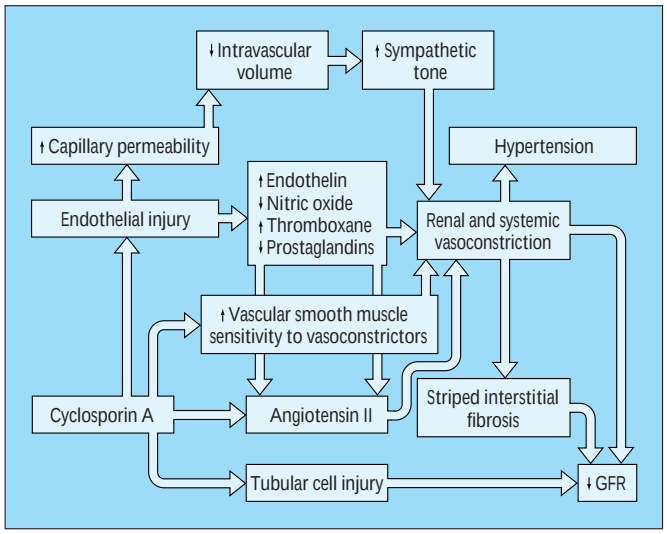

FIGURE 15-7

Renal injury from exposure to cyclosporin A. Cyclosporin A is one example of a toxicant that acts at several sites within the kidney. It can injure both endothelial and tubular cells. Endothelial injury results in increased vascular permeability and hypovolemia, which activates the sympathetic nervous system. Injury to the endothelium also results in increases in endothelin and thromboxane A_2 and decreases in nitric oxide and vasodilatory prostaglandins. Finally, cyclosporin A may increase the sensitivity of the vasculature to vasoconstrictors, activate the renin-angiotensin system, and increase angiotensin II levels. All of these changes lead to vasoconstriction and hypertension. Vasoconstriction in the kidney contributes to the decrease in glomerular filtration rate (GFR), and the histologic changes in the kidney are the result of local ischemia and hypertension.

Renal Cellular Responses to Toxicant Exposures

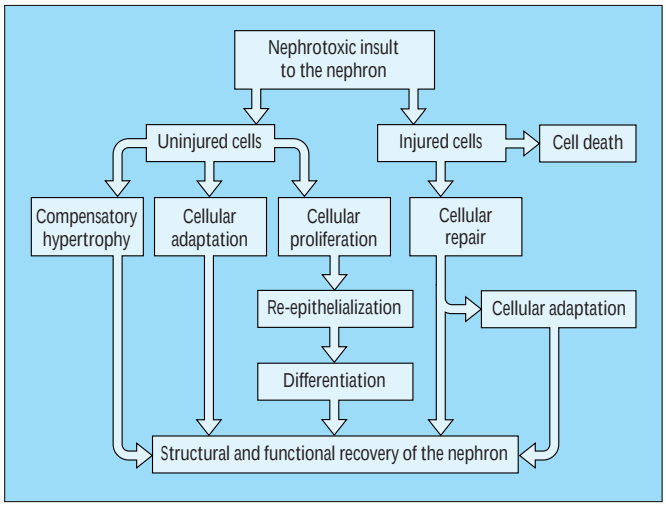

FIGURE 15-8

The nephron's response to a nephrotoxic insult. After a population of cells are exposed to a nephrotoxicant, the cells respond and ultimately the nephron recovers function or, if cell death and loss is extensive, nephron function ceases. Terminally injured cells undergo cell death through oncosis or apoptosis. Cells injured sublethally undergo repair and adaptation (*eg*, stress response) in response to the nephrotoxicant. Cells not injured and adjacent to the injured area may undergo dedifferentiation, proliferation, migration or spreading, and differentiation. Cells that were not injured may also undergo compensatory hypertrophy in response to the cell loss and injury. Finally the uninjured cells may also undergo adaptation in response to nephrotoxicant exposure.

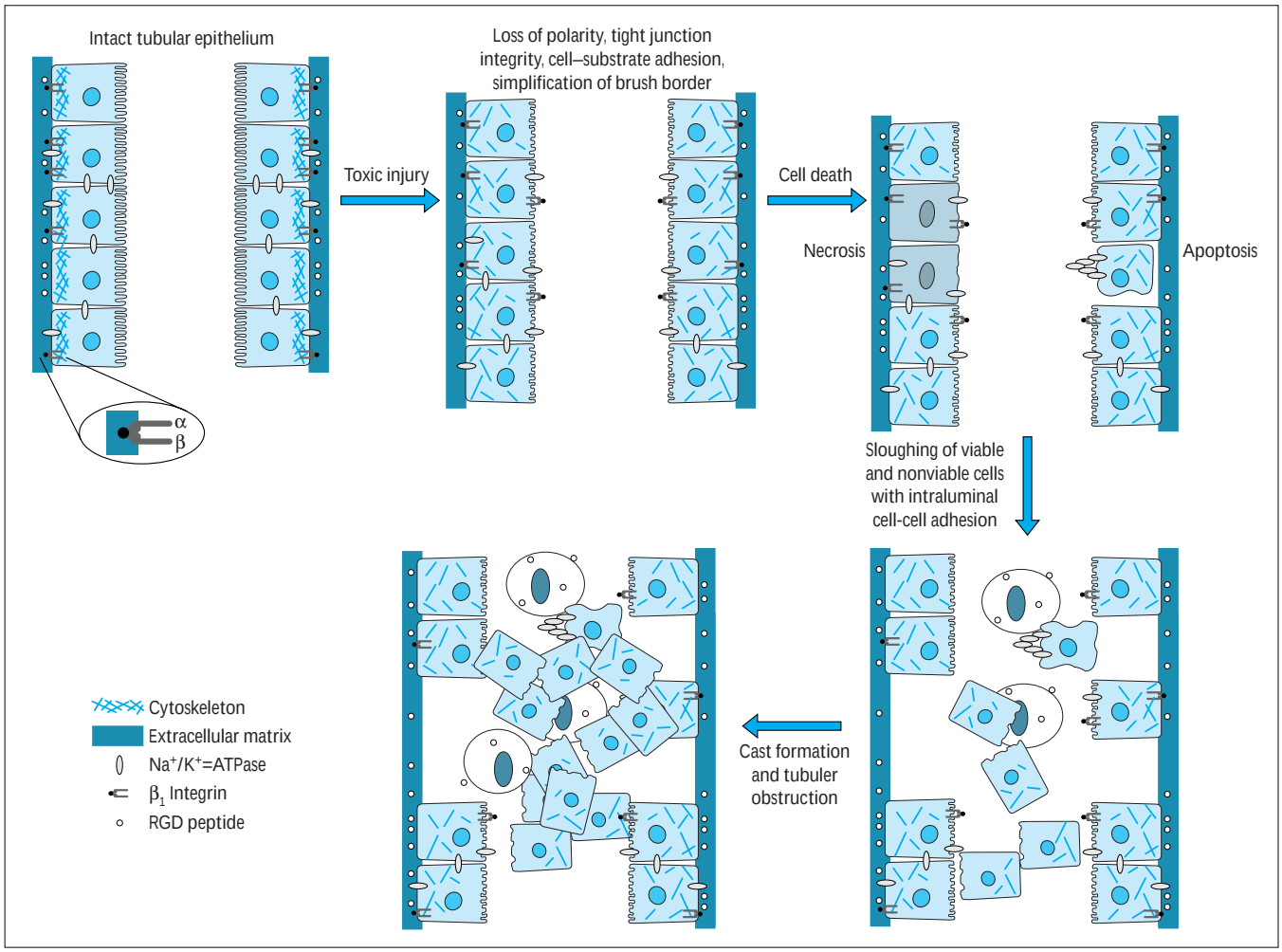

FIGURE 15-9

After injury, alterations can occur in the cytoskeleton and in the normal distribution of membrane proteins such as Na^+, K^+-ATPase and β_1 integrins in sublethally injured renal tubular cells. These changes result in loss of cell polarity, tight junction integrity, and cell-substrate adhesion. Lethally injured cells undergo oncosis or apoptosis, and both dead and viable cells may be sloughed into the tubular lumen. Adhesion of sloughed cells to other sloughed cells and to cells remaining adherent to the basement membrane may result in cast formation, tubular obstruction, and further compromise the glomerular filtration rate. (*Adapted from* Fish and Molitoris [1], and Gailit *et al.* [2]; with permission.)

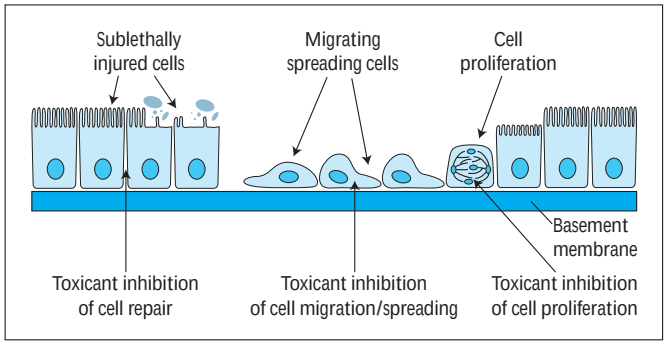

FIGURE 15-10

Potential sites where nephrotoxicants can interfere with the structural and functional recovery of nephrons.

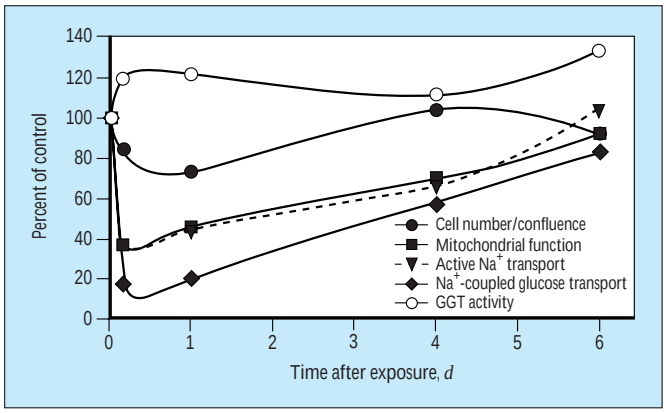

FIGURE 15-11

Inhibition and repair of renal proximal tubule cellular functions after exposure to the model oxidant *t*-butylhydroperoxide. Approximately 25% cell loss and marked inhibition of mitochondrial function active (Na^+) transport and Na^+-coupled glucose transport occurred 24 hours after oxidant exposure. The activity of the brush border membrane enzyme γ-glutamyl transferase (GGT) was not affected by oxidant exposure. Cell proliferation and migration or spreading was complete by day 4, whereas active Na^+ transport and Na^+-coupled glucose transport did not return to control levels until day 6. These data suggest that selective physiologic functions are diminished after oxidant injury and that a hierarchy exists in the repair process: migration or spreading followed by cell proliferation forms a monolayer and antedates the repair of physiologic functions. (*Data from* Nowak *et al.* [3].)

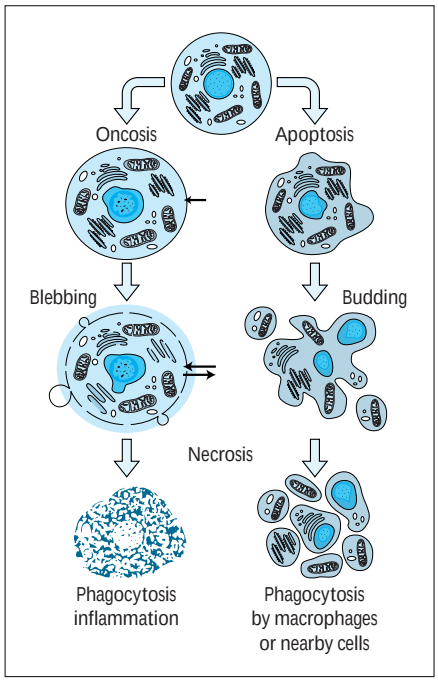

FIGURE 15-12

Apoptosis and oncosis are the two generally recognized forms of cell death. Apoptosis, also known as programmed cell death and cell suicide, is characterized morphologically by cell shrinkage, cell budding forming apoptotic bodies, and phagocytosis by macrophages and nearby cells. In contrast, oncosis, also known as necrosis, necrotic cell death, and cell murder, is characterized morphologically by cell and organelle swelling, plasma membrane blebbing, cell lysis, and inflammation. It has been suggested that cell death characterized by cell swelling and lysis not be called necrosis or necrotic cell death because these terms describe events that occur well after the cell has died and include cell and tissue breakdown and cell debris. (*From* Majno and Joris [4]; with permission.)

Mechanisms of Toxicant-Mediated Cellular Injury

Transport and biotransformation

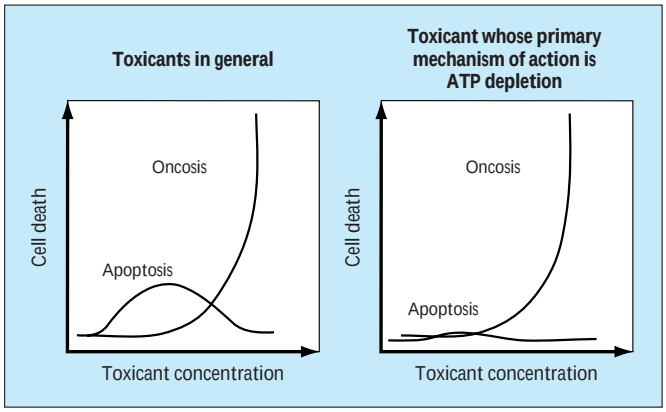

FIGURE 15-13

The general relationship between oncosis and apoptosis after nephrotoxicant exposure. For many toxicants, low concentrations cause primarily apoptosis and oncosis occurs principally at higher concentrations. When the primary mechanism of action of the nephrotoxicant is ATP depletion, oncosis may be the predominant cause of cell death with limited apoptosis occurring.

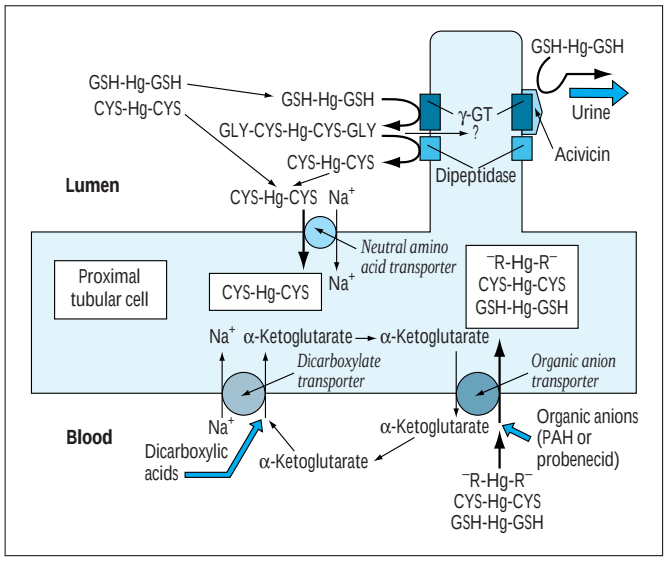

FIGURE 15-14

The importance of cellular transport in mediating toxicity. Proximal tubular uptake of inorganic mercury is thought to be the result of the transport of mercuric conjugates (*eg*, diglutathione mercury conjugate [GSH-Hg-GSH], dicysteine mercuric conjugate [CYS-Hg-CYS]). At the luminal membrane, GSH-Hg-GSH appears to be metabolized by (-glutamyl transferase ((-GT) and a dipeptidase to form CYS-Hg-CYS. The CYS-Hg-CYS may be taken up by an amino acid transporter. At the basolateral membrane, mercuric conjugates appear to be transported by the organic anion transporter. (-Ketoglutarate and the dicarboxylate transporter seem to play important roles in basolateral membrane uptake of mercuric conjugates. Uptake of mercuric-protein conjugates by endocytosis may play a minor role in the uptake of inorganic mercury transport. PAH—*para*-aminohippurate. (*Courtesy of* Dr. R. K. Zalups.)

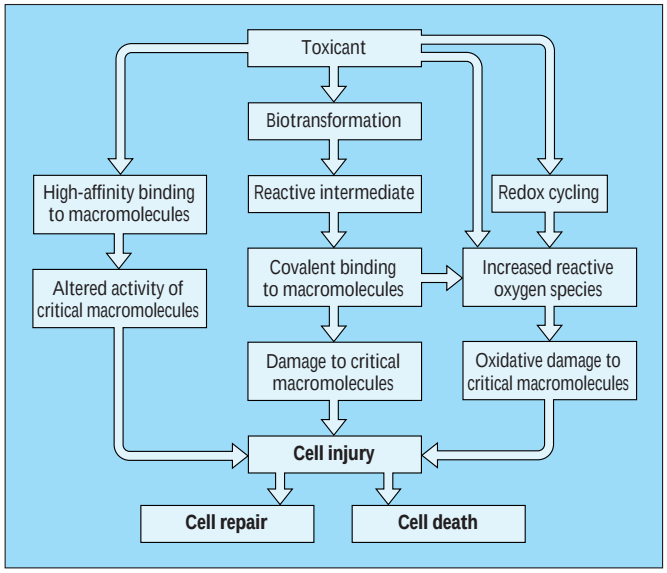

FIGURE 15-15

Covalent and noncovalent binding versus oxidative stress mechanisms of cell injury. Nephrotoxicants are generally thought to produce cell injury and death through one of two mechanisms, either alone or in combination. In some cases the toxicant may have a high affinity for a specific macromolecule or class of macromolecules that results in altered activity (increase or decrease) of these molecules, resulting in cell injury. Alternatively, the parent nephrotoxicant may not be toxic until it is biotransformed into a reactive intermediate that binds covalently to macromolecules and in turn alters their activity, resulting in cell injury. Finally, the toxicant may increase reactive oxygen species in the cells directly, after being biotransformed into a reactive intermediate or through redox cycling. The resulting increase in reactive oxygen species results in oxidative damage and cell injury.

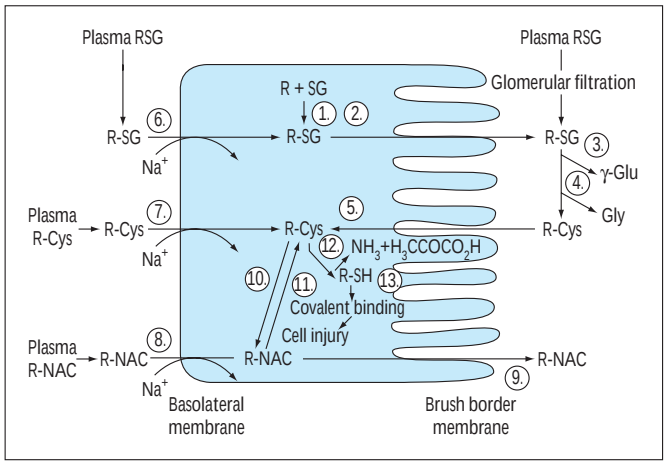

FIGURE 15-16

This figure illustrates the renal proximal tubular uptake, biotransformation, and toxicity of glutathione and cysteine conjugates and mercapturic acids of haloalkanes and haloalkenes (R). 1) Formation of a glutathione conjugate within the renal cell (R-SG). 2) Secretion of the R-SG into the lumen. 3) Removal of the γ-glutamyl residue (γ-Glu) by γ-glutamyl transferase. 4) Removal of the glycinyl residue (Gly) by a dipeptidase. 5) Luminal uptake of the cysteine conjugate (R-Cys). Basolateral membrane uptake of R-SG (6), R-Cys (7), and a mercapturic acid (*N*-acetyl cysteine conjugate; R-NAC)(8). 9) Secretion of R-NAC into the lumen. 10) Acetylation of R-Cys to form R-NAC. 11) Deacetylation of R-NAC to form R-Cys. 12) Biotransformation of the penultimate nephrotoxic species (R-Cys) by cysteine conjugate β-lyase to a reactive intermediate (R-SH), ammonia, and pyruvate. 13) Binding of the reactive thiol to cellular macromolecules (*eg*, lipids, proteins) and initiation of cell injury. (*Adapted from* Monks and Lau [5]; with permission.)

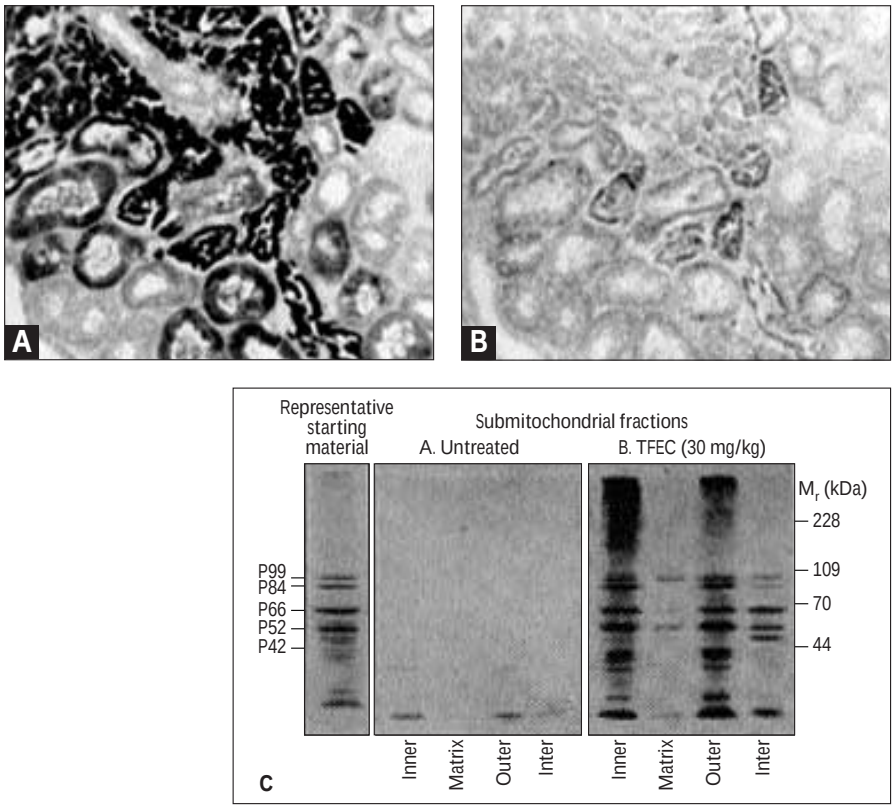

FIGURE 15-17

Covalent binding of a nephrotoxicant metabolite in vivo to rat kidney tissue, localization of binding to the mitochondria, and identification of three proteins that bind to the nephrotoxicant. **A**, Binding of tetrafluoroethyl-L-cysteine (TFEC) metabolites in vivo to rat kidney tissue detected immunohistochemically. Staining was localized to the S3 segments of the proximal tubule, the segment that undergoes necrosis. **B**, Immunoreactivity in untreated rat kidneys. **C**, Isolation and fractionation of renal cortical mitochondria from untreated and TFEC treated rats and immunoblot analysis revealed numerous proteins that bind to the nephrotoxicant (inner-inner membrane, matrix-soluble matrix, outer-outer membrane, inter-intermembrane space). The identity of three of the proteins that bound to the nephrotoxicant: P84, mortalin (HSP70-like); P66, HSP 60; and P42, aspartate aminotransferase. M_r—relative molecular weight. (*From* Hayden *et al.* [6], and Bruschi *et al.* [7]; with permission.)

Lipid peroxidation and mitochondrial dysfunction

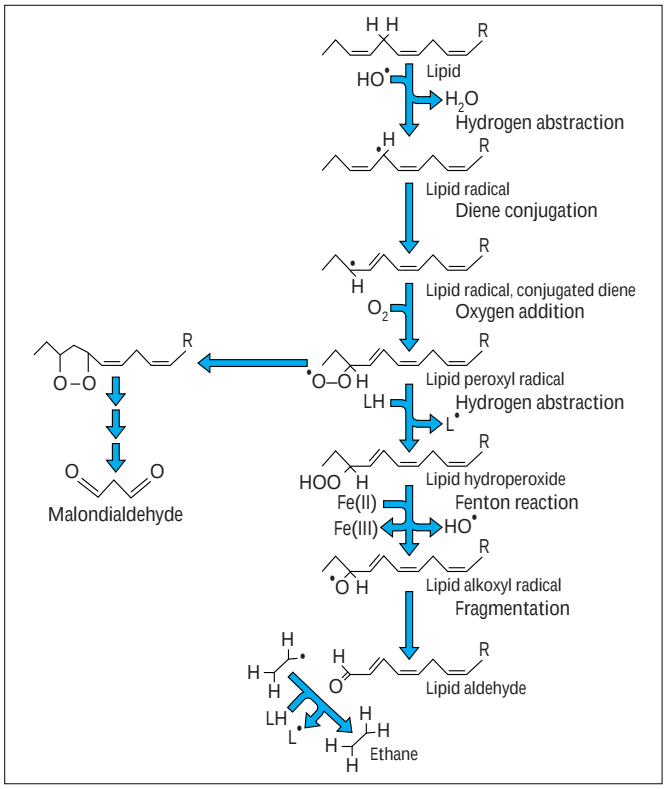

FIGURE 15-18

A simplified scheme of lipid peroxidation. The first step, hydrogen abstraction from the lipid by a radical (*eg*, hydroxyl), results in the formation of a lipid radical. Rearrangement of the lipid radical results in conjugated diene formation. The addition of oxygen results in a lipid peroxyl radical. Additional hydrogen abstraction results in the formation of a lipid hydroperoxide. The Fenton reaction produces a lipid alkoxyl radical and lipid fragmentation, resulting in lipid aldehydes and ethane. Alternatively, the lipid peroxyl radical can undergo a series of reactions that result in the formation of malondialdehyde.

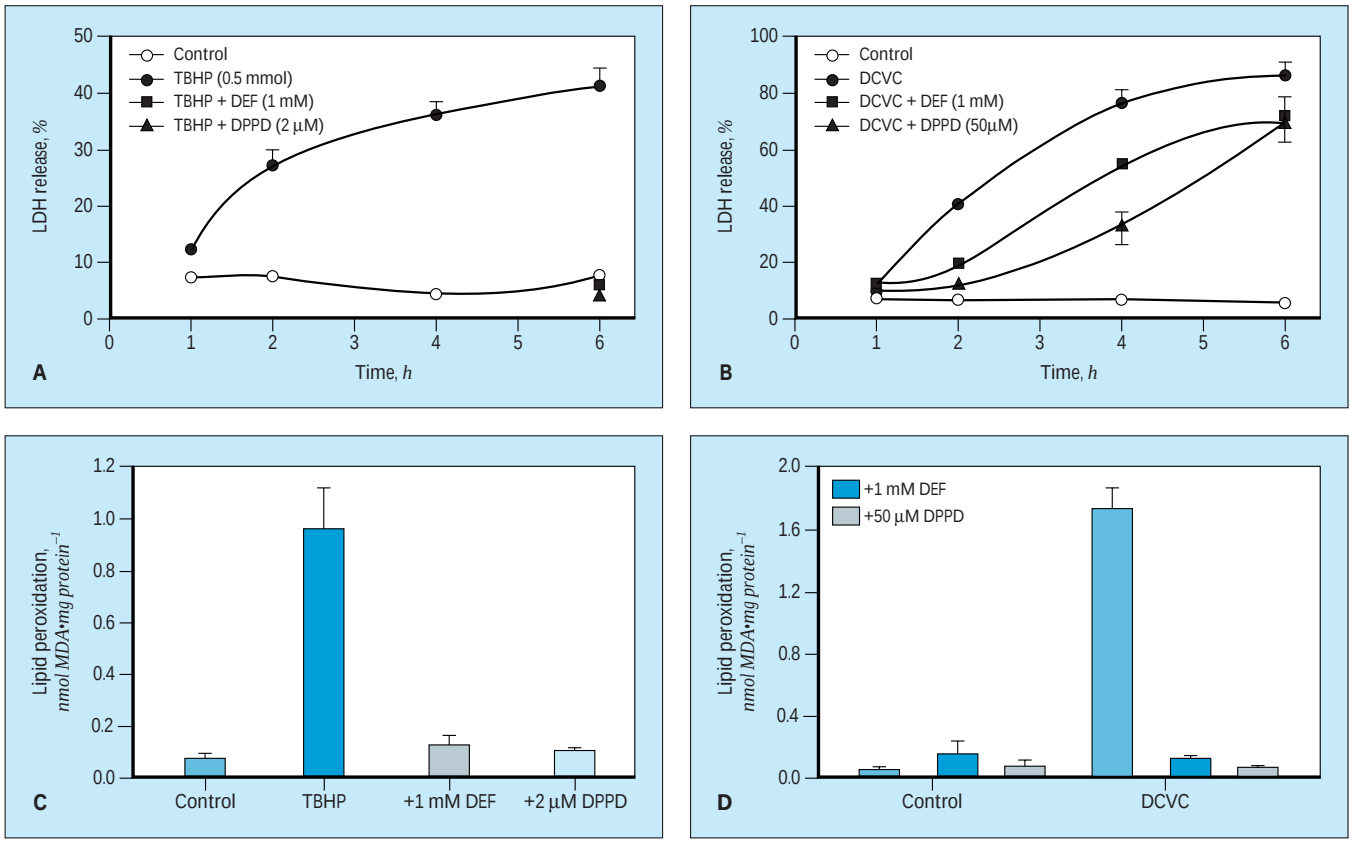

FIGURE 15-19

A–D, Similarities and differences between oxidant-induced and halocarbon-cysteine conjugate–induced renal proximal tubular lipid peroxidation and cell death. The model oxidant *t*-butylhydroperoxide (TBHP) and the halocarbon-cysteine conjugate dichlorovinyl-L-cysteine (DCVC) caused extensive lipid peroxidation after 1 hour of exposure and cell death (lactate dehydrogenase (LDH) release) over 6-hours' exposure. The iron chelator deferoxamine (DEF) and the antioxidant N,N'-diphenyl-1, 4-phenylenediamine (DPPD) completely blocked both the lipid peroxidation and cell death caused by TBHP. In contrast, while DEF and DPPD completely blocked the lipid peroxidation caused by DCVC, cell death was only delayed. These results suggest that the iron-mediated oxidative stress caused by TBHP is responsible for the observed toxicity, whereas the iron-mediated oxidative stress caused by DCVC accelerates cell death. One reason that cells die in the absence of iron-mediated oxidative stress is that DCVC causes marked mitochondrial dysfunction. (*Data from* Groves *et al.* [8], and Schellmann [9].)

ALTERATION OF RENAL TUBULAR CELL ENERGETICS AFTER EXPOSURE TO TOXICANTS

Decreased oxygen delivery secondary to vasoconstriction
Inhibition of mitochondrial respiration
Increased tubular cell oxygen consumption

FIGURE 15-20

Mechanisms by which nephrotoxicants can alter renal tubular cell energetics.

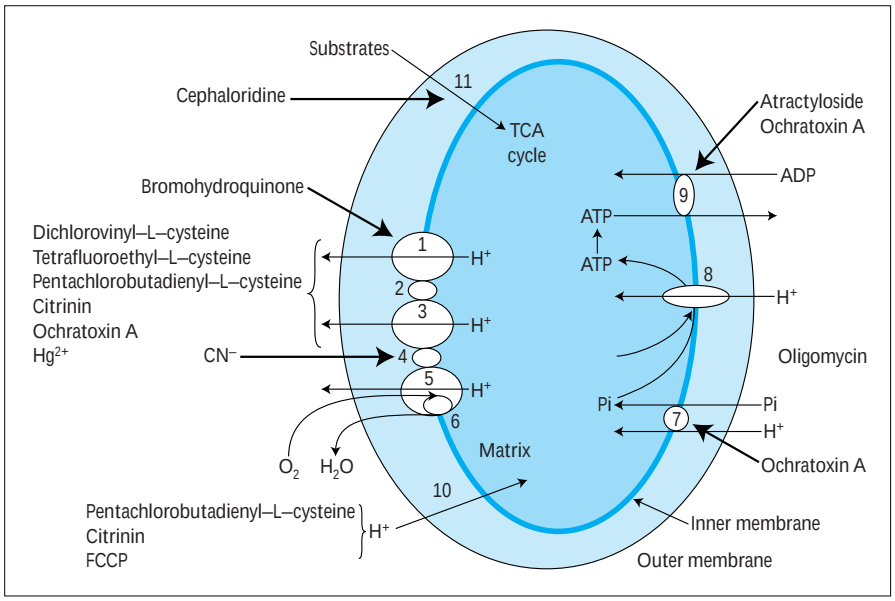

FIGURE 15-21

Some of the mitochondrial targets of nephrotoxicants: 1) nicotinamide adenine dinucleotide (NADH) dehydrogenase; 2) succinate dehydrogenase; 3) coenzyme Q–cytochrome C reductase; 4) cytochrome C; 5) cytochrome C oxidase; 6) cytochrome Aa_3; 7) H^+-Pi contransporter; 8) F_0F_1-ATPase; 9) adenine triphosphate/diphosphate (ATP/ADP) translocase; 10) protonophore (uncoupler); 11) substrate transporters.

Disruption of ion homeostasis

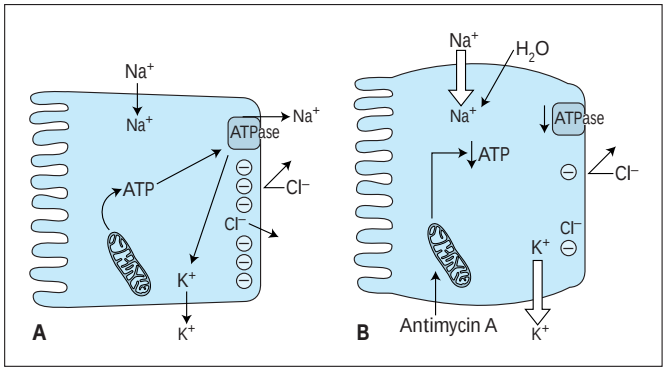

FIGURE 15-22

Early ion movements after mitochondrial dysfunction. **A**, A control renal proximal tubular cell. Within minutes of mitochondrial inhibition (*eg*, by antimycin A), ATP levels drop, resulting in inhibition of the Na^+, K^+-ATPase. **B**, Consequently, Na^+ influx, K^+ efflux, membrane depolarization, and a limited degree of cell swelling occur.

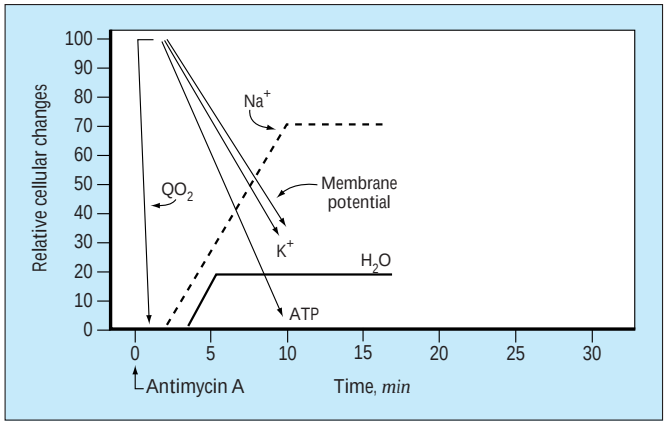

FIGURE 15-23

A graphic of the phenomena diagrammed in Figure 15-22.

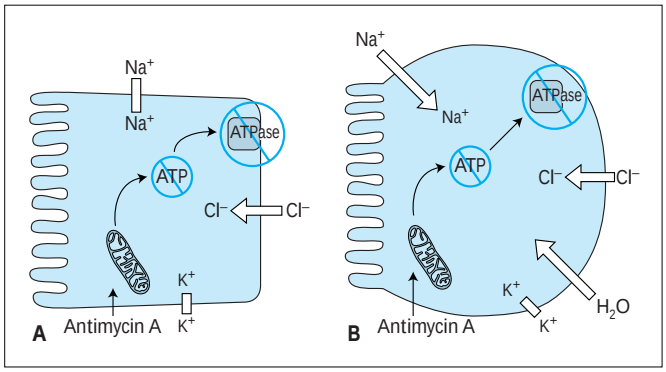

FIGURE 15-24

The late ion movements after mitochondrial dysfunction that leads to cell death/lysis. **A**, Cl^- influx occurs as a distinct step subsequent to Na^+ influx and K^+ efflux. **B**, Following Cl^- influx, additional Na^+ and water influx occur resulting in terminal cell swelling. Ultimately cell lysis occurs.

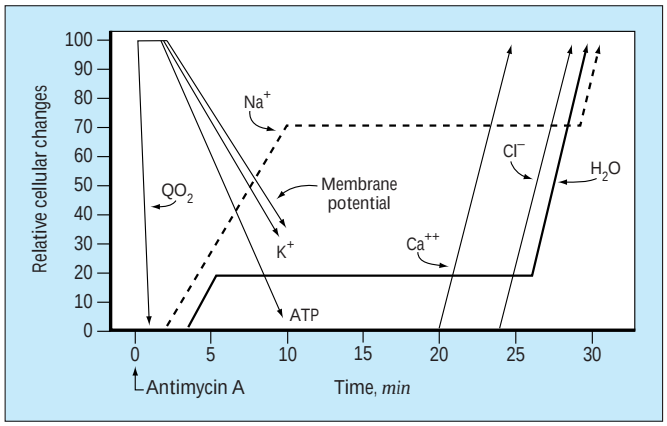

FIGURE 15-25
A graph of the phenomena depicted in Figures 15-22 through 15-24, illustrating the complete temporal sequence of events following mitochondrial dysfunction. QO_2—oxygen consumption.

Disregulation of regulatory enzymes

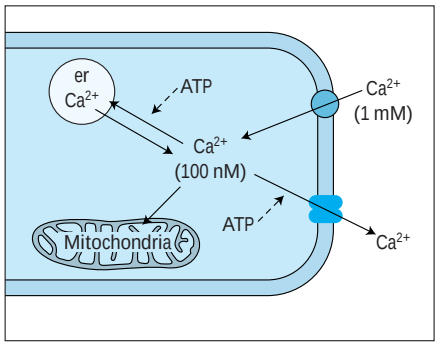

FIGURE 15-26
A simplified schematic drawing of the regulation of cytosolic free Ca^{2+}.

BIOCHEMICAL CHARACTERISTICS OF CALPAIN

Endopeptidase
Heterodimer: 80-kD catalytic subunit, 30-kD regulatory subunit
—Calpain and μ-calpain are ubiquitously distributed cytosolic isozymes
—Calpain and μ-calpain have identical regulatory subunits but distinctive catalytic subunits
—Calpain requires a higher concentration of Ca^{2+} for activation than μ-calpain
Phospholipids reduce the Ca^{2+} requirement
Substrates: cytoskeletal and membrane proteins and enzymes

FIGURE 15-27
Biochemical characteristics of calpain.

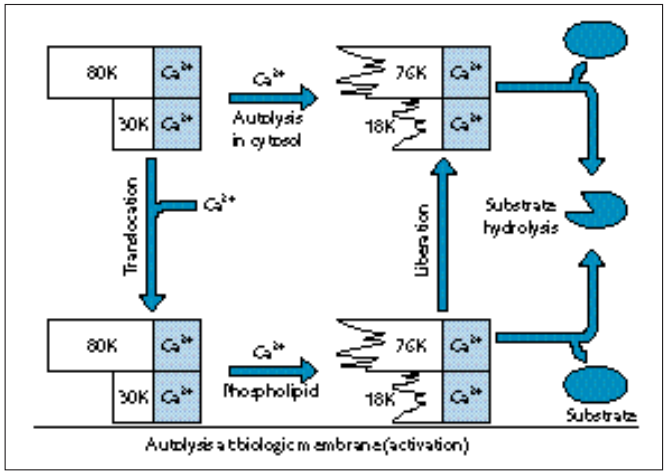

FIGURE 15-28
Calpain translocation. Proposed pathways of calpain activation and translocation. Both calpain subunits may undergo calcium (Ca^{2+})-mediated autolysis within the cytosol and hydrolyze cytosolic substrates. Calpains may also undergo Ca^{2+}-mediated translocation to the membrane, Ca^{2+}-mediated, phospholipid-facilitated autolysis and hydrolyze membrane-associated substrates. The autolyzed calpains may be released from the membrane and hydrolyze cytosolic substrates. (*From* Suzuki and Ohno [10], and Suzuki *et al.* [11]; with permission.)

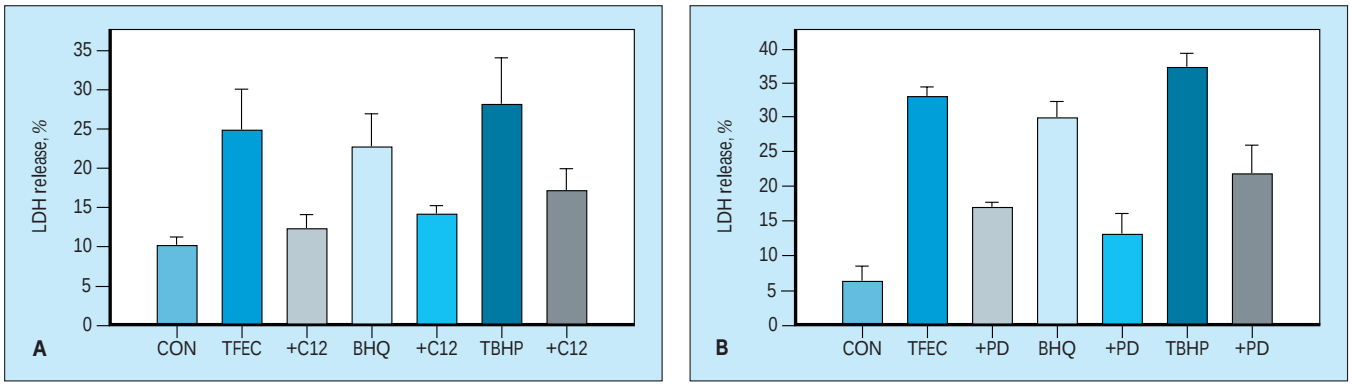

FIGURE 15-29

A, **B**, Dissimilar types of calpain inhibitors block renal proximal tubular toxicity of many agents. Renal proximal tubular suspensions were pretreated with the calpain inhibitor 2 (CI2) or PD150606 (PD). CI2 is an irreversible inhibitor of calpains that binds to the active site of the enzyme. PD150606 is a reversible inhibitor of calpains that binds to the calcium (Ca^{2+})-binding domain on the enzyme. The toxicants used were the haloalkane cysteine conjugate tetrafluoroethyl-L-cysteine (TFEC), the alkylating quinone bromohydroquinone (BHQ), and the model oxidant *t*-butylhydroperoxide (TBHP). The release of lactate dehydrogenase (LDH) was used as a marker of cell death. CON—control. (*From* Waters *et al*. [12]; with permission.)

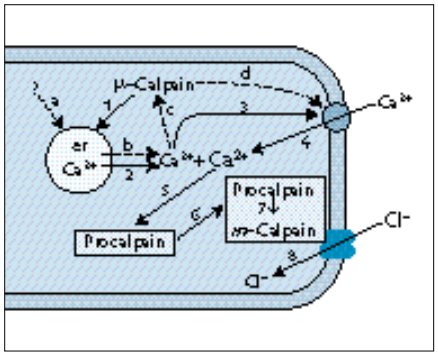

FIGURE 15-30

One potential pathway in which calcium (Ca^{2+}) and calpains play a role in renal proximal tubule cell death. These events are subsequent to mitochondrial inhibition and ATP depletion. 1) μ-Calpain releases endoplasmic reticulum (er) Ca^{2+} stores. 2) Release of er Ca^{2+} stores increases cytosolic free Ca^{2+} concentrations. 3) The increase in cytosolic free Ca^{2+} concentration mediates extracellular Ca^{2+} entry. (This may also occur as a direct result of er Ca^{2+} depletion.) 4) The influx of extracellular Ca^{2+} further increases cytosolic free Ca^{2+} concentrations. 5) This initiates the translocation of nonactivated *m*-calpain to the plasma membrane (6). 7) At the plasma membrane nonactivated *m*-calpain is autolyzed and hydrolyzes a membrane-associated substrate. 8) Either directly or indirectly, hydrolysis of the membrane-associated substrate results in influx of extracellular chloride ions (Cl^-). The influx of extracellular Cl^- triggers terminal cell swelling. *Steps a–d* represent an alternate pathway that results in extracellular Ca^{2+} entry. (*Data from* Waters *et al*. [12,13,14].)

PROPERTIES OF PHOSPHOLIPASE A_2 GROUP

Characteristics	Secretory	Cytosolic	Ca^{2+}-Independent	
Localization	Secreted	Cytosolic	Cytosolic	Membrane
Molecular mass	~14 kDa	~85 kDa	~40 kDa	unknown
Arachidonate preference	–	+	+	+
Ca^{2+} required	mM	(M	None	None
Ca^{2+} role	Catalysis	Memb. Assoc.	None	None

FIGURE 15-31

Biochemical characteristics of several identified phospholipase A_2s.

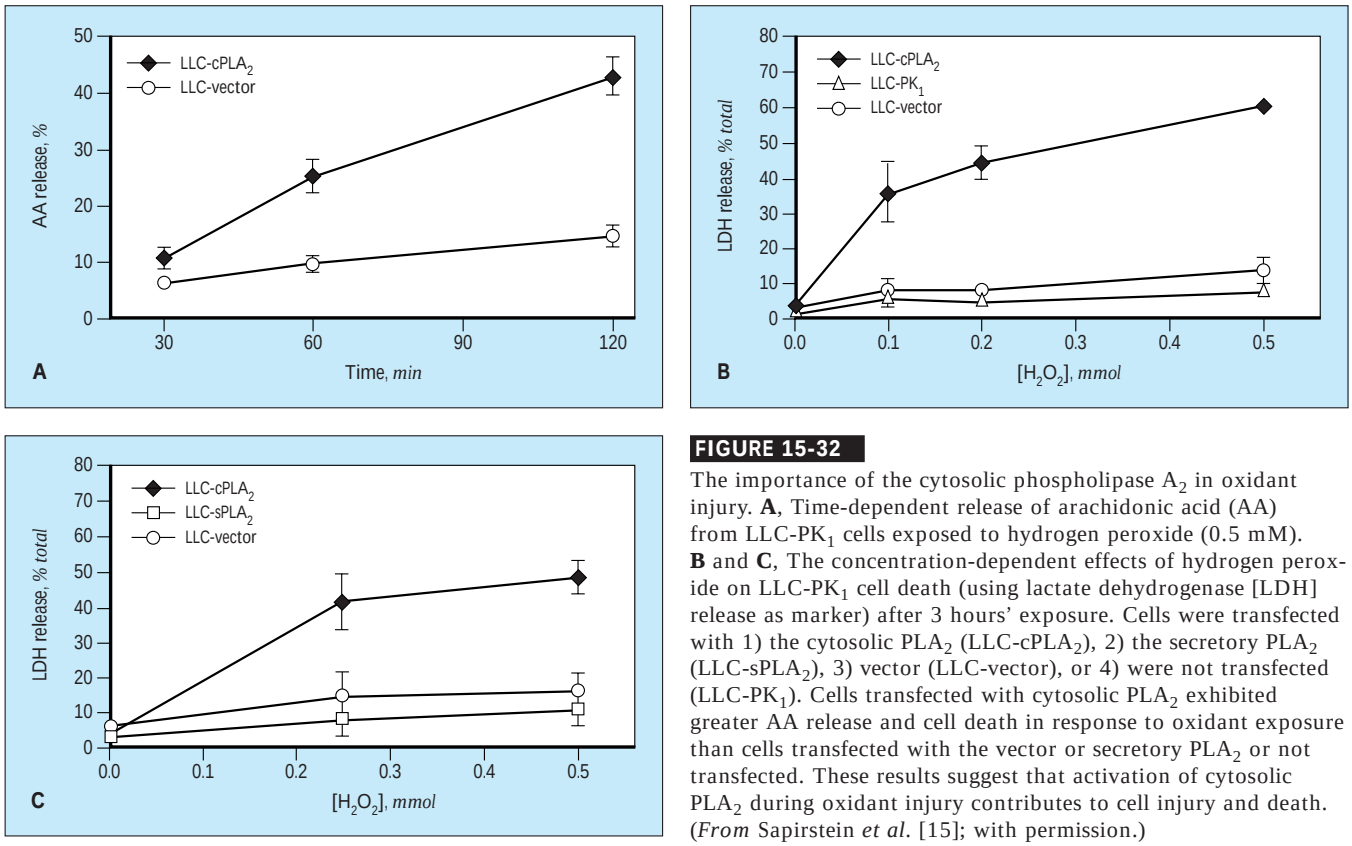

FIGURE 15-32

The importance of the cytosolic phospholipase A_2 in oxidant injury. **A**, Time-dependent release of arachidonic acid (AA) from LLC-PK_1 cells exposed to hydrogen peroxide (0.5 mM). **B** and **C**, The concentration-dependent effects of hydrogen peroxide on LLC-PK_1 cell death (using lactate dehydrogenase [LDH] release as marker) after 3 hours' exposure. Cells were transfected with 1) the cytosolic PLA_2 (LLC-$cPLA_2$), 2) the secretory PLA_2 (LLC-$sPLA_2$), 3) vector (LLC-vector), or 4) were not transfected (LLC-PK_1). Cells transfected with cytosolic PLA_2 exhibited greater AA release and cell death in response to oxidant exposure than cells transfected with the vector or secretory PLA_2 or not transfected. These results suggest that activation of cytosolic PLA_2 during oxidant injury contributes to cell injury and death. (*From* Sapirstein *et al.* [15]; with permission.)

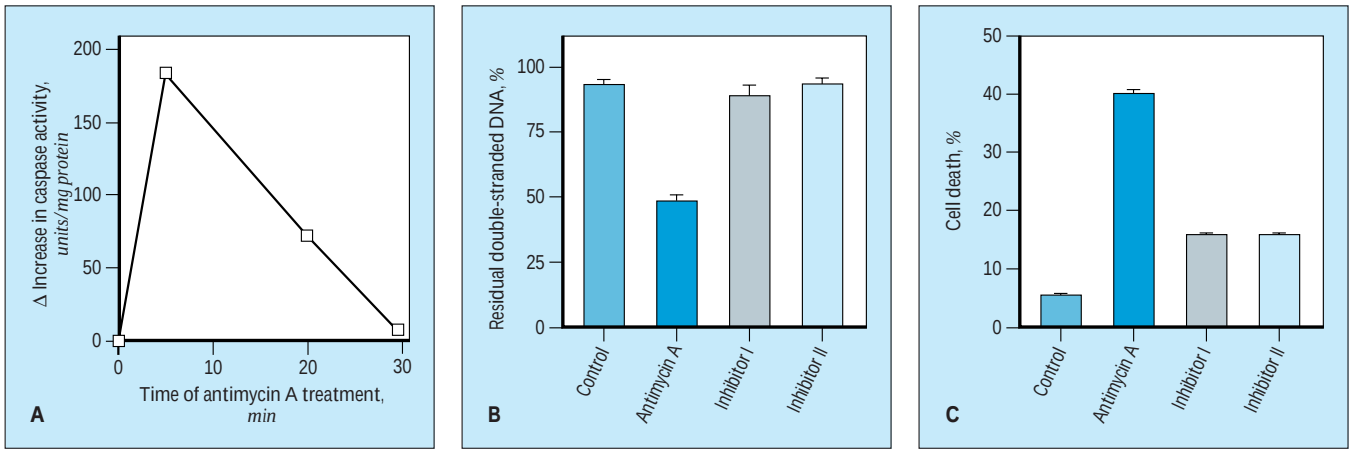

FIGURE 15-33

Potential role of caspases in cell death in LLC-PK_1 cells exposed to antimycin A. **A**, Time-dependent effects of antimycin A treatment on caspase activity in LLC-PK_1 cells. **B, C**, The effect of two capase inhibitors on antimycin A–induced DNA damage and cell death, respectively. Antimycin A is an inhibitor of mitochondrial electron transport. Inhibitor 1 is IL-1β converting enzyme inhibitor 1 (YVAD-CHO) and inhibitor II is CPP32/apopain inhibitor (DEVD-CHO). These results suggest that caspases are activated after mitochondrial inhibition and that caspases may contribute to antimycin A–induced DNA damage and cell death. (*From* Kaushal *et al.* [16]; with permission.)

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