General toxicology 4th stage

Toxic Responses of the Kidney

The functional integrity of the mammalian kidney is vital to total body homeostasis because of the kidney's role in the excretion of metabolic wastes, synthesis and release of the hormones renin and erythropoietin, and regulation of extracellular fluid volume, electrolyte composition, and acid–base balance.

Renal Vasculature and Glomerulus

The renal artery branches into afferent arterioles that supply the glomerulus, then blood leaves the glomerular capillaries through the efferent arteriole. Both the afferent and efferent arterioles control glomerular capillary pressure and the glomerular plasma flow rate. These arterioles are innervated by the sympathetic nervous system and respond to nerve stimulation, angiotensin II, vasopressin, endothelin, prostanoids, and cytokines, The glomerulus is a complex, specialized capillary bed that filters a portion of the blood into an ultrafiltrate that passes into the tubular portion of the nephron. Although the glomerular capillary wall permits a high rate of fluid filtration, it provides a significant barrier to the transglomerular passage of macromolecules; thus, small molecules such as inulin [molecular weight 5500] are freely filtered, whereas large molecules such as albumin (MW 56,000 to 70,000) are restricted. Filtration of anionic molecules tends to be restricted compared with that of neutral or cationic molecules of the same size.

Proximal Tubule

The proximal tubule consists of three discrete segments, The volume and composition of the glomerular filtrate are altered progressively as fluid passes through each of the different tubular segments. The proximal tubule reabsorbs approximately 60 to 80 percent of solute and water filtered at the glomerulus, mostly by means of numerous transport systems that are capable of driving the concentrative transport of many metabolic substrates. The proximal tubule also reabsorbs virtually all the filtered low-molecular-weight proteins by means of specific endocytotic protein reabsorption processes. Water reabsorption is through a passive iso-osmotic process, driven primarily by Na+ reabsorption, mediated by the Na+,K+-ATPase localized in the basolateral plasma membrane. In addition to active Na+ reabsorption, the proximal tubule reabsorbs other electrolytes, such as K+, HCO3 −, Cl–, PO43−, Ca2+, and Mg2+.

Loop of Henle

Approximately 25 percent of the filtered Na^+ and K^+ and 20 percent of the filtered water are reabsorbed by the segments of the loop of Henle

Distal Tubule and Collecting Duct

The macula densa contains specialized cells located between the end of the thick ascending limb and the early distal tubule, in close proximity to the afferent arteriole. Under normal physiologic conditions, increased solute delivery or concentration at the macula densa triggers a signal that results in afferent arteriolar constriction, leading to decreases in the glomerular filtration rate (GFR) (and hence decreased solute delivery). This regulatory mechanism is a volume-conserving mechanism that is designed to decrease GFR and prevent massive losses of fluid/electrolytes as a result of impaired tubular reabsorption. The renin-angiotensin system and other substances may be involved. The early distal tubule reabsorbs most of the remaining intraluminal Na^+, K^+, and Cl^- but is relatively impermeable to water.

The late distal tubule, cortical collecting tubule, and medullary collecting duct perform the final regulation and fine-tuning of urinary volume and composition. The remaining Na^+ is reabsorbed in conjunction with K^+ and H^+ secretion in the late distal tubule and the cortical collecting tubule. The combination of medullary and papillary hypertonicity generated by countercurrent multiplication and the action of antidiuretic hormone (vasopressin, ADH) enhances water permeability of the medullary collecting duct.

Acute Renal Failure

One of the most common manifestations of nephrotoxic damage is acute renal failure (ARF), which is characterized by an abrupt decline in GFR with resulting azotemia or a buildup of nitrogenous wastes in the blood

GFR depends on four factors:

(1) adequate blood flow to the glomerulus;

(2) adequate glomerular capillary pressure;

(3) glomerular permeability; and

(4) low intra tubular pressure.

Any decline in GFR is complex and may result from prerenal factors (renal vasoconstriction, intravascular volume depletion, and insufficient cardiac output), postrenal factors (ureteral or bladder obstruction), and intrarenal factors (glomerulonephritis, tubular cell injury, death, and loss resulting in back leak; renal vasculature damage, interstitial nephritis). Afferent arteriolar constriction decreases GFR by reducing blood flow, resulting in diminished capillary pressure,also Obstruction of the tubular lumen by cast formation increases tubular pressure; when tubular pressure exceeds glomerular capillary pressure, filtration decreases or ceases. In other hand Back-leak occurs when the paracellular space between cells

increases and the glomerular filtrate leaks into the extracellular space and bloodstream

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 and obstruction due to precipitation of a drug or endogenous compound within the kidney. Intrarenal factors include direct tubular obstruction and dysfunction,with or without inflammation, resulting in tubular back-leak and increased tubular pressure. Vascular damage, with or without inflammation, leading to hemodynamic changes. Alterations in the levels of a variety of vasoactive mediators may result in decreased renal perfusion pressure or efferent arteriolar tone and increased afferent arteriolar tone, leading to decreased glomerular hydrostatic pressure

Adaptation after Toxic Insult

The kidney has a remarkable ability to compensate for a loss in renal functional mass. After unilateral nephrectomy, GFR of the remnant kidney increases by approximately 40 to 60 percent. Compensatory increases in single-nephron GFR are accompanied by proportionate increases in proximal tubular water and solute reabsorption; glomerulotubular balance therefore is maintained, and overall renal function appears normal on standard clinical tests. Consequently, chemically induced changes in renal function may not be detected until these compensatory mechanisms are overwhelmed by significant nephron loss and/or damage.

There are a number of cellular and molecular responses to a nephrotoxic insult. After a population of renal cells is exposed to a toxicant, a fraction of the cells will be severely injured and undergo cell death by apoptosis or oncosis . Cells with nonlethal injuries may undergo cell repair and/or adaptation, which contribute to the structural and functional recovery of the nephron. In addition, there is a population of uninjured cells that may undergo compensatory hypertrophy, cellular adaptation, and cellular proliferation. The cellular proliferation and compensatory hypertrophy contribute to the structural and functional recovery of the nephron. Two of the most notable cellular adaptation responses are metallothionein induction and stress protein induction. Heat-shock proteins (Hsps) and glucose-regulated proteins (Grps) are two examples of stress protein families that are induced in response to a number of pathophysiologic states such

as heat shock, anoxia, oxidative stress, toxicants, heavy metal exposure, and tissue trauma. The distribution of individual heat-shock proteins (Hsps) and glucose-regulated proteins (Grps) varies between different cell types in the kidney and within subcellular compartments. These proteins are involved in the maintenance of normal protein structure and the degradation of damaged proteins and provide a defense mechanism against toxicity by facilitating recovery and repair.

Chronic Renal Failure

Progressive deterioration of renal function may occur with long-term exposure to various chemicals. After nephron loss, adaptive increases in glomerular pressures and flows increase the single-nephron GFR of remnant viable nephrons, which serve to maintain whole-kidney GFR. With time, these alterations are maladaptive, and a focal glomerulosclerosis eventually develops that may lead to tubular atrophy and interstitial fibrosis. Compensatory increases in glomerular pressures and flows of the remnant glomeruli may result in mechanical damage to the capillaries, leading to altered permeabilities

Glomerular Injury

The glomerulus is the initial site of chemical exposure in the nephron, and a number of nephrotoxicants alter glomerular permeability to proteins.

Cyclosporine, amphotericin B, and gentamicin impair glomerular ultrafiltration without a significant loss of structural integrity and decreased GFR. Amphotericin B decreases GFR by causing renal vasoconstriction and decreasing the glomerular capillary ultrafiltration coefficient (K_f). Gentamicin interacts with the anionic sites on the endothelial cells, decreasing K_f and GFR. Finally, cyclosporine not only causes renal vasoconstriction and vascular damage but is injurious to the glomerular endothelial cell.

Proximal Tubular Injury

The proximal tubule is the most common site of toxicant-induced renal injury. The proximal tubule has a leaky epithelium that favors the flux of compounds into proximal tubular cells. More importantly, tubular transport of organic anions and cations, low-molecular-weight proteins and peptides, GSH conjugates,and heavy metals is localized primarily if not exclusively to the proximal tubule. Thus, transport of these molecules will be greater in the proximal tubule than in other segments, resulting in proximal tubular accumulation and toxicity. drugs such as aminoglycosides, β-lactam antibiotics,and cisplatin; environmental chemicals such as ochratoxin and metals such as cadmium and mercury. The nephrotoxic potential of xenobiotics depends on the intrinsic reactivity of the drug with subcellular or molecular targets. Both cytochrome P450 and cysteine conjugate B-lyase are localized almost exclusively in the proximal tubule, and bioactivation contributes at least in part to the proximal tubular lesions produced by chloroform (via cytochrome P450) and haloalkene S-conjugates (via cysteine B-lyase). Finally, proximal tubular cells appear to be more susceptible to ischemic injury than are distal tubular cells

Loop of Henle/Distal Tubule/Collecting Duct Injury

Functional abnormalities at these sites manifest primarily as impaired concentrating ability and/or acidification defects. Amphotericin B, cisplatin, and methoxyflurane induce an ADH-resistant polyuria, suggesting that the concentrating defect occurs at the level of the medullary thick ascending limb and/or the collecting

duct. Amphotericin B is highly lipophilic and interacts with lipid sterols such as cholesterol, resulting in the formation of transmembrane channels or pores and disrupting membrane permeability. Thus, amphotericin effectively transforms the tight distal tubular epithelium into one that is leaky to water and ions and impairs reabsorption at these sites. The mechanisms mediating cisplatin-induced polyuria are not completely understood, but the first phase is responsive to vasopressin and inhibitors of prostaglandin synthesis. Methoxyflurane nephrotoxicity is associated with the inhibitory effects of the metabolite fluoride on solute and water reabsorption. Fluoride inhibits sodium chloride reabsorption in the thick ascending limb and inhibits ADH-mediated reabsorption of water, possibly due to disruption in adenylate cyclase

Papillary Injury

The renal papilla is susceptible to the chronic injurious effects of abusive consumption of analgesics. The initial target is the medullary interstitial cells, followed by degenerative changes in the medullary capillaries, loops of Henle, and collecting ducts. The important factor may contribute to this site-selective injury, including high papillary concentrations of potential toxicants and inhibition of vasodilatory prostaglandins,compromising RBF to the renal medulla/papilla and resulting in tissue ischemia.

ASSESSMENT OF RENAL FUNCTION

Evaluation of the effects of a chemical on the kidney can be accomplished using a variety of both in vivo and in vitro methods. Initially, nephrotoxicity can be assessed by evaluating serum and urine chemistries following treatment with the chemical in question. The standard battery of noninvasive tests includes measurement of urine volume and osmolality, pH, and urinary composition (e.g., electrolytes, glucose, and protein). Although specificity is often lacking in such an assessment, urinalysis provides a relatively easy and noninvasive assessment of overall renal functional integrity and can provide some insight into the nature of the nephrotoxic insult. Urinary excretion of high-molecular-weight proteins, such as albumin,is suggestive of glomerular damage, whereas excretion of low-molecular-weight proteins, such as β2-microglobulin, suggests proximal tubular injury. Urinary excretion of enzymes localized in the brush border (e.g., alkaline phosphatase, γ -glutamyl transpeptidase) may reflect brush-border damage, whereas urinary excretion of other enzymes (e.g., lactate dehydrogenase)mayreflect more generalized cell damage.

GFR can be measured directly by determining creatinine or inulin clearance. Creatinine is an endogenous compound released from skeletal muscle at a constant rate under most circumstances.Further, it is completely filtered with limited secretion. Inulin is an exogenous compound that is completely filtered with no reabsorption or secretion. Indirect markers of GFR are serial blood urea nitrogen (BUN) and serum creatinine concentrations. Chemically induced increases in BUN and/or serum creatinine may not necessarily reflect renal damage but rather may be secondary to dehydration, hypovolemia, and/or protein catabolism.

Biochemical Mechanisms/Mediators of Renal Cell Injury

Cell Death

cell death is thought to occur through either oncosis or apoptosis. apoptosis is a tightly controlled, organized process that usually affects scattered individual cells the cell breaks into small fragments that are phagocytosed by adjacent cells or macrophages without producing an inflammatory response. In contrast, oncosis often affects many contiguous cells;the organelles swell, cell volume increases, and the cell ruptures with the release of cellular contents, followed by inflammation. With many toxicants, lower but injurious concentrations produce cell death through apoptosis .As the concentration of the toxicant increases, oncosis plays a predominant role. However,because apoptosis is an ATP-dependent process, for those toxicants that target the mitochondrion, oncosis may be the predominant pathway with only limited apoptosis occurring.

Mediators of Toxicity

Nephrotoxicants generally are thought to produce cell injury and death through a variety of 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 and 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 causes oxidative damage and cell injuryAmphotericin B reacts with plasma membrane sterols, increasing membrane permeability; fumonisin B1 inhibits sphinganine (sphingosine) *N*-acyl transferase; and Hg2+ binds to sulfhydryl groups on cellular proteins. In contrast, some chemicals are not toxic until they are biotransformed to a reactive intermediate. Biologically reactive intermediates, also known as alkylating agents, are electron-deficient compounds (electrophiles) that bind to cellular nucleophiles (electron-rich compounds) such as proteins and lipids.For example, acetaminophen and chloroform are metabolized in the mouse kidney by cytochrome P450 to the reactive intermediates, *N*-acetyl-*p*-benzoquinoneimine and phosgene, respectively. Finally, chemicals may initiate injury indirectly by inducing oxidative stress via increased production of ROS, such as superoxide anion, hydrogen peroxide, and hydroxyl radicals. Oxidative stress has been proposed to contribute, at least in part, to the nephrotoxicity associated with ischemia/reperfusion injury, gentamicin, cyclosporine,cisplatin, and haloalkene cysteine conjugates.

Cell Volume and Ion Homeostasis

Cell volume and ion homeostasis are tightly regulated and are critical for the reabsorptive properties of the tubular epithelial cells. Toxicants generally disrupt cell volume and ion homeostasis either by increasing ion permeability or by inhibiting

energy production. The loss of ATP results in the inhibition of membrane transporters that maintain the internal ion balance. Following ATP depletion, Na+, K+-ATPase activity decreases, resulting in K+ efflux, Na+ and Cl– influx, cell swelling, and ultimately cell membrane rupture.

Cytoskeleton and Cell Polarity

Toxicants may cause early changes in membrane integrity such as loss of the brush border, blebbing of the plasma membrane, or alterations in membrane polarity. These changes can result from toxicant-induced alterations in cytoskeleton components and cytoskeletal-membrane interactions or may be associated with perturbations in energy metabolism or calcium and phospholipid homeostasis. Under control conditions, the tubular epithelial cell is polarized with respect to certain transporters and enzymes. During in vivo ischemia and in vitro ATP depletion, there is a dissociation of Na^+, K^+-ATPase from the actin cytoskeleton and redistribution from the basolateral membrane to the apical domain in renal proximal tubule cells.

Mitochondria

Numerous nephrotoxicants cause mitochondrial dysfunction through compromised respiration and ATP production or another cellular process. It is clear that mitochondria play a critical role in determining whether cells die by apoptosis or oncosis, depending on cellular ATP levels. For example,pentachlorobutadienyl-l-cysteine initially uncouples oxidative phosphorylation in renal proximal tubular cells by dissipating the proton gradient, whereas TFEC does not uncouple oxidative phosphorylation but rather inhibits state 3 respiration by inhibiting sites I and II of the electron transport chain. Whether toxicants target mitochondria directly or indirectly, it is clear that mitochondria play a critical role in determining whether cells die by apoptosis or oncosis. The mitochondrial permeability transition (MPT) is characterized by the opening of a high conductance pore that allows solutes of <1500 molecular weight to pass. It is thought that the MPT occurs during cell injury and ultimately progresses to apoptosis if sufficient ATP is available or oncosis if ATP is depleted. Further, the release of apoptotic proteins such as apoptosis inducing factor (AIF), cytochrome c, EndonucleaseG following MPT play a key role in activating downstream caspases and executing apoptosis.

Lysosomes

Lysosomes, which are key subcellular targets of aminoglycosides, unleaded gasoline, and d-limonene, are believed to induce cellular injury through rupture and release of lysosomal enzymes and toxicants into the cytoplasm after excessive accumulation of reabsorbed toxicant(s) and lysosomal overload.

Ca^{2+} Homeostasis

Ca2+ is a second messenger and plays a critical role in a variety of cellular functions. The distribution of Ca2+ within renal cells is complex and involves binding to anionic sites on macromolecules and compartmentation within subcellular organelles.. The critical cellular Ca^{2+} pool for regulation is the free Ca^{2+} present in the cytosol at a concentration of approximately 100 nM. This level is maintained by a series of pumps and channels located on the plasma membrane and the endoplasmic reticulum (ER). Sustained elevations or abnormally large increases in cytosolic free Ca2+ can exert a number of detrimental effects on the cell. For example, an increase in cytosolic free Ca2+ can activate a number of degradative Ca2+-dependent enzymes, such as phospholipases and proteinases (e.g., calpains), and can produce aberrations in the structure and function of cytoskeletal elements

Phospholipases

The phospholipase A_2 (PLA_2) family of enzymes hydrolyzes phospholipids. A supraphysiologic increase in PLA_2 activity can result in the loss of membrane phospholipids and consequently impair membrane function.

Endonucleases

Endonuclease activation and the associated DNA cleavage have been suggested to play a role in renal cell oncosis and apoptosis after hypoxia/reoxygenation.

Proteinases

Calpains are likely candidates for a role in cell death because they are cysteine proteinases; they are activated by calcium; and they have cytoskeletal proteins, membrane proteins, and enzymes as substrates. Supraphysiologic activation of proteinases can disrupt normal membrane and cytoskeleton function and lead to cell death. Under conditions of cell injury, the lysosomal membrane can rupture, releasing hydrolases into the cytosol to degrade susceptible proteins. In addition, caspases are another class of cysteine proteinases that play a role in renal cell death.

Specific Nephrotoxicants

Mercury

The kidneys are the primary target organs for the accumulation of Hg^{2+}. The acute nephrotoxicity induced by $HgCl_2$ is characterized by proximal tubular necrosis and ARF within 24 to 48 h after administration. Early markers of $HgCl_2$-induced renal dysfunction include an increase in the urinary excretion of brush-border enzymes such as alkaline phosphatase and gamma-GT. Subsequently, when tubular injury becomes severe, intracellular enzymes such as lactate dehydrogenase and aspartate aminotransferase increase in the urine. As injury progresses, tubular reabsorption of solutes and water decreases. Changes in mitochondrial morphology and function are very early events after $HgCl_2$ administration, supporting the hypothesis that mitochondrial dysfunction is an early and important contributor to inorganic mercury-induced cell death along the proximal tubule

Tetrafluoroethylene

Tetrafluoroethylene is conjugated with glutathione in the liver, and the glutathione conjugate is secreted into the bile and small intestine, where it is degraded to the cysteine *S*-conjugate , reabsorbed, and transported to the kidney. Although several metabolites are formed, the cysteine *S*-conjugate is the penultimate nephrotoxicant. After transport into the proximal tubule, the cysteine *S*-conjugate is a substrate for the cytosolic and mitochondrial forms of the enzyme cysteine conjugate b-lyase. The products of the reaction are ammonia, pyruvate, and a reactive thiol that is capable of binding covalently to cellular macromolecules, causing cellular damage. Functionally, increases in urinary glucose, protein, cellular enzymes

Nonsteroidal Anti-Inflammatory Drugs

At least three different types of nephrotoxicity have been associated with NSAID administration. ARF may occur within hours after a large dose of a NSAID, is usually reversible upon withdrawal of the drug, and is characterized by decreased RBF and GFR and by oliguria. When the normal production of vasodilatory prostaglandins is inhibited by NSAIDs, vasoconstriction induced by circulating catecholamines and angiotensin II is unopposed, resulting in decreased RBF and ischemia.

In contrast, chronic consumption of NSAIDs and/or APAP (>3 years) results in an often irreversible nephrotoxicity. The primary lesion in this nephropathy is papillary necrosis with chronic interstitial nephritis. The mechanism by which NSAIDs produce analgesic nephropathy is not known, but the process may result from chronic medullary/papillary ischemia secondary to renal vasoconstriction or from the genesis of a reactive intermediate that in turn initiates an oxidative stress or binds covalently to critical cellular macromolecules.

The third, type of nephrotoxicity associated with NSAIDs is an interstitial nephritis. These patients normally present with elevated serum creatinine and proteinuria. If NSAIDs are discontinued, renal function improves in 1 to 3 months

Aminoglycosides

Renal dysfunction caused by aminoglycosides is characterized by nonoliguric renal failure with reduced GFR, an increase in serum creatinine and BUN, and polyuria. Polyuria is an early event following aminoglycoside administration and may be due to inhibition of chloride transport in the thick ascending limb. Aminoglycosides are highly polar cations; they are almost exclusively filtered by the glomerulus and excreted unchanged. Filtered aminoglycosides undergo proximal tubular reabsorption by binding to anionic phospholipids in the brush border, followed by endocytosis and sequestration in lysosomes of the S1 and S2 segments of proximal tubules. The earliest lesion observed following clinically relevant doses of aminoglycosides is an increase in the size and number of lysosomes. These lysosomes contain *myeloid bodies*, which are electron-dense lamellar structures containing undergraded phospholipids. The renal phospholipidosis produced by the

aminoglycosides is thought to occur through their inhibition of lysosomal hydrolases, such as sphingomyelinase and phospholipases. Whereas phospholipidosis plays an important role in aminoglycoside nephrotoxicity, the steps between the phospholipid accumulation in the lysosomes and tubular cell death are less clear.

Cyclosporine

Cyclosporine-induced nephrotoxicity may manifest as (1) acute reversible renal dysfunction, (2) acute vasculopathy, and (3) chronic nephropathy with interstitial fibrosis. Acute renal dysfunction is characterized by dose-related decreases in RBF and GRF and increases in BUN and serum creatinine. The decrease in RBF and GFR is related to marked vasoconstriction induced by cyclosporine; and it is probably produced by a number of factors, including an imbalance in vasoconstrictor and vasodilatory prostaglandin production. In particular, increased production of the vasoconstrictor thromboxane A2 appears to play a role in cyclosporine-induced ARF. Endothelin may contribute to constriction of the afferent arteriole because endothelin receptor antagonists inhibit cyclosporine-induced vasoconstriction. Acute vasculopathy or thrombotic microangiopathy is a rather unusual nephrotoxic lesion that affects arterioles and glomerular capillaries, without an inflammatory component, following cyclosporine treatment. Hyaline and/or fibroid changes, often with fibrinogen deposition, are observed in arterioles, whereas thrombosis with endothelial cell desquamation affects the glomerular capillaries. Long-term treatment with cyclosporine can result in chronic nephropathy with interstitial fibrosis and tubular atrophy. Modestelevations in serum creatinine and decreases in GFR occur alongwith hypertension, proteinuria, and tubular dysfunction. These lesions may not be reversible if cyclosporine therapy is discontinued and may result in end-stage renal disease. Whereas the mechanism of chronic cyclosporine nephropathy is not known, vasoconstriction probably plays a contributing role.

Mycotoxins

Mycotoxins are products of molds and fungi and a number of mycotoxins produce nephrotoxicity such as aflatoxin B1, citrinin, ochratoxins, fumonisins, and patulin. Citrinin nephrotoxicity is characterized by decreased urine osmolality, GFR and RBF, and increased urinary enzyme excretion. Interestingly, the location of citrinin-induced tubular damage (proximal, distal) varies among species. Whereas the mechanism of citrinin toxicity to the tubules remains unresolved, citrinin enters the cells through the organic anion transporter and causes mitochondrial dysfunction. While acute exposures of ochratoxin A produce similar effects on the kidney as citrinin, chronic exposures result in progressive tubular atrophy and fibrosis. Fumonisins B1 and B2 are commonly found on corn and corn products and produce nephrotoxicity in rats and rabbits .Histologic examination of the kidney revealed disruption of the basolateral membrane, mitochondrial swelling, increased numbers of clear and electron-dense vacuoles, and apoptosis in proximal tubular cells at the junction of the cortex and medulla. Changes in renal function included increased urine volume, decreased osmolality, and increased excretion of low- and high-molecular-weight proteins. The fumonisins are structurally similar to sphingoid bases and are thought to produce their toxicity through the inhibition of

sphinganine (sphingosine) *N*-acyltransferase. Inhibition of this enzyme results in an increase in the ratio of free sphinganine to free sphingosine and a decrease in complex sphingolipids.