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Metabolic interactions between drugs and renal tubulo-interstitial cells: Role in nephrotoxicity

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A growing number of drugs exhibit selective toxicity for the kidney, the clinical expression of which may range from a mild disturbance of a discrete function to a generalized depression of all functions. The magnitude of the problem has stimulated research into the mechanisms underlying the susceptibility of the kidney to drug-induced alterations of function and structure. From such studies has emerged new insights into the metabolic interactions between specific drugs and renal tubulo-interstitial cells that underlie the development of toxic injury. This paper will review current concepts of the selective susceptibility of the renal tubulointerstitium to drug-induced toxicity. Space does not permit a comprehensive treatment of this complex subject; therefore a limited number of drugs or other xenobiotics to serve as examples of generic mechanisms have been selected for discussion.

Determinants of drug cytotoxicity

The toxic as well as the therapeutic activity of a drug is critically dependent on the concentration of the active moiety reaching sensitive target sites. In the case of the kidney, exposure to increased concentration of a drug or its metabolite typically derives from the fact that the kidney is the major route by which the offending agent is eliminated from the body. Depending on the specific mechanisms mediating drug elimination by the kidney, different portions of the nephron may be exposed to the drug or its metabolite. For example, the renal excretion of certain drugs is accomplished by tubular secretion via the organic anion transport system of proximal tubular cells. Consequently these cells may be exposed to high intracellular concentrations of potentially toxic agents. In other instances active tubular absorption may generate toxic concentrations of the offending agent. Any time the fractional absorption of water exceeds the fractional absorption of drug the lumenal concentration of drug will rise. This pattern is commonly seen with polar drugs or their derivatives, and during antidiuresis high concentrations of these compounds may be generated in distal tubular fluid, so that despite low permeability of the lumenal membrane, collecting duct cells may be exposed to toxic intracellular concentrations of drug.

In other instances the susceptibility of the kidney to the toxic effect of a drug derives from the fact that during the process of renal excretion the drug undergoes bioactivation to a reactive metabolite [1]. Xenobiotics are metabolized by a wide variety of enzymes and these have been classified under two broad categories [2, 3]. Phase I reactions include oxidations, reductions and hydrolyses, the products of which may be highly reactive and potentially toxic because of their ability to induce oxidative stress and/or to bind to macromolecules. Phase I reactions are commonly coupled to phase II reactions, which in general are conjugation or synthetic mechanisms such as glucuronidation, sulfation, acylation, methylation and glutathionation. Phase II reactions usually generate highly polar, water soluble, and biologically inactive products which can be readily excreted in the urine. However, in some cases phase II reaction products prove to be toxic. The optimal arrangement for protecting the cell against injury from phase I reactions is to have the metabolic machinery for phase I and phase II reactions in the same cell. As will become apparent this arrangement does not exist for all regions of the kidney. Moreover, even when present these pathways may become saturated or critical components may be depleted so that toxic intermediates may accumulate.

The largest capacity for the metabolic transformation of drugs resides in the liver. The same enzymatic pathways present in the liver are also found in the kidney, although the specific activity of these pathways in the kidney are, as a general rule, substantially lower than those in the liver [2, 3]. Of toxicological significance these metabolic pathways are not uniformly distributed throughout the kidney; rather they are localized to specific nephron segments or regions of the kidney (Table 1). This heterogenous metabolic profile of the kidney explains the susceptibility of certain areas of the kidney to injury from certain xenobiotics.

Figure 1 summarizes the major pathways by which a drug (X), a stable metabolite (M_x) or a reactive metabolite (M_x^*) may interact adversely with a cell. In general the toxic effect of a drug on an intracellular target is determined by the concentrationz of the toxicant at the target site, and the duration of exposure of the target to the offending drug or metabolite. Major determinants for pathways 1 and 2 (Fig. 1) include: the quantity of drug ingested; the rate of drug metabolism at an extrarenal site, typically the liver; the rate at which drug or metabolite is delivered to the kidney; and the rate at which drug or metabolite is transported into and out of the cell. The activity of pathway 6 (Fig. 1), in addition to the factors just mentioned,

Table 1. Intrarenal distribution of enzymes that participate in the biotransformation of xenobiotics

	RC or PT	OM	IM	Reference
Cytochrome P-450 MFO	4+	2+	tr	[34–36]
NADPH cytochrome reductase	4+	2+	2+	[34, 36]
UDP-glucuronosyl transferase	4+	2+	–	[95]
Sulfotransferase	4+	2+	–	[95]
GSSG reductase	4+	2+	2+	[80]
γGT	4+	2+	tr	[80, 84, 86]
GPx-I	4+	2+	tr	[80]
GPx-II	4+	2+	1+	[80]
PGES	tr	2+	4+	[70, 72–74]

The highest activity for a specific enzyme among the three regions of the kidney was designated 4+ irrespective of the absolute activity. Abbreviations are: RC, renal cortex; PT, proximal tubule; OM, outer medulla; IM, inner medulla; γGT, γ-glutamyltranspeptidase; GPx-I, glutathione peroxidase-I; GPx-II, glutathione peroxidase-II; PGES, prostaglandin endoperoxide synthetase; tr, trace.

will be determined by the rate at which the reactive metabolite (M_x) is generated (pathways 3 and 4) and the rate at which the reactive metabolite is inactivated (pathway 5). The activity of each pathway may be influenced by genetic factors, by drugs which induce or inhibit the pathway, and by the availability of co-factors required for optimal function of the pathway. In subsequent sections representative examples of these different pathways will be discussed.

Role of active transport

Aminoglycoside antibiotics (AGs)

AGs exhibit the capacity to disrupt the metabolism, function and structure of renal proximal tubular cells [4, 5]. In humans even therapeutic doses of these drugs commonly cause proximal tubular cell necrosis. The toxicity of AGs is determined by the interaction of two processes: the extent to which these drugs are accumulated by proximal tubular cells and the potential of these drugs to react with intracellular targets [4, 6]. AGs are excreted unchanged almost exclusively via the kidneys, and the dominant mechanism of renal excretion is glomerular filtration [7]. Of relevance to the toxicology of AGs is the fact that a small fraction of filtered drug is transported by a low affinity but high capacity system into proximal tubular cells, and due to the slow rate at which drug is released from these cells, toxic intracellular concentrations of drug can be attained even during standard therapy [7]. The transport of AGs across the apical membrane of proximal tubular cells is mediated, at least in part, by adsorptive endocytosis, following which the drug is translocated to the lysosomal compartment where it may reach mM concentrations [7]. Indirect evidence also implicates a small component of uptake across the basolateral membrane by an as yet undefined mechanism [7].

The relevance of cellular transport and accumulation of drug to the pathogenesis of toxicity is supported by the following: 1) necrosis is confined to cells of the nephron segment where the drug is absorbed [4, 6]; 2) for a given antibiotic the risk of toxicity increases as the renal cortical concentration of drug increases [4, 6, 7]; 3) rats with diabetes mellitus are resistant to aminoglycoside nephrotoxicity due to the fact that these rats accumulate less drug in the renal cortex than do sensitive non-diabetic rats [8, 9]; and 4) this resistance can be overcome by augmenting the renal cortical concentration of drug [9]. However, the ranking of individual AGs according to the extent to which they are transported and accumulated by renal proximal tubular cells does not predict the clinical nephrotoxicity potentials of these drugs because this classification method fails to take into consideration the second major determinant AG toxicity, that is, the potential of these drugs to interact with and disrupt one or more critical intracellular targets.

Insight into the fundamental mechanism underlying AG toxicity has been gleaned from studies of the interaction of these drugs with phospholipids. It has been established that AGs, which are organic polycations and carry a net cationic charge ranging from +3.10 for tobramycin to +4.37 for neomycin [10], preferentially bind to anionic phospholipids, especially phosphatidylinositol-4-5′-bisphosphate (PIP_2), whereas little or no binding to anionic non-lipid compounds is observed [11, 12]. This has lead to the hypothesis that anionic phospholipids function as membrane receptors for these drugs [5, 7, 11]. It also has been established that AGs induce a phosphatidylinositol (PI)-enriched phospholipidosis in the renal cortex and in cells grown in culture [5, 13] due primarily to impaired degradation of phospholipid at the level of the lysosome, the subcellular site where AGs accumulate in high concentration in association with phospholipid in the form of myeloid bodies, the typical ultrastructural lesion of AG toxicity. The impaired degradation of phospholipids is due to AG-induced inhibition of lysosomal phospholipases, presumably consequent to drug-substrate complexation [14]. Binding of the cationic AGs to anionic PIP_2 is probably responsible for the disruption of the PI cascade by these agents [15].

These perturbations of phospholipid metabolism have stimulated investigations into the molecular mechanisms by which AGs bind to anionic phospholipids of model membranes and the effects of such binding on the biophysical properties of the membranes [11, 16–18]. We recently reported that binding of AGs to anionic phospholipid is mediated by an electrostatic interaction between the positively-charged amino groups of the drug and the negatively charged phosphate of the lipid as well as by hydrogen bonding between the amino groups of the drug and the carbonyl esters linked to glycerol [17]. Furthermore, binding of drug to anionic PI altered the biophysical properties of the liposome; for example, it depressed the permeability to glycerol and stimulated membrane aggregation [17, 18]. Of particular interest is that the rank order of AGs, with respect to their ability to depress glycerol permeability and stimulate aggregation of PI-containing liposomes (neomycin > gentamicin > tobramycin > netilmicin), coincides precisely with their established clinical nephrotoxicity potentials [18]. The toxicological significance of AG binding to membrane phospholipid is supported by other experiments involving polyaspartic acid (PAA), a polymer of the anionic aspartic acid, which protects rats against experimental AG nephrotoxicity without inhibiting the renal accumulation of drug [19]. We have shown recently that PAA completely inhibits gentamicin-induced perturbations of phospholipid metabolism in cultured renal tubular cells and prevents gentamicin from altering the biophysical properties of PI-containing liposomes [20]. The mechanism of protection involves inactivation of the AG by electrostatic complexation with PAA.

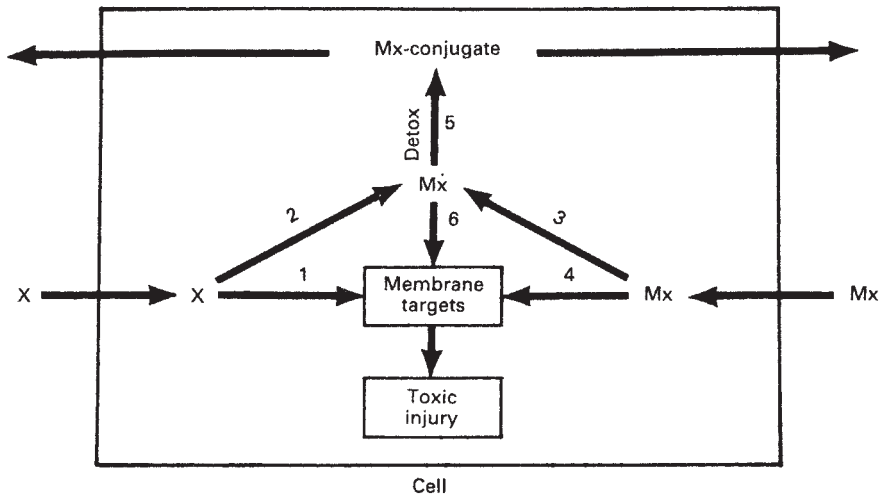

Fig. 1. *Major pathways by which drugs or drug metabolites interact with cells to cause toxic injury.* Drug X or a stable metabolite (Mx) may interact directly with sensitive intracellular targets after attaining threshold concentrations (pathways 1 and 4). X and Mx may be enzymatically converted to a toxic metabolite (M_x) which if it forms at a rate that exceeds the detoxication pathway (5) reacts with sensitive targets to cause injury. (Adapted with permission from figure 1 in HOOK JB; SMITH JA: Biochemical mechanisms of nephrotoxicity. *Transplant Proc* 17 (Suppl 11):41–50, 1985).

Thus AGs provide an example of toxicity due to the intracellular transport and accumulation of a drug that requires no metabolic transformation for expression of toxicity (Fig. 1, pathway 1). Although these drugs bind to and disrupt the function and metabolism of multiple intracellular membranes and organelles including plasma membranes, lysosomes, microsomes and mitochondria [5], the primary membrane target of AGs has not been unequivocally established. The leading hypothesis states that toxicity begins at the level of the lysosome where drug and phospholipid accumulate until a critical concentration is reached that causes labilization of the lysosomal membrane [21]. Presumably the resulting redistribution of drug and possibly potent lysosomal hydrolases to other cell membranes and organelles propagates the injury cascade. Increased oxidative stress accompanies AG nephrotoxicity, but it is a consequence rather than a causal mechanism of toxicity [22, 23].

Beta-lactam antibiotics

The beta-lactam antibiotics include the penicillins, the cephalosporins and the thienamycins. Most of these drugs, being organic anions, are actively transported by a carrier mediated process across the basolateral membrane of renal proximal tubular cells where they may attain high intracellular concentrations, and several have the potential to cause selective injury and necrosis of these transporting cells [24]. The importance of the organic anion transport system to the nephrotoxicity of beta-lactam antibiotics is supported by the following: 1) toxicity can be prevented by inhibitors of organic anion transport; 2) toxicity is restricted to those cells of the proximal tubule where the organic anion transport system resides; and 3) toxicity is not observed with beta-lactams which are not secreted by the organic anion transport system [24].

Cephaloridine was the first cephalosporin to be recognized as nephrotoxic in humans. Detailed studies of the renal transport of this drug in experimental animals have established that it is readily taken up across the basolateral membrane by the organic anion transport system; however, its rate of egress across the lumenal membrane is significantly slower than that of other secreted cephalosporins due to the fact that being a zwitter ion, the cationic moiety impedes its passage across the membrane [24]. Consequently at equivalent doses cephaloridine reaches proximal tubular cell concentrations substantially higher than do other secreted cephalosporins. While high intracellular concentration is a requirement for the expression of toxicity, it may not be the most important determinant. Comparative studies have established that cephaloglycin is slightly more nephrotoxic than cephaloridine, yet peak and sustained renal cortical concentrations of cephaloglycin are significantly lower than those observed with equivalent doses of cephaloridine and only slightly higher than those obtained with non-nephrotoxic cephalosporins [24]. Clearly cephaloglycin must have a greater potential than cephaloridine for interacting at the molecular level with its intracellular target(s).

Two theories have been proposed to explain the molecular toxicity of these agents: 1) lipid peroxidation of cell membranes and 2) mitocondrial injury. Cephaloridine has been shown to induce lipid peroxidation accompanied by depletion of reduced glutathione (GSH) and increased levels of oxidized glutathione (GSSG) in the kidney of the rat and rabbit, and the nephrotoxicity is potentiated by selenium deficiency and vitamin E deficiency which augment lipid peroxidation [25]. The pyridinium side chain of cephaloridine has been postulated to generate superoxide anion via a redox reaction involving NADPH and catalyzed by cytochrome P-450 reductase. The observation that cephaloridine-induced stimulation of MDA formation is concentration and time dependent and precedes the nephrotoxic related depression of transport function in renal cortical slices suggests that lipid peroxidation is causally linked to the pathogenesis of toxicity. However, this mechanism does not explain the toxicity of cephaloglycin as a nephrotoxic dose of this agent failed to induce in vivo the changes of lipid peroxidation and oxidative stress observed with cephaloridine [26].

A growing body of evidence implicates mitochondrial respiratory depression in the pathogenesis of cephalosporin nephrotoxicity. Nephrotoxic cephalosporins inhibit mitochondrial respiration in vivo, the extent of which is proportional to their nephrotoxicity potential [26–28]. The onset of mitochondrial respiratory depression can be detected 0.5 to 1 hour after administration of a toxic dose of drug, it is irreversible, and it

precedes by 5 to 10 hours the appearance of ultrastructural mitochondrial damage which resembles that seen with ischemic injury [26–28]. Many cephalosporins, both non-toxic as well as toxic, have the potential to inhibit succinate-dependent mitochondrial respiration in vitro [29] by blocking succinate uptake into the inner mitochondrial matrix [30]. In contrast to the delayed onset after in vivo exposure, inhibition of mitochondrial respiration after in vitro exposure to drug occurs within five minutes and can be completely reversed during the early phase by increasing the concentration of substrate. Tune [24] proposed a model, derived from a synthesis of these observations, to explain the mitochondrial toxicity of cephalosporins. The initial reversible depression of succinate-dependent mitochondrial respiration after in vitro exposure to cephalosporins is mediated by competitive inhibition of the mitochondrial carrier of anionic substrate. Irreversible depression is postulated to be due to acylation of these carriers by reactive cephalosporins. Beta-lactam antibiotics are known to form covalent complexes with bacterial penicillin-binding proteins (PBPs), and analysis of molecular structure indicates that reactivity is determined by the stability of the bond linking the R substituent to the 3-carbon position of the cephalosporin ring [24]. In vitro studies have established the rank order of various beta-lactam antibiotics with respect to their relative potencies to acylate PBPs: cefaclor > cephaloglycin ≥ cephaloridine > cephalothin ≥ cefazolin > several penicillins > cephalexin. This order is at variance with the nephrotoxicity potentials of beta-lactam antibiotics established from in vivo studies: cephaloglycin > cephaloridine >> cefaclor > cefazolin > cephalothin >> penicillin and cephalexin. The differences between the rank order for reactivity and that for nephrotoxicity potential can be explained by differences in the concentrative uptake of these agents by renal proximal tubular cells. Although cefaclor is a highly reactive acylator, the product of renal cortical drug concentration and time, defined as the area under the curve (AUC), is only 7% of that of cephaloridine and 37% of that of cephaloglycin [24]. The nephrotoxicity potentials of beta-lactam antibiotics, therefore, is determined both by their relative reactivity as acylators of target proteins and by the amount of drug (AUC) available to react with target proteins. This model accurately predicts the rank order of the nephrotoxic cephalosporins. Recent studies indicate that a similar mechanism of mitochondrial injury is a major factor in the pathogenesis of imipenem nephrotoxicity [31]. Thus, the toxicity of these agents, similar to AGs, is mediated by pathway 1 in Figure 1. However, the mechanism of concentrative uptake, the primary intracellular target and the mechanism of drug-target interaction obviously differ from those associated with AG toxicity.

Biotransformation of drugs to toxic metabolites

Role of microsomal cytochrome P-450-dependent mixed function oxidases (MFO)

Cytochrome P-450-dependent MFO comprise a family of microsomal enzymes of different substrate specificity that catalyze a variety of oxidative reactions such as aromatic and aliphatic hydroxylation, N-, O- and S-dealkylations, sulfoxidation, N-oxidation and epoxidation [1–3, 32, 33]. In addition to cytochrome P-450, the oxidative system includes a reductase, usually NADPH-cytochrome P-450 reductase (identical to NADPH-cytochrome c reductase) but occasionally NADH-cytochrome b_5 reductase, and phosphatidylcholine. In the kidney the cytochrome P-450 MFO system is found primarily in the renal cortex; significantly less activity is located in the outer medulla and no activity is detected in the inner medulla [34–36]. Within the renal cortex cytochrome P-450 MFO activity is confined to cells of the proximal tubule, principally S2 and S3 segments (Table 1). NADPH cytochrome c reductase is also distributed asymmetrically within the kidney and the highest concentration is found in proximal tubular cells (Table 1) [36]. Endogenous substrates for the microsomal cytochrome P-450 system include fatty acids, steroids, fat soluble vitamins and eicosanoids. This enzyme system also catalyzes the oxidation of a wide variety of drugs as well as other xenobiotics and may generate highly reactive and toxic metabolites [1–3, 32, 33]. The metabolism of acetaminophen will be discussed to illustrate bioactivation by the cytochrome P-450 system.

Acetaminophen, acetyl-p-aminophenol or APAP, is an effective and safe antipyretic and analgesic drug when used in therapeutically recommended doses. In the clinical setting of drug overdose APAP can cause hepatic injury often accompanied by acute renal failure [37–39]. The risk of hepatic necrosis and renal failure increases as a function of the magnitude of the ingested dose of drug. In therapeutic doses APAP is readily metabolized in the liver to glucuronyl and sulfate conjugates which subsequently are eliminated in the urine; however, a small fraction of APAP is metabolized by cytochrome P-450 MFO to a reactive intermediate that is inactivated by GSH and is excreted as a conjugate of cysteine or mercapturic acid [40–42]. Usually less than 2% of APAP is excreted unchanged in the urine [40, 41]. When an overdose (typically greater than 15 grams) is ingested, the capacity of the liver to metabolize APAP to glucuronyl and sulfate conjugates is exceeded and a larger fraction is metabolized via the cytochrome P-450 system to a reactive metabolite which is detoxified by reacting with GSH (Fig. 2) This is reflected by the increased urinary excretion of cysteine and mercapturic acid conjugates [41]. However, as GSH levels become depleted, the reactive metabolite binds covalently to macromolecules (Fig. 2) [43] and it is the arylation of critical intracellular proteins that appears to be causally linked to the induction of cellular injury [44].

Another consequence of ingesting an overdose of APAP is that a larger quantity of unmetabolized drug is delivered to the kidney as can be inferred from the elevated plasma concentration of APAP and from the increased excretion of unchanged drug [41, 42]. The renal handling of APAP involves glomerular filtration—only 13% is bound to plasma protein—followed by passive tubular absorption which, depending on the diuretic state, ranges from 60% to 74% of the filtered load in the dog [45]. The kidney can metabolize APAP to glucuronyl and sulfate conjugates but its capacity to do so is substantially less than that of the liver [46]. Similar to the liver the kidney can also metabolize APAP to an arylating intermediate via the cytochrome P-450 system [47, 48]. The intrarenal distribution of this enzyme system (Table 1) explains the finding that tubular cell necrosis from acute APAP toxicity is restricted to the proximal tubule [39, 47].

The conclusion that metabolism of APAP to a toxic metabolite by cytochrome P-450-dependent MFO mediates acute toxicity is based primarily on animal studies of hepatotoxicity in

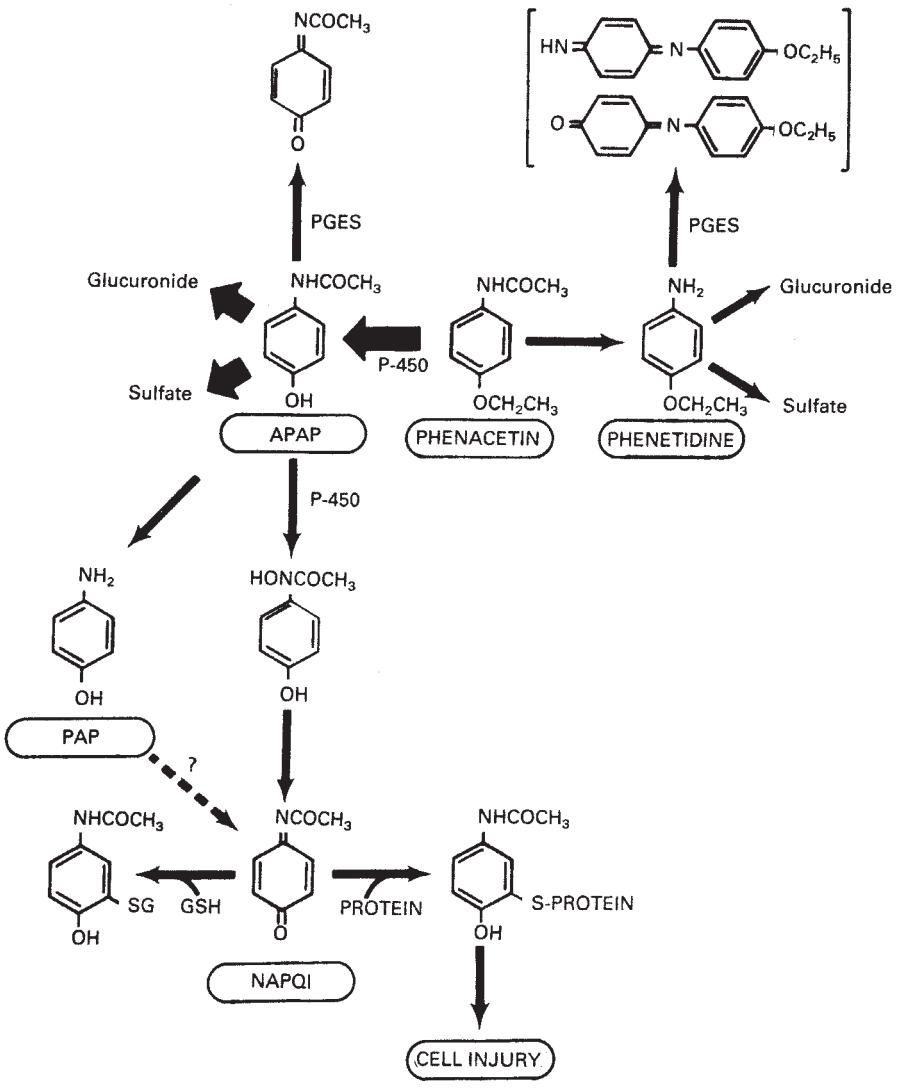

Fig. 2. *Pathways for the metabolism of phenacetin and its major metabolites, acetaminophen (APAP) and phenetidine, to toxic intermediates.* The dominant pathway is indicated by the size of the arrows. PAP, para-aminophenol; NAPQI, N-acetyl-p-benzoquinoneimine; P-450, cytochrome P-450 mixed function oxidase; PGES, prostaglandin endoperoxide synthetase. Consult text for details.

which it was shown that: 1) induction of cytochrome P-450 augments toxicity and is accompanied by a dose-dependent increase in covalent binding of radiolabeled APAP to macromolecules [43, 44, 49]; 2) inhibition of cytochrome P-450-dependent MFO decreases covalent binding of radiolabeled APAP to protein and prevents cellular necrosis [43, 44, 49]; and 3) maneuvers which promote GSH depletion augment covalent binding of APAP and toxicity whereas maneuvers which maintain GSH levels have the opposite effects [50–53]. That a similar cytochrome p-450 dependent mechanism mediates the acute renal toxicity of APAP was inferred from the following observations in the Fischer (F) 344 rat: 1) APAP causes dose-dependent depletion of renal GSH accompanied by reciprocal increases in covalent binding of [^{14}C]APAP to renal proteins; 2) similar to the findings in liver the covalent binding of [^{14}C]APAP to renal protein is enzyme dependent and requires NADPH and O_2; 3) pretreatment with cobalt chloride, an inhibitor of cytochrome P-450-dependent MFO activity, protected rats against APAP induced acute tubular necrosis, prevented depletion of GSH, and greatly reduced APAP binding to renal proteins in vivo and in vitro [47].

Recent studies by Newton et al [54–58] support the view that an additional metabolic pathway involving para-aminophenol (PAP) formation participates in the bioactivation of APAP. PAP is the deacetylated derivative of APAP (Fig. 2) and when administered to experimental animals causes renal functional and histopathologic lesions identical to those induced by APAP [54]. Of interest, PAP is 5 to 10 times as potent a nephrotoxicant as APAP whereas it is has little toxicity for the liver. PAP, similar to APAP, depletes renal cortical GSH and arylates renal cortical proteins [55, 59], but the metabolic pathway for generating the reactive intermediate appears to be independent of the microsomal cytochrome P-450 MFO system [56, 60]. Importantly, in the F344 rat PAP has been identified as a urinary metabolite of APAP and is derived at least in part from the deacetylation of APAP in the kidney [54, 55]. Newton, Pasino and Hook [57] have presented evidence that the deacetylase pathway plays no appreciable role in the metabolic activation of

APAP in the liver. The significance of the deacetylase pathway in the renal toxicity of APAP in the F344 rat is supported by the demonstration that inhibition of deacetylase activity partially protected rats against APAP but not PAP-induced functional and histopathological lesions [58]. No direct information exists about whether the deacetylase pathway is operational in humans. However, it is noteworthy that PAP was not identified as a urinary metabolite in humans [40].

N-acetyl-p-benzoquinoneimine (NAPQI) has been proposed as the toxic arylating cytochrome P-450-derived metabolite of APAP (Fig. 2) [61–63]. In support of this view NAPQI has been shown to cause dose-dependent cytotoxicity in isolated hepatocytes [64]. Initially NAPQI is detoxified by conjugation with GSH, but as GSH is depleted it interacts with cellular macromolecules causing oxidation or arylation of proteins, principally at thiol groups [64, 65]. At this time it remains uncertain whether protein arylation or oxidative stress is the more important determinant of NAPQI cytotoxicity as support can be found for both possibilities [66, 67]. Disruption of intracellular calcium homeostasis may be linked to the injury cascade triggered by NAPQI [66]. Benzoquinoneimine has been proposed as the reactive intermediate generated from PAP [59], but no evidence in support of this hypothesis has been presented.

The Sprague-Dawley (SD) rat, in contrast to the F344 rat, is resistant to APAP-induced nephrotoxic injury [49, 55] and is less sensitive than the F344 rat to PAP nephrotoxicity [54]. Compared to the F344 rat the SD rat has lower renal cortical cytochrome P-450 and deacetylase activities [68] and lower rates of covalent binding of reactive metabolite to renal microsomal protein [55]. The homozygous Gunn rat suffers from a genetic deficiency of UDP-glucuronyl transferase and consequently exhibits increased susceptibility to APAP hepato- and nephrotoxicity [69]. These observations serve to emphasize that genetic factors are an important determinant of the metabolic pathways and the metabolic rates at which xenobiotics undergo bioactivation and/or detoxification and, hence, may influence the susceptibility of the kidney to injury from potential nephrotoxicants.

In summary, acute APAP nephrotoxicity represents an example of injury due to metabolic activation of a drug (Fig. 1, pathway 3) at a rate that exceeds the capacity of the detoxifying mechanisms (Fig. 1, pathway 5).

Role of prostaglandin endoperoxide synthetase

The first step in the synthesis of prostaglandins from arachidonic acid is catalyzed by prostaglandin endoperoxide synthetase (PGES), which is also known as prostaglandin H synthase [70]. PGES is comprised of two enzymatic activities, a cyclooxygenase and a hydroperoxidase [71]. The cyclooxygenase catalyzes the conversion of arachidonic acid to PGG_2, a 15-hydroperoxy PG cyclic endoperoxide. The hydroperoxidase catalyzes the reduction of PGG_2 to PGH_2, a process that requires reducing equivalents. Previous studies using PGES from seminal vesicles have demonstrated that xenobiotics can serve as electron donors for the hydroperoxidase reaction and in the process undergo co-oxidation to either a stable or occasionally to a reactive metabolite (Fig. 3) [72, 73]. A similar mechanism of co-oxidation of xenobiotics has been shown to occur in the inner medulla, the renal site highest in PGES activity (Table 1). Moreover, this mechanism of co-oxidation can generate carcinogens and nephrotoxicants [73, 74].

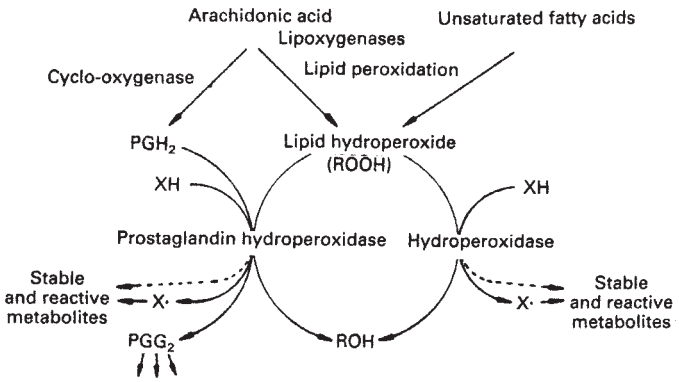

Fig. 3. *Peroxidase-catalyzed co-oxidation of xenobiotics.* The catalytic reduction of PGG_2 or other lipid hydroperoxides requires an electron donor which usually is an endogenous substrate. However, certain xenobiotics may substitute as electron donors for this reductive reaction and in the process are oxidized to toxic intermediates. (Reproduced with permission from MOLDEUS P, ROSS D, LARSSON R: Interrelationships between xenobiotic metabolism in lipid biochemistry. *Biochem Soc Trans* 13:847–850, 1985.)

Phenacetin has been implicated in the pathogenesis of renal papillary necrosis associated with chronic analgesic abuse [75]. The major metabolite of phenacetin is APAP which is formed in the liver via the cytochrome P-450 system (Fig. 2) [76]. Of interest, phenacetin overdosage does not cause hepatic or renal failure because the rate of APAP generation does not exceed the rate at which APAP can be conjugated with glucuronide, sulfate or GSH [76]. Chronic abuse of phenacetin containing analgesics, however, results in the long-term exposure of the papilla to low concentrations of APAP. Several investigators have demonstrated that APAP can undergo co-oxidation by the hydroperoxidase activity of prostaglandin endoperoxide synthetase [35, 72, 77–79] to form the reactive intermediate, NAPQI [65–67]. Whereas the inner medulla has the highest PGES activity of the kidney [34, 35, 70], the cells of this region have low concentrations of GSH and a limited capacity to regenerate or synthesize GSH [80]. This unique distribution of enzymes involved in the bioactivation and detoxification of reactive metabolites, together with the fact that APAP is concentrated in this region of the kidney [75], has been advanced as the explanation for the vulnerability of the inner medulla to injury from long-term exposure to low dose APAP or phenacetin [80].

Aspirin (acetylsalicylic acid), a common component with phenacetin in compound analgesics, has also been implicated in the pathogenesis of papillary necrosis [75]. Aspirin in sufficient concentration can inhibit the cyclo-oxygenase component of PGES; but it has no effect on the hydroperoxidase component [79]. Aspirin undergoes deacetylation to salicylic acid which during antidiuresis accumulates in high concentration within cells of the inner medulla [75]. Salicylic acid has no inhibitory effect on either component of PGES [79]; however, it has been shown to deplete the kidney of GSH [75], an effect which would magnify the nephrotoxic potential of APAP.

Recent studies raise the possibility that p-phenetidine, the deacetylated derivative of phenacetin (Fig. 2), may also play a

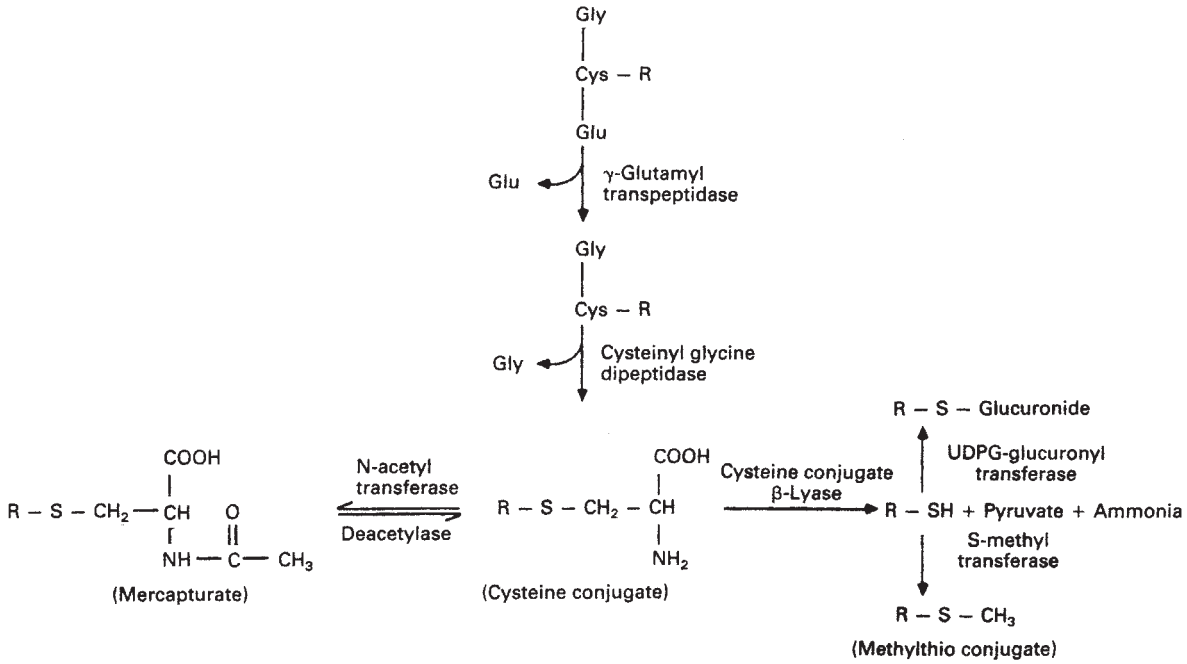

Fig. 4. *Pathways for the metabolism of glutathione conjugates.* Some cysteine conjugates are directly toxic following uptake by renal proximal tubules or are metabolized by cysteine conjugate β-lyase to toxic thiol metabolites. (Reproduced with permission from PICKETT B, LU AYH: Glutathione S–transferases: Gene structure, regulation and biological function. *Ann Rev Biochem* 58:743–764, 1989.)

role in the pathogenesis of papillary necrosis associated with chronic phenacetin abuse. Like APAP, p-phenetidine has been shown to be an excellent substrate for co-oxidation by PGES from the renal medulla, the products of which are N-(4-ethoxyphenyl)p-benzoquinoneimine (NEPBQI) and N-(4-ethoxyphenyl)p-benzoquinonedi-imine (Fig. 2) [81]. NEPBQI binds GSH, arylates microsomal proteins [81, 82] and is cytotoxic [83]. In addition, an unidentified primary oxidation product of p-phenetidine is genotoxic [82]. Genetic or drug-induced variation in the rate of p-phenetidine formation in the liver or possibly within cells of the renal medulla may be an important determinant of phenacetin nephrotoxicity.

In summary, the nephrotoxicity associated with phenacetin abuse represents an example of injury mediated by the metabolic activation in the renal inner medulla of a stable metabolite formed in the liver (Fig. 1, pathway 4).

Role of γ glutamyltranspeptidase and the formation of cysteine S-conjugates

Glutathione (GSH) is a ubiquitous tripeptide (L-γ-glutamyl-L-cysteinylglycine) that participates in many biological processes including the synthesis of proteins and DNA, transport of amino acids, modulation of enzyme activity, metabolism of endogenous and exogenous substrates and the detoxication of reactive metabolites [84]. The first step in the metabolism of xenobiotics and their metabolites involves the formation of GSH S-conjugates. This reaction can occur spontaneously in the case of highly electrophilic compounds or it can be catalyzed by GSH S-transferases, a family of enzymes with broad and overlapping substrate specificities [85]. GSH S-transferases are present in many cells; however, impressively high levels are found in the liver. Sequential steps in the metabolism of GSH S-conjugates include splitting of the γ-glutamyl bond by γ-glutamyltranspeptidase (γGT) to form S-cysteinylglycine conjugates, followed by dipeptidase degradation to cysteine S-conjugates which can be N-acetylated to mercapturic acids or further metabolized by β-lyase to thiol derivatives (Fig. 4) [84–86]. As a general rule the formation of GSH S-conjugates represents a detoxication pathway for many reactive xenobiotics and metabolites. However, for certain substrates, mainly halogenated hydrocarbons, formation of GSH S-conjugates represents a bioactivation pathway that generates nephrotoxic, hepatotoxic, mutagenic and/or carcinogenic metabolites [85–88]. The susceptibility of the kidney to toxic injury by this pathway relates to the major role the kidney plays in the formation and processing of toxic cysteine S-conjugates. Several reviews of this subject have appeared recently [86–88].

Halogenated alkanes and alkenes are substrates for liver GSH S-transferases which catalyze the formation of GSH S-conjugates. For example, the reaction of trichloroethylene with GSH generates S-(1,2 dichlorovinyl)glutathione (DCVG), the precursor of a nephrotoxic derivative [89]. The formation in the liver of S-(pentachloro-1,3-butadienyl) glutathione (PCBG) from the GSH S-transferase catalyzed reaction of GSH with hexachloro-1,3-butadiene represents another example [90]. GSH S-conjugates are transported from hepatocytes into plasma and bile, following which they are degraded sequentially by γGT and dipeptidases to form the corresponding cysteine S-conjugates. γGT is present on the surface membranes of many cells including biliary tract epithelium, jejunal epithelium and renal proximal tubular epithelium, both lumenal and basolateral membranes [84, 86]. Of toxicological significance the kidney exhibits the highest γGT activity of any organ or tissue [84, 86]. GSH S-conjugates transported into plasma are delivered to the kidney, where after glomerular filtration they are

catabolized by γGT and cysteinylglycine dipeptidase to form the cysteine S-conjugates which are transported across the apical membrane [85]. An unknown quantity of the GSH S-conjugates may be degraded by γGT on the basolateral membrane or transported directly into the cell by a sodium-dependent probenecid inhibitable pathway [91]. This pathway appears to be distinct from that which mediates the active uptake of cysteine S-conjugates [91, 92]. Conflicting data exists concerning the role of organic anion transport in mediating the active uptake of these conjugates [92, 93]. The fate of GSH S-conjugates which are transported from hepatocytes into bile has not been studied in detail, but it seems that an unknown quantity is returned to the circulation and processed by renal proximal tubular cells [86].

Nephrotoxic cysteine S-conjugates can cause proximal tubular cell injury by two major pathways. Certain nephrotoxic conjugates, such as S-(2-chloroethyl)cysteine, undergo spontaneous rearrangement to form an episulfonium ion which can cause toxic injury by interacting with nucleophilic groups of critical macromolecules [87, 88]. In other cases the cysteine S-conjugate is the penultimate nephrotoxin that becomes activated by renal β-lyase [87, 88]. S-(1,2 dichlorovinyl)cysteine exemplifies this mechanism [89]. Mitochondria appear to be a major target of the toxic metabolites generated by renal β-lyase [94]. In vitro studies have demonstrated that incubation of isolated renal cortical mitochondria with known toxic cysteine S-conjugates results in disruption of the function, metabolism and structural integrity of these organelles. The susceptibility of mitochondria to injury from cysteine S-conjugates can be explained by the observation that most of the β-lyase activity in renal tubular cells is found in the mitochondrial fraction [89]. Thus, the nephrotoxicity associated with cysteine S-conjugates of halogenated hydrocarbons represent examples of injury mediated by pathway 2 and pathway 4 in Figure 1.

Conclusion

In this brief review the generic pathways by which drugs interact with cells of the tubulo-interstitium to cause toxicity have been emphasized. Although the potential number of toxicants is large and rapidly expanding, the basic pathways by which they exert their toxic effect are few in number. The selective vulnerability of specific cells of the kidney, for example, proximal tubular cells, to certain drugs and xenobiotics is a predictable consequence of the unique transport and/or metabolic profile of such cells that results in the generation and/or accumulation of the offending toxicant. At the present time huge gaps exist in our knowledge of the ultimate toxic species of many agents, the specific targets of the toxicant and how the interaction of the toxicant with its target eventuates in cellular injury or death. As our understanding of specific molecular mechanisms mediating toxicity expands, it should be possible to devise strategies for protecting the kidney from a major cause of injury.

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