Phase III Elimination: Another Two-Edged Sword

Excretion into bile is well recognized as a major pathway for the elimination of amphiphatic, hydrophobic, high molecular weight xenobiotics and thus complements the renal elimination of hydrophobic compounds of low molecular weight. For many xenobiotics, excretion in bile, which has been termed a phase III elimination reaction (1), begins with an oxidation reaction (phase I), followed by conjugation (phase II), usually to glucuronide or glutathione conjugates, which are good substrates for biliary excretory pathways. Until recently, there was little understanding regarding the mechanisms by which substrates are concentrated in bile or regarding what determines the substrate specificity of the transporters. The identification of distinct ATP-dependent and voltage-dependent transporters in the canalicular membrane over the last few years has begun to provide answers to these questions. Important questions that have not been addressed, and which are critical for toxicology, relate to the regulation of the activities of these transporters by endogenous, or more importantly, environmental agents. Two examples illustrate the importance of concentrative transport into bile for toxicity and how regulation of this process and the subsequent fate of these conjugates might influence toxicity.

Technical-grade dinitrotoluene (DNT) consists primarily of 2,4-DNT (75.8%) and 2,6-DNT (19.5%) and is hepatocarcinogenic when fed to Fischer-344 rats. After administration of 35 mg DNT/kg/day for 55 weeks, the incidence of hepatocellular carcinomas in female rats was half that in males (2). In an elegant series of studies, Rickert and colleagues suggested a bioactivation pathway involving hepatic metabolism to dinitrotosylglucuronic acid followed by biliary excretion, deconjugation, and nitroreduction by intestinal microflora, and finally reabsorption of the resulting aminonitrobenzyl alcohol, which was then oxidized to reactive metabolites which were genotoxic. A key rate-determining step in the development of hepatocarcinoma was the rate and extent to which the glucuronide conjugate was delivered to the microflora in the gastrointestinal tract, which was several-fold lower in female rats.

Why is biliary excretion of this substrate lower in female rats? Based on the decreased biliary excretion of many substrates during pregnancy, and following treatment with estradiol, ethinyl estradiol, or diethylstilbestrol, it seems most likely that estrogens are important regulators of phase III elimination. The mechanism by which estrogens inhibit this process is not known, but the selective changes induced by treatment of rats with ethinyl estradiol (i.e., inhibition of some transporters; ATP-dependent transport of bile acids) but stimulation of others (Na+/glycine cotransport) in rat canalicular membrane vesicles suggest estrogen-receptor-mediated events, rather than a generalized "toxic" response (3,4).

Are environmental estrogens (e.g., chlodecone, DDT) able to interact with hepatic estrogen receptors and simulate the inhibitory effects of classic estrogens on biliary excretion? Interestingly, the biliary excretion of several substrates is increased postpartum in lactating rats; this effect has recently been shown to be mediated by the anterior pituitary hormone prolactin (5). Estrogens increase gene transcription and secretion of prolactin. Do environmental estrogens also stimulate prolactin release and thus blunt the inhibitory effects of estrogens on biliary excretion? The inhibitory effect of estrogens on the phase III elimination pathway in the rat parallels the decreased bile secretory function seen in normal women in the third trimester of pregnancy and during treatment with estrogen-containing oral contraceptives. However, the female rat liver has an exceptionally high number of prolactin receptors so that the rat may not be a good predictor of the human hepatic response to increased prolactin secretion.

A second example illustrates the toxicological importance of hydrolysis of glutathione conjugates following their excretion into bile. The nephrotoxicity of cysteine-S conjugates is well-known (6), and has been shown to require formation of an S-glutathione conjugate in the liver, followed by secretion into bile. The cysteine-S conjugates are then able to catalyze its hydrolysis to cysteine-S conjugates. The high concentrations of GGT in the intestine and kidney relative to the liver led initially to the concept that these organs were required for formation of cysteine-S conjugates. Ballatori and colleagues (7) have shown that this may be true for the rat and mouse, which have very low levels of hepatic GGT, but is unlikely for species like the guinea pig and man, which have relatively high concentrations of GGT in the biliary tree. In the guinea pig, glutathione conjugates are readily hydrolyzed within the biliary tree to the cysteine-S conjugates which can then be reabsorbed from the bile back into the liver. This GGT-dependent biliary-hepatic recycling has been shown to contribute to the long biologic half-life and toxicity of methyl mercury. Methyl mercury is thought to be transported into bile across the canalicular membrane as a glutathione complex which is hydrolyzed by GGT and dipeptidases to the relatively lipid-soluble methyl mercury-cysteine complex (8). Changes in the activity of hepatic GGT could therefore influence the retention and thus the toxicity of methyl mercury. What regulates GGT activity, and is this influence by environmental factors? Lieberman and colleagues (9) have recently cloned the 5' region of the mouse GGT gene and have shown that a single gene codes for six different mRNAs with alternative 5' sequences, suggestive of 5-6 distinct promoters in the upstream region of the gene. Differential tissue expression and developmental patterns of the six GGT mRNAs indicate a complex regulation of this important enzyme.

Thus, like phase I and phase II elimination reactions, phase III elimination has both detoxification and bioactivation roles, so that
either inhibition or stimulation of this pathway can modulate toxicity. The challenge will be to begin to unravel the role endogenous factors, such as pregnancy and lactation, and environmental agents play in regulating phase III elimination and toxicity in humans.

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