TOXICOLOGICAL HIGHLIGHT
The Road to Elucidating the Mechanism of Manganese-Bilirubin–Induced Cholestasis
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The article highlighted in this issue is “Synergistic Role of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase and Cholesterol 7α-Hydroxylase in the Pathogenesis of Manganese-Bilirubin–Induced Cholestasis in Rats,” by Marie-Yvonne Akoume, Shahid Perwaiz, Ibrahim M. Yousef, and Gabriel L. Plaa (pp. 331–338).

A major function of the liver is the formation of bile and hepatobiliary excretion of endogenous substances and xenobiotics, such as chemicals, drugs, and their metabolites. Cholestasis is a general condition of multiple etiologies, hereditary and acquired, in which bile excretion from the liver is attenuated or blocked. In some forms of cholestasis, cholesterol metabolism is perturbed, resulting in cholesterol accumulation in liver bile canicular membranes (BCM).

Intrahepatic cholestasis can be caused by drugs, sepsis, total parenteral nutrition, lymphomas, tuberculosis, sarcoidosis, and amyloidosis. Other causes of this form of the disorder include primary biliary cirrhosis, primary sclerosing cholangitis, viral hepatitis, alcoholic liver disease, pregnancy, and inborn errors or mutations of bile acid metabolism. Extrahepatic cholestasis can be caused by bile duct tumors, strictures, cysts, diverticula, and various forms of injury. Potential causes for this specific form also include stones in the common bile duct, pancreatitis and pancreatic tumor, primary sclerosing cholangitis, and compression due to a mass or tumor on a nearby organ.

The clinical use or abuse of drugs, including gold salts, nitrofurantoin, anabolic steroids, estrogens and oral contraceptives, chlorpromazine, prochlorperazine, sulindac, cholesterol-lowering “statins,” cimetidine, erythromycin, tobutamide, imipramine, and some penicillin-based antibiotics and herbal remedies, can cause cholestasis (Chitturi and Farrell, 2001). Exposure to these agents may reduce or block bile flow and precipitate liver injury, in part due to the toxicity of bile acids and other bile constituents. Thus, there is significant clinical relevance for conducting studies examining mechanisms or modes of action of chemical-induced cholestasis.

Cholestasis and perturbations in cholesterol metabolism have been the subjects of an exciting and intriguing path of inquiry undertaken by investigators from the laboratory that produced the highlighted paper in this issue (Akoume et al., 2003). In this article, the authors have extended their earlier work defining mechanisms associated with the manganese-bilirubin (Mn-BR) model of experimentally-induced intrahepatic cholestasis. The authors have been using this model for over 20 years because of the similarities in the pathophysiological changes in BCM to those observed in cases of cholestasis in humans. In the Mn-BR model, cholestasis is achieved through the sequential intravenous injection of Mn and BR, neither of which is cholestatic when administered alone. The onset of cholestasis is remarkably rapid—Mn is injected followed by injection of BR 15 min later, and maximum decreases in bile flow are observed 30 min after BR injection. Through a series of investigations, including the highlighted paper, the authors have contributed a body of work that illustrates a logical and rational approach to elucidate the mechanisms involved in Mn-BR–induced cholestasis.

In previous studies, the authors provided evidence that accumulation of cholesterol in the BCM and cytosol, increases in the BCM cholesterol/phospholipids ratio, and changes in BCM membrane fluidity play a role in the pathogenesis of Mn-BR cholestasis (Duguay et al., 1998). While narrowing the focus of action to the BCM, an unresolved question from that study was: What is the source of cholesterol that accumulates in the BCM? Or, in other words, is the elevated cholesterol derived from the existing cellular pool or from an increase in the de novo synthesis of cholesterol? This question was answered in a subsequent investigation (Duguay et al., 2000) in which the authors employed an experimental design utilizing two radio-labeled compounds that would discriminate between the existing cellular pool of cholesterol and cholesterol synthesized de novo. H3-cholesterol was injected to label the existing intracellular cholesterol pool followed by subsequent injection of

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14C-mevalonate, a cholesterol precursor, to label newly synthesized cholesterol. Interestingly, in Mn-BR cholestasis, 14C-labeled cholesterol levels were greater than 3H-labeled cholesterol levels in all hepatic subcellular fractions, but most dramatically in the BCM. These results indicated that newly synthesized cholesterol, and not cholesterol derived from previously synthesized pools, accounted for cholesterol accumulation in the BCM under conditions of Mn-BR cholestasis.

A logical explanation for the elevated levels of newly synthesized cholesterol would be for the Mn-BR treatment to enhance the rate of cholesterol biosynthesis through increasing the activity of microsomal 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme responsible for cholesterol biosynthesis. Because of their observation that the activity of this enzyme was decreased by over 50% after the Mn-BR treatment regimen (Duguay et al., 2000), the authors concluded that factors other than an enhanced rate of cholesterol synthesis must account for the accumulation of newly synthesized cholesterol. Thus, prior to the present highlighted study, confounding data sets existed that could not explain the accumulation of newly synthesized cholesterol. The question left unanswered was this: What is the metabolic basis that accounts for the newly synthesized cholesterol in BCM in the Mn-BR cholestasis model?

The investigators address this important question in the highlighted paper. In this study, the investigators measured the activities of HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis, as well as, for the first time, cholesterol-7α-hydroxylase, a key enzyme involved in cholesterol degradation to bile acids. These analyses were achieved by employing a mass spectrophotometric method developed in their laboratory for the simultaneous and rapid assay of the two enzymes (Ndong-Akoume et al., 2002). The two enzymes have opposing actions—any condition or intervention that results in

![Diagram of cholesterol metabolism](https://academic.oup.com/toxsci/article-abstract/73/2/216/1674334)
the activation of HMG-CoA reductase with concurrent inhibition of cholesterol-7α-hydroxylase will result in cholesterol accumulation and cholestasis (Fig. 1). The simultaneous evaluation of both enzymes using the new assay was a major breakthrough in and of itself. Prior to the development of this assay, the investigators measured HMG-CoA reductase using a laborious and time-consuming thin-layer plate chromatography assay, which limited them to evaluating samples from the Mn-BR treatment group only. Additionally, they had not looked at cholesterol-7α-dehydrogenase activity prior to this study. Not only was the analysis of the two enzymes critical, but the new two-enzyme mass spectrophotometric assay facilitated the evaluation of greater numbers of samples. Experimental designs could now be employed in order to tease out the effects of Mn and BR alone on enzyme activities and cholestasis parameters at critical times in the Mn-BR injection regimen (Fig. 2). These two key factors of methodology and experimental design were paramount to the success of the study, and as a result, the data sets obtained allowed the authors to further define the mechanism of Mn-BR-induced cholestasis.

Based on the evidence presented in the highlighted paper, the authors propose that Mn injected first stimulates HMG-CoA reductase activity and results in an enhanced rate of de novo cholesterol synthesis; however, the subsequent injection of BR inhibits cholesterol-7α-hydroxylase activity which prevents metabolism of cholesterol to bile acids, specifically cholic acid (Fig. 1). The actions of Mn and BR acting alone—but necessarily in proper and timed sequence—on cholesterol synthesis and degradation, respectively, result in the accumulation of newly synthesized cholesterol in the BCM, with ensuing toxic sequelae including alterations in BCM membrane fluidity and impairment of bile formation and flow. A remaining conundrum with the proposed mechanism is the observation of decreased activities of both enzymes, particularly HMG-CoA reductase, after sequential treatment with Mn and BR. The authors postulate, based on the report of Hwa et al. (1992), that elevated levels of intracellular cholesterol exert a negative feedback on HMG-CoA reductase (Fig. 1), a mechanism that would account for inhibition of enzyme activity observed after the Mn-BR treatment regimen (Fig. 1), a mechanism that would account for inhibition of enzyme activity observed after the Mn-BR treatment regimen (Fig. 2). While each subsequent hypothesis was carefully addressed in the authors’ studies of Mn-BR cholestasis, further laboratory investigations could test the validity of their hypothesis (e.g., by using specific inhibitors of the two enzymes). The authors also raise an important consideration in their discussion of the possibility that perturbations in biliary lipid membrane transporters, such as multidrug resistance P-glycoproteins (e.g., mdr2), may play a role in the mechanism of Mn-BR cholestasis. Indeed, disruption of the function of hepatobiliary transporters, especially those associated with the canalicular membrane, may form the underlying basis for drug-induced or hereditary cholestasis conditions (for a review, see Pauli-Magnus and Meier, 2003). Revelations from the research team based on additional mechanistic studies of Mn-BR cholestasis, especially of the role of biliary lipid membrane transporter dysfunction, are no doubt on the horizon.

**FIG. 2.** Experimental design for elucidation of mechanism of action of manganese (Mn) and bilirubin (BR) injected sequentially on the enhanced rate of new cholesterol synthesis in liver bile canalicular membranes. Symbols within the gray box indicate data reported for the first time in the highlighted paper (Akoume et al., 2003). In the Mn-BR model of intrahepatic cholestasis, Mn is injected iv followed 15 min later by BR injection, and parameters are evaluated 30 min after BR administration. Other treatment groups evaluated are: MnBR-15 = 15 min after sequential compound treatment; Mn-15 = 15 min after injection of Mn; Mn-45 = 45 min after Mn injection; BR-30 = 30 min after BR injection; control = injection of compound vehicles. (+) = cholestasis; (-) = no cholestasis or no change in enzyme activity; up arrow = enhanced enzyme activity; down arrow = inhibition of enzyme activity.
The highlighted paper represents the culmination of a series of discoveries on the part of the investigators as they moved from an interesting observation on the hepatotoxicity of two chemicals when administered sequentially to characterize now the cellular and biochemical events that provide a mechanistic basis for the Mn-BR model of cholestasis. Although there are other mechanisms of cholestasis, the highlighted paper advances our understanding of the pathogenesis of intrahepatic cholestasis.

REFERENCES


