

these blood donors frequently do not harbor HCV RNA and are not infectious. This is borne out by the absence of PTNANB hepatitis in the five recipients of anti-C100-3 HCV RNA-negative blood, whereas the only recipient of anti-C100-3-positive, HCV RNA-positive blood did contract PTNANB hepatitis. It would be of interest to know the subsequent clinical course of the donor of the latter blood. At present it is unclear whether HCV infection can occur without hepatitis, either acutely or chronically. If chronically infected, this donor could be analogous to a benign chronic carrier of HBsAg. Conversely, in view of Weiner's data, one may question why this donor did not have hepatitis if viral replication and hepatitis appear to go hand in hand. Clearly there is much to be learned about the mechanisms of cellular injury in HCV infection.

Garson's study does not indicate whether there were any anti-HCV-negative cases of PTNANB hepatitis among the recipients of the 1,100 units of blood tested. In a previous study by Van der Poel et al. (6), of 5,150 units of blood transfused into 383 recipients, 6 of 34 (18%) recipients of anti-C100-3-positive blood contracted NANB hepatitis. This result is similar to that of Garson—one of six recipients of anti-C100-3-positive blood. Interestingly, in Van der Poel's study, 3 of 349 recipients of anti-C100-3-negative blood contracted NANB hepatitis. In contrast, Esteban et al. (7) have reported on 280 transfusion recipients of 1,109 units of blood among whom 27 (9.6%) contracted PTNANB hepatitis. In this study anti-HCV status was assessed by anti-C-100 ELISA and also by the more recently developed recombinant immunoblot assay, which recognizes antibodies to two HCV epitopes, C-100 and 5.1.1. HCV RNA was not measured. Sixteen of the 27 PTNANB patients in Esteban's study received anti-HCV-positive blood, whereas 11 received anti-HCV-negative blood. However, 24 of 27 patients with PTNANB hepatitis became anti-HCV-positive during 52 wk of follow-up. Only two recipients of anti-HCV-positive blood did not contract PTNANB hepatitis. It is not clear why there was so marked a disparity in infectious potential of anti-HCV-positive blood between the studies by Garson and Van Der Poel and that of Esteban (18% vs. 88%). However, in agreement with Weiner's data, one can infer from Esteban's study that infectious HCV may be present in blood without detectable anti-HCV. The application of PCR technology to identification of HCV RNA in potentially infected sera is one possible solution to this clinical conundrum.

MICHAEL LUCEY, M.D.
PETER G. TRABER, M.D.
*Department of Internal Medicine
University of Michigan Medical School
Ann Arbor, Michigan 48109*

REFERENCES

1. Kuo G, Choo Q-L, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science* 1989;244:362-364.
2. Innis MA, Gelfand DH, Sninsky JH, White TJ. PCR protocols: a guide to methods and application. San Diego: Academic Press, Inc., 1990.
3. Enomoto N, Takada A, Nakao T, Date T. There are two major types of hepatitis C virus in Japan. *Biochem Biophys Res Commun* 1990;170:1021-1025.
4. Choo Q-L, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989;244:359-362.
5. Alter HJ, Purcell RH, Shih JW, Melpolder JC, Houghton M, Choo Q-L, Kuo G. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A non-B hepatitis. *N Engl J Med* 1989;321:1494-1500.
6. Van der Poel CL, Reesink HW, Schaasberg W, Leentvaar-Kuypers A, Bakker E, Exel-Oehlers PJ, Lelie PN. Infectivity of blood seropositive for hepatitis C virus antibodies. *Lancet* 1990;335:558-560.
7. Esteban JI, Gonzalez A, Hernandez JM, Viladomill L, Sanchez C, Lopez-Talavera JC, Lucea D, et al. Evaluation of antibodies to hepatitis C virus in a study of transfusion-associated hepatitis. *N Engl J Med* 1990;323:1107-1112.

NEW INSIGHTS INTO CANALICULAR ORGANIC ANION SECRETION

Oude Elferink RPJ, Ottenhoff R, Liefting WGM, Schoemaker B, Groen AK, Jansen PLM. ATP-dependent efflux of GSSG and GS-conjugate from isolated rat hepatocytes. *Am J Physiol* 1990;258:G699-G706.

EDITOR'S ABSTRACT

The effect of partial and complete dissipation of the membrane potential and partial depletion of cellular ATP content on the efflux of dinitrophenyl-glutathione and oxidized glutathione was examined in hepatocytes isolated from normal and mutant (TR⁻) rats exhibiting defective organic anion transport. Whereas alterations in the membrane potential difference had no effect on the initial efflux rate of dinitrophenyl-glutathione and oxidized glutathione, depletion of cellular ATP inhibited dinitrophenyl-glutathione and oxidized glutathione efflux and a linear relationship between the cellular ATP content and the initial efflux rate of dinitrophenyl-glutathione was observed in normal isolated rat hepatocytes. In contrast, depletion of cellular ATP content had no significant effect on the slower rate of dinitrophenyl-glutathione efflux from TR⁻ rat hepatocytes. These findings implicate an ATP-dependent hepatic transport system for oxidized glutathione and glutathione conjugates that is absent in TR⁻ mutants.

Kitamura T, Jansen P, Hardenbrook, Kamimoto Y, Gatmaitan Z, Arias IM. Defective ATP-dependent bile canalicular transport of organic anions in mutant (TR⁻) rats with conjugated hyperbilirubinemia. *Proc Natl Acad Sci USA* 1990;87:3557-3561.

EDITOR'S ABSTRACT

Fluorescence image analysis reveals normal secretion of a fluorescent bile acid (fluorescein isothiocyanate glycocholate) into the canalicular lumen of isolated normal and TR⁻ mutant rat hepatocyte couplets, but negligible canalicular accumulation of a non-bile-acid organic anion (carboxydichlorofluorescein diacetate) in TR⁻ hepatocyte couplets. Canalicular mem-

brane vesicles derived from normal rats exhibited saturable temperature- and ATP-dependent transport of sulfobromophthalein and sulfobromophthalein-glutathione that was absent in canalicular membrane vesicles from TR⁻ rats. However, ATP-dependent daunomycin transport, reflecting transport mediated by the multidrug resistance gene product, p-glycoprotein, was present in canalicular membrane vesicles from both normal and TR⁻ rats. Canalicular membrane vesicles from normal and TR⁻ rats contained equal amounts of p-glycoprotein on immunoblots. These studies demonstrate that the conjugated hyperbilirubinemia in TR⁻ mutant rats is the result of a functional absence of an ATP-dependent organic anion transport system on the canalicular membrane.

COMMENTS

The biliary excretion of organic anions, derived from either endogenous metabolism or xenobiotics, represents one of the major functions of the liver. Inborn errors of metabolism demonstrate that separate canalicular membrane transport processes exist for the excretion of these organic anions. Thus in Dubin-Johnson syndrome and in mutant Corriedale sheep, biliary excretion of conjugated bilirubin is defective, whereas bile acid secretion is intact. Yet, in comparison with our understanding of the mechanisms of canalicular bile acid transport, little was known about the driving forces of canalicular secretion of other organic anions such as bilirubin and glutathione. Recently a mutant rat strain (TR⁻) was discovered that has autosomal, recessive, conjugated hyperbilirubinemia (1) and defective biliary excretion of conjugated bilirubin, bromosulphthalein (BSP), dibromosulphthalein and ouabain but normal biliary excretion of taurocholate, cholate and a quaternary organic cation (1, 2). In addition, glutathione and glutathione conjugates were not transported into the bile of the TR⁻ rat (3). Because of the information from these descriptive studies, these two reports now characterize the nature of this selective defect in organic anion excretion at the membrane level. The findings clearly represent an important advance in our understanding of the hepatic handling of non-bile-acid organic anions.

In the first report, hepatocytes were loaded with S-dinitrophenyl glutathione (DNP-SG) by incubation in 1-chloro-2,4-dinitrobenzene, a compound rapidly taken up by the cells and conjugated to glutathione. As previously reported (3), DNP-SG efflux from TR⁻ hepatocytes was markedly slower than that from normal hepatocytes. Depolarization of the plasma membrane potential, estimated by the intracellular and extracellular equilibrium distribution of chloride by several means, including replacement of K⁺ for Na⁺ and inhibition of Na⁺, K⁺-ATPase activity, had little effect on DNP-SG efflux from normal hepatocytes. Next, the effect of depletion of cytosolic ATP on DNP-SG efflux was examined under various experimental conditions. Incubation of cells with fructose or glycerol or glycerol with ethanol has been reported to result in rapid intracellular phosphorylation of these substrates at the

expense of cytosolic ATP. Incubation with atractyloside, antimycin A and valinomycin have all been shown to inhibit separate steps in the production and maintenance of cytosolic ATP. Each condition resulted in significant inhibition of DNP-SG efflux from normal hepatocytes, with a linear correlation established between ATP depletion by atractyloside and DNP-SG efflux. No significant inhibition of DNP-SG efflux was observed in TR⁻ hepatocytes after depletion of cellular ATP with fructose incubation or with atractyloside. In similar experiments, the ATP dependence of oxidized glutathione (GSSG) efflux in normal hepatocytes was established.

Both the plasma membrane potential and cellular ATP content have been shown previously to play a role in the secretion of reduced glutathione (GSH) in isolated hepatocytes (4). The apparent discrepancy between these and the above findings can be resolved if one accepts that GSH efflux is a predominantly sinusoidal event, whereas GSSG and DNP-GS efflux occurs in the canalicular membrane domain. Furthermore, it should be noted that there is even a lack of consensus on the role of the membrane potential in sinusoidal GSH efflux (5).

The inherent difficulty in establishing the membrane localization for this and any transport process using isolated hepatocytes as an experimental model is overcome in the second study. Using isolated hepatocyte couplets that retain a canalicular lumen, Kitamura et al. demonstrate by conventional and confocal fluorescence microscopy normal fluorescein isothiocyanate glycocholate secretion but no carbodichlorofluorescein diacetate secretion into the canalicular space in TR⁻ hepatocyte couplets, despite similar cytoplasmic accumulation relative to normal hepatocyte couplets. Then, using isolated canalicular membrane vesicles (CMV) free of basolateral membrane contamination as assessed by absent Na⁺, K⁺-ATPase activity, ATP-dependent transport of BSP and BSP-GSH present in normal CMV was shown to be absent in TR⁻ CMV. The presence of ATP-dependent daunomycin transport in TR⁻ CMV both functionally and immunologically points further to the selective nature of the transport defect in TR⁻ CMV.

Previous work in a canalicular CMV vesicle model had suggested that transport of GSSG (6) and glutathione S-conjugates using DNP-SG as a model compound (7) was an electrogenic carrier-mediated process. More recently, however, ATP-dependent transport of DNP-SG was demonstrated in the liver, although the membrane localization of this transport system was uncertain (8, 9). Similar ATP-dependent transport systems for glutathione S-conjugates have been described in the heart and in erythrocytes (20, 11).

Like all important work, these studies raise additional questions. Is GSH efflux at the sinusoidal membrane an ATP-dependent process as well and, if so, is the defect in TR⁻ rats and in Dubin-Johnson syndrome the result of defective sorting from the basolateral to the canalicular membrane of a functional transport protein or normal sorting of a functionally defective transport protein to the canalicular membrane? The finding that BSP and

BSP-GSH binding to TR⁻ CMV was similar to that observed in normal CMV would suggest the latter hypothesis.

Clearly the combined effect of the findings from these two studies is more than just a better description of canalicular non-bile-acid organic anion secretion. Because the transport defect has been characterized in a mutant animal model, it is only a matter of time before the membrane protein involved in the canalicular secretion of non-bile-acid organic anions will be identified. Radioactive photoaffinity labels are available for the detection of glutathione S-conjugate-binding membrane proteins (12). Once isolated, further studies including molecular cloning of this protein will follow.

REFERENCES

1. Jansen PLM, Peters WH, Lamers WH. Hereditary chronic conjugated hyperbilirubinemia in mutant rats caused by defective hepatic anion transport. *HEPATOLOGY* 1985;5:573-579.
2. Jansen PLM, Groothuis GMM, Peters WHM, Meijer DFM. Selective hepatobiliary transport defect for organic anions and neutral steroids in mutant rats with hereditary-conjugated hyperbilirubinemia. *HEPATOLOGY* 1987;7:71-76.
3. Oude Elferink RPJ, Ottenhof R, Liefing W, De Haan J, Jansen PLM. Hepatobiliary transport of glutathione and glutathione-conjugate in rats with hereditary hyperbilirubinemia. *J Clin Invest* 1989;84:476-483.
4. Fernandez-Checa JC, Ren C, Aw TY, Ookhtens M, Kaplowitz N. Effect of membrane potential and cellular ATP on glutathione efflux from isolated rat hepatocytes. *Am J Physiol* 1988;255:G403-G408.
5. Wright TL, Fitz JG, Boyer TD. Hepatic efflux of glutathione by the perfused rat liver: role of membrane potential difference. *Am J Physiol* 1988;255:G547-G555.
6. Akerboom T, Inoue M, Sies H, Kinne R, Arias IM. Biliary transport of glutathione disulfide studied with isolated rat-liver canalicular-membrane vesicles. *Eur J Biochem* 1984;141:211-215.
7. Inoue M, Akerboom TPM, Sies H, Kinne R, Thao T, Arias IM. Biliary transport of glutathione S-conjugate by rat liver canalicular membrane vesicles. *J Biol Chem* 1984;259:4998-5002.
8. Kobayashi K, Sogame Y, Hara H, Hayashi K. Mechanism of glutathione S-conjugate transport in canalicular and basolateral rat liver plasma membranes. *J Biol Chem* 1990;265:7737-7741.
9. Kunst M, Sies H, Akerboom TPM. ATP-stimulated uptake of S-(2,4-dinitrophenyl) glutathione by plasma membrane vesicles from rat liver. *Biochim Biophys Acta* 1989;983:123-125.
10. Ishikawa T, Sies H. Cardiac transport of glutathione disulfide and S-conjugate: studies with isolated perfused rat heart during hydroperoxide metabolism. *J Biol Chem* 1984;259:3838-3843.
11. Kondo T, Murao M, Taniguchi N. Glutathione S-conjugate transport using inside-out vesicles from human erythrocytes. *Eur J Biochem* 1982;125:551-554.
12. Kunst M, Sies H, Akerboom TPM. S-(4-azidophenacyl)[³⁵S] glutathione photoaffinity labeling of rat liver plasma membrane-associated proteins. *Biochim Biophys Acta* 1989;982:15-23.

RICHARD H. MOSELEY, M.D.
Veterans Affairs Medical Center and
University of Michigan Medical Center
Ann Arbor, Michigan 48109

OMEPRAZOLE AND ARYL HYDROCARBON HYDROXYLASES: SHOULD WE BE WORRIED?

Diaz D, Fabre I, Daujat M, Saint Aubert B, Bories P, Michel H, Maurel P. Omeprazole is an aryl hydrocarbon-like inducer of human hepatic cytochrome P450. *Gastroenterology* 1990;99:737-747.

EDITOR'S ABSTRACT

Diaz and colleagues have carefully studied the effects of omeprazole on the expression of cytochrome P-450 in primary cultures of human hepatocytes. When omeprazole was added to the culture medium in varying concentrations, there was an increase in P-450IA2 protein and mRNA concentrations, an increase in *de novo* synthesis of P-450IA2 protein, and an increase in microsomal catalytic activities characteristic of P-450IA2 (phenacetin deethylase and acetanilide hydroxylase). Omeprazole treatment also resulted in an increase in both enzymatic activity characteristic of P-450IA1 (ethoxyresorufin deethylase and benzpyrene hydroxylase) and concentration of P-450IA1 mRNA. In contrast, omeprazole appeared to have no significant effect on expression of other P-450s within the P-450II or P-450III families in the hepatocytes. To validate these *in vitro* observations, liver biopsy specimens were obtained from five patients before and after a 4-day course of pharmacological doses of omeprazole. In each patient, omeprazole treatment appeared to result in a two- to eightfold increase in P-450IA2 immunoreactive protein and P-450IA1 and P-450IA2 enzymatic activities.

The authors conclude that omeprazole is an inducer of P-450IA2 and probably P-450IA1 in human liver. Induction of these enzymes could potentiate the bioactivation of carcinogens or the hepatotoxicity of some drugs such as acetaminophen.

COMMENTS

This is an important article likely to spark considerable controversy. The data presented and the accompanying editorial (1) appear to raise new concerns about the safety of omeprazole (Losec; Merck, Sharp & Dohme, Division of Merck & Co., Inc., West Point, PA). This drug is the latest addition to the peptic ulcer disease therapeutic arsenal, which accounts for sales well in excess of \$1 billion annually. As the most potent inhibitor of gastric acid secretion clinically available, omeprazole is already used widely in the treatment of severe esophagitis, Zollinger-Ellison syndrome and refractory peptic ulcer disease.

Diaz et al. investigated the effects of omeprazole on the regulation of phase I drug metabolizing enzymes termed cytochromes P-450 (P-450s). P-450s often play critical roles in the metabolism of drugs and other xenobiotics found in the environment (2, 3). The P-450 literature has recently been simplified by the adoption of nomenclature that classifies individual P-450 enzymes by gene family (designated by roman numerals) (4). Three major P-450 families appear to be involved in xenobiotic metabolism, each consisting of proteins that share >40% amino acid sequence homology. Each family contains subfamilies of proteins (designated by capital letters) that share >60% amino acid sequence homology. This nomenclature replaces other classifications that often designated P-450s by substrate specificity or by response to known inducers. The enzymes demonstrated to be induced in the hepatocytes by Diaz et al. (P-450IA1 and P-450IA2) are "aryl hydrocarbon hydroxylases" and correspond to what has also been termed "P-448."