Decreased hepatic accumulation and enhanced esterification of cholesterol in mice deficient in *mdr1a* and *mdr1b* P-glycoproteins

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Abstract **Class I P-glycoproteins [Pgp; *MDR1* (*ABCB1*) in humans, *mdr1a* and *mdr1b* in mice] confer resistance to structurally diverse chemotherapeutic drugs in cultured cells and intact animals, but the function of these proteins in normal physiology remains poorly characterized. Based on studies in cell culture, a putative role for class I Pgp in absorption and intracellular trafficking of sterols has been proposed. We examined wild-type and *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice to determine whether class I Pgp affects cholesterol absorption and esterification in vivo. Using a dual-isotope protocol, absorption of orally administered radiolabeled cholesterol into serum did not differ between wild-type and *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice, demonstrating that class I Pgp is not essential for overall absorption of cholesterol through the intestine. However, the ratio of oral to intravenous labeled cholesterol in liver was decreased significantly in *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice. In the liver, but not other tested organs, deletion of class I Pgp enhanced kinetics of esterification of an oral bolus of radiolabeled cholesterol without affecting esterification of cholesterol administered intravenously. Steady-state hepatic content of cholesterol and esterified cholesterol also were unaffected by absence of *mdr1a* and *mdr1b*. Thus, in normal animals, class I Pgp functions to kinetically increase hepatic accumulation and decrease esterification of orally administered cholesterol in vivo.**—Luker, G. D., J. L. Dahlheimer, R. E. Ostlund, Jr., and D. Piwnica-Worms. **Decreased hepatic accumulation and enhanced esterification of cholesterol in mice deficient in *mdr1a* and *mdr1b* P-glycoproteins. *J. Lipid Res.* 2001.** 42: **1389–1394.**

Supplementary key words ATP-binding cassette transporters • multidrug resistance • liver • transgenic animals

Class I P-glycoproteins [Pgp; *MDR1* (*ABCB1*)[2] in humans, *mdr1a* and *mdr1b* in mice] are ~170-kDa integral membrane proteins that originally were identified in multidrug-resistant (MDR) tumor cells, where these proteins function to reduce intracellular concentrations of structurally diverse chemotherapeutic drugs (1). In addition to tumors, class I Pgp is expressed normally in a number of different tissues including liver, adrenal, placenta, intestine, kidney, choroid plexus, and endothelial cells at the blood-brain barrier (2, 3). Mice with genetic disruption of one (*mdr1a*$^{-/-}$) or both (*mdr1a*$^{-/-}$/*1b*$^{-/-}$) class I Pgp appear phenotypically normal under standard laboratory conditions, although these animals show altered pharmacokinetics and increased sensitivity to a variety of drugs (4, 5). Based on studies performed in these mice, one proposed biological function of class I Pgp is protection from xenobiotics encountered in the environment. Currently, no other physiological role for class I Pgp is established in vivo.

Based on cell culture systems, *MDR1* Pgp has been associated with uptake and trafficking of sterols. Nonspecific inhibitors of class 1 Pgp inhibit synthesis and esterification of cholesterol putatively by blocking trafficking of sterols from plasma membrane to endoplasmic reticulum (6–8). Relative increases in *MDR1* Pgp within a given cell type are associated with increased accumulation of cholesterol (9) and enhanced kinetics of cholesterol esterification (10), further supporting a physiologic function for Pgp in homeostasis of cholesterol. Many organs in which class I Pgp is expressed (liver, placenta, adrenal, kidney, intestine) are involved in the synthesis, metabolism, or absorption of sterols. Thus, a role for Pgp in trafficking of sterols would be consistent with these sites of expression in vivo.

To further test the hypothesis that class I Pgp has a physiologic function in metabolism of cholesterol, we studied effects of deficiency of *mdr1a* and *mdr1b* on absorption and distribution of cholesterol administered orally and intravenously to mice fed a standard chow diet. Data showed that intestinal absorption of cholesterol was not affected by absence of class I Pgp. However, the kinetics of cholesterol distribution and esterification in liver differed between

[2] ATP-binding cassette (ABC) transporters are also named within parentheses in accord with the Human Gene Nomenclature Committee.

Abbreviations: ABC, ATP-binding cassette; AUC, area under the curve; MDR, multidrug resistance; Pgp, P-glycoprotein.

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mdr1a$^{-/-}$/*1b*$^{-/-}$ and wild-type mice, showing that class I Pgp affects hepatic processing of orally administered cholesterol.

EXPERIMENTAL PROCEDURES

Materials

[1,2-^{3}H]cholesterol (45 Ci/mmol) and [4-^{14}C]cholesterol (51 mCi/mmol) were obtained from NEN Life Science Products (Boston, MA). Intralipid-10%, a triglyceride emulsion stabilized with phospholipids, was purchased from Baxter Healthcare (Deerfield, IL). Free Cholesterol C and Cholesterol CII assay kits were obtained from Wako Biochemicals (Osaka, Japan). All other reagents were from Sigma (St. Louis, MO).

Animals

Six- to 7-week-old male wild-type and *mdr1a*$^{-/-}$/*1b*$^{-/-}$ FVB male mice were obtained from Taconic (Germantown, NY) and maintained on a standard chow diet ad libitum. Protocols were approved by the Animal Studies Committee at Washington University.

Cholesterol absorption

Absorption of cholesterol was determined by the dual-isotope method as modified for rodents (11–13). Briefly, mice were given an oral dose of [^{3}H]cholesterol (~1 μCi in 150 μl) by gavage immediately followed by an intravenous dose of [^{14}C]cholesterol (~0.1 μCi in 50 μl) by tail vein injection. For both routes of administration, labeled cholesterol was complexed with Intralipid-10% by adding tracer in a volume of ethanol equal to 1% of final emulsion volume and warming for 10 min at 37°C. Animals were sacrificed by cervical dislocation at various times after administration of cholesterol as indicated in figure legends. Blood was obtained by cardiac puncture, and the serum fraction was separated using Samplette tubes (Sherwood Medical, St. Louis, MO) according to the manufacturer's instructions. Organs were removed and blotted to remove adherent blood. Cholesterol absorption into the wall of the small and large intestine was analyzed after extensive washing with water to clear retained contents. The rectus abdominus was dissected as a representative sample of muscle. Samples were weighed prior to extraction of lipids (14) and then ^{3}H- and ^{14}C-labeled cholesterol counts were determined by liquid scintillation counting. Ratio of oral to intravenous radiolabeled cholesterol counts was calculated using the following formula (11):

$$\frac{\text{oral cholesterol}}{\text{intravenous cholesterol}} = \frac{\text{tissue } (^{3}\text{H dpm} / ^{14}\text{C dpm})}{\text{administered } (^{3}\text{H dpm} / ^{14}\text{C dpm})}$$

Lipid analyses

Amounts of total and free cholesterol in lipid extracts were measured using enzymatic assays (Wako) according to the manufacturer's instructions, and were normalized to gram of liver or deciliter of serum. The difference between total and free cholesterol was used to determine the amount of esterified cholesterol. To determine conversion of ^{3}H- and ^{14}C-labeled cholesterol to the respective cholesteryl esters, lipid extracts were separated by TLC (10, 15). Spots corresponding to cholesterol and cholesteryl ester were quantified by liquid scintillation counting. Data for ^{3}H- and ^{14}C-labeled cholesteryl ester were expressed as dpm/g tissue for tracer content or (dpm/g tissue)h for area-under-the-curve (AUC) analysis of cholesterol esterification over time.

Statistical analysis

Data are reported as mean values + or ± SEM for the number of animals indicated in the figure legends and tables. AUC for cholesteryl ester was determined with Kaleidagraph (Synergy Software, Reading, PA). Pairs were compared by two-way analysis of variance, and values of $P \leq 0.05$ were considered significant (16).

RESULTS

Absorption of orally administered cholesterol

We used the dual-isotope protocol to determine whether class I Pgp affects absorption of cholesterol from the intestine into serum and selected organs. Previous data established that relative levels of orally and intravenously administered cholesterol become stable by 72 h (11). Ratios of oral to intravenous cholesterol counts were determined at 24, 48, and 72 h after bolus administration of radiolabeled cholesterol, allowing us to quantify effects of class I Pgp both before and at steady state (**Fig. 1**). Orally absorbed cholesterol found in serum did not differ significantly between wild-type and *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice (Fig. 1A). Although the ratio of oral to intravenous cholesterol counts in liver did not differ between geno-

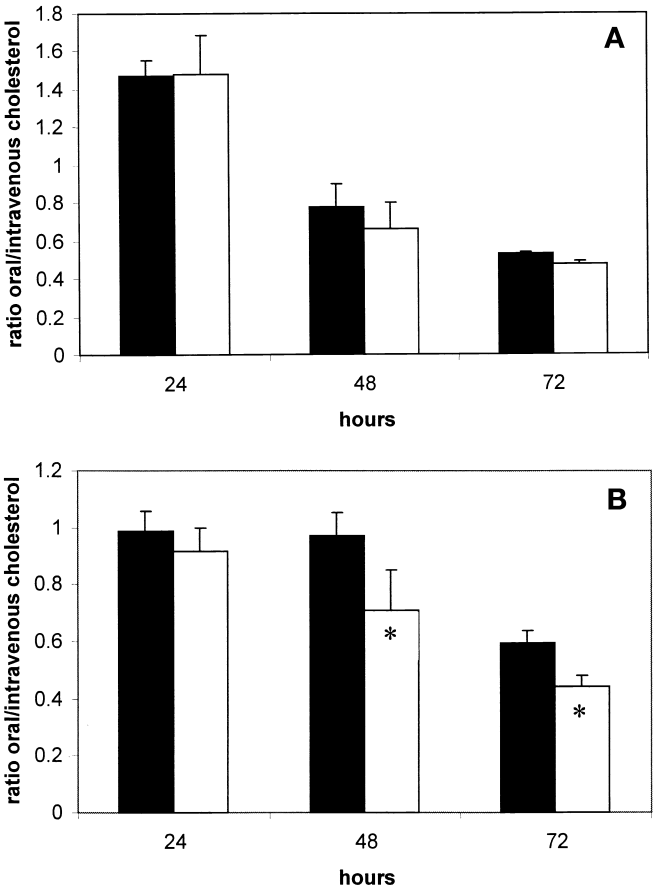

Fig. 1. Ratios of oral [^{3}H]cholesterol to intravenous [^{14}C]cholesterol in serum (A) and liver (B) were determined 24, 48, and 72 h after administration of tracers in wild-type FVB (closed bars) and *mdr1a*$^{-/-}$/*1b*$^{-/-}$ (open bars) mice as described in Experimental Procedures. Each data column is the mean value for n = 3 (24 h), n = 5 (wild-type mice at 48 h), or n = 6 (all other time points) mice. Bars represent + SEM. * $P < 0.03$ relative to wild-type mice.

types at 24 h, absence of class I Pgp significantly affected ratios in liver at 48 h and 72 h (Fig. 1B). At the latter time points, ratios of oral to intravenous labeled cholesterol counts were 0.97 ± 0.08 versus 0.71 ± 0.14 at 48 h and 0.60 ± 0.04 versus 0.44 ± 0.03 at 72 h for wild-type versus *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice, respectively. Over the course of the experiment, the ratio of oral to intravenous cholesterol in liver was significantly less in *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice ($P < 0.03$). Because ratios of labeled cholesterol in serum did not differ between genotypes, these differences are not due to blood retained within liver parenchyma. We also determined ratios of orally and intravenously administered cholesterol in muscle and all layers of the wall of the intestine at 72 h. In these sites, the ratio of oral to intravenous cholesterol did not differ significantly between wild-type and *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice (data not shown). These data do not exclude a function for class I Pgp in epithelia of the intestine because mucosa was not isolated from the remaining components of the wall of the intestine. Overall, in normal animals, these data suggested that class I Pgp increased the kinetics of hepatic accumulation of orally administered cholesterol in liver without altering overall absorption of cholesterol from intestine into serum.

Kinetics of esterification of orally administered cholesterol

Previous studies have shown that *MDR1* Pgp enhances esterification of cholesterol in cultured cells in vitro (10). To determine whether class I Pgp also affects cholesterol esterification in vivo, we quantified radiolabeled cholesterol and esterified cholesterol by TLC at different time points following pulses of orally and intravenously administered ^{3}H- and ^{14}C-labeled cholesterol, respectively (**Fig. 2**). The kinetics for [^{3}H]cholesteryl ester or [^{14}C]cholesteryl ester in serum did not differ significantly between wild-type and *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice (Fig. 2A and C). In addition, serum content of [^{3}H]- and [^{14}C]cholesteryl ester by AUC analysis over the course of the experiment did not differ significantly (285,027 ± 40,928 and 264,741 ± 50,214 ^{3}H (dpm/g tissue)h and 55,338 ± 4,636 and 52,617 ± 8,211 ^{14}C (dpm/g tissue)h for [^{3}H]- and [^{14}C]cholesteryl ester in wild-type and *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice, respectively ($P > 0.7$). Serum levels of unesterified oral and intravenous labeled cholesterol also did not differ significantly between the two genotypes of mice over the course of this experiment (data not shown). These data indicate that serum levels of cholesteryl ester were not affected significantly by class I Pgp, and further establish that the protein was not essential for absorption of cholesterol from the intestine into the serum compartment.

We also quantified the kinetics of labeled cholesteryl ester in liver following single boluses of oral ^{3}H- and intravenous ^{14}C-labeled cholesterol. Content of [^{3}H]cholesteryl ester in liver over time was affected by the presence or absence of class I Pgp (Fig. 2B). At the 6-h time point, values for [^{3}H]cholesteryl ester dpm/g liver were approximately 2-fold greater in *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice compared with wild-type mice ($P < 0.01$). Differences in [^{3}H]cholesteryl ester dpm/g liver were progressively less by 24 h and 72 h after feeding [^{3}H]cholesterol. Over the course of the experiment, hepatic content of [^{3}H]cholesteryl ester by AUC was 80,241 ± 18,297 and 122,700 ± 33,050 (dpm/g tissue)h for wild-type and *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice, respectively ($P < 0.01$). Unlike data for [^{3}H]cholesteryl ester, hepatic [^{14}C]cholesteryl ester dpm/g tissue was not affected by expression of class I Pgp (Fig. 2D). Consistent with counts for total cholesterol determined previously (Fig. 1), 72-h steady-state ratio of recovered hepatic ^{3}H/^{14}C counts {([^{3}H]cholesterol + [^{3}H]cholesteryl ester)/([^{14}C]-cholesterol + [^{14}C]cholesteryl ester)} was lower in *mdr1a* $^{-/-}$/*1b*$^{-/-}$ mice (data not shown). These results demonstrated that *mdr1a* and *mdr1b* Pgp functionally enhanced hepatic content of total cholesterol, but reduced

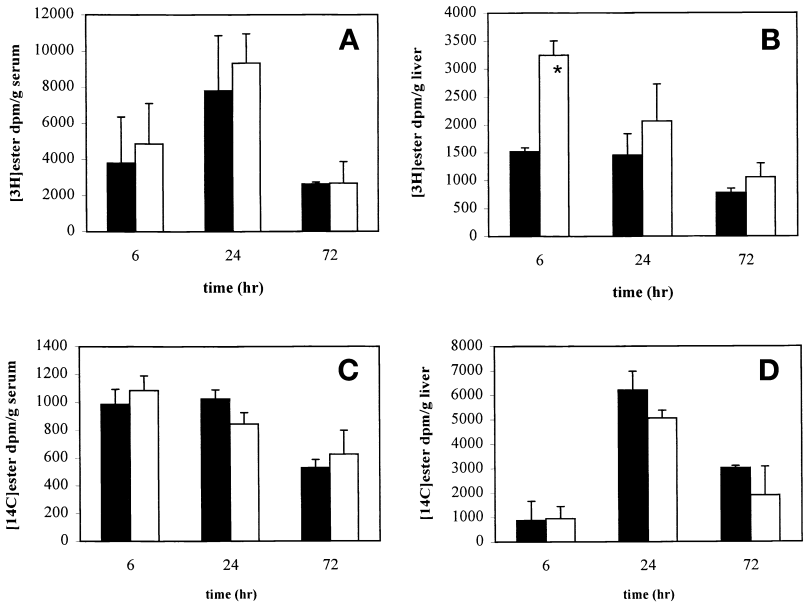

Fig. 2. Content of radiolabeled cholesteryl ester in serum and liver. Mice were sacrificed at the indicated time points following oral administration of [^{3}H]cholesterol and intravenous administration of [^{14}C]cholesterol. Lipids were extracted and then separated by TLC as described in Experimental Procedures. Cholesteryl esters were quantified by liquid scintillation counting. [^{3}H]cholesteryl ester (A and B) and [^{14}C]cholesteryl ester (C and D) were determined in serum (A and C) and liver (B and D) from wild-type FVB (closed bars) and *mdr1a*$^{-/-}$/*1b*$^{-/-}$ (open bars) mice, with n = 3, n = 2, and n = 2 mice of each genotype for the 6-, 24-, and 72-h time points, respectively. Data are expressed as mean dpm cholesteryl ester/g tissue. Bars represent + SEM (n = 3) or range (n = 2). * $P < 0.01$ relative to wild-type mice.

hepatic content of cholesteryl ester derived from cholesterol administered orally but not intravenously.

We also analyzed content of radiolabeled cholesterol and cholesteryl ester in other selected tissues following boluses of oral [^{3}H]- and intravenous [^{14}C]cholesterol. AUC or content of [^{3}H]- or [^{14}C]cholesteryl esters in muscle, spleen, lung, heart, or wall of intestine did not differ between wild-type and *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice (**Table 1**). In these organs, accumulation of [^{3}H]- or [^{14}C]cholesterol also was not affected by expression of class I Pgp (data not shown).

Tissue content of cholesterol and cholesteryl ester

Data from the time-course experiment with radiolabeled cholesterol suggested that class I Pgp affects the kinetics of cholesterol esterification in the liver without altering steady-state levels of cholesteryl ester in this organ. On a normal chow diet, tissue content of total cholesterol or cholesteryl ester did not differ significantly between the two genotypes of mice: 1.12 ± 0.26 versus 1.03 ± 0.18 mg cholesterol/g liver and 0.29 ± 0.08 versus 0.25 ± 0.11 mg esterified cholesterol/g liver for wild-type versus *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice, respectively. Similar to these data from liver, amounts of total cholesterol and cholesteryl ester in serum were not affected by expression of class I Pgp: 110 ± 12 versus 104 ± 18 mg cholesterol/dl and 83.6 ± 13.7 versus 75.9 ± 11.2 mg esterified cholesterol/dl for wild-type versus *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice, respectively. These data indicated that *mdr1a* and *mdr1b* Pgp affected kinetics of absorption and esterification of a pulse of orally administered cholesterol without affecting steady-state content of these lipids in liver.

DISCUSSION

Possible physiologic functions for class I Pgp in oral absorption and intracellular trafficking of cholesterol have been proposed based on studies with cultured cells. However, a role for class I Pgp in metabolism of cholesterol in vivo has not been established. The data presented herein demonstrate that class I Pgp affects kinetics of cholesterol accumulation and esterification in vivo, but the effects are confined to the liver.

Using a dual-label protocol for pulses of oral and intravenous cholesterol, overall absorption of orally administered cholesterol into the blood compartment and selected tissues was not affected by the presence or absence of class I Pgp. Specifically, ratios of oral to intravenous cholesterol in serum, muscle, and intestine did not differ between wild-type and *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice. Thus, class I Pgp is not required for normal absorption of cholesterol through the intestine. However, following gavage with radiolabeled cholesterol, significantly less oral cholesterol was detected in livers of *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice. Because the data in vivo imply that the kinetics of hepatic delivery of orally administered cholesterol were not affected by class I Pgp, the differences between genotypes in hepatic accumulation of oral cholesterol would appear intrinsic to liver. Nonetheless, differences between wild-type and knockout mice were relatively modest, suggesting that redundant pathways exist for hepatic accumulation of cholesterol. This conclusion is supported further by data showing equivalent amounts of nonradiolabeled cholesterol in livers of both genotypes of mice fed a standard chow diet.

The *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice had increased kinetics of accumulation of cholesteryl ester in liver following bolus administration of oral cholesterol, but effects on cholesteryl esters were not observed when cholesterol was injected intravenously as a lipid emulsion. These data suggest that physiologic steps in absorption of cholesterol through the intestine and delivery to the liver are necessary for class I Pgp to affect intracellular trafficking and metabolism of cholesterol in hepatocytes. In this regard, previous studies in cultured macrophages have shown that endocytic processing and intracellular transport of cholesterol are determined by type of lipoprotein particle (17, 18). Recent data also suggest that trafficking of cholesterol in hepatocytes in vivo may be altered by the absence

TABLE 1. Tracer content and AUC for [^{3}H]cholesteryl esters and [^{14}C]cholesteryl esters in selected tissues

	FVB				*mdr1a*$^{-/-}$/*1b*$^{-/-}$			
Organ	6 h	24 h	72 h	AUC	6 h	24 h	72 h	AUC
		dpm/g tissue		*(dpm/g tissue)h*		*dpm/g tissue*		*(dpm/g tissue)h*
[^{3}H]Cholesteryl ester								
Muscle	4,888 ± 195	10,497 ± 1,155	10,017 ± 466	630,801 ± 57,204	5,338 ± 393	8,172 ± 1,560	7,071 ± 1,556	487,422 ± 96,077
Heart	3,270 ± 913	6,686 ± 966	5,754 ± 119	388,164 ± 50,458	3,111 ± 417	7,116 ± 93	6,063 ± 3,131	408,339 ± 125,162
Intestine	2,978 ± 1,662	2,031 ± 72	899 ± 27	115,401 ± 40,606	3,454 ± 1,129	1,574 ± 241	776 ± 49	101,652 ± 29,756
Lung	6,205 ± 862	9,559 ± 626	9,325 ± 573	595,092 ± 42,594	5,918 ± 287	9,269 ± 783	8,951 ± 2,265	573,963 ± 97,563
Spleen	503 ± 86	980 ± 78	896 ± 207	58,371 ± 9,251	546 ±61	892 ± 40	716 ± 228	51,534 ± 9,367
[^{14}C]Cholesteryl ester								
Muscle	3,550 ± 182	4,149 ± 104	3,993 ± 476	264,699 ± 19,571	3,893 ± 227	3,646 ± 376	3,578 ± 102	241,227 ± 18,840
Heart	3,293 ± 180	2,144 ± 304	2,007 ± 120	148,557 ± 15,598	3,004 ± 45	2,343 ± 139	2,308 ± 14	159,747 ± 6,602
Intestine	339 ± 16	398 ± 14	392 ± 2	23,613 ± 765	398 ± 8	400 ± 11	370 ± 28	25,662 ± 1,243
Lung	4,063 ± 473	4,130 ± 153	4,173 ± 4	273,009 ± 13,548	3,916 ± 178	4,443 ± 125	4,357 ± 234	286,431 ± 11,816
Spleen	493 ± 38	377 ± 24	282 ± 52	23,646 ± 2,529	561 ± 89	456 ± 20	357 ± 1	28,665 ± 2,359

Orally (^{3}H) and intravenously (^{14}C) administered cholesterol were given to FVB and *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice (n = 7 for each genotype) as described in the legend to Fig. 2. Data represent mean values for [^{3}H]cholesteryl ester and [^{14}C]cholesteryl ester, expressed as dpm/g tissue ± SEM at a given time point and AUC [(dpm/g tissue)h ± SEM]. $P > 0.05$ for all data points relative to their respective controls.

or presence of apolipoprotein E (19). Thus, differing effects of class I Pgp on esterification of orally or intravenously administered cholesterol may reflect the context in which cholesterol is presented to hepatocytes. Kinetics and total accumulation of cholesteryl ester from both oral and intravenous sources were unaffected by class I Pgp in serum and all other sampled tissues. Thus, effects on esterification are not consistent with changes in activity of LCAT in blood or delivery of cholesteryl esters to liver. Rather, these data support our conclusion that differences in esterification of orally administered cholesterol in liver are due to hepatocellular-specific effects of class I Pgp on trafficking and processing of cholesterol by ACAT.

The decreased esterification observed in wild-type mice in vivo stands in contrast to studies with cultured cells wherein expression of class I Pgp actually facilitates esterification of cholesterol added directly to the plasma membrane (10). However, the current results do not allow us to determine whether these effects represent alterations in hepatocellular influx or efflux kinetics of cholesterol and metabolites, complicating analysis of the differences between the in vivo and in vitro systems. Potentially, the contrasting results may be due to the polar nature of hepatocytes in vivo, wherein class I Pgp trafficks directly from Golgi to bile canaliculus without traversing the basolateral membrane (20). Thus, cholesterol enters from the basolateral membrane of hepatocytes in vivo, and crosses the cell before reaching the apical surface where class I Pgp is expressed. Conversely, in vitro studies of the role of class I Pgp in esterification of cholesterol have used cell lines with absent or partial polarization (6, 10, 21) or cells in which cholesterol is presented initially to the Pgp-containing (apical) surface (8, 9). To better define in vivo effects of class I Pgp on cholesterol trafficking in liver, further studies using systems in vitro that maintain the physiologic presentation of cholesterol and polarization of class I Pgp in hepatocytes will be required.

Many different ABC transporters are associated with transport of sterols and lipids both in vitro and in vivo. MDR3 (*ABCB4*) Pgp functions as a flippase for phosphatidylcholine at the bile canaliculus (22); genetic deficiency of this protein causes secondary abnormalities in postprandial formation of chylomicrons, absorption of cholesterol from the intestine, and low levels of HDL (12, 23). Mutations in the liver-specific ABC transporter, sister of Pgp (*ABCB11*), cause defects in export of bile salts from hepatocytes in patients with progressive familial intrahepatic cholestasis (24). Another ABC transporter, *ABCG5*, has been identified as the abnormal gene in sitosterolemia, a disorder in which patients absorb excessive amounts of cholesterol and other sterols (25). Recent work by Repa et al. (26) showed that increased expression of *ABC1* (*ABCA1*) reduces absorption of cholesterol from the intestine, and mutations in *ABC1* have been identified in patients with Tangier disease, a disorder characterized by absence of plasma HDL and accumulation of cholesteryl esters in the reticulo-endothelial system (27–30). Klucken et al. (31) showed that *MDR1* (*ABCB1*) was among 20 different ABC transporters in macrophages whose levels of expression changed in response to cellular content of cholesterol. Both *ABC1* and the related *ABC8* (*ABCG1*) are up-regulated in response to cholesterol loading, consistent with their proposed functions as efflux transporters for cholesterol and phospholipids (31). Of note, *MDR1* expression was regulated in the opposite direction to that of *ABC1*, in support of a role for *MDR1* Pgp in cholesterol uptake. In addition, in a study of atherosclerotic lesions from human arteries, mRNA levels for *MDR1* and *ACAT* were correlated positively with content of cholesteryl ester (32). These observations suggest that a physiologic function of class I Pgp in cholesterol homeostasis may be more apparent after chronic feeding of a high fat/high cholesterol diet rather than standard chow. Breeding *mdr1a*$^{-/-}$/*1b*$^{-/-}$ mice with genotypes that are susceptible to atherosclerosis likely will be necessary to establish whether class I Pgp has a role in development or progression of atherosclerotic lesions.

In summary, the present data demonstrate an in vivo function for class I Pgp in hepatic processing of cholesterol, although our results cannot exclude the possibility that the effects are indirect. Trafficking of cholesterol mediated by Pgp appears to require physiologic absorption of cholesterol through the intestine. Consistent with studies in cell culture, class I Pgp does not impact hepatic content of cholesterol or cholesteryl ester under steady-state conditions, but specifically affects kinetics of accumulation and esterification of an oral bolus of cholesterol. However, the effects on esterification of cholesterol determined in vivo and in vitro are in opposite directions, perhaps reflecting different polarities of the model systems. Further studies in vivo with specific radiolabeled lipoprotein particles may better define the molecular mechanisms and relations with other ABC transporters in cellular trafficking of cholesterol mediated by class I Pgp.

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