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Does P-Glycoprotein Play a Role in Gastrointestinal Absorption and Cellular Transport of Dietary Cholesterol?

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ABSTRACT This commentary discusses the potential role of p-glycoprotein (Pgp) on the gastrointestinal absorption and cellular transport of dietary cholesterol. This is currently a controversial issue due to the conflicting evidence about the role of this ABC transporter in cholesterol transport. During the preparation of this commentary, several key publications on this topic arguing for and against this mechanism have been published. If true, this mechanism of Pgp could represent a novel role for Pgp and provide a potentially new molecular target for drug design and development.

KEYWORDS P-glycoprotein, mdr1, Gastrointestinal absorption, Cholesterol, Cellular transport

Cardiovascular disease, brought about by atherosclerosis, remains one of the leading causes of death in North America (Chow et al., 2005; Bourgault et al., 2005). It is the leading cause of death and disability in the Western world, resulting in more than one million deaths each year in North America and costing the health care system in excess of US $200 billion annually in direct costs. The annual worldwide market for prescription pharmaceuticals aimed at reducing cholesterol reached $12.3 billion (U.S.) in 2003 and is growing by approximately 7.5 percent annually. High plasma cholesterol levels, smoking, and high blood pressure are some of the major contributing risk factors in this fatal disease. However, the risk factor which has received the most attention over the past two decades is cholesterol. An elevated level of plasma cholesterol has been demonstrated to play an important role in the development of atherosclerosis. In man, cholesterol transport into the small intestinal cell contributes to the plasma cholesterol pool and to maintain whole body cholesterol balance. Unlike any other cell type within the body, the intestinal absorptive cell is bathed at its apical surface with dietary and biliary cholesterol (Field et al., 1998). This constant influx of cholesterol clearly adds to the total cholesterol pool of the enterocyte. In this regard, the intestinal cell is quite unique. With the continuous influx of cholesterol at the apical membrane, cholesterol synthesis and low-density lipoprotein receptor expression are suppressed. Since absorbed cholesterol is likely the major source of cholesterol

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for the intestinal cell, understanding its transport and utilization is of importance. Cholesterol homeostasis in the cell is regulated by a complex set of mechanisms that include cholesterol biosynthesis, hydrolysis from lipoproteins internalized into lysosomes, and bidirectional transport of cholesterol to the endoplasmic reticulum (ER) where cholesterol undergoes esterification, and from the ER to the plasma membrane (PM) (Hui & Howles, 2005; Turley, 2004). Although the transport of cholesterol between the PM and ER has been demonstrated, the mechanisms involved in this transport remain poorly understood.

Class I P-glycoproteins [Pgp; encoded by the mdr1 (human) & mdr1a/1b (mice) genes are integral cell membrane proteins that were originally identified in multidrug-resistant tumor cells (Glavinas et al., 2004; Breier et al., 2005; Chang, 2003). The original functions elucidated were to reduce intracellular concentrations of structurally diverse chemotherapeutic agents and to act as efflux transporters of xenobiotics from the small intestine enterocytes back into the intestinal lumen resulting in decreased drug bioavailability (Kunta & Sinko, 2004; Drescher, 2003).

Possible physiologic functions for class I Pgp in oral absorption and intracellular trafficking of cholesterol have been suggested based on preliminary studies in cell culture. Previous studies have reported that agents that were used to interfere with cholesterol intracellular transport from the plasma membrane to the endoplasmic reticulum (ER) were also used to reverse the multidrug-resistant phenotype in cells having amplified expression of Pgp. For example, progesterone, verapamil, and trifluoperazine, agents that interact with Pgp, inhibit the arrival of plasma membrane cholesterol to the ER in Caco-2, an intestinal cell line (Field, 1998). In contrast, methotrexate, an antimetabolyte that does not interact with Pgp, has no effect on this process. Moreover, progesterone, verapamil, and trifluoperazine also inhibit the secretion of newly synthesized apolipoprotein (apo) B and apoB mass and decreased the secretion of newly synthesized triacylglycerols, triacylglycerol mass, and cholesteryl esters. Taken together these observations suggest that these agents not only interfere with cholesterol transport from the plasma membrane but also interfere with the normal secretion of lipoprotein particles. Not only is the number of secreted lipoprotein particles decreased by these agents, but also affected is the amount of lipid that is carried in these particles.

To further support the hypothesis that Pgp is involved in cholesterol intracellular transport within Caco-2 cells, other recognized inhibitors of Pgp activity, including amiodarone, colchicines, cyclosporine A, forskolin, and tamoxifen were tested. These compounds were found to interfere with both cholesterol transport from the plasma membrane to the ER and apoB secretion.

Further studies by Wang et al. (2000) reported that cholesterol increased the Pgp-mediated ATP hydrolysis by approximately 1.6-fold with a K_s of 5 μM suggesting that cholesterol may directly interact with Pgp (Wang et al., 2000). Tessner and Stenson (2000) reported that mdr1-transfected IEC-18 rat intestinal epithelial cells exhibited increased Pgp expression reduced accumulation of vinblastine and increased uptake of [3H]cholesterol from cholesterol/monolein/taurocholate micelles. Verapamil, a Pgp inhibitor and UIC2, an antibody against mdr1, both diminished cholesterol absorption in these cells, but did not completely inhibit cholesterol cellular uptake. Recently we have observed that incubation of Caco-2 cells with increasing concentrations of cholesterol resulted in a significant decrease in mdr1 gene expression compared to untreated controls (Wasan et al., unpublished results). These data suggest that cholesterol uptake may regulate Pgp expression.

In a recent study Le Goff et al. (2005) examined the role of Pgp in cholesterol efflux. In kidney cells transfected with Pgp cholesterol efflux to methyl-beta-cyclodextrin (CD) was fourfold higher as compared to control cells, suggesting that the accessible pool of plasma membrane cholesterol was increased by Pgp expression. However, when cholesterol efflux to CD was examined in a HeLa cell model of inducible Pgp expression, there was no increased efflux associated to Pgp expression. The limitation of this study and others involving drug-selected cell lines is that these conditions may affect the expression of other ABC transporters. For instance, in the sPgp (sister of Pgp) knockout mice, Lam et al. (2005) reported the up regulation of Pgp but not of mdr2, mrp2, or mrp3. Another important issue is the selection of the cell line as kidney and HeLa cells may not resemble the characteristics of intestinal cells.

However, although the data reported in these studies are very suggestive that within intestinal cells, Pgp is involved in cholesterol transport, the evidence remains circumstantial. For example, the Pgp inhibitors used to demonstrate decreased cholesterol transfer from the plasma membrane to the ER themselves also

induce nonspecific (i.e., Pgp independent) effects on the cell. Thus, it is possible that these agents may interfere with cholesterol transport from the plasma membrane to the ER by mechanisms other than Pgp inhibition.

To date, few *in vivo* studies have been published trying to establish the role of class I Pgp on the absorption, distribution and metabolism of cholesterol.

Plosch et al. (2002) reported that following 15 days of a standard mouse diet to mdr1a/b deficient mice resulted in a significant decrease in total/plasma cholesterol and liver cholesteryl ester concentrations compared to wild-type mice. These observations are in concordance with other studies that demonstrate that Pgp is abundant in small intestinal absorptive cells located in the upper villous and further support the role of class I Pgp being involved in the intestinal absorption of cholesterol. In addition, Tous et al. (2005) recently reported that Turpentine-induced inflammation reduces the hepatic expression of mdr1 gene, the plasma cholesterol concentration and the development of atherosclerosis in apolipoprotein E deficient mice. They concluded that mdr1 may be an additional factor to consider in the complexity of alterations in cholesterol metabolism that occur in this model.

However, contrary to Plosch's findings, Luker et al. (2001) have reported absorption of orally administered radiolabeled cholesterol did not differ between wild-type and mdr1a and mdr1b Pgp deficient mice, suggesting that class I Pgp is not essential for overall absorption of cholesterol through the intestine, although they did show a decrease in hepatic cholesterol, but not due directly to absorption. They suggested that the lack of mdr1 appears to reduce the transport/trafficking of orally administered cholesterol to the liver. A potential limitation of this study was that the mice were fed a standard chow diet rather than a high-cholesterol and fat diet. It is possible that a physiologic function of class I Pgp in cholesterol homeostasis may be more apparent after chronic feeding of a high-cholesterol diet rather than standard chow. However the mdr1 high-fat/high-cholesterol experiment is not easy to carry out since the control mice, FVB, carry some genetic defect that renders them obese-resistant and thus complicates the interpretation of the results (Hu et al., 2005).

Recently Rodrigues et al. (2005) studied a group of hypercholesterolemic patients and found that a high baseline serum total and LDL cholesterol levels were associated with a specific mdr1 haplotype, suggesting that it may contribute to increased plasma cholesterol levels. Interestingly, this polymorphism presented a significant decreased duodenal mdr1 expression and increased digoxin plasma concentration (Hoffmeyer et al., 2000).

One component of the Pgp-cholesterol story that has not yet been explored extensively is the presence of cholesterol binding sites on Pgp. Recently, Wang et al. (2005) proposed that cholesterol interacted with the daunorubicin binding site in P-glycoprotein. The specific site of interaction between daunorubicin and Pgp is unknown and its identification could shed further light on the cholesterol binding site in Pgp. Taken together these *in vitro* and *in vivo* findings suggest that Pgp may be involved in the cellular homeostasis of cholesterol.

In summary, this commentary has discussed the potential role of p-glycoprotein on the gastrointestinal absorption and cellular transport of dietary cholesterol. This is currently a controversial issue due to the evidence in favor and against the role of this ABC transporter in cholesterol transport. During the preparation of this commentary, several key publications on this topic arguing for and against this mechanism have been published. If true, this mechanism of Pgp could represent a novel role for Pgp and provide a potentially new molecular target for drug design and development.

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