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The Structure and Function of Systemic Circulatory Lipoproteins and their Influence on Drug Disposition and Pharmacological Activity

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Lipoproteins are particles of lipids and proteins that primarily transport lipids through the vascular and extravascular body fluids to cells that require them for energy purposes. However, recent evidence has suggested that lipoproteins may act as transporters of different drug compounds and this interaction could lead to alterations in the biopharmaceutical and pharmacological action of the drug. In this paper, the important aspects of lipoprotein structure and function are discussed. Furthermore, the role of lipoproteins as transporters of hydrophobic drug compounds within the systemic circulation and the factors that influence the interaction with lipoproteins will be reviewed.

Lipoproteins are a heterogeneous population of macromolecular aggregates of lipids and proteins that are responsible for the transport of lipids through the vascular and extravascular fluids from their site of synthesis or absorption to peripheral tissues[1,2]. These lipids, which include triacylglycerols (TG), cholesteryl esters (CE) and phospholipids (PL) are delivered from the liver and intestine to other tissues in the body for storage or catabolism in the production of energy. Lipoproteins are also known to be involved in a number of other biological processes including coagulation of blood, tissue repair and act as carriers for a number of hydrophobic drug compounds within the systemic circulation[3-5].

It has been well documented that the circulating lipoprotein content and composition is significantly influenced by pathological conditions[5]. Therefore, it appears possible that alterations in the lipoprotein profile would not only affect the ability of a compound to associate with lipoproteins but also affect its distribution within the lipoprotein subclasses, resulting in alterations of the pharmacokinetic and pharmacological properties of the drug.

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Lipoprotein Structure and Function:

Lipoproteins are spherical particles consisting of a nonpolar lipid core (TG and CE) surrounded by a surface monolayer of amphipathic lipids (PL and unesterified cholesterol) and specific proteins called apolipoproteins[1]. A number of different phospholipids are incorporated into the coat of the lipoprotein, the most abundant of these phospholipids is phosphatidylcholine. Phosphatidylcholine is also utilized as a substrate in the esterification of cholesterol to cholesterol ester by the enzyme lecithin cholesterol acyltransferase[1]. Since lipids, in general, have lower buoyant densities than proteins, lipoproteins with a larger amount of lipid relative to protein will have a lower density than lipoproteins with a smaller lipid-to-protein ratio[1].

Plasma lipoproteins are classified and separated according to their densities and are divided into five main categories: triglyceride-rich lipoproteins which includes chylomicrons and very low-density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL)[1].

Chylomicrons are the largest of the lipoproteins (diameter of approximately 100-1000 nm) and are found in greatest abundance after a meal. They are synthesized by the intestine and are core-rich in TG derived from dietary fat. VLDL is the next largest lipoproteins (diameter of approximately 30-80 nm) and is also rich in TG. They are synthesized mainly by the liver but may also be synthesized to a lesser degree by the intestine. IDL, whose lipid core is comprised mainly of CE with some TG, are the resultant products of VLDL metabolism. However, LDL are the products of IDL metabolism in which almost all of the remaining TG have been hydrolyzed to produce a lipoprotein with a core comprised almost entirely of CE. LDL is the second smallest lipoproteins (diameter of approximately 18-25 nm) and the major carrier of cholesterol (mostly in the form of CE). HDL is the smallest of the lipoproteins with a diameter of approximately 7 to 12 nm.

Lipoproteins as Novel Carriers of Cyclosporine (CsA):

A number of laboratories, including ours, have shown CsA to associate with lipoproteins upon incubation in human plasma[6-8], resulting in a modification of its pharmacological activity. de Kippel *et al*[9]. and Nemunaitis *et al.*[10] have reported decreases in CsA activity in patients that have elevated plasma triglyceride levels, while de Groen and coworkers have observed an increase in CsA toxicity in patients with hypolipidemia[11]. Studies have demonstrated that the antiproliferative effects of CsA are enhanced when the drug is associated with LDL, but not VLDL or HDL[12]. In heart transplant patients, with high total plasma cholesterol levels the association of CsA with plasma LDL as well as CsA-induced renal toxicity is increased when compared to normolipidemic controls[13]. The phenomenon of aggravated CsA-induced renal toxicity is also observed in kidney transplant patients who have elevated plasma cholesterol levels[14]. These studies provide preliminary evidence suggesting that plasma lipoprotein lipid levels have a major impact on the efficacy and toxicity of CsA.

Factors that Influence CsA Interactions with Plasma Lipoproteins:

Since CsA is often administered to patients with abnormal lipid metabolism (i.e. hypo and hypercholesterolemia and/or hypertriglyceridemia), its association with plasma lipoproteins and the impact on the drug's efficacy and safety is a cause of concern in CsA therapy. Our laboratory has investigated two characteristics of dyslipidemic plasma, which may influence the association of CsA with plasma lipoproteins and thus modify its pharmacokinetics and pharmacodynamics. These are (1) changes in the rate of transfer of esterified cholesterol and triglycerides between different lipoprotein classes and (2) changes in plasma lipoprotein lipid and protein content.

(1) Influence of Cholesteryl Ester Transfer Protein (CETP):

A lipid transfer protein, often referred to as cholesteryl ester transfer protein (CETP) facilitates the transfer of cholesterol and triglyceride *in vivo*[15]. An alteration in CETP-mediated transfer of these endogenous substrates between different lipoprotein classes is an important feature of dyslipidemic plasma[15]. Our laboratory has hypothesized, that, since the human body recognizes hydrophobic compounds as lipid-like particles, an increase in CETP concentration and activity may facilitate the movement of compounds, such as CsA, among different lipoprotein classes[16]. We have demonstrated in some *in vitro* experiments, that increasing the CETP concentration results in an increase in the percentage of CsA recov-

TABLE 1: EFFECT OF CETP ON THE DISTRIBUTION OF CsA INTO PLASMA LIPOPROTEINS

Amount of CETP[a] (ug protein)	HDL/LPDP (%)[b]	LDL/VLDL (%)[b]
0	51+/-1	49+/-5
0.5	57+/-4*	43+/-4
1.0	59+/-3*	40+/-1*
2.0	61+/-1*	38+/-1*

Data were expressed as mean +/- standard deviation (n=6). *$p<0.05$ vs. HDL/LPDP or LDL/VLDL fraction at CETP = 0. CsA, cyclosporine; CETP, cholesteryl ester transfer protein; HDL, high density lipoproteins; LPDP, lipoprotein deficient plasma; LDL, low density lipoproteins; VLDL; very low density lipoproteins. [a]amount of exogenous CETP added to 1 ml of human plasma. Endogenous CETP concentration was 1 µg protein/ml of human plasma for all test samples. All incubations were carried out for 60 minutes in pooled human plasma. [b]percent of initial cyclosporine incubated in human plasma. Total recovery >98%. Adopted from reference 15.

ered in the HDL/lipoprotein deficient plasma (LPDP) fraction Table 1[16]. Studies from our laboratory have also demonstrated that CETP, perhaps, has the inherent ability to transfer CE and drug separately.

In experiments that were designed to directly measure the potential role of CETP to facilitate CsA transfer, it was shown that CETP-mediated percent transfer of CE among HDL and LDL particles in human plasma was significantly different from that of CsA[16]. Besides the capability of independently transferring lipids and drugs, these differences could also be attributed to the ability of HDL and LDL particles to accumulate a higher amount of CE than CsA[16].

Furthermore, when CETP-mediated transfer of CE between HDL and LDL was inhibited by a monoclonal antibody directed against CETP, TP2, only the transfer of CsA from LDL to HDL was significantly decreased[16]. These results suggest that the transfer of CsA from LDL to HDL but not from HDL to LDL is partially facilitated by CETP. The transfer of CsA from HDL to LDL is probably facilitated by other plasma factors and/or due to spontaneous transfer. This notion is supported by the work of Hughes and co-workers[17], who hypothesized that the plasma distribution of CsA is determined by factors other than simple diffusion between lipoprotein particles.

These findings suggest that the distribution/redistribution of CsA among plasma lipoproteins facilitated by CETP may serve as a possible mechanism for determining the ultimate biological fate of these compounds.

(2) Influence of Lipoprotein Concentration and Composition:

Besides alterations in CETP-mediated transfer of endogenous lipids and drugs, the dyslipidemic plasma is also characterized by an increase and/or decrease in plasma lipoprotein cholesterol and triglyceride concentrations. Since CsA associates with plasma lipoproteins *in vivo*, our laboratory has hypothesized that changes in lipoprotein concentration and composition would alter the lipoprotein distribution of CsA.

Studies from our laboratory have reported that dyslipidemic plasma and specific increases in LDL and VLDL lipid plasma levels results in an increasing amount of CsA recovered in these fractions and a corresponding decrease in the amount of CsA recovered in the HDL fraction[6]. The amount of drug recovered in the non-lipoprotein fraction however remains unchanged. These findings suggest that the lipoprotein distribution of CsA may be partially regulated by plasma lipoprotein cholesterol and to a lesser extent by triglyceride concentrations. These observations further suggest that the redistribution of drug from one lipoprotein class (HDL) to another (LDL or VLDL) could be influenced by different disease states and adjunct therapies such as Intralipid infusion, where lipoprotein plasma concentrations and composition are altered[18].

In other studies, we have observed that increasing the TG:total cholesterol (TC) ratio within VLDL and HDL, results in increased recovery of CsA in the VLDL fraction, whereas the recovery of the drug in the HDL fraction is decreased[6]. These findings suggest that not only lipid mass (TC and TG) and lipoprotein composition but also the type of lipoprotein in which these changes occur is another possible factor that determines the association of CsA to lipoproteins. Since patients with organ transplant exhibit lipid disturbances, including decreased cholesterol levels and/or elevated triglyceride levels, these results may provide an explanation for the unpredictable and inconsistent pharmacokinetics and pharmacodynamics of CsA following administration.

Compounds Incorporated into Lipid-Based Vesicles:

A number of studies have investigated the interaction of liposomes and lipid-complexes with plasma lipoproteins. Surewicz *et al.* (1986) have reported the formation of thermally stable complexes when anionic phospholipids (such as dimyristoylphosphatidylglycerol [DMPG]) are co-incubated with apolipoprotein AI[19]. Earlier Scherphof (1983) demonstrated the transfer of phosphatidylcholine from small unilamellar vesicles to HDL[20]. Our laboratory has recently investigated the interaction of three different compounds incorporated into lipid-based vesicles with plasma lipoproteins[5,22,23]. The observations from all these studies unambiguously indicate that there is a preferential distribution of hydrophobic drugs into the HDL fraction of plasma lipoproteins.

(1) Amphotericin B (AmpB) Lipid Complex (ABLC):

Preliminary studies were conducted in which ABLC (composed of dimyristoylphosphatidylcholine [DMPC] and DMPG) was incubated in human serum for 60 min at 37°. Following incubation the serum was separated into its lipoprotein and lipoprotein-deficient fractions by density gradient ultracentrifugation and percent of AmpB and DMPG recovered in each fraction was determined by

HPLC. Greater than 90% of the original concentration of AmpB and 80% of DMPG from the original formulation incubated in serum were recovered in the HDL fraction[5].

Recent studies have shown that when ABLC was incubated in human plasmas of varying lipid concentration and lipoprotein composition, the majority of AmpB was recovered in the HDL fraction[21]. We further showed that differences in lipid coat content (free cholesterol and PL) carried by HDL influenced the distribution of ABLC within plasma of different human subjects[21].

(2) Liposomal Annamycin:

Studies from our laboratory have demonstrated that when liposomal annamycin (composed of DMPC and DMPG) is incubated in human plasma at increasing concentrations (5-20 µg annamycin per ml) for 60 min at 37°, more than 67% of the drug is recovered in the HDL fractions[22]. We further observed that an increase in the concentration of plasma LDL-cholesterol or VLDL-triglyceride concentrations increased the percent recovery of annamycin in these lipoprotein fractions[22].

(3) Liposomal Nystatin:

When liposomal nystatin (composed of DMPC and DMPG) at 20 µg nystatin per ml was incubated in human plasma with varying lipid and lipoprotein concentrations for 5 min at 37°, 24 to 53% of the original nystatin concentration was recovered in the HDL fraction. Furthermore, it was observed that as the amount of HDL protein decreased the percent of nystatin recovered within this fractions decreased following the incubation of liposomal nystatin[23]. These findings suggest that the preferential distribution of nystatin into plasma HDL may be a function of the HDL protein concentration.

Taken together, our observations with ABLC, liposomal annamycin and liposomal nystatin suggest that lipid-based vesicles containing DMPG may have the ability to target compounds specifically to HDL due to the ability of DMPG to complex with apolipoprotein AI (Fig. 1). Therefore, any DMPG-drug complex introduced into human plasma, initially either as an intact liposome or as a lipid-drug complex would likely increase the level of distribution of that drug in to HDL. However, additional factors such as HDL particle size, charge and total surface area may also influence the distribution of the drug into HDL.

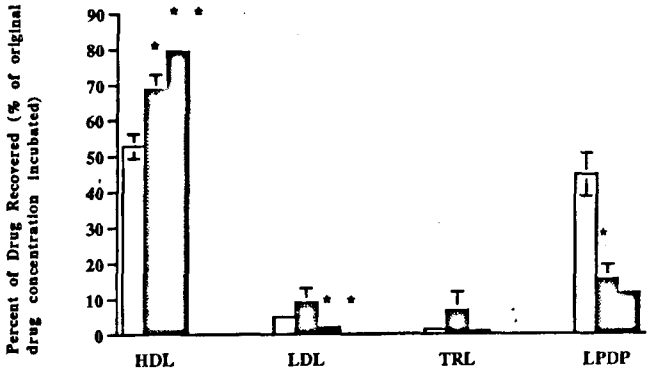

Fig. 1 : Distribution within human plasma of Liposomal Nystatin, Lyposomal Annamycin and Amphotericin B lipid complex

Liposomal Nystatin (□), Lyposomal Annamycin (▨) and Amphotericin B lipid complex (20 µg of drug/ml) (■) were incubated in human plasma for 60 min at 37°. Data represented as mean±standard deviation (n=3 for L-Nys and ABLC, n=5 for L-Ann). *Denotes $p<0.05$ vs. L-Nys and **denotes $p<0.05$ vs. L-ann. HDL stands for high-density lipoproteins; LDL, low-density lipoproteins; TRL, triglyceride-rich lipoproteins and LPDP for lipoprotein deficient fraction.

Pharmacological Implications of Elevated Serum Cholesterol Concentration:

AmpB and ABLC are examples of drug formulations that bind to serum lipoproteins *in vivo* and *in vitro*[24-28]. We believe that this property has a major impact on the efficacy and safety of these compounds since they are often administered to patients with abnormal serum cholesterol metabolism[29-32]. Disease-related changes in liver and kidney function and blood flow may also alter the pharmacokinetics and toxic effects of these drugs. However, it is our contention that understanding the mechanisms by which dyslipidemia (abnormal serum lipid concentrations) affects the action of these compounds is essential prior to administration of AmpB and ABLC formulations.

There is growing evidence that supports our hypothesis that increases in serum cholesterol concentrations increase the renal toxicity of AmpB. Specifically, we have previously observed that when AmpB is administered to hypercholesterolemic insulin-dependent diabetic rats, the magnitude of nephrotoxicity was more compared to control non-diabetic rats. Furthermore, the serum half-life and volume of distribution of AmpB were increased when

administered to the diabetic rats as compared to nondiabetic control rats[33].

Koldin and coworkers demonstrated that AmpB-induced nephrotoxicity was elevated when the drug bound to LDL was administered to hypercholesterolemic rabbits[34]. Preliminary studies recently completed by our laboratory have shown that, upon administration of a single dose to cholesterol-fed rabbits, AmpB was more nephrotoxic than when administered to control rabbits[35]. The enhanced nephrotoxicity of AmpB is probably mediated through drug binding to the LDL receptor as demonstrated by Krieger and coworkers and from our laboratory[36,37]. Recent studies from our laboratory on kidney cells have also shown that, when the number of LDL-receptors expressed on these cells were reduced, AmpB bound to LDL was less toxic compared to unbound AmpB[37]. These findings suggest that increases in AmpB binding with serum LDL enhance the ability of AmpB to damage kidney cells.

This notion is supported by *in vivo* studies in humans. For example, when AmpB was administered to patients with leukemia[38] and immunocompromised patient who exhibited lower serum cholesterol concentrations (<100 mg/dl)[39], AmpB-induced renal toxicity was decreased and serum half-life of AmpB was decreased. Similarly, Chabot and coworkers observed no measurable renal toxicity when AmpB was administered to cancer patients who exhibited hypocholesterolemia[40]. In addition, our preliminary findings suggest that patients with a higher percentage of AmpB bound to serum LDL are more susceptible to AmpB-induced kidney toxicity[24].

However, unlike AmpB, an increase in serum cholesterol concentration does not effect the pharmacokinetics or modify the renal toxic effects of ABLC[33]. Specifically, we have previously observed that when ABLC is administered to hypercholesterolemic insulin-dependent rats, the pharmacokinetics and renal toxic effects of ABLC is not markedly altered compared to nondiabetic rats[41]. Furthermore, it has been suggested that the renal toxicity of ABLC bound to serum lipoproteins may differ from that of AmpB alone. Whereas AmpB alone binds preferentially to LDL and can be internalized into renal cells expressing LDL receptors resulting in toxicity[37], ABLC predominantly binds to HDL[42] and remains in the bloodstream which renders it devoid of toxicity. Preliminary findings from our research group also suggest that AmpB bound to HDL is less toxic to kidney cells than AmpB bound to LDL possibly due to the low number of HDL receptors present on these cells[37]. Taken together these findings suggest that decreasing the ability of AmpB to bind serum LDL by incorporating the drug into a phospholipid vesicle (ABLC) diminishes its ability to damage kidney cells. These studies provide compelling evidence that serum lipoprotein levels have a major impact on the toxicity and pharmacokinetics of AmpB formulations.

Implications of Altered Lipid and Lipoprotein Metabolism:

Disturbances in lipid metabolism (e.g. hypertriglyceridemia and hypocholesterolemia) commonly occur during infection or when the immune system is compromised. Studies have indicated several mechanisms by which infection causes an increase in TG[29].

(1) increased hepatic *de novo* synthesis of fatty acids leading to increased secretion of VLDL,

(2) Increased adipose tissue lipolysis with the mobilized fatty acid being reesterified into TG in the liver and then resecreted as VLDL rather than being oxidized and

(3) Decreased levels of lipoprotein lipase (an enzyme responsible for the hydrolysis of TG into free fatty acids and glycerol) leading to decreased clearance of TG-rich VLDL.

Furthermore, cancer cells appear to require additional cholesterol for the formation of new membrane material and metabolism requirements, as evidenced by the development of hypocholesterolemia and a decrease in plasma LDL cholesterol concentration in leukemia patients[31].

SUMMARY

Since a number of hydrophobic compounds such as CsA, AmpB and several others, predominantly associate with plasma lipoproteins upon incubation in plasma, changes in plasma TG and cholesterol concentrations would not only affect the plasma distribution of these compounds, but may also have a bearing on their pharmacokinetics and pharmacodynamics. Understanding how variations in plasma lipid concentrations affect hydrophobic drug interactions with lipoproteins could help explain the changes in the pharmacokinetic and pharmacodynamic profiles of these compounds when administered to patients that exhibit modifications in their lipid and lipoprotein metabolism.

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