LIPOPROTEIN BINDING
OF DRUGS

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Compared to the extensive reports on plasma binding of drugs to albumin and $\alpha_1$-acid glycoprotein, little interest was shown in the possibility of lipoprotein binding. Some authors mentioned that selected drugs were partly bound to lipoproteins, but since the fundamental report of Vallner and Chen (1977) no general consideration was given to this possibility. However, it was clearly shown that drug binding to albumin and $\alpha_1$-acid glycoprotein involve mainly hydrophobic bonds and require lipophilic compounds. Since lipoproteins have high lipid contents they seem to be good challengers for the plasma binding of such drugs. Recent studies (Glasson, Zini, and Tillement 1982; Glasson et al. 1980) using isolated lipoproteins effectively showed that drugs providing enough liposolubility could bind to these macromolecules with high affinities. However, as the molar lipoprotein plasma concentrations are low if compared to those of albumin and $\alpha_1$-acid glycoprotein, the question remains whether lipoprotein binding may be significant.

The present article will discuss the characteristics of the drug lipoprotein interactions as well as the lipoproteins' significance in drug plasma binding. Finally, the pharmacological consequences of the lipoprotein binding of drugs will be considered, although they are not fully understood.

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CHARACTERISTICS OF THE DRUG LIPOPROTEIN INTERACTIONS

Some common features may be drawn from the analysis of drug lipoprotein interactions. The binding is reversible and apparently non-saturable; the drug bound amount is roughly proportional to the lipid content of the lipoprotein. A drug solubilization in the lipid core of the lipoprotein seems to be the main determinant of the interaction but not always the unique one.

The reversibility of the binding to lipoproteins was demonstrated with probucol (Urien et al. 1984), cyclosporin A (Mraz et al. 1983), and Δ⁹-tetrahydrocannabinol (Klausner, Wilcox, and Dingell 1975); it was shown that after incubation with a definite lipoprotein the drugs can be transferred to other lipoproteins or to blood cells. The binding process appears to be non-saturable over a large range of concentrations. Figure 7-1 shows the binding of propranolol to isolated α₁-acid glycoprotein, high density lipoprotein, low density lipoprotein, and very low density lipoprotein (Glasson et al. 1980); α₁-AGP showed saturable binding, whereas a non-saturable binding was observed with the different lipoproteins. No signs of saturation were found in the binding of lipoproteins to cyclosporin A (Lemaire and Tillement 1982), pindolol (Lemaire and Tillement 1982), digoxin and digitoxin (Brock 1976). It is obviously possible to reach lipoprotein

![Figure 7-1](image)

FIGURE 7-1
Degree of binding of propranolol to isolated α₁-AGP (0.9 g/l); HDL (3 g/l); LDL (3 g/l); and VLDL (1.5 g/l).
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saturation, with very high drug concentrations. This fact suggests a high number of binding sites for each macromolecule.

The amount of bound ligand generally correlates with the serum lipid concentration. As a matter of fact, the correlations between lipoprotein uptakes of probucol (Urien et al. 1984) or benzo[a]pyrene (Shu and Nichols 1981) and lipoprotein lipid concentrations suggest that these compounds are essentially dissolved in the lipid core of the lipoproteins. The interaction of tetracycline with human serum lipoproteins was studied by Powis (1974); rather than being bound to specific sites, the drug appears to be associated with lipoproteins in a manner similar to its distribution between phosphate buffer and chloroform. Moreover, the liposolubilization hypothesis is strengthened by the following fact: closed variations of free enthalpy were observed with several insecticides either in their interaction with lipoproteins or in their solubilization in organic solvents (Maliwal and Guthrie 1981).

These general findings, however, have some exceptions. Shireman and Schneider (1982) have found that the hydrophobic compound aflatoxin does not partition into the plasma lipoproteins. Moreover, studies of the interactions between lipoproteins and quinidine (Nilsen 1976) or vitamin A (Krinsky, Cornwell, and Oncley 1958) also concluded that the transport of these compounds by lipoproteins cannot be considered only as solubilization within the lipid moiety. Quinidine and propranolol exhibit a positive cooperative binding effect with high density lipoprotein and low density lipoprotein suggesting that drug binding and distribution to lipoproteins may be affected by the nature and concentration of some ions present in serum (Ho Ngoc-Ta Trung and Sirois 1984) and probably by a direct interaction with the corresponding apoproteins. The relative role of specific binding versus dissolution in the lipid portions of the lipoproteins remains to be elucidated.

Serum lipoproteins have distinct structural domains with which drugs can interact. These spherical pseudomicellar complexes have hydrophobic core regions surrounded by a phospholipid monolayer. Triglycerides and cholesteryl esters are contained in the apolar core whereas free cholesterol and proteins, known as apoproteins, are associated with the phospholipid membrane. Lipoproteins are defined by their physical characteristics and chemical compositions. They are classified in four major groups called chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). VLDL are the major carriers for triglycerides whereas in LDL and HDL, cholesterol is the predominant lipid. The highest apoprotein content is found in HDL, followed by LDL and VLDL.
The binding between a platelet antiaggregant agent, ticlopidine, and isolated apoproteins was found to be less than 10 percent of that observed for the native lipoproteins (Glasson, Zini, and Tillement 1982). Thus, the protein moiety of lipoproteins appears to play a role, even if a minor one, in the uptake of drugs by lipoproteins. Shu and Nichols (1981) found that the lipophilic carcinogen, 3-hydroxybenzo[a]pyrene, reacts primarily with the apoprotein-rich HDL fraction. Moreover, a linear correlation was found between the distribution of Δ⁸-tetrahydrocannabinol and the phospholipids of the lipoprotein catabolase, whereas no correlation was observed with other lipoprotein constituents such as cholesterol or triglycerides. Capelle et al. (1976) suggested a particular binding of the compound to polypeptide C present in VLDL and LDL. There was also some evidence for the binding of imipramine to apoprotein B, representing the LDL, whereas there was no indication of binding to apoprotein A representing the HDL (Kristensen 1983). Apoprotein C, a component of LDL, has been shown to have a separate region for the binding of lipid distinct from the ionic binding to lipoprotein lipase. Becker and Gamble (1982) suggested that the same sites could be available for the binding of hydrophobic molecules. The HDL fractions bound 3–6 times more dolichol, a polyisoprenoid alcohol, than VLDL or LDL; this result argues for specific uptake into the macromolecules rather than non-specific association with the lipids in the lipoproteins (Keenan, Kruczek, and Fischer 1977). In fact, it is also possible that the lipid and protein fractions may cooperate in the binding process, the drug first binding to a number of sites on the protein moiety and then dissolving in the lipid phase (Sellers et al. 1981).

Triglycerides represent an important binding compartment in the uptake of drugs by lipoproteins. The plasma protein binding of phenytoin (Guelen and Deimann 1980), reserpine (Chen and Danon 1979), and quinidine (Nilsen et al. 1978) were well related to serum levels of triglycerides but not to cholesterol. Sager et al. (1981) found that a subcutaneous injection of heparin lowered the triglyceride levels and consequently the plasma protein binding of propranolol. These different results indicate that the serum levels of triglyceride-rich VLDL are an important cause of binding variability in human sera.

The role of cholesterol in lipoprotein binding was also demonstrated in different experiments. Significant correlations were apparent between the plasma protein binding of perazine, amitriptyline, nortriptyline, and the cholesterol concentration present in plasma (Brinkschulte and Breyer-Pfaff 1980). Moreover, cholesterol was found to be the most important plasma component for the plasma binding of amphotericin B and haloperidol. Since HDL have lower cholesterol contents than LDL, the preference of haloperidol for the
HDL was explained by Rice and Makuku (1981) by the cholesterol being present on the surface of the HDL in contrast to the phospholipid-covered cholesterol present in the LDL. Brajtburg et al. (1984) demonstrated that the binding site for amphotericin B in lipoprotein is nonesterified cholesterol, 4 to 10 cholesterol molecules being bound to one molecule of the drug.

The lack of common binding sites on the different lipoproteins fractions could be checked by attempting competitive displacement experiments. Glasson et al. (1980) found no interaction between propranolol and imipramine for the binding to the different lipoproteins; they concluded that each drug was bound to a great number of binding sites without any mutual inhibition. The question remains, however, whether binding sites are effectively available on lipoproteins, or if the sometimes observed saturation is a part of the liposolubilization process.

Finally, the binding of drugs to lipoproteins seems to be species dependent. The size of the different lipoprotein fractions can differ between species. For instance, the amount of lipoproteins in human plasma is several times that in rat plasma. Moreover, the predominant lipoprotein class of human plasma is LDL, whereas that of rat plasma is HDL. Conformation differences were also found by Rosenkranz, Schlossmann, and Scholtan (1974) between human and dog lipoproteins. Subsequently, the authors found that nifedipine was bound to a greater extent by the beta-lipoproteins of human serum than of dog serum.

From all these characteristics of the lipoprotein binding, we can conclude that a common binding process involves a partitioning phenomenon, the drugs being dissolved in the lipid core of the lipoproteins. Nevertheless, some compounds were demonstrated to bind more specifically to different lipoprotein fractions.

THE LIPOPROTEIN SIGNIFICANCE IN DRUG TOTAL PLASMA BINDING

The significance of lipoproteins on drug plasma binding varies greatly among drugs. The percentages of overall plasma binding corresponding to the lipoprotein binding of some drugs are given in Table 7.1; the lipoproteins represent 5–95 percent of the total plasma protein binding. This list of drugs is not exhaustive. Thus, lipoprotein binding represents more than 40 percent of total serum binding for ticlopidine (Glasson, Zini, and Tillement 1982), amitriptyline and nortriptyline (Brinkschulte and Breyer-Pfaff 1980), $\Delta^9$-tetrahydrocannabinol (Klausner, Wilcox, and Dingell 1975) and tetracycline (Powis 1974).
TABLE 7.1
Lipoproteins Significance in Drug Plasma Binding

<table>
<thead>
<tr>
<th>Drug</th>
<th>Nature</th>
<th>% of overall plasma binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glafenec acid</td>
<td>Acid</td>
<td>≤ 5</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Acid</td>
<td>≤ 5</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Basic</td>
<td>5–15</td>
</tr>
<tr>
<td>Quinidine</td>
<td>Basic</td>
<td>40–50</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>Neutral</td>
<td>≥ 70</td>
</tr>
<tr>
<td>Probucol</td>
<td>Neutral</td>
<td>95</td>
</tr>
</tbody>
</table>

The magnitude of the drug binding to lipoproteins can be expressed by a comparison of the percent bound in plasma and in isolated lipoproteins. Thus Verbeeck et al. (1983) found that the plasma binding of phenothiazine neuroleptics was just slightly higher than the binding to isolated lipoproteins. However, since the lipoprotein binding is generally a non-saturable and reversible phenomenon, it may be quantitated by the total binding constant nK (product of the number of binding sites n by the corresponding affinity constant K). Since molar concentrations of lipoproteins are some two to three orders of magnitude lower than that of albumin, it is not surprising that the nK values corresponding to lipoproteins are generally higher than those for the binding to albumin or α1-acid glycoprotein. Thus, high total binding constants were found, ranging from $10^7 \text{ M}^{-1}$ for hexachlorobiphenyl (Becker and Gamble 1982) to $10^5 \text{ M}^{-1}$ for quinidine (Nilsen and Jacobsen 1975), propranolol (Glasson et al. 1980), and $10^3 \text{ M}^{-1}$ for chlorpromazine (Bickel 1975). Most of these total binding constants surpass the corresponding values for albumin and α1-acid glycoprotein and could be increased in some disease states. Similar binding constants were found for LDL and HDL with chlorpromazine (Bickel 1975) or quinidine (Nilsen and Jacobsen 1975). However, for propranolol (Glasson et al. 1980) and ticlopidine (Glasson, Zini, and Tillement 1982), the total binding constants seem to be dependent on lipid content (VLDL > LDL > HDL).

The variation in lipoprotein concentration is large within the normal population. There are also many individuals with primary (unknown aetiology) elevation of one or more classes of lipoproteins, as well as numerous causes of secondary hyperlipoproteinemia such as hypothyroidism, obstructive liver disease, alcoholism, etc. (Piafsky 1980). In genetic or acquired disorders of lipoprotein metab-
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olism, the plasma content of cholesterol and triglycerides may be increased by as much as 10- to 200-fold, respectively (Weinkam et al. 1980). Moreover, drugs can induce a modification of the lipid and lipoprotein profiles. Antihypertensive drugs induce lipid and lipoprotein changes generally by a moderate increase in total cholesterol, a marked increase in triglycerides and a decrease in HDL cholesterol (Sauvanet and Rouffy 1981).

It is, therefore, likely that variations in lipoprotein content could contribute to variations in the plasma distribution of drugs. Using the data of Glasson, Zini, and Tillement (1982), we have simulated in Figure 7-2 the plasma distribution of ticlopidine in the serum of normal subjects and hyperlipoproteinemic patients. These data indicate that ticlopidine is more highly bound to plasma in hyperlipoproteinemic patients (fraction free $f_u = 1.7$ percent) than in the normals (fraction free $f_u = 2.7$ percent). Moreover, the distribution of bound ticlopidine between lipoproteins, $\alpha_1$-acid glycoprotein, and albumin differs in the hyperlipoproteinemic situation: the bound fraction in the LDL is about twice that in normal patients.

However, studies of binding to plasma from hyperlipoproteinemic patients led to different conclusions. The overall binding of quinidine was relatively insensitive to changes in lipoproteins (Nilsen et al. 1978; Kates, Sokoloski, and Comstock 1978). Moreover, a linear correlation was found between the ratio of bound/free and the lipid concentrations for amitriptyline and nortriptyline, but not for quinidine (Pike et al. 1982). Likewise, removal of lipoproteins reduced the total serum binding of amitriptyline and nortriptyline, but not that of quinidine (Pike et al. 1983). These studies demonstrated that

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**FIGURE 7-2**
Plasma distribution of ticlopidine in the serum of normal and hyperlipoproteinemic subjects.
lipoproteins have little influence in the total serum binding of quinidine, whereas the binding of amitriptyline and nortriptyline depends on the lipid content.

An identical result to quinidine was obtained with reserpine. Chen and Danon (1979) also found that, in spite of a strong binding to lipoproteins, the total serum binding of reserpine did not vary between hyperlipoproteinemic and normal subjects. On the other side, a significantly higher binding of imipramine was found in hyperlipoproteinemic patients compared to normals (Danon and Chen 1979). The exact importance of the binding of imipramine to lipoproteins remains, however, to be elucidated. Imipramine is bound essentially to LDL (Kristensen 1983). However, this fraction represents less than 15 percent of the drug present in plasma (Shireman and Remsen 1983).

In conclusion, lipoprotein binding is likely to be most important in situations where lipoproteins have the highest binding constants and/or are present in increased concentration, and when the levels of other major binding components—i.e., α₁-acid glycoprotein and albumin—are normal or decreased. In such cases, they dominate the binding pattern in such a way that changes in their concentration are directly reflected in changes of total drug binding. Unexpected consequences of the influence of plasma lipids on the protein binding of phenytoin were found by Guelen and Deimann (1980). An expected positive correlation between the bound fraction of drug and the triglycerides—i.e., mainly VLDL—was observed whereas a negative correlation between the total bound fraction and the HDL concentration was found. The authors concluded that HDL probably competes with phenytoin for the binding sites on the proteins, as known for the fatty acids.

Another consequence of the particular binding of bupivacaine to HDL may be observed in paired maternal and fetal plasma samples taken at delivery. Differences in HDL concentrations in both plasmas have been suggested as being responsible for the concentration difference observed for bupivacaine. As a matter of fact, since this drug is strongly bound to HDL, its fraction bound is higher in HDL-rich maternal plasma than in fetal plasma (Mather and Thomas 1978).

Since the binding of drugs to lipoproteins involves a partitioning phenomenon, the physicochemical properties of the drugs represent an important factor for the significance of lipoproteins in drug binding. The importance of liposolubility was illustrated by a positive correlation \( r = 0.97 \) between the binding constants to lipoproteins and the partition coefficients octanol-phosphate buffer of 9 beta blockers (Lemaire and Tillement 1982). Rudman et al. (1972) also found a correlation between the extent of uptake by lipoproteins and the partition coefficients chloroform-phosphate buffer of 13 drugs, hor-
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mones, and fatty acids. Moreover, Shu and Nichols (1981) demonstrated that after incubation in plasma, the metabolites of benzo[a]-pyrene exhibited a decreasing distribution into the lipoproteins and an increasing uptake by the albumin as the degree of hydroxylation of the metabolites increased.

As shown in Table 7.1, lack of ionization represents an important factor in the binding to lipoproteins. The role of lipoproteins as a transport system for drugs was first recognized for basic, lipophilic molecules. Using ultraviolet spectroscopy, Vallner and Chen (1977) demonstrated that a number of basic drugs associated with lipoproteins, while acidic or neutral drugs appeared to have less or no affinity. Moreover, it was found (Pike et al. 1983) that removal of the lipoproteins from serum did not affect the serum binding of the acidic (phenytoin) and neutral drugs (digitoxin) tested, but reduces the binding of the basic drugs amitriptyline and nortriptyline. In another experiment, Bickel (1975) found no binding of lipoproteins with salicylic acid. However, some acidic drugs such as diclofenac and glafenic acid can also bind to lipoproteins, although the plasma lipoprotein bound fraction is, in these cases, relatively small as compared to the albumin one.

On the other hand, such neutral drugs as probucol (Urien et al. 1984) or cyclosporin A (Lemaire and Tillement 1982) are extensively bound to lipoproteins. Even if an ionic interaction was found between heparin and LDL (Pan, Kruski, and Elbein 1978), the particularly high binding of neutral drugs to lipoproteins suggests that only the un-ionized drug can be taken up by lipoproteins. The importance of binding of neutral drugs to lipoproteins underlines the role of vehicles of these proteins for water insoluble compounds.

PHARMACOLOGICAL CONSEQUENCES OF THE LIPOPROTEIN BINDING OF DRUGS

A consequence of a high affinity binding to lipoproteins could be a protection of the drug from plasma enzymatic biotransformation. Thus, Weinkam et al. (1980) found that addition of lipoproteins to serum stabilizes chloroethylnitrosoureas from degradation. The drugs partition into the hydrophobic core regions of the lipoproteins where the chloroethylnitrosoureas are chemically stable. The affinity of these compounds for lipoproteins being higher than for the decomposition enzyme, the lipoproteins linked chloroethylnitrosoureas are protected from biodegradation. The magnitude of the increase of stability of chloroethylnitrosoureas is greater for the more lipophilic analogs—i.e., for tightly bound compounds. In vitro binding experiments (Brajtburg et al. 1984) between amphotericin B and
plasma lipoproteins demonstrated that the drug associated to lipoproteins selectively maintains its biological activity. This stabilization may explain the persistence of the antifungal effects of amphotericin B in the body in spite of its rapid decomposition in media or buffers.

The pharmacokinetic significance of drug binding to lipoproteins is, however, not fully understood. The plasma half-lives of human lipoproteins are relatively short: chylomicrons 5 min, VLDL 1–3 hr, LDL 3–4 days, HDL 5–6 days (Counsell and Pohland 1982); therefore, kinetics of drugs and lipid turnover may be linked.

One can expect some relations between lipoprotein binding and tissue distribution. Based on the analogy of the albumin bound drugs (Tillement 1979), a possible retention of lipoprotein bound drugs in the diffusion compartments of these lipids can be considered if the corresponding binding capacities are higher than those of other body tissues. However, as lipoproteins' half-lives are shorter than those of albumin, it is clear that the previous concept cannot be directly applicable. Nevertheless, lipoprotein bound drugs exhibit large apparent volumes of distribution that could correspond to the important distribution of lipids in the body.

The role of lipoproteins binding in drug elimination was also demonstrated in some studies. Shu and Bymun (1983) suggested that differential biliary excretions of benzo[a]pyrene by the VLDL, LDL, and HDL carriers may be due to differences in metabolism of the lipoprotein classes; since plasma half-lives are approximately 10 min for rat VLDL and 1 hr for rat HDL, benzo[a]pyrene transported by the VLDL would be expected to be cleared by the liver at a higher rate than that transported by the HDL. In another experiment, Vauhkonen, Kuusi, and Kinnunen (1980) found that in rats injected intravenously with chylomicrons containing benzo[a]pyrene, the disappearance of the compound paralleled the removal of chylomicrons; as a consequence, the tissue distribution of benzo[a]pyrene tends to be primarily determined by the catabolism of these lipoproteins.

Finally, the most stimulating hypothesis is the possible role of some lipoproteins for the transport and delivery of hydrophobic drugs to specific cells and tissues. MacCoss et al. (1983) also suggested that phospholipid–nucleotide conjugates—i.e., release forms of the antileukemic agent 1-β-D-arabinofuranosylcytosine—may be carried by LDL and HDL as a site-specific drug delivery system. Lipoprotein receptors in the liver and extrahepatic tissues acquire their cholesterol by LDL–receptor mediated process; the receptors bind LDL by interacting with its apoprotein B component and were found to be located in eight tissues, mostly in the membranes of the adrenal gland and the gonads (Kovanen 1983). Since certain tumor
cells might have a higher affinity for LDL than normal tissues, LDL could be considered as a potential vehicle for cytotoxic drugs linked to this lipoprotein (Gal et al. 1981).

On the other hand, HDL has also been shown to bind to cells at specific sites that are separate from those that bind LDL. Drugs bound to HDL might be preferentially transported to tissues like adrenal and testes. Thus, the preferential transport of chlordecone by HDL could explain the high concentration of this pesticide found in rat adrenal and/or its toxic effects (Soine and Blanke 1982).

All these results provide further evidence for a possible role of lipoproteins in the transport of lipophilic drugs, and suggest the use of lipoproteins as a site-specific drug delivery system. However, only the results of further investigations will determine the possibilities of this approach.

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