Factors affecting the lipoprotein distribution of cyclosporine: Lipoprotein lipid concentration, lipoprotein composition and cholesteryl ester transfer protein activity

by

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Abstract

The purpose of this study was to determine factors that influence the lipoprotein distribution of cyclosporine (CSA). Cyclosporine is an immunosuppressant drug that has been effective in preventing graft rejection but the use of cyclosporine is limited by its renal toxicity. The majority of the cyclosporine (~95%) is found in the high density lipoprotein (HDL) and low density lipoprotein (LDL) fractions with the remainder in the very low density lipoprotein fraction (VLDL) following incubation in human plasma.

It has been clinically observed that patients with hypertriglyceridemia show decreases in CSA activity while in hypocholesterolemic patients, increases in CSA toxicity occur. Such dyslipidemias represent changes in lipoprotein lipid concentration and composition which may result in changes in the lipoprotein distribution of CSA. We studied such changes and found that as lipid concentration of LDL and VLDL increased, more CSA was found to be associated with this lipoprotein fraction. A parallel decrease in CSA was found to be associated with HDL when the triglyceride concentration of HDL was increased.

Another factor that was found to affect the lipoprotein distribution of cyclosporine was cholesteryl ester transfer protein (CETP), which is normally involved in the exchange of cholesteryl ester for triglyceride between HDL and LDL or VLDL. The transfer of CSA from LDL to HDL was shown to be dependent on CETP activity while the reciprocal transfer was independent of CETP.
Thus we have demonstrated that the lipoprotein distribution of cyclosporine can be influenced by both lipoprotein lipid composition and the activity of CETP. Because diseased and transplant patients have been shown to have changes in both lipid profile and CETP activity, this may alter the lipoprotein distribution of cyclosporine and possibly alter cyclosporine activity. By monitoring a patient's lipid profile and CETP activity it may be possible to more accurately dose cyclosporine in order to maximize its efficacy while minimizing cyclosporine toxicity.
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Abbreviations

ACAT - acyl-coenzyme A cholesterol acyl transferase
CD3 - cell surface molecule 3
CE - cholesteryl ester
CETP - cholesteryl ester transfer protein
CSA - cyclosporine
DG - diacylglycerol
GM-CSF - granulocyte-monocyte colony stimulating factor
HDL - high density lipoprotein
HMG CoA - 3-hydroxy-3-methylglutaryl coenzyme A
IDL - intermediate density lipoprotein
IFN - interferon
IL - interleukin
IP3 - inositol-1,4,5-trisphosphate
LCAT - lecithin cholesterol acyl transferase
LDL - low density lipoprotein
LPDF - lipoprotein deficient fraction
LPL - lipoprotein lipase
NF-AT - nuclear factor from activated T-cells
NF-ATc - nuclear factor from activated T-cells, cytoplasmic component
NF-ATn - nuclear factor from activated T-cells, nuclear component
PAI-1 - plasmin protease inhibitor 1
PIP2 - phosphatidylinositol bisphosphate
PKC - phosphokinase C
TCR - T cell receptor
TC - total cholesterol
TG - triacylglycerol or triglyceride
TP - total protein
TGF-β - transforming growth factor β
VLDL - very low density lipoprotein
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Dedication

I would like to thank the following people:

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guinivere, for whom i do battle every day

skippy

and to all those struggling to find meaning on this small blue speck in space called earth
Chapter 1

Introduction
Cyclosporine

Background

Transplantation research has furthered our knowledge of the immune system and fueled the development of drugs that modulate the immune response. There are four types of transplants [Roitt 1997]: 1) autografts, in which the donor and the recipient are the same individual; 2) isograft, in which the donor and recipient are genetically identical; 3) allograft or homograft, in which the donor and recipient are of the same species but genetically dissimilar, this is the most common type of transplant; and 4) xenograft, in which the donor and recipient are of different species. The objective of any organ transplant is to provide the patient with a healthy organ in order to live a useful life and avoid premature death.

Although the concept of organ transplantation dates back several centuries, it was only at the turn of the century that the field of transplantation truly developed. In 1908, an autotransplant of a dog kidney was successfully accomplished while the equivalent allotransplant resulted in eventual renal failure [Carrel 1908]. It was recognized that the failure of allografts was due to a process separate from necrosis, infection, or inflammation but the concept of rejection did not fully emerge until the second world war with the work of Drs. Peter Brian Medawar and Thomas Gibson [Medawar 1945].

Medawar and Gibson observed in rabbits that when a skin graft from Animal A to Animal B was performed, the skin graft survived approximately 7 days. When a second graft was performed in the same manner, the rejection occurred in half the time. This was termed the “second set response”. This second response was recognized to be caused by the previous graft but the basis for such a reaction was unknown. Medawar’s continuing work in the field revealed the role of the immune system in the phenomenon of rejection and in 1960
he was awarded a Nobel Prize in medicine for his contributions to tissue immunology [Gard 1960].

From Medawar’s work it was learned that graft rejection could be prevented by the use of treatments that suppress the immune response [Hasek 1962, Dresser 1968]. Early attempts to suppress the immune response exposed the patient to high dose levels of radiation [Dixon 1955, Taliaferro 1957, Talmage 1955]. However, such treatment was often at near lethal doses and was associated with high patient morbidity. This raised the importance of the quest for immunosuppressive drug therapy which would be more specific and less toxic than irradiation therapy.

The discovery of 6-mercaptopurine introduced a new era in immunosuppression and organ transplantation [Schwartz 1958, 1959]. A derivative of 6-mercaptopurine with decreased toxicity, azathioprine also known as Imuran® (Glaxo Wellcome), was synthesized and continues to be used [Hitchings 1963]. Other compounds found to have some immunosuppressive properties were adrenal steroids [Gabrielson 1967, Malmgren 1952]. Azathioprine, in combination with the adrenal steroid prednisone, was the mainstay of immunosuppressive therapy through the 1960’s and 1970’s [Starzl 1963, Lieberman 1996]. However, this treatment was only useful for renal transplants as the transplant of other organs such as the heart and liver still resulted in low graft and patient survival rates [Jones 1989]. The search for more new immunosuppressants led to the discovery of cyclosporine which was found to be effective in all types of transplants [Kahan 1989].

Cyclosporine, a cyclic peptide (Figure 1) isolated from a Norwegian fungus (Tolypocladium inflatum Gams), was discovered as part of a screening program for novel antifungal compounds at Sandoz by Borel et al. in the early 1970’s [Borel 1991]. The
Figure 1: The structure of cyclosporine A

Cyclosporine (MW 1202)
compound was found to have no significant antifungal activity but other screening assays revealed its relatively potent immunosuppressant activity. Further *in vivo* testing confirmed this activity and the compound was designated cyclosporin A. This compound is also known as ciclosporin by the World Health Organization and cyclosporine by its United States Adopted Name. Initially cyclosporine was deemed to have little clinical value and the economic viability of the drug in the limited market of transplantation was questioned. However Borel’s group at Sandoz pursued their studies and in 1976, in collaboration with White and Calne, cyclosporine was demonstrated to have beneficial effects on graft survival in several animal studies [Kostakis 1977, Dunn 1978, Calne 1977 & 1978].

These promising data led to the next stage of drug development, which was to determine the pharmacokinetic parameters of cyclosporine in human volunteers. This resulted in some initial difficulties as the drug alone was poorly absorbed but formulations containing olive oil were found to be effective in achieving detectable blood levels [Borel 1991]. These data combined with the animal data resulted in further development of the compound by Sandoz in hopes of discovering the mechanism of action. In 1983, FDA approval was given for the use of cyclosporine as an immunosuppressant.

**Mechanism of Action**

Cyclosporine acts by suppressing T-lymphocyte activation [Wiesinger 1979, Kay 1983]. T-cells contribute to the immune response by recognising foreign antigens, such as graft tissue. The binding of a foreign antigen results in the transcription of cytokines which activate other T-cells and B-cells which are necessary for an effective immune response. The current understanding of the process of T-cell activation emerged largely from the research
into the mechanism of action of cyclosporine. The activation of T-lymphocytes is summarized in Figure 2. T-cell activation begins with the binding of the antigen by the T-cell receptor (TCR) which is coupled to a specific cell surface protein designated CD3. The binding of antigen to this TCR/CD3 complex leads to the hydrolysis of phosphatidylinositol bisphosphate (PIP$_2$) to produce the secondary messengers inositol-1,4,5-trisphosphate (IP$_3$) and diacylglycerol (DG) [Imboden 1985a]. IP$_3$ stimulates the release of intracellular calcium stores and DG activates protein kinase C (PKC) [Truneh 1985, Imboden 1985b, Freedman 1975, Woodrow 1993]. The increased calcium levels and activated PKC results in the activation of the transcription factors necessary for T-lymphocyte activation. Some of these factors, including AP-1, NF-κB Oct-1 and the octamer associated protein (OAP), are found in cells other than T-lymphocytes and are ubiquitous transcription activation factors [Dumont 1996]. In contrast, the nuclear factor from activated T-cells (NF-AT) is only found in T-cells and is involved only in the activation of T-lymphocytes [Crabtree 1994]. All of these transcription factors work to initiate the transcription of lymphokines, including interleukins 2, 3, 4 (IL-2, IL-3, IL-4); granulocyte-monocyte colony stimulating factor (GM-CSF) and interferon-γ (IFN-γ). These lymphokines trigger the proliferation of T-cells and the activation of other components of the immune response such as B-lymphocytes and natural killer cells. Cyclosporine is known to inhibit the activation of NF-κB, Oct-1, OAP and NF-AT, which results in a diminished immune response [Crabtree 1994, Mattila 1990, Baumann 1991].

The binding of the antigen to the TCR/CD3 complex is associated with an increase in intracellular calcium levels [Weiss 1984; Nisbet-Brown 1985; Gardner 1989]. This increase is comprised of two phases: a rapid increase within the first minute followed by a lower
**Figure 2:** T-lymphocyte activation and site of action of cyclosporine. See text for details.

sustained plateau. The rapid release is due to IP3 stimulated release of calcium from the sarcoplasmic reticulum while the sustained plateau phase is a result of calcium entering from the extracellular environment [Berridge 1989]. The role of calcium is essential in the activation of T-cells with both extra- and intracellular pools playing equally important roles in achieving the necessary intracellular concentration of calcium [Gelfand 1986, 1988]. The elevated level of intracellular calcium must be sustained for a period of 1-2 hours in order for activation of the T-cell to occur, indicating this plateau phase plays a critical role in T-cell activation [Goldsmith 1988].

The discovery of the effector of increased intracellular calcium to inhibit transcription of T-cell activation factors was aided by the use of cyclosporine. cyclosporine has a unique method of action which does not block antigen binding by the TCR/CD3 complex, does not inhibit second messenger generation nor does it interfere with the binding of the specific transcription factors [Emmel 1989; Shevach 1985; Bijsterbosch 1985; Isakov 1987; Trenn 1989]. Cyclosporine inhibits a step in the calcium signal transduction cascade, preventing the transcription of the cytokines necessary for T-cell activation [Mattila 1990, Lin 1991, Kay 1989, Gunter 1989]. Studies have shown that cyclosporine binds to cyclophilin [Bierer 1990a, Dumont 1990, Tropschug 1989] which belongs to a family of receptors known as immunophilins [Schreiber 1991]. Cyclophilin possesses peptidyl-prolyl cis-trans isomerase activity which is involved in protein folding and it is this isomerase activity that cyclosporine inhibits [Takahashi 1989, Fischer 1989, Harding 1989, Siekierka 1989]. It was originally hypothesized it was this inhibition that modulated the immunosuppressive effect of cyclosporine. However, such a mechanism of action was placed in doubt by two observations. Firstly, immunophilins are extremely abundant within the cytosolic milieu and
it would require much greater concentrations of drug than experimentally used to completely inhibit isomerase activity [Schreiber 1991 & 1992]. Secondly, studies using cyclosporine analogs result in an inhibition of isomerase activity when bound to cyclophilin but no immunosuppressant activity [Bierer 1990a & 1990b, Dumont 1990, Sigal 1991]. This indicated that the immunosuppressant effects of cyclosporine are not due to a loss of isomerase activity. Instead a gain of function model was proposed, in which the cyclosporine-cyclophilin complex inhibits the calcium dependent activation of transcription factors required for T-cell activation.

A search for cellular factors that associated with the cyclosporine-cyclophilin complex identified calcineurin (also known as protein phosphatase 2B) as the major cellular target [Liu 1991, Friedman 1991]. Calcineurin is a calcium regulated serine/threonine phosphatase that consists of a 59 kDa calmodulin binding catalytic unit and a 19 kDa calcium binding regulatory unit [Guerini 1997, Pallen 1985]. The cyclosporine-cyclophilin complex inhibits the phosphatase activity of calcineurin at the same concentrations that inhibit T cell activation, providing strong evidence that calcineurin is the target of the immunosuppressant activity of cyclosporine [Fruman 1992]. Further support for the theory that calcineurin was involved in T-cell activation was provided by the use of mutant T-cells that overexpressed calcineurin or had a constitutively active form of calcineurin which were both resistant to the immunosuppressive effects of cyclosporine [Clipstone 1992 & 1993, O'Keefe 1992].

Thus, calcineurin was determined to be the enzyme that cyclosporine inhibited in the calcium signal transduction cascade. This indicated that a transcription factor needed to be dephosphorylated by calcineurin in order for T-cell activation to occur. Several transcription factors have been identified in the activation of T-cells of which the nuclear factor of
activated T-cells (NF-AT) [Ullman 1990] is essential for the transcription of interleukin-2, the main cytokine in T-cell proliferation [Ullman 1990]. NF-AT consists of two subunits which are the products of different genes [Northrop 1994]. There is a constitutive cytoplasmic component, NF-ATc, and an inducible nuclear component, NF-ATn. In order for NF-AT to bind to regulatory regions on DNA, NF-ATc must enter the nucleus and bind to NF-ATn. This localization of NF-ATc to the nucleus is apparently regulated by calcineurin which dephosphorylates the protein, but the phosphorylation state of the protein does not affect the binding properties [Rao 1994]. The synthesis of NF-ATn is stimulated by compounds that activate PKC [Flanagan 1991]. Upon localization to the nucleus NF-ATc complexes with NF-ATn to form an active transcription factor which can bind to the regulatory region of IL-2 and other cytokines to induce the transcription of these T-cell activating factors [Rao 1994].

Toxicity

Despite the effectiveness of cyclosporine to suppress the immune response, the use of cyclosporine is limited by its adverse effects which include nephrotoxicity, hepatotoxicity, hypertension, and lipid abnormalities [Bennett 1990, Starling 1990, Bennett 1988, Chan 1981]. The major obstacle for cyclosporine is its nephrotoxicity which is most often reversible upon cessation of cyclosporine therapy. Acute cyclosporine nephrotoxicity often occurs within the first week of therapy and is seen in patients that are on cyclosporine therapy for all types of solid organ transplantation [Bennett 1983]. Acute nephrotoxicity results in renal vasoconstriction occurring mainly at the afferent arteriole entering the glomerulus [English 1987, Curtis 1986]. This leads to a decrease in glomerular filtration
rate, an increase in serum creatinine levels and an increase in blood pressure. The underlying mechanism by which vasoconstriction occurs is unknown but some potential mediators have been indicated. Cyclosporine is known to induce endothelin release [Bunchman 1991] in vitro and in a rat model cyclosporine has been shown to increase circulating endothelin levels [Lanese 1993]. This association has also been seen in humans where peak endothelin levels parallel peak cyclosporine levels [Grieff 1993]. Further evidence for this relationship was provided by using an endothelin receptor antagonist which resulted in decreased vasoconstriction [Lanese 1993]. Cyclosporine has also been shown to increase thromboxane release in the kidney and an improvement in renal hemodynamics occurs when a thromboxane A2 antagonist has been administered [Rogers 1988, Smeesters 1988]. Another factor which might be involved in cyclosporine induced vasoconstriction is the observation that nitric oxide synthesis/release is inhibited by cyclosporine [Gardner 1996]. Thus, a number of possibilities that cause vasoconstriction associated with acute cyclosporine toxicity have been proposed, but the exact underlying mechanism is unknown.

Other indicators of impaired renal function associated with cyclosporine are hyperkalemia and hyperchloremic metabolic acidosis [Kamel 1992, Stahl 1986, Adu 1983]. Serum potassium, chloride and bicarbonate all return to normal only upon cessation of cyclosporine therapy and improve with a decrease in dosage, indicating a dose dependent effect. Increased serum potassium is most likely due to the inhibition of Na+/K+-ATPase activity by cyclosporine [Tumlin 1993]. However, as with the vasoconstrictive effects of cyclosporine, the exact mechanism by which this occurs is unknown.

Chronic cyclosporine toxicity results in the destruction of the arterial wall, progressive narrowing of the lumen wall and the appearance of tubulointerstitial fibrosis [Feutren 1992,
Mihatsch 1995], all of which contribute to renal failure. It would appear that such morphological damage is a result of cyclosporine related vasoconstriction as the decrease in renal blood flow seems to be a stimulus for fibrogenesis [Truong 1992] but such damage is not seen with other vasoconstrictors [Bennett 1996]. Such damage is seen in all types of patients being treated with cyclosporine, whether they have received a renal transplant, nonrenal transplant or are being treated for an autoimmune disorder [Myers 1984]. The progression of damage has been shown to progress even when cyclosporine is withdrawn [Elzinga 1993] indicating either an irreversible process was initiated or that chronic nephrotoxicity is not related to the vasoconstrictive effects of cyclosporine. The latter hypothesis is supported by the observation that the angiotensin I receptor antagonists losartan and enalapril protect against chronic cyclosporine nephropathy without improving renal blood floor or glomerular filtration rate [Bennett 1996]. Khanna et al. have shown that cyclosporine increases the expression and production of transforming growth factor β (TGF-β) and that this expression is reduced by antagonists of the renin-angiotensin system [Khanna 1994]. TGF-β has been shown to stimulate fibrosis by increasing the synthesis of matrix components and by decreasing the degradation of matrix [Border 1994]. One potential mediator of this is plasminogen activator inhibitor-1 (PAI-1), a plasmin protease inhibitor. PAI-1 synthesis is stimulated by TGF-β and have been shown to have elevated activity in rats treated with cyclosporine [Shihab 1996]. Although TGF-β has been shown to involved in cyclosporine nephrotoxicity, the cause of elevated TGF-β expression is unknown.

One possible mechanism of cyclosporine nephrotoxicity that was considered is the binding of cyclophilin by cyclosporine and the resultant inactivation of peptidyl-prolyl cis-trans isomerase. It is possible that the loss of isomerase activity could lead to the inactivation
of other proteins other than calcineurin resulting in nephrotoxicity. Sigal et al studied this possibility by assessing the relationship between cyclophilin binding, immunosuppression and nephrotoxicity of cyclosporine and 61 cyclosporine analogs [Sigal 1991]. Although cyclophilin binding is necessary for immunosuppressive activity, this study showed that 13 of the analogs bound cyclophilin but had no immunosuppressive activity. Their data suggested that the nephrotoxicity of cyclosporine or the analogs was related to immunosuppression rather than cyclophilin binding. This is not unexpected as the nephrotoxic effects of cyclosporine are unlikely to be due to cyclophilin binding for the same reason such binding is not directly responsible for immunosuppression: cyclophilin is extremely abundant in the cytosolic milieu and that the concentrations of cyclosporine used clinically are insufficient to inhibit all isomerase activity.

It has been proposed that the effector of cyclosporine nephrotoxicity is most likely calcineurin [Su 1995] which loses its phosphatase activity when bound to the cyclosporine-cyclophilin complex. Inactivation of phosphatase activity by cyclosporine has been shown to affect the activity of the transcription factor Elk-1 [Sugimoto 1997]. Elk-1 is involved in gene regulation and is activated by phosphorylation [Yang 1998] while phosphatases, primarily calcineurin, negatively regulate Elk-1 [Sugimoto 1997]. The inhibition of calcineurin activity by cyclosporine may perturb the phosphorylation state of Elk-1 and possibly other yet unidentified proteins, resulting in nephrotoxicity. Further research is obviously required in order to determine the mechanism of cyclosporine toxicity. If it is determined that calcineurin is the mediator of cyclosporine nephrotoxicity and that this is a requirement for effective immunosuppression it is unlikely that the development of new cyclosporine analogs will have any less nephrotoxicity. Instead, development of analogs
with different tissue distribution, plasma lipoprotein distribution and metabolism may prove to be an alternative to reduce the occurrence and severity of nephrotoxicity.

**Pharmacokinetics**

Due to the narrow therapeutic window of cyclosporine it is critical to administer the proper dose in order to achieve a therapeutic effect yet minimize toxicity. This has been difficult as there are significant inter- and intra-individual differences in cyclosporine blood levels after oral or i.v. administration [Ptachinski 1986]. The pharmacokinetic parameters of cyclosporine are, like other drugs, affected by various factors including disease state, age, the type of organ transplant and other drugs being taken concurrently. By studying these factors it may be possible to better characterize their effect on cyclosporine pharmacokinetics and achieve more standardized dosing regimens.

Absorption of cyclosporine is affected by food intake which has been shown to increase absorption [Ptachinski 1985] while other reports have indicated an impairment of absorption [Keown 1982]. These studies did not take into consideration fat content of the meal and a subsequent study by Gupta [Gupta 1990] indicated that cyclosporine when ingested with a meal high in fat content had both increased bioavailability and volume of distribution. Because of these conflicting reports and the poor understanding of the effect of food on cyclosporine absorption it has been suggested that patients should be taking cyclosporine consistently with regards to time and meal content in order to minimize variability in absorption [Honcharik 1991]. By doing so, it may be possible to ensure the proper therapeutic dose of cyclosporine while minimizing the toxic effects.
Cyclosporine bioavailability following oral administration can vary from 10-70% [Grevel 1986, Kahan 1983] and has been shown to be due more to variability in absorption and bile flow rather than a first pass effect [Gupta 1990, Grevel 1989]. This variability is seen in the use of Sandimmune®, an oral dosage form of cyclosporine that is available as a soft gelatin capsule containing 25, 50 or 100 mg of cyclosporine or an oral solution of cyclosporine dissolved in olive oil, emulsifier (Labrafil®) and ethanol at a concentration of 100 mg/mL. In 1995, a new microemulsion formulation of cyclosporine, Neoral®, was approved for use. Neoral is a mixture of cyclosporine 100 mg/mL with DL-α-tocopherol (antioxidant), castor oil, propylene glycol, corn oil and ethanol to form a mixture that spontaneously forms a microemulsion on contact with gastrointestinal fluids. The micelles formed are absorbed in the small bowel and this absorption is not dependent on the presence of bile nor is it influence by food [Friman 1996]. Neoral has been shown to increase the bioavailability of cyclosporine by 40-60% over the conventional Sandimmune formulation and has less variability in absorption when compared to the conventional formulation [Kovarik 1993, Mueller 1994a, Whipple 1994, Winkler 1994, Kovarik 1994]. Neoral, with a more consistent absorption profile, provides improved dose linearity enabling more accurate dose titration [Mueller 1994b] which is desirable for a drug with such a low therapeutic window. Overall, this allows cyclosporine to be administered in a manner that ensures therapeutic effect while minimizing the toxic side effects.

Following oral administration of a dose of 20 µg/kg, cyclosporine reaches a concentration maximum of ~1300 mg/L in ~3 hours [Keown 1982]. The volume of distribution of cyclosporine is very high with values of 4.5 ± 3.6 L/kg reported in renal transplant patients [Ptachcinski 1985]. Cyclosporine exhibits multicompartmental behaviour [Follath 1983, Yee
which is due to its highly lipophilic nature but is not distributed uniformly in the body. The majority of cyclosporine can be found in the liver, pancreas, adipose, kidney and blood [Kahan 1983]. Despite its lipophilic nature, cyclosporine does not readily cross the blood brain barrier [Cefalu 1985], making it difficult to explain the central nervous system side effects of cyclosporine. In the blood fraction, cyclosporine is found mainly in erythrocytes (58%) and plasma (33%) with the remainder in granulocytes and lymphocytes [LeMaire 1982]. Within the plasma over 80% of cyclosporine is associated with lipoproteins [LeMaire 1982, Niederberger 1983, Wasan 1997] with the majority of the drug associated with the high density lipoprotein and low density lipoprotein fractions (~50% and ~30% respectively) [Niederberger 1983, Wasan 1997]. The free fraction of cyclosporine in plasma is estimated to be between 1.0-2.4% [Henricsson 1987].

Table 1: Pharmacokinetic parameters of cyclosporine (Sandimmune® formulation)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioavailability</td>
<td>10-70%</td>
</tr>
<tr>
<td>C_{max} (µg/L)</td>
<td>538-1361</td>
</tr>
<tr>
<td>t_{max} (hr)</td>
<td>2.8-6.0</td>
</tr>
<tr>
<td>VDss (L/kg)</td>
<td>1.3-13.8</td>
</tr>
<tr>
<td>t_{1/2} (hr)</td>
<td>10-24</td>
</tr>
<tr>
<td>Clearance (mL/min/kg)</td>
<td>4.0-11.8</td>
</tr>
</tbody>
</table>

C_{max} = maximum concentration reached; t_{max} = time to reach C_{max}; VDss = volume of distribution at steady state; t_{1/2} = half life of elimination

**Metabolism**

Greater than 95% of cyclosporine is metabolized by cytochrome P450 enzymes with subsequent excretion in the bile [Maurer 1985]. Renal excretion does not play a large role in cyclosporine elimination with only small amounts of cyclosporine or cyclosporine
metabolites found in the urine [Maurer 1985]. In addition, it has been observed that reduced renal function does not significantly alter cyclosporine elimination [Follath 1983].

Cytochrome P450 enzymes represent a superfamily of enzymes that are involved in the biotransformation of drugs, environmental chemicals, and endogenous compounds [Benet 1996]. Cytochrome P450s are heme containing proteins that catalyze a variety of oxidative and reductive reactions, which usually result in a compound that is more hydrophilic and thus more easily eliminated. There are 12 cytochrome P450 gene families that have been identified in humans based on sequence homology. Cytochrome P450s are commonly abbreviated as CYP(x)(y). 'X' is the number representing the family and 'y' is a letter representing a further division of subfamily. In humans CYP1, 2, and 3 are the main cytochrome P450 enzymes involved in the biotransformation of drugs.

CYP3A has been identified as the major enzyme involved in the metabolism of cyclosporine [Kronbach 1988]. CYP3A activity has been detected in the liver, small intestine, heart and kidney [Gonzalez 1992]. Cyclosporine is primarily metabolised in the liver and small intestine [Christians 1993]. The metabolism of cyclosporine by the small intestine has been suggested as a possible source of the variability seen in cyclosporine absorption [Hoppu 1991, Gridelli 1986]. This is due to the inter-individual variations in CYP3A enzyme activity which has been shown to vary 10-fold [Guengerich 1989].

More than 30 cyclosporine metabolites have been isolated from the bile, blood and urine of various species [Christians 1993]. The nomenclature for cyclosporine metabolites is based upon the structure of the metabolites which replaces the previous nomenclatures based on HPLC retention times of the metabolites [Maurer 1985, Christians 1988]. The name of the metabolite begins with 'A' for cyclosporine A, 'M' to indicate it is a metabolite and a
sequence of numbers and letters to identify the amino acid position and type of modification [Consensus Document 1990].

The major factor influencing cyclosporine metabolism are other drugs that can induce or inhibit CYP3A activity (See Table 2) [Pichard 1990]. Inhibitors of CYP3A activity can lead to a rise in cyclosporine trough levels which can result in an increased incidence of nephrotoxicity. Inducers of CYP3A activity can lead to an increased metabolism of drugs biotransformed by CYP3A, including cyclosporine. If appropriate dose changes are not made, cyclosporine blood concentrations may decrease to sub-therapeutic levels with an associated increased risk of graft rejection. Conversely, when therapy of the CYP3A inducer is ceased, cyclosporine blood concentrations may increase with an associated increase of nephrotoxicity. Thus, when a patient is on cyclosporine therapy, close attention to the other drugs that the patient is receiving must be noted in order to ensure that appropriate doses of cyclosporine are being administered.
Table 2: List of drugs tested to determine their interaction with cyclosporine metabolism (Adapted from Pichard 1990)

<table>
<thead>
<tr>
<th>Drugs that affect the rate of CSA metabolism</th>
<th>Drugs that do not affect the rate of CSA metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inducers</strong></td>
<td><strong>Inhibitors</strong></td>
</tr>
<tr>
<td>rifampicin</td>
<td>triacetyloseandomycin</td>
</tr>
<tr>
<td>sulfadimidine</td>
<td>erythromycin</td>
</tr>
<tr>
<td>phenobarbital</td>
<td>josamycin</td>
</tr>
<tr>
<td>phenytoin</td>
<td>midecamycin</td>
</tr>
<tr>
<td>phenylbutazone</td>
<td>ketoconazole</td>
</tr>
<tr>
<td>dexamethasone</td>
<td>miconazole</td>
</tr>
<tr>
<td>sulfinpyrazone</td>
<td>midazolam</td>
</tr>
<tr>
<td>carbamazepine</td>
<td>nifedipine</td>
</tr>
<tr>
<td>diltiazem</td>
<td>verapamil</td>
</tr>
<tr>
<td>nicardipine</td>
<td>diltiazem</td>
</tr>
<tr>
<td>ergotamine</td>
<td>nifedipine</td>
</tr>
<tr>
<td>dihydroergotamine</td>
<td>diclofenac</td>
</tr>
<tr>
<td>glibenclamide</td>
<td>prostaglandin</td>
</tr>
<tr>
<td>ethynylestradiol</td>
<td>progestone</td>
</tr>
<tr>
<td>bromocriptine</td>
<td>cortisone</td>
</tr>
<tr>
<td>prednisone</td>
<td>methylprednisolone</td>
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<tr>
<td>prednisolone</td>
<td>methylprednisolone</td>
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<tr>
<td>methylprednisolone</td>
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</table>
**Lipoproteins**

Lipoproteins are aggregates of lipid and protein which serve to transport phospholipids, cholesteryl ester (CE), and triacylglycerols (TG) through the vascular and extravascular fluids [Davis 1996, Harmony 1986]. Both cholesterol and TG are derived either from dietary sources or, in the case of cholesterol, synthesized *de novo*. Lipoproteins transport these cellular nutrients from the site of absorption (small intestine) or synthesis to other tissues for storage or catabolism. Lipoproteins have also been shown to be involved in other biological processes including coagulation, tissue repair, atherogenesis, and immune reactions [Mbewu 1990, Durrington 1989, Basile-Borgia 1997, Edgington 1981].

Lipoproteins are roughly spherical and are made of a hydrophobic core containing CE and TG surrounded by a monolayer of phospholipid embedded with unesterified cholesterol and apolipoproteins (Figure 3). The types of phospholipids found in lipoproteins include phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine and sphingomyelin with phosphatidylcholine being the most abundant.

Lipoproteins are traditionally classified according to their density with those have a high protein to lipid ratio having a higher density [Davis 1991]. There are four main categories of lipoproteins: triglyceride rich lipoproteins which includes chylomicrons and very low density lipoprotein (VLDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL), and high density lipoprotein (HDL) (See Table 3). These are broad classifications and within each group there is heterogeneity in terms of particle size and composition.
**Figure 3:** The structure of a lipoprotein particle. Lipoproteins are approximately spherical macromolecular aggregates of lipid and protein consisting of a neutral lipid core and a polar surface coat consisting of amphipathic phospholipids, unesterified cholesterol and lipoprotein specific proteins (apolipoproteins).
Table 3: The density, size, and physical composition of human plasma lipoproteins (Adapted Davis 1996 and Havel 1995).

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>CHYLOMICRONS</th>
<th>VERY LOW DENSITY LIPOPROTEINS</th>
<th>INTERMEDIATE DENSITY LIPOPROTEINS</th>
<th>LOW DENSITY LIPOPROTEINS</th>
<th>HIGH DENSITY LIPOPROTEINS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (g/mL)</td>
<td>&lt; 0.95</td>
<td>0.95 - 1.006</td>
<td>1.006 - 1.019</td>
<td>1.019 - 1.063</td>
<td>1.063 - 1.210</td>
</tr>
<tr>
<td>Diameter (nm)</td>
<td>75 - 1200</td>
<td>30 - 80</td>
<td>25 - 35</td>
<td>18 - 25</td>
<td>5 - 12</td>
</tr>
<tr>
<td>Composition (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>1 - 2</td>
<td>8</td>
<td>19</td>
<td>22</td>
<td>47</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>86</td>
<td>55</td>
<td>23</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5</td>
<td>19</td>
<td>38</td>
<td>50</td>
<td>19</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>7</td>
<td>18</td>
<td>20</td>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td>Apoproteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AI, AII</td>
<td>B-100</td>
<td>B-100</td>
<td>B-100</td>
<td>AI, AII</td>
<td></td>
</tr>
<tr>
<td>B-48</td>
<td>CI, CII, CIII</td>
<td>CI, CII, CIII</td>
<td>E</td>
<td>Ci, CII, CIII</td>
<td>E</td>
</tr>
<tr>
<td>CI, CII, CIII</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td></td>
</tr>
</tbody>
</table>

Chylomicrons are the largest and least dense lipoproteins ranging from 100-1000 nm and are most abundant post prandially. They are synthesized and secreted by the intestine and are rapidly catabolised with a half life of minutes. VLDL are the next largest lipoproteins and like chylomicrons are rich in TG. VLDL are synthesized in the liver and to a lesser extent in the intestine. The TG in both chylomicrons and VLDL are hydrolyzed by lipases, releasing free fatty acids which are used as a cellular energy source or stored in adipose tissue.

IDL are what remains when the TG in a VLDL particle have been hydrolyzed. IDL are still rich in TG and undergo further hydrolysis resulting in the formation of a LDL particle which is rich in cholesteryl ester. LDL are the major cholesterol carrying lipoproteins and are the second smallest lipoproteins (18 to 25 nm). HDL are the smallest lipoproteins ranging from 7 to 12 nm and have the highest protein content of all the lipoproteins. HDL have varying ratios of CE and TG and are involved in a process known as reverse cholesterol transport [Tall 1998].
Triglyceride-rich lipoproteins

Upon consumption of a meal, the fat in the form of triacylglycerols is hydrolyzed to release monoacylglycerols and fatty acids which diffuse into enterocytes. Within the enterocytes, the triacylglycerols are resynthesised and packed with apolipoprotein B-48 and other phospholipids to form chylomicrons which are secreted into the mesenteric lymph [Davis 1996]. As the chylomicron enters the systemic circulation, cholesterol, apolipoprotein E and apolipoprotein C’s are exchanged from HDL, resulting in the maturation of the chylomicron. Within minutes this mature chylomicron is metabolized, with the triacylglycerols being hydrolysed by lipoprotein lipase (LPL). LPL is located on the surface of capillary endothelial cells and are activated by the apolipoprotein CII on the chylomicron particle. The residual lipoprotein is termed a chylomicron remnant and is removed from the circulation by the liver.

VLDL is synthesized and secreted by the liver and is involved in the transport of endogenously synthesized triacylglycerols from the liver to peripheral tissue. The VLDL particle that is initially secreted by the liver is in a nascent form and acquires cholesteryl ester, apolipoprotein CII, CHIII and E from HDL. Like chylomicrons, VLDL TG is hydrolyzed by LPL, leaving a VLDL remnant, which is also known as IDL. This IDL particle has a CE rich core and is subject to two possible fates. IDL can be taken up by the liver via the LDL receptor mediated pathway or converted to LDL by interaction with hepatic lipase. The interaction with hepatic lipase further hydrolyzes the remaining TG and the apolipoprotein C’s and apolipoprotein E are also lost. The remaining particle, still bearing the apolipoprotein B is now a LDL particle.
**Low density lipoprotein and the LDL receptor.**

In humans, LDL consists of approximately 50% cholesterol and is the major cholesterol carrying lipoprotein. This is in contrast to ruminants and some rodents in which HDL is the major cholesterol transporting lipoprotein [Davis 1991]. LDL is the product of the interaction of IDL with hepatic lipase although it has been suggested that LDL can also be synthesized and secreted directly by the liver [Myant 1990].

LDL is removed from the circulation primarily via the LDL-receptor pathway (See Figure 4). The LDL receptor was discovered in the early 1970’s and is one of the best understood receptor models [Brown 1976, Goldstein 1977]. The receptor is initially synthesized in the rough endoplasmic reticulum and undergoes post translational modification. This mature receptor is then translocated to the cell surface where it is localized into specialized regions known as coated pits [Harrison 1983]. These coated pits occupy only 2% of the cell surface but contain 80% of the surface LDL [Goldstein 1985]. These pitted regions undergo constitutive invagination, thus both LDL-receptors bound to a LDL particle and free receptors are internalized to form coated vesicles, also known as endosomes [Goldstein 1979]. These vesicles have surface associated (intracellular side) ATP-driven proton pumps [Stone 1983] which decreases the pH within the endosome, resulting in the dissociation of the LDL particle from the LDL receptor. The receptor recycles to the cell surface while the LDL particle is delivered to and degraded by lysosomes, releasing its lipid and protein components.
**Figure 4:** The LDL Receptor Pathway.
The release of cholesterol has three major regulatory effects on the cell [Myant 1990, Goldstein 1977]: i) the down regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCoA reductase), the rate limiting enzyme involved in \textit{de novo} cholesterol synthesis; ii) activation of acyl-coenzyme A cholesterol acyl transferase (ACAT), the enzyme responsible for the esterification of cholesterol to cholesteryl ester, which is stored as cholesteryl ester droplets within the cell; and iii) the down regulation of LDL-receptor synthesis. These combined effects allow the cell to selectively utilize either dietary cholesterol or synthesized cholesterol.

**High density lipoprotein and reverse cholesterol transport**

High density lipoproteins are comprised of a heterogeneous population that is subdivided into different subclasses based on apoprotein and phospholipid content. The main function of HDL is the transport of cholesterol from the peripheral tissues to the liver in a process known as reverse cholesterol transport [Fielding 1995]. The precursor to HDL, also known as nascent HDL, is formed by the association of apolipoprotein AI, AII and E with phospholipid and free cholesterol to form disc shaped particles [Eisenberg 1984, Fielding 1996]. These particles are good acceptors of free cholesterol transferred from peripheral cells [Fielding 1995]. The cholesterol in the nascent HDL are esterified through the action of lecithin cholesterol acyl transferase (LCAT). LCAT transfers a fatty acid moiety from phosphatidylcholine (lecithin) to the cholesterol, producing a cholesteryl ester which is more hydrophobic than cholesterol and is located in the core of the HDL particle. This accumulation of cholesteryl ester in the nascent HDL produces a hydrophobic core that
changes the disc shaped particle into a spherical shaped particle which is known as HDL\textsubscript{3} [Davis 1996, Eisenberg 1984, Fielding 1995, Fielding 1996].

The HDL\textsubscript{3} particle is rich in cholesteryl ester but deficient in triacylglycerol. Triacylglycerols are acquired through the action of cholesteryl ester transfer protein (CETP) which exchanges cholesterol for triacylglycerol between the HDL\textsubscript{3} particle and the TG-rich lipoproteins [Fielding 1996]. This leads to the maturation of the HDL\textsubscript{3} particle to what is termed an HDL\textsubscript{2} which has a hydrophobic core consisting of both cholesteryl ester and triacylglycerol. The TG-rich VLDL undergoes its normal life cycle which ends in uptake by the liver and thus removal of the cholesterol obtained from the HDL\textsubscript{3} particle, thus completing the cycle of reverse cholesterol transport. The HDL\textsubscript{2} particle interacts with hepatic lipase which hydrolyzes the triacylglycerol, regenerating the HDL\textsubscript{3} particle. This HDL\textsubscript{3} can acquire further cholesterol from peripheral tissue and interact with TG-rich lipoproteins again, thus the cycle of cholesterol removal can continue.

**Lipoproteins and Drugs**

A large number of drugs associate with the plasma proteins albumin and \(\alpha\)-1-glycoproteins. However, there are also a number of drugs that bind to lipoproteins such as amphotericin B [Wasan 1998], cyclosporine [Lemaire 1982], probucol [Urien 1984], delta-9-tetrahydrocannabinol (THC) [Klausner 1975] and nystatin [Wasan 1998]. Such binding and transport of these drugs may influence the pharmacokinetics, tissue distribution and pharmacological activity of these compounds [Wasan 1996].

The maximum tolerated dose and pharmacokinetics of drugs are commonly determined in healthy controls but it is known that such parameters are often different when the drug is
given to diseased patients [Gibaldi 1982, Rowland 1989]. In some cases, a dose that is determined effective and non-toxic can be found to be ineffective or toxic in the diseased patient [Sgoutas 1986, Rowland 1980, Zini 1986]. One factor that may cause such a difference between healthy and diseased patients is the lipoprotein distribution of such individuals.

Researchers have shown that the activity of cyclosporine may be dependent on the lipoprotein fraction to which it is bound. In one study it was demonstrated that when cyclosporine was bound to LDL there was greater antiproliferative effects observed than when cyclosporine was bound to VLDL or HDL [Lemaire 1988]. This supports the observation that the LDL receptor pathway is involved in the uptake of cyclosporine by T-lymphocytes [de Groen 1988a, Sanghvi 1989]. The factors that affect the lipoprotein distribution of cyclosporine are not well defined but include the lipid concentration and lipid composition of the various lipoprotein fractions. It has been reported that as plasma triglyceride and cholesterol concentration increases, the lipoprotein distribution of cyclosporine shifts from the HDL fraction to the LDL and VLDL fractions [Gardier 1993, Wasan 1997].

Another complicating factor that may affect the lipoprotein distribution of cyclosporine is the effect of cyclosporine on plasma lipoproteins. In a study by Ballantyne, patients on cyclosporine had significant increases in total cholesterol, primarily in the LDL fraction, after two months of therapy [Ballantyne 1989]. This change in total lipids and lipoprotein composition could shift the lipoprotein distribution of cyclosporine and be manifested as a change in effectiveness and toxicity.
The mechanism by which cyclosporine is distributed between the different lipoprotein fractions is unknown. In work by Hughes et al with isolated lipoprotein fractions it was hypothesized that the distribution of cyclosporine amongst lipoproteins is unlikely to be by simple diffusion [Hughes 1991]. The distribution of cyclosporine in these isolated systems was significantly different from the plasma distribution of cyclosporine. This indicated that there was probably some plasma factors that played a role in the lipoprotein distribution of cyclosporine but such factors were not identified. One such factor that has been shown to influence the distribution of hydrophobic drugs is lipid transfer protein I (LTP I), also known as cholesteryl ester transfer protein (CETP) [Wasan 1998].
**Cholesteryl Ester Transfer Protein**

CETP is a 476 amino acid hydrophobic glycoprotein with a molecular weight of 74 kD [Hesler 1987, Drayna 1987]. CETP, along with PLTP, are believed to have evolved from a common ancestor belong to the family of lipopolysaccharide binding proteins [Schumann 1990]. The two proteins share 20% homology and have homologous regions to lipopolysaccharide binding proteins [Day 1994, Schumann 1990]. CETP expression between mammalian species is variable, with undetectable levels in rats and mice and moderate levels in humans and rabbits [Ha 1982]. The majority of CETP in humans is synthesized in the liver, with lower levels produced in the adipose, kidney, heart and spleen [Moulin 1996].

CETP facilitates the transfer of cholesteryl esters from HDL to apo B-containing lipoproteins (VLDL & LDL) with a reciprocal transfer of triglyceride [Tall 1983, Lagrost 1994, Morton 1982, Morton 1983]. CETP, along with phospholipid transfer protein (PLTP), plays an important role in the metabolism and remodeling of plasma lipoproteins [Tall 1986, Morton 1990]. CETP may also play a role in certain disease processes such as atherosclerosis by redistributing cholesterol from the antiatherogenic HDL to the proatherogenic LDL. Conversely, CETP is also implicated in the process of reverse cholesterol transport which removes cholesterol from peripheral tissues and is viewed as antiatherogenic. The exact role of CETP in atherogenesis is uncertain and is the subject of various studies [Moulin 1996].

CETP is regulated by cholesterol, with an increase in activity and expression seen in response to cholesterol [Quig 1990]. In studies using human-CETP transgenic mice it was determined that the increased activity could be attributed to an increase in transcription of the
CETP gene [Jiang 1992]. Conversely, CETP activity has been shown to be reduced in response to corticosteroids and lipopolysaccharides [Masucci-Magoulas 1995].

The CETP binding domain for neutral lipids (CE and TG) is located in the 26 amino acid residues that comprise the carboxy-terminal end of CETP [Swenson 1989]. This was shown by the use of a monoclonal antibody, TP2, which blocks this binding domain and preventing the transfer of neutral lipid by CETP [Hesler 1988, Wang 1992]. The domain is apparently comprised of an amphipathic helix which consists of a charged/polar residue face and a hydrophobic residue face to which binding of neutral lipid occurs [Wang 1993]. This binding of neutral lipids may induce a conformational change in the protein which enhances its binding to lipoproteins [Swenson 1989].

CETP forms complexes with VLDL, LDL, and HDL with the HDL being the most stable complex [Pattnaik 1979, Morton 1985]. In plasma, CETP is recovered mainly in the HDL fraction [Groener 1984]. The binding of CETP to lipoproteins is due to an electrostatic interaction between positively charged regions of CETP with negatively charged components on the lipoprotein [Sammett 1985]. The stability of the lipoprotein-CETP complex is enhanced as the negative charge density of the lipoprotein is increased [Nishida 1993]. It has been shown that there is an optimal affinity of CETP for both the donor and acceptor particle in order to obtain maximal lipid transfer activity [Nishida 1993]. Thus changes in lipoprotein distribution and composition as a result of disease state, diet or drug therapy could affect lipid transfer rates.

CETP facilitated lipid transfer has been proposed to occur by two methods: 1) a carrier mediated process [Barter 1980] in which CETP acts a shuttle between the donor and acceptor particle and 2) a ternary mechanism [Ihm 1982] in which a donor-CETP complex collides
with the acceptor forming a ternary complex (Figure 5). In the carrier mediated process CETP binds to the donor particle (HDL), which may result in a conformation change, exposing a neutral lipid binding site on CETP. A molecule of cholesteryl ester is located to the binding site and CETP dissociates from the HDL. The CETP-cholesteryl ester complex then diffuses and eventually binds to an acceptor particle (VLDL or LDL) and the cholesteryl ester is exchanged for a molecule of triacylglycerol. The CETP dissociates from the receptor particle and the process repeats.

In the ternary mechanism of lipid exchange, the CETP-HDL complex does not dissociate. Instead this complex will collide and bind to the acceptor particle forming a ternary complex. Exchange of a cholesteryl ester for a triacylglycerol is then facilitated by CETP. Once the exchange of neutral lipids is completed, the three components dissociate and the process starts over.

Many studies into the mechanism of lipid transfer have been performed to determine whether the shuttling or the collision mechanism predominates in humans. From these studies it is generally accepted that both mechanisms most likely coexist in vivo and play a role in the transfer of neutral lipids amongst lipoproteins [Lagrost 1992].
Figure 5: Two models of CETP-mediated transfer of cholesteryl ester (CE) and triglyceride (TG). In the shuttle model CETP interacts with HDL and pick up one CE molecule. A transient CE-CETP complex would form and this would interact with an acceptor LDL or VLDL molecule. The CE is then transferred to the acceptor in exchange for a TG molecule. A transient TG-CETP complex would result and this could interact with an HDL molecule and the cycle would repeat. In the ternary collision model, HDL, CETP and LDL or VLDL would interact to form a macrocomplex. CETP would then facilitate the exchange of CE and TG between the two lipoproteins.
Chapter 2

Aims
**Specific Aims**

1) To determine the plasma lipoprotein distribution of cyclosporine by density gradient ultracentrifugation, gel filtration chromatography and affinity chromatography.

2) To determine the effect of changes in lipid concentration and composition on the lipoprotein distribution of cyclosporine.

3) To determine the role of CETP in the regulation of the plasma lipoprotein distribution of cyclosporine.

**Rationale**

*Aim 1:* The plasma lipoprotein distribution of cyclosporine as reported by various groups was determined by ultracentrifugation techniques [LeMaire 1982, Niederberger 1983, Sgoutas 1986]. However, ultracentrifugation was intended for the preparative fractionation of plasma lipoproteins [De Lalla 1954, Havel 1955] and was not designed for the study of drug associated with lipoproteins. The high shear forces experienced during ultracentrifugation may disturb the drug-lipoprotein complex in an unpredictable manner. Thus, the distribution observed may be an artifact of the technique. We shall verify these previous ultracentrifugal studies by studying the plasma lipoprotein distribution of cyclosporine by two additional techniques: gel filtration chromatography and affinity chromatography coupled with ultracentrifugation.
Aim 2: Previous studies have shown that increases in total lipid can alter the plasma lipoprotein distribution of cyclosporine [Wasan 1997, Gardier 1993]. Such changes may have pharmacologic implications and could affect the efficacy and toxicity of cyclosporine. We shall study the effect of changes in lipoprotein lipid concentration on the lipoprotein distribution of cyclosporine.

Aim 3: In previous work by Wasan et al, it was shown that CETP can influence the lipoprotein distribution of amphotericin B (Amp B) [Wasan 1993, 1996]. Cyclosporine, like Amp B, is a hydrophobic drug that associates with lipoproteins and thus may also be subject to CETP facilitated transfer. We shall study the role of CETP in the transfer of cyclosporine in an in vitro system that utilizes isolated $^3$H-cyclosporine-lipoprotein complexes. We shall also investigate if CETP plays a significant role in determining the lipoprotein distribution of cyclosporine.
Chapter 3

Materials and Methods
Materials and Methods

A. Chemicals and Reagents

Radiolabeled cyclosporine ([mebmβ-3H]-cyclosporine, specific activity 8.00 Ci/mmol) was purchased from Amersham (Buckinghamshire; UK). Radiolabeled cholesteryl ester ([1α,2α(n)-3H]-cholesteryl oleate, specific activity 50.0 Ci/mmol) was purchased from Amersham (Buckinghamshire; UK). Human plasma was obtained from the Vancouver Red Cross (Vancouver; BC). The LDL-Direct Plus kits were purchased from Isolab Inc. (Akron, OH). Sodium bromide was purchased from Sigma Chemical Co. (St. Louis, MO; USA). Sodium chloride was purchased from Fisher Scientific (Vancouver, BC). The cholesterol reagent, cholesterol standards, triglyceride reagent, triglyceride standards were purchased from Sigma Diagnostics (St. Louis MO; USA). Purified CETP was kindly provided by R Morton (Cleveland Clinic). TP2 monoclonal antibody was kindly provided by Y Marcel (Ottawa Heart Institute).

B. Lipoprotein Separation

I. Density Gradient Ultracentrifugation

Plasma was separated into its VLDL, LDL, HDL and lipoprotein deficient fraction (LPDF) by density gradient ultracentrifugation [Chapman 1981, Aviram 1983]. 2.8 mL of human plasma samples were adjusted to a density of 1.25 g/mL with the addition of sodium bromide and were placed in centrifuge tubes. Once the sodium bromide had dissolved completely in the plasma, 2.8 mL of the highest density sodium bromide solution (1.21 g/mL) was carefully layered on top of the plasma. 2.8 mL of the next highest density solution (1.063 g/mL) was then carefully layered on top, followed by the 2.8 mL of the
lowest density solution (1.006 g/mL). The plasma and all density solutions were kept at 4°C during the layering process.

The centrifuge tubes were placed into titanium rotor buckets (SW41 Ti rotor buckets; Beckman Instruments Inc.; Palo Alto, CA; USA), balanced and capped. The buckets were then hung in their respective positions on the rotor (Beckman Instruments Inc.; Palo Alto, CA; USA) and centrifuged at 40000 rpm (288000 × g), at a temperature of 15°C for 18 hr in a Beckman L8-80 (Beckman Instruments Inc.; Palo Alto, CA; USA). After ultracentrifugation the centrifuge tubes were carefully removed in order to minimize disturbing the gradient layers. Figure 6 illustrates how the layers would appear in the centrifuge tube. Each density layer was carefully removed using a pasteur pipette and the volume of each lipoprotein fraction measured. The samples were stored at 4°C until further analysis.

II. Affinity Chromatography

Lipoproteins were separated into an HDL/LPDF (alpha) and LDL/VLDL (beta) fractions by the LDL-Direct Plus Cholesterol Ratio System (Isolab Inc; Akron OH; USA) [Bentzen 1982, Whitaker 1986]. This chromatographic column consists of a heparin-agarose matrix which separates lipoproteins based on their apolipoprotein content. Lipoproteins bearing apolipoprotein B and E (ie: LDL and VLDL) are retained on the matrix while all other components (ie: HDL and LPDF) are eluted. Briefly, the virgin column is fully hydrated with the alpha eluting solvent (0.02% sodium chloride) before 200 μL of sample is added to the column. 50 μL of the alpha eluting agent is added and the eluent is collected. The sample is allowed to sit for five minutes before adding an additional 1.0 mL of the alpha
Very low density lipoproteins

Low density lipoproteins

High density lipoproteins

Lipoprotein-deficient plasma

Figure 6: A representation of the separation of plasma lipoprotein fractions in an Ultraclear® ultracentrifuge tube following ultracentrifugation in a Beckman SW41-Ti swinging bucket rotor at 40,000 rpm and 15°C for 18 hours in a Beckman L8-80M ultracentrifuge. Separation is based on the density of the plasma components; therefore the constituents of low density will rise to the top and those of heavier density will remain at the bottom.
eluting agent. Thus, the alpha fraction consists of a 200 µL of sample and 1050 µL of alpha eluting agent, for a total of 1.25 mL. To desorb the beta fraction, 2.5 mL of the beta eluting agent (2.9% sodium chloride) was added and the eluent collected. The beta fraction was further separated into its respective LDL and VLDL fractions by sequential ultracentrifugation (Havel 1955). All experimentation was performed at ambient temperature.

The beta fraction was placed in a QuickSeal ultracentrifuge tube (Beckman) and density solution (1.006 g/mL) was added to fill the tube. The tube was heat sealed and the contents thoroughly mixed. A balance tube of the same weight was also prepared. The ultracentrifuge tubes were placed in a TY65 fixed angle rotor (Beckman Instruments, Inc; Palo Alto, CA; USA) and centrifuged at 60000 rpm for 18 hours at 4°C in a Beckman L8-60M Ultracentrifuge (Beckman Instruments, Inc; Palo Alto, CA; USA). Upon completion of the spin, the tubes were carefully removed from the rotor and the top layer of the solution was carefully removed by pasteur pipette and collected in a separate test tube. This comprised the VLDL portion of the beta fraction.

III. Fast Protein Liquid Chromatography (FPLC)

Lipoprotein separation was performed by use of a gel filtration column connected to a fast protein liquid chromatography (FPLC) system. The FPLC system consisted of a Biorad econo pump (Biorad; Hercules; CA) and system recorder interfaced to a Biorad econo UV monitor, a Biorad 1325 econo-recorder, and a Biorad 2110 fraction collector. 2 mL of plasma were injected onto a Superose 6 column [1.6 cm × 50 cm] (Pharmacia; Montreal) which was serially connected to a Superose 12 column [1.6 cm × 50 cm] (Pharmacia;
Montreal). A mobile phase consisting of a buffer of 0.15M NaCl, 1 mM EDTA and 0.03% (w/v) sodium azide flowing at 0.25 mL/min was used to elute the sample. 0.5 mL fractions were collected by a sample collector for 75 minutes and UV detection was at a wavelength of 280 nm. The column was standardized with respect to elution times for VLDL, LDL, HDL and human serum albumin. See Figure 7 for a representative chromatogram of the separation of the lipoproteins.

C. Cholesteryl Ester Transfer Activity

I. CETP transfer of cyclosporine in plasma

To determine the ability of CETP to facilitate the movement of cyclosporine in plasma, the lipoprotein distribution of cyclosporine was determined in plasma which had been supplemented with exogenous CETP. In separate experiments, cyclosporine was incubated in plasma at a concentration of 1 μg/mL for 60 min at 37°C with increasing amounts of exogenous CETP (0.5 μg/mL, 1.0 μg/mL, and 2.0 μg/mL). The plasma was then separated into the α and β fractions using affinity chromatography. The amount of cyclosporine in each fraction was then quantitated by liquid scintillation counting.

II. CETP transfer of cyclosporine and cholesteryl ester

Cholesteryl ester transfer activity was measured following the protocol of Morton and Pattnaik [Pattnaik 1978, 1979; Morton 1982, 1983]. Briefly, 10 μg (based on cholesterol) of radiolabeled donor lipoprotein (either HDL or LDL) and corresponding cold acceptor lipoprotein were incubated in delipidated human plasma or T150 buffer (50 mM tris-HCl,
Figure 7: Representative chromatogram of lipoprotein separation by fast protein liquid chromatography
150 mM NaCl, 0.02% NaN3, 0.01% disodium EDTA; pH 7.4) that had been supplemented with CETP (1 μg/mL) for 60 min at 37°C.

The experiments were done in the presence and absence of TP2 (4 μg protein/mL), a monoclonal antibody to CETP which inhibits the lipid transfer activity of CETP [Hesler 1988]. The degree of CETP inhibition by TP2 was determined by studying effect of increasing amounts of TP2 on the transfer of radiolabeled cholesteryl ester from HDL to LDL after incubation for 120 min at 37°C. Percent inhibition of transfer was determined by comparing percent transfer of cholesteryl ester to a control in which no TP2 was added.

At the end of the incubation, the LDL fraction was precipitated using a 0.1 M manganese chloride solution. The sample was then centrifuged and the radioactivity of the supernatent was determined by liquid scintillation counting. The extent of lipid transfer was calculated by the following calculation:

\[ k_t = -\ln\left(1 - \frac{A_t}{D_0}\right) \]

Where \( D_0 \) and \( A_t \) are the radioactivity of the donor lipoprotein at time 0 and in the acceptor at time \( t \). The constant \( k \) is the fraction of radiolabeled compound that is transferred per unit time (t). Calculations assume steady-state conditions where lipid and drug transfer is a result of an exchange process and not mass transfer.
D. Lipoprotein Lipid Determination

I. Determination of Total Cholesterol

Total cholesterol was determined by utilizing an enzymatic procedure that is a modification of the method of Allain et al [Allain 1974]. This method provides a measurement of free cholesterol and cholesteryl ester (ie, total cholesterol). The enzymatic reactions are as follows:

\[
\text{cholesteryl esters} + \text{H}_2\text{O} \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acids}
\]

\[
\text{cholesterol} + \text{O}_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholesterol-4-en-3-one} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-aminoantipyrine} + \text{p-hydroxybenzene sulfonate} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + 4\text{H}_2\text{O}
\]

Initially cholesteryl esters are hydrolyzed by cholesterol esterase to produce cholesterol and fatty acids. All the free cholesterol is now oxidized in the presence of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide is coupled with 4-aminoantipyrine and p-hydroxybenzene sulfonate by the catalyst peroxidase to yield a quinoneimine dye, which has an absorbance maximum at 505 nm.

The cholesterol reagent was reconstituted in distilled water and mixed thoroughly by inversion. Stock solutions of 12.5, 25, 50, 100 mg/dL cholesterol were prepared by serial dilution of a 200 mg/dL cholesterol standard. 10 µL of each stock solution, sample and blank solution was aliquoted to an appropriately labeled test tube. To each of the test tube 1 mL of the cholesterol reagent was added. The mixture was vortexed for 10 seconds and incubated in a 37°C water bath for 5 minutes. Absorbance readings were performed at 506
nm using a Hewlett Packard 8452A Diode Array Spectrophotometer interfaced to a computer running a Hewlett Packard 89532A UV-Vis software package. The absorbance values of the standards were used to generate a standard curve (Figure 8). The total cholesterol concentration of each sample was subsequently determined from the equation derived from the standard curve.

II. Determination of Total Triglyceride

Total triglyceride was determined by utilizing an enzymatic procedure that is a modification of the method of Bucolo and David [Bucolo 1973]. Triglycerides are hydrolyzed and the glycerol produced is quantitated. The enzymatic reactions involved in the process are as follows:

\[
\text{triglycerides} \xrightarrow{\text{lipoprotein lipase}} \text{glycerol} + \text{fatty acids}
\]

\[
\text{glycerol} + \text{ATP} \xrightarrow{\text{glycerol kinase}} \text{glycerol-1-phosphate} + \text{ADP}
\]

\[
\text{glycerol-1-phosphate} + \text{NAD} \xrightarrow{\text{glycerol-1-phosphate dehydrogenase}} \text{DAP} + \text{NADH}
\]

\[
\text{NADH} + \text{INT} \xrightarrow{\text{diaphorase}} \text{formazan} + \text{NAD}
\]

The triglycerides are hydrolyzed by lipoprotein lipase to produce glycerol and free fatty acids. The glycerol is phosphorylated (ATP) glycerol kinase, to produce glycerol-1-phosphate (G-1-P) and adenosine-5-diphosphate (ADP). G-1-P is oxidized by glycerol-1-phosphate dehydrogenase to form dihydroxyacetone phosphate with the concomitant reduction of nicotinamide adenine dinucleotide. The NADH is oxidized to NAD while 2-[p-iodophenyl]-3-p-nitrophenyl-5-phenyl-tetrazolium chloride (INT) is reduced to produce
**Figure 8:** A representative cholesterol standard curve used for quantitating the total cholesterol in both lipoprotein and plasma samples.
formazan (INTH). This last step is catalyzed by diaphorase. The formazan is quantitated by UV spectrophotometry at an absorbance of 500 nm.

The triglyceride reagent was reconstituted with distilled water and mixed thoroughly by inversion. Stock solutions of 15.625, 31.25, 62.5, 125 mg/dL triglyceride were prepared by serial dilution of a 250 mg/dL triglyceride standard. 10 mL of each stock solution, sample and blank was aliquoted to an appropriately labeled test tube. The mixture was vortexed for 10 seconds and incubated in a 37°C water bath for 5 minutes. Absorbance readings were performed at 500 nm using a Hewlett Packard 8452A Diode Array Spectrophotometer interfaced to a computer running a Hewlett Packard 89532A UV-Vis software package. The absorbance values of the standards were used to generate a standard curve (Figure 9). The total triglyceride concentration of each sample was subsequently determined from the equation derived from the standard curve.

III. Determination of Total Protein

Total protein was determined by using Peterson’s modification of the micro-Lowry method [Peterson 1977]. Sodium dodecylsulfate is used to aid in the dissolution of insoluble proteins. The Lowry reagent consisting of an alkaline cupric tartrate reagent then complexes with the peptide bonds of the protein and forms a bluish-purple color when the Folin and Ciocalteu’s phenol reagent is added [Lowry 1951]. Absorbance is measured at a wavelength of 750 nm and the sample concentrations were determined by use of a standard curve (Figure 10).

The Lowry reagent was reconstituted with distilled water and mixed thoroughly by inversion. Folin and Ciocalteu’s phenol reagent was prepared by diluting 5 mL of 2N Folin
Figure 9: A representative triglyceride standard curve used for quantitating the total triglyceride in both lipoprotein and plasma samples.
and Ciocalteu’s phenol reagent with 25 mL of distilled water (i.e. a 1:5 ratio). The protein standard solution was prepared by reconstitution with water producing a solution equivalent to 400 µg/mL bovine serum albumin. A protein standard curve was prepared by diluting the protein standard solution in distilled water as follows:

<table>
<thead>
<tr>
<th>Protein Standard Solution (mL)</th>
<th>Distilled Water (mL)</th>
<th>Final Protein Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.000</td>
<td>0</td>
</tr>
<tr>
<td>0.125</td>
<td>0.875</td>
<td>50</td>
</tr>
<tr>
<td>0.250</td>
<td>0.750</td>
<td>100</td>
</tr>
<tr>
<td>0.500</td>
<td>0.500</td>
<td>200</td>
</tr>
<tr>
<td>0.750</td>
<td>0.250</td>
<td>300</td>
</tr>
<tr>
<td>1.000</td>
<td>0</td>
<td>400</td>
</tr>
</tbody>
</table>

**E. Radiolabeling of Plasma Lipoproteins**

Radiolabeling of human HDL and LDL was performed by the lipid dispersion technique (Morton 1982, 1983). Human plasma was incubated with egg phosphatidylcholine (0.1 µmol/mL) and $^3$H-cholesteryl ester (13.9 ng/mL) or $^3$H-cyclosporine (1000 ng/mL), dissolved in ethanol, at 37°C for 24 hours. The HDL and LDL fractions were separated by step gradient ultracentrifugation as described above. The fractions were then dialyzed for 24 hours in order to remove the sodium bromide and free $^3$H-cholesteryl ester or cyclosporine. Dialysis was done with a 1000 molecular weight cutoff dialysis tubing in normal saline (0.9% NaCl). Samples were then quantitated by comparison to a $^3$H-cyclosporine standard curve for the appropriate lipoprotein fraction (Figure 11).
Figure 10: A representative protein standard curve used for quantitating the total protein in both lipoprotein and plasma samples.
Figure 11: Representative standard curves of $^3$H-cyclosporine ($^3$H-CSA). The counts per minute (CPM) of fixed amounts (50, 250, 500 ng) of $^3$H-cyclosporine in 0.5 mL of isolated fractions of very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL), lipoprotein deficient plasma (LPDF) or plasma was determined by liquid scintillation counting.
F. Liquid Scintillation Counting

Quantitation of radiolabeled compounds was performed by liquid scintillation counting. Briefly, samples to be counted were transferred to the appropriate sized scintillation vial and CytoScint scintillation fluid (ICN; Costa Mesa, CA) was added. Appropriate standards were also prepared in the same fashion. The samples and standards were then placed in a Packard Tri-Carb 4530 liquid scintillation counter to detect tritium for a period of 5 min per sample.

G. Statistical Analysis

Differences in the plasma distribution following incubation were calculated using two-way analysis of variances (PCANOVA; Human Systems Dynamics). Critical differences were assessed by Neuman-Keuls posthoc tests and considered significant if p was < 0.05. Correlation coefficients were calculated using Pearson’s Test and were considered significant if p was < 0.05.
Chapter 4

Results
Lipoprotein distribution of cyclosporine

To determine if the lipoprotein distribution of cyclosporine is affected by the lipoprotein separation technique used, \(^{3}\)H-CSA was incubated at a concentration of 1 \(\mu\)g/mL in human plasma for 60 minutes at 37°C. Following incubation the plasma was separated by density gradient ultracentrifugation, affinity chromatography coupled with ultracentrifugation and FPLC. The distribution of CSA in the HDL, LDL and VLDL fractions is represented in Figure 12. Over 95% of the CSA was found in these fractions, the remainder being in the lipoprotein deficient fraction (LPDF). As seen in Figure 12, the majority (over 80% of the cyclosporine) associated with the HDL and LDL fractions. Within the VLDL fractions, more cyclosporine was recovered when FPLC was utilized. Thus, there are small but significant differences in cyclosporine lipoprotein distribution depending on the separation technique used. However, for all three methods, the overall pattern of cyclosporine distribution is similar, with the majority found in HDL and LDL. Based on these findings, subsequent studies were performed using the density ultracentrifugation technique as it is best suited to handling large volumes (ie: mL vs \(\mu\)L of sample).

Lipoprotein distribution of cyclosporine in different plasmas

The lipoprotein and plasma total cholesterol and total triglyceride was determined for three different plasma samples (Table 4). Plasma II had a significantly greater total plasma and lipoprotein cholesterol and triglyceride concentration when compared to Plasma I except for HDL triglyceride levels. Similarly, Plasma III also had significantly higher total plasma
**Figure 12**: Distribution of $^3$H-cyclosporine in plasma as separated by affinity chromatography, density gradient ultracentrifugation and FPLC following incubation at 1 µg/mL for 60 min at 37°C. Data expressed as mean ± S.D. (*p < 0.05 vs Affinity Chromatography, n = 3)
and lipoprotein cholesterol and triglyceride concentrations than Plasma I and II except for HDL-cholesterol levels.

**Table 4: Cholesterol and triglyceride concentrations from three different plasma samples**

<table>
<thead>
<tr>
<th></th>
<th>VLDL (mM)</th>
<th>LDL (mM)</th>
<th>HDL (mM)</th>
<th>Total (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cholesterol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma I</td>
<td>0.48 ± 0.04</td>
<td>1.63 ± 0.00</td>
<td>0.81 ± 0.00</td>
<td>2.92 ± 0.04</td>
</tr>
<tr>
<td>Plasma II</td>
<td>1.34 ± 0.06*</td>
<td>2.21 ± 0.03*</td>
<td>1.09 ± 0.13*</td>
<td>4.64 ± 0.16*</td>
</tr>
<tr>
<td>Plasma III</td>
<td>1.91 ± 0.17* **</td>
<td>3.32 ± 0.09* **</td>
<td>0.84 ± 0.11</td>
<td>6.08 ± 0.06* **</td>
</tr>
<tr>
<td><strong>Triglyceride</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma I</td>
<td>0.41 ± 0.02</td>
<td>0.24 ± 0.00</td>
<td>0.25 ± 0.00</td>
<td>0.90 ± 0.02</td>
</tr>
<tr>
<td>Plasma II</td>
<td>1.29 ± 0.06*</td>
<td>0.41 ± 0.05*</td>
<td>0.19 ± 0.07</td>
<td>1.89 ± 0.05*</td>
</tr>
<tr>
<td>Plasma III</td>
<td>3.23 ± 0.11* **</td>
<td>0.79 ± 0.04* **</td>
<td>0.45 ± 0.13* **</td>
<td>4.47 ± 0.14* **</td>
</tr>
</tbody>
</table>

Note: Data is expressed as mean ± standard deviation (n=6)
* p < 0.05 vs Plasma I profile, ** p< 0.05 vs Plasma II profile

Furthermore, plasma lipoprotein composition based on total cholesterol (TC), total triglyceride (TG) and total protein (TP) ratios was calculated for the three plasma samples (Table 5). Plasma II had a significantly lower LDL TC:TP than Plasma I. Plasma III had a significantly higher VLDL TG:TP and TG:TC and HDL TG:TC when compared to Plasma I. However, Plasma III had a lower HDL and LDL TC:TP and TG:TC than patient I. In comparison to Patient II, Patient III had a significantly higher HDL TG:TC.

Following incubation of ^3^H-CSA at a concentration of 1 µg/mL for 60 min at 37°C, a significantly greater amount of cyclosporine was recovered within the LDL fraction of Plasma III versus Plasma I (Table 6). However, lower levels of cyclosporine were recovered within the HDL fraction of Plasma III following incubation when compared to Plasmas I and II (Table 6).
Table 5: Lipoprotein composition of three different plasmas

<table>
<thead>
<tr>
<th></th>
<th>Plasma I</th>
<th>Plasma II</th>
<th>Plasma III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Very Low Density Lipoprotein</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC:TP</td>
<td>2.4 ± 0.6</td>
<td>2.2 ± 0.5</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>TG:TP</td>
<td>4.0 ± 1.9</td>
<td>4.7 ± 1.2</td>
<td>6.1 ± 0.4*</td>
</tr>
<tr>
<td>TG:TC</td>
<td>1.6 ± 0.4</td>
<td>2.2 ± 0.2</td>
<td>3.5 ± 0.5*</td>
</tr>
<tr>
<td><strong>Low Density Lipoprotein</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC:TP</td>
<td>2.0 ± 0.4</td>
<td>1.0 ± 0.4*</td>
<td>0.9 ± 0.4*</td>
</tr>
<tr>
<td>TG:TP</td>
<td>0.7 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>TG:TC</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.7 ± 0.1*</td>
</tr>
<tr>
<td><strong>High Density Lipoprotein</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC:TP</td>
<td>0.21 ± 0.04</td>
<td>0.15 ± 0.04</td>
<td>0.09 ± 0.03*</td>
</tr>
<tr>
<td>TG:TP</td>
<td>0.16 ± 0.02</td>
<td>0.11 ± 0.06</td>
<td>0.16 ± 0.06</td>
</tr>
<tr>
<td>TG:TC</td>
<td>0.80 ± 0.10</td>
<td>0.80 ± 0.50</td>
<td>2.1 ± 0.50**</td>
</tr>
</tbody>
</table>

Note: Data is expressed as mean ± standard deviation (n = 6); all ratios are weight:weight comparisons
* p < 0.05 vs Plasma I, ** p < 0.05 vs Plasma II
TC = total cholesterol (esterified + unesterified); TG = total triglyceride; TP = total protein

Table 6: Distribution of $^3$H-cyclosporine in three different plasmas

<table>
<thead>
<tr>
<th></th>
<th>VLDL (%)a</th>
<th>LDL (%)</th>
<th>HDL (%)</th>
<th>LPDF (%)</th>
<th>Percent Recoveryb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma I</td>
<td>12.6 ± 6.0</td>
<td>26.8 ± 4.4</td>
<td>47.2 ± 5.4</td>
<td>7.6 ± 2.7</td>
<td>94.1 ± 6.5</td>
</tr>
<tr>
<td>Plasma II</td>
<td>16.6 ± 6.1</td>
<td>33.9 ± 4.9</td>
<td>40.6 ± 7.8</td>
<td>5.3 ± 1.1</td>
<td>96.5 ± 4.7</td>
</tr>
<tr>
<td>Plasma III</td>
<td>19.4 ± 6.6</td>
<td>41.1 ± 2.5*</td>
<td>28.2 ± 2.3* **</td>
<td>6.9 ± 1.4</td>
<td>95.5 ± 5.9</td>
</tr>
</tbody>
</table>

Note: Data expressed as mean ± standard deviation (n = 6)
* p < 0.05 vs Plasma I, ** p < 0.05 vs Plasma II
a: percent of initial $^3$H-CSA; b: percent of initial drug incubated

When correlations between the amount of cyclosporine recovered and the amount of cholesterol and triglyceride in each individual lipoprotein fraction were calculated for the three different plasmas, the following observations were made: as VLDL cholesterol and triglyceride concentration increase, the amount of cyclosporine recovered in this fraction also increases (Figure 13; r = 0.78 and 0.78 respectively, p < 0.05). Similarly, as LDL cholesterol and triglyceride increase, the amount of cyclosporine in this fraction also increases (Figure 13; r = 0.95 and 0.90 respectively, p < 0.05). However, as HDL triglyceride concentrations
Figure 13: The amount of $^3$H-cyclosporine recovered within the very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) fraction versus total cholesterol or total triglyceride after incubation at 1 µg/mL for 60 min at 37°C in different human plasmas. Data represents incubations within three different plasmas (n = 6 for each plasma); 'r' represents the Pearson correlation coefficient; * p < 0.05
increase, the amount of cyclosporine recovered in this fraction was shown to proportionally decrease (Figure 13; \( r = 0.86, p < 0.05 \)).

When correlations between the amount of cyclosporine recovered and the composition of the individual lipoprotein fractions were calculated, the following observations were made (see Table 7): as the TG:TC ratio increased in the VLDL fraction, the amount of cyclosporine increased \( (r = 0.82, p < 0.05) \), while in the HDL fraction the amount of cyclosporine recovered correlated to an increasing TC:TP ratio \( (r = 0.87, p < 0.05) \).

**Table 7:** Correlation coefficients between the amount of cyclosporine recovered in each lipoprotein fraction with plasma lipoprotein composition

<table>
<thead>
<tr>
<th></th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC/TP</td>
<td>-0.43</td>
<td>-0.55</td>
<td>0.87*</td>
</tr>
<tr>
<td>TG/TP</td>
<td>0.57</td>
<td>-0.34</td>
<td>-0.30</td>
</tr>
<tr>
<td>TG/TC</td>
<td>0.82*</td>
<td>0.56</td>
<td>-0.81</td>
</tr>
</tbody>
</table>

Data represents incubations within three different plasmas \( n = 6 \) for each plasma. * \( p < 0.05 \)

**Influence of CETP on the lipoprotein distribution of cyclosporine**

To determine the influence of CETP on the lipoprotein distribution of cyclosporine, cyclosporine was incubated in human plasma at a concentration of 1 \( \mu \text{g/mL} \) for 60 minutes at 37°C which had been supplemented with exogenous CETP at concentrations of 0.5, 1 and 2 \( \mu \text{g} \text{ protein/mL} \) plasma. The plasma was separated by affinity chromatography and the cyclosporine quantitated by scintillation counting. As the CETP concentration increased, a greater percentage of cyclosporine was recovered in the HDL/LPDF with parallel decrease in the LDL/VLDL fraction (Figure 14).
Figure 14: Percent recovery of cyclosporine (CSA) in the high-density lipoprotein (HDL)/lipoprotein deficient fraction (LPDF) and very low density (VLDL)/low density lipoprotein (LDL) fractions within human plasma supplemented with exogenous CETP. 3H-cyclosporine was incubated at 1000 ng/mL for 60 min at 37°C. After incubation, plasma was separated into its HDL/LPDF and VLDL/LDL fractions by affinity chromatography and the percentage of CSA recovered in each fraction was determined by scintillation counting. Data was expressed as mean ± S.D. (n = 6); * p < 0.05 vs HDL/LPDF
The effect of TP2 on CETP transfer of cholesteryl ester

To determine the amount of TP2, a monoclonal antibody to CETP activity, required to significantly inhibit the transfer of cholesteryl ester from HDL to LDL by CETP, we studied the effect of increasing amounts of TP2 on this transfer (Table 8). We decided that 4.0 μg/mL of TP2 would be sufficient to provide significant inhibition of CETP activity and this was the concentration used in our other studies.

Table 8: The effect of TP2 on the transfer of cholesteryl ester (CE) by CETP

<table>
<thead>
<tr>
<th>Amount of TP2 added (μg/mL)</th>
<th>% Transfer of CE</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>23.5</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>20.2</td>
<td>14.0</td>
</tr>
<tr>
<td>1.0</td>
<td>17.5</td>
<td>25.5</td>
</tr>
<tr>
<td>2.0</td>
<td>15.3</td>
<td>34.9</td>
</tr>
<tr>
<td>4.0</td>
<td>9.6</td>
<td>59.1</td>
</tr>
</tbody>
</table>

Cholesteryl ester and cyclosporine transfer between HDL and LDL

To assess the ability of CETP to promote the transfer of cholesteryl ester (CE) and cyclosporine from HDL to LDL, radiolabeled CE or cyclosporine enriched HDL particles were incubated in T150 buffer which contained CETP (1 μg/mL) or in lipoprotein deficient plasma which had endogenous CETP (1 μg/mL). The percent transfer of CE from HDL to LDL was significantly greater than the percent transfer of cyclosporine in both buffer and plasma (Figure 15), with higher levels of transfer of both CE and cyclosporine observed in plasma (Figure 15). In order to determine if CETP produced the observed transfer, a monoclonal antibody to CETP, TP2 was utilized. When the experiment was repeated in the
Figure 15: Cholesteryl ester (CE) and cyclosporine (CSA) percent transfer from HDL to LDL in the presence or absence of a monoclonal antibody (TP2) directed against CETP after the incubation of radiolabeled CE- or CSA-enriched HDL with cold LDL in T150 buffer that was supplemented with CETP (1.0 μg protein/mL) or in delipidated plasma containing 1.0 μg protein/mL of CETP. (n = 3)
presence of TP2 (4 mg protein/mL), CE transfer significantly decreased. However, the transfer of CSA was not significantly different when TP2 was present (Figure 15).

The transfer of cholesteryl ester and cyclosporine from LDL to HDL was also performed, following the same procedure as above, except that LDL particles were enriched with either radiolabeled CE or cyclosporine. The percent transfer of CE from LDL to HDL was higher in the T150 buffer but in plasma the transfer of cyclosporine was higher (Figure 16). As with the transfer from HDL to LDL, the transfer of CE and CSA from LDL to HDL was significantly higher in plasma than in the T150 buffer system. When the experiment was repeated in the presence of TP2 monoclonal antibody, the transfer of CE and cyclosporine from LDL to HDL were both significantly decreased (Figure 16).
Figure 16: Cholesteryl ester (CE) and cyclosporine (CSA) percent transfer from LDL to HDL in the presence or absence of a monoclonal antibody (TP2) directed against CETP after the incubation of radiolabeled CE- or CSA-enriched LDL with cold HDL in T150 buffer that was supplemented with CETP (1.0 μg protein/mL) or in delipidated plasma containing 1.0 μg protein/mL of CETP (n = 3)
Chapter 5

Discussion
**Lipoprotein Distribution of Cyclosporine**

In our study, we chose to assess the lipoprotein distribution of cyclosporine by ultracentrifugation, gel filtration and affinity chromatography. The purpose of this study was two-fold: we wished to determine the lipoprotein distribution of cyclosporine and we wanted to demonstrate that the lipoprotein distribution of cyclosporine was independent of the lipoprotein separation technique used.

The classical method of lipoprotein separation is by ultracentrifugation which separates lipoproteins based on their buoyant density. Four major categories can be isolated: chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL). Chylomicrons and VLDL are often referred to as triglyceride rich lipoproteins (TRL). Ultracentrifugation was intended to be used for the separation of lipoproteins [De Lalla 1954, Havel 1955] and was not designed to study drug-lipoprotein interactions. The high forces (>250000 × g) created by ultracentrifugation may remove drug associated with the surface of lipoproteins and redistribute the drug amongst the lipoprotein fractions. Thus, the lipoprotein distribution of drugs, including cyclosporine, may be misrepresented when determined by ultracentrifugation.

In order to confirm that the lipoprotein distribution of cyclosporine as determined by ultracentrifugation was not an artifact of the technique, fast protein liquid chromatography (FPLC) was used. FPLC is an established technique for separating lipoproteins [Marz 1989] which utilizes a gel filtration column which separates lipoproteins based on size, and does not subject the lipoproteins to extreme physical forces. In FPLC separation of lipoproteins, VLDL elutes first followed by LDL and HDL, with the other plasma proteins eluting last. As seen in Figure 12, the lipoprotein distribution of cyclosporine as determined by FPLC is
virtually identical to that of ultracentrifugation. This confirms that ultracentrifugation is a valid technique for separating lipoprotein associated cyclosporine.

A third technique of lipoprotein separation, affinity chromatography, was also used to determine the lipoprotein distribution of cyclosporine. This technique utilized a heparin-agarose matrix, which separates lipoproteins based on apoprotein content. As with FPLC, affinity chromatography does not subject the lipoprotein-drug complex to extreme physical forces. The distribution obtained by this technique was similar to both ultracentrifugation and FPLC, with the majority of the drug located in the HDL and LDL fractions (Figure 12).

By the use of three different lipoprotein separation techniques, we have demonstrated that the cyclosporine predominantly associates with HDL and LDL. We have also shown that the lipoprotein distribution of cyclosporine is independent of the technique used to separate the lipoproteins.

**Lipoprotein composition and cyclosporine distribution**

The purpose of these studies was to determine if alterations in lipoprotein lipid composition modify the lipoprotein distribution of cyclosporine. Changes in the lipid composition of plasma lipoproteins are often seen in immunocompromised patients and transplant patients [Gardier 1993, Arnadottir 1991]. We observed that increases in LDL lipid concentration resulted in an increased amount of cyclosporine recovered in this fraction with a subsequent decrease of cyclosporine recovered in the HDL fraction. No significant alteration in drug recovered was seen in the VLDL or LPDP fractions (See Tables 4 and 6). This corresponds to the observations of Gardier et al, in which patients with higher plasma
lipid concentrations had higher binding of cyclosporine to the VLDL, IDL, and LDL fractions with lower binding in the HDL fraction [Gardier 1993].

Changes in lipid profiles have been shown to affect the serum concentration, efficacy and toxicity of cyclosporine. Patients with hypertriglyceridemia have been observed to have sustained concentrations of 1000 ng/mL without showing signs of severe nephrotoxicity [Nemunaitis 1986]. This is significantly greater than the desired blood level of cyclosporine of 100-400 ng/mL [Novartis Pharmaceuticals 1996]. Porres et al observed similar findings in renal transplant patients and showed that reducing the cyclosporine dose reduced cyclosporine associated nephrotoxicity while maintaining immunosuppressant activity. [Porres 1996]. Thus, in hypertriglyceridemic patients it appears that cyclosporine efficacy is increased with little or no change on nephrotoxicity.

While hypertriglyceridemia appears to improve cyclosporine efficacy, in hypercholesterolemic patients cyclosporine efficacy appears to be diminished [Ingulli 1992]. Ingulli et al observed that patients with a serum cholesterol >500 mg/dL were unresponsive to a standard cyclosporine dosages of 6 mg/kg, showing signs of allograft rejection [Ingulli 1992]. These patients were shown to have subtherapeutic levels of cyclosporine and had their cyclosporine dosage titrated to 10-14 mg/kg in order to achieve therapeutic levels. Despite this increase in dose, no increase in nephrotoxicity was observed. Based on these observations, it was recommended that in hypercholesterolemic patients it may be necessary to use higher than normal doses of cyclosporine.

Although hypercholesterolemia seems to attenuate the effects of cyclosporine, the opposite is seen in hypocholesterolemic patients. Liver transplant patients with hypocholesterolemia are more likely to show signs of cyclosporine toxicity and allograft
rejection [de Groen 1987, 1988b]. These patients proved difficult to treat as decreasing the dosage of cyclosporine resulted in allograft rejection. In order to minimize toxicity yet maintain immunosuppression these patients were switched to a regimen that included low dose cyclosporine, prednisolone and azathioprine.

It is evident from these studies that the lipid level of an individual can influence the pharmacology and pharmacokinetics of cyclosporine. As lipoproteins are the major carriers of lipid in plasma, changes in total lipid represent changes in lipoprotein lipid concentration. We have shown that as the triglyceride:total cholesterol ratio (TG:TC) within VLDL increased, the amount of cyclosporine recovered in that fraction increased [Wasan 1998]. The opposite was seen in HDL, with a decrease in cyclosporine recovered as the TG:TC ratio increased. However, as total cholesterol:total protein in cholesterol increased, a parallel increase in cyclosporine was seen (Table 5). These findings suggest that not only total lipid mass (cholesterol, cholesteryl ester, and triglyceride) influence the lipoprotein distribution of cyclosporine, but that the actual composition of the different lipoprotein fractions also plays a role. The clinical significance of such findings has been reported in the above studies, with increased efficacy and decreased toxicity associated with hypertriglyceridemia. While in hypocholesterolemic patients there seems to be a decrease in efficacy and increase in toxicity. However, these reports only indicate the role of total lipid levels while the importance of the individual lipoproteins is not mentioned.

Other factors that might affect the lipoprotein distribution of cyclosporine other than total lipid have only been speculated upon. In in vitro experiments, Hughes et al demonstrated that the transfer of cyclosporine between lipoproteins produces a different distribution than seen in plasma samples taken from patients [Hughes 1991]. Hughes postulated that such a
distribution seen in plasma is due to plasma factors that affect the transfer of cyclosporine but did not indicate any specific factor. One such plasma factor that has been shown to influence the transfer of drugs amongst lipoproteins is cholesteryl ester transfer protein (CETP) [Wasan 1996].

**CETP involvement in the lipoprotein distribution of drugs**

CETP is involved in the exchange of cholesterol for triglyceride from HDL to the apo-B containing lipoproteins LDL and VLDL. CETP has been shown to vary between individuals and it has also been shown that CETP activity can change in the diseased state [Asayama 1996, Hirano 1993, Tato 1997]. It has become apparent that CETP may be involved in determining the lipoprotein distribution of drugs such as amphotericin B [Wasan 1998, 1995, 1994]. It was concluded from these studies that CETP transfers amphotericin B due to the association of amphotericin B with cholesterol and cholesteryl ester rather than a direct transfer of amphotericin B between HDL and LDL.

Our studies have shown that CETP is partly involved in the transfer of cyclosporine from LDL to HDL. The mechanism of transfer is unknown but it appears to be partially independent of CETP because transfer rates were increased when the experiment was performed in plasma rather than the T150 buffer system (see Figure 16), indicating some other plasma factor was involved. These findings suggest that there are other factors involved in the transfer of cyclosporine and may include other endogenous plasma factors such as triglyceride or phospholipid transfer protein.

The transfer of cyclosporine from HDL to LDL does not appear to be CETP facilitated. When TP2 was added in amounts sufficient to significantly reduce the transfer of cholesteryl
ester from HDL to LDL, there was no significant change in the transfer of CSA (see Figure 15). This indicates that the transfer of cyclosporine from HDL to LDL is facilitated by other plasma factors.

Our model of the role of CETP in the transfer of cyclosporine between HDL and LDL is represented in Figure 17. Cyclosporine transfer from HDL to LDL appears to be independent of CETP activity. However, when the transfer of cyclosporine from LDL to HDL was measured, CETP activity played a partial role in transferring cyclosporine. The mechanism by which CETP affects cyclosporine lipoprotein distribution is unknown. Cyclosporine may interact with either cholesteryl ester or triglyceride and it is this lipid-cyclosporine complex which is transferred by CETP. Another possibility is cyclosporine may interact directly with CETP and is transferred independent of lipid. Further experimentation will be needed to determine the specific interaction of cyclosporine and CETP in order to fully understand the role of CETP in influencing the lipoprotein distribution of cyclosporine. Such information would further demonstrate the role of CETP in the interaction of drugs with lipoproteins and may provide an explanation for the differences in the lipoprotein distribution of a drug between different patients.

**Lipoproteins and cyclosporine pharmacology**

It has been shown that there is an association of cyclosporine with lipoproteins and that there are at least two factors which determine the lipoprotein distribution of cyclosporine: lipoprotein composition and the action of cholesteryl ester transfer protein (CETP). Changes in lipoprotein composition, reflected as changes in total lipid concentration have been shown to affect both the efficacy and toxicity of cyclosporine as reviewed above. These differences
**Figure 17:** Model of the influence of CETP on cholesteryl ester (CE) and cyclosporine (CSA) transfer between HDL and LDL.
in the pharmacology of cyclosporine may be due to the changes in the lipoprotein distribution of cyclosporine. However, the role of lipoproteins in cyclosporine uptake and how this may affect cyclosporine toxicity is poorly defined. Research into this aspect of lipoproteins and cyclosporine has been limited and has provided no clear mechanism on how cyclosporine crosses the cellular membrane or produces its toxic effects.

It has been hypothesized that the LDL-receptor is involved in the uptake and transport of cyclosporine across the cell membrane [de Groen 1988a]. The hypothesis was based on the observation that the tissue distribution of cyclosporine is similar to that of the LDL receptor (ie: liver, kidney and adipose) [Ried 1983, Kovanen 1979]. Sanghvi et al demonstrated that in peripheral blood lymphocytes that the uptake of LDL-associated cyclosporine increased when the cells were incubated in lipid free serum [Sanghvi 1989]. This provided support for the LDL-receptor uptake model but no indication of changes in toxicity were made in this study.

Rifai et al studied the uptake of cyclosporine in the HepG2 and JURKAT hepatic cell lines and found that free cyclosporine uptake decreased in the presence of LDL [Rifai 1996]. In the absence of serum and LDL, a rapid uptake of cyclosporine occurred in the first hour of incubation and approached a plateau at 4 hours. However, when increasing amounts of LDL were added, the amount of cyclosporine uptake decreased. It was concluded that LDL does not play a significant role in the uptake of cyclosporine in these two cell lines. The data suggested that the uptake of free cyclosporine may be LDL-receptor mediated but more specific experiments would need to be done to study this possibility.

In a study by Strong and Ueda it was shown in a perfused kidney model that indicators of cyclosporine toxicity were reduced in the presence of HDL or LDL [Strong 1997]. This was
attributed to the reduction of free drug when lipoproteins were present. Strong and Ueda argued that only unbound drug was able to interact with the kidney and concluded that lipoproteins can affect cyclosporine drug disposition and toxicity. This conflicts with the study by Sanghvi which indicated that LDL-associated cyclosporine can be taken up by cells.

It is evident from the literature that the role of lipoproteins in cyclosporine uptake, transport and toxicity is not fully understood. LDL-associated cyclosporine appears to be able to enter cells via the LDL-receptor pathway, but this mechanism has yet to be confirmed. Strong and Ueda contend that only the free fraction of cyclosporine is available to interact with tissue, but suggested no mechanism on how this may occur. Further experimentation is clearly needed to fully understand how cyclosporine enters the cell and causes its toxic effects. The role of lipoproteins in this process should also be further researched to determine if changes in the lipoprotein distribution of cyclosporine alters cyclosporine uptake and toxicity.

In conclusion, we have confirmed that cyclosporine predominantly associates with lipoproteins in the plasma fraction. We have also shown that changes in lipoprotein lipid concentration and composition alter the lipoprotein distribution of cyclosporine. We have also observed that CETP is another factor that affects the association of cyclosporine with lipoproteins. These results may provide an explanation for the changes seen in the efficacy and toxicity of cyclosporine in patients with different lipoprotein profiles and levels of CETP activity.
**Future Studies**

The role of lipoproteins in cyclosporine pharmacology is poorly understood and has not been well studied with respect to its nephrotoxicity, the main reason for cessation or modification of cyclosporine therapy. Cyclosporine accumulates in the renal proximal tubular segment which has been reported to be the site of damage caused by cyclosporine [Mihatsch 1985, Myers 1984]. Previous studies have used the renal proximal tubule cell line LLC-PK1 as a model to study cyclosporine uptake and toxicity [Walker 1989, Becker 1987]. However, these studies looked at the effect of cyclosporine alone and did not incorporate lipoproteins into their experiments. With the majority of cyclosporine in plasma associating with lipoproteins, there is a strong possibility that lipoproteins play a role in the uptake and toxicity of cyclosporine. Furthermore, as kidney cells express the LDL receptor, there is a possibility that this pathway may be involved in cyclosporine uptake. Thus, experiments to determine the role of lipoproteins and the LDL receptor in cyclosporine uptake into the LLC-PK1 cell line may provide insights into how changes in the lipoprotein distribution of cyclosporine may affect cyclosporine nephrotoxicity.

We demonstrated in our work that CETP is one factor that influences the lipoprotein distribution of cyclosporine. Our data also suggested that there are other plasma factors involved in the transfer of cyclosporine between lipoproteins. Further experimentation could be done to determine the role of other lipid transfer proteins, such as phospholipid transfer protein and triglyceride transfer protein, in the movement of cyclosporine between lipoproteins. These two proteins are likely candidates that may influence the movement of cyclosporine but there may be other, yet identified, plasma factors that affect the lipoprotein distribution of cyclosporine.
Conclusions

We have determined that within plasma, cyclosporine associates predominantly with lipoproteins and that lipoprotein lipid concentration and composition can affect the lipoprotein distribution of cyclosporine. In our study, we have observed that increases in VLDL and LDL lipid results in an increase in the amount of cyclosporine recovered in these fractions while in HDL, an increase in cholesterol content results in a decrease in the amount of cyclosporine recovered in this fraction. These findings indicate that cyclosporine lipoprotein distribution may be regulated by cholesterol and to a lesser extent triglyceride concentrations. Thus, redistribution of the drug may occur in transplantation patients who demonstrate changes in lipid profile [Lopez-Miranda 1992, Kuster 1994] or are receiving other therapies that affect lipid levels such as Intralipid® infusion [Wasan 1994b]. We also showed that changes in lipid levels does not effect the amount of cyclosporine recovered in the lipoprotein deficient fraction (Tables 4 & 6) confirming that cyclosporine binding to albumin and a-1-glycoprotein does not play a significant role in regulating the lipoprotein distribution of cyclosporine [Lemaire 1982].

Our studies also showed that CETP plays a role in determining the lipoprotein distribution of cyclosporine. As CETP activity increased, a greater amount of cyclosporine was recovered in the HDL fraction. Specifically, we showed that the transfer of cyclosporine from HDL to LDL is apparently independent of CETP activity while the transfer from LDL to HDL seems to be partially dependent on CETP activity. As CETP activity is increased in patients with hyperlipidemia [Tall 1995], changes in the lipoprotein distribution of cyclosporine could be due both to changes in lipoprotein lipid concentration and CETP activity.
It has been demonstrated that patients undergoing cyclosporine therapy may undergo changes in lipid concentration. Such changes are reflected as changes in lipoprotein lipid concentration and composition which affects the lipoprotein distribution of cyclosporine. Such changes could result in changes in both cyclosporine efficacy and toxicity. Thus monitoring of the lipid concentration of patients who shall be or are undergoing cyclosporine therapy may be a useful tool in determining the dose necessary to achieve adequate immunosuppression while minimizing nephrotoxicity. The exact mechanism by which lipoproteins affect cyclosporine activity is unknown and requires further investigation.
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