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Research Paper

Feasibility of Weekly HIV Drug Delivery to Enhance Drug Localization in Lymphoid Tissues Based on Pharmacokinetic Models of Lipid-Associated Indinavir

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Purpose. Compare the simulated pharmacokinetics of lipid-associated and soluble indinavir (IDV) to determine the potential for greater control of virus replication in the lymphoid tissues.
Methods. Two-compartment mathematical models were developed to simulate the human pharmacokinetics of soluble and lipid-associated forms of IDV in the central compartment and the lymphoid tissue. The lipid-associated IDV model was then used to determine the minimum dosing schedule needed to attain central or lymph drug concentrations comparable to the soluble form.
Results. Association of IDV to lipid nanoparticles has a favorable half-life and tissue distribution and allows comparable minimum drug concentration in the lymph (where the majority of viral replication occurs) to be achieved with a dosing schedule of every 95.5 h (~4 days).
Conclusions. Presuming pharmacodynamics of lipid-associated IDV are similar to soluble IDV, estimations based on the proposed kinetic model suggest the novel delivery system could have a tremendous impact on the current standard of HIV treatment, particularly for therapy targeted to clear virus sanctuaries in lymphoid tissues. With less frequent and more effective dosing, lipid-associated indinavir delivery as an adjunct to conventional antiviral therapy could lead to better suppression of viral replication, increased immunological benefit, and fewer treatment failures.

KEY WORDS: antiviral therapy; HIV; indinavir; liposomes; pharmacokinetics.

INTRODUCTION

An important objective of anti-HIV treatment is to reduce virus levels below the level of clinical detection in the peripheral blood. However, a considerable amount of viral replication occurs in the lymphatic tissues (1,2), with the amount of free virus (3) and infected cells (4) in the lymph nodes being orders of magnitude greater than the quantities in the periphery. Furthermore, with current treatment methods the lymphoid tissue (LT) may be exposed to lower antiviral drug concentrations than the plasma (5). These data and the observation that many patients continue to have viral breakthrough despite even the most potent therapy (6) strongly suggest that residual replication must still occur in the LT or other sanctuary sites of infection even when plasma viral levels are undetectable.

It is likely that new drugs or drug delivery mechanisms with greater access to the LT have the potential for superior suppression of virus replication, which could lead to fewer therapeutic failures and fewer instances of drug resistance compared to the current standard. Recent development of a novel, pH responsive lipid-indinavir formulation (of about 50 nm in diameter) that, when administered subcutaneously, greatly increased the available drug concentration in the lymph nodes compared to the soluble form as well as sustained a significant amount of drug in the plasma beyond 24 h (>20 *versus* <2 μg/l) when administered to HIV-2-infected macaques (7). Treatment of HIV-2-infected macaques with lipid-associated indinavir was also encouraging. Animals treated with the lipid-associated, but not the soluble form, showed increases in CD4 T cells and undetectable plasma virus after two weeks of daily treatment. Furthermore, upon examination of the animals' lymph nodes, only those treated with soluble IDV showed appreciable localizations of HIV-2 RNA in lymph node germinal centers. The objective of the current study is to compare the simulated pharmacokinetics of lipid-associated and soluble IDV to determine the potential for achieving greater control of virus replication in the lymphoid tissue with infrequent dosing of the lipid-drug complexes due to their superior LT distribution and elimination properties.

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MATERIALS AND METHODS

We developed two mathematical models to predict the human pharmacokinetics of orally administered indinavir and the subcutaneous (SC) administration of lipid-associated indinavir (of size 50–80 nm in diameter), which has been studied previously in macaques (7). Two-compartment models were developed using the commercial software VisSim32, version 3.0 (Visual Solutions, Inc.), and simulated with routines created in C programming language incorporating both the central compartment and LT.

Here, the central compartment is considered to be a well-mixed pool of plasma and other non-lymphoid tissues. Soluble IDV is considered to be absorbed into the central pool, from where drug elimination also occurs. When SC administered, lipid-associated IDV is absorbed into the lymph and eliminated from both the lymph and central compartments (Fig. 1) (8,9). Some studies demonstrate distribution of SC-administered liposomes to be confined only to the regional lymph nodes involved in drainage of the injection site (9). However, macaque data showed IDV distribution in several lymphatic structures after SC administration of the lipid-drug complex (7), indicating the assumption that the lymph compartment represents a well-mixed pool of lymph nodes and other lymphoid tissue is reasonable.

All model parameters were derived from either human clinical results or experiments using animal models, or chosen in conjunction with established parameters to approximate the known concentrations and dynamics of IDV in the central and lymph compartments. The essential model characteristics are a) the maximum plasma concentration (C_{max}) and time to C_{max} of the soluble model (10); b) the plasma concentration of the lipid-associated model at 24 h is three-fold lower than the peak concentration (7); and c) the peak plasma concentration of the lipid-associated model is ten-fold lower than the peak plasma concentration of the soluble model (7). Because complete human data of the pharmacokinetics of lipid-associated indinavir are unavailable, it was necessary to supplement with data from other animals. For the unknown parameters estimated within the current model, we performed sensitivity analyses of both models to determine relative influence of these parameter choices.

The volume of distribution of the central compartment for soluble IDV was estimated to be 0.65 l/kg, which is an average value of the reported data (10,11). Volumes of distribution for the lymphoid compartment and the lipid-associated IDV are currently unknown, so the approximate physiological volumes were used to determine drug concentration. That is, we used a plasma volume of 5 L and a lymphoid mass of 700 g (1% of total body weight for a 70 kg individual (12)).

Two drug elimination terms are incorporated into the models. The first, K_e, represents elimination from the central compartment and was obtained for soluble IDV from prescription information provided by the manufacturer (13). This term in the lipid-associated model was determined by the plasma decay kinetics of lipid-associated IDV in macaques (7). The second term, K_{et}, represents drug elimination from the LT from processes such as intracellular uptake of the complexes. Assuming elimination of IDV occurs exclusively through blood and low or no drug elimination directly from the LT, this term will be zero for the soluble model. K_{et} was estimated for the lipid-associated model so that the plasma concentration at 24 h after administration is three-fold lower than the peak plasma concentration, consistent with previous observations (7).

Drug is absorbed into both models at the rate K_a. This parameter was estimated to approximate the time to maximum central compartment concentrations given by the manufacturer (13) and Kinman *et al.* (7) for the soluble and lipid-associated models, respectively. Transport to and from the lymph is represented by the variables K_1 and K_2. For both models, these parameters were estimated in conjunction with one another so that a) the plasma concentration of the soluble model is consistent with observed kinetics in both human and macaque; b) the plasma concentration of the lipid-associated model at 24 h is three-fold lower than the peak concentration; and c) the peak plasma concentration of the lipid-associated model was ten-fold lower than the peak plasma concentration of the soluble model (7).

The drug dose was 800 mg for both soluble and lipid-associated IDV models. The bioavailability of orally administered IDV has previously been determined to be 65% (14). We used an estimate of 60% of the injected dose of lipid-associated IDV taken up from the injection site; the amount determined by SC-administration of similarly sized liposomes injected into rats (9).

RESULTS

Using parameter values listed in Table I, the soluble model (Fig. 2) is consistent with previously observed plasma (13) and LT (15) dynamics (reference (7), Fig. 3). The lipid-associated kinetics are also consistent with the observation that the maximum lipid-associated IDV plasma concentration

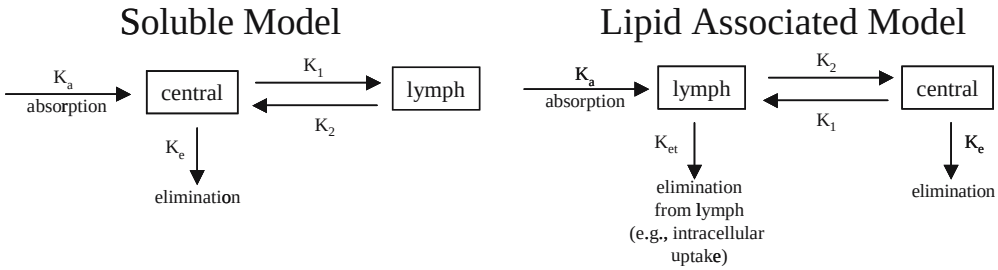

Fig. 1. Schematic diagram of pharmacokinetic models for soluble IDV and lipid-associated IDV. The soluble form first enters *via* the central compartment, whereas the lipid-drug complexes enter *via* the LT. No lymphatic elimination of soluble IDV is modeled.

Table I. Parameter Descriptions and Values Used in Simulations

Parameter	Description	Value		Reference
		Soluble Model	Lipid-Associated Model	
K_a	Absorption rate into the central compartment	4.0 h^{-1}	0.7 h^{-1}	Kinman *et al.* (7); Merck & Co. (13)
K_e	Elimination from the central compartment	0.385 h^{-1}	0.0081 h^{-1}	Kinman *et al.* (7); Merck & Co. (13)
K_{et}	Elimination from the LT compartment	0 h^{-1}	0.06 h^{-1}	None
K_1	Drug transport to/from the lymph and central compartments	0.1 h^{-1}	2.0 h^{-1}	None
K_2		2.0 h^{-1}	0.025 h^{-1}	None
V_d-central	Volume of distribution for soluble IDV	0.65 l/kg	–	Boyd *et al.* (10); Clotet *et al.* (11)
Bioavailability	Percent of drug that enters the central compartment	65%	60%	Oussoren *et al.* (9); Khoo (14)

Parameter values are adapted from indicated references. Unreferenced parameters are chosen in conjunction with parameters to produce pharmacokinetics similar to those previously observed.

is approximately 10% of that for soluble IDV. Differences between soluble and lipid associated IDV delivery include a nearly 120-fold increase in drug exposure to the lymph compartment, at the expense of a third less exposure in the central compartment. Steady-state pharmacokinetic parameters for 8-hour soluble dosing and 24-hour lipid-drug complex dosing are listed in Table II.

Subcutaneous injection of soluble indinavir was shown to have kinetics similar to orally administered drug with a single-dose time to maximum plasma concentration of 0.6 h and elimination of detectable drug levels by 6 h post-injection (7,13). These data indicate enhanced drug exposure to the lymph compartment is a result of the lipid-drug complexes and not solely the route of administration.

To determine the influence of parameters not determined experimentally on model outcomes, we varied the soluble model estimates for K_a and K_1, and the estimates of K_a, K_{et}, K_2, and bioavailability of the lipid-associated model by +25 or −25% of their original values. The soluble model was most sensitive to the drug transport parameter K_1 with steady-state minimum and maximum lymph concentrations ranging from 2.0 to 2.4 and 19.0 to 31.3 μg/g, respectively. Concentration changes of the central compartment were negligible. The lipid-associated model was most sensitive to the drug transport parameter K_2, the elimination parameter K_{et}, and the lipid-drug complex bioavailability term. Changes in K_2 result in a range of central compartment minimum and maximum concentrations of 0.3–0.5 and 0.9–1.6 μg/ml, respectively, with very little change in lymph concentrations. As expected, variations in bioavailability result in concentration changes in both the central and lymph compartments of −25 to +25%. The lipid-drug complex elimination term, K_{et}, also has a considerable effect on both the central and lymph compartments with minimum and maximum concentration changes ranging from −33 to +62 and −12 to +21%, respectively. Varying the parameter K_a showed little effect on concentrations of either model. These data indicate that bioavailability, elimination, and transport parameters are of greatest importance to experimentally identify in order to determine more exact pharmacokinetics of lipid-associated indinavir.

Despite the large difference in soluble antiviral concentrations between the plasma and the lymph, many patients have been able to achieve long-term central compartment (plasma) viral suppression with soluble IDV-containing therapy. Therefore, the drug concentration in the central compartment achieved with oral administration is sufficient to suppress the majority of detectable viral replication activity. However, this suppression is incomplete as evidenced by low,

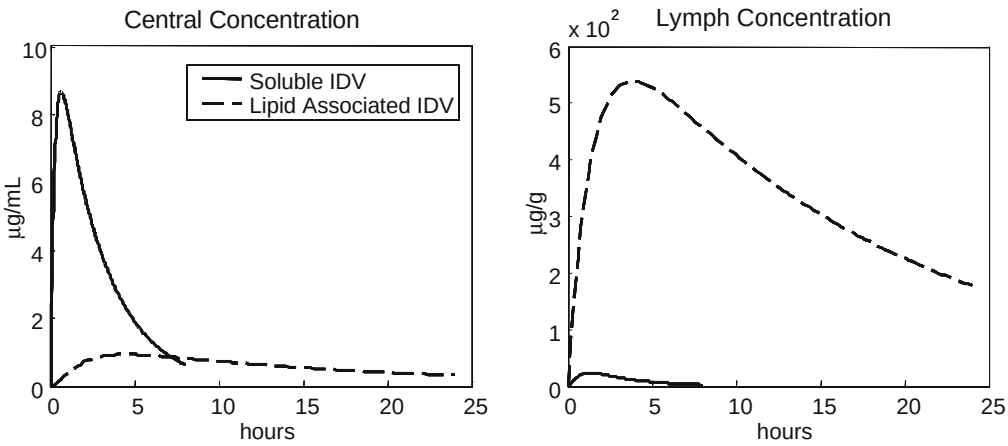

Fig. 2. Central compartment and lymph concentrations for single soluble and lipid-associated doses of 800 mg of IDV.

Table II. Comparison of Steady-State Pharmacokinetic Parameters for Soluble and Lipid-Associated Indinavir

	Soluble Model (8 h oral dosing)		Lipid-Associated Model (24 h SC dosing)	
Parameter	Central	LT	Central	LT
t_{max}	0.6 h	1.3 h	4 h	3.4 h
C_{max}	9.2 μg/ml	25.1 μg/g	1.3 μg/ml	728.0 μg/g
C_{min}	0.7 μg/ml	2.7 μg/g	0.4 μg/ml	234.4 μg/g
Avg. Conc.	3.7 μg/ml	12.1 μg/g	0.8 μg/ml	475.4 μg/g
AUC	29.6 μg h/ml	96.5 μg h/g	19.9 μg h/ml	11,409.0 μg h/g

800 mg dose for both models.

but detectable levels of gag protein production by *in situ* hybridization of lymph nodes (7) indicating a higher drug concentration in the lymph compartment is needed to eliminate this activity. The elevated IDV concentration achieved locally with lipid-IDV administration is likely to be within the therapeutic range required to eliminate residual virus replication. Using the drug concentrations of central and lymph compartments of the soluble model as targets, we determined the minimum lipid-associated IDV dosing schedule needed to attain these concentrations.

To reach an average central compartment drug concentration of 3.7 μg/ml with lipid-associated IDV, the models show lipid-drug complex dosing would need to occur more frequently than every 8 h. If the average soluble lymph concentration is to be achieved, the dosing schedule need only be once every 945.5 h (~39 days). However, with this schedule, the drug concentration from hours 60 to 945.5 is less than 1/10th the minimum lymph concentration for soluble IDV (2.7 μg/g) even though the *average* for the entire period is approximately 12.1 μg/g. This long period of very low concentration is due to both the lipid-associated drug in the lymph compartment sustained at much higher concentrations compared to the soluble form immediately after administration and the complexes' more favorable decay kinetics.

It is reasonable to expect this low level of drug would be ineffective for the final 885 h of the treatment period. To avoid time periods with potentially inadequate concentrations in the lymph that have greater potential to select for drug resistant strains, the optimal dosing schedule was determined so that the minimum lymph concentration for the lipid-associated model is no smaller than the minimum lymph concentration for the soluble model. With this target, lipid-associated IDV need only be administered every 95.5 h (~4 days) to attain an average lymph concentration of 119.5 μg/g, ten-fold greater than the concentration seen with the soluble model.

Table III. Sensitivity Analysis of 2,000 Dosing Schedule Outcomes Generated by Randomly Assigning Values to Parameters K_2, K_{et}, K_e, and Bioavailability of the Lipid-Associated Model

	Mean (SD)	Minimum	Maximum
Time between doses (hours)	96.8 (14.5)	73.5	130
Average LT concentration (μg/g)	119.4 (14.3)	93.9	144.5

Outcomes generated so the LT drug concentration of the lipid-associated model is no smaller than the minimum LT concentration of the soluble model.

The dependence of these conclusions on the most influential parameter values of the lipid-associated model was assessed by randomly assigning values from uniform distributions to the parameters K_2, K_{et}, and bioavailability from a range of −25 to +25% of the initial estimate. We also examined the influence of the parameter K_e, which was randomly assigned values from a normal distribution with a mean of 0.0081 and standard deviation of 0.0015, as determined previously (7). Two thousand simulations with these randomly chosen parameter values were performed to find the minimum doing schedule of the lipid-drug complex needed to attain a minimum lymph concentration similar to that of the soluble model. This sensitivity analysis demonstrates minimum lipid-associated dosing schedules range from 3 to 5.4 days with variations in these parameters (Table III). Specifying most conservative and least conservative parameter combinations into the lipid-associated model result in average lymph concentrations of 93.9 and 144.5 μg/g, respectively, eight-fold and 12-fold greater than with the soluble model. Minimum dosing schedules with these parameter combinations are 73.5 and 130 h, respectively.

DISCUSSION

The pharmacokinetic models presented here describe the kinetics of orally administered soluble IDV and subcutaneously administered lipid-associated IDV in the central and lymph compartments. The models describe the potential for infrequent dosing of the lipid-drug complexes to achieve higher drug concentrations in the lymphoid tissue where the majority of viral replication occurs and has been shown to persist in the lymphatic systems of patients receiving highly active antiretroviral therapy (HAART), despite the successful suppression of circulating virus in the blood and other well-perfused tissues readily accessible to drug in plasma (6). The results derived from our pharmacokinetic model are in good agreement with previously reported experimental results where HIV-2-infected macaques treated with lipid-associated IDV showed greater inhibition of virus replication in the lymph nodes than animals treated with soluble IDV (7). Clearly, a therapeutic strategy targeted to overcome drug insufficiency in lymph nodes should be considered as an adjunct to daily oral HAART, which reduces the plasma viral load. Therefore, one could envision that periodic (i.e., once weekly

or monthly) subcutaneous administration of lipid-associated drug in HIV patients along with concurrent HAART may provide further suppression of virus load in these lymphatic sanctuaries.

All human-specific parameter estimates needed for the models were not available from published literature. Missing parameters were derived from results obtained from experiments using other animals or estimated in the current study to reproduce previously observed dynamics of soluble and lipid-associated IDV in the central compartment. The purpose of this study is to provide a working model to predict the outcome of a dosing regimen of SC-administered lipid-associated indinavir. In order to achieve this model, estimates of human parameters had to be inferred from experimental data of other animals. The validity of these assumptions should be tested as more human data become available.

Two parameters of particular importance that have not yet been experimentally determined in any animal are the volumes of distribution in the lymph compartment of soluble IDV and the volumes of distribution of lipid-associated IDV in either compartment. In the absence of any experimental estimate for these volumes, we used physiological volumes to determine concentrations. Because drug concentration is a function of V_d, more accurate results and conclusions are dependent on more precise estimates of these volumes. It is also important to note that the use of physiological volumes makes the implicit assumption that the V_d is the same in the lymph for both soluble and lipid forms of IDV, although this may not be the case.

Other parameters determined to be important for an accurate assessment of lipid-drug complex kinetics were the elimination rate from the lymph, the drug transport ratio between the two compartments, and the bioavailability of the complexes relative to the injection site. However, even when using the most conservative estimates of these variables (i.e., bioavailability = 45%, K_2 = 0.3125, K_{et} = 0.075, and K_e = 0.0111), minimum complex dosing was still found to be on the order of days rather than hours as with current treatments.

To derive the potential minimum dosing schedule of lipid-associated IDV, we supposed that equal concentrations of soluble and lipid-associated IDV would have similar efficacy, thus implicitly assuming the pharmacodynamics of lipid-associated IDV would remain similar to the soluble form, a fact yet to be determined. However, *in vitro* data suggest that lipid association does not inhibit drug efficacy, and may even be three- to six-fold more potent than the soluble form (7), indicating the supposition of similar efficacy is rational.

We considered the concentration of lipid-drug complexes in the lymph to be a well-mixed compartment, although differential distribution in various macaque lymph nodes has been observed. The model also assumed the increased concentration of lipid-associated IDV in the lymph would not saturate its elimination mechanisms, thus maintaining a constant clearance rate, and that higher levels of the lipid-drug complexes in these tissues would not be toxic to the patient. Given the high therapeutic index, and low cytotoxicity to cell, the absence of increased toxicity is a reasonable belief. For further refinement of these models, additional clinical studies are necessary to determine the pharmacokinetics of lipid-associated IDV, the maximum tolerable and optimal lipid-drug complex dosage, and the distribution of the complexes relative to the injection site.

Subcutaneous administration of lipid-mediated nanoparticles containing protease inhibitors or other anti-HIV drugs could provide greater exposure of drug into the lymphoid tissues where low levels of virus replication persists, which may lead to better suppression of viral replication, increased immunological benefit and fewer treatment failures due to antiviral resistance. However, one disadvantage of SC administration can be that with repeated injections, abscesses can develop blocking the proper drainage of the lipid drug complexes, thereby reducing their beneficial effects with respect to LT saturation.

CONCLUSIONS

Although preliminary, the models presented here demonstrate the lipid-drug particles' potential for much less frequent dosing while still maintaining effective drug concentrations in the lymphoid tissues. We envision that subcutaneous lipid-associated IDV therapy may be particularly suitable as an adjunct to an oral regimen of highly active antiretroviral therapy, which controls systemic viral replication, while the lipid-associated therapy further reduces or eliminates residual replication in lymphoid tissues. As a result, the lipid-associated drug delivery system may extend the utility of current antivirals by providing greater control of virus replication in the lymphatic system as well as make effective anti-HIV therapy accessible to difficult-to-treat populations.

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REFERENCES

1. D. Finzi and R. F. Siliciano. Viral dynamics in HIV-1 infection. *Cell* **93**:665–671 (1998).
2. A. T. Haase. Population biology of HIV-1 infection: viral and CD4+ T cell demographics and dynamics in lymphatic tissues. *Annu. Rev. Immunol.* **17**:625–656 (1999).
3. W. Cavert, D. W. Notermans, K. Staskus, S. W. Wietgrefe, M. Zupancic, K. Gebhard, K. Henry, Z. Q. Zhang, R. Mills, H. McDade, C. M. Schuwirth, J. Goudsmit, S. A. Danner, and A. T. Haase. Kinetics of response in lymphoid tissues to antiretroviral therapy of HIV-1 infection. *Science* **276**:960–964 (1997).
4. G. Pantaleo, C. Graziosi, J. F. Demarest, L. Butini, M. Montroni, C. H. Fox, J. M. Orenstein, D. P. Kotler, and A. S. Fauci. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* **362**:355–358 (1993).
5. F. Mammano, V. Trouplin, V. Zennou, and F. Clavel. Retracing the evolutionary pathways of human immunodeficiency virus type 1 resistance to protease inhibitors: virus fitness in the absence and in the presence of drug. *J. Virol.* **74**:8524–8531 (2000).
6. J. Martinez-Picado, M. P. DePasquale, N. Kartsonis, G. J. Hanna, J. Wong, D. Finzi, E. Rosenberg, H. F. Gunthard, L. Sutton, A. Savara, C. J. Petropoulos, N. Hellmann, B. D. Walker, D. D. Richman, R. Siliciano, and R. T. D'Aquila. Antiretroviral resistance during successful therapy of HIV type 1 infection. *Proc. Natl. Acad. Sci. USA* **97**:10948–10953 (2000).

7. L. Kinman, S. J. Brodie, C. C. Tsai, T. Bui, K. Larsen, A. Schmidt, D. Anderson, W. R. Morton, S. L. Hu, and R. J. Ho. Lipid-drug association enhanced HIV-1 protease inhibitor indinavir localization in lymphoid tissues and viral load reduction: a proof of concept study in HIV-2287-infected macaques. *J. Acquir. Immune Defic. Syndr.* **34**:387–397 (2003).
8. T. Lian and R. J. Ho. Trends and developments in liposome drug delivery systems. *J. Pharm. Sci.* **90**:667–680 (2001).
9. C. Oussoren, J. Zuidema, D. J. Crommelin, and G. Storm. Lymphatic uptake and biodistribution of liposomes after subcutaneous injection. II. Influence of liposomal size, lipid composition and lipid dose. *Biochim. Biophys. Acta.* **1328**:261–272 (1997).
10. M. A. Boyd, R. E. Aarnoutse, K. Ruxrungtham, M. Stek Jr., R. P. van Heeswijk, J. M. Lange, D. A. Cooper, P. Phanuphak, and D. M. Burger. Pharmacokinetics of indinavir/ritonavir (800/100 mg) in combination with efavirenz (600 mg) in HIV-1-infected subjects. *J. Acquir. Immune Defic. Syndr.* **34**:134–139 (2003).
11. B. Clotet, L. Menéndez-Arias, L. Ruiz, C. Tural, A. Vandamme, D. Burger, J. Schapiro, C. A. Boucher, R. D'Aquila, and D. Richman. *Guide to management of HIV resistance and pharmacokinetics of drug therapy* 94–96 (2000).
12. Yoffey and Courtice. *Lymphatics, lymph and lymphoid tissue*, 2nd edn. London, 26–27 (1956).
13. Merck & Co., Inc. Crixivan® Prescribing Information, (2004).
14. B. D. M. C. Khoo S. Pharmacology. In G. G. J. Boucher C. A. (ed.), *Practical Guidelines in Antiviral Therapy*, Elsevier, 2002.
15. C. Solas, A. Lafeuillade, P. Halfon, S. Chadapaud, G. Hittinger, and B. Lacarelle. Discrepancies between protease inhibitor concentrations and viral load in reservoirs and sanctuary sites in human immunodeficiency virus-infected patients. *Antimicrob. Agents Chemother.* **47**:238–243 (2003).