Review

Antiretroviral drug concentrations in semen of HIV-1 infected men

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Because semen is a major vehicle for the sexual transmission of HIV-1, control of viral replication within the sanctuary of the male genital tract should be a goal of antiretroviral therapy. Local immune responses, virus specific factors, and the degree of viral and cellular trafficking all appear to be important in controlling viral replication and evolution. However, the most important factor influencing viral replication and evolution within the male genital tract may be the disposition of antiretroviral agents into genital tissues and fluids. This review proposes possible mechanisms of antiretroviral distribution into the male genital tract by using other sanctuary barriers; such as the placenta, renal tubules, and blood-brain barrier; as models. In addition, this review summarises recent clinical studies regarding the disposition of currently available antiretroviral drugs into the seminal plasma and discusses some of the difficulties in interpreting drug concentration in the genital tract.
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website
extra

A table detailing antiretroviral drugs appears on the STI website.

www.sextransinf.com

HIV-1 spreads primarily from HIV-1 infected men to their sexual partners via contact with infected semen.[1 2] Evidence is accumulating that the genital tract may represent a separate compartment or "sanctuary site" in which HIV may be subject to unique selective pressures.[3 4] Therefore, it is important that factors influencing viral replication and evolution within the male genital tract be fully understood.

Recently, the positive effects of highly active antiviral therapy (HAART) and treatment of concurrent sexually transmitted diseases on seminal shedding have been described.[5–9] Whether this will be reflected by a reduced sexual transmission of HIV in developed countries has yet to be proved. However, there are discouraging reports of drug resistant mutants within semen,[9–11] and sexual transmission of drug resistant HIV-1.[12–14]

An antiviral drug concentration within the male genital tract is one important factor influencing viral replication and the development of resistant virus. In this article, we review the basic pharmacokinetic mechanisms that determine the disposition of antiviral drugs into the male genital tract and summarise the current data on antiviral drug concentrations in the semen of HIV-1 infected men

This review addresses the following topics:
(1) Components and properties of seminal plasma
(2) Basic principles of drug accumulation and distribution
(3) Passive distribution of antiretrovirals into the male genital tract
(4) Distribution mechanisms of antiretroviral agents into other sanctuary sites
(5) Drug disposition of currently available antiretrovirals into semen
(6) Factors limiting interpretation of drug concentrations in seminal plasma.

Seminal plasma

Seminal plasma is the cell-free fraction of ejaculate. It contains contributions from the testis, epididymis, prostate, seminal vesicles, and urethral (Littre's) and bulbourethral (Cowper's) glands. Thus, seminal plasma represents a conglomerate of all secretory organs within the male genital tract, and is a common surrogate for studying the male genital tract. The average ejaculate is slightly alkaline (pH 7.2–7.6) with an average volume of 2.5–5 ml.[15 16] Sixty per cent (v/v) of the ejaculate originates from the seminal vesicles and 30% from the prostate. The remainder is a combination of secretions from the urethral and bulbourethral glands, testis, and epididymis.[17] A split, or fractionated, ejaculate can be used to study drug penetration into the male genital tract through specific tissues and glands.

The fraction of semen from the testis/epididymis is sperm rich with a protein poor fluid. Prostatic fluid is protein rich, has a pH of 6.6, and is the main source of seminal acid phosphatase, citric acid, and zinc. Fluid from the seminal vesicles is also protein rich, but is more alkaline (pH 7.8) and is the main source of seminal fructose and prostaglandins.[16–18] Some seminal proteins, like seminin, are proteases. These proteases degrade other seminal proteins and may release any bound or associated drugs.

It is important to remember seminal plasma is a dynamic entity, with nutrients for sperm development entering and waste materials leaving. Thus, its composition can vary between different individuals and between different ejaculates from the same individual. In addition, it is important to remember ejaculation in essence is a process of elimination. Frequent ejaculation can temporarily deplete the various fractions and may influence the phar-

macokinetics of antiretrovirals into seminal plasma.

Drug disposition and accumulation

Once entering the blood stream, drugs travel throughout the body via the systemic circulation. Leaving the circulation and entering other compartments is drug disposition. In general, disposition from the capillary lumen to the extravascular compartments occurs by diffusion and/or active transport. In many organs, diffusion occurs through and between capillary endothelial cells. However, tight junctions exist between testicular (and perhaps prostatic and vesicular) endothelial cells. These junctions prevent diffusion between endothelial cells.[15]

Drug accumulation, or concentration, in a tissue or biological fluid occurs when the influx of a drug is greater than its efflux. Because the rate of diffusion decreases as the concentration gradient decreases diffusion alone cannot lead to accumulation. However, concentration gradients do not govern the rate of active transport. Thus, active transport can play a part in accumulation. Drug sequestration is also responsible for accumulation. Sequestration involves either drug ionisation or drug binding to proteins and other macromolecules, preventing efflux by diffusion and/or transport.[15]

Antiretroviral diffusion into seminal plasma

Diffusion can be either simple (non-mediated) or carrier mediated. Carrier mediated diffusion has some characteristics that are similar to active transport—specificity, saturation kinetics, and susceptibility to competitive inhibition. Simple diffusion requires no transporters; thus, it is non-specific, non-saturable, and not susceptible to competitive inhibition. A drug will only diffuse in the direction that reduces its concentration gradient. Thus, at equilibrium, concentration of diffusible drug on both sides of the membrane will be equal.[19] The major physiochemical determinants of diffusion are degree of ionisation, lipid solubility, and size.[20] Frick's law of diffusion mathematically describes how these variables determine the rate of simple diffusion.[19 20]

LIPID SOLUBILITY

Endothelial cells are essentially lipid membranes, and Frick's law applies to both. The partition coefficient (K) describes the equilibrium distribution of a drug between organic and aqueous phases in a two layer system. In short, the coefficient predicts the likelihood of a drug diffusing across a membrane, or endothelial cell.[19] There is a hypothetical bell-shaped relation between the ability of a drug to diffuse across a membrane and its lipid solubility. Only drugs with some degree of lipophilicity and hydrophobicity should partition into and out of biological membranes.[21] Figure 1 describes the effects of lipid solubility on membrane diffusion.

ANTIRETROVIRALS AND LIPOPHILICITY

Glynn and Yazdanian[21] experimentally determined the partition coefficient between octanol and phosphate buffered saline (pH 7.4) of various antiretrovirals, including didanosine (ddI), stavudine (d4T), zalcitabine (ddC), zidovudine (ZDV), nevirapine (NVP), indinavir (IDV), saquinavir (SQV), and amprenavir (APV). They also studied the diffusion, or permeability, of these antiretrovirals across bovine brain endothelial cells. As predicted, they found a bell-shaped relation between lipid solubility and drug diffusion/permeability. Nevirapine is moderately lipophilic and most permeable. The nucleosides, although similar to nevirapine in size and molecular weight, are more hydrophilic and less permeable. The protease inhibitors (PIs) are more lipophilic than the other antiretrovirals in the study. Amprenavir is both slightly less lipophilic and more permeable than the other PIs studied. Table w1 (on the *STI* website) describes the lipid solubility of select antiretrovirals.

IONISATION

The degree of ionisation is dependent on the pKa of the drug and the pH of its environment.[22 23] Because the pH on one side of a membrane can differ from the pH on the other, ionisation may differ between membrane separated compartments. Because ionisation increases hydrophilicity, only non-ionised drugs diffuse through biological membranes. Ion trapping (or sequestering) occurs when ionisation differs on the two sides of the membrane. At equilibrium, non-ionised drug concentration on each side of the membrane will be equal while total drug concentration (ionised and non-ionised) on the two sides can be unequal[24] (fig 1). Modified versions of the Henderson-Hasselbalch equation predict the ratio, at equilibrium, between drug concentrations in two different solutions of known pH.[22 23]
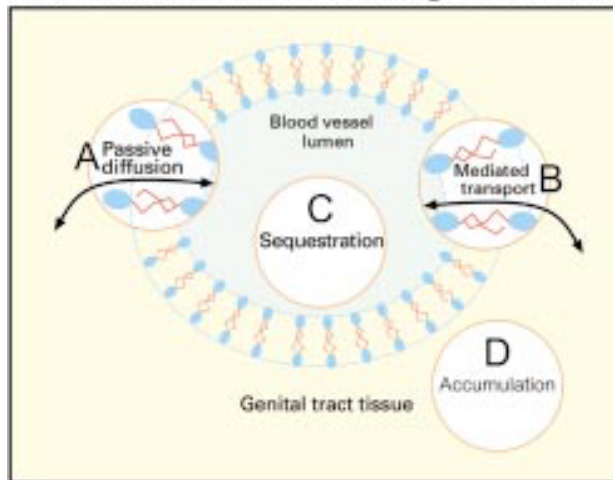
ANTIRETROVIRALS AND IONISATION

Ion trapping is an important concept, since the pH of blood plasma (7.4) is almost a log different from the pH of prostatic fluid (6.6). Thus, weak bases may accumulate in prostatic fluid. In fact, Henry *et al*[25] suggested ion trapping as the mechanism for zidovudine accumulation (pKa 9.68) in seminal plasma. The pH gradient between blood plasma and vesicular fluid (pH 7.4 versus pH 7.8) is less dramatic; thus, it is unlikely that there will be any significant ion trapping for any drug in the seminal vesicles. Table w1 (on website) contains pKa values of selected antiretrovirals.
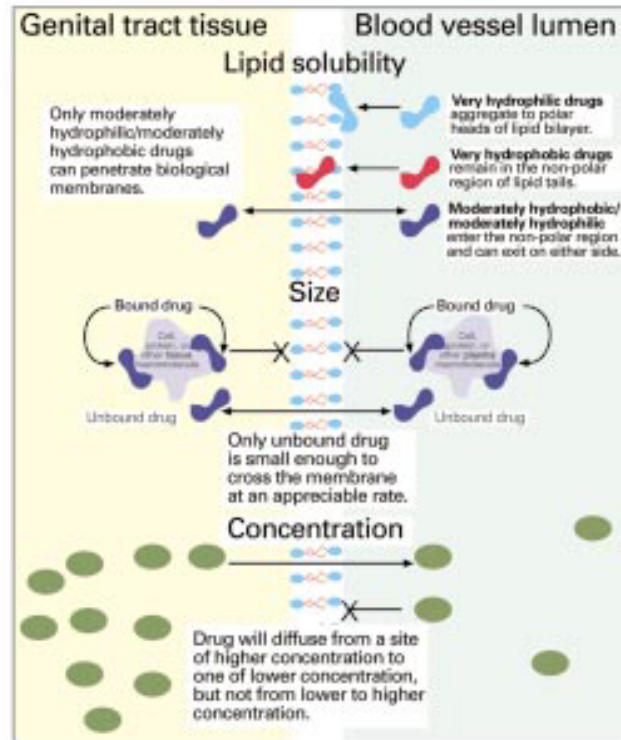
PROTEIN BINDING—SIZE

According to Frick's law, small drugs diffuse across membranes faster than large drugs. The effective size of a drug increases if it binds to macromolecules, including DNA and proteins. Thus, when a drug is bound its diffusion rate becomes so slow that diffusion is negligible and only free (unbound) drug appreciably diffuses across biological membranes.[26] Such binding is usually reversible with on/off rates and association constants. Drug accumulation can occur
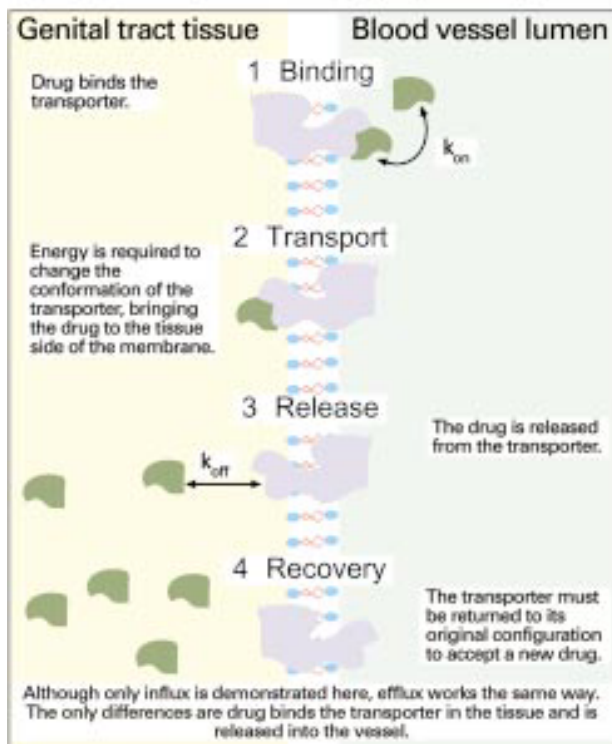
Overview of drug distribution between the systemic circulation and the male genital tract
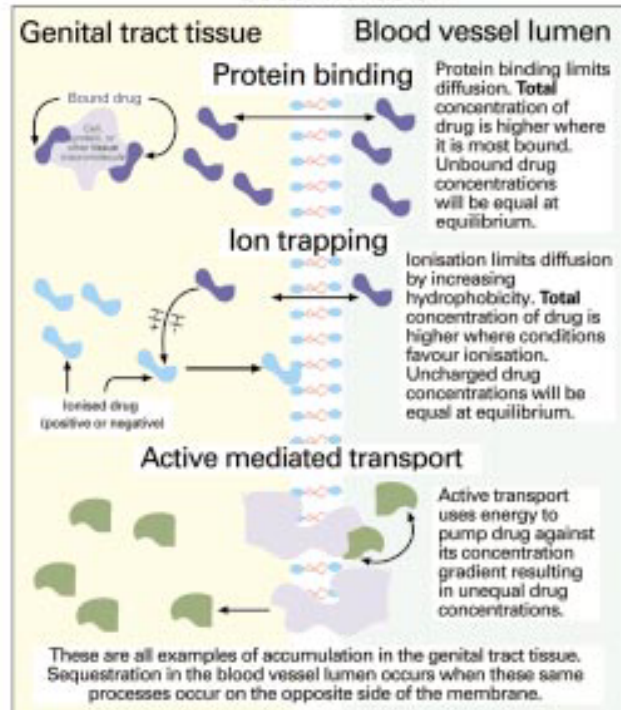
A Passive diffusion

Figure 1   Overview of drug distribution between the systemic circulation and the male genital tract. Drug distribution is the result of drug deposition by either (A) passive diffusion or (B) mediated transport. The partition coefficient, size, and concentration of the drug determine the rate of diffusion. Mediated transport requires recognition between the transporter and drug and energy to move the drug against its concentration gradient. Mediated transport, and not diffusion, is saturable and susceptible to competitive inhibition. Both (C) sequestration and (D) accumulation lead to concentration of drug in the systemic circulation and male genital tract, respectively. It is likely that protein binding, ion trapping, and active mediated transport have a role in sequestration and accumulation. A combination of passive diffusion, protein binding, ion trapping, and active transport are all likely mediators of drug disposition into the male genital tract. At the genital tract barrier, organic anion and organic cation transporters may transport nucleoside analogues and p-glycoprotein may transport protease inhibitors, as they do at other sanctuary barriers. By studying the mechanism of drug disposition at other barriers, scientists may understand the process of drug disposition into the male genital tract.

when the amount of drug binding differs on each side of the membrane. When binding differs, total drug concentration on each side of the membrane will be unequal while unbound drug concentration will be equal, at equilibrium (fig 1). It has been hypothesised that greater than 80% binding is required to affect drug disposition.[26–29]

ANTIRETROVIRALS AND PLASMA PROTEIN BINDING
Protein binding also affects the diffusion of antiretrovirals across membranes, including into target cells. Because current antiretrovirals only act within cells, it has been hypothesised that by studying the effects of extracellular proteins on intracellular antiviral activity one can indirectly determine the effects of protein binding on drug distribution.

As organic bases, protease inhibitors are attracted to α_1-acid glycoprotein (AAG). Zhang et al[30] studied the effect of AAG on the antiviral efficacy of select antiretrovirals: SQV, ritonavir (RTV), IDV, nelfinavir (NFV), APV, and ZDV. As predicted, increasing AAG concentration causes a decrease in PI activity. Addition of AAG to PIs affects IDV activity the least; NFV, RTV, APV the most; and has a moderate effect on SQV. This corresponds to the degree of PI-AAG binding, with IDV being least bound (60%). Increasing AAG had no effect on ZDV efficacy, consistent with lack of ZDV-AAG binding. Table w1 (on website) describes the plasma protein binding characteristics of currently available antiretrovirals.

Distribution mechanisms of antiretroviral agents into other sanctuary sites
More is known about antiretroviral drug disposition across the placenta, into urine, and across the blood-brain barrier than about drug disposition into semen. It is likely that the mechanisms regulating antiretroviral drug disposition and accumulation in the male genital tract are similar to the mechanisms described here.

PLACENTA
Maternal to fetal transport of ZDV and lamivudine (3TC) were studied in two separate laboratories using ex vivo human term placentas. Transfer was non-saturable with no accumulation on the fetal side. Therefore, diffusion is one mechanism by which ZDV and 3TC cross the placenta. Because addition of nucleoside transport inhibitors did not alter the transfer of ZDV, this transport system does not appear to be involved with the placental disposition of ZDV. However, active transport, by mechanisms other than the nucleoside transport system, could not be excluded.[31 32]

KIDNEY
Griffiths et al[33 34] were the first to study antiretroviral renal clearance. They found ZDV clearance was greater than the glomerular filtration rate, indicative of tubular secretion of ZDV. Using rat membrane vesicles, they showed that ZDV uses both the basolateral organic anion and the brush border organic cation transport systems, and suggested the azido moiety of ZDV might result in a pseudo-zwitterion capable of using both transport systems.

Bendayan et al[35] studied tubular secretion of ZDV in cultured renal epithelium. The flux of ZDV was greater than that of diffusion limited mannitol, indicative of active transport. Several organic bases (cimetidine, trimethoprim, quinine, and quinidine) limit ZDV flux, further evidence that ZDV uses the organic cation transport system. Again, the azido group was presumed to be the component allowing ZDV to act as an organic base.

Aiba et al[36] and Chatton et al[37] infused rats with ZDV, with and without co-administration of either probenecid (an anionic drug) or cimetidine (a cationic drug). Both drugs inhibit ZDV renal secretion. Rats given *p*-amino-hippurate or imipramine, putative organic anion and organic cation transport inhibitors, respectively, also demonstrated reduced ZDV renal secretion. This confirms renal secretion of ZDV by organic anion transporters and organic cation transporters.

CENTRAL NERVOUS SYSTEM (CNS)
Protease inhibitors
Protease inhibitors are substrates of p-glycoprotein (P-gp),[38 39] a 170 kDa transmembrane ATP dependent efflux pump found in brain capillary endothelial cells and in the epithelia of testis and prostate.[40 41] At the blood-brain barrier, P-gp pumps PIs out of the CNS, preventing drug accumulation.[42 43] Other tissues that contain P-gp (adrenal cortex, kidneys, intestines, liver, pancreas, placenta, and haematological cells—including CD4+ and CD8+ cells, monocytes, and macrophages) also have reduced penetration by protease inhibitors.[38 39]

Using P-gp expressing Caco-2 monolayers, Lee et al[39] showed protease inhibitors bind to P-gp and compete for P-gp mediated efflux. For example, RTV, SQV, and IDV compete with iodoarylazidoprazosin (a photoaffinity substrate of P-gp) and inhibit transport of cyclosporin A (a substrate of *p*-glycoprotein transport). Other experiments demonstrate P-gp mediates transport of IDV, NFV, SQV, RTV, and APV across Caco-2 cells unidirectionally.[42 43] In fact, the basolateral to apical flux is 2–23 fold higher than apical to basolateral flux for APV, IDV, RTV, and SQV. Other evidence of P-gp involvement in PI transport includes prevention of basolateral to apical flux by the P-gp inhibitor GF-120918.[43]

Kim et al[42] and Polli et al,[43] working separately, used mice to study whether P-gp prevented PI accumulation within the central nervous system. In mice, both mdr-1a and mdr-1b genes express P-gp. Kim used a single knockout (mdr-1a −/−) mouse strain; Polli used a double knockout (mdr-1a −/− and mdr-1b −/−) mouse strain and genetically normal mice pretreated with GF-120918. The amount of IDV, NFV, and SQV increased in the CNS of single knockout mice, compared with control mice. The double knockout mice and normal mice pretreated with GF-120918 had increased CNS concentrations of APV

when compared with normal mice without pretreatment. These mice studies confirm in vivo efflux of PIs from the CNS by P-pg.

Reverse transcriptase inhibitors
The CNS concentration of nucleoside reverse transcriptase inhibitors (NRTIs) is less than their concomitant blood plasma concentration. This suggests active efflux and/or sequestration of NRTIs in blood plasma. It was shown that the transport of ZDV across the rabbit blood-brain barrier is asymmetric, with efflux greater than influx and no evidence of sequestration in plasma.[44–46] This differs from disposition mechanisms of thymidine, which enters and accumulates in the CNS via multiple nucleoside transporters.[44 45] However, ribonucleosides or deoxyribonucleosides modified in the 2', 3', or 5' position will not be transported by the sodium dependent nucleoside uptake systems found in the CNS.[47] The general conclusion, after in vitro and in vivo experimentation, is that nucleosides enter the CNS by diffusion where they are pumped out by organic acid transporters.

Evidence of entry into the CNS by diffusion is based on the $[Drug]_{CNS}:[Drug]_{Plasma}$ ratios of ddI and ZDV, which are best explained by differences in their diffusion rates. The only diffusion related difference between the two drugs is their partition coefficient. The more lipid soluble ZDV has a higher $[Drug]_{CNS}:[Drug]_{Plasma}$ ratio than the less lipid soluble ddI, as would be predicted by Frick's law.

Evidence of active efflux of reverse transcriptase inhibitors is based on saturation and inhibition studies. Takasawa et al[48 49] first demonstrated that the efflux of ZDV and ddI was saturable, indicating mediated transport. This, as well as evidence that this efflux transfers ZDV and ddI against their concentration gradients, signifies active transport. There is in vivo evidence that this transport occurs via an organic anion transport system.[48 49] Probenecid or *p*-aminohippurate increases ZDV and ddI $[Drug]_{CNS}$ and $[Drug]_{CNS}:[Drug]_{Plasma}$ in rats and rabbits.[48] Thymidine co-administration did not affect the efflux of ZDV or ddI, again ruling out similar transport mechanisms for the drugs and this intrinsic nucleoside.

Efflux of NRTIs from the CNS by P-gp needs to be studied as there are currently no data to suggest ZDV and other nucleosides are substrates of this transport system.

Drug disposition of antiretrovirals into semen
NUCLEOSIDE ANALOGUES
Zidovudine (AZT, ZDV)
Henry *et al* first described ZDV concentrations in semen in 1988, while studying six patients receiving ZDV monotherapy.[25] They calculated $[ZDV]_{Semen}:[ZDV]_{Plasma}$ ratios approximately 1 hour and 3–4 hours post-drug ingestion and these ranged from 1.3–20.4 and 2.3–16.8, respectively. They found the ratios, although variable at both time points, suggested that ZDV, and perhaps other antiretrovirals, can accumulate within semen. Pereira *et al*[50] determined ZDV and 3TC concentrations in the

blood and seminal plasma of nine men commencing therapy. In this study, 70 paired seminal and blood plasma samples were obtained. The median $[ZDV]_{Semen}:[ZDV]_{Plasma}$ ratio was 5.9, with 81% of the samples having higher $[ZDV]_{Semen}$ than $[ZDV]_{Plasma}$.

More recently Anderson *et al*,[51] using a timed dose administration and timed single sample strategy, were able to create an area under the concentration time curve (AUC) for ZDV in semen and blood plasma. They found that zidovudine and zidovudine-gluconeride concentrations were uniformly higher in semen than in plasma at all time points except at 1 hour after the dose. Finally they found the $[ZDV]_{semen\ AUC}:[ZDV]_{plasma\ AUC}$ was 3.31 which is consistent with previous findings.

Lamivudine (3TC)
In the same study by Pereira *et al*,[50] the median $[3TC]_{Semen}:[3TC]_{Plasma}$ ratio was 5.9, with 81.4% of the seminal plasma samples having a higher $[3TC]_{Semen}$ than concomitant $[3TC]_{Plasma}$. Two other studies also suggest accumulation of 3TC in semen. One study looked at eight patients and found $[3TC]_{Semen}$ were approximately five times $[3TC]_{Plasma}$. Interestingly, the ratios changed over time; ratios at 2–4 hours and 8–12 hours post drug ingestion were 4.6 (range 2.5–6.4) and 8.7 (4.0–16.3), respectively.[52] In a separate study involving eight patients, median $[3TC]_{Semen}$ was 1062 ng/ml compared with a median $[3TC]_{Plasma}$ of 329 ng/ml.[53]

Stavudine (d4T)
Data on $[d4T]_{Semen}$ are confined to two small studies. The first study involved 10 patients receiving d4T. In some patients, d4T was undetectable in seminal and blood plasma samples. However, when detected, $[d4T]_{Semen}$ was similar in magnitude to $[d4T]_{Plasma}$. When detectable, the median $[d4T]_{Semen}:[d4T]_{Plasma}$ ratios at 0–2 hours, 2–4 hours, and 8–12 hours were 0.46 (range 0.19–0.73), 2.7 (0.47–4.80), and 3.5 (1.0–6.0), respectively.[52] The second study, in eight patients showed that $[d4T]_{Semen}$ (median 104 ng/ml) was again similar to $[d4T]_{Plasma}$ (78 ng/ml).[53]

At the time of writing there were no data on the concentrations of didanosine (ddI), zalcitabine (ddC), or abacavir (ABC) in seminal plasma.

NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NNRTIS)
Nevirapine (NVP)
There has been one published paper describing nevirapine penetration into the seminal plasma of 12 HIV-1 infected men receiving NVP containing regimens. It was found that NVP penetrates into semen well, with $[NVP]_{Semen}:[NVP]_{Plasma}$ ratios consistently between 0.6–1. Interestingly, these ratios were not dependent on the time post drug ingestion. The authors of the report estimated that the free $[NVP]_{Semen}$ should remain greater than 40 times the EC_{90} for wild type HIV-1 at all time points. (The EC_{90} is the effective concentration of drug required to inhibit viral replication by

90% in an vitro cell culture assay. This figure may, or may not, be corrected for drug/plasma protein binding interactions (see below)). In this study, 10/12 patients had seminal viral loads below the limit of detection at the time of the study.[52]

Efavirenz (EFV)

There have been no published studies on $[EFV]_{semen}$; however, recently Kim *et al*[54] have presented data suggesting that EFV also achieves therapeutic concentrations in semen. In this study they were able to obtain semen samples over the 24 hour dosing period and found that 24 hour $[EFV]_{semen}:[EFV]_{plasma}$ ratios more predictive than individual semen:plasma ratios.

Similarly, we have data on 19 HIV-1 positive individuals who commenced EFV containing regimens. All patients in this study had seminal plasma viral load suppressed by 24 weeks and $[EFV]_{semen}$ at 12 and 24 hours post drug ingestion were approximately 10% of $[EFV]_{plasma}$ (unpublished data).

Data on delavirdine $[Drug]_{Semen}$ were unavailable at the time of writing.

PROTEASE INHIBITORS (PIS)

Indinavir (IDV)

Two recent studies have investigated IDV penetration into semen. Taylor *et al*[55] reported that $[IDV]_{Semen}$ was high and similar to concurrent $[IDV]_{Plasma}$. The median $[IDV]_{Semen}:[IDV]_{Plasma}$ ratios 0–3, 3–6, and 6–9 hours post drug ingestion were 0.62, 0.8, and 1.4, respectively. Trough $[IDV]_{Semen}$ was above the protein corrected EC_{95} for IDV in blood plasma at all times. All seven patients in this study reached an undetectable viral load in blood and seminal plasma.

A second study by Van Praag *et al*[56] has recently been published. In this study low dose RTV (100 mg) was added to 13 patients already taking IDV containing regimens. Semen and CSF samples were available at baseline and after addition of RTV in six of these patients. Interestingly they found that addition of RTV increased $[IDV]_{Semen}$ by 8.2 times (95% confidence interval (CI) 5.2–11.6) and $[IDV]_{CSF}$ by 2.4 times (95% CI 1.8–3.9). They concluded that the increases in $[IDV]_{plasma}$ alone were not sufficient to explain the large increases in $[IDV]_{Semen}$ and $[IDV]_{CSF}$ and postulated modulation of P-gp as a potential mechanism.

Ritonavir (RTV) and saquinavir (SQV)

$[RTV]_{Semen}$ has only been determined in seven patients receiving RTV, SQV, and stavudine. Median $[Drug]_{Semen}:[Drug]_{Plasma}$ ratios were less than 0.05 at 2 and 12 hours post administration. $[RTV]_{Semen}$ fell below the protein corrected EC_{95} in all seven patients. Median $[SQV]_{Semen}:[SQV]_{Plasma}$ ratios were also less than 0.05 at 2 and 12 hours post administration. Despite high $[SQV]_{Plasma}$, $[SQV]_{Semen}$ was undetectable (less than 25 ng/ml) in 7/12 samples. In this study, viral load became undetectable in both seminal and blood plasma. However, because of the complex regimen, it is impossible to make a correlation between $[Drug]_{Semen}$ and viral suppression.[57]

Saquinavir (SQV) and nelfinavir (NFV)

A study by Reijers *et al*[53] measured $[SQV]_{Semen}$ and $[NLF]_{Semen}$ in eight patients taking part in the four drug ADAM study (NFV, SQV, d4T, and 3TC). SQV was only detected in 3/8 semen samples at low concentrations. NFV could not be detected in any of the seminal plasma samples.

Amprenavir (APV)

Pereira *et al*[58] determined $[APV]_{Semen}$ in 31 men. In this study, median $[APV]_{Semen}$ was 269 ng/ml with an interquartile range (IQR) of 73–929 ng/ml. The concomitant median $[APV]_{Plasma}$ was 1210 ng/ml with an IQR of 326–4696 ng/ml. Time post drug ingestion was approximately 6 hours. Seminal viral load was measured in all samples obtained. $[APV]_{Semen}$ were above the protein corrected EC_{95} for APV in blood plasma in some but not all patients. Seminal viral load was less than 400 copies per ml in 14/19 and 7/9 men receiving APV monotherapy and APV/ZDV/3TC, respectively.

Factors limiting interpretation of seminal plasma drug concentrations

WHAT TO MEASURE: TOTAL $[DRUG]_{SEMEN}$ VERSUS FREE $[DRUG]_{SEMEN}$

Currently, drug concentrations in semen are reported in a variety of ways. These include $[Drug]_{Semen}:[Drug]_{Plasma}$ ratios and simply $[Drug]_{Semen}$. In either case only the total $[Drug]_{Semen}$, not free $[Drug]_{Semen}$ has been measured. As described above, protein binding can affect the amount of drug available for antiretroviral activity. This needs to be taken into account when interpreting data. There are currently no analyses of seminal antiretroviral protein binding or free $[Drug]_{Semen}$.

In blood plasma, free drug concentration can be inferred from protein corrected EC_{50} and EC_{95}. For example, Molla *et al*[59] added 50% human blood plasma to cell cultures and determined EC_{50} values for PIs both before and after the addition of human blood plasma. Unfortunately, seminal plasma is toxic to cultured cells and similar experiments have not been performed with seminal plasma.

Some authorities believe that for adequate free drug concentrations to be available in vivo, drug concentrations should be targeted above "protein corrected EC_{95}s" in all compartments. However, establishing the minimum drug concentrations in plasma remains an area of controversy. The minimum concentrations in extravascular compartments are even less clear.

WHEN TO MEASURE

Most researchers express drug deposition into semen as a ratio, $[Drug]_{Semen}:[Drug]_{Plasma}$. Use of ratios can be misleading. They rely on single time point measurements, which give static observations on the dynamic processes of drug accumulation and drug elimination. Ratios also compare drug concentrations between compartments that probably have different rates of accumulation and elimination. Table w1 (see

website) lists the plasma half life of various antiretroviral drugs. This can lead to ratios that vary with time. These issues regarding the use of ratios lead to the question of when to sample blood and semen to get a realistic idea of the pharmacokinetics of antiretrovirals in the male genital tract.

Several groups report $[\text{Drug}]_{\text{CSF}}$:$[\text{Drug}]_{\text{Plasma}}$ ratios vary with time post dose. For example, ZDV is cleared rapidly from blood plasma and penetrates slowly into CSF. Thus, over time, ZDV blood plasma concentrations decrease and CSF concentrations increase, resulting in $[\text{Drug}]_{\text{CSF}}$:$[\text{Drug}]_{\text{Plasma}}$ ratios that increase with time post-dose.[60] Lamivudine, d4T, and IDV also have $[\text{Drug}]_{\text{CSF}}$:$[\text{Drug}]_{\text{Plasma}}$ ratios that are time dependent.[61 62] Interestingly, $[\text{NVP}]_{\text{Semen}}$:$[\text{NVP}]_{\text{Plasma}}$ ratios are relatively constant over time.[52] Ideally a ratio of the semen AUC to plasma AUC would give the best indication of overall drug exposure. However, obtaining AUC measurements for semen is technically and practically very difficult.

STATISTICAL PARAMETERS TO ESTIMATE GENITAL TRACT EXPOSURE

To make an informed interpretation of $[\text{Drug}]_{\text{compartment}}$:$[\text{Drug}]_{\text{Plasma}}$ ratios, the time post drug ingestion should be stated. If possible, the time between sampling the two compartments should be as close as possible. For the male genital tract, time since last ejaculation may also be relevant. Recently, several groups have attempted to estimate mathematically viral exposure to various antiretrovirals in various compartments. For example, population based pharmacokinetic modelling, using a NONMEM program instead of isolated ratios, has recently been applied to the study of antiretroviral disposition into CSF. It is probable that similar population modelling has applications in the field of studying drug penetration into the male genital tract.[63]

INTRACELLULAR DRUG CONCENTRATIONS

The study of drug penetration into sanctuary sites is in its infancy. So far, researchers have only reported $[\text{Drug}]_{\text{Extracellular}}$ in semen, not $[\text{Drug}]_{\text{Intracellular}}$. However, because current antiretrovirals act intracellularly $[\text{Drug}]_{\text{Intracellular}}$ is more meaningful. Further, because NRTIs require intracellular triphosphorylation for activity, $[\text{NRTI-triphosphate}]_{\text{Intracellular}}$ concentrations and not $[\text{NRTI}]_{\text{Intracellular}}$ are the more relevant measurements.[64] Since several studies have demonstrated a poor correlation between plasma concentrations of nucleoside analogues and intracellular concentrations, estimates of $[\text{Drug}]_{\text{Intracellular}}$ based on $[\text{Drug}]_{\text{Extracellular}}$ may be misleading.

Conclusions

Given that drug penetration into semen appears to be drug specific, it is surprising that most of the studies looking at the efficacy of HAART on genital tract shedding have shown a variety of regimens to be effective. There may be several reasons for this. In most of the studies, combinations of drugs have been used. In addition, most studies have included at least one or two drugs shown to reach therapeutic concentrations in semen. It is interesting to note that studies of monotherapy regimens failed to suppress seminal viral load in a substantial proportion of cases.[65 66] Currently, it is unknown what drugs and what concentrations are required to suppress genital tract viraemia.

The study of drug disposition into anatomical sanctuary sites is becoming increasingly important. The male genital tract is of particular relevance as semen represents the major vehicle for sexual transmission. Early reports suggest that drug penetration into semen is drug specific and may depend on a variety of drug specific physiochemical properties and host specific biological mechanisms. More studies are needed to determine how antiretrovirals in seminal plasma affect clinical outcome or the sexual transmission of HIV-1.

1 Royce RA, Sena A, Cates W Jr, *et al.* Sexual transmission of HIV. *N Engl J Med* 1997;**336**:1072–8.
2 Vernazza PL, Eron JJ, Fiscus SA, *et al.* Sexual transmission of HIV: infectiousness and prevention. *AIDS* 1999;**13**:155–66.
3 Byrn RA, Zhang D, Eyre R, *et al.* HIV-1 in semen: an isolated virus reservoir. *Lancet* 1997;**350**:1141.
4 Delwart EL, Mullins JI, Gupta P, *et al.* Human immunodeficiency virus type 1 populations in blood and semen. *J Virol* 1998;**72**:617–23.
5 Winter AJ, Taylor S, Workman J, *et al.* Asymptomatic urethritis and detection of HIV-1 RNA in seminal plasma. *Sex Transm Inf* 1999;**75**:261–3.
6 Tachet A, Dulioust E, Salmon D, *et al.* Detection and quantification of HIV-1 in semen: identification of a subpopulation of men at high potential risk of viral sexual transmission. *AIDS* 1999;**13**:823–31.
7 Vernazza PL, Gilliam BL, Flepp M, *et al.* Effect of antiviral treatment on the shedding of HIV-1 in semen. *AIDS* 1997;**11**:1249–54.
8 Zhang H, Dornadula G, Beumont M, *et al.* Human immunodeficiency virus type 1 in the semen of men receiving highly active antiretroviral therapy. *N Engl J Med* 1998;**339**:1803–9.
9 Taylor S, Cane PA, Drake SM, *et al.* Multi-drug resistant HIV-1 in semen: treatment of concurrent gonorrhoea may limit the spread of drug resistant virus. *Antiviral Ther* 2000;**5**:Abstract 40.
10 Eron JJ, Vernazza PL, Johnston DM, *et al.* Resistance of HIV-1 to antiretroviral agents in blood and seminal plasma: implications for transmission. *AIDS* 1998;**12**:F181–9.
11 Kroodsma KL, Kozal MJ, Hamed KA, *et al.* Detection of drug resistance mutations in the human immunodeficiency virus type 1 (HIV-1) pol gene: differences in semen and blood HIV-1 RNA and proviral DNA. *J Infect Dis* 1994;**170**:1292–5.
12 Boden D, Hurley A, Zhang L, *et al.* HIV-1 drug resistance in newly infected individuals. *JAMA* 1999;**282**:1135–41.
13 Little SJ, Daar ES, D'Aquila RT, *et al.* Reduced antiretroviral drug susceptibility among patients with primary HIV infection. *JAMA* 1999;**282**:1142–9.
14 Hecht FM, Grant RM, Petropoulos CJ, *et al.* Sexual transmission of an HIV-1 variant resistant to multiple reverse-transcriptase and protease inhibitors. *N Engl J Med* 1998;**339**:307–11.
15 Tortora GJ. *Principles of human anatomy*. 6th ed. New York: Harper Collins, 1992.
16 Mann T, Lutwak-Mann C. Passage of chemicals into human and animal semen: mechanisms and significance. *Crit Rev Toxicol* 1982;**11**:1–14.
17 Pichini S, Zuccaro P, Pacifici R. Drugs in semen. *Clin Pharmacokinet* 1994;**26**:356–73.
18 Frick J, Aulitzky W. Physiology of the prostate. *Infection* 1991;**19**(Suppl 3):S115–8.

19 Voet D, Voet G. *Biochemistry*. New York: John Wiley, 1990.
20 Routledge PA. Factors contributing to variability in drug pharmacokinetics. II. Drug distribution. *J Clin Hosp Pharm* 1985;**10**:15–23.
21 Glynn SL, Yazdanian M. In vitro blood-brain barrier permeability of nevirapine compared to other HIV antiretroviral agents. *J Pharm Sci* 1998;**87**:306–10.
22 Gallicano K. Antiretroviral drug concentrations in semen. *Antimicrob Agents Chemother* 2000;**44**:1117–18.
23 Kashuba AD, Dyer JR, Kramer LM, *et al*. Antiretroviral-drug concentrations in semen: implications for sexual transmission of human immunodeficiency virus type 1. *Antimicrob Agents Chemother* 1999;**43**:1817–26.
24 Madsen PO, Aagaard J. Pharmacokinetics of quinolone derivatives in the prostate. *Infection* 1991;**19**(Suppl 3): S154–6.
25 Henry K, Chinnock BJ, Quinn RP, *et al*. Concurrent zidovudine levels in semen and serum determined by radioimmunoassay in patients with AIDS or AIDS-related complex. *JAMA* 1988;**259**:3023–6.
26 Shargel L, Yu A. *Applied biopharmaceutics and pharmacokinetics*. 4th ed. Connecticut: Appleton and Lange, 1999.
27 Kwong TC. Free drug measurements: methodology and clinical significance. *Clin Chim Acta* 1985;**3**:193–216.
28 Chan S, Gerson B. Free drug monitoring. *Clin Lab Med* 1987;7:279–87.
29 Luzier A, Morse GD. Intravascular distribution of zidovudine: role of plasma proteins and whole blood components. *Antiviral Res* 1993;**21**:267–80.
30 Zhang XQ, Schooley RT, Gerber JG. The effect of increasing alpha1-acid glycoprotein concentration on the antiviral efficacy of human immunodeficiency virus protease inhibitors. *J Infect Dis* 1999;**180**:1833–7.
31 Bloom SL, Dias KM, Bawdon RE, *et al*. The maternal-fetal transfer of lamivudine in the ex vivo human placenta. *Am J Obstet Gynecol* 1997;**176**:291–3.
32 Boal JH, Plessinger MA, van den Reydt C, *et al*. Pharmacokinetic and toxicity studies of AZT (zidovudine) following perfusion of human term placenta for 14 hours. *Toxicol Appl Pharmacol* 1997;**143**:13–21.
33 Griffiths DA, Hall SD, Sokol PP. Interaction of 3'-azido-3'-deoxythymidine with organic ion transport in rat renal basolateral membrane vesicles. *J Pharmacol Exp Ther* 1991; **257**:149–55.
34 Griffiths DA, Hall SD, Sokol PP. Effect of 3'-azido-3'-deoxythymidine (AZT) on organic ion transport in rat renal brush border membrane vesicles. *J Pharmacol Exp Ther* 1992;**260**:128–33.
35 Bendayan R, Georgis W, Rafi-Tari S. Interaction of 3'-azido-3'-deoxythymidine with the organic base transporter in a cultured renal epithelium. *Pharmacotherapy* 1995;**15**:338–44.
36 Aiba T, Sakurai Y, Tsukada S, *et al*. Effects of probenecid and cimetidine on the renal excretion of 3'-azido-3'-deoxythymidine in rats. *J Pharmacol Exp Ther* 1995;**272**: 94–9.
37 Chatton JY, Odone M, Besseghir K, *et al*. Renal secretion of 3'-azido-3'-deoxythymidine by the rat. *J Pharmacol Exp Ther* 1990;**255**:140–5.
38 Van Asperen J, Mayer U, van Tellingen O, *et al*. The functional role of p-glycoprotein in the blood-brain barrier. *J Pharm Sci* 1997;**86**:881–4.
39 Lee CG, Gottesman MM, Cardarelli CO, *et al*. HIV-1 protease inhibitors are substrates for the MDR1 multidrug transporter. *Biochemistry* 1998;**37**:3594–601.
40 Kim AE, Dintaman JM, Waddell DS, *et al*. Saquinavir, an HIV protease inhibitor, is transported by p-glycoprotein. *J Pharmacol Exp Ther* 1998;**286**:1439–45.
41 Stewart PA, Beliveau R, Rogers KA. Cellular localization of p-glycoprotein in brain versus gonadal capillaries. *J Histochem Cytochem* 1996;**44**:679–85.
42 Kim RB, Fromm MF, Wandel C, *et al*. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. *J Clin Invest* 1998;**101**:289–94.
43 Polli JW, Jarrett JL, Studenberg SD, *et al*. Role of P-glycoprotein on the CNS disposition of amprenavir (141W94), an HIV protease inhibitor. *Pharm Res* 1999;**16**: 1206–12.
44 Thomas SA, Segal MB. Saturation kinetics, specificity and NBMPR sensitivity of thymidine entry into the central nervous system. *Brain Res* 1997;**760**:59–67.
45 Thomas SA, Segal MB. The passage of azidodeoxythymidine into and within the central nervous system: does it follow the parent compound, thymidine? *J Pharmacol Exp Ther* 1997;**281**:1211–18.
46 Wu D, Clement JG, Pardridge WM. Low blood-brain barrier permeability to azidothymidine (AZT), 3TC, and thymidine in the rat. *Brain Res* 1998;**791**:313–6.
47 Spector R, Huntoon S. Specificity and sodium dependence of the active nucleoside transport system in choroid plexus. *J Neurochem* 1984;**42**:1048–52.
48 Takasawa K, Terasaki T, Suzuki H, *et al*. In vivo evidence for carrier-mediated efflux transport of 3'-azido-3'-deoxythymidine and 2',3'-dideoxyinosine across the blood-brain barrier via a probenecid-sensitive transport system. *J Pharmacol Exp Ther* 1997;**281**:369–75.
49 Takasawa K, Terasaki T, Suzuki H, *et al*. Distributed model analysis of 3'-azido-3'-deoxythymidine and 2',3'-dideoxyinosine distribution in brain tissue and cerebrospinal fluid. *J Pharmacol Exp Ther* 1997;**282**:1509–17.
50 Pereira AS, Kashuba AD, Fiscus SA, *et al*. Nucleoside analogues achieve high concentrations in seminal plasma: relationship between drug concentration and virus burden. *J Infect Dis* 1999;**180**:2039–43.
51 Anderson PL, Noormohamed SE, Henry K, *et al*. Semen and serum pharmacokinetics of zidovudine ans zidovudine-glucuronidein men with HIV-1 infection. *Pharmacotherapy* 2000;**8**:917–22.
52 Taylor S, Van Heeswijk R, Hoetelmans RMW, *et al*. Concentrations of nevirapine, lamivudine and stavudine in semen of HIV1 infected men. *AIDS* 2000;**14**:1979–84.
53 Reijers M, Van Heeswijk R, Jurriaans S, *et al*. The concentrations of D4T, 3TC, nelfinavir, and saquinavir in plasma cerebrospinal fluid, and semen. In: Program and Abstracts of 7th Conference on Retroviruses and Opportunistic Infections, 2000;abstract 316.
54 Kim J, Gotzkowsky K, Eron J, *et al*. Penetration of efavirenz into the male genital tract as measured by seminal plasma to blood plasma concentration and AUC_{24h} ratios. In: Program and abstracts of 40th International Conference on Antimicrobial Agents and Chemotherapy, 2000;abstract 1171
55 Taylor S, Back D, Drake S, *et al*. Antiretroviral drug concentrations in semen of HIV-1 infected men: differential penetration of indinavir, ritonavir and saquinavir. In: Program and Abstracts of 7th Conference on Retroviruses and Opportunistic Infections, 2000;abstract 318.
56 Van Praag R, Wavering G, Portegies P, *et al*. Enhanced penetration of indinavir in cerebrospinal fluid after addition of low dose ritonavir. *AIDS* 2000;**14**:1187–94.
57 Taylor S, Back DJ, Workman J, *et al*. Poor penetration of the male genital tract by HIV-1 protease inhibitors. *AIDS* 1999;**13**:859–60.
58 Pereira A, Eron J, Dunn J, *et al*. Analysis of amprenavir, 3TC/AZT in blood and seminal plasma: penetration of amprenavir into the male genital tract. In: Program and Abstracts of 7th Conference on Retroviruses and Opportunistic Infections, 2000;abstract 317.
59 Molla A, Vasavanonda S, Kumar G, *et al*. Human serum attenuates the activity of protease inhibitors toward wild-type and mutant human immunodeficiency virus. *Virology* 1998;**250**:255–62.
60 Rolinski B, Bogner JR, Sadri I, *et al*. Absorption and elimination kinetics of zidovudine in the cerebrospinal fluid in HIV-1-infected patients. *J Acquir Immune Def Syndr* 1997;**15**:192–7.
61 Foudraine NA, Hoetelmans RM, Lange JM, *et al*. Cerebrospinal-fluid HIV-1 RNA and drug concentrations after treatment with lamivudine plus zidovudine or stavudine. *Lancet* 1998;**351**:1547–51.
62 Martin C, Sonnerborg A, Svensson JO, *et al*. Indinavir-based treatment of HIV-1 infected patients: efficacy in the central nervous system. *AIDS* 1999;**13**:1227–32.
63 Blaschke AH, Capparelli EV, Ellis RJ, *et al*. A population model-based approach for determining lamivudine cerebrospinal fluid penetration in HIV-infected adults. In: Program and Abstracts of 7th Conference on Retroviruses and Opportunistic Infections, 2000;abstract 310.
64 Moore KH, Barrett JE, Shaw S, *et al*. The pharmacokinetics of lamivudine phosphorylation in peripheral blood mononuclear cells from patients infected with HIV-1. *AIDS* 1999;**13**:2239–50.
65 Anderson DJ, O'Brien TR, Politch JA, *et al*. Effects of disease stage and zidovudine therapy on the detection of human immunodeficiency virus type 1 in semen. *JAMA* 1992;**267**:2769–74.
66 Krieger JN, Coombs RW, Collier AC, *et al*. Recovery of human immunodeficiency virus type 1 from semen: minimal impact of stage of infection and current antiviral chemotherapy. *J Infect Dis* 1991;**163**:386–8.