was greater with higher drug exposure and the rate of appearance of resistance mutations during monotherapy was found to correlate inversely with the trough plasma drug concentrations.[5] This observation, in addition to later studies demonstrating the important role of protein binding in the determination of necessary drug exposure,[6] laid the foundation for the design of an effective regimen of antiretroviral therapy.

When ritonavir is used at the therapeutic dose of 600 mg bid, many patients experience dose-limiting adverse effects, including gastrointestinal (GI) intolerance, headaches and circumoral numbness.[7] High-dose ritonavir can also cause serum lipid disturbances, including significant increases in serum cholesterol and marked increases in serum triglycerides.[7,8] Owing to these side effects and the subsequent availability of better tolerated PIs, the use of ritonavir as a therapeutic PI is now relatively insignificant.

The combination of ritonavir and saquinavir, the first approved PI that has distinct *in vivo* resistance mutant selections from ritonavir, was brought into clinical studies with the hope that the absence of cross-resistance would delay or prevent the emergence of resistance. These studies resulted in the discovery that co-administration of saquinavir with ritonavir significantly enhanced the plasma exposure of saquinavir and improved efficacy, thus opening a new era of PK enhancement.

### 13.2.3 Drug-resistant Mutations and Pharmacokinetic Profiles

As mentioned earlier, resistance development is a major obstacle to antiviral therapy and all active antiviral agents have been shown to select for resistance mutations. A successful antiviral agent should be aimed at suppressing all existing viral variants, thus preventing the selection of drug-resistant quasi-species and their subsequent evolution. This implies that the number of mutations required for the first escape of the antiviral agent should be greater than the expected number of mutations present in the viral population. In addition, the need to achieve target-site drug concentrations sufficient to suppress viral replication is another key factor in successful antiviral therapy and the prevention of resistance development. For most drugs, plasma concentration can be used as a surrogate marker for the drug concentration at the target site. For example, in HCV or HIV infection, for which the major drug target site is the liver and cells of immune system, respectively, it is the plasma drug concentration, rather than target-site drug concentration, which is monitored to be reached and maintained in order to achieve an antiviral response in the form of viral load reduction. Hence PK parameters that ensure adequate target-site drug concentration become important issues for antiviral drugs used in therapy.

Figure 13.1 helps explain the importance of drug exposure in preventing the emergence of drug-resistant variants. $IC_{50}$ is defined as the *in vitro* drug concentration necessary to inhibit viral replication by 50%. $WIC_{50}$ and $QIC_{50}$ represent the $IC_{50}$ of a drug against heterogeneous virus populations of wild-type and of highly populated quasi-species, respectively; the quasi-species has a