medium-, or high-permeable drugs and to produce transport results that correlate to established human absorption values.

Several modifications of Caco-2 cell model have been tested; for example, CYP3A4-transfected Caco-2 cells are also used to define the biochemical absorption barriers. Oral bioavailability and intestinal drug absorption can be significantly limited by metabolizing enzymes and efflux transporters in the gut. The most prevalent oxidative drug-metabolizing enzyme present in the intestine is cytochrome P4503A4 (CYP3A4). Currently, more than 50% of the drugs in the market metabolized by P450 enzymes are metabolized by CYP3A4. Oral absorption of CYP3A4 substrates can also be limited by the MDR transporter Pgp, as there is extensive substrate overlap between these two proteins. Pgp is an ATP-dependent transporter on the apical plasma membrane of enterocytes that functions to limit the entry of drugs into the cell. There is a significant interaction between CYP3A4 and Pgp in the intestine. Although Caco-2 cells express a variety of uptake and efflux transporters found in the human intestine, a major drawback to the use of Caco-2 cells is that they lack CYP3A4. As such, no data regarding the importance of intestinal metabolism on limiting drug absorption can be obtained from normal Caco-2 cells. Caco-2 cells pretreated with 1,25-dihydroxyvitamin-D3 (vitamin D3) express higher levels of CYP3A4 compared with Caco-2 but still underestimate the amount of CYP3A4 in the human intestine. In the CYP3A4-transfected Caco-2 cells, Pgp can enhance the metabolism of orally dosed drugs by repeated cycling of the drug at the apical membrane.

5.3.4 Animal Model Testing

The quantity of substance available at the preformulation stages is generally small; however, in some instances, early animal testing for absorption potential is needed, particularly if the solid form of the new drug offers many options, such as amorphous forms, solvates, and so on. The absorption models used in animals are well described and will not be discussed here. Establishing good in vitro–in vivo correlation (IVIVC) at this stage proves useful because of a limited access to sufficient compounds to run the entire absorption profiles. The IVIVC analysis can be made extensive, or general conclusions can be drawn from limited studies; the choice depends on the amount of compound available and the nature or robustness of correlation observed.

5.3.5 In Vitro–In Vivo Correlation

The selection of a drug candidate marks the most crucial stage in the life cycle of drug development. Such selection is primarily based on the drug "developability" criteria, which include physicochemical properties of the drug and the results obtained from preliminary studies involving several in vitro systems and in vivo animal models, which address the efficacy and toxicity issues. During this stage, exploring the relationship between in vitro and in vivo properties of the drug in animal models provides an idea about the feasibility of the drug delivery system for a given drug. In such correlations, study designs, including study of more than one formulation of the modified-release dosage forms and a rank order of release (fast/slow) of the formulations, should be incorporated. Even though the formulations and methods used at this stage are not optimal, they prompt better design and development efforts in the future.