chamber. Three portions of this solution are dosed to Caco-2. Monolayer integrity was measured with an epithelia voltohmmeter, from which TEER (transepithelial electrical resistance) is calculated. Aliquot samples are taken from the receiver chamber over a period of 120 min and are analyzed by HPLC. The experiments are performed in both apical to basolateral (A->B) and basolateral to apical (B->A) directions under non-gradient pH conditions (pH7.4 on both sides) and pH-gradient conditions (pH5.5 apically, pH7.4 basolaterally) (Koljonen et al. 2006). Good recovery is important to determine drug permeability accurately.

Metoprolol, propranolol, and atenolol are commonly used as control in permeability measurement. Among them, metoprolol is generally used in manual method, and propranolol in automated method. If the apparent permeability coefficient (Papp) of one drug, in the apical (A) to basolateral (B) direction, is higher than that of metoprolol, the drug may have good permeability. If Papp of (B->A)/(A->B) is higher than 1, the drug may have efflux in the Caco-2 system.

Rat Jejunal Perfusion

Under several conditions, it is preferred to measure drug permeability again with the rat jejunal perfusion method. For example, when drug recovery is low in Caco-2 measurement, the measured drug permeability data may not be accurate. When measured drug permeability is medium to low from Caco-2 study, it is important to confirm the drug permeability with the rat perfusion method. For insoluble drugs, the measured permeability from Caco-2 study may contain large experiment error, and rat perfusion study is often used to evaluate their intestinal adsorption. For several insoluble new chemical entities (NCEs) in clinical development, Caco-2 studies showed they have medium to low permeability, but rat perfusion studies confirmed they have high permeability. The rat perfusion measurements were consistent with the animal and human PK studies.

In one rat jejunal perfusion study, the jejunum was exposed via a midline incision from fasted rats (Swenson et al. 1994). The intestine was gently rinsed with 20 mL warm saline to remove residual contents, then securely fastened at both the proximal and distal incisions. The perfused segment was moistened with saline and covered with Parafilm. The solutions containing 0.25 mg/mL drug of interest, with some surfactants if needed, in isotonic pH6.5 sodium phosphate/sodium sulfate buffer was used to perfuse the intestine. The perfusion solution was kept at 37°C. Perfusate samples were collected over four 15 min intervals. An adsorption rate constant was calculated for each 15 min interval, based on the measured flow rate, the volume of the perfused intestinal segment, as well as the ingoing and outgoing drug concentration. Similarly, the reversibility experiments were executed, except that perfusion was carried out for 4 h, with perfusate collection in 15 min increments. GI stability study was executed at the same time to make sure that the drug did not degrade during the rat perfusion study. Overall, permeability data were average over 3 or 4 rats. The effective intestinal permeability (Peff) of drugs in jejunum will be compared with the value of metoprolol to see whether they have suitable permeability or not.

Biopharmaceutics Classification System Classification

Biopharmaceutical classification depends on both the solubility and permeability of a given drug, and provides a basis for predicting the oral absorption of drugs. In the FDA's Biopharmaceutics Classification System (BCS) Guidance, a drug substance is considered *highly soluble* when the highest dose strength is soluble in <250 mL water over a pH range of 1–7.5, and is considered *highly permeable* when the extent of absorption in humans is determined to be >90% of an administered dose, based on mass-balance or in comparison to an intravenous reference dose. For oral dosage forms of BCS II drugs which have good permeability but poor solubility, the drug bioavailability is mainly limited by the dissolution process; however, the drug bioavailability of BCS IV drugs which have low permeability and poor solubility is limited by not only the dissolution process but also the adsorption process. Based on pH-dependent permeability and solubility, the BCS concepts