Intestinal Permeability of Lamivudine Using Single Pass Intestinal Perfusion

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The intestinal transport of lamivudine, a nucleotide reverse transcriptase inhibitor, was investigated using the single pass intestinal perfusion technique in male Wistar rats. Single pass intestinal perfusion was performed in small intestine at a flow rate of 0.20 ml/min. Lamivudine exhibits a high intestinal permeability over the length of the small intestine indicative of compounds that are well absorbed. The P_{eff} of lamivudine is in the range of drugs with high intestinal permeability and high fraction of dose absorbed indicating that lamivudine readily crosses the intestine. This also suggests that lamivudine belongs to biopharmaceutics classification system class I and is a good candidate for biopharmaceutics classification system based biowaiver. The permeability values obtained from this study may be useful in models of exposure assessment.

Key words: Biopharmaceutic classification system, drug permeability, lamivudine, single pass intestinal perfusion

Lamivudine is a potent inhibitor of the DNA polymerase/reverse transcriptase of hepatitis B virus (HBV). The intracellular triphosphate, which is the active anabolite, acts competitive inhibitor of reverse transcriptase and is incorporated into HIV DNA to cause chain termination. Lamivudine has low affinity for human DNA polymerase, explaining its low toxicity to the host[1-5].

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The pharmacokinetics of lamivudine is well established and is found to be similar in patients with chronic HBV and in HIV-positive patients[6,7].

The biopharmaceutics classification system (BCS) is a drug development tool for the estimation of the contributions of three major factors, solubility, dissolution and intestinal permeability, affecting oral drug absorption from immediate release solid oral products. A biowaiver for clinical bioequivalence or bioavailability studies may be obtained for a class I drug substance whose drug product has rapid and similar dissolution. For a drug like lamivudine which shows good solubility and rapid dissolution the only factor that remains to be determined is the intestinal permeability to apply for a biowaiver status[8].

The intestinal permeability is the propensity of a compound to move across the epithelial barrier of the intestine. Permeability values from animal or cell culture experimental models offer legitimate alternatives to human intestinal permeabilities when human studies are not feasible. There are several standard methods for determining intestinal permeability commonly used for testing xenobiotics. These include (1) diffusion studies with intestinal segments from various species (e.g., rat and rabbit) or with cultured cell monolayers (e.g., Caco-2 cells); (2) uptake studies in brush-border membrane vesicles prepared from intestinal segments of various species and (3) the single pass intestinal perfusion (SPIP) in small mammals, most commonly rats. Whole animal pharmacokinetic studies also provide extremely useful information with respect to the overall exposure to a chemical. The complexity of the *in vivo* models due to the confounding processes of metabolism, distribution, protein binding, gastric emptying and food effects, however, generally precludes determination of the intestinal permeability from *in vivo* studies[9-12].

The main advantage of the *in situ* SPIP technique is the presence of an intact blood and nerve supply in the experimental animals. This methodology is found to be simple and highly accurate for predicting intestinal absorption in humans[13]. The aim of this study is to characterise and classify the intestinal permeability of lamivudine in rats using SPIP model.

Lamivudine was procured from Getz Pharma Research, Navi Mumbai, India. Propranolol hydrochloride and atenolol were obtained from ROAQ Chemicals Pvt. Ltd., Vadodara, India. Urethane was obtained from Dr. Reddy's Lab, Hyderabad, India. Phenol red was obtained from Qualigens Fine Chemicals, Navi Mumbai, India.

Male Wistar rats (250-300 g) were procured from Haffkine Institute, Mumbai, India. Animals were acclimated for a week before use either in the SPIP study or for harvesting intestinal tissue for homogenate preparation. All animal experiments were carried out in accordance with guidelines of Committee for the Purpose of Control and Supervision of Experiments of Animals (CPCSEA) and the study was approved by the Institutional Animal Ethical Committee.

Rats were fasted for 12 h (water *ad libitum*) prior to each experiment. Anaesthesia was induced with urethane (1.25 g/kg, i.p.). To maintain normal body temperature, rats were placed on a heated slide warmer and under a heating lamp. The abdomen was opened with a midline incision and an intestinal segment of approximately 10 cm was measured, isolated and cannulated with plastic tubing. Care was taken to avoid disturbance of the circulatory system and the exposed segment was kept moist with body tempered saline. Initially, the intestinal segment was rinsed with isotonic saline (37°) until the outlet solution was clear. The preparation time took approximately 30 min. The solution containing the substance of interest was given and thereafter followed by a constant perfusion at flow rate (Q_{in}) of 0.2 ml/min was administered. Each perfusion experiment lasted for 60 min and perfusate was quantitatively collected 0, 15, 30 45 and 60 min. The collected samples were then analysed by ultraviolet spectroscopy[14-18]. Krebs–Ringer buffer solution was used as blank perfusion solution[19]. Because water absorption and secretion during the perfusion may cause errors in the calculated P_{eff} values, phenol red was added at a concentration of 100 μg/ml as a nonabsorbable marker to calculate the net water flux (NWF)[20]. Lamivudine was administered at a concentration of 200 μg/ml, whereas propranolol hydrochloride and atenolol were administered at a concentration of 20 μg/ml. The doses of the above mentioned drug were calculated for rat from existing human dose.

Calculations were based on outlet perfusate steady state concentrations achieved after the selected time points. The steady state intestinal effective permeability (P_{eff}) was calculated according to a parallel tube model[21,22].

$$P_{eff,\ rat} = -Q \cdot \{\ln (C_{out}/C_{in})/60 \cdot 2\pi rl\}$$

where Q is the perfusion rate (0.2 ml/min), r is the radius of the intestinal segment (0.18 cm), l is the length of the intestinal segment (10 cm) and C_{in} and C_{out} are the inlet and fluid transport corrected outlet solute concentrations, respectively.

In vivo drug intestinal permeability in humans ($P_{eff,\ man}$) can be predicted from rat $P_{eff,\ rat}$ values according to following formula[13]:

$$P_{eff,\ man} = 3.6 \times P_{eff,\ rat} + 0.03 \times 10^{-4}$$

Compounds with $P_{eff} < 0.03 \times 10^{-4}$ cm/s in the rat small intestine are classified as poorly absorbed whereas compounds with $P_{eff} > 0.2 \times 10^{-4}$ cm/s are completely absorbed. The same classification of *in vivo* absorption may be defined in humans; poorly absorbed compounds have $P_{eff} < 0.1 \times 10^{-4}$ cm/s whereas completely absorbed compounds have $P_{eff} > 0.7 \times 10^{-4}$ cm/s[12,16].

Effective permeability values were calculated from the steady state concentrations of compounds in the perfusate collected from the outlet. Steady state was confirmed by the ratio of the outlet to inlet concentrations (corrected for water transport) versus time. Table 1 shows the intestinal permeability of propranolol hydrochloride, atenolol and lamivudine. In rats P_{eff} for propranolol hydrochloride and atenolol was found out to be 1.14×10^{-4} cm/s and 0.106×10^{-4} cm/s, respectively. Correspondingly, P_{eff} in man for propranolol hydrochloride and atenolol was calculated to be 4.20×10^{-4} cm/s and 0.389×10^{-4} cm/s. For lamivudine, the $P_{eff,\ rat}$ was found out to be 0.33×10^{-4} cm/s and $P_{eff,\ man}$ was found out to be 1.36×10^{-4} cm/s.

Intestinal permeability relates the ability of a compound to move across the epithelial barrier of the intestine and represents a direct measurement of the local absorption rate and reflects the transport velocity across the epithelial barrier, expressed in centimetres per second. In this study, compounds with different physicochemical properties and reported P_{eff} were chosen to test the method suitability for determination of permeability of lamivudine. The intestinal permeability of propranolol hydrochloride and atenolol in rats was found out to be 1.14×10^{-4} cm/s and 0.106×10^{-4} cm/s, respectively. Correspondingly, P_{eff} in man for propranolol hydrochloride and atenolol was calculated to be 4.20×10^{-4} cm/s and 0.389×10^{-4} cm/s. These values are in close agreement with the reported values and hence it indicates the validity of our SPIP procedure to determine the P_{eff}[23,24].

TABLE 1: EFFECTIVE PERMEABILITY OF PROPRANOLOL HYDROCHLORIDE, ATENOLOL AND LAMIVUDINE

Effective permeability (P_{eff}) ($\times 10^{-4}$ cm/s±SD*)					
Lamivudine		Propranolol hydrochloride		Atenolol	
$P_{eff,\ rat}$	$P_{eff,\ man}$	$P_{eff,\ rat}$	$P_{eff,\ man}$	$P_{eff,\ rat}$	$P_{eff,\ man}$
0.33±	1.36±	1.14±	4.20±	0.106±	0.389±
0.010	0.015	0.082	0.236	0.012	0.041

*n, total number of animals=6. SD=Standard deviation

Phenol red was used as a nonabsorbable marker along with compounds under investigation during the *in situ* intestinal perfusion for calculating NWF. Any leakage across the jejuna mucosa makes changes in the intestinal barrier function which is probably one of the main reasons for several contradictory results obtained by the *in situ* model. A stable barrier function was maintained in all the experiments.

For SPIP techniques drugs with $P_{eff,\ rat} > 0.2 \times 10^{-4}$ cm/s and $P_{eff,\ man} > 0.7 \times 10^{-4}$ cm/s can be considered as highly permeable[25,26]. For lamivudine, the $P_{eff,\ rat}$ was found out to be 0.33×10^{-4} cm/s and $P_{eff,\ man}$ was found out to be 1.36×10^{-4} cm/s. These results suggest that lamivudine is a highly permeable drug substance.

In situ SPIP technique provides several advantages over the other methods for determination of effective permeability. This technique provides conditions closer to what is faced following the oral administration, preserved microclimatic condition and less sensitive to pH variations. It provides the unique advantages of the experimental control (compound concentration and intestinal perfusion rate), the ability to study regional differences, factors that may influence the intestinal absorption of the compound. It has an advantage that transporter activity also gets counted in the same experimental condition, this will also help to predict accurate human intestinal permeability and classify the drug candidate according to BCS[26]. But, SPIP technique cannot completely elucidate the transport mechanism, especially for the highly permeable compounds and further studies with cultured cells or isolated tissues (Caco-2 of Madine darby canine kidney epithelial cells (MDCK)) are necessary for determination of both influx and efflux permeability.

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