Study on the intestinal permeability of lamivudine using Caco-2 cells monolayer and Single-pass intestinal perfusion

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Background: The aim of this work is to investigate the intestinal permeability of lamivudine and explore its absorption mechanism.

Method: Caco-2 cells monolayer and single-pass intestinal perfusion (SPIP) were selected for the investigation of lamivudine under different conditions, such as different concentration, absorption time, bidirectional transportation, and transportation with efflux transporters inhibitor. The concentration of lamivudine both in Caco-2 cells monolayer samples and SPIP samples was detected by HPLC-UV. Then the permeability parameters were calculated.

Results: The established HPLC-UV method reach the requirements for detection. There is no statistically difference between absorption parameters of lamivudine both in Caco-2 cells monolayer and SPIP (P > 0.05) under different dose groups. After transportation with efflux transporters inhibitor, the efflux rate of lamivudine in three dose groups was significantly decreased from 2.67, 2.59 and 2.59 to 1.78, 1.61, and 1.81 respectively. Lamivudine exhibits an absorption mechanism of passive diffusion.

Conclusion: The absorption of lamivudine may be related to efflux transporters. In addition, lamivudine is a moderate-permeability drug in Biopharmaceutics Classification System.

1. Introduction

A drug in oral administration is mainly absorbed in intestine. The intestinal permeability is the ability of a compound to pass through intestine and enter the blood circulation, which can directly affect the bioavailability of a compound (Vaidya et al., 2019). Thence, the permeability of drug to pass through intestinal tissue is a key factor for its effectiveness. In the early stages of development new drugs, many drug candidates appeared after synthesis and screening, but many of the new drug candidates are poorly absorbed in body (Tran et al., 2021). Therefore, in the early development of new drugs, investigating the intestinal absorption characteristics of new drugs has excellent significance for further development.

After oral administration, a drug may be absorbed through intestine by single or combination of several mechanisms, many known transporters, including uptake and efflux, are related with drug absorption (Eriksson et al., 2021, Macheras et al., 2020). Three majors of models, which is in-vitro, in-situ and in-vivo, are commonly used for evaluating of permeability. In vitro models generally experiment with cells, artificial membranes, or isolated intestinal tissue (Dahlgren et al., 2018, Fagerholm et al., 1996, Dubbelboer et al., 2019). For drugs transported by passive diffusion, the experimental results obtained by in vitro models are highly relevant to the human body. In-situ model generally refers to the perfusion of drug solution in rat’s intestine with a fixed flow rate, and then permeability values are calculated to evaluate the absorption characters. In vivo method is to establish the pharmacokinetic model in research objective after oral administration of investigation drug; then bioavailability and absorption fraction are estimated by pharmacokinetic parameters.

In previous reports, lamivudine exhibits the absorption mechanism of passive diffusion and carrier-mediated transportation (Gualdesi et al., 2012). In this study, we combination of Caco-2 cells monolayer and SPIP to investigate the intestinal permeability of lamivudine and explore its absorption mechanism.
2. Materials and methods

2.1. Chemicals

Lamivudine (purity 99.7%), metoprolol tartrate (purity 99.9%), and acyclovir (purity > 99.8%) were purchased from the China National Institute for the Control of Drugs (China). Digoxin (purity 99.9%), L-glutamine (purity 99.8%), Lucifer yellow (purity 99.8%) and Elacridar (GF120918, purity 99.8%) were obtained from Sigma-Aldrich. Acetonitrile, methanol (USA) were HPLC grade. Experimental water was made by Milli-Q system (USA). HPLC systems were purchased from Shimadzu (Japan). Processing and analysis of chromatogram were performed on LabSolutions Essentia software. Fluorescence spectrophotometer was obtained from HITACHI (Japan). All other reagents are chromatographically grade.

2.2. Caco-2 cells model

2.2.1. Caco-2 cells culture materials

Caco-2 cells obtained from Shanghai Academy of Life Sciences (China), Trypsin-0.25% EDTA, 100 × Penicillin-Streptomycin Solution, hanks buffer (HBSS), fetal bovine serum (FBS), Dulbecco’s Modified Eagle’s medium (DMEM), HEPES and PBS solution were obtained from Gibco (USA). DMEM was obtained from Sigma-Aldrich (USA). Resistance meter was purchased from Millipore (USA). 24-well transwell plates and 25 cm² cell culture flask were purchased from Corning (USA). CO₂ incubator was obtained from Thermo Scientific (USA).

2.2.2. Cytotoxicity of lamivudine

The cytotoxicity of lamivudine on Caco-2 cells was investigated by MTT assay. In brief, incubation plates (96-well) were seeded with cells at 2 × 10⁴ cells each well and incubated for 24 h. Then, incubation solution was replaced with different concentrations of lamivudine (12, 120 and 1200 µg/mL). After incubation of 24 h, incubation plates were added with 10 µL MTJ solution before another 4 h of incubation. After the upper layer solution was aspirated, 150 µL of DMSO solution was added to the plates, and the absorbance was detected at 630 nm.

2.2.3. Caco-2 cells culture

DMEM (containing 20% FBS, I0 IU/mL penicillin, 2 mM L-glutamine, and 100 µg/mL streptomycin) was used for culture of Caco-2 cells at 37 °C, 5% CO₂ and 90% humidity. After cells grow to 80–90% density, Trypsin-0.25% EDTA was added to digest the cells. 200 µL of cells solution was added to AP side of 24-well transwell and ensure per cm² has 1 × 10⁵ cells, 600 µL of blank DMEM was added to the BL side. Then cells were cultured to 21 days, DMEM was replaced every 48 h in the first week and 24 h in later. TEER value of cells monolayer was monitored (>300 Ω·cm²2 can be employed).

2.2.4. Caco-2 cells transport study

Cells monolayer was washed twice by transport buffer (37 °C HBSS, containing 10 mM HEPES), AP side and BL side was added with 200 µL and 600 µL transport buffer to preincubate for 30 min. Donor side was added 200 µL (AP) or 600 µL (BL) of drug solution, receiver side was added with HBSS. Transportation samples were taken after a 2 h of transport experiment, and then centrifuged at 16000 rpm for 5 min, and injected to HPLC or Fluorescence spectrophotometer (for lucifer yellow only), then the Papp and efflux rate was calculated. The transport study of high-permeability control drug metoprolol tartrate (800 µg/mL) and low-permeability control drug acyclovir (600 µg/mL) was investigated in absorption direction from AP to BL without efflux transporters inhibitor elacridar (GF120918). Lucifer yellow (20 µg/mL) was studied to verify the tight junction of Caco-2 cells and transported without GF120918 on the absorption direction. Digoxin was studied as the efflux control drug, a concentration of 30 µg/mL digoxin was investigated in bidirectional transport with and without GF120918. The bidirectional transport of lamivudine was investigated under 12, 120 and 1200 µg/mL, with and without GF120918.

2.2.5. Data analysis

\[ P_{\text{app}} = \frac{V_R}{A_r \times T \times C_o} \]  
\[ \text{ER} = \frac{P_{\text{app}}(\text{BL} - \text{AP})}{P_{\text{app}}(\text{AP} - \text{BL})} \]  
\[ \%\text{lucifer yellow} = \frac{V_{\text{BL}} \times RFU_{\text{BL}} + V_{\text{AP}} \times RFU_{\text{AP}}}{V_{\text{BL}} \times RFU_{\text{BL}} + V_{\text{AP}} \times RFU_{\text{AP}}} \times 100 \]

where \( V_R \) is the volume of receiver side (200 µL in AP side and 600 µL in BL side); \( C_o \) is the concentration of source side; \( A_r \) is the area surface of 24-well plates (0.33 cm²); \( T \) is the transport time (7200 s); \( C_i \) is the investigated concentration; \( P_{\text{app}}(\text{BL} - \text{AP}) \) and \( P_{\text{app}}(\text{AP} - \text{BL}) \) represent the \( P_{\text{app}} \) in the direction of efflux and uptake respectively; \( V_{\text{BL}} \) and \( V_{\text{AP}} \) are the volume in BL side and AP side respectively; \( RFU_{\text{BL}} \) and \( RFU_{\text{AP}} \) are the relative fluorescence units of lucifer yellow in BL side and AP side respectively.

2.3. The in-situ single-pass intestinal perfusion in rats

2.3.1. Animal

All experimental protocols were supervised and managed by Jiangsu Provincial Drug Safety Evaluation Center Agency Committee and the experiments were conducted in accordance with the guide for the related laws and regulations and Institutional Animal Care and Use Committee (IACUC). SD rats (half male and half female) weighing 280–320 g (Zhejiang Vital River Laboratory Animal Technology Co., Ltd., Zhejiang, China), 30 of SD rats were raised with standard condition for one week before, and free access to diet and water. Rats are divided into five groups by computer-generated random numbers according to their weight.

2.3.2. Buffer solution (K-R solution)

Krebs – Ringer (K-R) buffer solution was used as perfusion solution (Adjust pH to 6.8 with phosphoric acid). K-R solution was prepared each day.

2.3.3. SPiP in rats

Before perfusion experiment, rats were fasted for 12 h (with free access to water). Pentobarbital solution (30 mg/kg, i.p.) was used for anaesthesia of rats. Rats were fixed on an operating plate, and kept normal body temperature with warm light. The abdomen of rats was opened follow midline incision, about 10 cm of intestine segment was measured, separated and intubated with a plastic tube. The exposed intestine was kept moist by 37°C saline to keep the normal circulatory system. Isotonic saline was used to flush the experiment intestine until the solution at the outlet becomes clear, then the drug perfusion experiment was conducted. Warmed K-R buffer solution (37°C) containing the investigated drug was used as perfusion solution. The perfusion concentration of metoprolol tartrate and acyclovir is 120 µg/mL; lamivudine was perfused at concentration of 12, 120 and 1200 µg/mL. The experiment intestine was perfused with drug solution for 110 min, 0.2 mL of drug solution was pumped at inlet in every min-
ute. After 30 min perfusion, the solution at outlet was collected in every 20 min. The weight of solution at inlet and outlet was recorded for the correction of concentration. After perfusion, recording the length and radius of perfusion intestine. Before HPLC analysis, drug solution was centrifuged for 5 min at 16000 rpm.

2.3.4. Data analysis
  The following equations are used for the calculation of $P_{eff}$ and $K_{eff}$:

$$P_{eff} = \frac{-Q \times \ln \left( \frac{C_{out} \cdot V_{out}}{C_{in} \cdot V_{in}} \right)}{2\pi l} \tag{4}$$

$$K_{eff} = \frac{Q \times \left( 1 - \frac{C_{out} \cdot V_{out}}{C_{in} \cdot V_{in}} \right)}{\pi l^2} \tag{5}$$

where $C_{in}$ is the experiment concentration of a drug, $C_{out}$ refers to the concentration at outlet, $V_{out}$ and $V_{in}$ refer to the volume that collected at the outlet and the volume that perfusion solution reduced at the inlet, $Q$ is the flow rate (0.2 mL/min) pumped into intestine, $r$ and $l$ are the recorded radius and length after perfusion.

2.4. Determination of experimental samples

The determination of metoprolol tartrate, acyclovir, digoxin, and lamivudine both in Caco-2 and SPIP was performed on HPLC-UV. Same mobile phase was used for separation of drug in samples. HPLC-UV conditions are shown in Table 1. Digoxin was eluted by gradient procedure, as shown in Table 2.

The determination of lucifer yellow was performed on fluorescence spectrophotometer. The concentration of lucifer yellow in transport samples of Caco-2 transport study was calculated by the relative fluorescence units (RFU), then the permeability of lucifer yellow ($\%$lucifer yellow) was calculated.

2.5. Statistical analysis

Values are expressed as mean ± SD, $n = 3$ in Caco-2 cells monolayer transportation; $n = 6$ in SPIP perfusion. Differences between groups were assessed by t-test and ANOVA using SPSS 23. Differences was considered statistically significant at $P < 0.05$.

3. Results

3.1. HPLC-UV analysis method validation

The determination of metoprolol tartrate, acyclovir, digoxin, and lamivudine both in Caco-2 and SPIP was performed on HPLC-UV. The retention time of metoprolol tartrate, acyclovir and lamivudine under the HPLC-UV conditions described in “2.4” was 6.628, 7.295 and 5.785 min for SPIP perfusion samples; 5.948, 7.867 and 6.463 min for Caco-2 cells transport samples, respectively. The retention time of digoxin was 6.654 min in Caco-2 cells transport samples. The HPLC-UV chromatograms of lamivudine are shown in Fig. 1. The validation results of HPLC-UV are shown in Table 1. Results showed that the established HPLC-UV methods were specific, stable, and sensitive.

3.2. Cytotoxicity test

MTT assay results shows that the growth ability of Caco-2 cells did not significantly affect by different concentrations of lamivudine. After 24 h incubation with 1200 µg/mL lamivudine, the cell viability was higher than 80%. Therefore, we conducted the transportations at concentrations of 12, 120, and 1200 µg/mL lamivudine.

3.3. Caco-2 cells monolayer transport studies

The transport results of control drugs are shown in Table 4. Without 10 µM GF120918, the $P_{app}$ of metoprolol tartrate and acyclovir on the absorption direction (AP-BL) was (160.10 ± 6.52) and (0.81 ± 0.11) $\times 10^{-7}$ cm/s (mean ± SD, $n = 3$); with the presence of efflux transporters inhibitor GF120918, the $E_{p}$ of digoxin decreased from 132.81 to 1.43. The transport result of lucifer yellow ($\%$lucifer yellow) was calculated to be (0.81 ± 0.03) %, indicating that the tight junction between Caco-2 cells had been formed.

As shown in Table 5 and Fig. 2, with the absence of GF120918, $P_{app}$ values in uptake direction were (2.96 ± 0.05), (2.97 ± 0.15), and (2.85 ± 0.44) $\times 10^{-7}$ cm/s at 12, 120, and 1200 µg/mL lamivudine. Table 5 also shows the efflux rate of lamivudine was 2.67, 2.59 and 2.59 at different concentrations. After transport with efflux transporters inhibitor GF120918, the efflux rate decreased to 1.78, 1.61, and 1.81 at the three dose concentrations, respectively, as shown in Fig. 3.

The $P_{app}$ difference of lamivudine in the three dose groups at the direction of absorption is not statistically significant ($P > 0.05$), the independency on $P_{app}$ and concentration indicating lamivudine exhibits an absorption mechanism of passive diffusion. The $P_{eff}$ and $K_{eff}$ of lamivudine after perfusion of 12, 120 and 1200 µg/mL through rats intestine are shown in Table 6. $P_{app}$ values of lamivudine at different perfusion times under different dose

### Table 1
HPLC-UV conditions for determination drugs.

| Drug               | Metoprolol tartrate | Acyclovir | Digoxin | Lamivudine |
|--------------------|---------------------|-----------|---------|------------|
| Mobile phase       | A: acetonitrile: B: 60 mM ammonium acetate containing 3.0 mL of phosphoric acid, 2.0 mL of trimethylamine and 10.0 mL of glacial acetic acid (A:B = 25:75) | A: methanol; B: water (A:B = 10:90) | A: acetonitrile : water (10:90); B: acetonitrile : water (60:40) | A: methanol; B: 25 mM ammonium acetate (adjusted pH to 3.8 with glacial acetic acid) (V/V = 15: 85) |
| Flow-rate (mL/min) | 1.0                 | 1.0       | 1.5     | 1.0        |
| Wavelength (nm)    | 280                 | 254       | 230     | 277        |
| Injection volume (µL) | 10                  | 10        | 10      | 20 µL of transportation solution; 10 µL of perfusion solution |
| Column temperature | Room temperature    | Room temperature | 50 °C   | Room temperature |

### Table 2
Gradient elution procedure of digoxin.

| Run time (min) | A(%) | B(%) |
|---------------|------|------|
| 0             | 60   | 40   |
| 5             | 60   | 40   |
| 15            | 0    | 100  |
| 15.1          | 60   | 40   |
| 20            | 60   | 40   |
Table 3
Validation results of HPLC-UV.

| Drug                | Standard curve                      | Accuracy (\(\%\), \(n = 6\)) | Precision (RSD, \(n = 6\)) | Stability (\(\%\)) |
|---------------------|-------------------------------------|--------------------------------|-----------------------------|---------------------|
|                     |                                     | Con (\(\mu g/mL\)) | In Caco-2 | In SPIP | In Caco-2 | In SPIP | In Caco-2 | In SPIP |
| Metoprolol tartrate | \(y = 2542.7x + 40.503\) (0.25 ~ 25 \(\mu g/mL\), \(R^2 = 0.999\)) | 0.5 | 99.98 | 98.24 | 0.76 | 1.43 | 100.79 | 98.34 |
|                     |                                     | 10 | 100.54 | 99.63 |                |                |                |                |
|                     |                                     | 20 | 100.32 | 98.34 |                |                |                |                |
| Acyclovir           | \(y = 39966x - 1140.8\) (0.1 ~ 5 \(\mu g/mL\), \(R^2 = 1\)) | 0.25 | 100.03 | 98.32 | 0.83 | 1.75 | 99.68 | 98.64 |
|                     |                                     | 1 | 98.92 | 98.54 |                |                |                |                |
|                     |                                     | 4 | 99.43 | 99.26 |                |                |                |                |
| Digoxin             | \(y = 13521x - 421.28\) (0.25 ~ 10 \(\mu g/mL\), \(R^2 = 0.999\)) | 0.5 | 97.75 | ND | 0.88 | ND | 98.55 | ND |
|                     |                                     | 2.5 | 100.84 |                |                |                |                |                |
|                     |                                     | 8 | 99.59 |                |                |                |                |                |
| Lamivudine          | \(y = 47840x + 1128.7\) (0.025 ~ 2.5 \(\mu g/mL\), \(R^2 = 0.999\)) | 0.05 | 100.55 | 98.64 | 1.29 | 0.98 | 99.56 | 99.45 |
|                     |                                     | 0.5 | 99.98 | 99.43 |                |                |                |                |
|                     |                                     | 2 | 99.54 | 98.45 |                |                |                |                |

*ND: not detection.

Table 4
Transport results of control drugs. ND: not detection.

| Control drug       | \(+\)/-10 \(\mu M\) GF120918 | \(P_{app} \times 10^{-7}\) cm/s | \(E_R\) |
|--------------------|--------------------------|-------------------------------|-------|
|                    |                          | AP-BL | BL-AP |                   |       |
| Metoprolol tartrate| –                        | 160.10 ± 6.52                | ND    | ND    |
| Acyclovir          | –                        | 0.81 ± 0.11                  | ND    | ND    |
| Digoxin            | –                        | 0.90 ± 0.05                  | 119.25 ± 3.12 | 132.81 |
|                    | +                        | 35.92 ± 1.28                 | 51.53 ± 0.58 | 1.43   |

Table 5
Transport results of different concentrations lamivudine in Caco-2 cells monolayer.

| Lamivudine(\(\mu g/mL\)) | \(+\)/-10 \(\mu M\) GF120918 | \(P_{app} \times 10^{-7}\) cm/s | \(E_R\) |
|---------------------------|--------------------------|-------------------------------|-------|
|                            |                          | AP-BL | BL-AP |                   |       |
| 12                         | –                        | 2.96 ± 0.05                  | 7.88 ± 0.44 | 2.67 |
|                            | +                        | 3.07 ± 0.08                  | 5.48 ± 0.11 | 1.78 |
| 120                        | –                        | 2.97 ± 0.15                  | 7.70 ± 0.27 | 2.59 |
|                            | +                        | 3.22 ± 0.07                  | 5.19 ± 0.08 | 1.61 |
| 1200                       | –                        | 2.85 ± 0.11                  | 7.37 ± 0.09 | 2.59 |
|                            | +                        | 2.93 ± 0.04                  | 5.31 ± 0.12 | 1.81 |

Fig. 1. HPLC-UV chromatograms of lamivudine. A: blank solution; B: sample solution in SPIP; C: sample solution in Caco-2 cells monolayer.
groups are shown in Fig. 4. $P_{\text{eff}}$ values of lamivudine at different perfusion times under different dose groups.

Table 6

| Lamivudine (µg/mL) | Perfusion times (min) | $P_{\text{eff}}$ ($\times 10^{-5}$ cm/s) | $P_{\text{eff}}$ ($\times 10^{-5}$ cm/s) | $K_a$ ($\times 10^{-4}$/s) | $K_a$ ($\times 10^{-4}$/s) |
|------------------|----------------------|----------------------------------------|----------------------------------------|--------------------------|--------------------------|
| 12               | 50                   | 1.84 ± 0.55                            | 2.16 ± 0.68                            | 1.44 ± 0.32              | 1.69 ± 0.40              |
|                  | 70                   | 1.22 ± 0.29                            | 1.45 ± 0.38                            | 1.44 ± 0.32              | 1.69 ± 0.36              |
|                  | 90                   | 1.44 ± 0.32                            | 1.46 ± 0.17                            | 1.44 ± 0.46              | 1.53 ± 0.44              |
| 120              | 110                  | 1.24 ± 0.14                            | 1.46 ± 0.17                            | 1.44 ± 0.46              | 1.53 ± 0.44              |
| 1200             | 50                   | 1.45 ± 0.36                            | 1.61 ± 0.50                            | 1.64 ± 0.25              | 1.64 ± 0.41              |
|                  | 70                   | 1.29 ± 0.26                            | 1.41 ± 0.32                            | 1.64 ± 0.25              | 1.64 ± 0.41              |
|                  | 90                   | 1.45 ± 0.41                            | 1.60 ± 0.47                            | 1.64 ± 0.25              | 1.64 ± 0.41              |
|                  | 110                  | 1.37 ± 0.46                            | 1.49 ± 0.44                            | 1.64 ± 0.25              | 1.64 ± 0.41              |

between 0.03 and 0.2 ($\times 10^{-4}$ cm/s), indicating that lamivudine exhibits a moderate permeability in BCS.

4. Discussion

Among all the models for permeability study, data from in to vivo model can truly reflect the absorption of drugs in the body system, but it is difficult to get. Caco-2 cells monolayer and in-situ SPIP are commonly selected as experimental models for investigation of intestinal permeability (Sirisha et al., 2018, Chen et al., 2020, Yang et al., 2018). The retention time of metoprolol tartrate, acyclovir and lamivudine under the HPLC-UV conditions showed that the established HPLC-UV methods were specific, stable, and sensitive. MTT assay results shows that the growth ability of Caco-2 cells did not significantly affect by different concentrations of lamivudine.

Lamivudine structurally like nucleotides, generally used as a treatment for hepatitis B virus (He et al., 2018). After oral administration, the active metabolite—lamivudine triphosphate acts as a competitive inhibitor of reverse transcriptase, can incorporate into the replication process of hepatitis B virus DNA to prevent chain elongation (Yap et al., 2019, Woo et al., 2020). Lamivudine has a low affinity in human DNA polymerase, does not interfere the normal DNA synthesis in human (Reis et al., 2013). The bioavailability of lamivudine was reported to exceed 80%, and the pharmacokinetic characteristics show a linear kinetic process (Du et al., 2019, Yuen et al., 1995). About 70% of lamivudine is eliminated unchanged by kidneys, with little metabolism in the liver (Sutton et al., 2001). The permeability of lamivudine is reported to be high, low, and borderline in different publications, whereas the reported
permeability classification of lamivudine is investigated by single method or predicated on its bioavailability in human (Dezani et al., 2016, Lindenberg et al., 2004, Wu et al., 2005).

With the absence of efflux transporters inhibitor, lamivudine transport was observed to have a significant efflux effect (efflux rate higher than 2), with the presence of efflux transporters inhibitor, the efflux rate was significantly decreased, which indicated that the absorption of lamivudine may be related to efflux transporters. In addition, the $P_{app}$ of lamivudine on Caco-2 cells monolayer is between high-permeability and low-permeability, which indicated the permeability of lamivudine is moderate-permeability in BCS.

During perfusion, the intestine is absorbing and secreting water, which affects the accurate determination of concentration changing. It is necessary to correct the net water flux (NWF). Method for NWF correction during the SPIP perfusion usually involves the simultaneous perfusion with phenol red, it is conventionally considered to be not absorbed in the intestine, thus it has long been used as a correcting substance for correcting the NWF. However, during the pre-experiment process, we found that phenol red may affect the calculation of $P_{eff}$. For high permeability drugs, the effect phenol red is negligible, but for drugs with low permeability, phenol red may have an obvious effect. Therefore, gravimetric method was used to correct the NWF during the perfusion in rat intestine in this study.

After perfusion through rat intestine, the $P_{eff}$ value of high-permeability control drug (metoprolol tartrate) and low-permeability control drug acyclovir was $(6.13 \pm 2.49) \times 10^{-6}$ cm/s and $(0.282 \pm 0.208) \times 10^{-5}$ cm/s (mean $\pm$ SD, $n = 6$) respectively. A drug with $P_{eff} > 0.2 \times 10^{-4}$ cm/s in SPIP, it can be considered as high permeability drug in BCS, and it is absorbed completely whereas drugs with $P_{eff} < 0.03 \times 10^{-4}$ cm/s is classified as low permeability in BCS, which are poorly absorbed (Fagerholm et al., 1996). The $P_{eff}$ of selected control drug in this study is in accordance with the above standard, which indicated the reliability of SPIP model in this work. Transport results of control drug shown that the Caco-2 cells monolayer of this experiment expresses the functions of permeability and efflux transportation, can be used for the permeability investigating of lamivudine.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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