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Determination of Site of Absorption of Simvastatin in Rat Gut Using In Situ Single-Pass Intestinal Perfusion

Author Name – Mr. Akhil M. Shaikh[1*] Prof. Dr. Dinesh Kumar Upadhyay[2] Prof. Dr. Quaizi Md Aamer[3], Dr. Anasuya Patel[4]

1* Research Scholar, School of Pharmaceutical Science at Jaipur National University, Jaipur, India.

2. Professor, School of Pharmaceutical Science at Jaipur National University, Jaipur, India.

3. Professor, K. T. Patil College of pharmacy, Osmanabad, India

4. Group Leader at Wockhardt Research Center Aurangabad, India

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Abstract

Simvastatin is a widely prescribed drug for management of dyslipidemia. It is reported to have low oral bioavailability possibly due to poor oral absorption or extensive first pass metabolism. In the present study, beneficial effect of bioenhancer piperine on the intestinal model in rats. Abdomen of overnight fasted anaesthetized rats (3 per group) was cut common and the jejunum segment (10 cm) of small intestine was exposed and cannulated at both the ends using PE tubings. Through one end buffer solutions containing either simvastatin (30 µg/mL) or simvastatin (30 µg/mL) in combination with piperine (20 µg/mL) were perfused at a flow rate of 0.2 ml/min and the perfusate samples were collected at different time points up to 2 h. Phenol red (30µg/ml) was added to both the solutions as non-absorbable biomarker to calculate net water flux. The samples were estimated for simvastatin levels and effective permeability (Peff) was calculated. The Peff value estimated for the combination group was significantly improved compared to alone simvastatin group, suggesting beneficial role of piperine in the oral absorption of simvastatin. Thus piperine can be combined with simvastatin to improve its oral bioavailability, however further studies are required to confirm this findings.

Key words: *Piperine; simvastatin; bioavailability; Black pepper; SPIP*

Introduction

The biopharmaceutics classification system (BCS) can be used to estimate the three main parameters that affect oral drug absorption: intestinal permeability, dissolution, and solubility. According to BCS classification, the medicines are divided into four classes: Class I (high permeability and solubility), Class II (high permeability and low solubility), Class III (low permeability and high permeability), and Class IV (low permeability and low solubility). Using this method, several of the most widely used antihyperlipidemic medications are classified under Class II.[1] Simvastatin is mostly absorbed in the small intestine, where the presence of esterase's and the pH cause some of the drug to hydrolyze into simvastatin acid.[2] Simvastatin undergoes significant hepatic first-pass metabolism following absorption. Simvastatin enters the liver through passive diffusion, while the produced simvastatin acid is carried into the human liver via both passive diffusion and uptake transporter OATP1B1. [3] Simvastatin is significantly metabolized by CYP3A4 and CYP3A5. The bioavailability of simvastatin in rats as well as human is reported to be low. Co-administration of bioenhancer can increase the permeability of such low-permeability medicines. When bioenhancer are combined with a therapeutic medicine, the pharmacological action of the drug is enhanced. Such enhancers have been found to increase the bioavailability of a number of medicines at extremely low dosages. [4] In general, they can improve bioavailability by increasing passive diffusion or inhibiting efflux transporters such as P-gp and other multidrug resistance proteins (MRPs). Piperine, an active constituent of Black pepper *(piper nigrum)* is known to enhance the bioavailability of many drugs.[5] In the present study, the effect of piperine on the intestinal permeability of simvastatin is studied using in vitro SPIP models.[6]

Material and Methods

Material

Simvastatin and urethane was obtained from Wockhardt research center. Propranolol hydrochloride was obtained as gift samples from IPCA laboratories, Mumbai. Phenol red was procured from Sigma Aldrich, India and other chemicals and solvents were of analytical grade and procured from commercial sources. Black pepper was obtained from the local market and extracted.

Animals

Male Wistar rats of body weight 200-250 g and age 6-8 weeks were procured from Wockhardt Research Center, Aurangabad. All animal experiments were carried out in accordance with guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the study protocols were approved by Wockhardt's Animal Ethics Committee. The animals were kept under well-regulated room condition with 12 h light/dark cycle maintained. The rats were provided pelleted diet and drinking water *ad libitum.* Only during the experimentation period, the animals were made to fast, while they were allowed free access to drinking water.

Extraction of piperine: Approximately 350 g of powdered black pepper was extracted in a Soxhlet extractor at 60–70 °C using 1.5 liters of 95% ethanol. At 60 °C, the solution was filtered and vacuum-concentrated on a water bath. The filtrate residue was mixed with 10% w/v alcoholic potassium hydroxide and refrigerated overnight at 2–8 °C. Following their deposit in the flask's

bottom, the yellow piperine crystals were cleaned and purified using organic solvents. Using acetone, recrystallization was carried out. Crystals of piperine were kept for further use.

Analysis of piperine: The isolated piperine was analyzed by high performance liquid chromatography (HPLC) with a Microsorb-MV 100-5 C18 column maintained at 37 °C, a mobile phase of acetonitrile and 0.1% orthophosphoric acid (60:40), a UV-visible detector set at 340 nm, and a flow rate of 1 ml/min.

In vivo studies using single pass intestinal perfusion method (SPIP): The intestinal permeability of Simvastatin was investigated using SPIP technique in Wistar rats. [7] Rats (n=9) were acclimatized for a week before use in the SPIP study. Rats were fasted for 12 h (water ad libitum) prior to each experiment. Anesthesia was induced with urethane (1.25 gm/kg, i.p.). The abdomen was opened with a midline incision and jejunum segment of the intestine was exposed. Approximately 10 cm of the segment was measured and cannulated at both end with plastic tubing (PE350 tubing). Initially, the intestinal segment was rinsed with isotonic saline until the outlet solution was clear. In 3 cannulated rats, simvastatin solution (30 µg/ml) prepared in Krebs-Ringer buffer and in other 3 rats, simvastatin (30 µg/ml) and piperine (20 µg/ml) solution prepared in Krebs-Ringer buffer was infused by a constant perfusion at a flow rate of 0.2 ml/min. Phenol red (30µg/ml) was added to both the solutions as non-absorbable biomarker to calculate net water flux. Each perfusion experiment lasted for 60 min and the perfusate was quantitatively collected at 15, 30, 45 and 60 minutes after the achievement of steady state. The collected perfusate samples were then analyzed by HPLC for estimation of simvastatin and phenol red. Propranolol hydrochloride (80 µg/ml) was used as high permeability markers to validate the experimental procedure. Effective intestinal permeability was calculated from the steady-state concentrations of simvastatin in the perfusate collected from the outlet using the below formula

$$P_{eff}\,(cm/s) = [-Q\ In\ (C_{out} / C_{in})] / 2\pi RL$$

Where,

P_{eff} = Effective Permeability coefficient

Q = Perfusion Flow rate (0.2ml/min)

L = Length of perfused intestinal segment (Approximately 10 cm)

R = Radius of the rat small intestine (jejunum = 0.18 cm)

C_{out} = corrected outlet concentration of the drug),

C_{in} = Inlet drug Concentration

The intestinal Net Water Flux was calculated using following formula:

$$NWF = (1-[ph.red_{out}/\ ph.\ red_{in}] \times Q_{in}) / L$$

Where, ph. Red_{out} and ph. Red_{in} are the inlet and outlet concentrations.

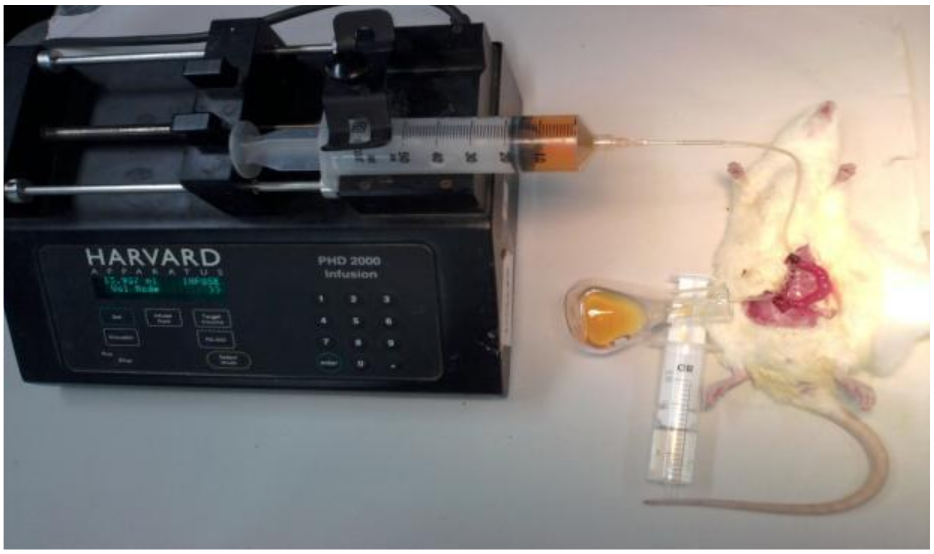


Fig. 1: Experimental SPIP Model

Determination of Simvastatin by HPLC

All the samples were analyzed for simvastatin concentration using HPLC. The HPLC system consisted of an Agilent 1100 series with a UV detector. The data integration was done using chemstation software. The mobile phase comprising of 10 mm ammonium formats and Acetonitrile (10:90 v/v %) were passed through the column at a flow rate of 1ml/min. The column used was Zorbax SB C18 (75 x 4.6mm, 3.5µm) with Fortis C8, 4mm guard column and column oven temperature was adjusted at 30 °C. The run time was set at 15 minutes. Detection was done by UV Detector set at a wavelength of 340 nm.

Statistical analysis: Statistical significance between simvastatin alone and simvastatin in combination with piperine was determined through student's t-test using Graph pad prism software (version 5). $P<0.05$ was considered as statistically significant value.

Results

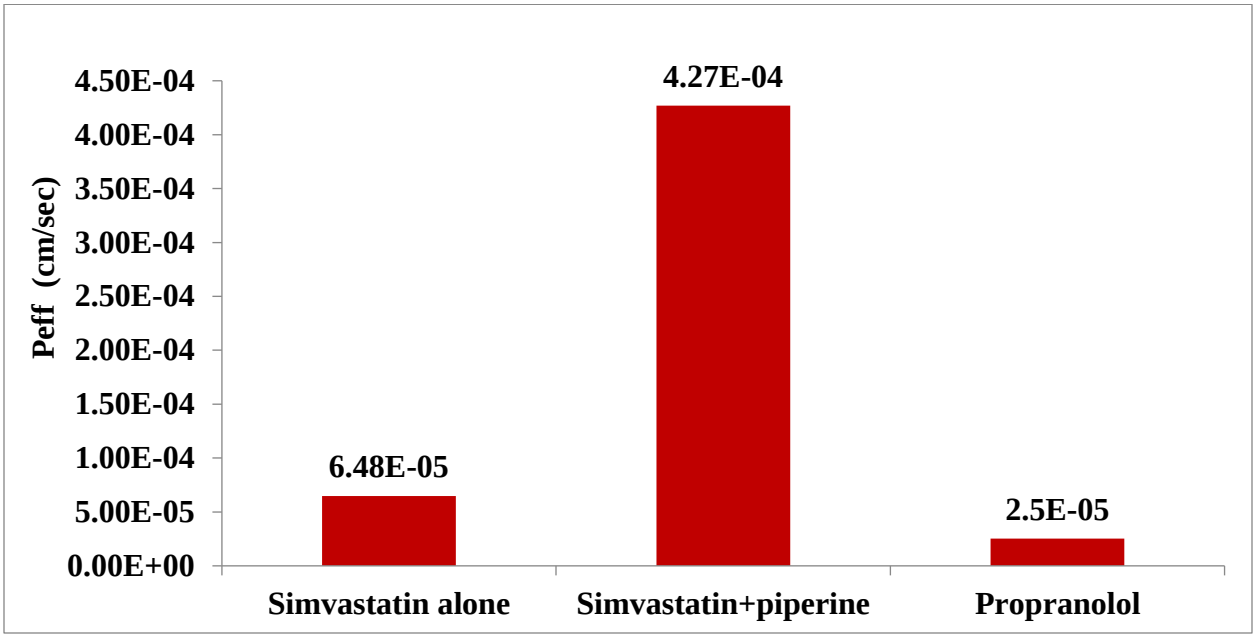
In the present study (Peff) effective intestinal permeability of simvastatin was determined through rat jejunum segment using in situ single pass intestinal perfusion technique and the samples were analyzed by reverse phase HPLC. Effective permeability values were calculated from the steady state concentration of compounds in the perfused collected from the outlet. Table 1 and Figure 1 provides the effective permeability of simvastatin alone and simvastatin in combination with piperine in rats. The Peff of Simvastatin alone in rats was found to be 6.48E-05 cm/sec and simvastatin in combination with piperine was found to be 4.27E-04 cm/sec in rats. This indicated that piperine significantly improved the intestinal permeability of simvastatin. The Peff value of propranolol used as references standard was found to be 2.51E-05 cm/sec which is in agreement with the reported studies. This indicates the validity of the SPIP method.

Table 1: Effective permeability (P_{eff}) of simvastatin alone and in combination with piperine in Wistar rats using the SPIP model.

P_{eff} (cm/sec)			
Rat No.	**Simvastatin alone**	**Simvastatin+Piperine**	**Propranolol**
1	9.209E-06	1.860E-04	5.374E-05
2	9.124E-05	2.568E-04	1.348E-05
3	9.393E-05	8.381E-04	8.198E-06
Mean	**6.48E-05**	**4.27E-04***	**2.51E-05**
SD	**4.82E-05**	**3.58E-04**	**2.49E-05**

*P<0.05 was considered as statistically significant effect compared to simvastatin alone group using the unpaired Student's t test.

Figure 2. Rat intestinal permeability of Simvastatin in the absence and presence of Piperine. ***** $P < 0.05$ as compared to Simvastatin



Discussion

In situ SPIP model is considered as a suitable model for predicting oral absorption of drug formulations. This model has several benefits, the most important being the undisturbed intestinal physiology that allows to study the role of transporters and intestinal CYP enzymes in the oral absorption of drugs. However, in this model role of hepatic enzymes cannot be determined. Simvastatin is reported to show low oral bioavailability and large variability possibly due to liver mediated first pass metabolism or poor oral absorption. Several formulation based approaches have been tried earlier with simvastatin to improve its oral bioavailability. [9] However, in the present study using SPIP model, the improvement in the simvastatin's intestinal permeability has been

determined in combination with herbal bioenhancer. Several herbal bioenhancer have been reported to improve the oral absorption of drugs and one of them is piperine.

In the present study, effective permeability was determined for simvastatin alone and simvastatin in combination with piperine. Effective permeability was also determined for propranolol to validate the model. In the study, simvastatin alone showed comparative Peff value to propranolol which is reported to have high permeability. [10,11] Thus simvastatin poor oral bioavailability does not seems to be due to poor oral absorption but could be mainly attributed to extensive metabolism through liver. However, surprisingly combination of simvastatin with piperine further significantly improved the oral absorption of simvastatin. [12] This suggests that there could be some involvement of intestinal transporters or intestinal metabolizing enzymes in the oral absorption of simvastatin.[13] Apart from being a P-glycoprotein substrate, simvastatin absorption from the gut is due to passive diffusion and active transport via the PEPT1 transporters.[14] Piperine is known to alter membrane dynamics and permeation properties, which could have possibly, enhanced simvastatin's absorption by passive diffusion. Additionally, piperine is known to block drug efflux pumps, one of which is the Pgp efflux transporter. Piperine could have lowered the efflux of simvastatin which is a Pgp substrate, thereby increasing simvastatin permeability. Simvastatin is also reported to be a substrate of CYP3A enzyme located in both liver and intestine. [16] Piperine due to its ability to inhibit CYP3A enzymes could have helped to increase the oral absorption of simvastatin. [18,19] Further mechanistic studies are required to ascertain the piperine's role in the oral absorption of simvastatin.

Conclusion

Piperine and Drug Absorption:

Piperine, a bio-enhancer, is known to improve the bioavailability of various drugs by inhibiting enzymes that metabolize drugs in the intestines and liver. This study focused on its effect on simvastatin, a cholesterol-lowering drug.

In Situ Single-Pass Intestinal Perfusion:

This technique involves perfusing a segment of the intestine with a drug solution and measuring the drug concentration entering and leaving the segment. It helps in determining the drug's permeability and absorption site.

Effective Permeability:

Effective permeability (Peff) is a measure of how easily a drug can pass through the intestinal wall. High Peff indicates better absorption and bioavailability.

Significant Findings:

The study found that piperine significantly increased the Peff of simvastatin. This suggests that piperine enhances the intestinal absorption of simvastatin, potentially leading to higher blood concentrations and improved therapeutic effects.

Predictive Value for New Drugs:

The method used in this study can be applied to other new chemical entities to predict their absorption profiles. It provides valuable insights during the drug development process to enhance bioavailability.

Implications for Drug Development:

Understanding how piperine and similar compounds affect drug absorption can lead to the development of more effective oral formulations. Enhancing drug absorption can reduce the required dosage and minimize side effects.

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