

Impact of Micellar Vehicles on *in situ* Intestinal Absorption Properties of Beta-Lapachone in Rats

Soung Baek Jang¹, Dongju Kim¹, Seong Yeon Kim¹, Changhee Park¹, Ji Hoon Jeong², Hyo-Jeong Kuh³, and Jaehwi Lee¹

¹College of Pharmacy, Chung-Ang University, Seoul 156-756, ²Department of Pharmacology, College of Medicine, Chung-Ang University, Seoul 156-756, ³Department of Biomedical Science, College of Medicine, The Catholic University of Korea, Seoul 137-701, Korea

The aim of the present study was to examine the effect of micellar systems on the absorption of beta-lapachone (b-lap) through different intestinal segments using a single-pass rat intestinal perfusion technique. B-lap was solubilized in mixed micelles composed of phosphatidylcholine and sodium deoxycholate, and in sodium lauryl sulfate (SLS)-based conventional micelles. Both mixed micelles and SLS micelles improved the *in situ* permeability of b-lap in all intestinal segments tested although the mixed micellar formulation was more effective in increasing the intestinal absorption of b-lap. The permeability of b-lap was greatest in the large intestinal segments. Compared with SLS micelles, the effective permeability coefficient values measured with mixed micelles were 5- to 23-fold higher depending on the intestinal segment. Our data suggest that b-lap should be delivered to the large intestine using a mixed micellar system for improved absorption.

Key Words: Beta-lapachone, Mixed micelles, Permeability, Single-pass intestinal perfusion

INTRODUCTION

Many drug candidates are poorly soluble in water. In the case of highly hydrophobic drugs, overall absorption process is quite slow due to limited solubility in gastrointestinal fluids and therefore the bioavailability of such drugs is generally low [1-3]. Several approaches have been employed to improve bioavailability including lipid-based formulations, micronization, solid dispersions, and use of colloidal carriers [4-8]. Among these, colloidal carriers such as liposomes, micelles, and nanoparticles are especially promising because they are able to increase both solubility of the drugs and drug transport across the absorptive membranes [9].

Micelles are frequently prepared with pharmaceutically acceptable surfactants and are considered to be one of the most promising absorption-enhancing vehicles because they combine solubilization of drugs within the micelle cores and strong interaction between micellar carriers and absorptive membranes to increase drug bioavailability. Mixed micelles, generally composed of bile salts and phospholipids, have been also been shown to be useful in increasing bio-

availability of poorly water-soluble drugs.

Beta-lapachone (b-lap) (Fig. 1) is currently under development for the treatment of obesity and for anti-tumor activities [10,11]. In our laboratory, we estimated the solubility of b-lap to be approximately 35 $\mu\text{g/ml}$ in water, which is regarded as insoluble. In previous studies, the formation of inclusion complexes with cyclodextrins including hydroxypropyl- β -cyclodextrin and methylated- β -cyclodextrin has led to an increase in the water solubility of b-lap and allowed the drug to be successfully administered intraperitoneally [12]. However, to our knowledge it has never been delivered via the oral route. Therefore, we have attempted to develop micelle-based oral delivery systems for b-lap because oral delivery offers numerous advantages

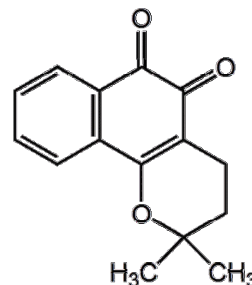


Fig. 1. Chemical structure of b-lap.

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Correspondence to: Jaehwi Lee, College of Pharmacy, Chung-Ang University, 84, Heuksuk-ro, Dongjak-gu, Seoul 156-756, Korea. (Tel) 82-2-820-5606, (Fax) 82-2-816-7338, (E-mail) jaehwi@cau.ac.kr



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ABBREVIATIONS: b-lap, beta-lapachone; SLS, sodium lauryl sulfate; HPLC, high performance liquid chromatography; ANOVA, analysis of variance.

over parenteral administration and micellar carriers provide the intrinsic advantages mentioned above [13-15].

To develop an oral formulation of b-lap, we examined the impact of micelle types on the absorption properties of b-lap across the small and large intestine using an *in situ* single-pass perfusion model that is frequently employed in the evaluation of absorption properties of drugs in the gastrointestinal tract. We also evaluated the effective permeability of b-lap in four different intestinal segments (duodenum, jejunum, ileum, and large intestine) to assess site dependency of absorption. The specific aim of the study was, therefore, to compare the intestinal permeability profiles of b-lap from conventional micelles prepared with SLS and mixed micelles prepared with a mixture of sodium deoxycholate and phosphatidylcholine together with evaluation of regional absorption variability.

METHODS

Materials

B-lap, phosphatidylcholine and sodium deoxycholic acid were purchased from Sigma-Aldrich Company (MO, USA). Sodium lauryl sulfate (SLS) and phenol red were purchased from Duksan Pure Chemical Industries (Seoul, Korea). Ketamine was obtained from Yuhan Corp. (Seoul, Korea). All other reagents were analytical grade and deionized water was used throughout.

Animals

Male SPF/VAF outbred rats weighing 240~260 g were obtained from Orient Bio Inc. (Sungnam, Korea). The rats were housed under constant temperature (22±2°C) in the animal facility at the College of Pharmacy, Chung-Ang University and were fed a commercial diet with tap water provided *ad libitum*. The rats were acclimated for at least 5 days before the experiments and fasted overnight prior to the perfusion study.

Preparation of micellar formulations of b-lap

The SLS-based micellar formulation (SLS micelles) was prepared by adding b-lap (20 mg) and phenol red (2 mg) as a non-absorbable marker to 100 ml SLS solution (1% SLS in 50 mM Tris-HCl, pH 7.4). This mixture was completely dissolved by constant stirring (500 rpm) on a hot plate for 16 hr.

Mixed micelles were composed of a mixture of phosphatidylcholine and sodium deoxycholate in a molar ratio of 1 : 2. Phosphatidylcholine and sodium deoxycholate were solubilized in chloroform:methanol (2 : 1 v/v) in a clean, dry, round-bottom flask and the solvent was evaporated in a rotary evaporator. The resulting thin film was hydrated with 50 mM Tris-HCl (pH 7.4) followed by sonication for 5 min. The drug was added to mixed micelles in a molar ratio of b-lap : mixed micelle of 1 : 6 [16] and vortexed for 30 sec. Phenol red (20 µg/ml) was also added to the above solution as a marker.

Characterization of micellar formulations

Colloidal properties of micellar formulations of b-lap such as average size, size distribution, and zeta-potential values

were determined by dynamic light scattering method with a Zetasizer Nano-ZS (Malvern Instrument, Worcestershire, UK).

Single-pass intestinal perfusion experiments

The rats were fasted overnight before the perfusion experiment with free access to tap water only. Rats were anesthetized with an intraperitoneal injection of ketamine (60 mg/kg) and then placed on a pad under a surgical lamp to maintain body temperature. After verification of loss of the pain reflex, a midline abdominal incision was made and each intestinal segment (duodenum, jejunum, ileum, or large intestine) of 10 cm was cannulated at both ends with silicone tubing (internal diameter 2 mm). Both cannulae were secured with surgical silk sutures. Each intestinal segment was identified as follows: duodenum segment beginning from pylorus, jejunum segment beginning 25 cm from the pylorus, ileum segment beginning 20 cm above caecum, large intestine segment beginning at caecum. Approximately 10 cm of the inlet tubing was placed inside the abdominal cavity to allow the perfusion solution to enter the intestinal segment at body temperature [17]. The segment was rinsed with saline (37°C) until a clear perfusate flowed and drops of saline were added to the surgical area then covered with wet gauze to avoid loss of fluid. The experiment was initiated by rapidly filling the segment with the perfusion solution and the solution was perfused with a peristaltic pump at a rate of 0.4 ml/min. After approximately 20 min, when steady-state was achieved, the outlet perfused samples were collected at 20 min intervals up to 120 min [18,19].

HPLC assay of b-lap and phenol red

Perfusion solution was filtered with 0.45 µm nylon membrane (Whatman Inc., Florham Park, NJ). Levels of b-lap and phenol red in perfusion samples were analyzed using a Hitachi HPLC system. b-Lap and phenol red were separated on a Capcell Park C₁₈ column (4.6×150 mm, 5 µm, Shiseido, Tokyo) with the mobile phase consisting of methanol : water (60 : 40 v/v) maintained at 30°C. The flow rate was set at 1.0 ml/min and the eluent was monitored at 254 for b-lap and 430 nm for phenol red, respectively.

Data analysis

Effective permeability coefficient (P_{eff}) was calculated from the steady-state concentrations achieved after approximately 120 min. The effective permeability at steady-state (P_{eff} , cm/s) was calculated according to a parallel tube model [17]:

$$P_{eff} = \frac{[Q_{in} \cdot \ln(C_{in} - C_{out})]}{2\pi r l}$$

Where Q_{in} is the perfusion flow rate (0.4 ml/min); the mass transfer surface area within the intestinal segment was assumed to be the area of a cylinder ($2\pi r l$), with a radius (r) of 0.18 cm and length (l) of 10 cm; and C_{in} and C_{out} are the respective inlet and fluid-transport-corrected outlet solution concentrations (corrected for water transport). The outlet concentration of b-lap was corrected by multiplying the inlet concentration by $[\text{phenol red}]_{in}/[\text{phenol red}]_{out}$ determined by HPLC [17]. Data are presented as mean±standard deviation ($n=3$).

Statistical analysis

The results reported were expressed as mean \pm SD. The statistical difference between test groups was evaluated using analysis of variance (ANOVA). $p < 0.05$ was considered statistically different.

RESULTS AND DISCUSSION

Characterization

We evaluated physical characteristics of mixed micelles and SLS micelles including mean particle size, polydispersity index, and zeta potential (Table 1). The mean diameter of micellar formulations was 110.5 \pm 8.7 nm for mixed micelles and 140.7 \pm 12.6 nm for SLS micelles. As a result of a polydispersity index of less than 0.3 for micellar formulations, the particle size distribution was estimated to be narrow. Although the zeta potential values of the mixed micelles were greater than that of SLS micelles, both micellar formulations were regarded as stable when considering the zeta potential values [20].

In situ absorption profiles of b-lap

The single-pass intestinal perfusion method was used to estimate the effective permeability of b-lap in different intestinal segments and compare the intrinsic absorptivity of b-lap formulated into mixed micelles and SLS micelles. Since b-lap is poorly soluble in water, an *in situ* perfusion study is not feasible without solubilization of the drug. In the present study, micellar formulations were selected as a delivery system for b-lap as they can solubilize b-lap and are expected to enhance absorption of the drug. Moreover, the *in situ* perfusion study was carried out with different segments of the intestinal tract to evaluate regional variability in absorption.

The profiles of percentage of b-lap remaining in mixed micelles and SLS micelles following perfusion through different intestinal segments are shown in Fig. 2. The drug was fairly rapidly absorbed from mixed micelle solutions for the initial 20 min. In particular, drug absorption from the large intestine was the greatest among the intestinal segments tested. Although the rate of b-lap absorption from SLS micelles was slower than that achieved with mixed micelles, drug absorption from SLS micelles was also greatest through the large intestine. Approximately 65% of the initial dose of b-lap from mixed micelles was absorbed through the large intestine over 120 min. The reason for the enhanced absorption of b-lap in micellar formulations through the large intestine may be related to the membrane properties of the intestinal tract. The unstirred water layer located adjacent to the intestinal wall may be a rate-limiting

barrier for absorption of lipophilic molecules in the *in situ* models [21,22]. This layer is known to be much thinner in the large intestine than in other intestinal segments. Another plausible explanation for the best absorption of b-lap at large intestine is that the lipid composition of the colonocytes favors the partitioning of lipophilic components such as phospholipids. The other study revealed that the colon is more permeable to lipophilic molecules than the small intestine [23]. The present study also revealed that micellar formulations might efficiently lower the barrier property of the large intestinal tract. The reason for the greater absorption from mixed micelles compared with SLS micelles remains unclear but we speculate that interaction between micellar components and the intestinal membrane is probably easier for micellar formulations containing a component of the intestinal membrane such as phosphatidylcholine [24]. The reason for the employment of *in situ* perfusion model for current study was to monitor the difference between the inlet and outlet concentrations that is attributed to absorption from different micellar vehicles. To test this drug was perfectly solubilized in micellar solution (perfusion solution) and therefore the pH effect could be minimized even though the physiological pH of the gut lumen such as duodenum, jejunum, ileum and large intestine is different and varied.

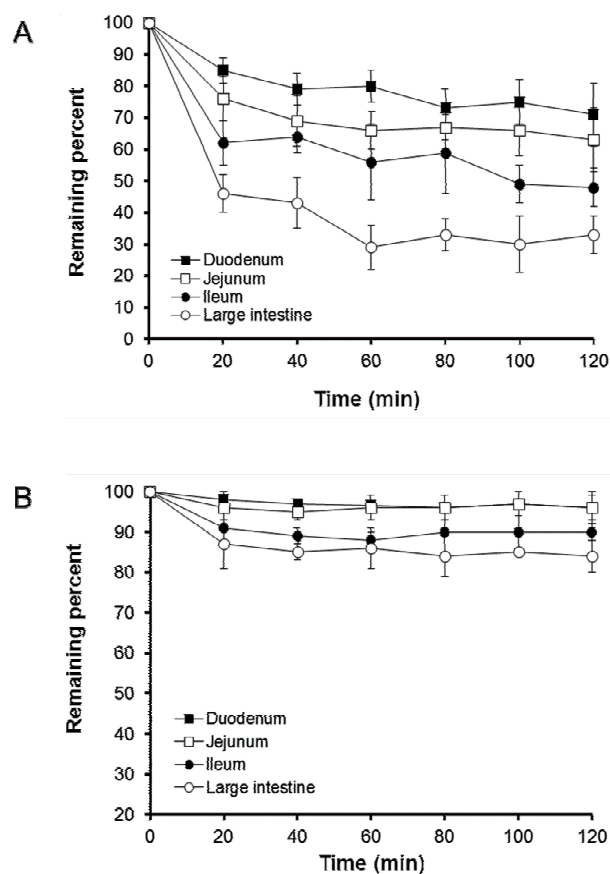


Table 1. Characterization of mixed micelles and SLS micelles (Mean \pm SD, n=3)

Formulation	Mean micellar size (nm)	Zeta potential (mV)	Polydispersity index
Mixed micelles	110.5 \pm 8.7	-34.6 \pm 4.2	0.162 \pm 0.006
SLS micelles	140.7 \pm 12.6	-21.8 \pm 6.8	0.251 \pm 0.014

Fig. 2. Plots showing remaining percentage of b-lap in mixed micelles (A) and SLS micelles (B) versus time in four intestinal segments of rats using an *in situ* single-pass perfusion method (Mean \pm SD, n=3).

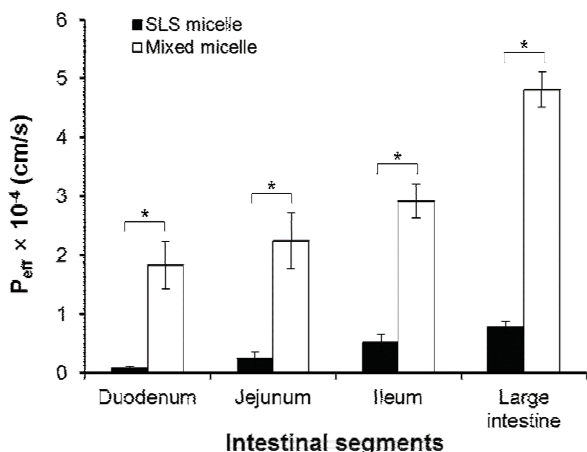


Fig. 3. Effective permeability, P_{eff} , of mixed micelles and SLS micelles at 120 min in the duodenum, jejunum, ileum, and large intestine. * $p < 0.05$ (Mean \pm SD, $n=3$).

Confirmation of steady-state concentrations and effective permeability of b-lap

Steady-state concentrations in collected samples can be obtained from *in situ* absorption profiles in Fig. 2. Steady-state was confirmed by the ratio of outlet to inlet concentrations versus time and was achieved when ($C_{\text{out}}/C_{\text{in}}$) of b-lap remained constant. For mixed micelles, the ratio of outlet to inlet concentrations decreased significantly, compared to that of SLS micelles as a function of time in all intestinal segments tested. The ratio of outlet to inlet concentrations for mixed and SLS micelles became approximately constant 20 min after the experiment was initiated. The reason for the gradual changes in $C_{\text{out}}/C_{\text{in}}$ values in mixed micelles might be due to the continuing absorption of b-lap. Thus, effective permeability coefficient values were calculated from the steady-state concentrations of b-lap in the perfusate collected from the outlet at 120 min.

As shown in Fig. 3, the effective permeability values of b-lap in mixed micelles were significantly higher ($p < 0.05$) than those of b-lap in SLS micelles. The estimated effective permeability values of b-lap in mixed micelles at 120 min in the duodenum, jejunum, ileum, and large intestine were 1.83, 2.24, 2.92, and 4.81×10^{-4} cm/s, respectively. These values were significantly greater than those measured with SLS micelles, which were 0.79, 2.37, 5.15, and 7.82×10^{-5} cm/s. These results imply that the permeability coefficient values measured with mixed micelles were almost 5- to 23-fold higher than those measured with SLS micelles, depending on the intestinal segment.

Both mixed micelles and SLS micelles improved the *in situ* permeability of b-lap in all intestinal segments tested. The mixed micellar formulation was more effective in increasing the intestinal absorption of b-lap and the permeability of b-lap was greatest in the large intestinal segments for both formulations. We therefore suggest that b-lap should desirably be delivered to the large intestine with mixed micellar systems for better absorption.

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