Pharmaceutical Formulation Design Laboratory[1], College of Pharmacy, Chung-Ang University, Seoul; Department of Food Science and Technology[2], Chung-Ang University, Anseong, South Korea

Impact of pharmaceutical excipients on *in vitro* association of saquinavir to chylomicrons

J. Ro[1,*], H. Kim[1,*], S. H. Hwang[1], G. Yun[2], J. Lee[1]

Received February 26, 2014, accepted June 13, 2014

J. Lee, Pharmaceutical Formulation Design Laboratory, College of Pharmacy, Chung-Ang University, Seoul 156-756, South Korea
jaehwi@cau.ac.kr

[*] *These authors contributed equally to this work.*

Pharmazie 69: 745–746 (2014) *doi: 10.1691/ph.2014.4034*

This study was performed to investigate the impact of pharmaceutical excipients commonly used for lymphatic transport on *in vitro* drug association with chylomicrons (CM). A CM association study was conducted using saquinavir solubilized in four different pharmaceutical excipients. We observed a linear relationship between saquinavir solubility and drug association, suggesting that the solubility of saquinavir in excipients is a key determinant for successful lymphatic delivery. Broadly, these results suggest that excipients with good solubilization properties may be advantageous for enhancing lymphatic drug delivery.

1. Introduction

Intestinal lymphatic drug delivery has attracted a great deal of interest as one of the more promising routes of administration for transporting drugs with low oral bioavailability due to hepatic first-pass metabolism (Porter and Charman 1997). Numerous approaches have been developed for efficient transport of such drugs *via* the intestinal lymphatic pathway, including lipid-based formulations such as liposomes (Kim et al. 2005), self-emulsifying drug delivery systems (Haus et al. 1998), and solid lipid nanoparticles (Manjunath et al. 2005). The lipid-based formulations, however, have shown partial increases in the lymphatic exposure of drugs (Porter et al. 2007). Chylomicrons (CM) are considered critical for enhancing lymphatic absorption of drugs using lipid-based formulations, because only drugs associated with CM are transported to the intestinal lymphatic system (Gershkovich and Hoffman 2005). Generally, lipidic materials supplied from foodstuffs are known to facilitate the secretion of CM which in turn enhances the fraction of CM associated drugs, leading to improved lymphatic delivery (Yáñez et al. 2011). However, to the best of our knowledge, little is known about the effect of pharmaceutical excipients preferred in lipid-based formulations for lymphatic delivery on the association of drugs with CM. Therefore, we examined several excipients commonly used in lymphatic formulations as solubilizers (propylene glycol, Transcutol HP and ethanol), and surfactant (Tween 80) to determine if they modify CM association with saquinavir. Saquinavir was selected as a model drug due to poor solubility and low oral bioavailability as a result of hepatic first-pass metabolism (Tam-Zaman et al. 2009).

2. Investigations, results and discussion

Solubility tests with saquinavir were performed using four common pharmaceutical excipients: ethanol, propylene glycol, Transcutol HP, and Tween 80. Figure 1 shows that ethanol had the greatest solubilizing capacity on saquinavir (250 ± 18.7 mg/g), followed in order by propylene glycol (228 ± 19.5 mg/g), Transcutol HP (158 ± 17.8 mg/g), and Tween 80 (37 ± 4.3 mg/g).

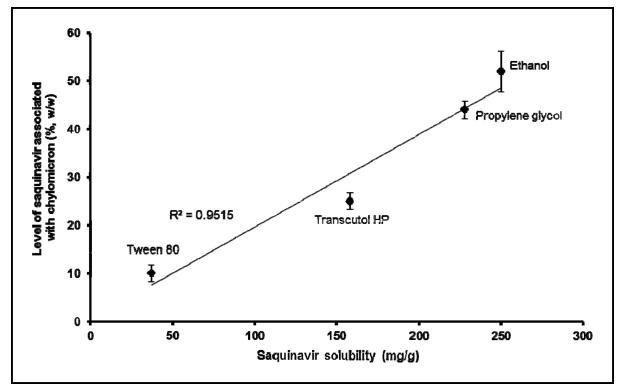

Fig. 1: Relationship between solubilities of saquinavir in pharmaceutical excipients tested and levels of saquinavir associated with chylomicron (n = 3).

A CM association study was performed using five different formulations (S1–S5) to investigate the effect of the pharmaceutical excipients selected in this study on the association of saquinavir with CM. An association of saquinavir with CM was not detected in a mixture of CM dispersion and saquinavir incubated without any pharmaceutical excipients (S1), which was considered to be the result of the poor solubility of saquinavir in water (about 35 μg/mL) (Boudad et al. 2001) and likely prevented dispersion of the drug at the molecular level. However, approximately 10-52% of the initially added saquinavir solubilized with pharmaceutical excipients (S2-S5) were associated with CM particles (Fig. 1). Further, the degree to which the saquinavir in S2-S5 associated with CM was in good agreement with the rank of saquinavir solubility in the pharmaceutical excipients, exhibiting a linear relationship (correlation

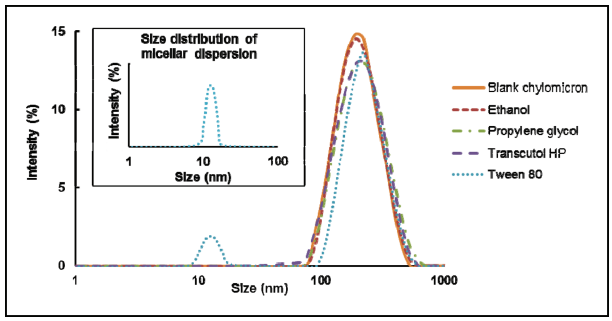

Fig. 2: Size distribution profiles of incubated mixtures (S2-S5), blank chylomicron dispersion, and micellar dispersion of saquinavir.

coefficient value = 0.9515). This trend was thought to be due to an increase in the molecular level of contact between saquinavir and CM as a result of solubilzation of saquinavir with pharmaceutical excipients, which in turn may have facilitated association of saquinavir with CM.

Next, the size distributions of a blank CM dispersion, S2-S5 formulations, and a micellar dispersion of saquinavir (0.25 μg/5% Tween 80) were determined to examine particle size changes after CM association. Figure 2 shows the size distribution data obtained from each sample. The incubated mixtures prepared with solubilizers (S2-S4) and blank CM dispersions exhibited one main peak with a mean particle size of approximately 210-230 nm. In contrast, the incubated mixture prepared with Tween 80 (S5) exhibited 2 peaks composed of one major peak similar to the peaks observed above, and a small secondary peak with a mean particle size of approximately 10 nm, which was also observed with the Tween 80 micellar dispersion containing saquinavir. Based on these results, we speculated that the entrapment of saquinavir in micelles might have prevented the association of the drug with CM, wherein the hydrophilic surface of the micelles acted as a barrier of the interaction between saquinavir and CM. In addition, the lower solubility of saquinavir in Tween 80 may have led to a lower association of the drug with CM.

In conclusion, the association of saquinavir with CM was enhanced by the pharmaceutical excipients tested in this study. Our results revealed a linear relationship between the solubilities of saquinavir in pharmaceutical excipients and levels of drug association with CM, suggesting that pharmaceutical excipients with good solubilizing properties may be potentially useful for enhancing intestinal lymphatic drug exposure. Therefore, lipid-based formulations containing solubilizers will likely be advantageous for increasing the association between drugs and CM for facilitated lymphatic delivery.

3. Experimental

Male Wistar rats (300-330 g, 8 weeks) were obtained from Hanlim Experimental Animals (Hwasung, Korea). Saquinavir was purchased from Vivagen Co., Ltd. (Sungnam, Korea). Peanut oil and Transcutol HP was obtained from Sigma-Aldrich Company (St. Louis, MO, USA). Diethyl ether, ethanol, propylene glycol, and Tween 80 were purchased from Daejung Chemicals & Metals Co., Ltd. (Siheung, Korea). All other chemicals used were pharmaceutical grade.

For solubility testing, an excess amount of saquinavir was added to each pharmaceutical excipient and the suspensions were agitated in a magnetic stirrer apparatus for 24 h at room temperature. The resulting suspensions were then centrifuged (2,000 g, 15 °C, 1 h), and levels of saquinavir in the supernatants were measured by HPLC with UV detection at 240 nm with appropriate dilutions performed using acetonitrile. Separation of saquinavir was achieved with a Hypersil GOLD® column (4.6 mm × 250 mm. 5 μm) and a mobile phase consisting of 0.1 % phosphoric acid and acetonitrile (60:40, v/v) set at a flow rate of 1.0 mL/min.

Animal experiments were performed in accordance with an animal protocol approved by Chung-Ang University Support Center for Animal Experiments. Animals were sacrificed using bicarbonate gas, and all efforts were made to minimize suffering. Male Wistar rats were used to obtain CM dispersion. Specifically, rats were fasted overnight with free access to water, and then gavage fed a total of 0.8 mL peanut oil. Whole blood was then collected from caudal vena cava of the rats using a syringe with a 22-gauge needle after anesthetization with diethyl ether. Blood obtained from animals was transferred to 15 mL centrifuge tubes containing 10% (w/v) EDTA-K_2-Na_2 (100 μL), and the plasma was separated from the blood by centrifugation (2,000 g, 15 min, 15 °C). Following centrifugation, the CM fraction was separated from the plasma by density gradient ultracentrifugation (DGU) as described previously with some modifications (Karpe and Hamsten 1994). Briefly, an appropriate amount of potassium bromide was added to the plasma to adjust the density of its continuous phase to 1.100 g/mL, followed by the serial addition of sodium chloride solutions with densities of 1.062, 1.018, and 1.005 g/mL, respectively, in order to establish a density gradient. The resulting mixture was then subjected to ultracentrifugation (38,000 rpm, 15 °C, 38 min) (Optima XE-100, Beckman Instruments Inc., Fullerton, CA, USA), and the top layer of the centrifuged mixture corresponding to CM fraction was carefully collected.

The CM association study was performed using five different formulations (S1-S5). To test S1, the formulation without any pharmaceutical excipients, 25 μL of acetonitrile containing 1 mg/mL saquinavir was taken and the solvent was evaporated. CM dispersion (0.2 mL) was then added to saquinavir. The same experimental process was used to analyze S2-25, except that instead of acetonitrile the evaporation procedure was repeated using 10 mg of ethanol, propylene glycol, Transcutol HP, or Tween 80, respectively, The resulting mixtures (S1-S5) were incubated with shaking at 80 rpm and 37 °C for 3 h, after which DGU was performed to separate the CM fraction followed by dissolving with acetonitrile and determination of saquinavir levels by HPLC as described above. Each of the studies described above was performed at least in triplicate. The size distributions of samples were analyzed with a Zetasizer Nano ZS® (Malvern Instruments Ltd., Malvern, UK).

Acknowledgments: This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012-0001499).

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