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論文内容の要旨

Introduction

The breast cancer resistance protein (BCRP) transporter is a second member of an ATP binding cassette (ABC) transporter subfamily G. It is an ATP driven efflux transporter that pushes its substrates to the secretory direction regardless of the concentration gradient. BCRP is highly expressed in the apical membrane of intestinal epithelial cells and was reported to be a significant factor that can reduce the intestinal absorption of its substrates. Presently, hundreds of drugs were identified as BCRP substrates and the number is still increasing. This low intestinal absorption of BCRP substrates should be overcome for the drug development in the pharmaceutical industries. For instance, US Food and Drug Administration (FDA) is currently recommending that all new drugs in the development pipelines should be evaluated by *in vitro* transport studies if their drug candidate is a potential substrate of BCRP. Meanwhile, several inhibitors have been developed to reduce the function of BCRP. Among these inhibitors, pharmaceutical excipients are considered to be promising candidates to inhibit the function of BCRP. In this study, sulfasalazine and topotecan were used as model substrates of BCRP and the effects of pharmaceutical excipients on the transport and absorption of these substrates were evaluated by *in vitro* and *in situ* experiments. Moreover, possible mechanisms of BCRP inhibition by these pharmaceutical excipients were elucidated by evaluating their interactions to the biomembranes. Lastly, the toxicity of these pharmaceutical excipients was studied.

Chapter I: Effects of various pharmaceutical excipients on the intestinal transport and absorption of sulfasalazine, a model substrate of BCRP transporter

Sulfasalazine, which is actively effluxed by BCRP and has less than 15% oral bioavailability, was used as a typical model for BCRP transporter studies. Our experiment showed that sulfasalazine was preferentially transported into a secretory direction (serosal to mucosal side of a membrane) by an *in vitro* diffusion chamber method using the isolated rat small intestinal membranes. Later, Ko143, a selective BCRP inhibitor, was used as a

positive control. Results showed that the directional transport of sulfasalazine was completely neutralized in the presence of Ko143 (Efflux ratio (ER)=1.17), suggesting that sulfasalazine is a substrate of Bcrp1 and Ko143 can be used as a typical inhibitor of rodent Bcrp1. Next, the effects of several pharmaceutical excipients on the intestinal absorption of sulfasalazine were examined. These pharmaceutical excipients including BL-9EX, Brij97, Labrasol, and Tween 20 significantly decreased the secretory transport of sulfasalazine, suggesting that Bcrp1 might be inhibited by these excipients. Because these pharmaceutical excipients were reported to open the tight junction and increase the transport of drugs via a paracellular route, the transport of 5(6)-carboxyfluorescein (CF), a non-BCRP substrate paracellular transport marker, was measured. CF was equally transported in both direction (ER=1.14). Ko143 as well as other pharmaceutical excipients did not affect the transport parameters of CF, suggesting that the decrease in sulfasalazine secretory transport might be due to the inhibition of Bcrp1-mediated efflux transport by these pharmaceutical excipients, while the paracellular permeation was not altered. Next, the qualified pharmaceutical excipients were further examined using an *in situ* closed loop intestinal absorption method. BL-9EX at 0.1% increased $AUC_{(0\text{-}4h)}$ value of sulfasalazine by over 2-fold without any significant alteration of CF absorption. Brij97 at the same concentration also increased $AUC_{(0\text{-}4h)}$ value of sulfasalazine by 1.79-fold, but the author observed that Brij97 also increased CF absorption, suggesting that Brij97 might increase the transport of sulfasalazine via a paracellular pathway. These results suggested that BL-9EX could be used as a BCRP inhibitor in an oral drug formulation.

Chapter II: Effects of various pharmaceutical excipients on the intestinal transport and absorption of topotecan, an orally active BCRP substrate

In this chapter, the author examined the effects of pharmaceutical excipients on the intestinal transport and absorption of topotecan, an orally active anticancer drug with low bioavailability (30%-40%). It was also reported to be a substrate of BCRP. The author first evaluated the transport of topotecan across Caco-2 cell monolayers. Topotecan was preferentially transported into a secretory direction (ER=16). This directional transport of topotecan was neutralized in the presence of 1 μM Ko143 but not with 50 μM verapamil, a P-gp inhibitor. These findings suggested that topotecan was mainly transported by BCRP. Results also showed that most pharmaceutical excipients significantly decreased the efflux transport of topotecan but had no effect on the transport of CF, suggesting that the decrease in the transport of topotecan might be caused by the inhibition of BCRP by the pharmaceutical excipients. Next, the intestinal absorption of topotecan was examined by the *in situ* closed loop method. Tween 20 and Cremophor EL at 0.05% significantly increased the $AUC_{(0\text{-}4h)}$ values of topotecan by over 2-fold. Labrasol and Pluronic F68 also increased the absorption of topotecan, although the increased absorption was not statistically significant. Therefore, their inhibitory effect against rodent Bcrp1 might be weaker than with human BCRP. Lastly, none of our tested excipient significantly increased the CF absorption across rat small intestine, suggesting that the pharmaceutical excipients might inhibit the function of Bcrp1 and increase the permeation of topotecan via a transcellular pathway.

Chapter III: Possible inhibitory mechanisms of BCRP transporters by the pharmaceutical excipients and their safety

All tested pharmaceutical excipients in this study have amphipathic characteristics. They tend to form a micelle at the concentration above their critical micellar concentration (CMC). The author found that at ≤0.1% concentration, interactions between sulfasalazine and pharmaceutical excipients were minimal (micellar ratio

<10%). Therefore, this finding indicates that the highest applicable concentration is up to 0.1% for all tested pharmaceutical excipients. Additionally, since each drug was entrapped into the micelle in a different degree, the author suggest that it should be evaluated for the micellar interaction to avoid the excessive micellar entrapment which could interfere with the absorption of the drug.

In general, changes in membrane fluidity are one of the most important mechanisms to inhibit the function of ABC transporters by pharmaceutical excipients. Therefore, the effects of pharmaceutical excipients on the membrane fluidity of Caco-2 cells were evaluated. In this study, tma-DPH and DPH were used as fluorescence probes to measure the changes in membrane fluidity. Interestingly, most pharmaceutical excipients increased the membrane fluidity in the inner lipid bilayers, while only Pluronic F68 decreased the membrane fluidity in the outer lipid bilayers. These changes are in an accordance with the changes in topotecan permeation across Caco-2 cell monolayers, suggesting that the key mechanism of these pharmaceutical excipients was to modify the membrane microenvironment which could subsequently disrupt the efflux ability of BCRP transporters.

Lastly, the membrane toxicity of these pharmaceutical excipients after their administration to the intestine was examined. The author did not observe any elevation in LDH activities or protein amount after the exposure to the pharmaceutical excipients in our *in situ* closed loop experiments, suggesting that these pharmaceutical excipients are safe and did not cause any significant damage to the small intestinal membrane.

Summary

In conclusion, the author demonstrated that several pharmaceutical excipients could inhibit the BCRP transporter and improve the intestinal absorption of its substrates. Especially, BL-9EX, Tween 20, and Cremophor EL, increased the intestinal absorption of BCRP substrates and did not cause any significant damage to the small intestine. These findings suggested that these pharmaceutical excipients could be used to improve the intestinal absorption of BCRP substrates.

審 査 の 結 果 の 要 旨

近年、消化管上皮細胞に発現する排出型輸送担体である breast cancer resistance protein (BCRP) が、BCRP の基質となる薬物の消化管吸収性を制御することが知られている。すなわち、BCRP の基質となる薬物は、一旦、消化管の上皮細胞に取り込まれた後、 BCRP の働きにより再度消化管管腔内に排出されるため、BCRP は、これら薬物の消化管吸収性を低下させる要因となる。したがって、 BCRP の基質となる薬物の消化管吸収性を改善するためには、消化管に発現している BCRP の機能を低下させることが重要である。

そこで本研究では、BCRP の機能を低下させる物質として、薬理効果がほとんど見られない各種製剤添加物に注目し、BCRP の基質となる薬物の腸管粘膜透過性及び吸収性に及ぼす各種製剤添加物の影響について検討した。

第 1 章　スルファサラジンの腸管粘膜透過性及び吸収性に及ぼす製剤添加物の影響

最初に、*in vitro* diffusion chamber 法により、代表的な BCRP の基質であるスルファサラジンの吸収方向と排泄方向の消化管粘膜透過性を評価したところ、排泄方向のスルファサラジンの透過性が、吸

収方向の透過性よりもきわめて高いことが認められた。また、スルファサラジンの透過の方向性は、BCRP の典型的な阻害剤である Ko143 の併用により、ほぼ完全に消失することが認められたことから、本実験系により BCRP の阻害作用を正確に評価できることが認められた。

次に、スルファサラジンの腸管粘膜透過性に及ぼす製剤添加物の影響について検討したところ、BL-9EX、Brij97、Labrasol、Tween 20 の併用により、スルファサラジンの透過の方向性が消失することが認められた。したがって、これら製剤添加物は、BCRP の機能を低下させる物質であることが示唆された。

さらに、スルファサラジンの消化管吸収性に及ぼす製剤添加物の影響について、*in situ* closed loop 法を用いて検討したところ、0.1% BL-9EX の併用により、スルファサラジンの消化管吸収性が増大することが認められた。したがって、BL-9EX は、スルファサラジンの消化管吸収の改善に有用であることが認められた。

第 2 章　トポテカンの腸管粘膜透過性及び吸収性に及ぼす製剤添加物の影響

Caco-2 細胞法により、スルファサラジンと同様に BCRP の基質であるトポテカンの吸収方向と排泄方向の消化管粘膜透過性を評価したところ、排泄方向のトポテカンの透過性が、吸収方向の透過性よりもきわめて高いことが認められた。

また、トポテカンの消化管吸収性に及ぼす製剤添加物の影響について、*in situ* closed loop 法を用いて検討したところ、Tween 20 及び Cremophor EL の併用により、トポテカンの消化管吸収性が増大することが認められた。したがって、これら製剤添加物は、トポテカンの消化管吸収の改善に有用であることが認められた。

第 3 章　製剤添加物による BCRP 輸送担体の機能抑制機構の解析

本研究で用いた製剤添加物は、界面活性剤と同様の性質を有するものが多く、これら製剤添加物がミセルを形成して、そのミセルと薬物が相互作用する可能性がある。そこで、平衡透析法を用いて各種製剤添加物が形成したミセル内へのスルファサラジンの取り込み率を評価した。その結果、今回用いた製剤添加物の濃度範囲では取り込み率の値は小さいことから、薬物のミセルとの相互作用は重要ではないことが示唆された。

次に、蛍光偏向解消法を用いて、消化管粘膜流動性に及ぼす各種製剤添加物の影響について検討したところ、これら製剤添加物は脂質内部相の膜流動性を増大させていることが認められた。したがって、製剤添加物による BCRP 輸送担体の機能抑制機構には、脂質内部相の膜流動性の増大が寄与している可能性が示唆された。

以上のことから、BCRP の基質となる薬物の消化管吸収性は、各種製剤添加物の併用により改善されることが認められた。したがって、これら製剤添加物は、BCRP の基質となる薬物の消化管吸収改善に有効かつ安全な添加物であることが認められた。

学位論文とその基礎となる報文の内容を審査した結果、本論文は博士（薬科学）の学位論文としての価値を有するものと判断する。