氏　名（生年月日）	ALAMA　TAMMAM（1987年6月2日）
学位の種類	博　士（薬科学）
学位記番号	博薬科　第8号
学位授与の日付	2017年3月18日
学位授与の要件	学位規則第4条第1項該当
学位論文題目	**Improvement of intestinal absorption of poorly absorbed dugs by gemini surfactant and sucrose fatty acid esters and their absorption enhancing mechanisms** (ジェミニ型界面活性剤及びショ糖脂肪酸エステルによる難吸収性薬物の消化管吸収改善ならびにその吸収促進機構)
論文審査委員	(主査)　教　授　山　本　　昌 (副査)　教　授　加　藤　伸　一 (副査)　教　授　矢　野　義　孝

論文内容の要旨

Oral administration represents the most convenient, safest, and least expensive way to deliver a drug and it is the route most often used. The lack of pain and discomfort associated with injections, and the elimination of possible infections caused by inappropriate use or reuse of needles are some of the advantages offered by the oral route. Moreover, most patients prefer swallowing medicines to other routes of administration. However, many obstacles have to be overcome in the gastrointestinal (GI) tract for better absorption of the target drugs. The physiological barriers, mainly represented by the presence of tight junctions (TJs), the biochemical barriers and the physico-chemical characteristics of the target drug, are the major challenges in enhancing the GI absorption of drugs. Various strategies have been evaluated to improve the intestinal absorption of such drugs, including the use of additives like absorption enhancers. Absorption enhancers, which help to achieve the efficiency-safety balance, are considered one of the most promising agents for the improvement the intestinal absorption of drugs.

Chapter 1. Absorption-enhancing effects and mechanisms of gemini surfactant on the intestinal absorption of poorly absorbed hydrophilic drugs including peptide and protein drugs in rats

Gemini surfactants, consisting of two monomeric surfactants and linked through a spacer, are an important class of functionalized amphiphilic molecules. These surfactants are superior to the single-chain conventional surfactants, owing to better wetting properties due to their greater surface activities. The intestinal absorption of drugs, with or without sodium dilauramidoglutamide lysine (SLG-30), a gemini surfactant, was examined by an *in situ* closed-loop method in rats. The intestinal absorption of 5(6)-carboxyfluorescein (CF) and fluorescein isothiocyanate-dextrans (FD4, FD10 and FD70) was significantly enhanced in the presence of SLG-30 at a concentration of 0.5% (v/v), with 16, 13, 6 and 3 times higher than the control groups, respectively. Such effect being reversible. Moreover, the plasma glucose levels significantly decreased when insulin was co-administered with SLG-30 at a concentration of 0.5% (v/v) into the large intestines (59% decrease of the initial value), suggestive of the increased intestinal absorption of insulin from the large intestines. Furthermore, the plasma calcium levels significantly decreased when calcitonin was co-administered with SLG-30 at a concentration of

0.5% (v/v) into small (29% decrease of the initial value) and large (46% decrease of the initial value) intestines, suggestive of the increased intestinal absorption of calcitonin from both small and large intestines. Comparison studies with conventional absorption enhancers including sodium laurate and sodium glycocholate suggested that SLG-30 was superior, effective as a whole compound and stable in the small intestines. In addition, no significant increase in the lactate dehydrogenase (LDH) activity or in protein release from the intestinal epithelium was observed in the presence of SLG-30, suggestive of the safety of this compound. Additionally, SLG-30 increased the membrane fluidity at the protein portion of the cells membranes. On the top of that, SLG-30 decreased the transepithelial electrical resistance (TEER) values of Caco-2 cells significantly, suggestive of loosening of the TJs. In this context, the expression levels of claudin-1 and claudin-4 significantly deceased with 57% and 64%, respectively, compared with the control group, in the presence of SLG-30 at a concentration of 0.5% (v/v), then started to recover after washing out SLG-30 from the intestines. These findings indicate that SLG-30 is an effective absorption enhancer for improving the intestinal absorption of poorly absorbed drugs, without causing serious damage to the intestinal epithelium. The mechanisms might be changing the membrane fluidity, and loosening of TJs by decreasing the expression of claudin-1 and claudin-4, enhancing by that the absorption through the transcellular and paracellular pathway, respectively.

Chapter 2. Enhanced oral delivery of alendronate by sucrose fatty acids esters in rats and their absorption-enhancing mechanisms

Bisphosphonates, carbon-substituted pyrophosphate analogs, are useful in reducing the hazard of future fractures in osteoporosis patients who have already sustained a fracture due to the disease. However, the intestinal absorption of alendronate (ALN), a bisphosphonate drug, after oral administration is very poor. In this study, sucrose fatty acid esters as promising absorption enhancers were used to enhance the intestinal absorption of ALN using an *in situ* closed-loop method in rats. The intestinal absorption of ALN was significantly enhanced in the presence of sucrose fatty acid esters. The best absorption enhancing effect was observed in the presence of L-1695 in a dose-dependent manner, with 11 times higher than the control group at a concentration of 5.0% (w/v). Moreover, there is almost no regional differences in the intestinal absorption of ALN in the small and the large intestines. In addition, no considerable increase was observed in the activity of LDH or in protein release from the intestinal epithelium in the presence of sucrose fatty acid esters at concentrations equivalent to or lower than 1.0% (w/v), suggesting that these compounds are safe. Furthermore, mechanistic studies revealed increased membrane fluidity in the presence of sucrose fatty acid esters at the inner portion between the phospholipids bilayers and at extracellular faces of the phospholipids bilayers of the membrane. Additionally, sucrose fatty acid esters at all studied concentrations significantly decreased the TEER values of Caco-2 cells. TEER values recovered to the baseline after removing sucrose fatty acid esters and ALN. Therefore, the loosening of the TJs might be another underlying mechanism by which sucrose fatty acid esters improve the intestinal absorption of ALN. L-1695 at a concentration of 2.0% (w/v) decreased the levels of claudin-1 and claudin-4, with 24% and 49%, respectively, compared with the control group. Such effect being reversible. These findings suggest that sucrose fatty acid esters are effective absorption enhancers for improving the intestinal absorption of ALN, without causing serious damage to the intestinal epithelium, through the transcellular and paracellular routes, respectively.

These findings give us basic information about enhancing the intestinal absorption of poorly absorbed drugs including peptide and protein drugs.

審 査 の 結 果 の 要 旨

一般に、ペプチド・タンパク性医薬品をはじめとする難吸収性薬物の消化管吸収性を改善する方法としていくつかの方法が試みられているが、このうち、吸収促進剤などの製剤添加物の利用は、多くの薬物に簡便に適用できるため汎用されている。そこで本研究では、2 種の添加物を用いて、難吸収性薬物の消化管吸収性の改善を試みた。

まず、難吸収性薬物の消化管吸収性に及ぼすジェミニ型界面活性剤 sodium dilauramide glutamide lysine (SLG-30) の影響について検討した。その結果、水溶性薬物である 5(6)-carboxyfluorescein (CF) 及び高分子薬物であるインスリン、カルシトニンの消化管吸収性は、SLG-30 の併用により濃度依存的に改善することが認められた。また、大腸における薬物の消化管吸収に対する SLG-30 の効果は、小腸に比べ顕著に観察された。また、SLG-30 の吸収促進効果は、従来から吸収促進剤として知られているグリココール酸ナトリウムやラウリン酸に比べ、強いことが明らかとなった。一方、形態学的観察により、SLG-30 は消化管上皮細胞に障害を惹起しないことが確認され、安全性の高い添加物であることが確認された。さらに、SLG-30 は膜流動性を増大させ、細胞内経路を介した薬物の透過性を改善することと共に、tight junction 関連タンパク質である claudin-1 及び claudin-4 の発現レベルを低下させることにより、tight junction を開口させ、細胞間経路を介した薬物の透過性も改善することが示唆された。

次に、bisphosphonates の一種であり、消化管吸収性がきわめて低いアレンドロネートの消化管吸収性に及ぼすショ糖脂肪酸エステルの影響について検討した。その結果、アレンドロネートの消化管吸収性は各種ショ糖脂肪酸エステルの併用により増大し、中でも L-1695 が最も促進効果が高いことが確認された。また、乳酸脱水素酵素 (lactate dehydrogenate、LDH) 活性値及びタンパク質放出量を指標として、小腸における各種ショ糖脂肪酸エステルの粘膜障害性を評価したところ、これら生化学的マーカーは、各種ショ糖脂肪酸エステルを併用しても有意な差は認められなかった。したがって、これらショ糖脂肪酸エステルは、比較的安全性の高い吸収促進剤であることが明らかとなった。

さらに、各種ショ糖脂肪酸エステルの細胞内経路を介した薬物透過性増大作用の寄与を検討するため、蛍光偏光解消法を用いた膜流動性の変化について評価した。その結果、各種ショ糖脂肪酸エステルは脂質膜内部及び外部脂質の流動性を増大させることが示唆された。このことから、各種ショ糖脂肪酸エステルの吸収促進機構には一部、細胞内経路を介する薬物透過性増大作用が寄与している可能性が示唆された。

また、細胞間経路を介した薬物透過性増大作用の寄与を検討するため、Caco-2 細胞単層膜を用いた薬物透過実験ならびに膜抵抗値測定実験を行った。その結果、各種ショ糖脂肪酸エステルの併用により、膜抵抗値は濃度依存的に低下し、CF の透過性は濃度依存的に増大することが明らかとなった。以上の結果より、各種ショ糖脂肪酸エステルの吸収促進機構には、一部 tight junction の開口による細胞間経路を介した薬物透過性増大作用が寄与している可能性が示唆された。さらに、tight junction 関連タンパク質である claudin-1 及び claudin-4 の発現量は、L-1695 の併用により低下することが確認された。

以上、これらの知見は、難吸収性薬物の消化管吸収を改善する上で有用な基礎的情報を提供するものと思われる。

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。