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学位論文題目	Improvement of the solubility and intestinal absorption of curcumin by *N*-acyl taurates and cyclodextrins （*N*-アシルタウリン塩及びシクロデキストリンによるクルクミンの溶解性ならびに消化管吸収性の改善）
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論文内容の要旨

Introduction

Curcumin is a polyphenolic compound named as (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione; CAS number: 458-37-7. The molecular formula is $C_{21}H_{20}O_6$ and it has a molecular weight of 368 g/mol. This compound is derived from *Curcuma longa L.* and has demonstrated versatile pharmacological effects including anti-inflammatory and antioxidant actions in extensive preclinical studies. In addition, the therapeutic effects, such as anti-tumors, were studied in human clinical trials over the last few decades. In terms of the high dose at 12 g per day in healthy volunteers, curcumin was well tolerated in the oral administration and appeared to be safe for the clinical use. However, based on the poor aqueous solubility and low intestinal permeability of curcumin, the natural product is classified as a biological classification system (BCS) Class IV molecule. The hydrolytic and light-sensitive properties also cause the rapid degradation of this natural polyphenol. Due to these characteristics, curcumin showed a low concentration in plasma after oral administration resulting in a poor bioavailability. Various approaches have been developed to overcome the bioavailability problem, such as nanoformulations. Because many ingredients in formulae are used for both solubilizers and permeation enhancers, it is of interest to investigate their multiple functions with respect to drug absorption. In our recent research, amorphous solid particles of curcumin showed an enhanced permeation across the absorptive membrane, while it was not observed in the presence of crystalline particles or supersaturated solution. Consequently, since the crystalline powder is more stable than the amorphous particles in dosage forms, the present study focused on the development of new curcumin formulations using crystalline particles with absorption enhancers and examined their absorption-enhancing mechanisms.

Chapter I Improvement of the solubility and intestinal absorption of curcumin by *N*-acyl taurates (NATs)

NATs are a subset of acylated amino acids which are surfactants with natural lipid-like structures that exhibit amphiphilic properties. In this chapter, the effects of NATs on the small intestinal absorption of curcumin were examined in rats by an *in situ* closed-loop method. Among these NATs, 1% (v/v) sodium methyl lauroyl taurate

(LMT) and sodium methyl cocoyl taurate (CMT) were the most effective in increasing the solubility and intestinal absorption of curcumin. The intestinal membrane toxicity of NATs was also evaluated by measuring the activity of lactate dehydrogenase (LDH), a cytotoxicity marker. All of them did not increase the activity of LDH in the luminal fluid, suggesting that they may be safely administered orally. The relationship between the solubility and absorption demonstrated that the drug solubility is an important factor contributing to the absorption of curcumin. However, the drug absorption was not changed when the solubility was higher than 5 μg/mL, which means that the rate-limiting step was shifted from the apparent solubility of curcumin to the permeation across the intestinal membrane. Thus, the absorption-enhancing mechanism was elucidated in the paracellular pathway using Caco-2 cells. In cellular transport studies, LMT and CMT reduced the transepithelial electrical resistance (TEER) values of Caco-2 cells and increased the transport of 5(6)-carboxyfluorescein (CF) and curcumin. Hence, besides of the increased solubility, the improved permeability of curcumin by LMT and CMT also contributed to the intestinal absorption.

Chapter II Improvement of the solubility and intestinal absorption of curcumin by cyclodextrins (CDs)

CDs are a unique type of macrocyclic carriers widely used in pharmaceutical formulations owing to their versatile functions as the solubilization, stabilization, and permeation enhancement. In this chapter, α-CD, β-CD, γ-CD, hydroxypropyl (HP)-β-CD, and dimethyl (DM)-β-CD were applied to the formulation of curcumin. The interaction between curcumin and CD molecules was investigated by phase-solubility diagrams, suggesting that 1:1 complex formation was observed in the solution except for γ-CD. The effects of various CDs on the intestinal absorption of curcumin were evaluated in rat intestine by the *in situ* closed-loop experiment. Among the tested CDs, 50 mM α-CD significantly enhanced the intestinal absorption of curcumin without causing any serious toxicity to tissues like intestinal membrane, liver, and kidney. In addition to curcumin, 50 mM α-CD increased the intestinal absorption of hydrophilic drugs including CF, fluorescein isothiocyanate-labeled dextrans with average molecular weights of 4000 (FD4), FD10, and salmon calcitonin, suggesting a molecular weight dependency of the absorption-enhancing ability. The analysis of cellular transport across Caco-2 cell monolayers showed that 50 mM α-CD reduced the TEER value of cell monolayers and improved the paracellular permeability of CF. Furthermore, in the western blotting analysis, α-CD decreased the expression of claudin-4, a tight junction-associated protein, in brush border membrane. Additionally, α-CD increased the membrane fluidity of lipid bilayers in brush border membrane vesicles and may also promote the permeation of drug molecules via the transcellular pathway. Upon these results, it is concluded that 50 mM α-CD is the optimal CD formulation to enhance the absorption not only by solubilizing curcumin but also by assisting its permeation across the intestinal membrane.

Summary

When the solubility was higher than 5 μg/mL, the rate-limiting step of curcumin absorption was shifted from the apparent drug solubility to the permeation across the intestinal membrane, which confirmed the drawbacks of curcumin in both solubility and permeability. Of tested absorption enhancers, 1% (v/v) LMT or CMT, and 50 mM α-CD improved the absorption of curcumin from the rat small intestine significantly without inducing any serious toxicity to intestinal tissue or organs. The absorption-enhancing effect of these materials on the paracellular pathway was evidenced by Caco-2 cell model. In particular, α-CD altered the barrier properties of both the paracellular and transcellular pathways. Therefore, the intestinal absorption enhancement by absorption enhancers might be attributed to the synergistic effect of increased solubility and permeability of curcumin in their presence.

審 査 の 結 果 の 要 旨

Curcumin はウコンの根茎 (*Curcuma longa* L.) から得られる黄色化合物であり、天然の食用色素として広く利用されている化合物である。近年、curcumin は、抗酸化作用、抗炎症作用、抗腫瘍作用等の様々な有用な薬理効果を有することが明らかとなっており注目されている。しかしながら、curcumin は、溶解性が低く膜透過性が低いこと、消化管内で不安定であることから消化管吸収性が極めて悪いことが知られており、臨床応用が制限されているのが現状である。そこで本研究では、 製剤添加物として界面活性剤である *N*-アシルタウリン塩及び包接化合物であるシクロデキストリン (CD) を用いて curcumin の溶解性ならびに消化管吸収性の改善を試みた。

N-アシルタウリン塩は胆汁中に含まれる生体内界面活性剤であるタウロコール酸に類似した構造を持つアニオン性界面活性剤である。本研究では、 curcumin の消化管吸収性に及ぼす 5 種類の *N*-アシルタウリン塩の影響について系統的に検討した。その結果、用いた *N*-アシルタウリン塩のうち、sodium methyl lauroyl taurate (LMT) 及び sodium methyl cocoyl taurate (CMT) が curcumin の消化管吸収性を最も改善することが明らかとなった。また、これら *N*-アシルタウリン塩の消化管粘膜障害性について、消化管管腔内における乳酸脱水素酵素 (lactate dehydrogenase, LDH) の活性を指標にして評価したところ、いずれの *N*-アシルタウリン塩も消化管粘膜障害性はほとんど見られないことが認められた。さらに、*N*-アシルタウリン塩の吸収促進機構について検討したところ、*N*-アシルタウリン塩の併用による curcumin の溶解度の上昇と、消化管上皮細胞における tight junction の開口に伴う細胞間経路の透過性の増大が寄与している可能性が示唆された。

次に包接化合物である各種 CD を用いて curcumin の溶解性及び消化管吸収性の改善を試みた。その結果、各種 CD が、curcumin の溶解性及び消化管吸収性を改善することが認められたが、中でも α-CD の吸収促進効果が高いことが明らかとなった。次に α-CD の消化管粘膜障害性について評価したところ、消化管粘膜障害性はほとんど見られないことが認められた。また、α-CD の消化管投与後の肝臓ならびに腎臓の毒性も見られず、α-CD の安全性が確認された。さらに、curcumin の消化管吸収に対する α-CD の吸収促進機構について検討したところ、α-CD の併用により、 Caco-2 細胞の膜抵抗値 (transepithelial electrical resistance, TEER) が低下し、主に細胞間経路を透過することが知られているマーカー物質である 5(6)-carboxyfluorescein (CF) の透過性が増大することが認められた。また、tight junction の開口に関与する claudin-4 の発現量が、α-CD の併用により低下することが認められた。従って、curcumin の消化管吸収に対する α-CD の消化管吸収促進機構には、消化管上皮細胞における tight junction の開口に伴う細胞間経路の透過性の増大が寄与している可能性が示唆された。一方、α-CD は消化管上皮細胞の脂質部分の膜流動性を増大させることから、細胞内経路を介した curcumin の消化管吸収性を改善する作用も有することが示唆された。

これらの知見は、難溶解性ならびに難吸収性化合物である curcumin の経口製剤開発に有用な基礎的情報を提供するものと考えられる。

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（薬学）の学位論文としての価値を有するものと判断する。