博士（薬　学）　何　　新

学 位 論 文 題 名

An in vitro system for prediction of drug absorption and pH-related changes in absorption

(In vitro システムを用いた経口製剤の吸収性評価に関する研究)

学位論文内容の要旨

Introduction

Oral administration is the easiest and most useful method for drug delivery, and prediction of drug absorption is therefore very important for the design of an oral preparation. In this study, we attempted to develop a system for predicting drug absorption, and also tried to establish several models of different gastric acidities for evaluating various kinds of oral preparations and drug-drug interactions.

Results and Discussion

1. Drug absorption prediction system[1]

An in vitro system for prediction of oral absorption in humans includes a drug-dissolving vessel (DDV; modeled stomach), a pH adjustment vessel (PAV; modeled intestine) and a side-by-side diffusion chamber mounted not only by Caco-2 monolayer but also by rat intestine.

2. Pharmacokinetic model[1]

The elution of a relatively water-soluble drug to the donor compartment of side-by-side diffusion chamber is expected to follow a one-compartment drug-distribution model. (flip–flop phenomenon).

$$C = \frac{k_2 X_0}{(k_2 - k_1)V_d}(e^{-k_1 t} - e^{-k_2 t}) \quad (1) \qquad AUC = \int_0^\infty C dt = \frac{k_2 X_0}{(k_2 - k_1)V_d}\left(\frac{1}{k_1} - \frac{1}{k_2}\right) = \frac{X_0}{k_1 V_d} \quad (2)$$

3. Models of different gastric acidities[2]

The normal gastric acidity model (model **A**) was used for prediction of drug absorption that takes into account the physiological conditions of the GI tract. We established a low gastric acidity model (model **B**, model of achlorhydria) and a temporarily elevated gastric acidity model (model **C**), in which gastric acidity was elevated temporarily by coadministration of an acidic drug. The weak antacid model (model **E**) is a model of a case in which a weak antacid drug was coadministered to temporarily elevate pH up to 6.0. The strong antacid model (model **F**) is a model of a case in which a strong antacid drug was coadministered to temporarily elevate pH up to 8.0. As a control experiment, a model of normal gastric acidity (model **D**) was established using a PAV solution with pH of 7.5.

4. Permeation of relatively water-soluble drugs across a Caco-2 monolayer or a rat intestine[1]

Drugs that permeated across a Caco-2 monolayer at cumulative permeation of more than 0.03% or

over 0.04% in the rat intestine, such as caffeine and theophylline, can be almost completely absorbed in humans. If the cumulative permeation across a Caco-2 monolayer is lower than 0.03% or below 0.04% in the rat intestine, there were good linear correlations between cumulative permeation across a Caco-2 monolayer and oral absorption in humans (R=0.967) and between cumulative permeation across a rat intestine and oral absorption in humans (R=0.959).

5. Evaluation of absorption of two preparations of ketoprofen[3]

We examined the release of commercial capsules and controlled-release granules of ketoprofen in model **A** and model **B**. The concentration after administration of a commercial capsule increased more rapidly and then decreased more rapidly in a state of low gastric acidity than in a state of high gastric acidity. No variation of absorption was observed after administration of controlled-release granules to high and low gastric acidity models because of the lower sensitivity to the surrounding pH.

6. Evaluation of pH-related changes in absorption of dipyridamole and glibenclamide[2, 4-7]

The purpose of this experiment was to compare the amounts of drugs absorbed and the degrees of absorption variation due to changes in pH in different models. Our results agree with results obtained in clinical studies showing that amounts of oral absorption of dipyridamole in subjects with low levels of gastric acidity are much lower than those in subjects with normal levels and that the oral absorption can be improved to some degree by coadministration of acidic drugs, which can temporarily increase gastric acidity. The cumulative permeation of glibenclamide in model **F** was significantly higher than that in model **D**, but the cumulative permeation in model **E** was almost the same as that in model **D**.

7. Studies of absorption mechanism and absorption prediction of ester prodrugs[1, 8]

The enzymatic activities in homogenates from the small intestines of rats or humans were higher than those in homogenates from Caco-2 cells. Ester hydrolysis showed that the disappearance occurs in accordance with the esterase activity of homogenates. Higher P_{app} values were obtained for prodrugs than for active acids. At the end of flux studies, the cell accumulation of ampicillin produced from pivampicillin in the Caco-2 monolayers was greater than that of ampicillin when ampicillin was added to the apical side. The same tendency was found in the case of cefcapene (CFPN). These results suggest that intracellular degradation of prodrugs resulted in intracellular accumulation, and extensive ester hydrolysis and increased transepithelial flux of the active metabolite occurred during transport.

Permeation of pivampicillin and cefcapene pivoxil (CFPN-PI), ester-type oral antibiotics, across a Caco-2 monolayer and a rat intestine using model **A** was also studied. A higher degree of oral absorption of a prodrug predicted from a Caco-2 or rat intestine model than that determined from a clinical experiment in humans was received.

Conclusions

(1) An *in vitro* system and several models of different gastric acidities for the prediction of oral absorption of drugs were established. (2) Not only Caco-2 monolayers but also other membranes, such as rat intestine, can be mounted on the chamber in this system. (3) Our system can also enable

evaluation of pH-related variation in absorption of more kinds of oral preparation and drug-drug interactions. (4) The absorption mechanism of ester prodrugs in Caco-2 and rat intestine models was elucidated.

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学位論文審査の要旨

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学位論文題名

An in vitro system for prediction of drug absorption and pH-related changes in absorption

(In vitro システムを用いた経口製剤の吸収性評価に関する研究)

経口投与製剤の開発初期においては、主薬の安定性、溶解性、膜透過性などの薬物の特性それぞれを評価し、動物実験等も含めた検討により吸収性の優れた化合物、製剤を選択して行く。これらの基礎的な検討に要する時間と労力は膨大であり、この課程をいかに簡便に、短期間で行うかが解決すべき問題点の一つになっている。これまで、経口投与製剤の吸収性試験は、薬物の製剤からの溶出性試験と動物を用いた吸収性試験、培養細胞を用いた膜透過性試験などが別々に行われてきた。本研究は、これまで多段階で行われてきた製剤評価を同時に実施し、ヒトにおける吸収性を予測できる実験装置の開発を目的として行われた。また、胃内pHの変動を想定した実験条件を確立し、消化管吸収与える胃内pHの影響を評価できる実験系の構築を目的とした。

まず、実験装置のデザインおよびその妥当性を検討した。実験装置は胃を想定した「薬物溶出槽」、腸への移行を想定した「pH 調整槽」および腸管での吸収を想定した「拡散チャンバー」から成る。チャンバー部分にはCaco-2 培養細胞膜あるいは腸管組織を装着可能である。このような装置は本研究で初めて検討されたものであり、新規性がある。本装置を吸収評価に適用するに当たって、薬物動態速度論的モデルを用いて装置内を灌流する液の流速などの妥当性を評価した。その後、本装置を用いて、すでにヒトにおける吸収率が明らかにされている薬物を用いて検討した結果、本装置を用いて得られた培養細胞膜透過量および腸管組織透過量とヒトにおける吸収率との間に良い相関が認められたことから、本実験装置はヒトにおける易溶性薬物の吸収性評価に有用であることが示された。

次に、本装置を用いて胃内 pH 変動が経口投与製剤の吸収性に与える影響について検討した。本装置は「薬物溶出槽」に流入する液の組成を変えることで、種々の胃内 pH を再現できた。すなわち、無酸症状態や一過性の低胃酸度状態、強制酸剤服用時の過度の高 pH 状態など、種々の胃内 pH 条件での吸収性評価が可能であった。そこで、胃内 pH に依存した吸収性を示すいくつかの薬物を用いて、製剤学的な手法により pH 非依存的吸収性を示す製剤を調製し、本装置の有用性を検討した。その結果、pH 依存的な吸収、非依存的な吸収いずれも *in vivo* での検討で得られた結果とよく一致していた。これらのことより、本実験装置は消化管内の pH 変動に伴う製剤の消化管内動態、吸収性の変化をよく反映するシステムであることが実証された。

口頭発表において、井関教授より本装置の適用可能な薬物の範囲および溶解せずに流れる粉末がチャンバーに装着した細胞に与える影響に関して質問があった。また、山崎助教授より、本装置が現在用いられている評価法に変わり得るものであるかとの質問がなされた。いずれの質問にも本装置の利点、欠点をもとに妥当な回答を行った。また、提出された論文の審査において、審査担当者よりいくつかの修正点を指摘され修正した。

本研究において確立した本装置は各種経口投与製剤間の吸収性の優劣を、一つの装置で判定できる *in vitro* 予測システムであり、医薬品開発を効率よく進める上で有用性の高い装置と考えられる。また、本研究の成果は、医薬品開発のみならず、医療現場における特殊製剤の検討などにも利用可能であるものと考えられ、臨床上の有用性も高い。

以上のことより、本研究は学位論文として価値あるものと認めた。