学位論文要旨

Summary

Development of prodrugs is one of the common approaches to overcome various defects of candidate drugs. The purpose of this study is to establish an *in vitro* system for selection of candidate prodrugs and appropriate experimental animals. First, by evaluating physicochemical/biopharmaceutical properties and metabolic profiles of 21 model prodrugs, which are hydrolyzed to their active metabolites, a novel strategy was established according to the following 4 viewpoints; 1) log D values can be used as an index of lipophilicity so that prodrugs with improved membrane permeability should be more lipophilic than its active metabolites, 2) in solubility assay, artificial intestinal fluids can be used to evaluate the improvement of solubility of prodrugs, 3) membrane permeability of prodrugs should be higher than that of its active metabolites, 4) in metabolism study, conversion efficiency of prodrugs to their active metabolites and metabolic profile in small intestine should be ideally similar between human and experimental animals for *in vivo* pharmacokinetic (PK) studies. All 21 prodrugs were efficiently converted to active metabolites with human matrices, although some of them were not with dog or monkey matrices. Then, the utility of the established strategy was evaluated using oseltamivir and its 23 analogues. It was demonstrated that oseltamivir and 16 out of 23 analogues would be capable for proceeding to *in vivo* PK studies. Since oseltamivir was selected to be one of the effective prodrugs, this study demonstrated the usefulness of the established strategy for selection of candidate compounds and experimental animals for preclinical study. Taken together, the present study successfully proposed a novel screening strategy for efficient selection of prodrug candidates.

Background

Development of prodrug is one of the useful strategies for overcoming various defects of compounds such as low bioavailability, short duration of action, and toxicity. The concepts of some marketed prodrugs have been published, but information regarding the processes to select efficient

candidate prodrugs has been limited. Furthermore, the species differences in the specific activities, substrate specificities, and tissue distributions of hydrolases are obstacles to predict drug disposition in humans from animal data. To increase the success rate in development of prodrugs, a systematic *in vitro* method that enables an appropriate selection of candidate prodrugs is desired. The purpose of this study is to establish a systematic approach for screening of prodrugs based on physicochemical/biopharmaceutical and ADME properties.

Physicochemical/biopharmaceutical properties and metabolic profiles of 21 clinically used prodrugs

For this purpose, physicochemical/biopharmaceutical properties (log D, solubility, and membrane permeability) and metabolic stabilities of 21 selected clinically used prodrugs with improved membrane permeability (azilsartan medoxomil, bacampicillin, benazepril, candesartan cilexetil, cefuroxime axetil, enalapril, fenofibrate, fesoterodine lenampicillin, mycophenolate mofetil, olmesartan medoxomil, moexipril, oseltamivir, ramipril, sultamicillin, and temocapril) or aqueous solubility (estramustine phosphate, etoposide phosphate, fosamprenavir, prednisolone phosphate, and tedizolid phosphate) and their pharmacologically active metabolites were characterized. All of these 21 prodrugs are subjected to the hydrolysis to produce their active metabolites. Log D values of the prodrugs with improved membrane permeability were higher than those of their active metabolites, whereas those of the prodrugs with improved aqueous solubility were lower than those of active metabolites. Solubility of the prodrugs with improved membrane permeability in artificial intestinal fluids was not extremely low compared with its active metabolites. Membrane permeability of the prodrugs was higher than that of its active metabolites.

After the absorption, prodrugs should be efficiently converted to an active metabolite, and metabolic profiles in small intestine should be ideally similar between human and experimental animals. To estimate the contribution of hydrolysis to overall metabolism of the prodrugs, two novel parameters were set. The ratio of the amount of active metabolite to amount of all metabolites after an *in vitro* metabolism assay using enterocytes, hepatocytes, or serum was defined as conversion

ratio (CR), as follows:

$$CR = [\text{Formation of active metabolite (\%)}] / \{100 - [\text{Remaining prodrug (\%)}]\}$$

The $CR_{\text{small intestine}}$, CR_{liver}, or CR_{serum} represent the rate of conversion in each tissue. The sum of $CL_{\text{int,small intestine}}$, $CL_{\text{int,liver}}$, and $CL_{\text{int,serum}}$ was defined as $CL_{\text{int,total}}$, representing the intrinsic clearance in body. CS was defined as the conversion efficacy in each tissue. $CS_{\text{small intestine}}$, CS_{liver}, or CS_{serum} represent the ratio of the intrinsic clearance of prodrug that was converted to active metabolite in each tissue to that in body, and was calculated using the following equations:

$$CS_{\text{small intestine}} = CR_{\text{small intestine}} \times CL_{\text{int,small intestine}} / CL_{\text{int,total}}$$

$$CS_{\text{liver}} = CR_{\text{liver}} \times CL_{\text{int,liver}} / CL_{\text{int,total}}$$

$$CS_{\text{serum}} = CR_{\text{serum}} \times CL_{\text{int,serum}} / CL_{\text{int,total}}$$

The sum of $CS_{\text{small intestine}}$, CS_{liver}, and CS_{serum} was defined as CS_{total}. CS_{total} should ideally be 1.0, if prodrugs were efficiently converted to active metabolites in human body. Almost all the prodrugs were rapidly hydrolyzed in enzymatic and/or non-enzymatic manner and the tested prodrugs showed high CS_{total} values in humans. However, they showed lower CS_{total} values in monkey and dog. It is generally recognized that monkey shows similar tissue distribution and substrate specificities of drug-metabolizing enzymes, but F (especially F_g) of monkey is not always close to that of humans. Dog shows similar absorption to humans, but shows lower hydrolase activities than humans, with lacking CES2. The CS_{total} values in rat were close to those in humans, although the numbers of CES isoforms and their expression levels in rat were different from those in humans. Thus, the CS_{total} value would be a good parameter for selection of animals for *in vivo* PK screening to evaluate bioavailability showing similarity to humans.

From the results of physicochemical/biopharmaceutical properties and metabolic profiles, a novel systematic approach for screening of prodrugs has been established by following concept (Fig. 1): In the 1st step, pharmacologically active compounds with low metabolic clearance are selected. In the 2nd step, the number of candidate prodrugs is narrowed down based on the criteria for log D, solubility in artificial intestinal fluids, membrane permeability, and human CS_{total}. In the 3rd step, animal species to be used in the subsequent *in vivo* PK studies are selected based on animal CS_{total}. In

the 4th step, the *in vivo* PK in the selected experimental animal is evaluated. Based on the results from the *in vivo* PK studies, the criteria for selection of compounds in the 2nd step, and, if necessary, in the 1st step, would be rearranged to select other candidate prodrugs. This scheme would streamline the development of prodrugs.

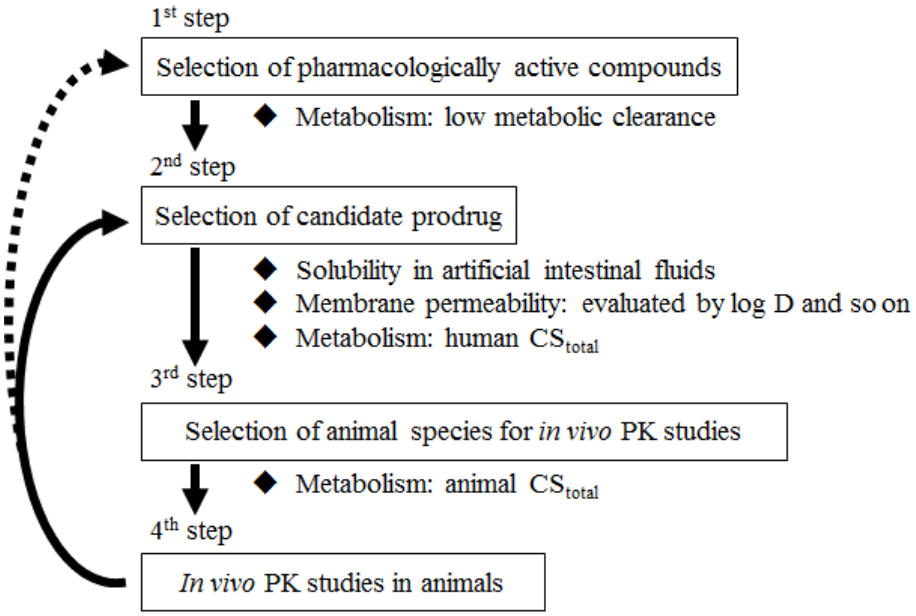

Fig. 1. Scheme for *in vitro* screening of candidate prodrugs designed to enhance oral absorption.

Physicochemical/biopharmaceutical properties and metabolic profiles of oseltamivir and its 23 analogues

The utility of the established systematic approach for screening of prodrugs was evaluated using oseltamivir and 23 kinds of oseltamivir analogues having various types of side chain as model compounds (Fig. 2). Oseltamivir analogues with varied lipophilicities were designed to show higher log D values than oseltamivir acid, an active metabolite. To investigate the effects of metabolism in small intestine on membrane permeability, some analogues were designed to be hydrolyzed by carboxylesterase (CES) 2, which is expressed in the human intestine and liver, by adding large acyl moiety, although oseltamivir is hydrolyzed by carboxylesterase (CES) 1 expressed in the liver. By using these analogues, physicochemical/biopharmaceutical properties (log D, solubility, and

membrane permeability) and metabolic stabilities were characterized.

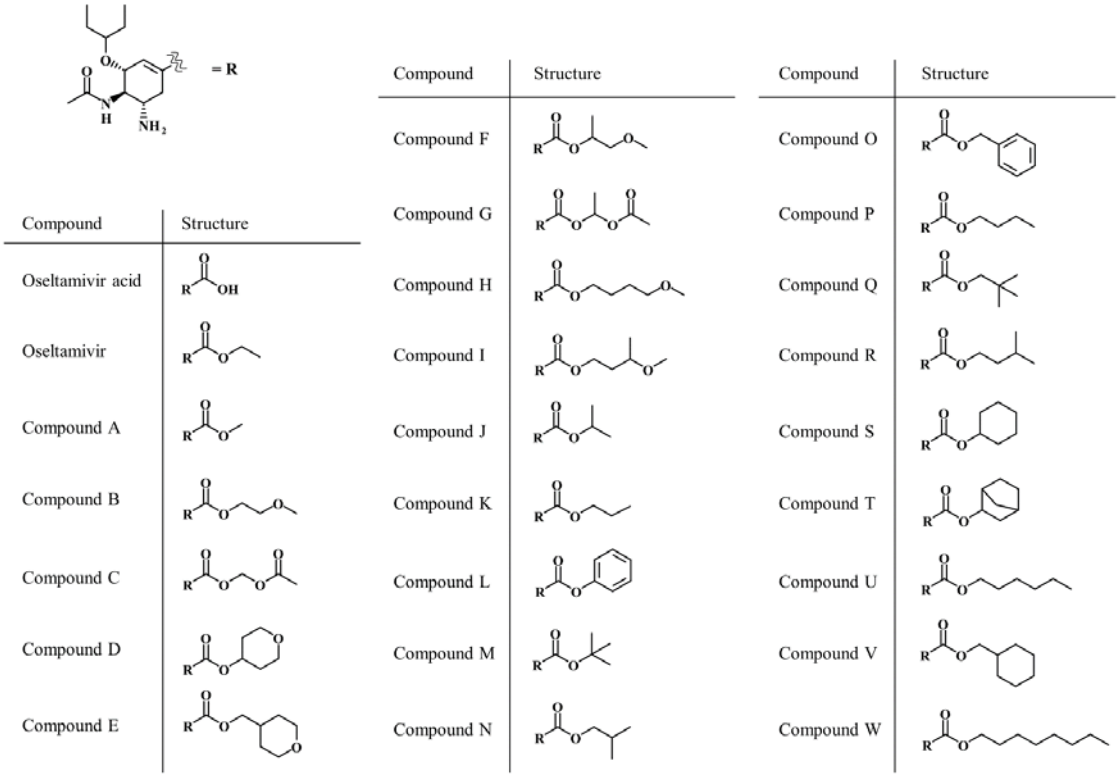

Fig. 2. Chemical structures of oseltamivir and its analogues used in this study.

Log D values of oseltamivir and analogues (2.0 to 4.9) were higher than that of oseltamivir acid (0.7), supporting a fact that oseltamivir had been developed to improve permeability of oseltamivir acid. Solubilities of analogues in artificial intestinal fluids were over 80% except compound W having the highest lipophilicity. The membrane permeability of oseltamivir and its analogues were higher than that of oseltamivir acid. In addition, a positive correlation was observed between membrane permeability and log D values of the analogues. By the analysis of metabolic profile, it was demonstrated that compounds S and T were subjected to metabolic reaction(s) other than hydrolysis in human, dog, and monkey. In addition, compounds E, Q, V, and W in dog and compounds E, M, and Q in monkey were subjected to metabolic reaction(s) other than hydrolysis. All of the analogues were efficiently hydrolyzed in rat. Thus, *in vitro* metabolism study would be necessary for the selection of experimental animals suitable for the prediction of PK in humans. Furthermore, to clarify whether oseltamivir and its analogues are hydrolyzed by CES1 or CES2,

metabolism study using recombinant enzymes was performed. Although some analogues were hydrolyzed by enzymes other than CES1 as intended, most of the analogues were mainly hydrolyzed by CES1 despite of varied functional groups. In addition, metabolism studies using recombinant enzymes and human matrices revealed that a functional group with larger steric hindrance might be unsuitable for the addition to oseltamivir acid, because hydrolase activity of the analogues was decreased and alternative metabolic reaction(s) proceeded.

By using the established systematic strategy, it was demonstrated that oseltamivir and 16 out of 23 analogues are appropriate prodrugs, which could be proceeded to *in vivo* PK studies, with selection of suitable experimental animals.

Conclusion

This study demonstrated that physicochemical/biopharmaceutical properties could be useful information to facilitate design of prodrugs and for selection of candidate prodrugs, and the *in vitro* evaluation of conversion efficiency to active metabolites would be helpful for selecting ideal prodrugs as well as appropriate animals for *in vivo* PK studies. This systematic strategy would be one of the tools to facilitate drug development.

Reference

Shimizu M, Fukami T, Taniguchi T, Nomura Y, and Nakajima M (2020) A novel systematic approach for selection of prodrugs designed to improve oral absorption. *J Pharm Sci* **109**: 1736-1746.

審査結果の要旨

プロドラッグ化は，消化管吸収性や薬効の持続性改善，副作用の軽減を目的に用いられる有用な手法の一つである。市販されているプロドラッグの中にはその薬効体をプロドラッグ化した目的を公開しているものもあるが，化合物選抜のプロセスについては情報がほとんどない。また，プロドラッグは代謝されて薬効体に変換されるが，変換を担う薬物代謝酵素の活性や基質特異性，発現分布にヒトと実験動物で種差があり，動物実験で得られた *in vivo* PK 試験結果のヒトへの外挿性を困難にしている。本研究では，*in vitro* 試験に基づいたプロドラッグ創出スキームを構築することを目的とし，市販プロドラッグ 21 化合物とその薬効体の log D，溶解性，膜透過性，代謝安定性を評価した。脂溶性の指標である log D には，膜透過性と水溶性改善の開発目的別に明確な差が認められ、また、人工腸液を用いた溶解性評価は化合物の溶解性向上を捉えることに適していると考えられた。代謝安定性試験のデータより，プロドラッグからの薬効体への変換に関して新たなパラメータを創出し，ヒトと実験動物種の種差を明確に示した。これらの物性および動態学的特徴よりプロドラッグ創出スキームを構築した。次に、構築したスキームの有用性を検証するために，市販プロドラッグであるオセルタミビルをモデル化合物とし，その類縁体 23 化合物を合成して，log D，溶解性，膜透過性，代謝安定性を評価した。オセルタミビルは膜透過性を向上させることで吸収改善を目的に開発されたプロドラッグであることから，類縁体の log D は薬効体よりも高く設計した。その結果，最も log D の高い類縁体は他化合物よりも溶解性が悪く，また，膜透過性は全ての化合物で薬効体よりも高かった。変換効率はヒトとラットが類似しており，イヌやサルは薬効体以外への代謝物に変換されやすい傾向にあり，代謝される組織はラットが最もヒトと異なっていた。物性および薬効体への変換効率に基づき、オセルタミビルを除外することなく候補化合物を選抜でき，構築したプロドラッグ創出スキームの有用性が示された。本研究は、プロドラッグ開発における新たなスキームを提唱した点で創薬科学的意義が認められることから、博士(創薬科学)論文に値すると判定された。