An Assessment of the Oral Bioavailability of Three Ca-Channel Blockers Using a Cassette-Microdose Study: A New Strategy for Streamlining Oral Drug Development

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ABSTRACT: A cassette-microdose (MD) clinical study was performed to demonstrate its usefulness for identifying the most promising compound for oral use. Three Ca-channel blockers (nifedipine, nicardipine, and diltiazem) were chosen as model drugs. In the MD clinical study, a cassette-dose method was employed in which three model drugs were administered simultaneously. Both intravenous (i.v.) and oral (p.o.) administration studies were conducted to calculate the oral bioavailability (BA). For comparison, p.o. studies with therapeutic dose (ThD) levels were also performed. In all studies, blood concentrations of each drug were successfully determined using liquid chromatography–mass spectrometry with the lower limit of quantification of 0.2–2.0 pg/mL. Oral BA of nifedipine in the MD study was approximately 50% and in the same range with that obtained in the ThD study, whereas other two drugs showed significantly lower BA in the MD study, indicating a dose-dependent absorption. In addition, compared with the ThD study, absorption of nicardipine was delayed in the MD study. As a result, nifedipine was considered to be most promising for oral use. In conclusion, a cassette-MD clinical study is of advantage for oral drug development that enables to identify the candidate having desired properties for oral use. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:3154–3161, 2015

Keywords: clinical study; microdose; cassette dose; Nonlinear pharmacokinetics; bioavailability; first-pass metabolism; dose proportionality; oral absorption

INTRODUCTION

The number of new API (active pharmaceutical ingredients) that was approved as a new medicine in Japan, US, and Europe is decreasing every year after 2000.[1] The main causes of this critical problem in pharmaceutical industries include a low-success probability in the clinical trial. Clinical trial often terminated because of the insufficient efficacy and/or safety in human, although the test compound was selected as a potent candidate from the millions of newly synthesized compounds. Now, success probability of the new drug candidates in the clinical trial is reported to be less than 10%.[2]

Exploratory investigational new drug (eIND) studies have been proposed to help identifying potent candidates at the early stages of drug development to improve the efficiency or the success ratio in the drug development. In the guidance on M3(R2) of International Conference on Harmonization, five types of eIND studies were defined.[3] Among those eIND studies, Type I and Type II methods correspond to the "microdose (MD)" clinical study, in which investigational new drugs can be administered to humans at the dose less than 1/100th of the minimum dose of a test compound, at which its pharmacological or nontoxicological action appears, and at a maximum dose of $\leq$100 μg. An exploratory MD clinical study can provide useful inside into the pharmacokinetic (PK) behavior of a candidate drug, before entering expensive and potentially dangerous studies at therapeutic dose (ThD) levels. The MD clinical study can therefore help avoiding a failure from poor PK properties in human and consequently increase the overall success ratio.[4–6]

For oral use, the API is required to show the sufficient blood concentration profile for therapy by oral (p.o.) administration. In order to predict the oral absorbability of candidates in human, addition to the *in vitro* study for solubility, permeability, and enzyme stability of compounds, *in vivo* studies with experimental animals, such as rats, dogs, and monkeys, have been performed in the drug discovery and preclinical stages.[7–9] These information with kinetic model analysis for drug absorption have enabled the simulation of the blood concentration profiles of candidate compounds after p.o. administration and oral bioavailability (BA).[10,11] However, on the contrary, Sietsema[12] has reported that the oral BA of various drugs in rodents, dogs, and primates does not correlate with those in humans mainly because of the large species differences in the first-pass metabolism in the liver and/or small intestine. Furthermore,

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Musther et al.[13] have expanded the dataset of oral BA in experimental animals and humans and concluded that the lack of correlation highlighted that animal BA is not quantitatively predictive of BA in humans. Difficulties in animal scale up of oral BA sometimes resulted in the unpredicted low blood concentration of the test compound in human that might terminate the project during the clinical trial.

As one of the advantages of the MD clinical study, multiple compounds can be administered simultaneously to the same subject as a cassette if the total dose of compounds meets the requirement for the MD clinical study. The cassette-MD study is highly productive to select the most promising compound for Phase I trial having a most desirable PK profiles in human. Progress in the ultrasensitive liquid chromatography–mass spectrometry (LC-MS/MS) technology has enabled this method in which very low plasma concentration (pg/mL level) of multiple compounds should be detected with a high accuracy.[14] In our previous report, the cassette-dose method was successfully used for analyzing the rate-determining process of the hepatic elimination of atorvastatin where atorvastatin was given to subjects together with the probe drugs for drug transporter OATP (pravastatin), and drug metabolizing enzyme CYP3A4 (midazolam).[15]

In this study, to demonstrate the usefulness of the cassette-MD clinical study for selecting the most promising compound for oral use, three Ca-channel blockers (nifedipine, nicardipine, and diltiazem) were chosen as the model drugs as these drugs are reported to undergo the extensive first-pass metabolism in the liver and/or in the small intestine after p.o. administration.[16,17] In the protocol of the MD clinical study, three model drugs were administered simultaneously to human as a cassette. Both intravenous (i.v.) and p.o. administration studies were conducted as the cassette-MD studies to calculate the oral BA. In addition, to compare the blood–concentration profiles after administration of MD and ThD levels of these drugs, p.o. studies with ThD levels were also performed.

MATERIALS AND METHODS

Subject

A total of eight healthy male Japanese volunteers were recruited and registered for this clinical study. The inclusion criteria were age between 20 and 40 years and body mass index (BMI) between 18.0 and 30.0 kg/m^2. For screening the eligibility of subjects for this clinical study, the history of significant medical illness, alcohol dependence, or hypersensitivity to any drugs were obtained from each candidate subject before the study. The subjects were not permitted to consume beverages containing grapefruit juice or supplements containing St. John's wort or any drugs from 7 days before admission to the hospital until the end of the study. All subjects gave their written informed consent to take part in all phases of the study. In each phase, the subjects came to the hospital the day before the drugs were scheduled to be administered to check their health condition. All studies were carried out after an overnight fast. During the whole period of study, routine safety assessments consisted of collecting all adverse events and serious adverse events, along with their severity and relationship to study drug. Safety assessments also included regular monitoring of hematology, blood chemistry, and urinalysis as well as vital signs, and physical examination.

Model Drugs

From the category of antihypertensive drug, three Ca-channel blockers, nifedipine, nicardipine hydrochloride (nicardipine), and diltiazem hydrochloride (diltiazem), were selected as model drugs for this study. These three drugs are known to show relatively low oral BA because of the extensive first-pass metabolism.

Marketed products of three drugs for i.v. treatment were used for both MD-i.v. and MD-p.o. studies and ThD-p.o. studies except for the ThD-p.o. study with nifedipine. Used products of drugs were HERBESSER® 10 mg for injection (Mitsubishi Tanabe Pharma Co. Chuo-ku, Osaka, Japan) for diltiazem, and Perdipine® injection 2 mg (Asteras Pharma Inc. Chuo-ku, Tokyo, Japan) for nicardipine. In the case of nifedipine, Adalat® 5 mg/50mL pro infusione (Bayer AG. Leverkusen, Germany) was used for MD study, and Adalat® capsule 10 mg (Bayer AG. Leverkusen, Germany) was used for ThD study.

Study Design

The study protocol including blood sampling of human subject in accordance with GCP was approved by the internal review boards of the Oita University hospital. The study was performed in the Oita University hospital and was registered in the UMIN Clinical Trials Registry at http://www.umin.ac.jp/ctr/index.htm (UMIN000002578) before starting the study.

The protocol of this clinical study consists of five phases. First two phases are the MD study for i.v. and p.o. administration of drugs carried out as an open, parallel, randomized, cross-over clinical study. Latter, three phases are the p.o. administration study with ThD carried out as an open study (Fig. 1).

MD Study

In the MD clinical study, three drugs were administered as a cassette for both i.v. and p.o. According to the guidance for MD clinical trials, it is defined that a test compound allows administering less than 1/100 of the minimum dose, at which pharmacological or nontoxicological action appears, and at a maximum dose of ≤100 μg.[3] As the ThD of each drug is 10–20 mg (nifedipine), 20–40 mg (nicardipine), and 30 mg (diltiazem), the total dose of three drugs in the MD clinical study was

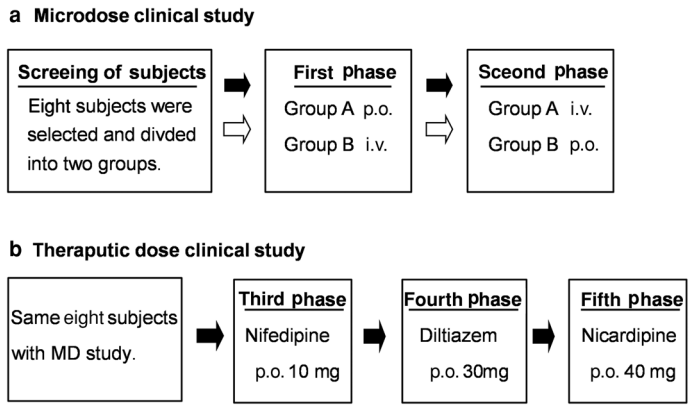

Figure 1. Protocol of the MD and ThD clinical studies. A total of eight healthy male Japanese volunteers were recruited and screened the eligibility for the study. At least 1-week interval was taken as a washout period before starting the next phase of the study.