Protocol Synopsis

Title of study	Global Clinical Study on Ethnic Differences in Drug Metabolism Based on the Joint Statement by the Japanese, Chinese and Korean Ministers of Health Clinical Pharmacokinetic Study of Simvastatin in Healthy Adult Male Subjects (Study number ID: UMIN000003644)
Objective	To investigate whether or not there are ethnic differences in the pharmacokinetics (PK) of simvastatin in healthy adult Japanese, Chinese, Korean and Caucasian male subjects based on the same protocol among the four countries.
Design	Open-label, single administration study
Subject [Inclusion criteria] [Exclusion criteria]	Healthy adult male volunteers are eligible. In Japan, China and Korea, the nationalities of these subjects should be the same as those of grandfathers, grandmothers, father and mother. In China, only the Han race is eligible. In the U.S., only European Caucasian is eligible. In this study, the above healthy adult male volunteers who satisfy all of the following inclusion criteria and none of the following exclusion criteria are eligible. 1) Those who are capable of providing written informed consent. 2) Men aged 20 to 35 years at the time of signing informed consent. 3) BMI of 18.5 to <30.0 and body weight of 50.0 to 100.0 kg at screening. 4) Those who are determined by the investigator to be healthy in the screening test and eligible for the study. 1) Organopathy involving the heart, lung, liver and/or kidney etc. 2) A history of diseases involving the heart, lung, kidney, blood (such as coagulation system disorder), central nervous system and metabolic system that may interfere with the study. 3) Hypothyroidism, genetic myopathy or family history of it, history of drug-induced myopathy 4) Hypersensitivity or allergies to drugs or food, etc. (Particularly, a history of allergy to or adverse reactions associated with statin-type antihyperlipidemia drugs) 5) Oral administration of drugs such as over-the-counter drugs within 1 week prior to the study drug administration, or taking any kind of health food/supplement, grapefruit, drink/food containing grapefruit from 2 week prior to the study drug administration to the day 2, or taking any kind of fruit juice, drink containing caffeine or green tea from 1day before the study drug administration to the day 2, or the necessity for using other medications before study completion. 6) Smokers or a smoking history within the last 6 months. (The cotinine test is performed, if necessary) 7) Drug abuse or suspicion of drug abuse. (The drug screening test is performed, if necessary) 8) Alcohol drinkers (daily alcohol intake* of 50 g or more). * Alcohol intake (g) = [alcohol content] × [amount of alcohol intake (mL)] × [0.8 (specific gravity: weight of 1 mL of alcohol)] Example: When drinking 1000 mL of beer (alcohol content: 5.5%), alcohol intake = 0.055 × 1000 × 0.8 = 44 g 9) Total bilirubin or direct bilirubin, AST, ALT and ALP is 1.5 times higher, or other liver and renal function tests items are 1.25 times higher than the upper limits of normal at the sites. 10) Blood sampling of 200 mL or more within 1 month prior to the study drug administration, blood component donation (plasma or platelet) within 2 weeks, or blood collection of 400 mL or more within 3 months. 11) Participation in a Phase I study of a drug containing a new active ingredient within 4 months prior to the study drug administration or another clinical study within 3 months, and being administered the investigational drug (within 1 month prior to the study drug administration for a patch test). However, even if the period is longer than these, those who are determined to be not eligible for enrollment in the study in consideration of the characteristics of the previous investigational drug. 12) Those who are determined by the investigator to be not suitable as subjects of the study.
Study drug	A film-coated white tablet containing simvastatin, equivalent to 20 mg simvastatin The same product lot will be used in Japan, China, Korea and the U.S.

Dosage and administration	One 20 mg tablet of the study drug is orally administered with 150 mL of soft mineral water (hardness <100, Volvic®, etc.) after fasting for at least 10 hours. Water drinking is prohibited up to 2 hours after taking the study drug. The subjects can drink soft mineral water (hardness <100, Volvic®, etc.) up to 500 mL during a period from 2 to 4 hours after administration. Soft mineral water (hardness <100, Volvic®, etc.) is given during the study period. Food intake is not allowed up to 4 hours after administration. The calories and the balance of three major nutrients (PFC balance) of the dinner on the day before administration and the first lunch and dinner after administration are unified among the countries as much as possible.
Endpoints	**PK endpoints** 1) Plasma concentrations of simvastatin and its open acid form [Blood sampling points] Before administration (baseline), 0.5,1, 1.5, 2, 3, 4, 5, 6, 8, 12 and 24 hours after administration (12 points in total) [PK parameters to be calculated] For each substance to be measured and total inhibitory substance concentration, model-independent parameters and parameters related to compartmental model are calculated. The following parameters should be calculated for model-independent parameters. Peak plasma concentration (C_{max}), time to peak plasma concentration (t_{max}), elimination half-life ($t_{1/2}$), area under the plasma concentration-time curve (AUC_{0-t}, $AUC_{0-\infty}$), mean residence time (MRT), apparent total clearance (CL/f) and volume of distribution (Vd/f) Maximum plasma concentration ($C_{max,\ norm}$) and AUC ($AUC_{0-t,\ norm}$, $AUC_{0-\infty,\ norm}$) normalized to dose per body weight of each substance to be measured and total inhibitory substance concentration. **Gene analysis for polymorphism** • Gene analysis for polymorphism of the CYP3A4, CYP3A5, ABCB1, OATP1B1 and ABCG2 related to the pharmacokinetics of simvastatin. **Safety endpoints** • Types, severity, onset date/time, duration, number of subjects, number of cases and incidence of adverse events that occurred after the study drug administration will be examined. • For laboratory values, vital signs (body temperature, blood pressure and pulse rate) and body weight, basic statistics (mean and standard deviation) should be calculated by each test point.
Observation and tests	1) Observation period: From the day of the study drug administration (Day 1) to discharge from the hospital (Day 2). 2) Tests: Physical examination findings (history taking, subjective symptoms, objective findings, auscultation, percussion), hematology, blood biochemistry, urinalysis, vital signs (body temperature, blood pressure and pulse rate) and body weight measurement
Target sample size	40 subjects for each country (160 subjects in total in Japan, China, Korea and the U.S.)
Planned study period	June 2010 to November 2010

Study Schedule

		Screening	Study												
Date of study		Within 30 days	Day -1	Day 1											Day 2
Time			Admission by 16:30	8:00	9:00	9:30	10:00	10:30	11:00	12:00	13:00	14:00	15:00	17:00	21:00	9:00
Time after oral administration (h)				-1	0	0.5	1	1.5	2	3	4	5	6	8	12	24
Admission/visit		Visit	Admission	←												→
Informed consent		○														
Subject background characteristics		○														
Study drug administration					○											
Physical examination by the investigator		○		○												○
Body weight		○		○												○
Height		○														
Vital sign		○		○												○
12-lead electrocardiography		○														
Adverse event			←													→
Blood sampling	Gene polymorphism examination															○[a)]
	Pharmacokinetics			○		○	○	○	○	○	○	○	○	○	○	○
	Laboratory test	○		○												○
	Infectious disease test	○														
Urine sampling	Laboratory tests	○		○												○

Meal time during hospitalization: Morning of Day 1 is fasting. Follow the instruction on the meal time by the site other than the following.

1 day before administration (Day -1: at 19:00)

Day of administration (Day 1: Having a lunch after completion of blood sampling and tests at 4 hours after administration.)

1 day after administration (Day 2: Having a breakfast after completion of blood sampling and tests at 24 hours after administration.)

a): Collected specimens (e.g., EDTA-2Na added) will be refrigerated at -60°C or less, and sent to the institute for gene polymorphism analysis using dry ices within 2 weeks after sampling, if possible.

List of abbreviations

Abbreviation	Full expression
ABC	ATP-binding cassette (ATP-binding cassette) ABC transporter: gene ABCB1, gene ABCG2
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve AUC_{0-t}: Area under the plasma concentration-time curve from time zero to the last blood sampling $AUC_{0-\infty}$: Area under the plasma concentration-time curve from time zero to infinity
CK	creatine kinase
CL/f	apparent total clearance
C_{max}	peak concentration
CRP	C-reactive protein
CYP	Cytochrome P450: Collective term of hydroxylase family
f	bioavailability
γ-GTP	gamma glutamyl transpeptidase
HBs antigen	hepatitis B surface antigen
HCV antibody	anti-hepatitis C virus antibody
HDL	high density lipoprotein
HIV antibody	human immunodeficiency virus antibody
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
LDH	lactate dehydrogenase
LDL	low-density lipoprotein
MDR	multidrug resistance protein
MedDRA	Medical Dictionary for Regulatory Activities
MRT	mean residence time
OATP	organic anion-transporting polypeptide
PFC balance	Protein- Fat- Carbohydrate balance
$t_{1/2}$	half-life
t_{max}	time of peak concentration
Vd/f	volume of distribution

List of definitions of terms

Terms	Definition
Anonymized number that is linkable to subjects	The numbers are given to the subjects (anonymized). The record of the linkage of the numbers and the subjects is kept, if necessary to identify the subjects.

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1. INTRODUCTION

Lately, global clinical studies have been promoted from the perspectives of effective and prompt development of new drug and of solving drug lag issue. At the "First Meeting of the Japanese, Chinese and Korean Health Ministers" held in April 2007 in Seoul, the 3 countries worked on the clarification of racial and ethnic differences in clinical study data and ultimately agreed to aim at the mutual acceptance of clinical research data.

In consideration of this circumstance, Japan has analyzed ethnic difference by using existing clinical pharmacokinetic study data in the "Study Group for Evaluation of Ethnic Factors in Clinical Data in Japanese, Chinese and Korean Populations (Tohkin Group)". In response to the result of this research, in the "Global Clinical Study on Ethnic Differences in Drug Metabolism Based on the Announcement by the Japanese, Chinese and Korean Ministers of Health, Labor and Welfare (Kawai Group)," a 2009 Health Labour Sciences Research Grants (Global-Scale Health Topic Promotion Research), implementation of a global pharmacokinetic study is planned with a study drug which has been selected based on evaluation results using existing data to collect supplemental data on ethnic difference or to improve data reliability.

Simvastatin, used as a study drug in this study, is a statin-type antihyperlipidemia drug which has been already marketed in all the countries. Its dosage is different, depending on countries, and the initial dose is 5 mg/day and maximum dose is 20 mg/day in Japan, while the initial dose range is 20-40 mg/day in Korea, China and the U.S. The initial doses in those countries are four times higher than that in Japan. The pharmacokinetic characteristics of simvastatin are as follows: Simvastatin is a prodrug, showing its pharmacological activity by changing to an open acid form in liver in humans. Simvastatin is metabolized mainly by CYP3A4/5 in the intestinal tract/liver, and its metabolites show their pharmacological activity, too. The pharmacokinetic parameters of unchanged simvastatin can be affected by functional change of CYP3A4/5 or OATP1B1 etc., drug transporters involved in hepatic uptake. Oral bioavailability is low, less than 5% as an open acid form. The elimination rate is about 60% in the feces and about 13% in urine, considered that simvastatin is mainly eliminated in bile. Comparison of existing pharmacokinetic data by the statistic process combining the results in Japanese and Western populations showed a large difference in the pharmacokinetics of simvastatin between Japanese and Western populations, but information on pharmacokinetics of simvastatin in East Asian population is insufficient due to the lack of the data on Chinese and Koreans. Considering these backgrounds, we decided to obtain the data on East Asian population and conduct a clinical pharmacokinetic study, according to the same protocol, in East Asian and Caucasian populations (as a reference), to examine the difference in pharmacokinetics of simvastatin among these populations.

2. STUDY OBJECTIVE

To investigate whether or not there are ethnic differences in the pharmacokinetics (PK) of simvastatin which has been already marketed in healthy adult Japanese, Chinese, Korean and Caucasian male subjects based on the same protocol among the four countries.

2.1 Endpoints

2.1.1 PK Endpoints

Plasma concentration of simvastatin and its open acid form.

[Blood sampling points]

Before administration (baseline), 0.5, 1, 1.5, 2, 3, 4, 5. 6, 8, 12 and 24 hours after administration (12 points in total)

[PK parameters to be calculated]

For each substance to be measured and total inhibitory substance concentration, model-independent parameters and parameters related to compartmental model are calculated.

The following parameters should be calculated for model-independent parameters.

Peak plasma concentration (C_{max}), time to peak plasma concentration (t_{max}), elimination half-life ($t_{1/2}$), area under the plasma concentration-time curve (AUC_{0-t}, $AUC_{0-\infty}$), mean residence time (MRT), apparent total clearance (CL/f) and volume of distribution (Vd/f)

Maximum plasma concentration ($C_{max,\ norm}$) and AUC ($AUC_{0-t,\ norm}$, $AUC_{0-\infty,\ norm}$) normalized to dose per body weight of each substance to be measured and total inhibitory substance concentration.

2.1.2 Gene polymorphism examination

- Gene analysis for polymorphism of the CYP3A4, CYP3A5, ABCB1, OATP1B1 and ABCG2 related to the pharmacokinetics of simvastatin.

[Reasons for choice]

Simvastatin is known to be metabolized by CYP3A4 and CYP3A5 [1]. In addition, it is reported that pharmacokinetics of simvastatin are different, depending on polymorphism of the OATP1B1, involved with hepatic uptake of simvastatin from the blood [2]. In addition, it is reported that pharmacokinetics are different, depending on polymorphism of the ABCB1 and ABCG2 that eliminate the drug from the liver to bile [3, 4]. Based on these, we will conduct the polymorphism analysis of these genes considered to affect pharmacokinetics of simvastatin.

2.1.3 Safety endpoints

- Types, severity, onset date/time, duration, number of subjects, number of cases and incidence of adverse events that occurred after the study drug administration will be examined.
- For laboratory values, vital signs (body temperature, blood pressure and pulse rate) and body weight, basic statistics (mean and standard deviation) should be calculated by each test point.

3. STUDY TYPE AND DESIGN

3.1 Study Type

An open-label and single administration study

3.2 Study design

The study design is described in **Table 3-1**.

Table 3-1 Study design

Method	Open-label, single administration study
Study drug	Simvastatin. The same product lot will be used in all the countries.
Target sample size	40 subjects for each country (160 subjects in total in Japan, China, Korea and the U.S.)
Dosage and administration	One 20 mg tablet of the study drug is orally administered with 150 mL of soft mineral water (hardness <100, Volvic®, etc.) after fasting for at least 10 hours. Water drinking is prohibited up to 2 hours after taking the study drug. The subjects can drink soft mineral water (hardness <100, Volvic®, etc.) up to 500 mL during a period from 2 to 4 hours after administration. Soft mineral water (hardness <100, Volvic®, etc.) is given during the study period. Food intake is not allowed up to 4 hours after administration. The calories and the balance of three major nutrients (PFC balance) of the dinner on the day before administration and the first lunch and dinner after administration are unified among the countries as much as possible.

[Rationales for selection]

Target sample size:

The number of subjects required to investigate ethnic differences in the pharmacokinetics was calculated [5]. Based on the integration of the data, the difference in $AUC_{0-\infty}$ revealed about 60% between Japanese and Western populations, and the difference in $AUC_{0-\infty}$ was not expected among East Asian population from the data of rosuvastatin. Considering this information, the number of the patients was calculated to detect 40% difference in $AUC_{0-\infty}$ at least between East Asian and Western populations with a power of 80%. As a result, under the null hypothesis "There is no difference in PK among Japanese, Chinese, Korean and Caucasian" and the alternative hypothesis that at least one ethnic group shows the difference in the mean of pharmacokinetics, the number of subjects was calculated to be at least 25 subjects based on information of a 40 % and 20% difference in $AUC_{0-\infty}$ with a power of 80 % between Japanese and Western populations, and between Japanese and Chinese/Koreans, respectively. Since the analysis of gene polymorphism is performed after PK analysis. Based on this, the target sample size of this study was determined to be 40 subjects from each ethnic population, considering the subjects excluded from the results of analysis of CYP3A5 to be CYP3A5*3 homo, dropouts by

discontinuation or withdrawals.

The reason why the target sample size was approximately 1.5 times the number of subjects necessary for statistical analysis was based on information on ethnic differences of CYP3A5 polymorphism. According to the investigation[6], the frequency of CYP3A5*3 polymorphism was 0.7 to 0.77 in East Asian population and 0.85 to 0.95 in Caucasian population .

Dosage and administration: The dosage and administration was chosen to be within the range of those approved, and single oral administration of 20 mg, with which the plasma concentrations of simvastatin and its open acid form can be measured for a sufficient period of time, was selected.

In order to avoid a wide variation in absorption of study drug from the digestive track, the intake of water after administration of study drug, and calories and PFC balance at the dinner on the day before and the first lunch and dinner after administration were to be unified among all the four countries as much as possible.

4. SUBJECTS

In Japan, China and Korea, the nationalities of the subjects should be the same as those of grandfathers, grandmothers, father and mother. In China, only the Han race is eligible. In the U.S., only European Caucasian is eligible.

In this study, subjects are healthy adult male volunteers who satisfy all of the following "**4.1 Inclusion criteria**" and none of the following "**4.2 Exclusion criteria**".

4.1 Inclusion criteria

1) Those who are capable of providing written informed consent.
2) Men aged 20 to 35 years at the time of signing informed consent.
3) BMI of 18.5 to <30.0 and body weight of 50.0 to 100.0 kg at screening.
4) Those who are determined by the investigator to be healthy in the screening test and eligible for the study.

[Rationales for selection]

1) The criterion was selected by giving ethical consideration to the study.
2) For voluntary participation in the study, the age was set at 20 years or older at which individual consent is legally established, and the upper limit of age was specified at 35 years for minimizing variations in laboratory values and changes in PK.
3) The criterion was chosen for further reducing interindividual changes.
4) Because the subjects are healthy adults, the criterion was specified for excluding people who are unsuitable for study enrollment in terms of health.

4.2 Exclusion criteria

1) Organopathy involving the heart, lung, liver and/or kidney etc.
2) A history of diseases involving the heart, lung, kidney, blood (such as coagulation system disorder), central nervous system and metabolic system that may interfere with the study.
3) Hypothyroidism, genetic myopathy or family history of it, history of drug-induced myopathy
4) Hypersensitivity or allergies to drugs and food, etc. (Particularly, a history of allergy to or adverse reactions associated with statin-type antihyperlipidemia drugs)
5) Oral administration of drugs such as over-the-counter drugs within 1 week prior to the study drug administration, or taking any kind of health food/supplement, grapefruit, drink/food containing grapefruit from 2 week prior to the study drug administration to the day 2, or taking any kind of fruit juice, drink containing caffeine or green tea from 1 day before the study drug administration to the day 2, or the necessity for using other medications before study completion.
6) Smokers or a smoking history within the last 6 months. (The cotinine test is performed, if necessary)
7) Drug abuse or suspicion of drug abuse. (The drug screening test is performed, if necessary)
8) Alcohol drinkers (daily alcohol intake* of 50 g or more).

   * Alcohol intake (g) = [alcohol content] × [amount of alcohol intake (mL)] × [0.8 (specific gravity: weight of 1 mL of alcohol)]

   Example: When drinking 1000 mL of beer (alcohol content: 5.5%), alcohol intake = 0.055 × 1000 × 0.8 = 44 g
9) Total bilirubin or direct bilirubin , AST, ALT and ALP is 1.5 times higher, or other liver and renal function tests items are 1.25 times higher than the upper limits of normal at the sites.
10) Blood sampling of 200 mL or more within 1 month prior to the study drug administration, blood component donation (plasma or platelet) within 2 weeks, or blood collection of 400 mL or more within 3 months
11) Participation in a Phase I study of a drug containing a new active ingredient within 4 months prior to the study drug administration or another clinical study within 3 months, and being administered the investigational drug (within 1 month prior to the study drug administration for a patch test). However, even if the period is longer than these, those who are determined to be not eligible for enrollment in the study in consideration of the characteristics of the previous investigational drug.
12) Those who are determined by the investigator to be not suitable as subjects of the study.

[Rationales for selection]

1) to 4) The criteria were specified for subject safety assurance.
5) to 9) The criteria were selected for safety assurance and because they might interfere with PK analysis and safety evaluation.
10) The criterion was specified for taking subjects' safety and ethics into consideration and satisfying the criteria for blood donation organized by the Study Group on Blood Donation (healthy adult men: 400 mL per donation, blood sampling interval: 3 months or more, total annual amount of blood sampling: 1200 mL or less, number of donations: 3 or less).
11) The criterion was set to exclude the influence of drugs having interactions with the study drug and

long-acting drugs and take subjects' safety and ethics into consideration.

12) The criterion was chosen for the principal investigator to be able to make determination in consideration of overall factors.

4.3 Discontinuation/withdrawal criteria

Subjects, who meet any of the following criteria, will be withdrawn or dropped out from the study:

1) When the subject requests to withdraw from study participation after providing informed consent.
2) When the investigator determined that the subject is incapable of respecting the protocol.
3) When the principal investigator judged that the study should be discontinued. (When the subject clearly developed a fever (37.5°C or higher), when the subject is affected by serious acute disease, etc.)

[Rationales for selection]

1) The criterion was set from the perspective of respecting the subject's free will.
2) The criteria were specified for giving consideration to subjects' safety.
3) The criterion was selected for the investigator to be able to determine the discontinuation of the study drug administration in consideration of overall factors.

4.4 Procedures for discontinuation/withdrawal

1) When discontinuing the study, the principal investigator should promptly explain such a fact to the subject (When the subject requested to terminate the study, the principal investigator should check the detailed reason whenever possible). The principal investigator should also enter information such as the timing of discontinuation/withdrawal and reason in the case report form (CRF).
2) When discontinuing the study or withdrawing the subject from the study after the study drug administration, the principal investigator should perform observation in accordance with the Section **"8.2.4 Evaluation items for the safety endpoints"** (However, 7. Body weight is excluded). Blood sampling for measurements of plasma drug concentrations of the subjects with discontinuation/withdrawn after administration should be carried out only if possible.
3) In the presence of adverse events, the principal investigator should investigate until the symptoms resolve or become stable (1 month at the longest).
4) When the drop outs take place by discontinuation or withdrawal, additional subjects will not be recruited.

5. STUDY DRUG

5.1 Supply of the Study Drug

The product of simvastatin that was manufactured by Merck China and marketed in China is used in the study (single lot).

5.2 Name of the Study Drug and Other Explanations

The brand name, manufacturer and indications are as follows:

Brand name ZOCOR®

Distributor Merck China

Indications
- Hyperlipidemia
- Coronary heart diseases
- Pediatric patients with heterozygous familial hypercholesterolemia

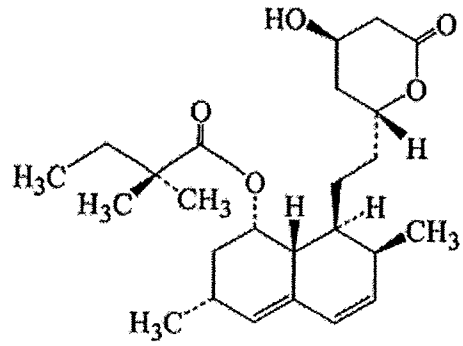
Simvastatin is a compound inhibiting HMG-CoA reductase, developed by Merck U.S.A, in 1979. Simvastatin, since approval in Sweden in 1988, is approved as an oral drug in 117 or more countries in March, 2010. Simvastatin is a prodrug (inactive form) and its active form is an open acid form.

Active ingredient:

[Nonproprietary name] Simvastatin

[Chemical name] (+)-(1S,3R,7S,8S,8aR)-1,2,3,7,8,8a-Hexahydro-3,7-dimethyl-8-[2-[(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl]ethyl]-1-naphthyl 2,2-dimethylbutanoate

[Chemical structure]



[Molecular formula] $C_{25}H_{38}O_5$

[Molecular weight] 418.57

Contents and dosage form:

A film-coated tablet containing 20 mg of simvastatin

Storage condition:

Store at room temperature

5.3 Control/Storage of the Study Drug

The executive investigator should provide the study drug that is sold in China and entrust the control and storage of the study drug to the storage personnel at the study site. The study drug should be delivered, controlled and collected in accordance with the "Procedure for Control of the Study Drug" provided by the executive investigator. When the study is completed, the study drug storage personnel should take a count of the remaining study drug. The study drug storage personnel should discard or dispose study drug after destruction under the direction of the principal investigator following the standard procedure at the study site.

6. PROHIBITED CONCOMITANT MEDICATIONS

Coadministration of any drugs will be prohibited during a period from 1 week before study drug administration to study completion.

When they are used due to inevitable reasons such as treatment of adverse events, the principal investigator should enter information such as the name, dosage/administration, duration of use and purpose of use of the drug in the CRF.

7. MANAGEMENT OF SUBJECTS

7.1 Management of Subjects in Each Period

1) Screening

   The principal investigator should perform the screening test within 30 days before the study drug administration after acquisition of informed consent.

2) Day before the study drug administration (Day -1, hospitalization) to 2 days after administration (Day 2, discharge)

   The principal investigator should hospitalize the subjects by 16:30 on the day before the study drug administration. The subjects should be kept fasted from after having a dinner on the day of hospitalization to 4 hours after the study drug administration. On the day of administration, after performing tests such as the physical examination, laboratory test, vital signs (blood pressure, pulse rate and body temperature), and blood sampling for measurements of drug concentrations before administration, the study drug administration should be started sequentially at 9:00 am. Thereafter, blood sampling for tests and measurements of drug concentrations should be implemented in accordance with the study schedule.

   On Day 2, the investigators should conduct physical examination, blood sampling for measurements of drug concentrations, and blood sampling for gene analysis for polymorphism of the CYP3A4, CYP3A5, ABCB1, OATP1B1 and ABCG2 related to the pharmacokinetics of simvastatin. After confirming that there is no safety (health condition) problem, the investigator should discharge the subjects from the hospital. However, when safety assurance is determined to be necessary, the principal investigator should prolong the hospitalization period and carry out a reexamination or

additional examination to implement a follow-up investigation. In such a case, the details of the actions taken should be recorded in the CRF.

7.2 Intake of health foods /supplement, grapefruit or drink/food containing grapefruit, fruit juice and drink including caffeine and green tea

The intake of any kind of health food/supplement, grapefruit or drink/food containing grapefruit from 2 week prior to the study drug administration to the day 2 (day of discharge), or taking any kind of fruit juice, drink containing caffeine or green tea from 1 day before the study drug administration to the day 2 will be prohibited.

[Rationales for selection]

It was reported that St. John's wort affected the metabolism and the excretion of simvastatin. Taking any kind of health food/supplement will be prohibited since it is difficult to identify the health food or supplement containing St. John's wort. Taking grapefruit or drink/food containing grapefruit will be prohibited since it was reported that some constituent of grapefruit showed the irreversible inhibition of the metabolism of simvastatin. Taking fruit juice will be prohibited. It cannot be denied that fruit juice might affect the metabolism of simvastatin. It was reported that taking green tea together with simvastatin affected the blood concentration of simvastatin. It cannot be denied that taking other drink containing caffeine affects the blood concentration of simvastatin. So, taking a drink containing caffeine or green tea will be prohibited.

7.3 Eating and Drinking

The intake of grapefruit or drink/food containing grapefruit from 2 week before the study drug administration to the day 2, or taking any kind of fruit juice, drink containing caffeine or green tea from 1 day before the study drug administration to the day 2 will be prohibited.

During the hospitalization period, intake of meals and water other than those supplied by the site will be prohibited. Water drinking will be prohibited for 2 hours after drug administration, and the subjects can drink soft mineral water (hardness <100, Volvic®, etc.) up to 500 mL during a period from 2 to 4 hours after administration. Soft mineral water (hardness <100, Volvic®, etc.) is given during study period. Food intake is not allowed up to 4 hours after administration.

Timing of hospitalization and meal time will be as follows.

Morning of Day 1 is fasting. Follow the instruction by the site on the meal time other than the following.

Day -1 (day of hospitalization):	19:00
Day 1 (day of administration):	Having a lunch after completion of blood sampling and tests at 4 hours after administration.
Day 2:	Having a breakfast after completion of blood sampling and tests at 24 hours after administration.

7.4 Smoking

Smoking will be prohibited during the hospitalization period (after hospitalization on the day before administration).

[Rationales for selection]

It was specified because nicotine contained in cigarette may affect test values such as blood pressure and pulse rate.

7.5 Physical exercise

Strenuous exercise and work giving a heavy physical burden will be prohibited during the hospitalization period (after hospitalization on the day before administration).

[Rationales for selection]

It was stipulated because physical exercise may increase enzyme levels such as AST, ALT and CK.

7.6 Posture

It will be prohibited to be in the supine from the study drug administration up to 3 hours after administration.

[Rationales for selection]

It was specified for avoiding changes in absorption caused by the physical position.

8. OBSERVATIONS AND ENDPOINTS

8.1 Procedure for Study Implementation

8.1.1 Screening

The investigator should perform the screening test in subjects who provided written informed consent. Observation and tests listed in **Table 8-1** should be implemented as the screening test to verify whether the subjects are eligible for the study. The investigator should also record the details in the CRF.

Table 8-1 Observation and tests at screening

Subject background and characteristics	Sex, height, body weight/BMI, birth day/month/year, current health condition, history of drug allergy, medical history, and smoking/drinking habits
Clinical observation	Physical examination by the investigator
Hematology	White blood cell count (WBC), differential WBC, red blood cell count (RBC), hemoglobin concentration, hematocrit value, platelet count and reticulocyte count
Blood biochemistry	Blood sugar, total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, total protein, albumin, uric acid, creatinine, total bilirubin, direct bilirubin, AST, ALT, γ-GTP, LDH, ALP, CK, Na, K, Cl and CRP
Urinalysis	Glucose, bilirubin, ketone bodies, occult blood, pH, protein, urobilinogen and sediment (to be conducted if protein or occult blood is positive)
Vital signs, electrocardiography	Blood pressure/pulse rate (sitting), body temperature and 12-lead electrocardiography
Infectious disease test	HBs antigen, HCV antibody, serologic tests for syphilis and HIV antibody

[Rationales for selection of observation and tests]

General items, which are found to be necessary for verification of the health condition of subjects to be enrolled in the study, were adopted. The infectious disease test was specified for the purpose of the prevention of infection to personnel handling blood.

8.1.2 Study

The investigator should perform the observation and tests listed in **Table 8-2** in the subjects, who provided consent for study participation, in accordance with the study schedule in **Table 8-3**.

Table 8-2 Observation and tests during the study

Clinical observation	Physical examination by the investigator
PK	Plasma drug concentrations
Hematology	WBC, differential WBC, RBC, hemoglobin concentration, hematocrit value, platelet count and reticulocyte count
Blood biochemistry	Blood sugar, total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, total protein, albumin, uric acid, creatinine, total bilirubin, direct bilirubin, AST, ALT, γ-GTP, LDH, ALP, CK, Na, K, Cl and CRP
Urinalysis	Glucose, bilirubin, ketone bodies, occult blood, pH, protein, urobilinogen and sediment (to be conducted if protein or occult blood is positive)
Vital signs, body weight measurement	Blood pressure/pulse rate (sitting), body temperature and body weight
Gene polymorphism examination	Collected blood specimens (e.g., EDTA-2Na Added) will be refrigerated at -60℃ or less, and sent to the institute for gene polymorphism analysis using dry ice within 2 weeks after sampling, if possible.

Table 8-3 Study Schedule

		Screening	Study												
Date of study		Within 30 days	Day -1	Day 1											Day 2
Time			Admission by 16:30	8:00	9:00	9:30	10:00	10:30	11:00	12:00	13:00	14:00	15:00	17:00	21:00	9:00
Time after oral administration (h)				-1	0	0.5	1	1.5	2	3	4	5	6	8	12	24
Admission/visit		Visit	Admission	←	—	—	—	—	—	—	—	—	—	—	—	→
Informed consent		○														
Subject background characteristics		○														
Study drug administration					○											
Physical examination by the investigator		○		○												○
Body weight		○		○												○
Height		○														
Vital sign		○		○												○
12-lead electrocardiography		○														
Adverse event			←	—	—	—	—	—	—	—	—	—	—	—	—	→
Blood sampling	Gene polymorphism examination															○[a)]
	Pharmacokinetics			○		○	○	○	○	○	○	○	○	○	○	○
	Laboratory test	○		○												○
	Infectious disease test	○														
Urine sampling	Laboratory tests	○		○												○

Meal time during hospitalization: Morning of Day 1 is the fast. Follow the instruction by the site on the meal time other than the following.
1 day before administration (at 19:00 on Day -1)
Day of administration (Day 1: Having a lunch after completion of blood sampling and tests at 4 hours after administration)
1 day after administration (Day 2: Having a breakfast after completion of blood sampling and tests at 24 hours after administration.)
a): Collected specimens (e.g., EDTA-2Na Added) will be refrigerated at -60°C or less, and sent to the institute for gene polymorphism analysis using dry ice within 2 weeks after sampling, if possible.

8.2 Investigations/evaluations

8.2.1 Evaluation items for the PK endpoints

Plasma drug concentrations

1) Test substance: Simvastatin and its open acid form

2) Blood sampling point:

Before administration, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12 and 24 hours after administration (time allowance at 0.5-8 hrs from administration: ± 5 minutes, 12 and 24 hrs from administration: ± 10 minutes). [12 points in total]

3) Processing method:

At the specified blood sampling points, 7 mL of blood should be taken from an antecubital vein, using a plastic vacutainer tube containing heparin sodium. Blood sample should be kept on ice, and the centrifuge should start within 1 hour after blood sampling in a refrigerated centrifuge at 1900×g (approx. 3000 rpm) for 10 minutes at 4℃. The obtained plasma should be divided into 3 plastic serum tubes containing at least 0.6 mL plasma each on ice. Two plastic serum tubes should be transferred to the measurement site and the remaining one serum tube should be stored at the study site as back-up sample. Within 1 hour after obtaining plasma, serum tubes should be stored in a refrigerator whose temperature is set at -60°C or less. (Do not use glass tube/containers). Since simvastatin in blood or plasma is easy to be hydrolyzed at high temperature or by sunlight, plasma samples should be handled on ice and don't expose them to direct sunlight or at high temperature.

4) Labeling and transport method of tubes for plasma specimens:

The example of the label is as follows. The format of the label can be designed freely. The label should describe for PK, name of study drug, the subject number (9 digits) that consists of the study site code (Japan: 00001, China: 00002, Korea: 00003 and the U.S.: 00004, 5 digits) and the number that is anonymized and linkable to the subjects (4 digits), date/time of blood sampling and specimen type. The label should be attached to the tubes for specimens.

Study drug	SIMVASTATIN
Subject No.	000010001
Time	0.5 h
Date	DD/MM/YY
Matrix	PLASMA <PK>

If possible, within 2 weeks after sampling, the frozen plasma specimens should be packed in dry ice and sent to the laboratory center for measurement of drug concentration from the study site. The procedure for sending should be given separately.

5) Back-up samples:

Back-up samples are stored to compensate the missing specimens, for example, the specimens might be broken during the transportation to the measurement site (Pharmacokinetics and Bioanalysis Center, Shin Nippon Biomedical Laboratories, Ltd.). Each study site should store the backup samples at -60℃ or less until the measurement of drug concentration has been completed. After measurement of drug concentration has been completed at the measurement site or re-measurement is considered not to be required, each study site should discard or dispose the back-up samples. However, when it