

# Innovative Strategies for Drug Development Using Microdosing Clinical Studies

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### The Japanese Society for the Study of Xenobiotics The 24<sup>th</sup> Annual Meeting

Date: November 27 (Fri.) - 29 (Sun.), 2009

Venue: Kyoto International Conference Center (ICC Kyoto)  
Takaragaike, Sakyo-ku, Kyoto, 606-0001, Japan

### Presentation by NEDO MicroDose Project Team

Oral Session Drug discovery 9:00 ~ 11:00 November 29, 2009 NEDO & APDD  
Venue: Kyoto International Conference Center (ICC Kyoto) Room A

Innovative Strategies for Drug Development Using Microdosing Clinical Studies (1)

## Ethics and Human Subjects Protection in the Project of Multiplex Studies

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### [Purpose]

To develop the system of managing multiplex clinical studies in the "Research Project for Establishment of Evolutional Drug Development with the Use of Microdose Clinical Trial", to assure quality of the studies and human subjects protection.

### [Methods]

A Clinical Research Management Team has been established and practical management, discussion meetings, and document development work have been conducted as part of the research project.

### [Results and Discussion]

The following items were identified as the essential elements in the management system of multiplex studies: (1) visiting and quality check of the clinical sites and of the product manufacturing sites; (2) organizing several

bodies for radiological protection of human subjects; (3) systematic literature search and inquiry for safety issues and error recovery procedures; (4) systematic documentation and SOP (standard operating procedure)/guidance development; and (5) Ethics Committees submission support. Some of these procedures seem to provide some hints to redesign clinical drug development in Japan.

### [Conclusions]

The clinical research management system we have developed would be useful especially for conducting new types of studies such as microdosing clinical studies and molecular imaging PET studies.

### [Acknowledgement]

This and the following 5 studies are sponsored by the New Energy and Industrial Technology Development Organization (NEDO).

### ● Microdose Clinical Trial

[Definition: MHLW (June 2008) → ICH-M3 (June 2009)]

- total dose ≤ 100 μg/human & ≤ 1/100th NOAEL and ≤ 1/100th pharmacologically active dose
- total dose ≤ 500 μg, maximum of 5 administrations AND each dose ≤ 100 μg AND each dose ≤ 1/100th of the NOAEL and ≤ 1/100th of the pharmacologically active dose

[Analysis]

- AMS (Accelerator Mass Spectrometry)
- LC/MS/MS (Liquid chromatograph Mass spectrometry)
- PET (Positron Emission Tomography)

◆ **NEDO MicroDose PJ** First conduct of microdosing in Japan

◆ **More than 20 protocols** in 2.5 years project

◆ **Clinical Research management Team** to assure ethical, reliable conducts of studies

⇒ **Development of "Package Tool"** to improve the predictability of pharmacokinetics of therapeutic dose from microdosing.

"Microdose Clinical Trial" is a study design aimed to predict pharmacokinetics/pharmacodynamics of therapeutic dose from minimal dose administration to human defined as "microdose", in order to select a compound for clinical development among several candidates. Following the Guidance by European Union and United States, Japanese Health Ministry issued Guidance on Microdose Clinical Trial in 2008, and then the definition of this study and requirements of non-clinical studies before it was harmonized by ICH initiative in 2009. This study design is remarked as newly emerging key strategy of drug development in 21 century. In this NEDO MicroDose Project, more than 20 protocols of clinical researches using authorized drugs are to be conducted and ethical, reliable conducts of studies are assured by "Clinical Research Management Team". The final goal of our project is to develop "Package Tool" to improve the predictability of pharmacokinetics of therapeutic dose from microdosing.

Innovative Strategies for Drug Development Using Microdosing Clinical Studies (2)

# Prediction of the Change in the Pharmacokinetics of Drugs by Genetic Polymorphisms in Transporters and Transporter-Mediated Drug Interactions from *in vitro* Studies and Microdosing Clinical Studies

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## [Purpose]

Recently the importance of transporters in the detoxification of drugs has been increasingly recognized by several *in vitro* and clinical studies on the genetic polymorphisms in drug transporters and transporter-mediated drug-drug interactions (DDIs). However, the evidences of the quantitative prediction of time profiles in the tissue and plasma concentrations of transporter substrate drugs from *in vitro* data with modeling and simulation have not been accumulated so far. Thus, in the present study, we validated our strategies to predict the pharmacokinetics (PK) of some drugs from *in vitro* data and mathematical modeling. We also tried to show the significance of microdosing clinical studies for this kind of prediction.

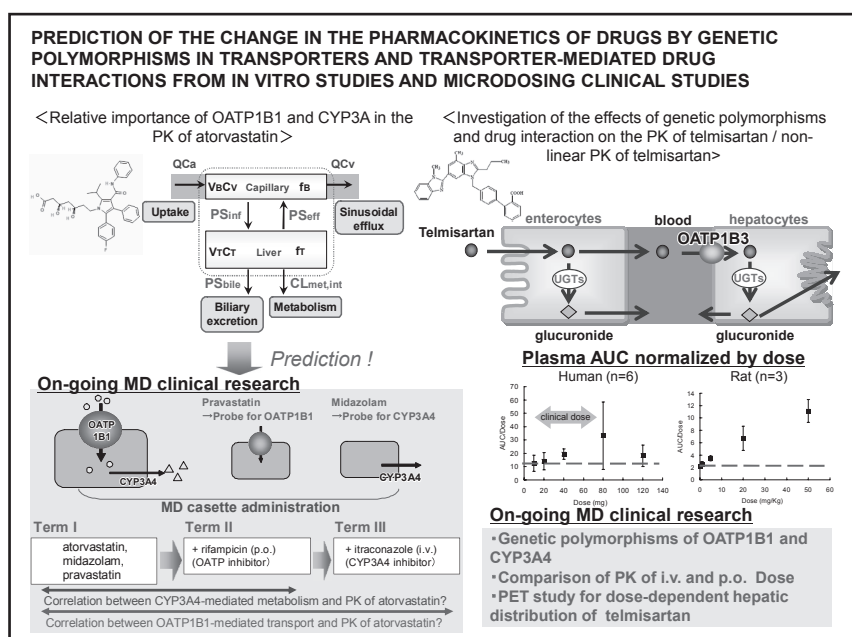
## [Method]

Atorvastatin (a bi-substrate of CYP3A4 and OATPs) and telmisartan (a bi-substrate of UGTs and OATP1B3) are chosen as a model compound for the PK prediction. In the clinical study, microdose of atorvastatin, midazolam (probe for CYP3A4) and pravastatin (probe for OATP1B1) is coadministered with rifampicin (inhibitor

for OATPs) and itraconazole (inhibitor for CYP3A4) to healthy volunteers. For telmisartan, the effect of genetic polymorphisms of OATP1B3 on its PK parameters is observed. Some *in vitro* and animal studies to obtain the intrinsic parameters for the PK modeling are also performed.

## [Results and Discussion]

The saturable uptake of atorvastatin was observed in rat and human hepatocytes and the *in vivo* hepatic intrinsic clearances of four kinds of statins were well correlated with their uptake intrinsic clearances rather than metabolic enzymes, suggesting that the rate-limiting step for the clearance of these statins is uptake process mediated by OATPs. Regarding the telmisartan, non-linear PK was observed when changing its dose in rats and its clearance after i.d. dose was largely decreased by the function defect of UGT1s in Gunn rats, suggesting that non-linearity of its PK was mainly caused by the saturation of intestinal UGTs, though species difference in the PK of telmisartan in humans and rats.



In this project, we try to establish the methodologies for the prediction of the change in the pharmacokinetics of drugs by genetic polymorphisms in transporters and transporter-mediated drug interactions from *in vitro* studies and microdosing clinical studies.

As shown in the left panel, we are now performing the microdose clinical study of atorvastatin, which is one of the example drugs recognized by both metabolic enzymes and transporters, to clarify the relative importance of OATPs (Organic anion transporting polypeptides) and CYP3A in the overall clearance of atorvastatin by observing the effects of their inhibitors on the pharmacokinetics of atorvastatin as well as cassette dose of probe drugs for OATPs and CYP3A.

In the right panel, we want to clarify the mechanisms (especially focused on OATP1B3 and UGTs (UDP-glucuronosyl transferases)) of non-linear pharmacokinetics of clinical dose of telmisartan by several kinds of microdose clinical studies in which the effects of genetic polymorphisms of OATP1B3 and UGTs on the pharmacokinetics of telmisartan and dose-dependent pharmacokinetics of telmisartan are investigated.

Innovative Strategies for Drug Development Using Microdosing Clinical Studies (3)

## Prediction of Nonlinear Pharmacokinetics of MDR1 Substrates in Humans from Nonclinical Data

Yuji Kumagai<sup>1</sup>, Akira Yamazaki<sup>1</sup>, Yasuhiko Ikeda<sup>1</sup>, Tomoe Fujita<sup>1</sup>, Noriyasu Kamei<sup>2</sup>, Kazuya Maeda<sup>2</sup>, Hiroyuki Kusahara<sup>2</sup>, Shinji Yamashita<sup>3</sup>, Yasunori Oyama<sup>4</sup>, Kohei Nozawa<sup>4</sup> and Yuichi Sugiyama<sup>2</sup>

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### [Purpose]

Clinical studies were performed to clarify whether nonlinear pharmacokinetics of drugs in humans can be predicted by nonclinical data. In the clinical study, verapamil and quinidine, good substrates of MDR1, were selected as test drugs and the pharmacokinetic parameters were compared between their microdose and therapeutic dose. The increase in the bioavailability due to the saturation of MDR1 in small intestine observed in this clinical study was also predicted based on the  $K_m$  values and estimated drug concentration in the small intestine.

### [Methods]

Studies were done in 2 separate open-label 4-stepwise dose-titration designs including 8 healthy male subjects for verapamil and quinidine. Doses for verapamil were 100  $\mu$ g, 3, 16 and 80 mg and doses for quinidine were 100  $\mu$ g, 1, 10 and 100 mg. For the prediction, the drug concentrations in small intestine were estimated by dividing the actual dose by the lower limit of apparent intestinal

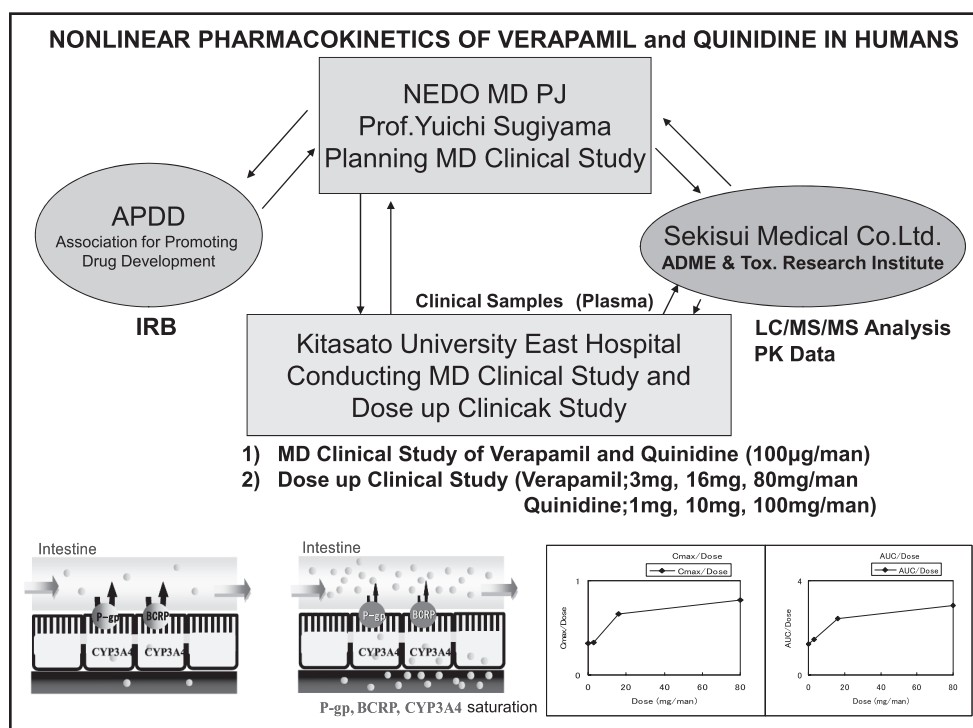
volume (3L) proposed by Tachibana et al. (Xenobiotica, 39, 430-43 (2009)).

### [Results and Discussion]

By increasing the dose from microdose to therapeutic dose, the bioavailabilities of quinidine and verapamil were altered from 0.197 to 0.504 and from 0.049 to 0.113, respectively. By using a simple mathematical model with estimated intestinal concentration, the apparent  $K_m$  values determined by fitting of clinical data were close to the *in vitro*  $K_m$  values for MDR1. The saturation of intestinal CYP3A4 also contributes to their nonlinearity and the similar type of prediction is now on going.

### [Conclusions]

Nonlinear pharmacokinetics of drugs can be explained by nonclinical data. Microdose clinical trials are a promising choice, even in cases with some concerns for nonlinear pharmacokinetics.



Clinical studies were conducted in 2 separate open-label 4-stepwise up dose from MD designs including 8 healthy male subjects for verapamil and quinidine. Nonlinear pharmacokinetics of drugs can be explained by non-clinical data. Microdose clinical trials are a promising choice, even in cases with some concerns for nonlinear pharmacokinetics.

Innovative Strategies for Drug Development Using Microdosing Clinical Studies (4)

## Assessment of Oral Bioavailability of Drugs by Cassete IV and PO Dosing

Shinji Yamashita<sup>1</sup>, Yoshie Masaoka<sup>1</sup>, Makoto Kataoka<sup>1</sup>, Shinji Sakuma<sup>1</sup>, Yuki Suzaki<sup>2</sup>, Hiromitsu Imai<sup>2</sup>, Tsutomu Kotegawa<sup>2</sup>, Takuya Morimoto<sup>2</sup>, Kyoichi Ohashi<sup>2</sup>, Akihiro Inano<sup>3</sup> and Yuichi Sugiyama<sup>4</sup>

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### [Purpose]

Purposes of this project are 1) to investigate the possibility of Microdosing (MD) clinical study to accelerate the development of oral drug products 2) to develop a rationale method to assess the oral absorbability of drugs by MD study and 3) to simulate the human BA of drugs based on the *in vitro* and *in vivo* (MD) studies.

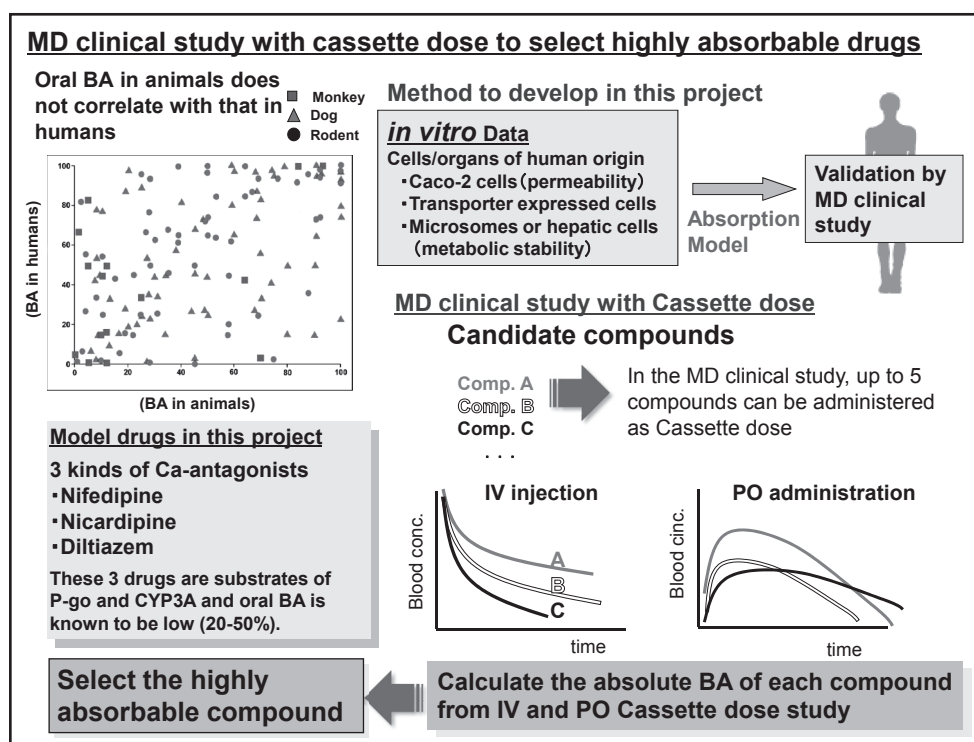
### [Methods]

In this study, three Ca-antagonists (nifedipine, nicardipine and diltiazem) were chosen as model drugs. These drugs are reported to undergo the extensive first-pass metabolism and P-gp mediated transport that makes it difficult to predict the oral BA from the preclinical animal study. In the protocol of MD study, cassette IV and PO dosing of three drugs were employed to develop a

rationale method to select the best candidate for oral products efficiently.

### [Results and Discussion]

Since a clinical study with cassette IV and PO dosing has not been reported yet, the method for cassette dosing was carefully searched before the clinical study. For IV administration, protocol was designed to inject the solution of each drug at 1-2 min intervals by using a tube with 3-way bulb to prevent a precipitation or degradation of drugs by mixing IV solutions of three drugs before administration. *In vitro* experiment revealed that 100% of dose can be injected by flushing the tub with 5-10 ml saline. In the presentation, results of MD clinical study will be reported with the *in vitro* prediction of human BA of these drugs.



In this study, three Ca-antagonists (nifedipine, nicardipine and diltiazem) were chosen as model drugs. These drugs are reported to undergo the extensive first-pass metabolism and P-gp mediated transport that makes it difficult to predict the oral BA from the preclinical animal study. In the protocol of MD study, cassette IV and PO dosing of three drugs were employed to develop a rationale method to select the best candidate for oral products efficiently.

Innovative Strategies for Drug Development Using Microdosing Clinical Studies (5)

# Identification of Human Metabolites by a Combinatorial Use of AMS and LC/MS/MS

Zenzaburo Tozuka<sup>1</sup>, Kohei Nozawa<sup>1</sup>, Yoshimi Hamabe<sup>2</sup>, Ippei Kijima<sup>3</sup>, Yuichi Sugiyama<sup>4</sup> and Toshihiko Ikeda<sup>4</sup>

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## [Aim]

To evaluate pharmacokinetics (PK), metabolism and drug interaction of <sup>14</sup>C-acetoaminophen (<sup>14</sup>C-AA) in human volunteers after a single oral microdose (MD) (100 µg/200 nCi/body) by AMS and LC/MS/MS.

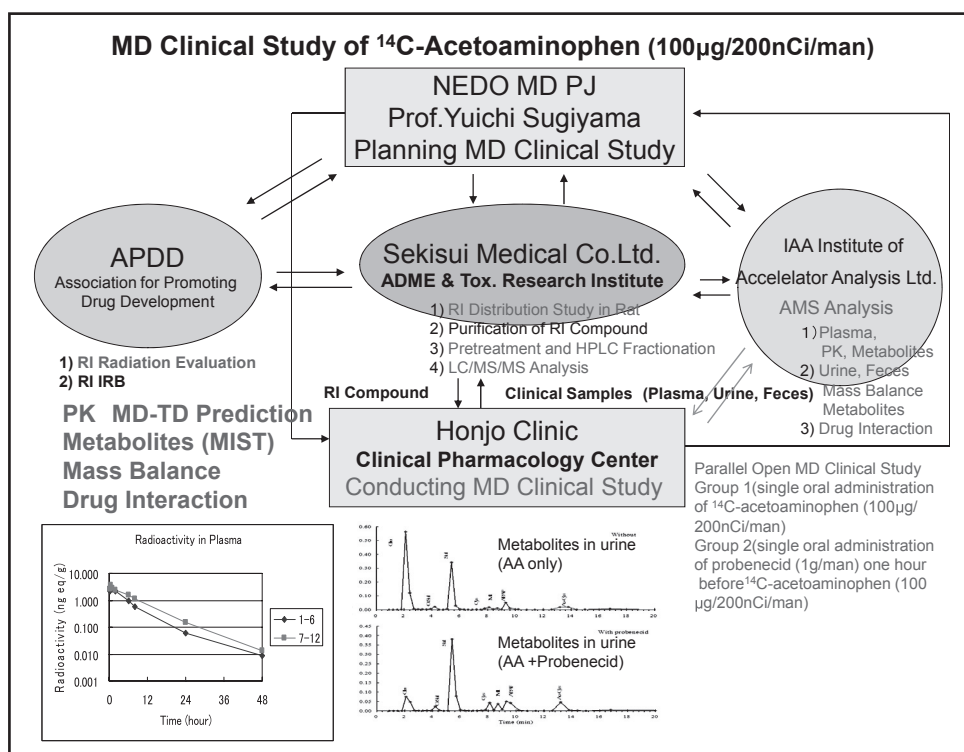
## [Methods]

Tissue distribution of radioactivity in male pigmented rats and isolation of metabolites in urine after therapeutic oral administration of AA were conducted before the MD clinical study. Drugs were administered to two groups (n=6, per group) of human volunteers (first group (<sup>14</sup>C-AA only) and second group (1g probenecid (PB) one hour after dosing of <sup>14</sup>C-AA). The single oral MD of <sup>14</sup>C-AA was 100 µg/200 nCi/body. Blood, urine and feces samples were collected up to 48 h, 168 h and 168 h respectively. The samples were then pretreated. After fractionation of

HPLC, Radioactivity of a parent drug (AA), sulphate (AA-Sul) and glucuronic acid (AA-Glu) is measured by AMS. The structure of metabolites is analyzed by two LC/MS/MS systems using high sensitive LTQ Orbitrap and Qtrap 5500.

## [Results and Discussion]

The human radiation dose calculated was 4.09E-04 mSv/MBq (0.015 µSv/µCi) from the tissue distribution of radioactivity in male pigmented rats. The retention times (min, the isolated metabolites) were (1.87, AA-Glu), (3.90, AA-3OSul), (4.91, AA-Sul), (7.66, AA-3Cys), (8.94, AA) and (13.81, AA-3MC) by UPLC (HSS T3 column). In 24<sup>th</sup> JSSX Annual Meeting we discuss the followings, 1) mass balance, 2) the linearity of AUC and Cmax between MD and TD, 3) the drug interaction between AA and PB, and 4) metabolites in the MD study.



This NEDO MD PJ evaluates pharmacokinetics (PK), metabolism and drug interaction (with probenecid) of <sup>14</sup>C-acetoaminophen in human volunteers after a single oral microdose (MD) (100 µg/200 nCi/body) by AMS and LC/MS/MS.

Innovative Strategies for Drug Development Using Microdosing Clinical Studies (6)

# Use of <sup>14</sup>C-labeled Compounds to Analyze Human Pharmacokinetics of Parent Drugs and Their Metabolites

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## [Purpose]

The aims of the study are 1) to validate the processes from manufacturing to administration of <sup>14</sup>C-labeled compounds in a domestic clinical environment, and 2) to develop a method to analyze the pharmacokinetics in microdosing (MD) clinical studies.

## [Methods]

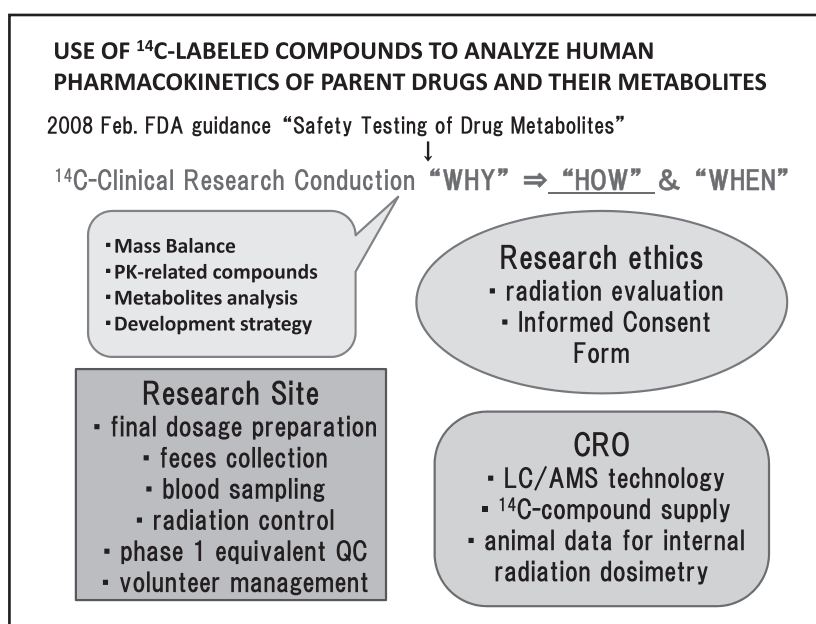
Acetaminophen (AA) and tolbutamide (TB) were chosen as model drugs and two separate MD clinical studies were scheduled with <sup>14</sup>C-labeled AA and TB. Accelerator mass spectrometry (AMS) was used for <sup>14</sup>C detection. All the feces and urine were collected throughout the study periods and plasma samples were collected at 9 time points during 0-48 hours.

## [Results and Discussion]

In both MD clinical studies, it was found that administration of 100 µg of drug (per person) as a single oral dose contains 7400 Bq of radioactivity. The radiation risks are much lower than generally accepted risks such as international flight and X-ray medical examination. <sup>14</sup>C-labeled compounds can be manufactured under current Good Manufacturing Practice and the final dosage preparation can be verified at the pharmacy at the study site. Total amount of radioactivity and concentration is under the limit of the domestic law.

## [Conclusions]

MD clinical studies with <sup>14</sup>C-labeled compound can be conducted under conditions meeting quality assured environmental standards.



There is no more debate for necessity of <sup>14</sup>C-clinical research after FDA guidance "Safety Testing of Drug Metabolites". The question is now focusing on "how" and "when" <sup>14</sup>C-clinical research should be considered. We confirmed that MD clinical studies with <sup>14</sup>C-labeled compound can be conducted under conditions meeting quality assured environmental standards.