ISSN: 2378-3419

Li et al. Int J Cancer Clin Res 2017, 4:082
DOI: 10.23937/2378-3419/1410082
Volume 4 | Issue 2
Open Access

International Journal of
Cancer and Clinical Research

RESEARCH ARTICLE

Radiolabeled APIs for the Conduct of Human ADME Studies of Oncology Compounds

Yuexian Li*, Mihaela Plesescu and Shimoga R Prakash

Department of Drug Metabolism and Pharmacokinetics, Takeda Pharmaceuticals International Co., USA

***Corresponding author:** *Yuexian Li, Department of Drug Metabolism and Pharmacokinetics, Takeda Pharmaceuticals International Co., 40 Landsdowne Street, Cambridge, MA 02139, USA, Tel: +1-617-444-1275, Fax: +1-617-444-1480, E-mail: yuexianfrank.li@takeda.com*

Abstract

Human ADME (Absorption, Distribution, Metabolism, and Excretion) studies of new chemical entities are an important part of the drug development process. These studies are normally performed by using a radioactive tracer (C-14 or H-3) blended with a therapeutic dose of non-radioactive drug in about four to six subjects. The radiolabeled API (Active Pharmaceutical Ingredient) is utilized to evaluate the recovery and track the metabolic fate and physiological disposition of the drug. More challenges are faced regarding subject and site selection and supply of C-14 labeled drug product when performing these studies with oncology compounds. If the compound is suitable to study in healthy volunteers, the strategy employed will be similar to what is normally conducted with non-oncology compounds. But if the compound is not suitable to study in healthy volunteers (e.g. a cytotoxic drug), different strategies including recruitment of patients and availability of pure radioactive drug product whenever a patient becomes available for the study must be employed. These studies generally extend over a period of six months to a year. Because of this, stability studies of manufactured radiolabled compound assume importance. Two approaches (Microtracer-AMS (Accelerator Mass Spectrometry) and the traditional method) used for these studies will be described. Detailed information will be explained by using three examples (Compounds A, B, and C, with microtracer - AMS method in healthy volunteers, microtracer - AMS method in patients, and traditional method in patients respectively).

Keywords

Human ADME, Oncology compounds, Radiolabeled API, Stability, AMS

Introduction

Human ADME study (Absorption, Distribution, Metabolism, and Excretion) is an important and essential part of the drug development process. It is completed by using a radioactive tracer (C-14 or H-3) blended with the therapeutic dose of non-radioactive test drug. Its objectives are to determine the route of excretion of compound related material, identify metabolites of a test compound, and describe the exposure of test compound metabolites [1-9].

Depending on non-clinical and clinical toxicological profiles of oncology compounds, human ADME studies can be conducted in either healthy volunteers or oncology patients. At Takeda, majority of the studies had to be conducted in patients. If human ADME studies are conducted in patients, we have to face difficult subject recruitment, limited options of clinical sites, preparation of radioactive drug for each patient, and a longer period of stability study for the radiolabeled API (Active Pharmaceutical Ingredient). But if conducted in healthy volunteers, we will have relatively easier subject recruitment, more options of clinical sites, one batch preparation of radiolabeled drug for all subjects at the same time, and a shorter period of stability study for the radiolabeled API.

Methods

There are two methods for human ADME studies. Method 1 is Microtracer dose-AMS (Accelerator Mass Spectrometry) method [2,3,10-13]. In this method, the radioactivity per subject is normally 0.2-1.0 µCi. The weight of radiolabeled compound per person is in µg level. The GMP (Good Manufacturing Practices) synthesis of labeled compound is generally not required. The rat dosimetry information is also not required. But AMS

Citation: Li Y, Plesescu M, Prakash SR (2017) Radiolabeled APIs for the Conduct of Human ADME Studies of Oncology Compounds. Int J Cancer Clin Res 4:082. doi.org/10.23937/2378-3419/1410082

Received: May 30, 2017; **Accepted:** August 28, 2017; **Published:** August 30, 2017

method might be sensitive to the contamination at the clinical site. Generally, the AMS analysis is more expensive and time consuming.

Method 2 is the Traditional (GMP radiolabeled compound-LSC (Liquid Scintillation Counting)) method. In this method, radioactivity per subject is 50-100 µCi. The weight of radiolabeled compound per person is normally in mg level. The GMP synthesis of labeled compound is required. This method is less sensitive to the contamination at the clinical site. But the tissue distribution data in rats and dosimetry information are required.

Results

We use three examples to explain these two methods. Two examples are from the AMS method either in healthy volunteers or patients. The third example is from the traditional method in patients.

Example 1 was a human ADME study of Compound A by using Microtracer-AMS method in healthy volunteers. Compound A is safe enough to be administered in healthy subjects. Human dosing considerations included 6 healthy volunteers, 0.5 µCi (32 ng) per subject, and single oral solution dose (100 mL of citrate buffer solution per subject). The large volume (100 mL) is needed because of its high dose. We used the ethanol solution of [^{14}C]-Compound A as the spiking solution for the radioactive formulation. This is because Compound A is very soluble in ethanol. Ethanol can stabilize the radiolabeled compound. It is also convenient for addition of a small amount of [^{14}C]-Compound A (32 ng) into the formulation. The concentration of the spiking solution was 0.5 µCi in 0.5 mL ethanol per subject. The radioactive formulation was prepared at the clinical site. The Clinical CRO (Contract Research Organization) transfer-related activities are summarized in Table 1.

[^{14}C]-Compound A formulation was stable at 5 °C for 24 hours plus at room temperature for 4 hours. These stability conditions mimicked the actual clinical operations. Three different higher concentrations (2.5 µCi/mL, 5 µCi/mL, 10 µCi/mL) of solutions were prepared for the stability study because the radiometric detector cannot be used for the low radioactive concentration (0.5 µCi in 100 ml of citrate buffer solution). This stability study assumed that the stability of a radioactive formulation should be same or even better with the lower concentration. The ethanol spiking solution of [^{14}C]-Compound A was stable for more than a year.

Example 2 was a human ADME study of Compound B by using Microtracer-AMS Method in patients. The ADME Study was in patients because Compound B is cytotoxic. This study was utilizing a single oral solution dose. Each subject was administered 0.4-0.6 µCi [^{14}C]-Compound B (21-37 ng API at specific activity of 16.6 µCi/mg) in 10 mL citrate buffer (pH 5.8). The specific pH (5.8) is required for a good solubility and stability. [^{14}C]-Compound B spiking solution was prepared by using a citrate buffer (pH 5.8) at a concentration of 0.83 µCi/mL. A higher concentrations (2 µCi/mL) of spiking solution was utilized for the stability study because 0.83 µCi/mL was too low to be analyzed by a radiometric detector. [^{14}C]-Compound B dosing solution was prepared by adding the spiking solution into the Compound B dissolved in citrate buffer (pH 5.8, 10 mL). Because the concentration (0.06 µCi/mL) of the dosing solution is also too low to be analyzed by radiometric detector, three higher concentrations (10 µCi/10 mL, 15 µCi/10 mL, and 20 µCi/10 mL) were prepared for the stability study.

Our clinical operations team, like others, always wants to know the timelines of the radioactive API and formulation related activities before selection of a clinical site that are summarized in Table 2. [^{14}C]-Compound B API was stable only for 26 weeks at -80 °C (Table 3).

Table 1: Clinical CRO transfer-related activities.

Activity	Organization
Transfer of radiolabeled formulation method	Takeda
Transfer of analytical method	Takeda
CRO visit for any trouble shooting during the mock run	Takeda
Generation of formulation batch records (mock and real runs)	CRO
QC data and release	CRO
Review of batch records	Takeda

Table 2: Timelines of microtracer dose-AMS method.

Activity	Time, month
Preparation of [^{14}C]-API	1-3
Develop and prepare spiking solution	1
Radiolabeled formulation and stability study	1-2
Write CMC radiolabeled portion for IND Amendment	1
Mock run, review of batch records	1
Total	5-8

Table 3: Stability of [^{14}C]-Compound B API (16.6 µCi/mg).

Time point at -80 °C, week	Chemical purity, %	Largest chemical impurity, %	Radiochemical purity, %	Largest radiochemical impurity, %
Initial	99.9	0.1	99.4	0.2
7	99.5	0.2	99.0	0.3
11	99.1	0.3	98.9	0.3
14	98.8	0.3	98.1	0.5
18	98.6	0.4	98.0	1.1
22	98.6	0.5	97.6	0.7
26	98.5	0.7	97.2	0.9

Table 4: Stability of [^{14}C]-Compound B spiking solution (2 µCi/mL).

Time point at room temperature, h	Radiochemical purity, %	Largest individual radiochemical impurity, %
Initial	97.29	1.00
2	97.18	1.00
4	97.00	1.21
6	96.93	1.70

Table 5: Stability of [^{14}C]-Compound B dosing solution (10 µCi/10 mL).

Time point at room temperature, h	Radiochemical purity, %	Largest individual radiochemical impurity, %
Initial	97.28	1.05
2	97.11	1.35
4	97.01	1.30
6	96.86	1.19
8	96.64	1.67

Table 6: CMO transfer-related activities.

Activity	Organization
Transfer radiolabeled synthetic methods	Takeda
Transfer analytical methods	Takeda/CMO
Test synthetic methods by unlabeled and labeled compounds	CMO
Technical batch API synthesis	CMO
GMP synthesis of radiolabeled API	CMO
QC data	CMO
Generation of batch records	CMO
Review of batch records and analytical data	Takeda
Prepare stability protocol and analyze samples	Takeda/CMO
Approve and release the radiolabeled API for clinical use	Takeda

Two batches of [^{14}C]-API were required to complete the human ADME study. Spiking solution (2 µCi/mL) was stable for 4 hours at room temperature (0.3% decomposition was within the specifications that require radiochemical purity should be larger or equal to 97.0% and individual impurity less or equal to 2.0%) (Table 4). [^{14}C]-Compound B dosing solution (10 µCi/10 mL) was also stable for 4 hours at room temperature (0.3% decomposition was within the specifications) (Table 5).

Example 3 was a human ADME study of Compound C by utilizing the Traditional Method in patients. The study was in patients because of Compound C's cytotoxicity. The study was designed by administering 4-6 Patients. Each patient was dosed 80-100 µCi with a single oral solution.

A CMO (Contract Manufacturing Organization) was required for the synthesis of GMP [^{14}C]-Compound C API. The selection of the CMO was according to the following considerations at Takeda:

– Technical capability and experience in radiolabeled synthesis.

– Analytical capability for in-process and release analysis.

Table 7: Timelines of traditional method in API and formulation portions.

Activity	Time, month
Develop radio-synthetic route	2
GMP synthesis at CMO	5-7
Radiolabeled formulation and stability study	1-2
Write CMC radiolabeled portion for IND Amendment	1
Mock run, review of batch records	1
Total	10-13

Table 8: Stability of [^{14}C]-Compound C API (2.23 µCi/mg).

Time point at -80 °C	Chemical purity, %	Radiochemical purity, %
Initial	99.7	100
1 Month	99.7	100
2 Month	99.7	100
…….		
12 Month	99.7	100

Table 9: Stability of [^{14}C]-Compound C dosing solution (6.0 µCi/mL).

Time point	Chemical purity, %	Radiochemical purity, %
Initial	99.7	100
24 h at 5 °C	99.7	100
24 h at 5 °C + 4 h at room temperature	99.7	100

– QC and QA to support GMP operations.

To make the GMP preparation of [^{14}C]-API smooth, we followed the last-step procedures of un-labeled GMP synthesis and crystallization with some minor modification. The advantages of doing so are the availability of validated analytical methods, impurity profile, and other synthetic and analytical information. Smoother transfer of manufacturing and analytical details to CMO is usually feasible because of prior optimization work at the existing radiochemistry unit at Takeda. Due to a requirement for GMP qualification of existing radiochemistry unit at Takeda with appropriate SOPs and instrument qualifications, working with a qualified CMO is preferred. This trend of using GMP qualified vendors for manufacture of radiolabeled API is more prevalent in the industry. Based on the survey conducted by IQ, for a radiolabeled human ADME study, a one-time event, question of GMP synthesis of API is still under debate [14].

The CMO transfer-related activities are summarized in Table 6. The timelines related to API and formulation portions are summarized in Table 7. The [^{14}C]-API was found stable at least 1 year at -80 °C (Table 8). Stability in general is compound dependent, and Compound C is much more stable than Compound B (Table 3). The dosing solution was stable 24 hours at 5 °C plus 4 hours at room temperature (Table 9). These stability conditions mimicked the actual clinical operations.

Conclusions

In general for oncology compounds, it is essential to

have capability to prepare a minimum of two batches of radiolabeled API during human ADME study because of delays in patient recruitment and stability of API. For the GMP preparation of radiolabeled API, it is preferable to follow the last-step procedures of unlabeled GMP synthesis and crystallization with some minor modification. Confirmation of stability for longer periods of time by appropriate storage is required.

Acknowledgements

Thanks are due to the Process Chemistry, Analytical Development, QA, QC, Formulation, Clinical Pharmacology, Clinical Operations, and DMPK departments of Takeda-Boston.

References

1. Marathe PH, Shyu WC, Humphreys WG (2004) The use of radiolabeled compounds for ADME studies in discovery and exploratory development. Curr Pharm Des 10: 2991-3008.
2. Beumer JH, Garner RC, Cohen MB, Galbraith S, Duncan GF, et al. (2007) Human mass balance study of the novel anticancer agent ixabepilone using accelerator mass spectrometry. Invest New Drugs 25: 327-334.
3. Beumer JH, Beijnen JH, Schellens JH (2006) Mass balance studies with a focus on anticancer drugs. Clin Pharmacokinet 45: 33-58.
4. Dain JG, Collins JM, Robinson WT (1994) A regulatory and industrial perspective on the use carbon-14 and tritium isotopes in human ADME studies. Pharm Res 11: 925-928.
5. Suri A, Pusalkar S, Li Y, Prakash S (2016) Absorption, distribution, and excretion of the investigational agent Orteronel (TAK-700) in healthy male subjects: a Phase 1, open-label, single-dose study. Clin Pharmacol Drug Develop 5: 180-187.
6. Lappin G (2016) Approaches to intravenous clinical pharmacokinetics: Recent developments with isotopic microtracers. J Clin Pharmacol 56: 11-23.
7. Penner N, Xu L, Prakash C (2012) Radiolabeled absorption, distribution, metabolism, and excretion studies in drug development: why, when, and how? Chem Res Toxicol 25: 513-531.
8. US Food and Drug Administration (FDA) (2010) The Radioactive Drug Research Committee: Human research without an investigational new drug application. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research.
9. European Medicines Agency (2013) Guideline on the investigation of drug interactions. Committee for Human Medicinal Products.
10. Comezoglu SN, Ly VT, Zhang D, Humphreus WG, Bonacorsi SJ, et al. (2009) Biotransformation profiling of [(14) C]ixbepilone in human plasma, urine and feces samples using accelerator mass spectrometry (AMS). Drug Metab Pharmacokinet 24: 511-522.
11. Boddy AV, Sludeden J, Griffin MJ, Garner C, Kendrick J, et al. (2007) Pharmacokinetic investigation of imatinib using accelerator mass spectrometry in patients with chronic myeloid leukemia. Clin Cancer Res 15: 4164-4169.
12. Arjomand A (2010) Accelerator mass spectrometry-enabled studies: Current status and future prospects. Bioanalysis 2: 519-541.
13. Bae SK, Shon JH (2011) Microdosing studies using accelerated mass spectrometry as exploratory investigational new drug trials. Arch Pharm Res 34: 1789-1798.
14. Faul MM, Slatter JG, Dean D, Smith K, Hesk D, et al. (2017) Industry practices for the synthesis of radioactive active pharmaceutical ingredient for use in human absorption, distribution, metabolism and excretion studies (A survey of current practices of 21 IQ consortium member companies).