Webinar Series
March 2015

xceleron

Successful clinical ^{14}C microtracer investigations

What can contemporary AMS do for you?

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Webinar Q&A Summary

Questions were received during the Webinar on 26th March 2015, following on from participants on the webinar and augmented by questions frequently asked of webinar presenters.

The Q&A is organized in sections as follows:

Our panel of industry experts spoke on the following topics:

GSK experience of application of AMS to clinical studies - the last 5 years & GBC (LC+AMS) Update

Graeme Young, GSK
Manager AMS, Biotransformation & Drug Disposition (BDD), RD Platform Technology & Science

Combining Accelerator Mass Spectrometry (AMS) with the Human ADME study

Ken Cassidy, Eli Lilly and Company
Senior Research Advisor, Drug Disposition

Intravenous Pharmacokinetics Enabled with Accelerator Mass Spectrometry

Graham Lappin, Lincoln University
Visiting Professor

#1

Types of biological investigations with ^{14}C and AMS

What types of investigations can AMS be used in?

Anything that benefits from the use of a ^{14}C tracer, very low analytical sensitivity, absence of matrix effects on the detector, a molecule that doesn't ionize or completely lacks a chromophore (e.g. a carbohydrate), where only small amounts of sample are available, or any combination of the above. For a summary see:

Lappin, G. and L. Stevens, *Biomedical accelerator mass spectrometry: recent applications in metabolism and pharmacokinetics.* Expert Opin Drug Metab Toxicol, 2008. **4**(8): p. 1021-33.

What types of life sciences investigations has AMS typically been used in?

AMS has been used in a wide range of applications. An early use was in the investigation of DNA adduct binding. More recently AMS application has been mostly pharmaceutical, mostly clinical, mostly with healthy volunteers and mostly small molecule. That said, as the industry gets more comfortable with the platform we are using it more now with patients particularly in oncology. We are also investigating very potent peptides and antibody-drug conjugates and more often in preclinical studies and even in research to investigate cell turnover and target kinetics.

Outside of pharmaceutical uses, we have used AMS in food metabolism research and to investigate crop protection products.

What types of patient studies has ^{14}C and AMS been used in?

Chronically ill cancer, and renally/hepatically impaired patients. We are aware of IV microtracer studies and oral microtracer studies. In both cases the microtracer was administered with a therapeutic dose of cold drug, to assess IV pharmacokinetics (absolute bioavailability, clearance, volume of distribution) and to metabolism (rates and routes of excretion, metabolite profiling and metabolite safety) respectively.

We are experiencing increased interest in the use of microtracers in oncology patient studies and this appears to be related to the safety of administering typically < 1,000 nCi radioactivity to patients on their experimental therapies. Also, levels of radioactivity are sufficiently low that after dosing the patients can go onto areas otherwise not designated for use with radioactivity. We have had patients return to hospital wards or even directly home. The IV microtracer study design also reduces patient impact because it uses only one dose occasion rather than a cross over design.

We expect also to conduct more oncology microtracer studies as therapeutic application becomes chronic in nature. Greater understanding of fundamental kinetics will become more important to defining appropriate doses.

#2

Important terminology

What is the difference between a microdose and a microtracer?

A microdose is defined by the mass of drug administered. To meet the definition for a microdose, the dose administered must be ≤100µg, or ≤100th of the pharmacologically active dose, whichever is the lower (see ICH M3 R2 Section 7.1). Subjects (healthy volunteers or patients) are not exposed to any higher dose by any route of administration in the study. A microdose may, therefore, be comprised of only unlabelled active substance or may be enriched with ^{14}C. It follows also that analysis of tissues from a microdosed clinical study may be accomplished with LC-MS/MS or AMS, respectively.

We think of a microtracer as a low level of tracer (e.g., ^{13}C or ^{14}C) associated with the mass of the dose administered. A microtracer may be administered with a microdose or a pharmacologically active dose of the drug of interest.

In terms of ^{14}C, it is worth addressing the level of radioactivity associated with the microtracer. Typically the clinics we work with assume a cut-off at ≤1µCi without the need for dosimetry and formal approval to dose this amount of radioactivity. However, we work with clinicians in Holland who will administer ≤2.7µCi. The difference arises from regulators governing the clinical jurisdiction.

The great majority of microtracer studies conducted today involve a pharmacologically active dose of drug.

How are microdoses and microtracers employed in pharmaceutical investigations?

Microdoses – often to assist with early phase (including perhaps Phase 0/exploratory FTIH) clinical investigations for progression/termination decisions based around absolute or relative pharmacokinetics, penetration of site of action and/or metabolism. Human microdose investigations have been used quite often to investigate drug disposition in those cases in which preclinical in vitro and in vivo results are equivocal. Later stage efforts have included penetration at site of action investigations.

Microtracers – for a whole variety of scientific and economic reasons microtracers have been used to investigate human PK (absolute bioavailability, CL and V) and human ADME.

The webinar presentations by Graeme Young and Graham Lappin provide many examples.

What is the consortium that was mentioned on the webinar?

GBC = Global Bioanalysis Consortium; AMS 10 is a harmonisation team formed mostly of AMS providers to the Pharmaceutical industry whose main objective has largely been to look at best practices for use of LC+AMS application to Bioanalysis and to try to encourage harmonisation towards best practices where feasible (see Graeme Young slide and reference for details)

#3

Administration of ^{14}C in biological investigations

Is GMP manufacturing required for ^{14}C study material?

There are three aspects of manufacture that we need to consider in answering this question, namely (1) manufacture of the ^{14}C-labelled drug material, (2) manufacture of the cold drug material if necessary and (3) manufacture (often referred to as compounding) of materials prior to dosing in the clinic.

We should state up-front that approaches to the manufacture and QP (in Europe) release of ^{14}C-labelled active substance are often company-specific. Some companies prefer to manufacture and release all drug material intended for clinical use in accordance with strict GMP requirements. In our experience, the majority follow the approach described below.

Manufacture of the ^{14}C-labelled active substance need not strictly comply with all GMP requirements if the dose administered is sufficiently low. In our experience, the dose administered is sufficiently low if it is considered a microdose or a microtracer administered in a mass dose which is <0.15% of a co-administered investigational medicinal product. In our experience, ^{14}C-labelled active substance intended for clinical administration under these circumstances may come from existing, re-purified preclinical material or may be synthesized specifically under GMP-like conditions. Our experience also shows that subsequent provision of a certificate of analysis showing 98% chemical and radio-chemical purity is sufficient.

Manufacture of drug material administered at a mass greater than a microdose or >0.15% of a co-administered investigational medicinal product should be manufactured and released in compliance with GMP standards.

Manufacture or compounding (e.g., dilution of ^{14}C-labelled drug material immediately prior to sterile IV administration) must be performed in compliance with GMP standards.

One presenter on the webinar mentioned manufacturing 5mCi of ^{14}C-labelled material. Isn't it cheaper to make less?

Isotope chemists have told us that it is easier to synthesize larger batches and dilute as necessary. In our experience, 1-5mCi is typical.

How do you see the potential issue of adsorption of ^{14}C-drug material to all materials used?

We are a little unclear on how to answer this question. It was submitted during the webinar and we did not have time to clarify. We will assume that the questioner is interested in understanding potential adsorption of microtracer during IV administration.

Non-specific binding to the dosing apparatus used for IV administration (sterile filters, lines etc.) has to be checked out in advance of the clinical study. This is often referred to as compatibility testing and is typically performed at the same concentration as the IV dose. It can be performed with cold material and LC-MS/MS or using higher amounts of radioactivity and LSC rather than AMS. If significant binding is found then different materials for the dosing apparatus and excipients which alter surface activity are explored in order to reduce the adsorption.

Is there the potential to administer a compound labelled with both ^{13}C and ^{14}C and differentiate between the two?

We are aware of one publication where the study design was a conventional human ADME using a ^{14}C-labelled oral dose concomitant to an intravenous ^{13}C-tracer dose of the same compound. The reference is as follows:

Schwab, D., et al., *A Novel Double-Tracer Technique to Characterize Absorption, Distribution, Metabolism and Excretion (ADME) of [(14)C]Tofogliflozin After Oral Administration and Concomitant Intravenous Microdose Administration of [(13)C]Tofogliflozin in Humans.* Clin Pharmacokinet, 2013. 52(6): p. 463-73.

AMS measures the isotope ratio between $^{12}C/^{13}C/^{14}C$ and so in theory it can measure the ^{14}C and ^{13}C content in dual labeled study in the single analysis. Xceleron recognize the potential in this and are currently researching the technique.

#4

Preclinical studies

What kind of preclinical studies have been conducted?

The utilization of AMS in regulatory preclinical studies is more limited due to the practical fact that doses of radioactivity in animal models are not limited by the same safety concerns as in humans. Hence larger doses of ^{14}C can be utilized (i.e. 50- 100 uCi/kg), and LSC and radiometric profiling methods can be employed.

IND enabling studies for highly potent cytotoxins in ADC constructs to assess systemic levels for safety assessments, and to undertake metabolite profiling investigations of cytotoxin and linker have been conducted. Animal studies have also been conducted with peptides and potent compounds such as some respiratory compounds.

In vitro studies of respiratory products at therapeutically relevant concentrations have been conducted.

^{14}C and AMS is also used to measure the turn-over of proteins in animal and disease models, with and without therapeutic intervention, to assess the 'druggability' of certain targets.

Why are ^{14}C and AMS used?

Sensitivity for a range of compound classes in a range of biological matrices in small samples.

#5

Microdose studies

What kind of studies have been conducted?

Often to assist with early phase (including perhaps Phase 0/exploratory FTIH) clinical investigations for progression/termination decisions based around absolute or relative pharmacokinetics, penetration of site of action and/or metabolism. Human microdose investigations have been used quite often to investigate drug disposition in those cases in which preclinical *in vitro* and *in vivo* results are equivocal. Later stage efforts have included penetration at site of action investigations.

Are there situations where microdosing studies may not be appropriate?

Yes, in situations in which significant non-linearity across the dose range is anticipated and for which a manageable solution is unknown. For example, target mediated disposition is a known cause of non-linearity for drugs such as Warfarin. A microdose investigation may not be of use for such drugs.

There are also situations in which non-linearity across the dose range is anticipated but in which the data can still be interpreted usefully. This may occur, for example, in certain cases with saturation of drug transporters, metabolizing enzymes, dissolution properties etc. Such situations do not automatically rule-out a microdose study but need to be assessed on a case-by-case basis.

It is worth pointing out that the majority of orally administered drugs have demonstrated scalable PK within a factor of two, and all iv administered drugs have shown excellent scalability. The latest review is:

Lappin, G., R. Noveck, and T. Burt, *Microdosing and Drug Development: Past, Present and Future*. Expert Opin Drug Metab Toxicol, 2013. **9**(7): p. 817-834.

For oncology drugs, tox studies are not done to enable healthy subject studies and I want to know if a microdose can give the ADME info in HV's without having to do the tox studies?

We are a little unclear on how to answer this question. It was submitted during the webinar and we did not have time to clarify. We will assume that the questioner is interested in understanding whether a microdose of ^{14}C labelled cytotoxic drug can be dosed to healthy volunteers in order to understand routes & rates of excretion and metabolism?

As defined earlier a microdose is ≤100µg, or ≤100th of the pharmacologically active dose, whichever is the lower. Such studies require a single dose rat toxicity study with 14-d recovery and limited histopathology to allow a microdose to humans, so it is possible to dose tiny amounts of ^{14}C labelled cytotoxic / carcinogenic compounds to healthy subjects (at levels inconsequential to health) and determine the human AME/PK properties of those compounds.

Examples of this approach are described in the recent paper by Madeen et al., where they used a microdosing approach (oral route) to investigate PK for a known carcinogen (dose of only 29ng; 5nCi). Human carcinogen microdose ›

Also Clavis recently used the same approach with an intravenous microdose of ^{14}C-elacytarabine: Clavis microdose ›

#6

Clinical studies

What kind of Phase I studies have been conducted?

Increasingly clients are using microtracers in early human studies (sometimes incorporated into SAD/MAD, sometimes into second line DDI / Food Effect) in order to get an early steer on fundamental PK and metabolism to better design later studies in terms of dose setting and formulation requirements

When are these studies conducted?

They have been conducted at different stages throughout development – see the webinar slides presented by G Young and G Lappin

Are they just volunteer studies or are patients also investigated?

See the slides by G Young; both healthy volunteers and patient studies have been conducted

What are the advantages of the IV tracer design over the traditional cross-over design?

See specifically slides by G Lappin

1. Removes the need for expensive and time-consuming IV toxicology (saving of $2M and 1 year).
2. Significantly simplifies the IV formulation, particularly for insoluble drugs.
3. The study is performed in one dose occasion and so saves clinical costs.
4. Scientifically a better approach - as it significantly reduces the risk of non-linear PK (see answer below).
5. Enables CL, V and F to be obtained under steady state conditions.

I don't understand why the cross-over study might suffer from non-linear PK, can you explain this please?

The plasma-drug concentration versus time data are acquired for both dose routes and the absolute bioavailability (F) is calculated by comparing the area under the drug-concentration time curve extrapolated to time-infinity ($AUC_{0-\infty}$) for the extravascular (AUC_{ex}) and i.v. (AUC_{iv}) doses, normalized to the doses administered (equation-1).

$$F = \left(\frac{AUC_{ex}}{AUV_{iv}}\right)\left(\frac{Dose_{iv}}{Dose_{ex}}\right) \qquad \text{Equation-1}$$

continued on the next page

Although equation-1 is commonly used in the determination of F, it should be remembered that it does not show the influence of CL. The two equations in respect to CL are shown in equations 2 and 3 below.

$$CL_{iv} = \frac{Dose_{iv}}{AUC_{iv}} \qquad \text{Equation-2}$$

$$CL_{ex} = \frac{(F \times Dose_{ex})}{AUC_{ex}} \qquad \text{Equation-3}$$

Thus F calculated from equation-1 assumes that CL is the same for both the oral and i.v. administrations. This is probably a reasonable assumption providing CL is not plasma drug-concentration dependent. The ideal cross-over study design therefore, is one where the plasma concentrations (and hence AUCs) attained for the oral and i.v. doses are the same, or at least as close as possible. In practice this is very difficult to achieve without already knowing F and V. In a recent study for example, the absolute bioavailability of tasimelteon was studied using a cross-over design with an oral dose of 20 mg and an i.v. dose of 2 mg (Torres, R., et al., *Absolute Bioavailability of Tasimelteon.* Am J Ther, 2015) where the $AUC_{0-\infty}$ for the oral dose was 5 times greater than that for the i.v. dose. In other cases, bioavailability in excess of 100% have been observed probably due to membrane transporter-mediated non-linear PK (Ward, K.W., et al., *Apparent absolute oral bioavailability in excess of 100% for a vitronectin receptor antagonist (SB-265123) in rat. II. Studies).* Non-equivalent clearance is also thought to be responsible for an approximate 10% error in the determination of F for phenytoin (Jusko, W.J., J.R. Koup, and G. Alvan, *Nonlinear assessment of phenytoin bioavailability.* J Pharmacokinet Biopharm, 1976. **4**(4): p. 327-36). Error in the calculation of F arising from non-equivalent CL is rarely considered in absolute bioavailability studies although the effect has been recognized for several decades. The issue may not be significant in many cases but nevertheless, the potential is there. The microtracer approach however, results in a single systemic concentration (a mixture of the oral and IV administered drug) and so CL will be the same for both dose routes during the elimination phase.

Is it necessary to have toxicology to support the administration of an IV microtracer in humans for an oral compound?

No, so long as the microtracer dose does not exceed the regulatory definitions of a microdose. That is to say, the IV dose cannot exceed a mass dose of 100 µg, or 1/100th of the therapeutic dose – whichever is the lower of the two.

The ICH M3 guideline allows that the IV dose be covered by the pre-IND toxicology studies to support the oral administration of the parent drug. See ICH M3 R2 Section 7.1. Systemic exposure data from the oral route is then used to support the IV administration without further toxicology studies by the IV route.

How many subjects are usually dosed in a microtracer absolute bioavailability study?

The studies presented in the webinar used 4-6 healthy volunteers or patients. Because the microtracer approach has only one dosing occasion then temporal effects between the IV and oral dose are virtually eliminated and so statistically fewer volunteers can be used. In addition, the dropout rate of volunteers is potentially higher in a traditional cross over design as the subjects have to return for a second dosing.

Are the regulatory authorities insistent on having absolute bioavailability data or can a case be made to avoid doing the study?

We are aware of only one jurisdiction, Australia, in which this data is a requirement. There is always the possibility that a regulatory authority can be convinced that a particular data set is unnecessary or impossible to generate.

Regulatory agencies in Europe, the United States and Japan may request absolute bioavailability data when bioavailability is apparently low or variable and there is a known relationship between pharmacokinetics and pharmacodynamics

Aside from questions from regulators, knowing important parameters such as absolute bioavailability, CL and V early can assist in understanding issues with absorption and first pass metabolism and the role that formulation may play in compound drugability.

Irrespective of whether interest is generated in absolute bioavailability by a regulator or an interest in development efficiency, the microtracer approach makes the study very affordable.

Do regulators accept IV microtracer data for Abs Bio determination and oral microtracer data for mass balance / metabolism?

Yes – there are several examples of both Regulatory input to clinical study design and successful submissions that have included such data.

The table in G Lappin's presentation listed published studies where the data were submitted to the regulatory agency.

What advantages have Eli Lilly and GSK experienced from using microtracer approaches?

GSK – particular advantage for the use of the microtracer (absolute bio. study) in cancer patients with the ability to reduce the time of the study, number of patients involved and allow those patients on study to move onto a protocol for treatment of their disease

Lilly – as detailed in Annes et al., *Journal of Pharmaceutical Sciences* 104:207–214, 2015 the utility of AMS microtracer technology in a two arm crossover study allowed the IV dosing of the prodrug and the active moiety in human subjects. This design was used to determine the contribution of pre systemic and systemic contribution of peptidases to the oral exposure of the prodrug.

Why is the IV dose given at tmax and not simultaneously with the oral dose?

If the drug shows plasma-concentration dependent CL (see answer to question above) then there is the possibility that the absorption phase will exhibit non-linear PK. By delaying the IV administration to the t_{max} - in fact by giving the IV dose as an infusion on either side of the t_{max} - this minimises non-linear effects. For most drugs of relatively short t_{max} of an hour or so, this is very much in the fine-tuning of the study design.

What is the approximate cost difference between a traditional absolute BA study vs an AMS absolute BA study?

In the reference below, AstraZeneca and Xceleron show in detail how we can achieve 6-months and $1.5MM savings. Using the same methodology, we have demonstrated elsewhere that the ^{14}C AMS study can reduce development time by at least one year and expenses by $2MM. The additional savings are realized by conducting the ^{14}C investigation as a component within a FIM protocol rather than a separate follow-on investigation.

Lappin, G., et al., *A Resource and Data Quality Comparison: Absolute Bioavailability Data from an Oral / IV Crossover and an IV Isotopic Tracer Clinical Design in AAPS.* 2011: Washington, DC USA.

What kind of Phase II/III studies have been conducted?

The studies are generally considered to be of Phase I design (small number of volunteers) but have occurred in Phase II/III of development. Human ADME and IV microtracer studies have been conducted

What are the advantages of the tracer AMS approach over the traditional ^{14}C LSC approach?

We are aware of three potential advantages, analytical sensitivity, reduction in expense and development time because GMP material is not needed and avoidance of compound radiochemical instability.

The sensitivity advantages of AMS lead to the ability to provide data where it would not be possible using LSC and this in turn leads to more flexibility in study design and support.

Time and expense is avoided using ^{14}C and AMS because there is no regulatory need for full GMP-grade material and animal dosimetry. We have been informed that this could save up to 40% off the expense of a traditional approach. Individual companies may decide to produce full GMP-grade material intended for clinical use, irrespective of this view.

Do you need dosimetry (e.g. QWBA) to support a microtracer study?

Generally no dosimetry is required so long as the microtracer dose of radioactivity does not exceed 1 µCi – 2.7 µCi depending on the jurisdiction

For Ken: What was the extraction recovery of ^{14}C from the faeces to generate samples for metabolite profiling?

The extraction recovery was >90%

Metabolite quantitation & HPLC fractionation: are stable isotope labelled compounds required for HPLC fractionation to control fraction collection?

No. It is often the case that unlabelled analyte (parent drug and sometimes metabolite standards) are spiked into samples to be fractionated so as to provide and internal standard in some instances and also chromatographic marker(s).

#6

Does the analysis a single AUC plasma pool for metabolite profiling mean that metabolite kinetics are not determined?

From Ken - There is always this trade off when choosing to conduct AUC pooling. Since we had bioA methods for 3 of the major metabolites we did not consider profiling plasma time points, however in the event these assays were not available we likely would have pooled subjects across time points and generated PK data for metabolites.

Producing a single profile from an AUC plasma pool will provide a clear understanding of exposure to parent and metabolites during the study, and specifically determine the presence of 'significant' (greater than 10% of total drug related exposure according to ICH M3) and potentially 'disproportionate' metabolites. If such metabolites are found in the single pooled profile then further investigation can be undertaken at different time points to establish kinetics if required.

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