CHAPTER 11

Isotope drug studies in man

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11.1 *Radiolabelled studies in man*

11.1.1 Introduction

One of the most important aspects of drug metabolism/pharmacokinetics in drug development is to show that the animals used in toxicology have been exposed to the same chemical species (drug and metabolites) as will your patient. Usually the metabolism and kinetics of the drug are studied in animals with radiolabelled compound and the quickest way of getting the comparative information from man is also to give a radioactive dose.

Ideally the human radiolabel study should be completed as early as possible in a drug's development (before long-term toxicology) if it is going to be useful in the selection of the animal species for toxicology.

To dose the radioactive compound in man you usually need:

1 Basic toxicology data.
2 Basic kinetics and metabolic information in animals.

3 Basic pharmacokinetics of non-radiolabelled compound in man (although assay sensitivity may be insufficient, and the label may be being given to obtain basic pharmacokinetic information).
4 Estimated organ radioactive exposure data from tissue distribution and excretion balance studies.
5 Ethical approval.
6 Administration of Radioactive Substances Advisory Committee (ARSAC) approval.

These studies are not for statistical evaluation. Therefore ethics dictates the use of a limited number of subjects (2–6). Women of child bearing age should not be used.

Normally a single route is used with a radioactive dose of approximately 2 MBq for ^{14}C or 4 MBq for ^{3}H. The exact amount that can be given is determined using ARSAC calculations (see below). Half the amount of radioactivity can be given each time if two routes of administration are used per subject. The dose formulation used should be the one which has the highest bioavailability normally, this will be a solution of the drug. It is often not possible to use a formulation which is equivalent to the proposed clinical formulation (if known) because the method of preparing the clinical formulation may not be practical with the small quantities of radiolabelled drug available. If a solution is not used, the non-radioactive and radioactive material should be dissolved together, crystallised and the resulting homogenous material used.

The choice of isotope should receive a mention here. The counting efficiency of tritium is only about one-third of that of carbon-14. However, the dose constant for tritium is only about one-ninth that of carbon-14, and consequently more Bq of tritium can be administered. The outcome is that the use of tritium affords a 3-fold increase in sensitivity of detection over carbon-14 for a given radiation exposure of a volunteer.

The main objective is to establish the route of elimination of radioactive products and to compare the metabolite pattern in excreta and plasma with those in animals (radio-HPLC/MS). This information can then be used to select/justify the animal species for long-term toxicology. It is not necessary to identify metabolites at this stage although eventually they will have to be identified. As stated above radiolabel can also assist, through the use of radio-HPLC, with conventional pharmacokinetic studies when the properties of the molecules preclude detection by conventional means.

To gain the maximum information:

1 Measure radioactivity in excreta (urine and faeces) and balance with dose. At a stroke this tells us if the biliary excretion seen in animals is replaced by renal excretion in man.
2 Compare metabolites in excreta and plasma with those in animal species used in/intended for toxicology (but what happens if they are different? How far

will a toxicologist go?). This aspect is becoming increasingly important as safety cover based on metabolites as well as parent compound is a developing issue.
3 Measure radioactivity in blood and plasma at different times after dosing. This can give useful information on the distribution in blood and can be supplemented by *ex vivo* protein-binding studies.
4 Measure intact drug in plasma and compare with total radioactivity.
5 If possible, measure a dynamic parameter as well.

If the intravenous route of administration (e.g. IV infusion) is used as well as oral, this can enhance the knowledge of the basic pharmacokinetics of the drug (clearance, volume of distribution, absorption, bioavailability), providing specific measurements of the drug are also included.

11.1.2 ARSAC

Radiolabelled studies in man must first be approved by the ARSAC of the DoH. The guidelines upon which the ARSAC made its decisions, until recently, are to be found in the WHO publication "Use of ionizing radiation and radionuclides on human beings for medical research, training, and non-medical purposes" (1977). There were no specific guidelines for volunteer studies.

The WHO defined three categories of radioisotope work, according to the radiation dose received, these are summarised in Table 11.1.
These exposure limits were recently reviewed on two counts:

1 The data is based on the effects of the bombs in Hiroshima and Nagasaki, and the degree of shielding between the blast and the victims is now considered to have been underestimated.
2 The statistical methods applied to those data are now being questioned.

TABLE 11.1 *Categories of radioisotope work*

	Category		
	I	**II**	**III**
Range of effective dose equivalent	<0.5 mSv	0.5–5 mSv	5–50 mSv
Level of risk	within variations of natural background radiation	within dose limits for members of the public	within dose limits for persons occupationally exposed to radiation

This has led to revised calculations which are to be found in ICRP publication 60 (1991).

The ICRP has now modified the WHO exposure categories to take these revisions into account (ICRP, 1992). The following categories have been drawn up specifically to cater for volunteer studies. They are presented in Table 11.2.

Category IIa involves a risk of one in one hundred thousand, and a benefit related to increases in knowledge leading to health benefit. Category IIb is associated with a risk of one in ten thousand, and the benefits will be more directly aimed at the cure or prevention of disease.

In addition to the above, the National Radiological Protection Board (NRPB) advise that the average annual intake over several years should be <15mSv for occupational exposure.

As a general rule, the use of radiolabel should comply with the principles of ALARA (As Low As Reasonably Acceptable), and as such the aim should be to keep volunteers within WHO category IIa or lower. Under these circumstances a volunteer will be exposed to no greater risk than they might be if they were to move from one part of the country to another. The principle of ALARA becomes even more important when we consider that these exposure limits are drawn up from historical data that is constantly being re-appraised.

The purpose of the following sections is to describe the calculations that must be performed on animal data to provide ARSAC with the information they require for a particular study. These calculations can be performed by the pharmaceutical company itself, or by the NRPB. Even if the NRPB route is adopted, a knowledge of the calculations will help to plan the most appropriate animal studies. In addition, it is clearly important to know if a volunteer study that will meet your scientific goals is going to be possible before even submitting the data to NRPB and ARSAC.

It is recommended that for current ARSAC submissions both old and new methods of calculation are performed and the most conservative results used.

The primary calculation required is that of the Committed Effective Dose (1991) or the Committed Effective Dose Equivalent (1977). These calculations are performed on data obtained from quantitative whole body autoradiography studies (Chapter 12) and excretion balance studies.

TABLE 11.2 *Categories for radiolabelled exposure for volunteers*

Level of risk	Risk category	Effective dose range (mSv)	Level of societal benefit
Trivial	I ($\sim<10^{-6}$)	<0.1	Minor
Minor to intermediate	IIa ($\sim10^{-5}$)	0.1–1	Intermediate to moderate
	IIb ($\sim10^{-4}$)	1–10	
Moderate	III ($\sim>10^{-3}$)	>10	Substantial

Both new and old methods of calculation and interpretation will now be covered.

Calculation of committed effective dose (CED); ICRP, 1991

(a) *Which tissues?* ICRP publication 60 specifies the tissues that *must* be taken because of their susceptibility to damage. These are shown in Table 11.3.

(b) *Relative sensitivity of tissues* The dose limits are defined in terms of whole body exposure. However, some organs, such as gonads, are more susceptible to radiation damage than others. Therefore each organ is given a weighting factor (W_T) which takes this into account when calculating total exposure.

The weighting factors take into account the probability of fatal and non-fatal cancer, severe hereditary effects and relative length of life lost.

The weighting factors laid down by the ICRP publication 60 are shown in Table 11.4.

(c) *Transformations per Bq* This is the exposure that an organ receives after administration of 1 Bq to the animal.

For tissues containing radioactivity it is the area under concentration time curve (*AUC*) of label in the organ. This is calculated in the same way as is a plasma level-time *AUC* except that the total radiolabel in the organ is used, and not the concentration.

Melanin binding may be a problem as the half-life may be extremely long and dedicated studies may be required to determine the *AUC*.

(d) Exposure from excreta (urine, bile, intestinal contents) which pass through the body is determined from the percentage dose passing through each route and the

TABLE 11.3 *Tissues specified by ICRP publication 60*

Gonads
Colon (LLI)
Lung
Red bone marrow
Stomach
Bladder
Breast
Liver
Oesophagus
Thyroid
Bone surface
Skin

TABLE 11.4 *Weighting factors*

	W_T
Gonads	0.2
Colon (LLI)	0.12
Lung	0.12
Red bone marrow	0.12
Stomach	0.12
Bladder	0.05
Breast	0.05
Liver	0.05
Oesophagus	0.05
Thyroid	0.05
Bone surface	0.01
Skin	0.01
Remainder	0.05*

* Weighting becomes 0.025.

mean residence time (MRT) in man. An additional factor must also be taken into consideration. Not all the label in, for example, faeces will irradiate the intestinal mucosa, as some radiation will be absorbed by the surrounding excreta. For this reason a 'geometric' factor is used to allow for the reduced exposure.

11.2 *Which isotope?*

The biological effects vary from isotope to isotope. A 'dose constant' is used to provide data for a specific isotope.

11.3 *Calculations*

With the above principles in mind we are now ready to perform the calculations themselves. The guidelines for these are laid down in ICRP publication 60. A number of approaches can be made; the method adopted by the NRPB will be described below.

The following calculations are performed for each organ:

$$H_{\mathrm{T}} = \frac{U\varepsilon\phi A}{m}$$

H_{T} = equivalent dose to the target organ (S_{v}).

U = transformation per Bq dose administered, e.g. the AUC of radiolabel in organ (Bq sec).

m = mass of the source organ in man (Kg) obtained from ICRP publication 23.

ε = dose constant for radioisotope in use

$= 9.12 \times 10^{-16}\,\text{kg Gy Bq}^{-1}\,\text{s}^{-1}$ for ^{3}H

$= 79.4 \times 10^{-16}\,\text{kg Gy Bq}^{-1}\,\text{s}^{-1}$ for for ^{14}C.

It is the mean energy of radiation per nuclear transformation. That is, a measure of how damaging a given isotope will be.

ϕ = Fraction of radioactive emissions absorbed ('geometric factor'). It is 1.0 (label in tissue) or 0.5 (label in excreta).

A = amount of radiolabelled drug administered (Bq).

The effective equivalent dose is the whole body dose which would produce the same risk as a non-uniformly distributed absorbed dose. It is calculated for each organ from the equivalent dose using the formula:

$$\text{Weighting factor} = H_{\text{T}} \times W_{\text{T}}$$

The effective equivalent dose of the remainder is a mass weighted mean of the equivalent doses of the contributing tissues.

$$\text{Effective equivalent dose} = \frac{\text{mean of } (H_{\text{T}} \times \text{mass})}{\text{mean of mass}}$$

The effective equivalent doses for all the organs are then summed to produce the 'committed effective dose'. It is this value that must be below 1.0 mSv to fall within a risk category IIa.

11.3.1 Source of data

All the information required for the above calculations is available from tables except for the value of U, and it is this that must be determined experimentally from quantitative tissue distribution, QTD (or quantitative whole body autoradiography, QWBA; see Chapter 12) and excretion balance studies.

The exact amount of data required is unclear, and it probably varies from compound to compound. At one end of the spectrum would be QTD in a rodent and dog, using perhaps nine or ten time points in the rodent and two in the dog. At the other end of the spectrum, it may be possible to use the rodent only and use quantitative WBA at only three or four time points. In both cases an excretion balance study in both species would also be required. If the latter course is adopted the only work involved is encompassed by the normal ADME package.

Quantitative WBA has now gained wide acceptance and clearly offers a great saving in labour over the conventional QTD. Standard organ weights must be used to convert concentrations to organ contents.

Whichever approach is adopted, the organs can be divided into 'tissues' for which QTD or QWBA provides the data, and 'excreta' for which excretion balance studies are the source data.

(a) *Tissues*

A common approach is to take four time points. One point would be the expected peak level, and the other three would be chosen to characterise the elimination. Depending on the compound, the last point may be 24 hours, 48 hours, or even as long as a week or two after dosing.

The value of U is readily obtained by the trapezoidal rule. Integration may also be used. The value of A_o in the formula A_o/λ would be either the peak amount of label in the organ or the value extrapolated to time zero, whichever is the greater. λ is the half-life of the biological elimination of radioactivity in the organ.

In either case, the exposure must be extrapolated beyond the end of the experiment by assuming that any label remaining has a half-life of 100 days. That is,

$$U = \frac{A_o}{\lambda} + \frac{A_Z \times (100 \times 24 \times 60 \times 60)}{0.693}$$

The value of U is normalised to a dose of 1.0 Bq.

(b) *Melanin*

Binding to ocular melanin may have a half-life in excess of 100 days. For this reason melanin binding must be treated as a special case, should it occur, and detailed pharmacokinetics should be obtained.

(c) *Excreta*

Because this has a finite residence time in the body, and therefore exposure to radiation is linked to bodily function as opposed to metabolite pharmacokinetics, a different approach is used.

$$U = F \times MRT \text{ (remember SI units)}$$

F is the fraction of the dose that passes through a particular excretion route (i.e. a fraction of the 1.0 Bq nominal dose).

MRT is the mean residence time for that route in man (Table 11.5)

NB If significant label resides in the walls of the intestines, bladder, etc. this must be calculated separately and added to the exposure from the excreta. It should be remembered that the GI tract is considered as four organs and that biliary excretion in rat might be replaced by urinary excretion in man.

TABLE 11.5 *MRT is the mean residence time for that route in man*

Tissue	MRT (hours)
Stomach	1
Small intestine	4
Upper large intestine	13
Lower large intestine	24
Urine	10
Bile	2.53

Typical calculations supplied by the NRPB are:

Thyroid

Mass of thyroid $= 0.02$ kg
Transformations per Bq $= 4.5$
$\phi = 1$
Equivalent dose $= 7.57 \times 10^{-7}$ Sv
Effective equivalent dose $= 2.27 \times 10^{-8}$ Sv

Small intestine (from contents)

Mass of contents $= 0.4$ kg
Fraction activity $= 1.0$ (p.o dose)
$MRT = 4$ hours
Transformations per Bq $= 14,400$
$\phi = 0.5$
Equivalent dose $= 6.02 \times 10^{-5}$ Sv
Effective equivalent dose $= 3.61 \times 10^{-6}$ Sv

The sum of all the effective equivalent doses gives us the CED, which must be $<$1mSv for a category IIa study.

11.3.2 Calculation of committed effective dose equivalent (CEDE); ICRP 1977

The calculation of the CEDE is similar to that of the CED. The major difference is that a different set of tissues is mandatory and the weighting factors are consequently different.

ICRP publication 26 specifies six tissues that *must* be taken because of their susceptibility to damage. These are:

Gonads
Breast
Bone marrow) usually combined, and assume all activity in marrow
Bone surface)
Lung
Thyroid

In addition, five other tissues must be selected. These should be the ones receiving most exposure.

The weighting factors, laid down by the WHO report number 611 are shown in Table 11.6.

The effective dose equivalents for the ten organs are then summed to produce the 'committed effective dose equivalent'. It is this value that must be below 0.5 mSv to comply with a WHO category I experiment.

The sum of all the effective equivalent doses (dose equivalents) gives us the committed effective dose (or committed effective dose equivalent) to a human volunteer for a given dose. For example, if a CED of 550 μSv is arrived at based on the administration of 1.0 MBq, then a maximum of 1.8 MBq could be administered to be within the 1 mSv limit of a WHO category IIa experiment. If the calculated CEDE is higher than the CED, the amount of radioactivity that could be administered would be reduced as this would be the more conservative estimate. Also, it should be remembered that the category IIa upper limit is twice that of the old category 1, and that exposure of greater than 0.5 mSv would need to be very carefully considered. Above all, remember ALARA!

The term ALARA may take on a whole new meaning when we consider the use of the technique of accelerated mass spectrometry in support of radiolabelled studies in man.

TABLE 11.6 *Weighting factors laid down by the WHO report number 611*

	W_T
Gonads	0.25
Breast	0.15
Red bone marrow	0.12
Bone	0.03
Lung	0.12
Thyroid	0.03
Five other organs	$\frac{0.06 \times 5}{1.00}$

11.4 *Accelerator mass spectrometry*

As indicated in the section above, in the United Kingdom, it is a pre-requisite for human radiolabelled studies to gain approval from the ARSAC. This can be a lengthy process and the conditions placed on the design of the study are very stringent. An exception to gaining this approval can be made by using an extremely low dosage of radioactivity which results in an ionising radiation exposure to the subject of $<1\mu Sv$. To ensure that the expected exposure to radiation will be $<1\mu Sv$, the calculations outlined above will still have to be performed. The analytical tool known as accelerator mass spectrometry (AMS) can make the use of such a low radioactive dose, a viable alternative. AMS is an established technology, which allows the measurement of extremely small quantities of rare and radioactive isotopes, such as radiocarbon (^{14}C), with high precision (Scott *et al.*, 1990; Vogel *et al.*, 1995). AMS is mainly used in the geochemical and archeological areas, such as in radiocarbon dating, but following use of AMS for biomedical applications by the Lawrence Livermore National Laboratory (LLNL), California, USA, the technique is being used more widely in this area of science. A facility known as the Centre for Biomedical Accelerator Mass Spectrometry (CBAMS) Ltd dedicated to the use of AMS for biomedical applications is now operational, near York, England. AMS can detect ^{14}C at concentrations 10^3–10^6 lower than the techniques currently used in drug metabolism studies, such as liquid scintillation counting. AMS differs from conventional radioisotope counting since it is a nuclear detection technique, rather than a decay counting technique.

With increased focus on the safety aspects of using humans in drug trials, AMS offers the advantage of lowering the amount of radiolabelled material administered from typically 2 MBq to circa 4 kBq and thus greatly reducing the exposure to ionising radiation.

Since the levels of exposure to radiation can be lowered to such a degree, it opens the way for carrying out experiments that are more difficult to arrange for ARSAC studies such as administration of both intravenous and oral administration to the same subject, i.e. it is possible to administer radiolabelled drug by two routes in an ARSAC study but the dosage on each administration would be half of the approved dosage.

The current AMS instruments are very large (see photograph of an instrument in Figure 11.1) and can cost in the region of £2million. AMS is currently limited in its application by the complexity of the sample preparation involved. Sample throughput is fairly low, being in the region of 100 samples that can be prepared per technician/day. Advances in automating the process, possibly by radical change, may be required in the future to enhance throughput.

The sensitivity of the instrument is such that ^{14}C contamination from areas where higher levels have been handled is a very real obstacle to obtaining meaningful results. There is therefore a need for controlled access to and use of facilities, to attempt to limit the cross-contamination potential.

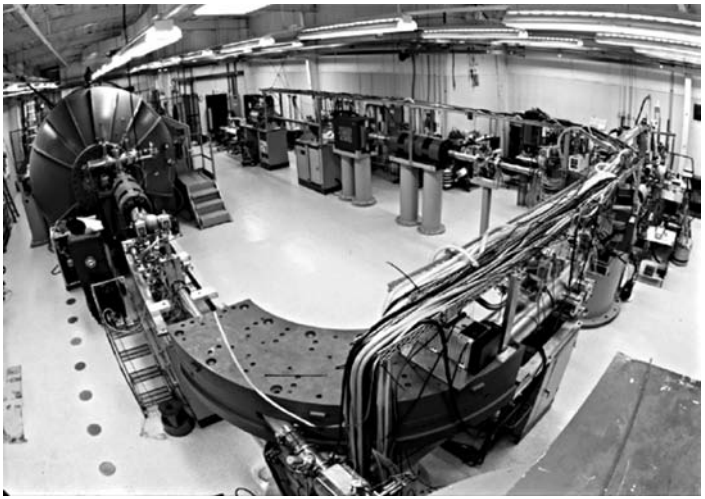

FIGURE 11.1 *Wide angle view of the 10 MV FN tandem accelerator and mass spectrometer located in the Center for Accelerator Mass Spectrometry (CAMS) at the Lawrence Livermore National Laboratory in Livermore, California, USA*. At the NIH NCRR National Resource for Biomedical Accelerator Mass Spectrometry the 10 MV AMS is used for the analysis of biomedical samples.

11.4.1 SAMPLE PREPARATION FOR AMS

The schematic representation in Figure 11.2 shows part of the process of sample preparation which involves sample combustion and reduction to graphite, prior to analysis by AMS.

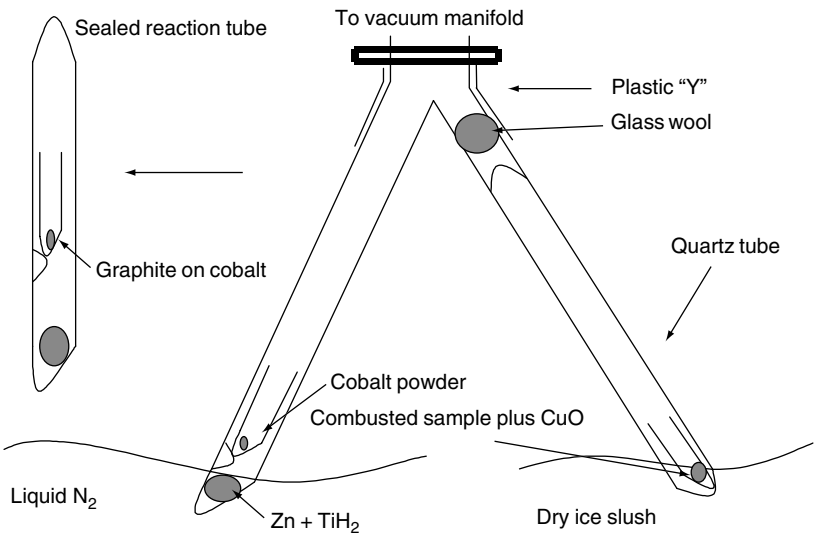

FIGURE 11.2 *Graphite production from biological samples.*

The carbon from the sample, typically 50μL of plasma for example, is isolated as CO_2 by oxidation for 2 hours at 6,500 °C in the presence of copper oxide (CuO). The CO_2 is isolated cryogenically from other oxidation products and reduced to graphite with H_2 and Zn on a cobalt catalyst by heating to 5,500 °C for 4 hours. The carbon/cobalt sample is then transferred into a sample holder which is introduced into the AMS via a sample wheel.

11.4.2 Instrumental details of AMS

Figure 11.3 shows a schematic representation of the nuclear physics involved in the technique of AMS. The graphite sample is bombarded by a caesium–sputter ion source which results in production of negatively charged nuclear and molecular isobars of carbon (e.g. $^{14}C^-$, $^{12}CH_2^-$). The carbon ions are then selected using a mass spectrometer and accelerated along a tandem electrostatic accelerator where charge conversion occurs to produce positively charged ions. The ion beam is then deflected through a second mass spectrometer resulting in further 'purification' of the ion beam and for a few milliseconds every second, the ^{13}C content is determined by means of a Faraday cup. The ion beam is then focussed towards a final ionisation detector

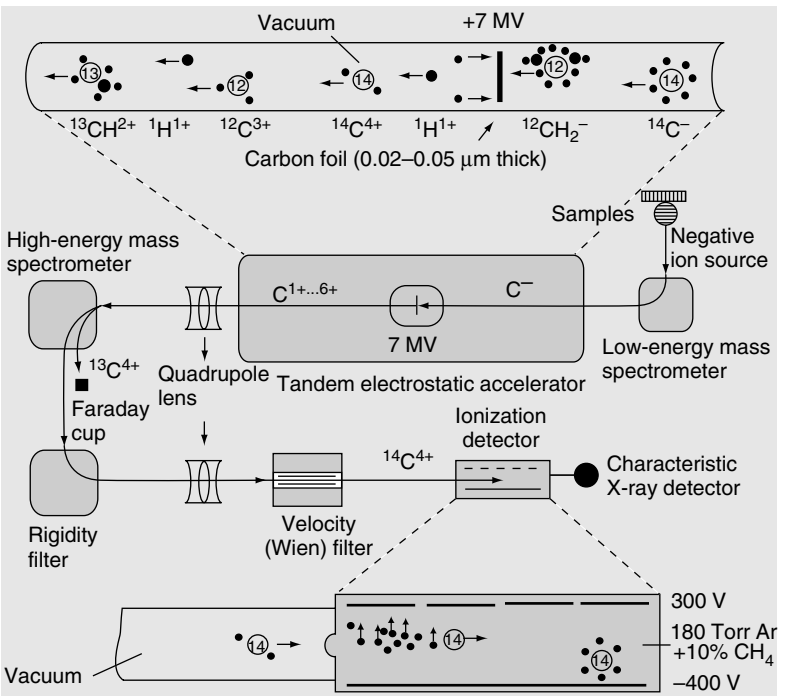

Figure 11.3 *Instrumental operation* (reproduced with permission from John S. Vogel and Lawrence Livermore National Laboratory).

where the ^{14}C content is measured. The ratio of ^{13}C to ^{14}C can be used to determine the amount of ^{14}C in the sample above the background level, which in turn allows determination of analyte concentration, i.e. drug and/or metabolite(s) of interest in the initial sample. In order to ensure that the AMS is operating correctly, an international standard of known ^{13}C:^{14}C is measured with each set of samples. There are a number of such standards in use, including the commonly used Australian National University sucrose standard.

The sensitivity advantage of AMS relative to decay counting is shown by the equation:

$$dN/dt = -(1/\tau) \times N$$

where (dN/dt) = the activity or the rate of decay, τ = the mean half-life of the isotope, and N = the total number of isotope nuclei present. Decay counting techniques such as liquid scintillation counting, indirectly predict N by measuring dN/dt, which is a very small percentage of N for all but the shortest lived isotopes. AMS counts N directly, independent of the mean life, resulting in sensitivity of detection increases relative to scintillation counting of 10^3 for ^3H and 10^6 for ^{14}C.

Elemental analysis of any samples submitted for AMS analysis is required in order to determine the %carbon content of the samples. Quantitation of ^{14}C content of samples relies on knowledge of the full carbon inventory of the samples i.e. the sources of carbon in the samples. If there is insufficient carbon in the samples to allow sufficient graphite to be prepared then addition of a carrier of known ^{14}C content, e.g. tributyrin, is required.

The technique of AMS could not only allow reduction of exposure of human subjects to radioactivity in drug development studies, but also make many experiments possible which are currently impossible. Examples of these are the monitoring of systemic exposures following administration of very small quantities of drug, e.g. when administered by the inhaled, intranasal or topical routes. Microsampling of tissues following administration at conventional doses may also be possible.

11.5 *Future of AMS*

A potential application for AMS may be to include the administration of radiolabelled material in Phase I human studies for all new chemical entities. This would increase greatly the amount of metabolism and pharmacokinetic information made available early in drug development, thereby enhancing the potential of the investigators to make good development decisions on the new chemical entity (NCE).

AMS may also be of use in the future in research experiments with extremely low dosages where there is little need for toxicology safety cover. There will however, always be the additional concerns of possible non-specific adsorption problems and lack of linearity to therapeutic dosages. Advances in sample preparation and/or

introduction will be very important if AMS costs are to be reduced and sample throughput increased. Both of these factors are likely to have great influence on the development and use of the technique in the future.

11.6 *Stable isotope studies*

Stable isotope labelling of the drug under development can be a useful alternative to studies using the radiolabelled isotopes or indeed stable and radioactive isotopes can be used in combination. Commonly carbon-13 or deuterium is used to produce stable labelled drug. The isotope pattern produced is very distinctive when samples from a study are analysed by mass spectrometry and this makes identification of metabolites facile. The disadvantage of using solely stable isotope labelling as opposed to radioactive labelling tends to be one of sensitivity, i.e. since the background levels of stable isotopes tend to be much higher than radioactive isotopes, it is a less sensitive technique to use.

All of the techniques and approaches mentioned in this chapter have both advantages and disadvantages. AMS offers an exciting new approach to the conduct of isotope studies in the future but there may still be a place for conventional radiolabel studies where AMS cannot supply all of the information required.

11.7 *Acknowledgements*

Lawrence Livermore National Laboratory, California.

11.8 *References*

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