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Commentary

A Regulatory and Industrial Perspective of the Use of Carbon-14 and Tritium Isotopes in Human ADME Studies

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INTRODUCTION

A radio-isotopically labeled modification of a new therapeutic agent (new chemical entity; NCE), usually carbon-14 or tritium, is commonly used to determine absorption, distribution, metabolism and excretion (ADME) parameters of the agent. Human ADME studies are often performed early in clinical development (phase I/II). The results of these studies (referred hereafter as ADME studies) provide information on mass balance, routes of excretion, biotransformation, identification of active metabolites, provide data to assist in the extrapolation of animal safety data to man, and provide information for the development of sensitive and specific analytical methodology for the parent drug and pharmacologically active metabolites (1). The consensus statement (2) from a recent workshop (1992) of scientists from academia, industry and FDA specifically mentions the value of radiotracer studies. The benefits (Table I) of an ADME study, from a drug development perspective, are apparent from the wealth of useful information that can be obtained. For chronically administered compounds, a multiple dose ADME study at the estimated common therapeutic dose is sometimes performed.

Regulations/Guidelines in the Conduct of Human ADME Studies

A synopsis of the regulations/guidelines with regard to the use of radiolabeled drugs in man for research purposes is shown in Table II. All human research must be approved by an Institutional Review Board (IRB). The current 21 CFR § 361.1 (3) gives additional administrative requirements such as establishment of a Radioactive Drug Research Committee (RDRC) associated with a medical institution operated for the care of patients or established by a state authority, qualifications of the members of the committee, and details on the submission of annual reports to the FDA.

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21 CFR § 361.1 states that a radiolabeled drug can be used for certain research purposes and provides the necessary criteria to assure safety to human subjects in terms of yearly exposure (dosimetry calculations) and protection from adverse side-effects of the drug to be studied. The most significant statement in this CFR which provides guidance to a sponsor with regard to amount of radioactivity (μCi/subject) that may be administered is as follows: "The amount of radioactive material to be administered shall be such that the subject receives the smallest radiation dose with which it is practical to perform the study without jeopardizing the benefits to be obtained from the study" [21 CFR § 361.1 (b)(3)]. Thus the objectives of the study determine the amount of radioactivity to be used and dosimetry calculations, extrapolated from animal data, assures that the exposure of the subject to radioactivity is in compliance with these regulations. Dosimetry calculations and justifications for the intended amount of radioactivity should be included in human ADME study protocols.

These regulations provide a substantial delegation of authority from the FDA to the local RDRC (4). For a marketed drug, a radiolabeled study can be conducted under most conditions (described in § 361.1) solely with the approval of the local IRB and the local RDRC. In these cases, FDA's involvement is limited to review of annual reports from the RDRC.

If a drug is not marketed in the U.S., then an Investigational New Drug (IND) application must be filed with the FDA for the unlabeled drug substance. However, a study of the radiolabled compound can proceed without prior FDA approval if the local RDRC determines that the drug and protocol are "safe" based upon what has been learned in other studies and "effective" for the purpose of defining mass balance, metabolism, etc. Although prior FDA approval is not required for this specific study, a current IND must be on file at FDA, and the results of any radiolabeled studies must be included in the annual reports required for all INDs.

Although oversight functions have been delegated to RDRCs, FDA staff have shown a continuing interest in the conduct of these studies. This has been apparent by correspondence of the FDA with sponsor companies regarding the design (age of subjects, quantity of radioactivity used, and animal species used for dosimetry calculations) of these studies.

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Table I. Benefits of ADME Studies

Single Dose Study
• Evaluate ADME parameters and mass balance
• Determine single dose pharmacokinetics of drug and metabolites
• Generate projections for steady state
• Provide samples for comparative metabolism
• Identify major metabolites for evaluation of pharmacological activity
• Validate assay specificity and detection limits
• Assist in interpretation of toxicity findings
• Extrapolation of animal safety data to man
Multiple Dose Study
• Validate single dose projections
• Identify accumulating metabolites
• Complete biotransformation investigations

Industrial Perspective of the Use of Radioisotopes in Human ADME Studies

Methods

In order to fully understand pharmaceutical industry needs so that the regulatory concerns can be properly addressed a survey was conducted to gather information on the use of radioisotopes in humans in the drug discovery/development process. The results are evaluated with emphasis on what constitutes an appropriate research study.

A questionnaire entitled "Practical and Ethical Concerns of the Use of Carbon-14 and Tritium Isotopes in Humans During Drug Discovery and Drug Development" was prepared by the authors (J.G.D. and W.T.R.) and sent to 40 pharmaceutical companies within the United States as well as to several major European Pharmaceutical concerns. Thirty completed questionnaires were returned and represent the data base for this presentation. In Table III is a summary of the key questions regarding the use of carbon-14 and tritium isotopes in humans during drug discovery and drug development. For those questions which requested responses in either "yes", "no", or numerical form, the results were tabulated as a percent of responses to that question. For questions that required a written response, the results were categorized and the percent response to each category was tabulated. Interpretations were made in terms of trends with no application of statistical inferences.

RESULTS AND DISCUSSION

Approximately 75% of the respondents used a historical basis for selecting an amount of radioactivity (μCi/subject); a lower percentage (ca. 25%) utilized animal ADME data and/or objective of the study (biotransformation study versus ADE study). Safety (exposure) in all cases was assured through dosimetry calculations and the rat (100%) was the common species used for this purpose; the dog (32%), primate (14%) and mouse (7%) were also used but to a lesser extent (Figure 1).

In general, respondents used one of the following approaches (see equations 1 and 2) in dosimetry calculations (D = rads; for β-emitters 1 rad = 1 rem) (5).

Table II. Regulations/Guidelines in the Design of a Human ADME Study[1]

Purpose of Study: a radiolabeled drug may be used to obtain basic information regarding its metabolism [§ 361.1 (a)].
Radiation Exposure: exposure is justified by the quality of the study and the importance of the information it seeks to obtain [§ 361.1 (b)(1)(iii)].
Dosimetry: calculations are based on an absorbed fraction method [§ 361.1 (b)(3)(iv)].
Maximum Yearly Exposure: exposure from a single dose or from cumulative studies within 1 year must include all sources [§ 361.1 (b)(3)(iii)].
Limits on Radiation Dose: the smallest radiation dose is used with which it is practical to perform the study without jeopardizing the benefits to be obtained from the study [§ 361.1 (b)(3)].
IND Content and Format: for radioactive drugs sufficient data from animal or human studies to allow a reasonable calculation of radiation absorbed dose to whole body and critical organs upon administration to a human subject [§ 312.23 (10)(ii)].
Route of Administration: there is no limit on route of administration; drugs intended for parenteral use must be in a sterile and pyrogen-free form [§ 361.1 (9)(f)(11) and (12)(ii)].
Regimen: There is no limit on the number of repeated doses but the total may not exceed maximum yearly exposure [§ 361.1 (c)(3); item 6 (d) in Report on Research Use of Radioactive Drugs].
Study Populations:
Age: subjects must be 18 years of age and legally competent; subjects under 18 years may receive only 10% of the amount listed in 21 CFR § 361.1 (b)(3)(i) [§ 361.1 (b)(3)(ii) and (d)(5)].
Gender: females of child bearing age are permissible with adequate justification and without significant risk [§ 361.1 (c)(3) and (d)(5)].
Population Size: If more than 30 subjects over the age of 18 a special summary is to be submitted to the FDA [§ 361.1 (c)(3) and (d)(5)].
Magnitude and Range of Pharmacological Dose: the total amount of active ingredients excluding the radionuclide may not exceed the dose limitations applicable to the separate administration of the active ingredients [§ 361.1 (b)(2)].

[1] All citations are from 21 CFR.

$$D = 73.8\overline{E_\beta}C_{max}\,t_{1/2} \quad (1)$$

$$\overline{D} = \Sigma\tilde{A}S \quad (2)$$

In equation 1 (6) 73.8 is a constant and E_β is the average energy of the radioisotope. In this calculation a 1-compartment model is assumed and for each tissue or organ the product of Cmax and $T_{1/2}$ (terminal radioactivity half life) give $AUC_{0-\infty}$ (7). In equation 2 (8), A is the cumulative radioactivity dose (μCi·h) for each tissue or organ and is based on an estimate of the $AUC_{0-\infty}$ (trapezoidal rule). The constant S (rad/μCi·h) is determined for each tissue or organ (9).

The amounts of radioactivity (carbon-14 and tritium) administered in recent human ADME studies approved by an IRB and RDRC are shown in Figure 2. About 50% of the respondents administered ≥100 μCi/subject of carbon-14 and 70% administered ≥200 μCi of tritium as a single dose. Respondents indicated that if lower than currently administered amounts of radioactivity were used, absorption, dis-

Table III. Practical and Ethical Concerns of the Use of Carbon-14 and Tritium Isotopes in Humans During Drug Discovery and Drug Development
Summary of the Key Questions from Questionnaire Sent to U.S. and European Pharmaceutical Companies.

- How are the quantities of radioactivity chosen for an ADME study and what is the relationship between dose level and study objectives?
- Which species are used for dosimetry calculations?
- What dosimetry calculations are used?
- What are the quantities of radioactivity currently employed in human ADME studies? If lower amounts were used, which ADME parameters could/could not be obtained?
- What is the role of alternative methods in the determination of the ADME parameters and mass balance?
- What is the frequency of single dose studies vs multiple dose studies?
- What subject populations are used in human ADME studies?
- When are human ADME studies (single and multiple dose studies) normally conducted? Would it be feasible to postpone the ADME study to a later date and what are the advantages/disadvantages of such a postponement?

position and mass balance could still be assessed (87%). In contrast, the use of lower amounts of radioactivity would seriously impede the determination of metabolite patterns (73%) and metabolite characterization, identification and quantification (87%).

Classical analytical methodologies (cold methods) were used by approximately half of the respondents as an alternative to radioactive methodology to assess absorption and distribution parameters. For the assessment of absorption, this suggests that investigators had sufficient information regarding the presence of parent drug and/or specific metabolites in urine so that quantitative analytical methodologies could be developed. Specific methodologies such as liquid chromatography, gas chromatography, mass spectrometry, and RIA, for example, were developed and applied in these cases. Cold methodologies were also used for the quantitation of parent drug/metabolites in blood (plasma) to support bioavailability studies. The terms absorption (a quantitative measure of all drug related materials that were transferred into the body) and bioavailability (a concentration of drug in blood) are often used interchangeably which may have contributed to the reported high use of specific methodologies

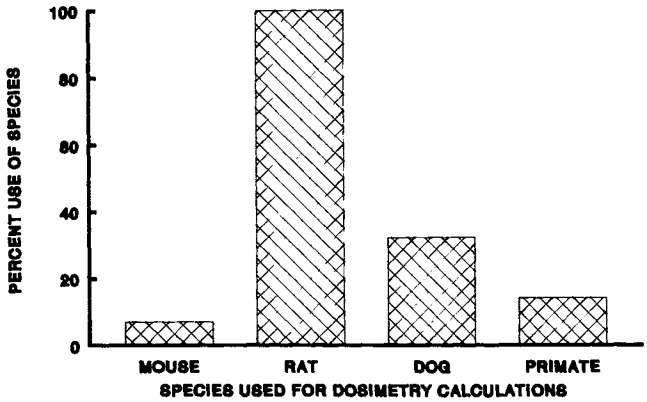

Fig. 1. Percent use of species for dosimetry calculations

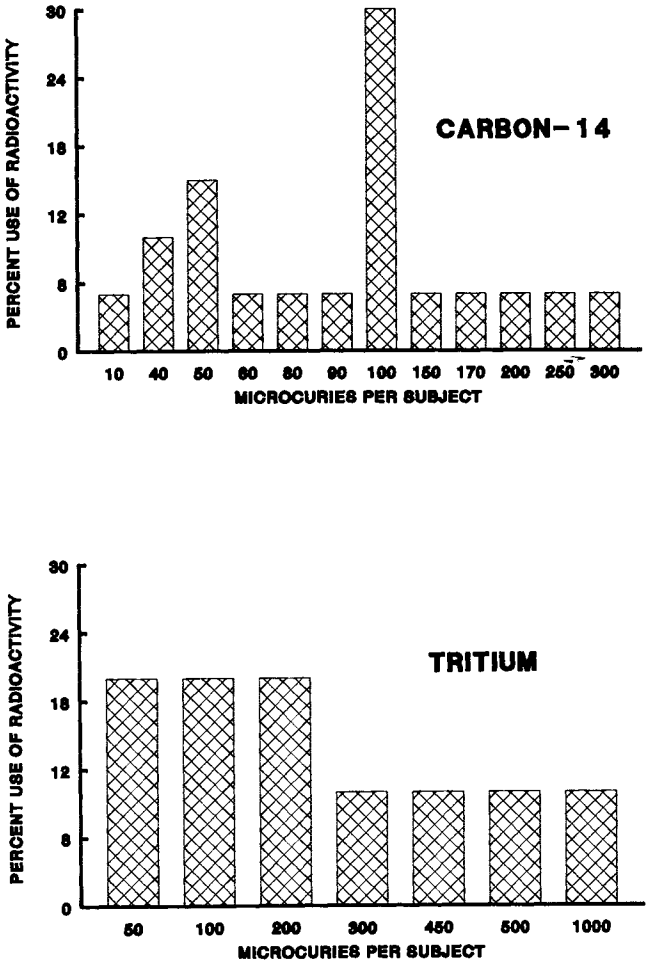

Fig. 2. Current usage, μCi/subject, of carbon-14 and tritium isotopes in human ADME studies.

for the determination of absorption. There was a greater reliance on radioactive methods to assist in the determination of the structures of metabolites (77%) and for the quantitative measurement of excretion (72%) which is expected when dealing with a NCE. The use of radioisotopes was recognized as the best or only method for the determination of mass balance by almost all respondents (96%).

All respondents performed single dose ADME studies while only 10% conducted multiple dose studies. In both types of studies, six subjects (86%) were routinely employed. Most respondents (70%) used normal healthy male volunteers; a smaller percentage (30%) used geriatric/patient populations.

Timing of the human ADME study relative to the rest of the development program for a NCE was considered important since 86% of the respondents indicated that this study must be done in phase I/II. Postponement would delay obtaining both metabolite identification (75%) and quantitative metabolite patterns which are necessary for comparative metabolism (67%) purposes (validating the extrapolation of safety data to man from the toxicity studies). In addition, potential delay in obtaining information regarding the specificity and sensitivity of analytical methodology (46%), the ADE parameters (42%) as well as mass balance (37%) were important concerns.

The survey confirms the importance of ADME studies with radio-isotopically labeled drugs as a research tool in the early phases of drug development and 21 CFR § 361.1 recognizes the need to perform these research studies. Specific statements in this CFR are made regarding safety as well as appropriate dosing to meet the objectives of the study while at the same time adhering to the principle of "as low as reasonably attainable".

The questionnaire indicated that there was a greater use of historical amounts of radioactivity administered per subject rather than an amount needed to meet the study objective. Based on the responses to the questionnaire it is not clear if the spirit of the regulations were being followed with regard to minimizing the amount of radioactivity administered per subject based on extrapolation of animal data to man. If the sponsor can furnish data (projections) that justify the proposed amount of radioactivity in man, it is believed that this will provide a better foundation for the support of the proposed ADME study. However, the assessment by 87% of the respondents that lower amounts of radioactivity would seriously impede their studies, indicates that minimal amounts are being used in actual practice.

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