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SCIENTIFIC COMMENTARY

Addendum 1. Use of Isotopes in Bioavailability Testing[1]

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INTRODUCTION

In the early studies of a new drug candidate, a specific assay for the intact drug may not be available at the time when one may wish to undertake pharmacokinetic studies in animals or in man. In other cases, the drug candidate may show pharmacological activity at relatively small dose levels where it is virtually impossible to establish a specific chemical assay. In these situations, many pharmaceutical scientists synthesize the compound with a radioactive atom at one or more sites on the molecule which do not readily exchange or do not result in loss of radioactivity when the compound undergoes metabolism in the animal species being investigated.

It is fairly common for such initial studies to be undertaken utilizing total radioactivity measurements. Such studies can be valuable in initial screening of a compound; however, one must be aware of the potential errors if one does not attempt to isolate the intact drug from its metabolites. An additional problem exists unless sufficient consideration has been given to the method of incorporation of the radioactive chemical into the dosage form prior to its administration in animal or human studies. Unless the radioactivity is incorporated into the basic crystal, serious complications can result. Discussion of the analytical problem and the bioavailability dosage form considerations is given below.

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[1]In 1972, the Academy of Pharmaceutical Sciences published a report entitled "Guidelines for Biopharmaceutical Testing in Man" which was prepared in response to the need for critical analysis of the current concepts related to this area of product evaluation. Several different aspects related to biopharmaceutical testing were not extensively discussed in this report. From time to time, the editors of this journal will attempt to develop addendum reports on various topics deemed important to biopharmaceutical testing which appear to warrant further comment. The material that follows is the first of these addenda.

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ANALYTICAL CONSIDERATIONS

The simplest analytical procedure to handle blood, urine, or tissue samples containing radioactive molecules is to utilize a total radioactivity assay. Procedures are available to solubilize the biological sample into an appropriate solution for assay with a liquid scintillation spectrometer. More recently, automated apparatus have been developed to combust the samples to carbon dioxide and water, which are separately trapped and assayed in a suitable scintillation solution as noted above.

There is no doubt that there is a degree of value in total radioactivity studies. For example, it offers the opportunity to obtain material balance information, presuming that the radioactivity assay is carried out accurately, i.e., quenching is corrected for with suitable sample processing or through the use of internal standards. Total radioactivity can also lead to information on the time span of exposure of specific organs to the drug and/or its metabolites.

The use of total radioactivity in pharmacokinetic studies to follow the time course of a drug is a common and most serious error. A total radioactivity analysis does *not* allow detection of the concentration of the intact drug in an independent fashion. Rather, it is a reflection of the total number of radioactive molecules of any chemical form in the sample. Unfortunately, each metabolite formed from the drug has its own volume of distribution and its own rate of formation and elimination and therefore concentrates and persists in the body for different periods of time. Total radioactivity is therefore a reflection of the sum of the radioactive molecules present. Even if the data appear to fit some pharmacokinetic model, this is a fortuitous fit and rarely, if ever, can be used with any certainty to define the biological half-life of the intact compound. Occasionally, when elimination of the metabolites are all rate limited by their rates of formation, they will all show the same terminal log linear slope as that seen with the intact compound. Then, the total radioactivity analysis will lead to a good estimate of the biological half-life of the intact compound. However, one will never know whether this is the case unless there is additional analytical evidence to support the presumption.

It is therefore essential to develop a separation procedure which will allow one to assay the intact drug separate from its metabolites before attempting to undertake detailed pharmacokinetic analysis of the data. Fortunately, separation techniques such as various chromatographic procedures can be utilized in the isolation of the intact compound. However, if this is not undertaken, much potential valuable information can be lost. It is, indeed, a waste of the compound, procured at considerable time and expense. Even worse, one can possibly interpret the total radioactivity data

improperly, arriving at fallacious conclusions, which are then utilized in planning additional experiments with the compound.

Studies on the extent of radioactivity recovered in the feces and/or excreted in the urine are frequently taken as measures of bioavailability. *Bioavailability* was defined in the "Guidelines for Biopharmaceutical Testing in Man" to be a term used to indicate the rate and relative amount of the administered drug which reaches the general circulation intact. Therefore, measurement of the percent excreted in the urine *vs.* the total drug administered is not a good measure of bioavailability and is subject to potential misinterpretation. For example, there always remains the distinct possibility that a considerable portion of the drug underwent metabolism during the absorptive phase. If this occurred, the percent recovered in the urine will reflect the total drug absorbed into the blood and will not indicate the percent of the drug available in the form of the active species. Additional complications are that some of the absorbed intact drug may have undergone biliary excretion and subsequent elimination in the feces. Animal studies and occasionally studies undertaken in man offer the opportunity to collect bile samples and to investigate the possibility of enterohepatic cycling of the compound and/or its metabolites. Unless the bile is appropriately analyzed, the possibility of cycling of the drug orally via metabolite back into the body may not be fully appreciated. An additional complication may occur if portions of the intact drug or its metabolites are excreted directly into the stomach or intestinal contents and could affect the urine to feces ratio. Each of these above complications introduces a degree of uncertainty into the interpretation of the percent of the drug excreted in the urine as measured by total radioactivity assay data. At times, a specific assay for the intact drug is used with an assay for total radioactivity in the biological sample. The total radioactivity found in urine or blood samples can be used with the data on the intact drug not only to define the bioavailability but also to estimate the degree of total absorption at the same time.

The availability of radiotagged drug offers one the theoretical opportunity to detect the terminal log linear portion of the concentration time curve. This, of course, presumes that one is able to administer the compound with adequate specific activity to allow adequate detection of the intact drug for a sufficient length of time, even in the presence of the accrual of metabolites. Unfortunately, the above circumstances may not be attainable. Some workers have resorted to the administration of multiple doses to allow accumulation of the drug prior to attempting to determine the terminal log linear kinetics. Unfortunately, the radioactive drug must be administered with each of the doses, since a single radioactive dose cannot distribute homogeneously into the same tissue spaces as occupied by the total dose. The single radioactive dose will not have been metabolized and distributed

into the tissues as seen after multiple dosing. The concentration time course of the metabolite will therefore be different in the two cases.

Some attempts have been made to use nonradioactive isotopes (^{13}C and ^{2}H) to tag the parent compound. A compound so tagged can be readily detected in a mass spectrograph and may allow the development of analytical procedures to follow not only the intact drug but also its metabolites, hopefully at low enough levels to allow these techniques to be used as an alternative to exposure to radioactive compounds. The use of isotopes, of course, leaves one open to the problems of the isotope effects as they may influence binding, metabolism, etc.

BIOAVAILABILITY AND DOSAGE FORM CONSIDERATIONS

When one is utilizing radioactive compounds in bioavailability testing, it is essential that the radioactive molecules be incorporated into the pure chemical prior to fabrication of the dosage form in a manner which is virtually identical in physical and chemical properties to the nonradioactive dosage form. It must be pointed out that one cannot apply the radioactive drug in an organic solvent on the surface of the drug crystal, the granulation, or final dosage form, since this will not suitably incorporate the drug into the individual crystals of the drug. In such systems, bioavailability studies on the radioisotope may not reflect the bioavailability of the cold drug.

Preparation and Testing of Pure Drug Substance

The radioactive chemical should be tested for purity by two-dimensional thin layer chromatography in several solvent systems and, if proper methods are available, by the use of gas chromatography with an appropriate radioactive detector. The radioactive drug should be recrystallized with sufficient nonradioactive drug to assure that it has similar physical properties to nonradioactive crystals or powder. This may require a particle size analysis (such as obtained using a Coulter counter), X-ray diffraction analysis, differential thermal analysis, differential scanning calorimetric analysis, and dissolution rate studies.

Fabrication of Dosage Form

The granulation procedures and other prefabrication processing must be duplicated insofar as possible. It is highly unlikely that the radioactive powder or granule can be processed through the finished dosage form in a manner which exactly duplicates that used with nonradiotagged drugs. However, every effort should be made to assure that the processing is as

similar as possible. If feasible, one should fabricate the final dosage form on similar equipment as used for the nonradiotagged drug, i.e., by the use of special feeds for one punch and isolation of the final tablets. Similar steps should be taken in capsule fabrication.

Tests of the Finished Dosage Form

Disintegration, disaggregation, and dissolution tests on the finished dosage form must be undertaken with sufficient care to verify that the tablets or capsules containing the radioactive drug duplicate insofar as possible the properties of the nonradiotagged dosage form. Initially, one should define the statistical parameters of disintegration, dispersion (disaggregation), and dissolution properties of the nonradioactive dosage form. Sufficient studies should have been made on the dissolution and disaggregation properties of the nonradioactive dosage form to define the effects of stirring, *p*H, surface activity, and exposure of the compound to bile and mucus. The radioactive dosage form should be shown to conform to the same statistical constraints.

While each and every one of the above tests may not be required, one must recognize that radioactivity is a valuable tool in bioavailability analysis only if suitable tests are done to verify that the radioactive drug has been properly incorporated into the drug and its final dosage form to be predictive of the nonradioactive dosage form.

RECOMMENDATION

It is recommended that plans for bioavailability and clinical studies using radiotagged drugs be carefully reviewed by competent scientists so that one is certain that data are available which verify that the physical-chemical properties of the radiotagged crystalline drug and its final dosage forms are statistically indistinguishable from those of the nonradiotagged drug. Further, one must be certain that the analytical procedures to be applied to the biological samples assure that one will be unambiguously analyzing for the intact drug. Until such precautionary steps are placed in the planning procedures, one may continue to generate ambiguous data fraught with potential for misinterpretation. What is particularly discouraging is to find a company or scientific group that does not recognize these implications and presumes that the data are satisfactory for statistical analysis. Statisticians are frequently not suitably trained to perceive the hazards listed above. Their manipulation of the final data may lead to beautifully statistical summaries. Unfortunately, they are often completely wrong, since the intrinsic presumptions needed to apply the statistical tests were not met by the data submitted.