DDR

DRUG DEVELOPMENT RESEARCH 59:208–226 (2003)

Research Overview

Imaging Studies of Biodistribution and Kinetics in Drug Development

Marc S. Berridge,[2,3]* Donald L. Heald,[4] and Zhenghong Lee[1]

[1]*University Hospitals of Cleveland, Cleveland, Ohio*
[2]*Case Western Reserve University, Cleveland, Ohio*
[3]*3D Imaging, Oakwood Village, Ohio*
[4]*Aventis Pharmaceuticals, Inc., Bridgewater, New Jersey*

Strategy, Management and Health Policy				
Venture Capital Enabling Technology	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT Although the intravenous route of administration is rarely used for drugs, it is by far the most common route for PET and SPECT radiotracers. This article discusses the use of planar and tomographic nuclear medicine technologies to image and quantify the distribution of drugs after local administration. In principle, this would include topical dermatologic, otic, ophthalmic, rectal, and vaginal administration, as well as the intramuscular, oral, and inhalation routes, although precedents do not yet exist for all of these. The studies reviewed focus mainly on oral ingestion and oral and nasal inhalation. The use of nondrug tracers for formulations is discussed, principally with planar imaging or SPECT using radionuclides such as ^{99m}Tc, as well as PET imaging where the active ingredient of a formulation can be labeled with ^{11}C or sometimes ^{18}F. An example of the latter type is a study of the deposition and kinetics in the lungs and airways of triamcinolone acetonide, an antiinflammatory steroid used for topical treatment of allergic rhinitis and asthma, dispensed from an inhaler. PET has high potential for evaluation of different formulations and delivery devices in the development of topically applied drugs. Drug Dev. Res. 59:208–226, 2003.

Key words: Imaging; drug development; radionuclide; oral; pulmonary

INTRODUCTION

Biodistribution and kinetics of radiopharmaceuticals is the basis of all PET investigations and is a key to imaging studies in drug development work. That can be seen in the other contributions to this volume. But the importance of biodistribution in most drug development imaging is limited to sufficient delivery of the radiotracer to the organ of interest to allow PET imaging. Kinetic calculations are done using PET and other measured data to solve for physiological parameters of interest. These parameters include metabolic rates, receptor concentrations, and rates of enzyme activity. In each case, the object is not to investigate the labeled molecule but to use the labeled molecule to investigate the biological system. In this way the effects of drugs are measured with sensitivity and objectivity in a relatively common use of imaging technology for drug development work.

It is more rare to see an investigation of the distribution of a drug for the purposes of drug development [Eichler and Muller, 1998]. There are good reasons for this. The biodistribution of a drug is often nearly irrelevant to its function. Consider β-adrenoceptor antagonists. Propranolol was an extremely successful drug by all measures. However, when it

*Correspondence to: Marc Berridge, Ph.D., 3D Imaging, 7650 First Place, Suite A, Building B, Oakwood Village, OH 44146.
E-mail: MSB5@PO.CWRU.EDU

Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/ddr.10220

and other successful β-adrenoceptor antagonists were labeled with ^{11}C for biodistribution studies [Berger et al., 1982, 1983; Prenant et al., 1987] they were found to be distributed in a general fashion throughout the body, with no evidence of specific receptor binding detectable by competition or displacement studies in vivo. With the clarity of hindsight, we see that this happened because only a small fraction of those injected drugs is normally bound to the receptor. There was rapid cycling of molecules on and off receptors from a larger pool of drug in the tissue. So receptor binding increased the amount of tracer localized in tissue by an unnoticed amount. The kinetics of receptor binding were also indistinguishable because the dissociation of drug from the receptor was not slower than the rate of clearance of drug from the tissue. Receptor binding, although substantial, remained invisible to imaging of the labeled drug. But the kinetic properties that made the drugs unusable as radiotracers do not adversely affect pharmacological performance. To some extent they contribute to the drug's good clinical performance. The widespread distribution provides a reservoir to regulate and prolong the drug concentration at the site of action. Rapid turnover at the receptor allows the action of the drug to be pharmacologically modified or interrupted if necessary. So receptor occupancy and clinical efficacy were achieved although the biodistribution did not provide evidence of receptor binding. Biodistribution was not very important for pharmacologic action. This is probably representative of the majority of drugs. For such drugs, the best way to use imaging is to probe their functional effects using radiopharmaceuticals chosen to measure the metabolic, functional, or receptor occupancy changes they cause. This is the topic treated by most of the other contributions to this issue. But sometimes biodistribution is a clinical variable that directly impacts the drug's effect. It is then a very important parameter, and one which can be difficult to measure by conventional means.

Traditional pharmacokinetics rely upon an assumption of a fairly constant relationship between the concentration of drug in different tissues. Plasma concentrations of drug as a function of time are almost universally measured to deduce the amount of drug at inaccessible sites of action. Studies of drug dosage rely on relationships between the amount of administered drug, the drug concentration in plasma, and the drug effect, which are fairly constant between individuals. In the case of an injected or even a typical ingested drug the assumption is quite reasonable because distribution is fairly predictable. Biodistribution data is only necessary when distribution is not consistent between administrations or individuals. This happens if plasma pharmacokinetics does not control bioavailability either because the drug is heavily sequestered from plasma or because specific organ-dependent uptake is important. Slow intestinal absorption might produce the former situation, and a drug which must penetrate across the blood–brain barrier is a good example of the latter. PET imaging is an effective way to measure uptake and organ distribution of radiolabeled drugs. However, local drug administration presents the major need for imaging measurements of biodistribution to contribute to drug development. When absorption of locally administered drug is rate-limiting, the drug concentration in plasma vs. time may not be reproducible between individuals, or even between subsequent administrations to one individual. When a drug is locally administered, the drug profile in plasma will most likely not correlate with its local delivery. In such a case the plasma curve may allow one to address issues of safety, but it will not be relevant to efficacy at the site of local delivery.

Drugs are locally administered for two primary reasons. The first is to maximize the efficacy of the drug by placing it directly at the site of action. Familiar examples are topical dermatologic, otic, and ophthalmic preparations. The second is to minimize the undesirable side effects of systemic administration. Topical application of drug reduces the dose necessary to achieve an effective concentration at the site of action, which directly reduces side effects [Rohatagi et al., 1999]. Local administration may also reduce the fraction of the administered dose that is absorbed into the systemic circulation. Without imaging, measurement of the drug that penetrates and remains in treated tissues would often require biopsy [Leong et al., 1998]. This presents obvious ethical and experimental problems. Some formulations have therefore been designed and tested based on drug concentrations in plasma or in vitro studies in spite of the large uncertainties such data leaves. Other efforts continue to refine mathematic models to predict deposition [Finlay et al., 1996; Finlay and Wong, 1998]. But for local methods of delivery the direct in vivo measurement remains the most reliable method. These include topical dermatologic, otic, and ophthalmic administrations, oral and nasal inhalations, and rectal and vaginal administration. They also include, for the purposes of this discussion, ingested drugs that may have variable release and absorption. This includes tablets with enteric coatings and other sustained release formulations. Not all of these routes of administration are currently represented in the literature of imaging for drug development, but all are areas where application of imaging techniques could be productive.

Perhaps surprisingly, the largest amount of imaging literature for investigation of drug distribution concerns planar gamma scanning of formulations containing ^{99m}Tc. Planar imaging is not generally associated with other drug evaluation studies because its nonquantitative and two-dimensional nature lacks an ability to provide necessary information. However, clinical pulmonary ventilation and perfusion studies laid the groundwork for its use to visualize airway distribution of inhaled materials. The same tracers used in clinical studies, ^{99m}Tc-DTPA [Carter et al., 1995; Terra-Filho et al., 1996], ^{99m}Tc-Teflon [Svartengren et al., 1995], and ^{99m}Tc-HSA [O'Riordan et al., 1995] have found use in studies of inhaled drugs. The methods are quite similar, with the only difference being that care is taken to adsorb the tracer onto the particles in a drug formulation, when applicable. The technique was originally, and most generally, used for qualitative or semiquantitative estimation of drug distribution and comparison of drug formulations. Several reviews of the topic have appeared [Davis et al., 1992; Newman and Pavia, 1985; Newman, 1993] which emphasize the use of planar imaging to show the extent of penetration of a tracer in the airways, primarily in the lung, and include claims of relative and even absolute quantification of the drug deposition in regions of interest. A recent review [Newman and Wilding, 1999] includes SPECT and PET, but largely dismisses them as having low added value and/or high difficulty.

RADIOTRACERS FOR BIODISTRIBUTION MEASUREMENTS

In contrast to functional imaging studies using PET, the only radiotracer of any interest for a drug distribution study is the drug in question. Because of the typical chemical composition of drugs, the only modality which has the general capability to measure biodistribution is PET. Although the use of PET in this area is relatively new, and tracer development is not trivial, several studies have demonstrated its usefulness. Tracers that have been used for biodistribution studies are [^{18}F]fluorodeoxyglucose [Dolovich et al., 1998, 1999; Ojima et al., 1998; Hatazawa et al., 1990], [^{11}C]triamcinolone acetonide [Berridge et al., 1994; 1997, 1998, 1999b, 2000], [^{18}F]1,1,1,2-tetrafluoroethane (HFA134a) [Pike et al., 1995a,b], [^{18}F]fluticasone propionate [Aigbirhio et al., 1997; Berridge et al., 1999b], [^{11}C]zanamivir [Bergstrom et al., 1999], and [^{11}C]nicotine [Bergstrom et al., 1995]. But SPECT and planar gamma imaging have been used for the majority of biodistribution studies found in the literature. Therefore, the majority of studies were done not with a trace of labeled drug of interest but with a small added amount of a different radiotracer. This requires care to avoid violation of the tracer principle, the requirement that a tracer behave identically to the material it traces as it is being measured and that it not perturb that behavior during the experiment by the fact of its addition. Terminology in the literature cited below is confused, especially the literature of ^{99m}Tc labeled radiotracers in drug formulations. For example, solution or suspension formulations of steroids or bronchodilators to which labeled ^{99m}Tc compounds (most commonly DTPA or pertechnetate [Summers et al., 1996], or albumin [Fok et al., 1999]) have been added as a tracer have often been called "labeled drug" although the drug has not been radiolabeled and the formulation does not normally contain the labeled molecule that is used as a tracer. Although it has not been used, nor is it likely to be, for drug development biodistribution studies, an elegant example of what can be done with true technetium labeling of drug molecules was provided in steroid structures that incorporated technetium and rhenium [DiZio et al., 1991; Hom and Katzenellenbogen, 1997; Spradau and Katzenellenbogen, 1998] while retaining estrogenic pharmacological activity. These publications provide a true example of a Tc-labeled estrogenic drug. However, there are no such examples in the drug development literature. In some dose distribution studies the chemical form of the tracer is unspecified, causing difficulty in interpreting results. A better term for the preparations that are used would be "labeled formulation" or more accurately "drug formulated with a tracer" or perhaps, if applicable, "labeled particles." This seems at first to be an innocuous error in terminology, but it readily leads to discussions of the "labeled drug" and of the behavior of the "drug" rather than the "tracer." One is tempted to forget the distinction to the point that absorption or redistribution kinetics of the "drug" have even been considered, something that is clearly inappropriate without careful validation. The observed distribution and kinetics of the tracer can strongly depend on physical chemical properties and processes at the molecular level which may be very different from those of the drug. But usually in such studies the quantity of interest is the initial deposition after inhalation, and for that purpose a tracer that is formulated and tested with due care can be relied on to be valid. Kinetic information must always be interpreted with caution, and its effect on the measurement of initial distribution must be considered. For example, we recently determined that the absorption rate of ^{99m}Tc pertechnetate adsorbed on triamcinolone acetonide drug particles in the human lung was approximately 0.07 min^{-1}, while the absorption rate of the triamcinolone acetonide itself was

0.03 min^{-1} under the same conditions [Berridge et al., 1999a, 2000; Lee et al., 1999a,c]. Such rapid tracer absorption could be a factor even in measurements of initial deposition if a scan of several minutes duration were used. A different tracer could produce different kinetics or even a different apparent initial distribution. The worst situation arises when the absorption rate in different regions of interest are not the same and the relative apparent distribution changes as a function of time. This could be the reason behind the observation that ^{99m} Tc-DTPA had anomalous kinetics, with 6% of the material remaining in a whole chest region after 24 h in spite of an initially rapid absorption rate [Foster et al., 1997]. As long as tracer properties are known and sufficient experimental detail is given, nondrug tracers can provide useful initial deposition data.

Development of a tracer for a dissolved preparation is relatively straightforward. A dissolved tracer will accurately represent a solution until a physical-chemical process, such as binding to proteins or absorption through membranes, occurs. Once the drug or tracer no longer behave as the bulk liquid, the reliability of the method will depend on the individual chemical properties and the process involved. Still, the only important limiting parameter for measurement of initial deposition is the ratio of the initial absorption rate to the time required to make a measurement.

Labeled particles in suspension are of considerable interest and present more of a challenge to tracer design. The particles are generally contained in a propellant, often chlorofluorocarbons (CFC) or hydrofluoroalkanes (HFA), possibly containing cosolvents, and are administered as an aerosol from a metered dose inhaler (MDI), nebulizer, or spray pump device. The physical chemistry involved in labeling particles has been considered [Farr, 1996]. The chemical nature of the drug and the tracer and the action of surfactants in the formulation all affect the tracer affinity for the drug particles, and for the internal surfaces of the canisters. Particle sizes change immediately after actuation of a device as the solvent and propellant evaporate and the surfactant structure rearranges. Failure of the tracer to adhere to the particles generally results in a spray containing tracer that has a different, generally smaller, particle size distribution than the drug. Differences in charge and hydrophilicity of tracer and drug particles can also occur. Because particle size is probably the most important parameter affecting the initial distribution of an inhaled material, this situation would violate the tracer principle. The particle size distribution of tracers for particulate drugs is therefore always measured, usually with a cascade impactor. The size distributions of the tracer and drug must be similar. One criterion that has been recently proposed based on empirical evaluation [Pitcairn and Newman, 1999] is that the F2 statistic for the comparison of the tracer and drug particle size distributions should be greater than 60, or greater than 75 if only the fine particle fraction is considered. Additional experiments are needed to fully characterize the forces that adhere technetium-labeled compounds to formulated drug particles and the criteria that define an acceptable radiotracer.

Labeled solids are also of interest because of the use of oral tablets and capsules. The considerations in tracer design and use are similar to those affecting labeled particles. The validity of the tracer depends on maintaining physical entrainment in the drug formulation being traced. If any quantitative measurement of the tracer is intended, there may be an implicit assumption that the dissolution of the drug and tracer is dependent only on breakup of the tablet, that there is no leaching of either the drug or the tracer from the solid tablet in vivo. The assumption can be validated by appropriate in vitro testing. But normally a tracer is used qualitatively to track movement through the GI tract and correlate pharmacokinetic measurements with tablet position. This gives valid results until the tracer is absorbed to the point that the tablet is no longer detectable. In an interesting example [Davis et al., 1988], ^{99m}Tc-DTPA was incorporated into a rubber adhesive attached to an insoluble osmotic pump capsule. Tracer integrity was validated by recovery of the labeled assembly from feces. A common method for tracing solids is to incorporate a small amount of an appropriate stable nuclide into the formulation and create the tracer by neutron activation of the finished solid formulation. Generally, an oxide of an appropriate isotopically enriched metal is used to achieve reasonable radionuclidic purity. This allows normal manufacturing methods to be used to ensure that the tablet is essentially identical to the commercial dosage form. It also minimizes radioactive waste and contamination of manufacturing equipment. Tracers that have been used are erbium-171 [Parr et al., 1987], and samarium-153 [Ishibashi et al., 1998; Kenyon et al., 1998a; Adkin et al., 1997]. A similar application was a study [Brown et al., 1997] of vaginal retention of a gel and a suppository as supports for slow-release drug delivery. ^{99m}Tc of unspecified chemical form, although most likely pertechnetate, was bound to quaternary ammonium ion exchange resin and mixed into the suppository, while Tc-HSA was added to the gel. The tracer technology was novel and interesting. But since initial distribution was not the experimental question, insufficient data was presented to establish that the tracer retention measured during 6 h was representative of retention of the support matrix. The problem is further

illustrated in a review [Davis et al., 1992] which compared two studies of ^{99m}Tc in suppositories. ^{99m}Tc-hydroxymethyldiphosphonate was used as a model for a water-soluble drug [Jay et al., 1985] showing limited distribution in the rectum. Another study [Williams et al., 1987] used "^{99m}Tc labeled 5-aminosalicylic acid suppositories" and found more extensive distribution. Interpretation of the facts is essentially impossible. Different time-dependent distributions were observed with different formulations of suppository and different radiotracers. Observed differences could reflect chemical or physical differences between the suppositories, or between the radiotracers. "Labeled suppository" and "labeled drug" in these cases were clearly misnomers. No component of the formulation was labeled, the kinetics of the tracers were not validated relative to the formulation, but an attempt to measure absorption kinetics was made. It is important to understand and respect the difference between labeled drugs or ingredients and formulations to which labeled compounds have been added. Terminology that makes the distinction clear should be used. A preparation with an added but foreign radiotracer will give valid results for some applications (enteric tablets traversing the intestines) but will violate the tracer principle for others (absorption kinetics of the same). To define the limits of a radiotracer in drug distribution applications is an important and not always simple task. Conversely, while a labeled drug will always give accurate drug distribution information, the labeling is not necessarily simple.

Labeled compounds that have been used as inhalation tracers are quite varied. [^{99m}Tc]erythromycin lactobionate [Durak et al., 1996] was used for ventilation imaging to take advantage of its slow lung clearance. Technetium-labeled endogenous natural surfactants ([^{99m}Tc]ENS) [Calmanovici et al., 1998, 1999] were produced for clinical aerosol lung imaging. Exogenous surfactants [Fok et al., 1999] were complexed with ^{99m}Tc and formulated with drug particles. Tetraphenylarsonium pertechnetate [Newman et al., 1989b] (uncharacterized, from tetraphenylarsonium chloride and [^{99m}Tc]pertechnetate) was used as a tracer for terbutaline sulfate particles, presumably making use of the relatively hydrophobic ion pair association. Preparations that were differently labeled only by the use of different reducing agents during preparation of the radiotracer produced different distributions of tracer in the lung. This underscores the potential for sensitivity of distribution studies to the chemical properties of a tracer. Hydroxypropylmethylcellulose was labeled with indium-111 [Feely and Davis, 1989] for gastrointestinal studies, but without full evaluation of the labeling chemistry or the potential for label dissociation in vivo. Interferon gamma-1β was covalently labeled with iodine-123 by the iodogen method and well characterized [Virgolini et al., 1997] as a tracer for inhaled therapeutic interferon in a well-controlled dose–response study. Basic research has been done on the behavior of inhaled aerosol particles or droplets for environmental interest or therapeutic delivery. A variety of labeled particles with defined sizes have been specifically produced for the purpose. These include such novel tracers as ^{99m}Tc-iron oxide [Bennett et al., 1999], ^{99m}Tc-polystyrene [Agnew et al., 1984], ^{99m}Tc-albumin microspheres [Olseni et al., 1994], ^{99m}Tc-dilauroylphosphatidylcholine in liposome form [Saari et al., 1998], and more common tracers such as ^{99m}Tc-DTPA aerosol [Smye and Unsworth, 1985; Chan et al., 1999].

DISTRIBUTION OF SOLIDS

Solid dosage forms comprise tablets, capsules, and suppositories. They are subject to a variety of environments in the body that affect the solid carrier and the drugs within it. The pharmacokinetic behavior of a drug is relatively predictable once it has been released from the solid. But the release itself can vary between individuals and according to the composition and timing of meals and other local variables. There are controlled-release and enteric formulations designed to modify the release profile of drug, or to provide for release of the drug at a desired location. Imaging has been used to provide noninvasive measurement of location and integrity of these solid dosage forms and relate these parameters to the pharmacokinetics of the drugs and the design goals of the formulations.

The literature of imaging colonic and vaginal dosage forms is severely limited, with considerable room for development in this area. In one study [Brown et al., 1997] of vaginal suppository and gel formulations, some novel strategies were employed to measure dissolution of the formula. Unfortunately, it was not clear that the tracer and formulation would remain together during the 6-h imaging period. There is considerable scope for use of similar techniques employing labeled drugs or formulation ingredients to measure distribution of locally administered drugs, whether they are administered by suppository or local injection. The technique would be especially useful to determine the factors that are most important for drug absorption or retention. Another similar and potentially effective application to local drug delivery is the development of formulations for radiation synovectomy. SPECT and planar imaging have both been used, although not heavily, in this area to measure drug retention. [Clunie et al., 1995, 1996; Clunie and Ell, 1995; Jia et al., 1996; Wang et al., 1995, 1998]. In most

cases, the (generally heavy metal) radionuclide used for radiation synovectomy also produces suitable gamma emissions for imaging. Isotopes of the same element or chemically similar elements have also made excellent tracers. In this application, low absorption into the surrounding tissues is the goal and high-resolution imaging is well-equipped for noninvasive assessment of dispersal over time.

For studies of oral systemic administration, planar imaging has mainly been used. In the most common study, a radionuclide is added to the orally ingested solid dose. The progress of the solid can then be followed through the GI tract. Plasma pharmacokinetic data is often collected and correlated with the movement and dispersion of the tracer. The resulting data allows the absorption and pharmacokinetic variability to be understood to evaluate enteric coatings and complex delivery systems. The method is similar in concept and execution to clinical radionuclide gastric emptying studies [Read et al., 1986; Harris et al., 1987] and to other studies which are conducted using a nonradioactive radiotelemetric device known as a Heidelberg capsule. [Chan et al., 1990; Groning and Berntgen, 1996; Henderson et al., 1995; Kosoglou et al., 1995; Shelton et al., 1998]. The capsule contains a small sensor and transmitter which transmits pH information and allows determination of position. The capsule is generally separate from the drug tablets, so pharmacokinetic correlations might not be as close as when tracer is incorporated into the administered drug formulation. In an isolated but similar application, ultrasound imaging [Amitai et al., 1992] has been used to individually follow ingested pills to the point of dissolution. This method has one conceptual advantage in that it removes any question of artifactual results due to premature leaching of tracer from a solid carrier. The advantage is minor because the evidence from published pharmacokinetic studies indicates the problem is not often a significant factor.

There are many examples of methods fitting this same general description. Little was learned from studies [Atherton et al., 1994; Graffner et al., 1990; Hardy et al., 1993; Kenyon et al., 1998a] in which the tracer was subject to dissolution in the stomach. In these cases, dissolution and subsequent drug absorption and tracer dissipation was rapid. The only observed effect of changing administration conditions was the expected prolongation of stomach residence time caused by a meal. However, studies of drugs which are enteric coated or otherwise designed for delayed or extended release gave interesting correlations of position and dissolution of the solid with the absorption of drug to show how well the formulation was achieving its design goals. Passage of the undissolved dosage into the small intestine, although the timing was widely variable, generally correlated with the initial appearance of drug in the plasma. Dissolution of the tablet correlated with the shape of the pharmacokinetic curve. Tablets which survived to reach the colon exhibited steady drug absorption throughout their intestinal transit. Several studies demonstrated this same general pattern in intestinal absorption [Marathe et al., 1998; Feely and Davis, 1989; Wilding et al., 1992; Borin et al., 1990]. Although some of the variability indicates there are factors that are independent of the tracer observations, imaging studies have been useful for explaining a large part of the experimental variability in clinical studies and for reducing the number of subjects that must be studied to reach valid conclusions.

The success of oral enteric delayed-release formulas designed to deliver drug to the colon has been proven by similar imaging studies correlating drug release with position [Parr et al., 1987; Hardy et al., 1987; Ishibashi et al., 1998]. Dissolution and release of drug generally occurred about 5 h after gastric emptying, although there is a wide meal-dependent variation in gastric emptying time. Similar studies have been conducted for development of delivery systems regardless of the drug they might eventually carry [Adkin et al., 1997; Davis et al., 1988]. In one interesting study [Sun et al., 1996], the radiotracer was also used quantitatively as an internal standard. The tracer ([^{153}Sm]samarium oxide) was essentially unabsorbed, and so was used to calculate total drug bioavailability from the ratio of drug to tracer in a sample taken from cumulatively collected feces, a simple and reliable calculation compared to the use of plasma concentration data.

INHALED DRUGS

Drugs that are administered by inhalation represent by far the most prolific area of drug biodistribution studies for drug development. Although the drug application is essentially topical, the tissues of the nasal turbinates and lungs are internal and inaccessible for direct measurements of drug distribution or local effect. Further, the immediate local effect, if measurable, is not necessarily proportional to the deposition of drug. Thus, imaging is the only effective method for observation of deposition and absorption in vivo. Many parameters affect deposition of any inhaled substance, including drugs. One important parameter is the size distribution [Clay et al., 1983; Kenyon et al., 1995; Newman, 1998; Svartengren et al., 1995; Edwards et al., 1998] of the particles being inhaled, whether they are drug particles, another solid used as a drug carrier, or liquid droplets of drug solution. For

example, essentially 100% of the drug administered by a nasal pump spray, with fairly large droplet size, is deposited on the nasal turbinates [Berridge et al., 1998], but a nebulizer which produces very fine droplets passes a substantial fraction through the nose and even into the peripheral lungs [Ogoshi and Usui, 1991]. PET studies of pulmonary distribution in normal and diseased subjects [Dolovich et al., 1998, 1999] have shown a quantitative dependence of the depth and pattern of lung deposition as a function of particle size. Particle charge and density are also strong factors in distribution, although this has received much more attention in the environmental science literature [Kenyon et al., 1998b] than in drug distribution. Velocity of airflow during inhalation [Anderson et al., 1999; Bennett et al., 1999; Svartengren et al., 1999a,b] along with the timing of the device activation and the degree of turbulence also can affect the quantity and distribution of drug deposited in target tissues. These are in turn affected by the device, including inhalation spacers, used to deliver the drug [Pauwels et al., 1997; Lipworth and Clark, 1998]. Studies to date have most heavily concentrated on determination of the characteristics of individual products in comparison to others, rather than on systematic study of the parameters that determine distribution. This is partly due to the immediate need in each case to evaluate the proposed drug, device, or formulation. It is also a result of the fact that the outcome is determined by multiple interacting variables so that the design of a good combination of drug and formulation retains a large empirical element.

The starting point for evaluation of a new inhaled formulation is in vitro testing. Although it is not the subject of this article, many references to it are found in the literature. Measurements of particle size distribution, deposition in casts and models of the airways, deposition on filters, and models of deposition based on theoretical treatments of air and particle flow, deposition, and sedimentation are used to predict the in vivo behavior of drug formulations and delivery devices. In vitro data is easy to obtain and the results can be used to explore the effect of new devices and formulations. Still, the extrapolation of these results to clinical use is only a prediction and eventually the most promising candidates must be evaluated in vivo [Fink et al., 1999]. If the first in vivo evaluation is to be a clinical trial, the step can be costly and time-consuming. In one example, a 26-site study with 397 enrolled subjects was used to compare the efficacy of three delivery devices [Fiel et al., 1995]. Imaging, particularly quantitative imaging, can provide a useful bridge between the in vitro data and the clinical trial. A larger number of devices, formulations, etc., can be screened in order to select the ones that are most likely to deliver drug to the pharmacological sites of action and increase the likelihood of a clinical success. Formulations or devices that do not deliver drug effectively to target regions could be removed more quickly from costly development programs. Perhaps a more important function of imaging, but one which has not yet been realized, is to provide the link between aspects of drug deposition and clinical efficacy. Quantitative imaging allows a dose–response relationship to be based on regional deposition, as opposed to the dose emitted by a device subject to variable efficiency in delivery to individual subjects. It should be possible to measure the minimum necessary drug deposition for clinical effect, identify the regions in which deposition is clinically important (or not important), determine the duration of action after wash-out, and the level at which maximal effect is achieved. Such information would allow the rational design of formulations and delivery devices to achieve success in the clinic. As a component of clinical trials, quantitative imaging can measure individual variation and allow it to be taken into account in data analysis to lead to a better understanding of results with fewer experimental subjects. Correlation of the clinical response with the quantity of drug actually applied to target regions of action is the most powerful future role for imaging in the design of inhaled drug products. The combination of distribution imaging, functional imaging, and clinical endpoints has the potential to provide rapid answers to the most fundamental drug design questions.

PET IMAGING OF INHALED DRUGS

The main strength of PET has been in quantitative functional imaging studies to measure physiological parameters, with a primary focus on brain, heart, and tumors. In the authors' experience functional imaging for drug evaluation [Bednarczyk et al., 1991, 1992] naturally led to use of PET for biodistribution studies as well. PET for biodistribution of inhaled drugs was preceded by its use for quantitative measurement of pulmonary ventilation, perfusion, vascular permeability, and inflammation using injected diffusible tracers, metabolic substrates, microspheres, and inhaled gases [Murata et al., 1986; Senda et al., 1986; Schuster, 1998; Treppo et al., 1997; Mijailovich et al., 1997; Rhodes and Hughes, 1995; Rhodes et al., 1989; Hughes, 1985; Selwyn et al., 1986; Taylor et al., 1996; Schuster et al., 1998]. These techniques serve as the basis for quantitative drug distribution studies. From the standpoint of pulmonary quantification, studies of perfusion and metabolic activity associated with the inflammatory response are considerably more demanding of the scan technique. Such methods generally

require a series of quantitative images and a quantitative input function (arterial blood curve) which are used to calculate the parameters of interest by solving mathematical modeling equations of the tracer kinetics. Drug biodistribution studies go no farther than the quantitative images, which constitute the desired information. Conversely, biodistribution studies are concerned with the entire organ and/or every region within it, and so have more stringent demands for precision of irregular regions of interest (Fig. 1), image analysis in three dimensions, and large-region scanning than the more typical functional studies.

FDG (2-deoxy-[^{18}F]fluoro-D-glucose) has been formulated as a powder and used for inhalation studies [Ojima et al., 1989], primarily with a clinical perspective [Ojima et al., 1998]. There have also been preliminary reports [Ojima et al., 1998; Dolovich et al., 1998, 1999] of its use as a tracer for particulate drugs, in much the same way as ^{99m}Tc-labeled compounds are used in planar imaging. That technique, which could employ any available positron-emitting tracer, has two key advantages. New labeling chemistry is not required and the use of PET provides true quantitative 3D initial distribution information. The same tracer considerations that are discussed in the context of planar imaging apply to this kind of PET study. The approach has not yet been widely used but it deserves more attention when the drug development problem is appropriate for its use.

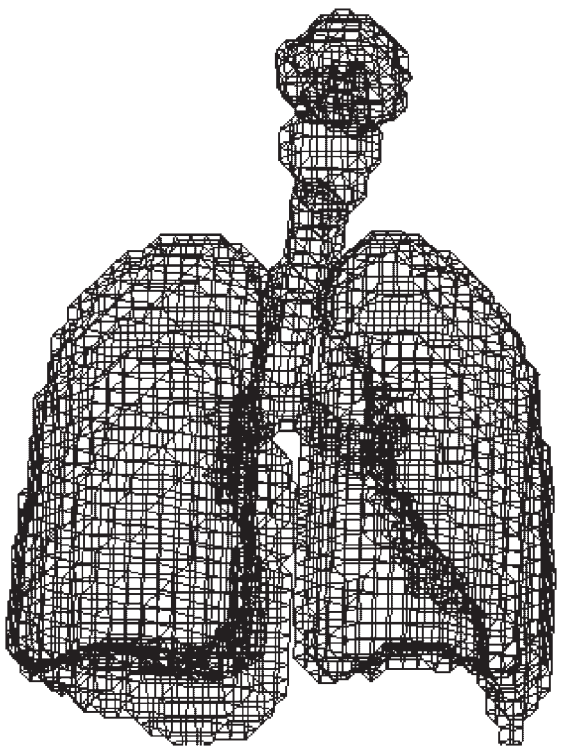

Fig. 1. A wire-frame representation of combined regions of interest (oral, airway, lung) as commonly applied to PET and SPECT studies. Regional contours can be seen as they conform to the shape of the structures.

PET studies applied to inhaled drugs have now been published by several groups. Distribution of nicotine was studied using [^{11}C]nicotine [Bergstrom et al., 1995]. Deposition was quantified in the oral cavity, lung, esophagus, and airway, and kinetics of deposition and elimination were measured to evaluate absorption from the different regions. Elimination/absorption of nicotine had a significant effect, which had to be taken into account in estimating the initial deposition even with short imaging frames. The same group also investigated [^{11}C]zanamivir [Bergstrom et al., 1999] to determine the deposition pattern and kinetics of the antiviral compound after nasal administration. Regions in the nasal passages and lungs were evaluated. The biodistribution of labeled 1,1,1,2-tetrafluoroethane (HFA134A) has also been studied [Pike et al., 1995a,b], which is being widely adopted as a substitute for banned chlorofluorocarbon propellants for aerosol canisters. The low absorption and subsequent rapid clearance from the body was found to justify the assumption that the compound presents a very low risk to users of aerosol products. Some of their imaging work, although done with a positron-emitting nuclide so that the compound of interest could be labeled, was done with an NaI-based whole-body imaging system. Although PET cameras also routinely perform whole-body imaging, the larger-field special purpose device allowed finer kinetic sampling for the rapid clearance kinetics. They have also prepared the antiinflammatory corticosteroid [^{18}F]fluticasone propionate [Aigbirhio et al., 1997] for use in inhalation studies. There have been several preliminary reports of the results of biodistribution experiments in the human lung, but at this writing the biodistribution work has not been published in abstract form. In similar work from our own group, triamcinolone acetonide, another antiinflammatory corticosteroid, was labeled with carbon-11 [Berridge et al., 1994] and formulated for investigations of nasally inhaled allergic rhinitis treatment products Nasacort® [Berridge et al., 1997] and NasacortAQ® [Berridge et al., 1998]. Deposition in the nasal cavity, turbinates, and sinuses was quantified and absorption rate of the drug in each region was measured, giving evidence to explain the clinically observed duration of action of the drug. Rapid imaging, using frames as short as 6 sec, allowed capture of the delivery of the nasal spray and subsequent clearance, and an accurate estimation of initial regional drug delivery with minimal concern for mucociliary clearance. Further work with the same compound in

different formulations included investigations into the antiasthmatic formulation Azmacort® [Berridge et al., 1999a, 2000; Berridge and Heald, 1999] to determine the effects on regional distribution and kinetics of such factors as the inhalation spacer device and particle size distribution. In particular, the spacer was shown to have a strong, favorable, and highly significant effect on the deposition pattern of the drug, something which had not previously been determined. Agreement was noted between regional deposition and absorption kinetics, in units of micrograms of active ingredient, and independent pharmacokinetic data [Argenti et al., 1999]. Additional nasal inhalation studies with the same drug and with [^{18}F]fluticasone propionate were reported in preliminary form [Berridge et al., 1999b].

SPECT IMAGING OF INHALED DRUGS

SPECT, like PET, is underused relative to planar imaging for inhaled drugs. This is a curious situation, since SPECT uses the same tracers in the same way, and is now more commonly used than planar imaging for clinical purposes. SPECT shares the tracer-related difficulties of planar imaging, but it has several advantages. It gives a true 3D image of the distribution of tracer (Fig. 2), which allows accurate differentiation between regions of interest. Although attenuation and scatter correction remain difficult, making quantification less than fully reliable, it does offer significant improvements over planar imaging in this regard [King et al., 1995, 1996]. A potential problem with SPECT is that it has generally required more time than planar imaging to acquire a scan. For initial deposition studies, which are all that can be done with nondrug tracers, this is not a serious problem. Research subjects usually have no difficulty remaining in the scanner long enough to acquire an image. However, if the tracer is subject to absorption or mucociliary clearance on a time scale of tens of minutes, the resulting image will be skewed by the differences between kinetics in individual regions. Tracers such as pertechnetate and technetium DTPA therefore present difficulties for long imaging sequences. The problem can be significantly reduced by using labeled compounds such as albumin or solid particles [Svartengren et al., 1995], or anything else that is not rapidly absorbed, leaving only mucociliary clearance as an omnipresent concern. In addition, faster SPECT imaging techniques [Chan et al., 1999a,b; Eberl et al., 1999] are being developed that will allow SPECT acquisitions to be done in a time similar to traditional planar studies. Similar to the other modalities, clinical studies [Smye and Unsworth, 1985] led the way for drug development work by demonstrating the advantages of the technique. Another clinical study of note [Watanabe et al., 1995] showed that a fine aerosol, although similar to gaseous ventilation images by planar imaging, had a heterogenous distribution in the lung when viewed by SPECT, compared to images obtained using the radioactive noble gas ^{81m}Kr (half-life = 13 sec). The finding demonstrated the spatial resolution offered by SPECT and its usefulness for assessment of aerosol deposition, even if the motivation in this case was clinical. In another study, the effect of aerosol particle size was measured with SPECT and the result compared with measurements by planar imaging [Summers et al., 1996]. The study showed that deposition of particles having a mass median aerodynamic diameter (MMAD) of 16 and 24 microns were not significantly different, but also showed that planar imaging significantly overestimated peripheral deposition. Small particle sizes were used to investigate airway function and new aerosol delivery devices [King et al., 1997; Chan et al., 1999] and probe the uniformity of deposition in the lungs, again demonstrating the advantages of 3D imaging. An interesting issue that has arisen from the limited number of SPECT studies of aerosols is that of standardization of analysis and expression of deposition patterns. In planar imaging, a penetration index (PI) has been defined as a ratio of deposition in the peripheral and central lung (or, more accurately, chest) regions (Fig. 3). The value of PI in planar imaging is sensitive to the shape of the regions drawn and necessarily includes the entire thickness of the chest in each region. In 3D imaging one can take advantage of the improved distribution data to exclude overlying peripheral lung regions from the central regions [Phipps et al., 1989], but only by radically changing the definition of the index and the values that

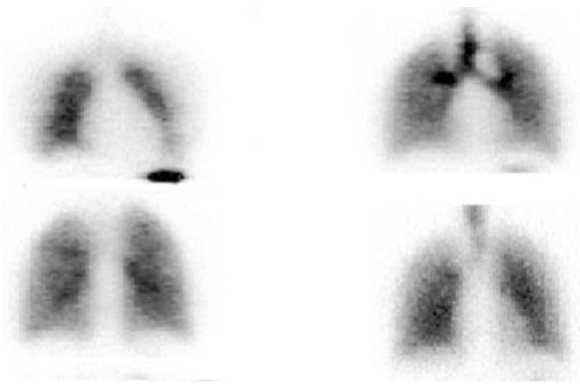

Fig. 2. A pulmonary aerosol SPECT study. The lower right image is a front projection of the whole dataset. The other three images show individual coronal planes (clockwise: posterior, anterior, and in the plane of the central airway). This is not the same drug as shown in the other figures. Courtesy of Dr. Stefan Eberl, Royal Prince Alfred Hospital and Dept. of Pharmacy, University of Sydney, Sydney, Australia.

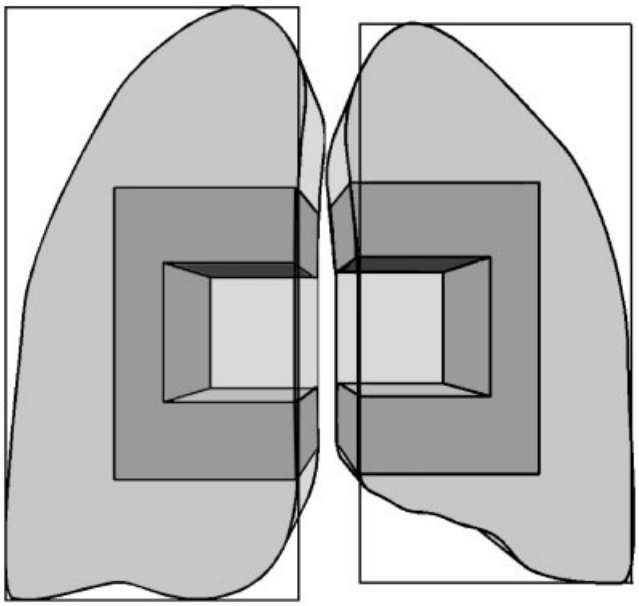

Fig. 3. Regions of interest commonly applied for analysis of planar images. The inner box, light color, represents the inner region, the dark box is the mid-lung region, and the outer box which extends beyond the body represents the peripheral region. Counts are typically averaged within the regions and compared.

are obtained. The question is not yet settled, and there is clearly a need for some standardized analysis method to allow comparison of work by different investigators.

PLANAR IMAGING OF INHALED DRUGS

There is a large body of literature concerning planar scans of inhaled materials, such that it is not practical to provide an exhaustive review. This discussion includes representative examples of the studies and issues in the area.

From the point of view of this review, with a concentration on methods and general results, many of the planar studies in the literature are difficult to evaluate. Details of the acquisition and data analysis methods are commonly sparse, and in some work the tracer that was used is not specified. Emphasis is naturally given to the distribution result as it pertains to a new drug, new device, etc. Crossover study designs [Olseni et al., 1994] permit complete comparisons in each volunteer and provide reliable qualitative evaluations. Other study designs require knowledge of the details of the tracer kinetics and the acquisition parameters and image correction methods to allow evaluation of the reliability of the results. Many of the techniques necessary for good execution of an inhaled drug study have been worked out using planar methods [Newman, 1995]. The importance of reproducible inhalation technique and inspiratory flow rate [Borgstrom et al., 1994], for example, has been well established in this work. The establishment of gross deposition features of new devices [Carter et al., 1998; Pitcairn et al., 1997; Newman et al., 1995a,b, 1996] in vivo has been a very productive area for planar imaging. Some devices are designed for upper respiratory deposition while others strive to place the dose deep in the lungs. The qualitative determination of the initial distribution pattern is easily made by planar imaging [Newman et al., 1998] even when quantitative results may not be reliable. In one study, deposition of an antiviral compound studied by planar imaging [Cass et al., 1999] was then investigated with a different formulation using PET [Bergstrom et al., 1999]. A comparison of the two methods with a single formulation [Lee et al., 1999c] showed qualitative as well as quantitative differences, but upheld the validity of properly controlled planar imaging qualitative comparisons. In exceptional planar studies, erythromycin lactobionate was labeled with technetium for studies of its clearance from the lung [Durak et al., 1996], demonstrating a very slow absorption or clearance rate, and superoxide dismutase (SOD) was labeled with Tc for investigation of a new nebulizer method in small pigs [Langenback et al., 1999]. In the latter study, it was determined that 46% of the dose penetrated to the lung. Even with a small animal and treatment of the entire lung, the implied degree of precision is probably not justified. However, the main conclusions of the study relevant to the evaluation of the nebulizer did not require a precise quantitative evaluation and made an important contribution to the development of SOD for clinical use. The effect of clinical parameters on deposition of aerosol drugs has also been studied [O'Riordan et al., 1995] and compared with ventilation patterns, with the conclusion that features of pathology which go beyond ventilation effects can alter particulate deposition. Comparative studies of devices and formulations have clearly demonstrated their effect on the deposition of particles in the lung [Vidgren et al., 1990; Newman et al., 1989a; Newman and Newhouse, 1996] although again the achievement of 1% precision, as often reported, is unlikely. Other studies have performed measurements of distribution and clearance of entities of interest using ^{99m}Tc labels. Liposomes were labeled with ^{99m}Tc [Saari et al., 1998] by chelation with phosphate groups attached to the lipids. The liposomes were verified to remain stable during the administration process, but the problem of clearance measurement is a difficult one. It was not possible to verify that clearance of radioactivity in this case was representative of liposomes, dissociated lipid, some other labeled species formed in vivo, or a combination of these.

Similarly, measurement of clearance of cromoglycate particles with [^{99m}Tc]pertechnetate incorporated by spray-drying [Vidgren et al., 1991] would require careful validation before multiexponential clearance could be assumed to represent absorption and clearance of the drug rather than just that of the tracer compound.

CHOICE OF IMAGING MODALITY FOR BIODISTRIBUTION STUDIES

Planar imaging (Fig. 4) has by far the most literature in the drug distribution field. But as much as planar imaging has been used for drug distribution studies, its usefulness is limited. As demonstrated above, there are few instances in which a qualitative or strictly relative result is all that is desired. Since the error in quantitative distribution measurement with planar imaging is a function of the distribution itself, even relative results are biased. This is further discussed below. Improvement of relative planar quantification is possible if correction methods for scatter and attenuation are applied, but direct comparison with quantitative PET imaging has shown that planar quantification is, at best, relative [Lee et al., 1999a–c, 2000b]. Planar imaging has only one real advantage to consider when choosing a study method: it is simple. A ^{99m}Tc-technetium compound can be added to any formulation. With moderate persistence a distribution of the tracer can be achieved that, for at least a short time, reflects distribution of the drug. Planar imaging devices are relatively inexpensive to acquire. But the simplicity advantage has greatly diminished in the last decade.

SPECT cameras now outnumber planar devices in many nuclear medicine departments, and the SPECT scanning-time disadvantage can be decreased. Since no change is needed in the radiochemistry, a SPECT study can be done essentially as easily or even more easily than a planar study, since it gives data that can be better corrected for attenuation and scatter and provides a valuable improvement in the data obtained, both qualitatively and quantitatively. Aside from providing 3D data with improved quantification, the issues surrounding a SPECT and a planar study are the same because they use the same radiotracers. To perform a SPECT study of this type is therefore essentially identical to performing a planar study. The advantage of SPECT is that the data obtained allows observations of 3D regions of interest to better distinguish deposition in various central and peripheral regions of the lung and airways, in defined regions in the nasal turbinates, and in the sinuses. Some of these regions of interest are difficult to locate on planar images and impossible to separate from overlying regions.

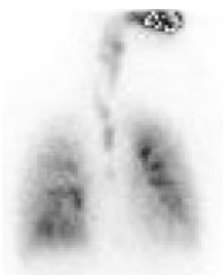

Fig. 4. Geometric mean of anterior and posterior planar images of [^{99m}Tc]pertechnetate formulated in Azmacort®. This is the same individual and drug formulation shown in Figure 5. Note the apparent difference in extent and pattern of lung distribution.

Similarly, PET is a very viable candidate now that it has become widely available. The drawback of PET is that it can be complicated and demanding if it is done as well as it can be done. But there are several strong advantages. Labeling of the active drug ingredient is a very desirable goal [Newman and Pavia, 1985] which can generally be achieved only with PET. It ensures that the correct drug distribution will be obtained and allows the drug kinetics or redistribution to be observed. Kinetics cannot be observed with a nondrug tracer without extensive case-by-case validation that would obviate the imaging measurement. The 3D nature of PET, like SPECT, allows the drug measurement to be made in 3D regions of interest of any size and shape within the body. Corrections for quantification are based on the simultaneous detection of the two photons that result from positron decay and are built into the PET technique. These corrections are done routinely by all PET cameras and their software and provide the only generally reliable quantification in nuclear imaging. During the past 30 years, clinical studies, other drug investigations, and other research with PET has demonstrated its quantitative reliability.

Because of the historical dominance of planar imaging the question of choice of imaging method is generally framed in terms of comparison to that technique. The strengths and weaknesses of each method are noted above. SPECT scanning is the same as planar in most respects, since any proposed study can adopt the same tracer, protocol, scan strategy, and analytical methods for either technique. The major difference, of course, is that SPECT provides 3D data, and attenuation and scatter corrections are easier and more accurate [King et al., 1995, 1996]. There is therefore a strong constant factor in favor of SPECT. The cost of SPECT is not substantially different from planar costs now, and availability in many locations is better.

In making a comparison with PET, it is important to compare like quantities. Planar imaging is generally done with minimal validation of a tracer aside from particle size measurements, with arbitrary region of interest definition, and with minimal or no correction for attenuation or scatter in the images. The majority of PET studies to date have been done using radiotracers that were the result of custom labeling efforts and with separate anatomic imaging for region definitions. A PET study could be done rapidly and cheaply in any PET center by adopting the strategies of planar imaging. Use of (nondrug) tracers in PET, as is done when planar imaging is used, provides a choice among hundreds of existing compounds with widely varied properties and would eliminate tracer development. Minimal data acquisition and analysis to measure only initial deposition and not kinetics would reduce consumption of camera time and speed data analysis. Elimination of CT or MRI scans would cause the use of somewhat more arbitrary 3D regions of interest for scan analysis and would further reduce the study cost and data analysis effort. But the major advantage of PET is the ability to label and observe the active ingredient and to acquire kinetic data. The major cost of a study is in processing volunteers and doing the scans and basic data analysis. The additional costs of doing a study well are not a major factor in the long run. Over the brief history of PET for inhaled drug evaluation, the monetary cost of full PET imaging studies has been amazingly comparable to that of planar imaging. The cost in time to complete the study is another factor that is very important in drug development work, and is higher for a full PET study than for any other, primarily because of new tracer development and opportunities for more detailed data analysis. There is no added time cost, compared to planar imaging, when existing tracers and simple initial distribution analyses are used. Time pressure will most likely be the major motivation for PET studies that use nondrug tracers in the future.

Conversely, to do planar studies well is not trivial. Attenuation and scatter corrections are obviously very important to quantitative imaging. They are also important in qualitative imaging because of the large effect they have on perceived tracer distribution within the body (note that Figs. 4 and 5 are images of a single drug formulation in a single person), but they are rarely done using sufficiently rigorous methods for the type of data being analyzed [Buvat et al., 1995, 1998; el-Fakhri et al., 1999; Perring et al., 1994; Lee et al., 1999a–c, 2000b]. The use of the simplest effective corrections in a planar study introduces a degree of complexity that rivals that of a full PET study. Accurate definition of anatomic regions poses a similar obstacle that also increases study complexity.

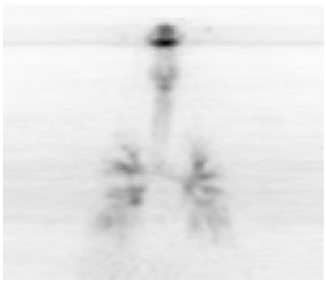

Fig. 5. Front projection of a PET volume set from [C-11]triamcinolone acetonide administered as Azmacort®. This is the same individual and drug formulation shown in Figure 4, taken within 1 week of each other. Note the apparent difference in extent and pattern of lung distribution.

Bergstrom et al. [1999] concluded that PET was quantitative and more spatially accurate than alternative techniques and that the measurement of the distribution and kinetics of the labeled drug was a significant advantage. The work done so far with triamcinolone acetonide and fluticasone propionate [Berridge and Heald, 1999; Berridge et al., 1997, 1998; 1999a,b, 2000; Lee et al., 2000a] has demonstrated the value of reliable quantification of regional deposition and of absorption/redistribution rate measurements. Three dimensional image displays (Fig. 6) allow kinetic and distribution data to be shown in three dimensions and, by using cine-loop displays, as a function of time. Rotating image cine-loops provide a strong sense of the actual 3D distribution of data. This has been very useful for rapidly understanding the data and to compare formulations. It has also been very useful for demonstrating study results to audiences that are not fully familiar with traditional display schemes for PET and SPECT data. The effects of delivery, formulation, and device parameters are now being quantitatively measured and statistical significance of results is being achieved using very few experimental subjects. The results of such studies are providing guides for the improvement of drug products that are already on the market and promise to do the same for products under development.

QUANTIFICATION ISSUES

Accurate quantification of any radiotracer in a body region first requires adequate spatial definition of the location of the detected radioactivity within that region. Then, compensation must be made for the efficiency of the detection device and for spatial variations in detection efficiency caused by losses of radiation due to absorption and Compton scattering in

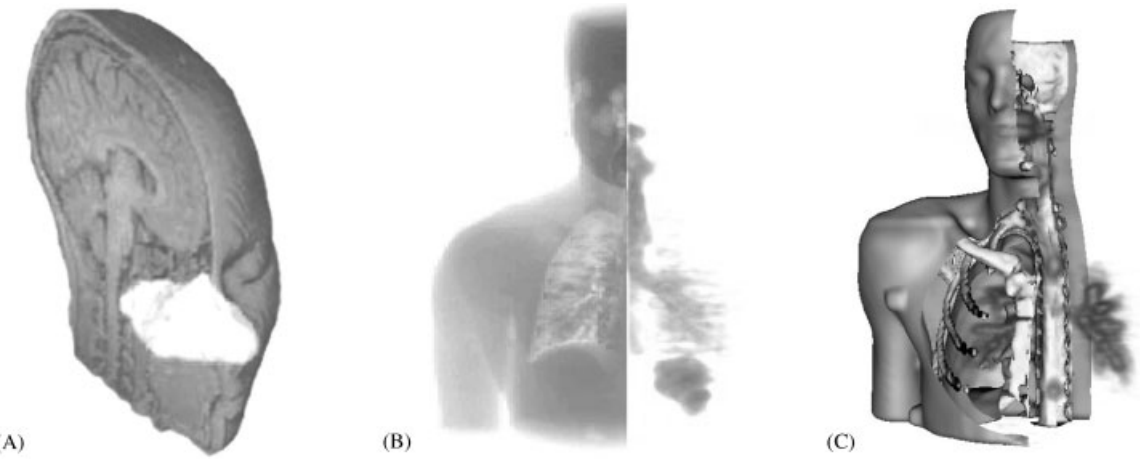

Fig. 6. Image displays of PET data with anatomic overlay. **A:** Nasal inhalation of TAA, surface rendering with MRI overlay. **B:** Oral inhalation of TAA, 3D projection with CT overlay of low resolution, contrast, and opacity. **C:** Oral inhalation of TAA, 3D projection with tissue-selective high resolution, contrast, and opacity CT overlay.

intervening tissues [el-Fakhri et al., 1999; Buvat et al., 1998, 1995; Miller et al., 1996; Pitcairn and Newman, 1997]. Quantification by PET has been extensively validated [Rhodes and Hughes, 1995; Korf, 1997; Leskinen, 1994; Czernin and Schelbert, 1994; Kuwert et al., 1992]. PET enjoys the ability to do analytical attenuation correction because dual-photon detection results in a constant attenuation between any pair of detectors. The main limitation to quantification accuracy in PET is due to camera spatial resolution and generally comes into play when regions of interest have volumes of a few milliliters or less [Herzog et al., 1991]. Scatter correction, although less than perfect, is made easier for PET by the high energy of the photons, the narrow energy window that can be used for detection, and the statistics of dual-photon detection. For SPECT and especially for planar imaging, none of the requirements for quantification are easy to satisfy in a general sense. Lateral spatial definition from a planar image is straightforward, but depth information is lost, and with it the information necessary to quantify the radioactivity by attenuation correction. Side views, and ultimately SPECT imaging, provide improvements. If deposition is noted on defined anatomic structures, such as the bronchi, then the depth of that deposition can be accurately inferred from an independent determination of anatomy and appropriate corrections can be done. The spatial distribution of the commonly diffuse-appearing deposition within a lung is impossible to determine from a planar image alone, and assumptions have to be made if quantification is to be attempted. Usually it is assumed that the distribution is uniform over the thickness of the body (or lung) and that attenuation is spatially uniform throughout the field of view. Neither assumption is generally valid for inhaled drugs in the human lung. To the extent that the assumptions are violated, planar quantification is in error.

Quantitatively, many studies have shown strong variability in planar imaging of doses administered by inhaler. A survey of particle size vs. planar imaging determinations of quantitative lung penetration [Newman, 1998] showed substantial scatter and anomalously high lung penetration of larger particles. Although a strong correlation of particle size with distribution is evident from the data, it is probable that the true correlation is better than it appeared to be, due to the artifactual character in much of the data. Another study that compared planar imaging quantification with independent measurements [Borgstrom et al., 1992] found that planar imaging overestimated the lung deposition fraction of a powder inhaler by 27% of the true value. Similarly, metered dose inhaler deposition [Newman et al., 1995a,b] was found to overestimate lung deposition of drug as compared to pharmacokinetic methods. All of these results are consistent with the variable quantitative errors of planar imaging and underestimation of central deposition and overestimation of peripheral lung deposition, especially when attenuation and scatter are not fully corrected (Figs. 4, 5) [Lee et al., 1999a–c, 2000b]. Other studies of planar imaging that show results consistent with independent measurements [Fok et al., 1999; Itoh et al., 1985] allow us to complete the understanding of the quantification problem. In these studies the particles inhaled were very small and the region of interest for deposition was the whole lung. The assumption of uniform distribution is not strongly violated by a small-particle aerosol that

distributes deeply into the smaller generations of the airways and large regions of interest help to cancel out some of the remaining error. In some scans, small animals or children were the subjects. This minimizes attenuation and scatter, along with the errors they cause. The quantitative success of planar imaging in these instances points clearly to the importance of accurate attenuation and scatter corrections since these studies were optimized for their correction by simple methods. Correction for attenuation is also done by using ventilation or perfusion tracers, which are uniformly distributed, as a reference standard, which also implicitly assumes a uniform tracer distribution. The approach is perfectly valid when the experimental tracer distributes like the reference tracer, but is invalid for a nonuniform tracer distribution that differs from that of the calibration scan. The distributions that are most poorly estimated are those with heavy central or other heavily nonuniform distributions. But since planar imaging strongly underestimates central relative to peripheral deposition, in one measured instance by 100% [Lee et al., 1999b, 2000b], there can be little visually noticeable difference between a scan of a distribution that is uniform and therefore reliably measured and one that is nonuniform and poorly quantified. The issue is important because the amount of central and peripheral lung deposition is often a key question in the evaluation of drug delivery. Use of large regions (whole or half lung) and small bodies (animals or children) greatly helps to minimize the errors, but it also reduces the usefulness of the data. The available literature shows that studies in which planar imaging seems quantitative have very high and uniform lung distribution and/or very low total attenuation and scatter. Distributions which are less predictable are also less measurable. Therefore, quantitative expressions of planar results must be interpreted with great care. Even regional ratios can be in error by large amounts [Lee et al., 1999b, 2000b]. Still, in many cases the experimental question is relative. Which formulation gives the more preferred deposition pattern? In these cases a qualitative interpretation of relative planar quantification, especially that which is obtained in sequential studies using the same individuals, or possibly between individuals that are reasonably well matched for body size and type, clearly provides valid and useful results.

The use of 3D imaging for pulmonary distribution raises a new quantification issue. Regional analysis has been somewhat standardized in planar imaging by use of a penetration index (PI), a ratio of average spatial deposition in arbitrary inner and outer chest ("central" and "peripheral") regions (Fig. 3). A similar approach can be taken with a 3D image [Perring et al., 1994; Phipps et al., 1989]. The 3D nature can be taken into account. Central and peripheral regions, or "shells" with various amounts of peripheral character can be defined by their distance from the main bronchial bifurcations. The resulting regions and ratios are similarly arbitrary and subject to the methods of each investigator, and would be more useful if they were standardized between studies and investigators. Another approach under development for PET and SPECT [Fleming et al., 1995, 1996, 1997; Lee et al., 1999d] is to use the detailed 3D distribution, at the resolution of the imaging device, together with transformed airway dimensions from human lungs and a mathematical model. Briefly and conceptually, measured anatomic dimensions from the scan subject are combined with the airway dimensions of a lung model to give an intermediate shell model of the lung that preserves the individual anatomy and includes the detailed airway generation contribution to each shell. The measured 3D distribution of tracer is fitted to that structure to solve for the deposition of tracer on airway generations in the lung. The resolution of the images does not provide sufficient data to uniquely fit all the airway generations. The assumption is made that the distribution of tracer on any given airway generation is not spatially dependent, and assumptions are made about the way the tracer deposits on the smallest airways. Although distribution patterns among the acinar (smaller) airways are very sensitive to the assumptions, the distribution in the conducting airways and the total fraction penetrating from the conducting to the acinar airways seem to provide detailed descriptions of the imaging data. The method has not been quantitatively validated and validation promises to be difficult. But it does provide results consistent with other quantitative measures and it has the strong advantage of providing an objective, detailed, and quantitative description of drug deposition in the airways, so it may be the most useful way that is available to qualitatively and semiquantitatively compare different formulations.

CONCLUSIONS

When the biodistribution of a drug is a major determinant of its action, measurement of biodistribution becomes an important part of the evaluation process. The measured distribution can show that the drug performs as expected, and from a regulatory viewpoint it shows that claims of the drug's method of action and its delivery are justified. Kinetic measurements of regional drug concentration are relevant for the design of long-acting topical formulations and for analysis of conventional pharmacokinetic data. Imaging during drug development allows these important

variables that affect the likelihood of clinical success to be directly assessed and optimized before making a commitment to a particular formulation. Quantitative biodistribution and kinetic data can be important to optimize dosage and dose frequency of distribution-dependent dosage forms. The same data may also allow a regulatory assessment of the bioequivalence of different formulations, but only if it is quantitative and inclusive of drug kinetics over a relevant time period. Planar imaging has been effectively used to assess gross initial drug deposition and thereby to screen a wide variety of drug formulations and delivery devices. The more recent emergence of 3D and quantitative scanning with SPECT and PET promises to add detail and anatomic and quantitative accuracy to these studies. The use of radiolabeled drugs, as opposed to foreign radiotracers, is now providing kinetic and regional redistribution information that was not previously available. We conclude that the use of imaging for drug biodistribution evaluation has matured to a point of usefulness for drug development work. Although it has provided, and will continue to provide, excellent evaluations of marketed devices and formulations, its most effective application will most likely be to evaluate formulations and delivery methods during the development phase so that the most effective ones will be discovered, refined, and brought to market.

REFERENCES

Adkin DA, Kenyon CJ, Lerner EI, Landau I, Strauss E, Caron D, Penhasi A, Rubinstein A, Wilding IR. 1997. The use of scintigraphy to provide "proof of concept" for novel polysaccharide preparations designed for colonic drug delivery. Pharm Res 14:103–107.

Agnew JE, Bateman JR, Pavia D, Clarke SW. 1984. Radionuclide demonstration of ventilatory abnormalities in mild asthma. Clin Sci 66:525–531.

Aigbirhio FI, Carr RM, Pike VW, Steel CJ, Sutherland DR. 1997. Automated radiosynthesis of no-carrier-added [S-fluoromethyl-18F]fluticasone propionate as a radiotracer for lung deposition studies with PET. J Label Comp Radiopharm 39:567–584.

Amitai Y, Silver B, Leikin JB, Frischer H. 1992. Visualization of ingested medications in the stomach by ultrasound. Am J Emerg Med 10:18–23.

Anderson M, Svartengren M, Camner P. 1999. Human tracheobronchial deposition and effect of a cholinergic aerosol inhaled by extremely slow inhalations. Exp Lung Res 25:335–352.

Argenti D, Shah B, Heald D. 1999. A pharmacokinetic study to evaluate the absolute bioavailability of triamcinolone acetonide following inhalation administration. J Clin Pharmacol 39:695–702.

Atherton JC, Washington N, Bracewell MA, Sutton LJ, Greaves JL, Perkins AC, Hawkey CJ, Spiller RC. 1994. Scintigraphic assessment of the intragastric distribution and gastric emptying of an encapsulated drug: the effect of feeding and of a proton pump inhibitor. Aliment Pharmacol Ther 8:489–494.

Bednarczyk EM, Green JA, Nelson AD, Leisure GP, Little D, Adler LP, Berridge MS, Panacek EA, Miraldi FD. 1991. Comparison of the effect of temafloxacin, ciprofloxacin, or placebo on cerebral blood flow, glucose, and oxygen metabolism in healthy subjects by means of positron emission tomography. Clin Pharmacol Ther 50:165–171.

Bednarczyk EM, Green JA, Nelson AD, Leisure GP, Little D, Adler LP, Berridge MS, Panacek EA, Miraldi FD. 1992. Comparative assessment of the effect of lomefloxacin, ciprofloxacin, and placebo on cerebral blood flow, and glucose and oxygen metabolism in healthy subjects by positron emission tomography. Pharmacotherapy 12:369–375.

Bennett WD, Scheuch G, Zeman KL, Brown JS, Kim C, Heyder J, Stahlhofen W. 1999. Regional deposition and retention of particles in shallow, inhaled boluses: effect of lung volume. J Appl Physiol 86:168–173.

Berger G, Maziere M, Prenant C, Sastre J, Syrota A, Comar D. 1982. Synthesis of carbon-11 labeled propranolol. J Radioanal Chem 74:301–304.

Berger G, Prenant C, Sastre J, Syrota A, Comar D. 1983. Synthesis of a beta-blocker for heart visualization: [C-11]Practolol. Int J Appl Radiat Isot 34:1556–1557.

Bergstrom M, Nordberg A, Lunell E, Antoni G, Langstrom B. 1995. Regional deposition of inhaled 11C-nicotine vapor in the human airway as visualized by positron emission tomography. Clin Pharmacol Ther 57:309–317.

Bergstrom M, Cass LM, Valind S, Westerberg G, Lundberg EL, Gray S, Bye A, Langstrom B. 1999. Deposition and disposition of [11C]zanamivir following administration as an intranasal spray. Evaluation with positron emission tomography. Clin Pharmacokinet 36(Suppl 1):33–39.

Berridge MS, Heald DL. 1999. In vivo characterization of inhaled pharmaceuticals using quantitative positron emission tomography. J Clin Pharmacol 39:25S–29S.

Berridge MS, Cassidy EH, Bordeaux KG. 1994. Preparation of [^{11}C] triamcinolone acetonide. Appl Radiat Isot 45:91–95.

Berridge MS, Muswick GJ, Lee Z, Leisure GP, Nelson AD, Muzic RF Jr, Miraldi F. 1997. PET Evaluation of Azmacort ([C-11]triamcinolone acetonide) dose administration. J Nucl Med 38:4.

Berridge MS, Heald DL, Muswick GJ, Leisure GP, Voelker KW, Miraldi F. 1998. Biodistribution and kinetics of nasal [11C]-triamcinolone acetonide. J Nucl Med 39:1972–1977.

Berridge MS, Lee Z, Heald D. 1999a. Pulmonary drug distribution and kinetics by positron tomography. J Aerosol Med 12:117.

Berridge MS, Lee Z, Zheng L, Heald D. 1999b. Nasal distribution of inhaled drugs determined by positron tomography. J Aerosol Med 12:117.

Borgstrom L, Newman S, Weisz A, Moren F. 1992. Pulmonary deposition of inhaled terbutaline: comparison of scanning gamma camera and urinary excretion methods. J Pharm Sci 81:753–755.

Borgstrom L, Bondesson E, Moren F, Trofast E, Newman SP. 1994. Lung deposition of budesonide inhaled via Turbuhaler: a comparison with terbutaline sulphate in normal subjects. Eur Respir J 7:69–73.

Borin MT, Khare S, Beihn RM, Jay M. 1990. The effect of food on gastrointestinal (GI) transit of sustained-release ibuprofen tablets as evaluated by gamma scintigraphy. Pharm Res 7:304–307.

Brown J, Hooper G, Kenyon CJ, Haines S, Burt J, Humphries JM, Newman SP, Davis SS, Sparrow RA, Wilding IR. 1997. Spreading and retention of vaginal formulations in post-menopausal women as assessed by gamma scintigraphy. Pharm Res 14:1073–1078.

Buvat I, Rodriguez VM, Todd PA, Benali H, Di-Paola R. 1995. Comparative assessment of nine scatter correction methods based on spectral analysis using Monte Carlo simulations. J Nucl Med 36:1476–1488.

Buvat I, De-Sousa MC, Di-Paola M, Ricard M, Lumbroso J, Aubert B. 1998. Impact of scatter correction in planar scintimammography: a phantom study. J Nucl Med 39:1590–1596.

Calmanovici G, Zubillaga M, Lysionek A, Hager A, De-Paoli T, Alak M, Degrossi O, Garcia-del RH, Nicolini J, Caro R, Boccio J. 1998. 99mTc-ENS, a new radiopharmaceutical for aerial lung scintigraphy: comparative studies in rats. Nucl Med Biol 25:511–513.

Calmanovici GP, Boccio JR, Lysionek AE, Salgueiro MJ, Hager A, De-Paoli T, Calcagno ML, Nicolini J, Tarlati M, Caro RA, Zubillaga MB. 1999. 99mTc-ENS: a new radiopharmaceutical for aerosol lung scintigraphy. Comparison between different freeze-dried formulations. J Nucl Med 40:1080–1083.

Carter NJ, Eustance CN, Kettle AG, Barrington SF, Page CJ, O'Doherty MJ, Coakley AJ. 1995. Ventilation and perfusion lung imaging–which nebulizer? Nucl Med Commun 16:489–493.

Carter NJ, Page CJ, Eustance CN, O'Doherty MJ. 1998. Evaluating the Swirler nebulizer: a user's perspective. Nucl Med Commun 19:599–604.

Cass LM, Brown J, Pickford M, Fayinka S, Newman SP, Johansson CJ, Bye A. 1999. Pharmacoscintigraphic evaluation of lung deposition of inhaled zanamivir in healthy volunteers. Clin Pharmacokinet 36(Suppl 1):21–31.

Chan KK, Mojaverian P, Ziehmer BA, John VA. 1990. Application of radiotelemetric technique in evaluating diclofenac sodium absorption after oral administration of various dosage forms in healthy volunteers. Pharm Res 7:1026–1032.

Chan HK, Daviskas E, Eberl S, Robinson M, Bautovich G, Young I. 1999a. Deposition of aqueous aerosol of technetium-99m diethylene triamine penta-acetic acid generated and delivered by a novel system (AERx) in healthy subjects. Eur J Nucl Med 26:320–327.

Chan HK, Eberl S, Daviskas E, Constable C, Young I. 1999b. Dynamic SPECT of aerosol deposition and clearance in healthy subjects. J Aerosol Med 12:135.

Clay MM, Pavia D, Newman SP, Clarke SW. 1983. Factors influencing the size distribution of aerosols from jet nebulisers. Thorax 38:755–759.

Clunie G, Ell PJ. 1995. A survey of radiation synovectomy in Europe, 1991-1993. Eur J Nucl Med 22:970–976.

Clunie G, Lui D, Cullum I, Edwards JC, Ell PJ. 1995. Samarium-153-particulate hydroxyapatite radiation synovectomy: biodistribution data for chronic knee synovitis. J Nucl Med 36:51–57.

Clunie G, Lui D, Cullum I, Ell PJ, Edwards JC. 1996. Clinical outcome after one year following samarium-153 particulate hydroxyapatite radiation synovectomy. Scand J Rheumatol 25:360–366.

Czernin J, Schelbert HR. 1994. Non-invasive quantification of myocardial blood flow and flow reserve using dynamic positron emission tomography. Wien Klin Wochenschr 106:478–486.

Davis SS, Washington N, Parr GD, Short AH, John VA, Lloyd P, Walker SM. 1988. Relationship between the rate of appearance of oxprenolol in the systemic circulation and the location of an oxprenolol Oros 16/260 drug delivery system within the gastrointestinal tract as determined by scintigraphy. Br J Clin Pharmacol 26:435–443.

Davis SS, Hardy JG, Newman SP, Wilding IR. 1992. Gamma scintigraphy in the evaluation of pharmaceutical dosage forms. Eur J Nucl Med 19:971–986.

DiZio JP, Fiaschi R, Davison A, Jones AG, Katzenellenbogen JA. 1991. Progestin-rhenium complexes: metal-labeled steroids with high receptor binding affinity, potential receptor-directed agents for diagnostic imaging or therapy. Bioconjug Chem 2:353–366.

Dolovich M, Inman M, Wahl L, Nahmias C, Hargreave F, Coates G. 1998. 3D Imaging of the lung: PET scanning and aerosols. Am J Respir Crit Care Med 157:A78.

Dolovich M, Nahmias C, Thompson M, Yuki S, Frietag A, Coates G. 1999. Positron emission tomographic (PET) imaging of the lung in cystic fibrosis: 3D assessment of the distribution of inhaled therapy. Am J Respir Crit Care Med 159:A687.

Durak H, Sayit E, Aktogu S, Degirmenci B, Yurekli Y, Ertay T, Guven H. 1996. Lung clearance of inhaled 99Tcm-erythromycin lactobionate in non-smokers and smokers. Nucl Med Commun 17:895–901.

Eberl S, Chan HK, Daviskas E, Constable C, Young I. 1999. Assessment of lung radioaerosol deposition and clearance with simultaneous emission-transmission dynamic SPECT. J Nucl Med 40:95p.

Edwards DA, Ben-Jebria A, Langer R. 1998. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol 85:379–385.

Eichler HG, Muller M. 1998. Drug distribution. The forgotten relative in clinical pharmacokinetics. Clin Pharmacokinet 34: 95–99.

El-Fakhri GN, Buvat I, Pelegrini M, Benali H, Almeida P, Bendriem B, Todd PA, Di-Paola R. 1999. Respective roles of scatter, attenuation, depth-dependent collimator response and finite spatial resolution in cardiac single-photon emission tomography quantitation: a Monte Carlo study. Eur J Nucl Med 26: 437–446.

Farr SJ. 1996. The physico-chemical basis of radiolabelling metered dose inhalers with 99mTc. J Aerosol Med 9(Suppl 1):S27–S36.

Feely LC, Davis SS. 1989. Correlation of phenylpropanolamine bioavailability with gastrointestinal transit by scintigraphic monitoring of 111In-labeled hydroxypropylmethylcellulose matrices. Pharm Res 6:274–278.

Fiel SB, Fuchs HJ, Johnson C, Gonda I, Clark AR. 1995. Comparison of three jet nebulizer aerosol delivery systems used to administer recombinant human DNase I to patients with cystic fibrosis. The Pulmozyme rhDNase Study Group. Chest 108:153–156.

Fink JB, Dhand R, Grychowski J, Fahey PJ, Tobin MJ. 1999. Reconciling in vitro and in vivo measurements of aerosol delivery from a metered-dose inhaler during mechanical ventilation and defining efficiency-enhancing factors. Am J Respir Crit Care Med 159:63–68.

Finlay WH, Wong JP. 1998. Regional lung deposition of nebulized liposome-encapsulated ciprofloxacin. Int J Pharm 167:121–127.

Finlay WH, Stapleton KW, Chan HK, Zuberbuhler P, Gonda I. 1996. Regional deposition of inhaled hygroscopic aerosols: in vivo SPECT compared with mathematical modeling. J Appl Physiol 81:374–383

Fleming JS, Nassim MA, Hashish AH, Bailey AG, Conway J, Holgate ST, Halson P, Moore E, Martonen TB. 1995. Description of pulmonary deposition of radiolabeled aerosol by airway generation using a conceptual three-dimensional model of lung morphology. J Aerosol Med 8:341–356

Fleming JS, Halson P, Conway J, Moore E, Nassim MA, Hashish AH, Bailey AG, Holgate ST, Martonen TB. 1996. Three-dimensional description of pulmonary deposition of inhaled aerosol using data from multimodality imaging. J Nucl Med 37:873–877.

Fleming JS, Hashish AH, Conway J, Hartley-Davies R, Nassim MA, Guy MJ, Coupe J, Holgate ST, Moore E, Bailey AG, Martonen TB. 1997. A technique for simulating radionuclide images from the aerosol deposition pattern in the airway tree. J Aerosol Med 10:199–212.

Fok TF, al-Essa M, Kirpalani H, Monkman S, Bowen B, Coates G, Dolovich M. 1999. Estimation of pulmonary deposition of aerosol using gamma scintigraphy. J Aerosol Med 12:9–15.

Foster WM, Stetkiewicz PT, Freed AN. 1997. Retention of soluble 99mTc-DTPA in the human lung: 24-h postdeposition. J Appl Physiol 82:1378–1382.

Graffner C, Wagner Z, Nilsson MI, Widerlov E. 1990. Plasma concentrations of remoxipride and the gastrointestinal transit of 111In-marked extended-release coated spheres. Pharm Res 7:54–58.

Groning R, Berntgen M. 1996. Estimation of the gastric residence time of magnetic dosage forms using the Heidelberg capsule. Pharmazie 51:328–331.

Hardy JG, Healey JN, Lee SW, Reynolds JR. 1987. Gastrointestinal transit of an enteric-coated delayed-release 5-aminosalicylic acid tablet. Aliment Pharmacol Ther 1:209–216.

Hardy JG, Harvey WJ, Sparrow RA, Marshall GB, Steed KP, Macarios M, Wilding IR. 1993. Localization of drug release sites from an oral sustained-release formulation of 5-ASA (Pentasa) in the gastrointestinal tract using gamma scintigraphy. J Clin Pharmacol 33:712–718.

Harris D, Fell JT, Sharma HL, Taylor DC. 1987. Assessment of gastric emptying using gamma scintigraphy. J Clin Pharm Ther 12:343–346.

Hatazawa J, Yanai M, Itoh M, Ido T, Ojima F, Takahashi T, Aikawa T, Sasaki H, Takishima T, Watanuki S, et al. 1990. Tracheobronchial mucociliary clearance and alveolar epithelial permeability measured by PET with 18FDG powder. J Comput Assist Tomogr 14:175–181.

Henderson LM, Brewer GJ, Dressman JB, Swidan SZ, DuRoss DJ, Adair CH, Barnett JL, Berardi RR. 1995. Effect of intragastric pH on the absorption of oral zinc acetate and zinc oxide in young healthy volunteers. J Parenter Enteral Nutr 19:393–397.

Herzog H, Kops ER, Schmid A, Feinendegen LE. 1991. Effects of varying physical parameters of a PET system on the accuracy of radioactivity quantitation in vivo. Med Prog Technol 17:193–198.

Hom RK, Katzenellenbogen JA. 1997. Technetium-99m-labeled receptor-specific small-molecule radiopharmaceuticals: recent developments and encouraging results. Nucl Med Biol 24: 485–498.

Hughes JM. 1985. Regional lung function: physiology and clinical applications. Clin Physiol (5 Suppl 3):19–31.

Ishibashi T, Pitcairn GR, Yoshino H, Mizobe M, Wilding IR. 1998. Scintigraphic evaluation of a new capsule-type colon specific drug delivery system in healthy volunteers. J Pharm Sci 87:531–535.

Itoh H, Smaldone GC, Swift DL, Wagner-HN J. 1985. Quantitative measurement of total aerosol lung deposition: comparison of three different techniques. J Aerosol Sci 16:367–371.

Jay M, Beihn RM, Digenis GA, Deland FH, Caldwell L, Mlodozeniec AR. 1985. Disposition of radiolabelled suppositories in humans. J Pharm Pharmacol 37:266–268.

Jia W, Ehrhardt GJ, Zinn K, Wang N, Ketring AR. 1996. Re-186/Re-188 Labeled polypeptide microspheres as a potential radiation synovectomy agent. J Radioanal Nucl Chem 206:107–117.

Kenyon CJ, Dewsbury NJ, Newman SP. 1995. Differences in aerodynamic particle size distributions of innovator and generic beclomethasone dipropionate aerosols used with and without a large volume spacer. Thorax 50:846–850.

Kenyon CJ, Brown F, McClelland GR, Wilding IR. 1998a. The use of pharmacoscintigraphy to elucidate food effects observed with a novel protease inhibitor (saquinavir). Pharm Res 15: 417–422.

Kenyon CJ, Thorsson L, Borgstrom L, Newman SP. 1998b. The effects of static charge in spacer devices on glucocorticosteroid aerosol deposition in asthmatic patients. Eur Respir J 11:606–610.

King MA, Tsui BM, Pan TS. 1995. Attenuation compensation for cardiac single-photon emission computed tomographic imaging. 1. Impact of attenuation and methods of estimating attenuation maps. J Nucl Cardiol 2:513–524.

King MA, Tsui BM, Pan TS, Glick SJ, Soares EJ. 1996. Attenuation compensation for cardiac single-photon emission computed tomographic imaging. 2. Attenuation compensation algorithms. J Nucl Cardiol 3:55–64.

King GG, Eberl S, Salome CM, Meikle SR, Woolcock AJ. 1997. Airway closure measured by a technegas bolus and SPECT. Am J Respir Crit Care Med 155:682–688.

Korf J. 1997. Imaging of receptors in clinical neurosciences. Acta Neurol Belg 97:200–205.

Kosoglou T, Kazierad DJ, Schentag JJ, Patrick JE, Heimark L, Radwanski E, Christopher D, Flannery BE, Affrime MB. 1995. Effect of food on the oral bioavailability of isosorbide-5-mononitrate administered as an extended-release tablet. J Clin Pharmacol 35:151–158.

Kuwert T, Sures T, Herzog H, Loken M, Hennerici M, Langen KJ, Feinendegen LE. 1992. On the influence of spatial resolution and of the size and form of regions of interest on the measurement of regional cerebral metabolic rates by positron emission tomography. J Neural Transm Suppl 37:53–66.

Langenback EG, Davis JM, Robbins C, Sahgal N, Perry RJ, Simon SR. 1999. Improved pulmonary distribution of recombinant human Cu/Zn superoxide dismutase, using a modified ultrasonic nebulizer. Pediatr Pulmonol 27:124–129.

Lee Z, Berridge MS, Nelson AD, Heald D. 1999a. Evaluation of planar imaging vs. PET for pulmonary deposition of inhaled aerosols: a validation study. J Nucl Med 40:197.

Lee Z, Berridge MS, Nelson AD, Heald D. 1999b. Corrections in gamma scintigraphy. J Aerosol Med 12:135.

Lee Z, Heald D, Berridge MS. 1999c. Comparison of PET and gamma scintigraphy: validation studies. J Aerosol Med 12:134.

Lee Z, Berridge MS, Finlay WH, Heald D. 1999d. Airway generation mapping of aerosol drug deposition data. J Aerosol Med 12:131.

Leong BK, Coombs JK, Sabaitis CP, Rop DA, Aaron CS. 1998. Quantitative morphometric analysis of pulmonary deposition of

aerosol particles inhaled via intratracheal nebulization, intratracheal instillation or nose-only inhalation in rats. J Appl Toxicol 18:149–160.

Leskinen KS. 1994. Positron emission tomography in oncology. Clin Physiol 14:329–335.

Lipworth BJ, Clark DJ. 1998. Early lung absorption profile of non-CFC salbutamol via small and large volume plastic spacer devices. Br J Clin Pharmacol 46:45–48.

Marathe PH, Sandefer EP, Kollia GE, Greene DS, Barbhaiya RH, Lipper RA, Page RC, Doll WJ, Ryo UY, Digenis GA. 1998. In vivo evaluation of the absorption and gastrointestinal transit of avitriptan in fed and fasted subjects using gamma scintigraphy. J Pharmacokinet Biopharm 26:1–20.

Mijailovich SM, Treppo S, Venegas JG. 1997. Effects of lung motion and tracer kinetics corrections on PET imaging of pulmonary function. J Appl Physiol 82:1154–1162.

Miller C, Filipow L, Jackson S, Riauka T. 1996. Planar imaging quantification using 3D attenuation correction data and Monte Carlo simulated buildup factors. Phys Med Biol 41:1401–1423.

Murata K, Itoh H, Senda M, Todo G, Yonekura Y, Torizuka K. 1986. Ventilation imaging with positron emission tomography and nitrogen 13. Radiology 158:303–307.

Newman SP. 1993. Scintigraphic assessment of therapeutic aerosols. Crit Rev Ther Drug Carrier Syst 10:65–109.

Newman SP. 1995. A comparison of lung deposition patterns between different asthma inhalers. J Aerosol Med 8(Suppl 3):S21–S26.

Newman SP. 1998. How well do in vitro particle size measurements predict drug delivery in vivo? J Aerosol Med 11(Suppl 1):S97–104.

Newman SP, Newhouse MT. 1996. Effect of add-on devices for aerosol drug delivery: deposition studies and clinical aspects. J Aerosol Med 9:55–70.

Newman SP, Pavia D. 1985. Aerosol deposition in man. In: Moren F, Newhouse MT, Dolovich M. Aerosols in medicine, principles, diagnosis and therapy. New York: Elsevier Science Publishers. p 193–215.

Newman SP, Wilding IR. 1999. Imaging techniques for assessing drug delivery in man. Pharm Sci Technol Today 2:181–189.

Newman SP, Clark AR, Talaee N, Clarke SW. 1989a. Pressurised aerosol deposition in the human lung with and without an "open" spacer device. Thorax 44:706–710.

Newman SP, Moren F, Trofast E, Talaee N, Clarke SW. 1989b. Deposition and clinical efficacy of terbutaline sulphate from Turbuhaler, a new multi-dose powder inhaler. Eur Respir J 2: 247–252.

Newman S, Steed K, Hooper G, Kallen A, Borgstrom L. 1995a. Comparison of gamma scintigraphy and a pharmacokinetic technique for assessing pulmonary deposition of terbutaline sulphate delivered by pressurized metered dose inhaler. Pharm Res 12:231–236.

Newman SP, Steed KP, Hooper G, Brickwell J. 1995b. Scintigraphic assessment of the oropharyngeal and nasal depositions of fusafungine from a pressurized inhaler and from a novel pump spray device. J Pharm Pharmacol 47:818–821.

Newman SP, Steed KP, Reader SJ, Hooper G, Zierenberg B. 1996. Efficient delivery to the lungs of flunisolide aerosol from a new portable hand-held multidose nebulizer. Pharm Sci 85:960–964.

Newman SP, Brown J, Steed KP, Reader SJ, Kladders H. 1998. Lung deposition of fenoterol and flunisolide delivered using a novel device for inhaled medicines: comparison of RESPIMAT with conventional metered-dose inhalers with and without spacer devices. Chest 113:957–963.

Ogoshi T, Usui N. 1991. Distribution of inhaled aerosol using nasal nebulizer. Nippon Jibiinkoka Gakkai Kaiho 94:51–56.

Ojima F, Ido T, Takahashi T, Hatazawa J, Ito M, Sasaki H, Yanai M, Aikawa T, Takishima T, Abiko K, et al. 1989. Preparation of a fine powder of 2-deoxy-2-[18F]fluoro-D-glucose suitable for inhalation to diagnose lung diseases by means of PET. Ann Nucl Med 3:143–147.

Ojima F, Ido T, Hatazawa J, Itoh M, Shinohara H, Watanuki S, Seo S, Kai H, Takahama K, Ishii T, Nakagawa Y, Miyata T. 1998. Development of a new method for simultaneously evaluating mucociliary clearance and pulmonary epithelial permeability in rabbit experiments by means of ^{18}FDG, three-dimensional positron emission tomography and rectilinear scan. Ann Nucl Med 12:231–235.

Olseni L, Palmer J, Wollmer P. 1994. Quantitative evaluation of aerosol deposition pattern in the lung in patients with chronic bronchitis. Physiol Meas 15:41–48.

O'Riordan TG, Iacono A, Keenan RJ, Duncan SR, Burckart GJ, Griffith BP, Smaldone GC. 1995. Delivery and distribution of aerosolized cyclosporine in lung allograft recipients. Am J Respir Crit Care Med 151:516–521.

Parr AF, Beihn RM, Franz RM, Szpunar GJ, Jay M. 1987. Correlation of ibuprofen bioavailability with gastrointestinal transit by scintigraphic monitoring of 171Er-labeled sustained-release tablets. Pharm Res 4:486–489.

Pauwels R, Newman S, Borgstrom L. 1997. Airway deposition and airway effects of antiasthma drugs delivered from metered-dose inhalers. Eur Respir J 10:2127–2138.

Perring S, Summers QA, Fleming JS, Nassim MA, Holgate ST. 1994. A new method of quantification of the pulmonary regional distribution of aerosols using combined CT and SPECT and its application to nedocromil sodium administered by metered dose inhaler. Br J Radiol 67:46–53.

Phipps PR, Gonda I, Bailey DL, Borham P, Bautovich G, Anderson SD. 1989. Comparisons of planar and tomographic gamma scintigraphy to measure the penetration index of inhaled aerosols. Am Rev Respir Dis 139:1516–1523.

Pike VW, Aigbirhio FI, Freemantle CA, Page BC, Rhodes CG, Waters SL, Jones T, Olsson P, Ventresca GP, Tanner RJ. 1995a. Disposition of inhaled 1,1,1,2-tetrafluoroethane (HFA134A) in healthy subjects and in patients with chronic airflow limitation. Measurement by ^{18}F-labeling and whole-body gamma-counting. Drug Metab Dispos 23:832–839.

Pike VW, Tanner RJ, Aigbirhio FI, Freemantle CA, Waters SL, Page BC, Rhodes CG, Olsson P, Ventresca GP, Jones T. 1995b. The disposition of a new drug propellant (HFA 134a) in humans studied by fluorine-18 labelling and whole-body gamma-counting. In: Comar D, editor. PET for drug development and evaluation. Boston: Dordrecht, Kluwer Academic Publishers. p 341–352.

Pitcairn GR, Newman SP. 1997. Tissue attenuation corrections in gamma scintigraphy. J Aerosol Med 10:187–198.

Pitcairn GR, Newman S. 1999. The F2 metric to validate radiolabelling methods used in lung deposition studies. J Aerosol Med 12:134.

Pitcairn GR, Lim J, Hollingworth A, Newman SP. 1997. Scintigraphic assessment of drug delivery from the Ultrahaler dry powder inhaler. J Aerosol Med 10:295–306.

Prenant C, Sastre J, Crouzel C, Syrota A. 1987. Synthesis of [C-11]pindolol. J Label Comp Radiopharm 24:227–232.

Read NW, Al-Janabi MN, Holgate AM, Barber DC, Edwards CA. 1986. Simultaneous measurement of gastric emptying, small bowel residence and colonic filling of a solid meal by the use of the gamma camera. Gut 27:300–308.

Rhodes CG, Hughes JM. 1995. Pulmonary studies using positron emission tomography. Eur Respir J 8:1001–1017.

Rhodes CG, Valind SO, Brudin LH, Wollmer PE, Jones T, Buckingham PD, Hughes JM. 1989. Quantification of regional V/Q ratios in humans by use of PET. II. Procedure and normal values. J Appl Physiol 66:1905–1913.

Rohatagi S, Rhodes GR, Chaikin P. 1999. Absolute oral versus inhaled bioavailability: significance for inhaled drugs with special reference to inhaled glucocorticoids. J Clin Pharmacol 39: 661–663.

Saari SM, Vidgren MT, Koskinen MO, Turjanmaa VM, Waldrep JC, Nieminen MM. 1998. Regional lung deposition and clearance of 99mTc-labeled beclomethasone-DLPC liposomes in mild and severe asthma. Chest 113:1573–1579.

Schuster DP. 1998. The evaluation of lung function with PET. Semin Nucl Med 28:341–351.

Schuster DP, Markham J, Welch MJ. 1998. Positron emission tomography measurements of pulmonary vascular permeability with Ga-68 transferrin or C-11 methylalbumin. Crit Care Med 26:518–525.

Selwyn AP, Shea MJ, Foale R, Deanfield JE, Wilson R, de-Landsheere CM, Turton DL, Brady F, Pike VW, Brookes DI. 1986. Regional myocardial and organ blood flow after myocardial infarction: application of the microsphere principle in man. Circulation 73:433–443.

Senda M, Murata K, Itoh H, Yonekura Y, Torizuka K. 1986. Quantitative evaluation of regional pulmonary ventilation using PET and nitrogen-13 gas. J Nucl Med 27:268–273.

Shelton MJ, Adams JM, Hewitt RG, Morse GD. 1998. Previous infection with Helicobacter pylori is the primary determinant of spontaneous gastric hypoacidity in human immunodeficiency virus-infected outpatients. Clin Infect Dis 27:739–745.

Smye SW, Unsworth J. 1985. The use of single photon emission computed tomography in lung imaging with aerosols. Nucl Med Commun 6:397–404.

Spradau TW, Katzenellenbogen JA. 1998. Ligands for the estrogen receptor, containing cyclopentadienyltricarbonylrhenium units. Bioorg Med Chem Lett 8:3235–3240.

Summers QA, Fleming JS, Dai Y, Perring S, Honeywell R, Gough KJ, Renwick AG, Clark AR, Nassim MA, Holgate ST. 1996. The pulmonary deposition of two aerosol preparations of nedocromil sodium delivered by MDI assessed by single photon emission computed tomography. J Aerosol Med 9 Suppl 1:S93–109.

Sun JX, Walter B, Sandefer EP, Page RC, Digenis GA, Ryo UY, Cipriano A, Maniara WM, Powell ML, Chan K. 1996. Explaining variable absorption of a hypolipidemic agent (CGP 43371) in healthy subjects by gamma scintigraphy and pharmacokinetics. J Clin Pharmacol 36:230–237.

Svartengren K, Philipson K, Svartengren M, Nerbrink O, Camner P. 1995. Clearance in smaller airways of inhaled 6-microm particles in subjects with immotile-cilia syndrome. Exp Lung Res 21: 667–682.

Svartengren K, Svartengren M, Philipson K, Barck C, Bylin G, Camner P. 1999a. Clearance in small ciliated airways in allergic asthmatics after bronchial allergen provocation. Respiration 66:112–118.

Svartengren M, Svartengren K, Aghaie F, Philipson K, Camner P. 1999b. Lung deposition and extremely slow inhalations of particles. Limited effect of induced airway obstruction. Exp Lung Res 25:353–366.

Taylor IK, Hill AA, Hayes M, Rhodes CG, O'Shaughnessy KM, O'Connor BJ, Jones HA, Hughes JM, Jones T, Pride NB, Fuller RW. 1996. Imaging allergen-invoked airway inflammation in atopic asthma with [^{18}F]-fluorodeoxyglucose and positron emission tomography. Lancet 347:937–940.

Terra-Filho M, Meneghetti JC, Cukier A, Teixeira LR, Soares JJ, Camargo EE, Vargas FS. 1996. Gallium-67 lung imaging and pulmonary clearance of 99mTc-DTPA aerosol in patients with amiodarone pneumonitis. Braz J Med Biol Res 29:1467–1471.

Treppo S, Mijailovich SM, Venegas JG. 1997. Contributions of pulmonary perfusion and ventilation to heterogeneity in V(A)/Q measured by PET. J Appl Physiol 82:1163–1176.

Vidgren M, Paronen P, Vidgren P, Vainio P, Nuutinen J. 1990. In vivo evaluation of the new multiple dose powder inhaler and the Rotahaler using the gamma scintigraphy. Acta Pharm Nord 2:3–10.

Vidgren P, Vidgren M, Paronen P, Vainio P, Karjalainen P, Nuutinen J. 1991. Removal of inhaled 99mTc-labelled particles of disodium cromoglycate from the lungs. Acta Pharm Nord 3:155–158.

Virgolini I, Kurtaran A, Leimer M, Smith-Jones P, Agis H, Angelberger P, Kletter K, Valent P, Linkesch W, Eichler HG. 1997. Inhalation scintigraphy with iodine-123-labeled interferon gamma-1b: pulmonary deposition and dose escalation study in healthy volunteers [see comments]. J Nucl Med 38:1475–1481.

Wang SJ, Lin WY, Hsieh BT, Shen LH, Tsai ZT, Ting G, Knapp-FF J. 1995. Rhenium-188 sulphur colloid as a radiation synovectomy agent. Eur J Nucl Med 22:505–507.

Wang SJ, Lin WY, Chen MN, Hsieh BT, Shen LH, Tsai ZT, Ting G, Chen JT, Ho WL, Mirzadeh S, Knapp FF J. 1998. Rhenium-188 microspheres: a new radiation synovectomy agent. Nucl Med Commun 19:427–433.

Watanabe N, Inoue T, Tomioka S, Yamaji T, Endo K. 1995. Discordant findings between krypton-81m gas and Tc-99m labeled ultrafine aerosol lung ventilation SPECT in two patients with idiopathic pulmonary fibrosis. Clin Nucl Med 20:315–317.

Wilding IR, Hardy JG, Sparrow RA, Davis SS, Daly PB, English JR. 1992. In vivo evaluation of enteric-coated naproxen tablets using gamma scintigraphy. Pharm Res 9:1436–1441.

Williams CN, Haber G, Aquino JA. 1987. Double-blind, placebo-controlled evaluation of 5-ASA suppositories in active distal proctitis and measurement of extent of spread using 99mTc-labeled 5-ASA suppositories. Dig Dis Sci 32:71S–75S.