PET and Drug Research and Development*

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The use of PET to examine the behavioral, therapeutic and toxic properties of drugs and substances of abuse is emerging as a powerful new scientific tool. PET provides a new perspective on drug research by virtue of its ability to directly assess both pharmacokinetic and pharmacodynamic events in humans and in animals. These parameters can be assessed directly in the human body both in healthy volunteers and in patients. Moreover, the new generation of high-resolution, small-animal cameras hold the promise of introducing imaging in the early stages of drug development and make it possible to carry out longitudinal studies in animals and to study genetically altered animals. This places PET in a unique position to contribute significantly to the process of drug development through understanding the molecular mechanisms underlying drug action while addressing some very practical questions such as determining effective drug doses for clinical trials for new drugs, determining the duration of drug action and examining potential drug interactions.

Key Words: PET imaging; fluorodeoxyglucose; drug development

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PET technology has been applied in creative ways to study drug action directly in humans and laboratory animals (*1–3*). Its strength is the ability to directly obtain quantitative knowledge of the movement and bioavailability of drugs (pharmacokinetics) and their therapeutic and toxic effects on living systems (pharmacodynamics) (*4*). The term "pharmacokinetics" refers to the dynamics of drug absorption, distribution and elimination that determine the fraction of the dose of a drug available for action. The term "pharmacodynamics" refers to the biological processes involved in the drug's effects. Because PET can be used to measure both drug pharmacokinetics and drug pharmacodynamics, radiotracer development both for labeled drugs and for labeled tracers is a key area for advancement of the field. In addition, because drug development begins with animal research, the new generation of small-animal PET imaging instruments represents a major advancement. These instruments will allow studies to be performed on living animals early in the process of drug development and will make it possible to take advantage of advances in genetics to probe specific drug mechanisms in genetically altered living animals (*5,6*). However, in addition to providing vital feedback in new areas of drug development, PET offers an opportunity to review drugs that are currently in the therapeutic armamentarium, to investigate their bioavailability, to understand their mechanisms of action and even to improve their use. However, much remains to be done, and the acquisition of new knowledge is critical to the development of better drugs. A special challenge is to use imaging technologies early in the drug development process.

This article is not intended to be a catalog of drug studies using PET but rather to illustrate principles and problems. Within this context, we discuss radiolabeled compounds for drug studies. This discussion is followed by examples in which PET has been used to understand drug action, to develop new treatment strategies and to monitor the therapeutic and toxic effects of drugs. We conclude with a summary of some of the special challenges of this field.

RADIOLABELED COMPOUNDS FOR DRUG STUDIES

An up-to-date critique of the state of the art and of current needs in the design and development of PET radiopharmaceuticals was published recently (*7*). To fully use PET technology in drug development, labeled compounds are needed to address both pharmacokinetic and pharmacodynamic properties of drugs. The direct measurement of drug pharmacokinetics depends on the ability to incorporate the short-lived positron emitters into drug molecules. PET imaging with the labeled drug is the most direct way to determine bioavailability and kinetics. The drug usually must be labeled with ^{11}C to avoid changing the characteristics of the parent compound. ^{18}F also is used if the drug has a fluorine atom, and $^{13}NH_3$ has been used to label some drugs.

There are important factors to consider in the interpretation of PET studies of labeled drugs, including the fact that the PET image may include labeled drug metabolites. In addition, PET studies typically use tracer quantities that are administered intravenously, whereas drugs usually are administered in milligram or gram amounts and typically are given orally (*8*). A limitation in studying the pharmacokinetics of a drug labeled with ^{11}C when administered orally is that the rate of absorption may be too slow and metabolism may be too rapid, relative to the 20.4-min half-life, to provide meaningful information; local dosimetry to the upper gastro-

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intestinal system also may be a limitation. Imaging in three-dimensional mode provides higher sensitivity, which is due to an increase in counting statistics and, thus, shorter imaging time or a lower administered dose. Thus, the three-dimensional mode may permit the measurement of drug absorption after oral administration in humans. A method for studying pharmacokinetics in humans at picomolar concentrations using a whole-body counter also has been proposed as a means for determining the human biodistribution of a new drug at an early stage in its development (*9*).

PET also has made it possible to assess the effects of drugs in various physiological and biochemical parameters. The most investigated ones have been the effects of drugs on brain glucose metabolism using 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG) (*10*) and on cerebral blood flow (CBF) using ^{15}O-labeled water (*11*). Under physiological conditions, glucose metabolism and CBF serve as markers of brain function. FDG is also important because its uptake is elevated in malignant tumors, and it is a useful marker for myocardial viability (*12*). This has made FDG a core radiotracer in pharmaceutical research. With PET and FDG, it has been possible to examine regional drug effects, to generate hypotheses regarding molecular targets and to gain new knowledge regarding drug toxicity. In addition, it has been possible to identify the role of specific neurotransmitters in the psychoactive effects of drugs by integrating PET with drug challenge studies coupled with behavioral measures.

The brain dopamine (DA) system clearly exemplifies how advances in radiotracer development have influenced drug research (*13*). DA is a key neurotransmitter system in movement, motivation and reward. Elements of the brain DA system are also important drug targets in drug abuse, neurodegenerative disease and neuropsychiatric disorders. From the early history of biochemical and molecular PET imaging, there have been radiotracers for imaging various elements of the DA system. The earliest was [^{18}F]fluorodopa, which measures DA metabolism and has been applied for many years to the study of neurodegenerative disorders affecting the DA system (*14*). Another class of PET tracers that have been developed are those binding to DA D_2 and D_1 receptors (*13*). Two classes, the butyrophenones and the benzamides, have been used widely to probe the effects of disease and drugs on D_2 receptors in the DA system (*15,16*). One of these, [^{11}C]raclopride, because of its moderate affinity for DA D_2 receptors, has become a major scientific tool for measuring drug-induced changes in synaptic DA and the effects of disease and aging on this process (*17,18*). After the development of tracers for the DA D_2 receptors were tracers for the DA transporter (DAT), which are presynaptic molecules that clear synaptic DA and are the target for drugs of abuse, e.g., cocaine, therapeutic drugs like methylphenidate (MP) and neurotoxic compounds like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (*19–22*). At the same time, tracers were developed for imaging the subtypes of monoamine oxidase (MAO A and B, enzymes that break down dopamine and other neurotransmitters) (*23*) and for examining the vesicular monoamine transporter within monoamine neurons (*24*).

However, much remains to be done, especially in the area of developing radiotracers that can be used to evaluate a variety of other molecular targets. In addition, synthetic methods for labeling drugs that cannot be labeled easily with present methods are needed. Indeed, every time that a new radiotracer whose binding properties and kinetics can be used to quantify a specific molecular target is developed, new opportunities arise for its use in drug development and mechanistic studies.

CENTRAL NERVOUS SYSTEM DRUGS

Over the past two decades, PET studies with radiolabeled drugs have provided new information on drug uptake, distribution and kinetics in the human brain and their influence on the relationship to the biochemical, behavioral, therapeutic and toxic effects of the drugs. This has been true particularly for antipsychotic and psychostimulant drugs (*2,3*). Although route of administration and slow biological processes may be limitations to imaging drug pharmacokinetics directly when a drug is administered orally, this information can be obtained indirectly by measuring the pharmacodynamic effects of the drug at various times after its administration. In this way, the time course of the drug's effects at a specific molecular target may be assessed over a span of hours to weeks. Studies on antipsychotic drugs, psychostimulant drugs, MAO B inhibitor drugs and opiate drugs illustrate this point.

Antipsychotic Drugs

The antipsychotic drug chloropromazine was visualized in the human brain 20 y ago (*25*). Since then, many antipsychotic drugs have been labeled with either ^{11}C or ^{18}F, and their distribution and kinetics have been measured in the human brain. For example, the distribution and kinetics of haloperidol have been measured in the human brain (*26*). Because antipsychotic drugs usually bind to DA D_2 receptors, many studies have used PET both to determine the degree of receptor occupancy by therapeutic doses and to relate receptor occupancy to drug dose, plasma drug levels and treatment response. Previous studies using [^{18}F]N-methylspiroperidol showed that receptor occupancy by haloperidol reached a plateau and that increasing doses did not result in increasing levels of receptor occupancy (Fig. 1) (*27,28*). Previous studies also showed that DA D_2 receptor occupancy by haloperidol did not differ between responders and nonresponders, demonstrating the futility of elevating drug doses in an attempt to achieve a therapeutic response (*29*). PET studies also have established that there is a threshold occupancy at which extrapyramidal effects occur (*3*). Recent studies have shown that higher levels of DA D_2 receptor occupancies occur after administration of typical (as opposed to atypical) antipsychotics and that atypical antipsychotics, such as clozapine and risperidone, also induce significant levels of 5-HT_{2A} occupancies (*3*).

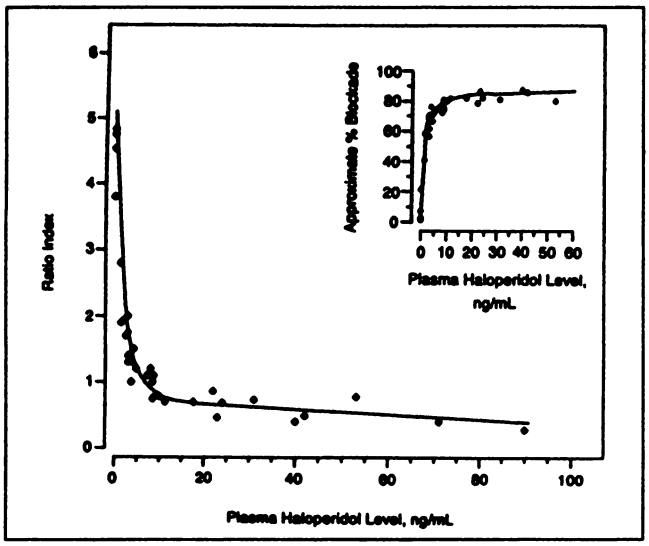

FIGURE 1. Relationship between plasma haloperidol level and receptor availability (ratio index) or approximate receptor blockade using [^{18}F]N-methylspiroperidol. (Reprinted with permission of [*28*].)

PET also has been used to examine the pharmacokinetics of BMY-14802, a potential antipsychotic drug that has moderate affinity for the sigma binding site and low affinity for the DA D_2 receptor (*30*). BMY-14802, which has a chiral center and contains a fluorine atom, was labeled with ^{18}F, and PET studies were conducted in baboons. The labeled racemic drug was subjected to chiral high-performance liquid chromatography to isolate the individually labeled enantiomers. PET studies in the baboon showed a rapid uptake into the brain and a rapid clearance, such that by 60 min only 10% of the peak value remained. There was no stereoselectivity, and the regional distribution did not parallel the distribution of sigma binding sites. This study indicated that rapid clearance from the brain would be a major limiting factor in the use of BMY-14802. Subsequent human studies in patients with schizophrenia showed no statistically significant clinical improvement, and clinical evaluation of the drug was no longer pursued.

PET also has been used to understand the pharmacological profile of new, atypical antipsychotic drugs such as clozapine and risperidone, which are effective in the treatment of some patients who do not respond to classic antipsychotic drugs and which produce fewer extrapyramidal side effects than typical antipsychotics such as haloperidol. Interestingly, in contrast to typical antipsychotic drugs, clozapine occupies only 20%–67% of DA D_2 receptors, yet it occupies DA D_1 receptors and 5-HT_{2A} receptors to a high degree (*31,32*).

On the basis of these observations, which were made directly in the human brain using radiotracers with selectivity for D_1, D_2 and 5HT2A receptors, several antipsychotic drugs are now being developed to determine the benefit of developing drugs with a high affinity for the 5HT2 receptor (*3*).

Psychostimulant Drugs

An area in which PET has been particularly valuable is in understanding the behavioral and toxic effects of psychostimulant drugs using both pharmacokinetic and pharmacodynamic studies (*2*). PET has been used to compare the psychostimulant drugs cocaine and MP. These two drugs bind to the DAT with similar affinities, yet cocaine is one of the most addictive substances, and MP is used in the treatment of children with attention deficit hyperactivity disorder. The behavioral and therapeutic effects of these drugs have been attributed to their ability to increase the synaptic concentration of dopamine by blocking the DAT and thus interfering with DA clearance from the synapse.

PET studies have been used to compare the pharmacokinetics of these two drugs and the degree to which they are blocked at behavioral and therapeutic doses. To compare pharmacokinetics, each drug was labeled with ^{11}C. Labeled cocaine and labeled MP were administered intravenously to assess their pharmacokinetics in the human brain. Both [^{11}C]cocaine and [^{11}C]MP rapidly reached the brain (peak uptakes were 4–6 min for cocaine and 8–10 min for MP). However, their clearance rates differed markedly: The clearance of [^{11}C]cocaine was much faster than that of MP ($t_{1/2}$ = 20 min versus 90 min, respectively). In the case of cocaine, its rapid uptake and clearance paralleled the short-lived, self-reported "high" induced by the drug (*33*). In the case of MP, the fast uptake of the drug paralleled only the ascending portion of the time course for the "high," which rapidly returned to baseline, even though MP had not cleared from the brain (*34*). The rapid uptake of intravenously administered [^{11}C]cocaine and [^{11}C]MP in the brain contrasts with the slow brain uptake of [^{11}C]MP when it is administered orally (*35*). Oral MP does not reach peak concentration in the brain until 60 min after administration. It has been postulated that this slow brain uptake is the reason why MP does not induce a high when administered orally, because the rapidity with which drugs of abuse exert their effects has been shown to be crucial in their reinforcing effects (*36*).

To determine whether DAT occupancy also might be a factor contributing to the different behavioral effects of cocaine and MP, DAT occupancy with pharmacologically effective doses was compared. Using [^{11}C]cocaine as a tracer for the DAT, it was found that an intravenous dose of 0.075 mg/kg MP was required to occupy 50% of the DAT (*37*). This is in the same range as intravenously administered cocaine, which occupies 50% of the DAT at a dose of 0.13 mg/kg. When MP was administered orally, a dose of 0.25 mg/kg was required to occupy 50% of the DAT (Fig. 2) (*35*). The latter dose is within the range of therapeutic doses used for attention deficit hyperactivity disorder. Despite the fact that oral MP induced levels of DAT blockade similar to those induced by reinforcing doses of cocaine, it did not induce a high. These comparative PET studies draw a compelling picture of the importance of drug pharmacokinetics and route of administration as major contributing factors in

abuse versus therapeutic potential (*36*). Indeed, when MP is administered intravenously, it produces a "high" that cocaine abusers cannot distinguish from that of cocaine (*34*).

Such studies that measure pharmacokinetics and DAT occupancy provide important knowledge in the continuing effort to develop a pharmacologic strategy for treating cocaine abuse (*38*).

Monoamine Oxidase B Inhibitor Drugs

The irreversible MAO B inhibitor drug L-deprenyl has been used for many years in the treatment of Parkinson's disease to reduce the MAO B catalyzed breakdown of brain dopamine (*39*). L-deprenyl also appears to have neuroprotective properties, although these properties do not appear to be associated with MAO B inhibition (*40*). Lazabemide is a new, reversible MAO B inhibitor (*41*). Before initiation of clinical trials, PET was used to determine the minimum effective dose and duration of action of lazabemide in a group of unmedicated patients in the early stages of Parkinson's disease. [^{11}C]L-deprenyl was used as a tracer for MAO B. A baseline PET scan with [^{11}C]L-deprenyl was obtained for each patient, and then each patient received 25, 50 or 100 mg lazabemide twice a day for 1 wk. Twelve hours after the last dose of lazabemide was administered, a second PET scan was obtained. Comparison of the second scan with the baseline scan showed that the 50-mg dose was sufficient to block >90% of the enzyme, whereas the 25-mg dose was inadequate. A third PET scan obtained 36 h after the last dose of lazabemide was administered showed that the inhibition was completely reversible after this short drug-free interval (Fig. 3) (*42,43*). This reversibility of inhibition of lazabemide contrasts sharply with the irreversible inhibition of the enzyme by L-deprenyl, which PET showed to have a half-time of 40 d for enzyme recovery after drug withdrawal (*44*).

From this PET study of lazabemide (*43*), it was possible to determine both the dose and the frequency of administration needed for clinical trials in Parkinson's disease. From the PET study with L-deprenyl (*44*), it is evident that MAO B inhibition can be maintained with lower or less frequent doses of L-deprenyl than are presently used.

Opiate Drugs

Opiate drugs have been of long-standing interest because of both their therapeutic applications to pain and their addictive liability. Several studies have documented changes in human brain metabolic activity in response to opioid drugs (*45*). Changes in CBF in response to fentanyl were found to be highly localized and to specifically affect brain regions associated with a range of pain-related responses (*46*). The sites of action of morphine also have been assessed in a study reporting increased blood flow in structures associated with the medial pain system both during acute pain and in relief of chronic pain (*47*).

An important problem in therapeutics is the use of opioid antagonists to reverse narcotic anesthesia within an appropriate time frame. Patients receiving naloxone to reverse the effects of opioid analgesics must be monitored carefully, because naloxone is short acting. In contrast, nalmefene, an opioid antagonist with a longer duration of action, has shown promise. Studies using [^{11}C]carfentanil (a radiotracer for μ opiate receptors) compared the duration of binding of naloxone with that of nalmefene and showed that naloxone and nalmefene have clearance half-times of 2 and 28 h, respectively. This study was conducted with a coincidence probe detector rather than a PET scanner (*48*). From this study, it was concluded that nalmefene's longer blockade of opioid receptors is advantageous in the clinical reversal of narcotic anesthesia and opioid side effects and the reversal of opioid overdose.

DRUG TOXICITY AND ADVERSE DRUG INTERACTIONS

PET has been used to examine potential central nervous system (CNS) side effects of non-CNS drugs. The fluoroquinolones (fleroxacin, lomefloxacin, ciprofloxacin, ofloxacin and temafloxacin) are a new class of antibiotics with a broad antibacterial spectrum and excellent tissue penetration (*49*). They act by inhibiting an essential bacterial enzyme, deoxyribonucleic acid gyrase. However, the use of these compounds has been associated with adverse CNS effects. Consequently, the Food and Drug Administration has mandated that, as part of the approval process, fluorinated quinolones must undergo PET evaluation to assess whether they affect either CBF or energy metabolism (*50*). Several PET studies have been performed with these compounds using FDG or ^{15}O-labeled water or oxygen to measure brain glucose metabolism, CBF or oxygen metabolism. These studies were performed before and after treatment with the drug to determine whether the drug changed these parameters (*51–53*). All studies reported that short-term administration of these drugs does not significantly alter CBF, brain glucose metabolism or oxygen metabolism. Interestingly, two studies reporting on the pharmacokinetics of two fluoroquinolone antibiotics, lomefloxacin and fleroxacin, labeled with ^{18}F indicated that peak concentrations of the drug occur in the liver and lungs, with low uptake in the brain (*54,55*).

PET studies also have revealed potentially toxic drug interactions. For example, recent PET studies with FDG have shown that active cocaine abusers have an exaggerated response to an acute dose of a benzodiazepine drug (*56*). This effect is manifested by severe drug-induced depression of brain glucose metabolism accompanied by intense sleepiness relative to nonabusing subjects. This intense response was interpreted as reflecting a disruption of γ-aminobutyric acid (GABA) neurotransmission from repeated cocaine administration. This study should alert clinicians of the potential toxicity from accentuated responses to sedative hypnotics in active cocaine-abusing subjects.

CANCER TREATMENT

A limited number of PET studies have been conducted to measure the accumulation of chemotherapeutic drugs in tumors. Some examples include [^{11}C]1,3-bis-(2-chloroethyl)-1-nitrosourea (*57*), cisplatin (labeled with $^{13}NH_3$) (*58*) and 5-fluorouracil (labeled with ^{18}F) (*59*). Chemotherapeutic drug accumulation was significantly improved using the intra-arterial route, and this strategy may serve to determine which patients may benefit from this route of administration. An important advancement in this area is the appreciation of multidrug resistance as a limiting factor in cancer chemotherapy and the development of imaging tools to probe this as a property of some tumors (*60,61*).

Pharmacokinetic strategies also have been investigated in boron neutron capture therapy in the treatment of brain tumors. Boron neutron capture therapy is based on the delivery of ^{10}B to tumor cells, followed by exposure to neutrons. When a neutron is absorbed by a ^{10}B atom, lithium ions and an α particle are produced, with tissue destruction occurring within 10 μ of the original boron atom. Because the success of boron neutron capture therapy depends on the delivery of adequate ^{10}B to the tumor, PET and an ^{18}F-labeled derivative of boronophenylalanine (the boron-containing drug) have been synthesized as a tracer to determine the accumulation of the drug in the tumor (*62,63*). Presently, ^{10}B accumulation in the tumor is measured by the analysis of surgical samples to plan for treatment.

PET has been widely used in detecting tumors and metastases and in monitoring their response to treatment. The most widely used radiotracer has been FDG, and its use for this purpose has been increasing rapidly (*64,65*). ^{11}C glucose also has been used to avoid using a lumped constant correction (*66*). In general, FDG uptake is elevated in actively growing tumors because of increased glycolysis (*67*). Because of the low body background from FDG, high-contrast images of tumors usually can be obtained (*10*). A patient's response to both chemotherapy and radiation therapy can be tracked using serial studies before and after the initiation of therapy, and several studies are underway to verify the role of FDG PET in cancer treatment.

Although FDG uptake has been an important tool in measuring cellular energetics in the diagnosis and treatment of cancer, other tracers have also been developed. [^{11}C]thymidine has been examined as an index of cellular proliferation in human cancers (*68*). Although, in principle, [^{11}C]thymidine should be the gold standard for measuring cellular proliferation, its rapid metabolism has been a limiting factor; thus, the recent development of [^{18}F]3′-deoxy-3′-fluorothymidine is an exciting new breakthrough (*69*). This tracer is metabolically stable and produces high-contrast images of normal bone marrow and tumors in canine and human subjects.

Other tracers have been developed to monitor additional properties of tumors, such as amino acid metabolism (*70*) and receptor status. [^{11}C]methionine is readily synthesized and has been used for many years as a marker for tumors (*71*). 16-α-[^{18}F]fluoro-17-β-estradiol has been used to identify estrogen-receptor-positive breast tumors that are likely to respond to antiestrogen therapy in patients (*72,73*). In addition, labeled compounds that bind to cell surface receptors, such as growth factors, are under development and may be of value in assessing other molecular properties of tumors and their responses to treatment (*74,75*). Similarly, labeled compounds that are sensitive to hypoxia (*76*) or those that can be used to assess multidrug resistance (*60,61*) are also valuable in planning and monitoring treatment.

CHIRAL DRUGS

Many drugs are marketed as racemic mixtures, which are defined as equal amounts of (S)- and (R)-enantiomers (*77*). For some of these drugs, the biological activity resides predominantly or totally in one enantiomer. However, whether the inactive enantiomer influences the behavior of the active enantiomer and whether there is a therapeutic advantage for single enantiomers are both important questions. For this reason, many older racemic drugs are being redeveloped as single enantiomer drugs. PET is an ideal tool for assessing the pharmacokinetics of individual enantiomers of drugs marketed as the racemic mixture. For example, the psychostimulant drug MP is marketed as the racemic mixture, although its pharmacological activity is known to reside in the *d-threo*-enantiomer. PET studies of individual enantiomers in the human brain show specific retention of the *d-threo*-enantiomer and no specific retention of the *l-threo*-enantiomer (*78*), indicating that *l-threo*-MP contributes little to DAT inhibition (Fig. 4). Thus, half of the weight of the administered drug is of no therapeutic value, although it may affect the enzymes involved in drug detoxification and could indirectly affect the pharmacokinetics of the *d-threo*-enantiomer. The availability of these labeled enantiomers sets the stage for pharmacodynamic studies addressing the therapeutic use of MP enantiomers.

NASAL ADMINISTRATION AND PULMONARY INHALATION

Nasal administration can be used to improve the bioavailability of drugs that undergo first-pass metabolism after oral dosing. It has been reported that nasal administration also may provide access to the brain for large molecules through areas that are not protected by the blood-brain barrier (*79*). A recent article (*80*) described the use of PET to track the biodistribution of tramcinolone acetonide, an adrenocortical steroid marketed to treat dermatoses, bronchial asthma, rheumatoid disorders and allergies. Tramcinolone acetonide was labeled with ^{11}C and was formulated and administered as a nasal inhalant in human volunteers. This study showed the immediate deposition of the majority of the dose on the target tissues: penetration into the sinuses along with moderate redistribution and slow migration to the throat. In addition, significant amounts of the drug remained in target regions for several hours. Although that study was techni-

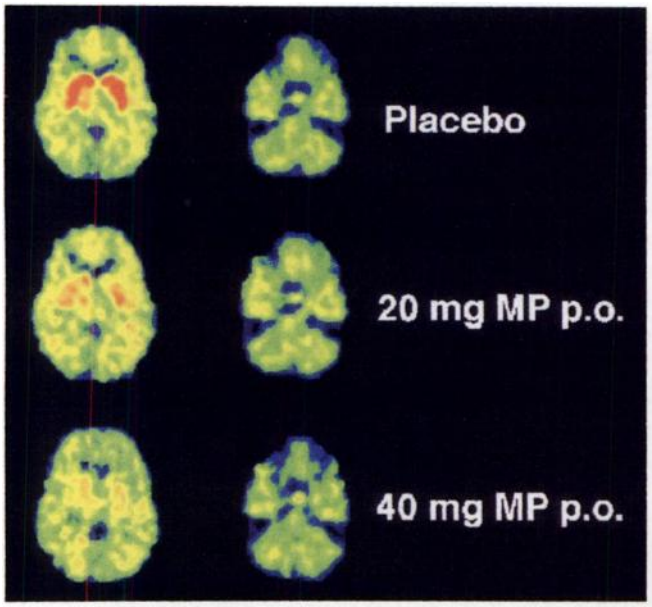

FIGURE 2. Images at level of basal ganglia and cerebellum obtained with [^{11}C]cocaine at baseline (placebo) and with 20 and 40 mg MP administered orally (p.o.) 2 h before labeled cocaine.

cally demanding, the information that was obtained is unique and demonstrates the ability of PET to screen locally administered drug formulations.

Another study examined the regional deposition of inhaled [^{11}C]nicotine vapor in the human airway, comparing shallow, frequent inhalations (buccal mode) with deep inhalations (pulmonary mode) (*81*). There was no significant difference in deposition between the shallow and deep modes of administration. The authors reported no statistically or clinically important differences between the buccal and pulmonary deposition patterns or elimination rates, placing the nicotine vaporizer in the same category as the nicotine polacrilex formulations. However, the authors pointed out that additional research is needed to assess whether the nicotine inhaler is better than the gum, because the inhaler is used in the same manner as cigarettes and may be preferred by individuals who want to quit smoking.

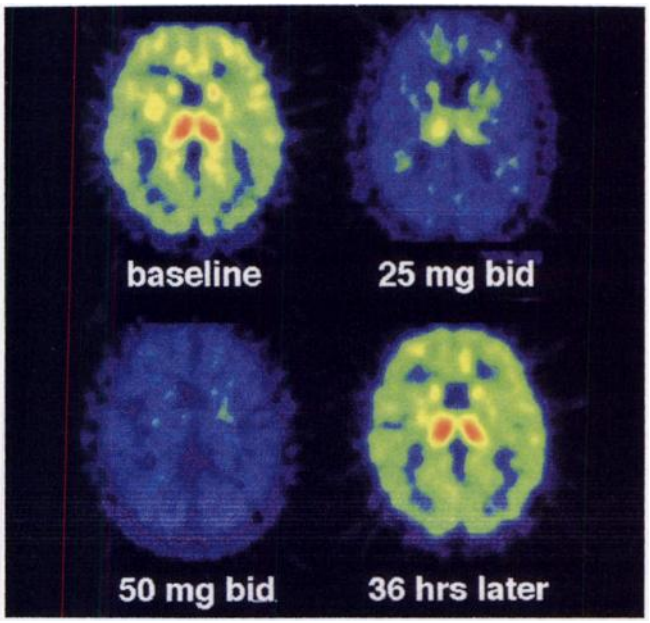

FIGURE 3. PET studies with [^{11}C]L-deprenyl to determine minimum effective dose of lazabemide needed to inhibit >90% of MAO B. Images are at baseline (upper left), after 50 mg twice a day (bid) for 1 wk (lower left), after 25 mg bid (upper right) and 36 h after administration of last dose of drug (lower right). Note that 50 mg bid produced high (>90%) inhibition. (Reprinted with permission of [*43*].)

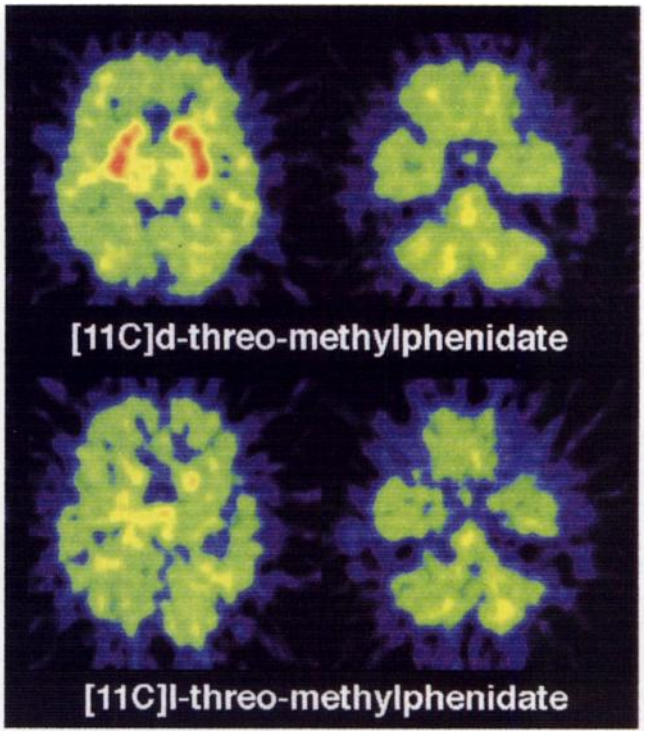

FIGURE 4. Images of human brain after injection of [^{11}C]*d-threo*-MP (top) and [^{11}C]*l-threo*-MP (bottom). Note absence of specific retention with *l-threo*-enantiomer. (Reprinted with permission of [*42*].)

TREATING ADDICTION

Addiction to both legal and illegal drugs is a major public health problem. When one considers the morbidity and mortality associated with cigarette smoking alone and the fact that smoking cessation at any age decreases the risk of lung cancer relative to a current smoker (*82*), it becomes clear that the understanding and successful treatment of addiction would represent a major advancement in health care. PET has provided information on the addicted human brain that may be valuable for developing new treatment strategies.

PET has shown that smokers have a reduction in both MAO A (*83*) and MAO B (*84*). This reduction may account for the reduced rate of Parkinson's disease in smokers (*85*) and also may contribute to some of the features of smoking epidemiology, including high rates of smoking in people with either psychiatric disorders, such as depression and

schizophrenia, or addictions to other substances. There is evidence that smokers are self-medicating in the case of certain psychiatric disorders and that they use smoking to reduce anxiety and to increase alertness and cognition. All of this evidence needs to be considered in the treatment of smoking addiction (*86*).

Drugs of abuse are thought to produce their rewarding effects by increasing DA in limbic brain regions. It is becoming more evident that the brain DA system is disregulated in both cocaine abusers (*87*) and alcoholics (*88*) and that reduced brain metabolism in the orbital frontal cortex and the cingulate gyrus are associated with low brain DA activity (*87*). Thus, there is a major effort in drug research on cocaine addiction to develop drugs that antagonize the ability of cocaine to increase DA concentrations by interfering with its reinforcing effects (*38*). However, if a decrease in DA brain function predisposes individuals to administer drugs of abuse, as suggested by the reward-deficiency-syndrome hypothesis of addiction (*89*), then cocaine "antagonist" drugs may not be sufficient to prevent relapse in these subjects. In this case, drugs that could help restore DA brain function could be therapeutically beneficial.

It has been shown that increases in DA caused by drugs of abuse can be modulated trans-synaptically by enhancing levels of the inhibitory neurotransmitter GABA with the anticonvulsant gamma vinyl GABA (GVG, a suicide inhibitor of GABA transaminase) (*90*). GVG significantly attenuates cocaine- and nicotine-induced DA release (*91,92*). This biochemical effect has been shown both by microdialysis in freely moving rats and by PET in baboons using [^{11}C]raclopride, whose binding is reduced by elevations in synaptic DA. GVG also abolishes cocaine- and nicotine-induced behaviors such as self-administration and conditioned place preferences in animals. This major new pharmacologic strategy for treating addiction has its roots in PET studies of neurotransmitter interactions.

AIDS DEMENTIA

Acquired immunodeficiency syndrome (AIDS) dementia complex is characterized by severe behavioral, cognitive and motor dysfunction and frequently occurs late in the course of the disease in patients with AIDS. However, subtle symptoms also can occur early in the disease and in children. PET studies have shown that functional brain impairment in AIDS dementia complex can be accompanied by regional or generalized reductions in brain glucose metabolism and that effective treatment with antiviral therapy (azidothymidine) can reverse these metabolic abnormalities. Thus, PET and FDG may be useful for assessing abnormalities and in tracking responses to antiviral therapy (*93*).

TRANSPLANTATION

Although the classic treatment of Parkinson's disease involves the replacement of brain DA using L-dopa, the restoration of DA production through tissue or cellular transplantation into the brain is also under investigation. To assess the efficacy of a transplant, it is necessary to monitor both behavioral and motor changes as well as the restoration of DA activity. PET with [^{18}F]fluoro-DOPA has been used to monitor the viability of transplants of fetal tissue (*94*). Recent PET studies of transplantation in Parkinson's disease have shown increased striatal [^{18}F]fluoro-DOPA uptake along with clinical improvement in some patients. An autopsy study of one patient who had received fetal nigral transplants demonstrated robust graft survival and striatal reinnervation, without immune rejection (*95*). The ability to objectively monitor noninvasively the efficacy of transplantation therapy may be an important aspect in the use of transplantation in the restoration of neurotransmitter function in several degenerative and neuropsychiatric disorders.

GENE THERAPY

The goal of gene therapy is to manipulate the expression of genes in human cells to treat genetically based diseases or to replace gene products that are lost as a result of disease processes. Targeting gene therapy to a specific tissue or to a tumor is a particularly active area of research, and there are many worldwide trials in gene therapy. Opportunities abound in this area because of the ultimate need to define the location, magnitude and persistence of gene expression over time in humans receiving gene therapy (*96*). These questions potentially can be answered with imaging methodologies. In addition, the development of antisense drugs that block gene expression at the ribonucleic acid level raises the need to develop methods for labeling oligonucleotides so that their pharmacokinetics can be tracked in vivo (*97*). Progress has been made in several areas.

In the area of imaging the expression of transfected genes in vivo, the concept of using a marker gene and a marker substrate has been validated in animals. The marker gene is not normally present in the host tissue, and the gene product is an enzyme that catalyzes a reaction with a labeled marker substrate. Thus far, the herpes simplex virus 1 thymidine kinase (HSV1-TK) gene has been used as a model, and radioiodinated 5-iodo-2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyluracil (*98*) and ^{18}F-labeled gancyclovir (*6*) have been used as marker substrates whose level of accumulation is sensitive to HSV1-TK enzyme levels determined in vivo.

In the area of imaging oligonucleotides for antisense therapy, the problem of rapidly labeling oligonucleotides with ^{18}F and measuring pharmacokinetics in vivo has been addressed and showed that PET nuclides are well suited for PET imaging in primates (*99*). However, the authors pointed to several hurdles (in addition to the synthesis of labeled antisense molecules) that limit the use of antisense therapy in vivo, including rapid breakdown by plasma nucleases, limitation in cellular penetration and potential toxicity. In the future, other delivery systems, such as liposomes and viral vectors, may serve to protect vulnerable drugs from systemic metabolism and may allow delivery of certain com-

pounds across the blood-brain barrier. PET can be envisioned as a tool for developing this area of therapeutics.

FUTURE STUDIES

Thus far, PET technology has not been applied widely in the initial stages of drug development. However, early studies could be extremely valuable in streamlining the drug development process, reducing the number of animals required and allowing early investigations of biodistribution and kinetics to be performed safely in humans. Early studies also may point out problems that were not anticipated, such as failure of a putative CNS drug to penetrate brain tissue (*100*). This will require closer collaboration between imaging scientists and scientists in the pharmaceutical industry. The organization of joint meetings and the publication of the results of imaging studies in meeting proceedings and pharmacology journals are important. Apart from this, the continued development of PET technology is crucial to progress. We have identified the following needs:

1. Basic research in radiotracer chemistry is needed to expand the range of labeled drugs and radiotracers available for application in drug research and development. A critical application of mechanistic biochemistry and pharmacology, as well as the principles of tracer kinetics, must be used to evaluate new tracers, paying special attention to their sensitivity to clinically relevant changes in different biological parameters (*101*). The development of tracers that are sensitive to changes in neurotransmitter concentration is of special importance in view of the need for quantitating the efficacy of drugs for treating neurodegenerative diseases.
2. In the drug design phase, it would be useful to incorporate either a fluorine atom or a carbon atom in a position or functionality amenable to labeling with either ^{18}F or ^{11}C. This would allow eventual PET imaging throughout the drug development process.
3. The development of safer methods for examining the efficacy of new drug delivery methods in humans is needed. Methods must be found for delivering drugs (e.g., growth factor) that do not normally pass the blood-brain barrier (either because of molecular weight or lipophilicity). The development of drugs that target endogenous transport systems within the capillary endothelium is a particularly exciting area (*102*).
4. Many drugs are still marketed as the racemic mixture. The development of synthetic methods and separations, which can be used to rapidly synthesize labeled single enantiomers, is needed to evaluate chiral drugs and to understand enantiomer-enantiomer interactions.
5. The development of tracers to image the molecular properties of tumors is needed to complement measures of tumor energetics and proliferative status and to guide the identification of new opportunities for treatment.
6. There is a need to develop methods for monitoring gene therapy, especially the development of radiotracers that can report on the synthesis of gene products, including those that can be used to track antisense therapies and those that can be used to track the delivery of viral substrates. This represents an enormous challenge, particularly when the restrictive time scale for synthesis is considered.
7. The development of methods for examining drug properties and a drug's ability to target multiple interacting systems rather than isolated systems is needed. Multidimensional approaches to drug development, including the combination of imaging with functional and behavioral measures, will optimize new knowledge from PET studies.
8. A sensitivity to important public health problems such as obesity, cancer, addiction and aging and degeneration, coupled with the need to develop new knowledge pertaining to mechanisms and therapeutic approaches, is vital.
9 Understanding biochemical variables that may predispose an individual to certain drug effects (i.e., toxicity, addiction) is needed.

CONCLUSION

Although PET is technologically complex, the payoff is that compounds labeled with positron-emitting isotopes can be used to track the distribution and kinetics of drugs and to map specific molecular targets in the human brain and body. Provided that appropriately labeled drugs and radiotracers are available, one can determine the amount of a drug that gets into the brain or other organs (or tumor), the minimum effective dose, the duration of action or the binding-site occupancy required to elicit a particular therapeutic or behavioral effect. This can be done with a relatively small number of PET studies. Because studies can be performed in humans, the relationship of pharmacokinetic and pharmacodynamic parameters to behavior and to therapeutic efficacy can be evaluated. The possibilities are enormous and are largely driven by advances in rapid synthesis that synergize with advances in pharmacology. However, the challenge of using imaging in the early stages of drug research and development remains an important one that is expected to be facilitated with the emerging use of small-animal scanners.

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