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PROOF OF CONCEPT: FUNCTIONAL MODELS FOR DRUG DEVELOPMENT IN HUMANS

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A drug developed for human use classically goes through a number of steps, including discovery, extensive preclinical studies for safety in experimental animals, and then human safety and efficacy studies. The Food and Drug Administration (FDA) requires successful completion of all the above tasks before approval for therapeutic use in human beings. The exciting advances in human genomics and combinatorial chemistry promise substantial applications to new drugs for human diseases; however, the time and expense associated with the processes necessary to bring a new drug to market are rising exponentially. Thus, there is a great need for functional models of disease progression, including animal models of human disease and biomarkers in human clinical trials.

BIOMARKERS AND SURROGATE MARKERS

The monitoring of biologic disease processes increasingly employs biomarkers (Table 34.1). At a recent National Institutes of Health (NIH) and FDA conference (1) biomarkers for clinical efficacy were divided into several groups including natural history markers, biological activity markers, and surrogate markers.

A relevant issue in clinical trials is the selection of an appropriate endpoint. Endpoints that are less deleterious than death or onset of a disease are desirable. Surrogate markers or endpoints are events of a more intermediate nature. These typically replace the final endpoints such as mortality. A surrogate marker is defined statistically as a ''response variable for which a test of the null hypothesis of no relationship to the treatment groups under comparison is also a valid test of the corresponding null hypothesis based on the true endpoint'' (2). A good biomarker, in contrast, provides information on the possible mechanisms of action of medications or the pathophysiology involved in a disease process. Surrogate markers that are helpful in early drug development (e.g., FDA Phase I and II trials) provide a prognostic indication of a clinical endpoint relevant to FDA Phase III trials.

Accelerated approval, which has become more common in FDA clinical trials, may occur based on surrogate marker effects although completion of longer-term clinical outcome and clinical endpoints may eventually be required. Prentice (1989) suggested that a surrogate marker must be both prognostic of disease progression and affected by treatment (2); the effect of the treatment on the surrogate marker should mediate the effect of the treatment on the clinical outcome or true outcome measure. There are not yet any surrogates for neuropsychiatric disorders that fully meet these criteria.

ROLE OF SURROGATES FOR DRUG DEVELOPMENT

The value of surrogates and biomarkers for drug development is recognized by the pharmaceutical industry. The needs to reduce development costs, improve the practical attainment of predictive outcomes, and expedite these endpoints are motivations for growing interest in this area. We present in the following an overview of the current state-of-the art regarding utilization of various surrogate markers, in a much broader sense of the term, which includes biomarkers, in drug discovery and development. We start with the most basic steps pertinent to initial studies in humans.

The integration of preclinical science and assessment of therapeutic potential in humans is done primarily through quantification of drug and active metabolites in accessible specimens (blood, urine, and cerebrospinal fluid). This is an advance on the maximum tolerated dose (MTD) approach, which necessarily prevailed before sufficiently sensitive assay

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TABLE 34.1. ABBREVIATIONS FREQUENTLY ENCOUNTERED WITH BIOMARKERS AND SURROGATE MARKERS

5HIAA	5-Hydroxyindoleacetic acid	MAO	Monoamine oxidase
5HT	Serotonin	MAOI	Monoamine oxidase inhibitor
5HT2A	Serotonin type 2A	MCCP	Meta-chlorophenylpiperazine
5HTP	5-Hydroxy-L-tryptophan	MCV	Mean red cell volume
ACTH	Adrenocorticotropic hormone	MDL	MDL 100,907
AD	Alzheimer's disease	MHPG	3-Methoxy-4-hydroxyphenylglyco
Ag	Agonist	MOI	Medical optical imaging
AIDS	Acquired immunodeficiency virus	MOS	Medical optical spectroscopy
ALS	Amyotropic lateral sclerosis	MRI	Magnetic resonance imaging
AMP	Amphetamine	MRM	Magnetic resonance microscopy
An	Antagonist	MRS	Magnetic resonance spectroscopy
APOE	Apolipoprotein E	MTD	Maximum tolerated dose
CNS	Central nervous system	NE	Norepinephrine
CSF	Cerebrospinal fluid	NERI	Norepinephrine reuptake inhibitor
D_2	Dopamine type 2	NIH	National Institutes of Health
D2R	Dopamine type 2 receptor	NIOS	Near-infrared optical spectoscopy
DA	Dopamine	NIRI	Near-infrared imaging
DAT	Dopamine transporter	NMR	Nuclear magnetic resonance
ECD	Ethylenediylbis-L-cystein diethylester	NMSP	N-methyl-spiperone
EEG	Electroencephalogram	OCD	Obsessive-compulsive disorder
EPR	Electron paramagnetic resonance	OCT	Optical coherence tomography
FDA	Food and Drug Administration	P-EEG	Pharmaco-electroencephalogram
FDG	Fluorodeoxyglucose	PET	Positron emission tomography
fMRI	Functional magnetic resonance imaging	PK	Pharmacokinetic(s)
GABA	Gamma aminobutyric acid	PRE AMP	Before amphetamine
GBR	GBR 12909	PRL	Prolactin
GGT	Gamma-glutamyl transferase	rCBF	Regional cerebral blood flow
GH	Growth hormone	SPECT	Single photon emission computed tomography
HCFA	Health Care Financing Administration	SSRI	Selective serotonin reuptake inhibitor
HIV	Human immunodeficiency virus	SWS	Slow wave sleep
HMPAO	Hexamethylpropyleneamine oxime	V_h	Volume of distribution
HVA	Homovanillic acid		
IV	Intravenous		

methodology was available. In current drug development, upper doses for early human studies are based on toxicokinetic studies in at least two animal species (most often rat and dog) whereby one does not exceed some predetermined ratio between the exposure (i.e., plasma concentration) for the dose in humans and the exposure associated with toxicity in the most sensitive species (3). Thus, depending on the ratio selected, exposures in humans may produce few side effects and be below those achieved in the older MTD approach.

In practice, one often embarks on clinical studies with a constricted range of exposures and an inability to address the question of whether therapeutic (or biochemical) effects are greater at the MTD until much later in development (4). If a compound does not produce significant side effects within this original range, doses much higher than those used in initial Phase I studies can be tested depending on the nature of the toxicity observed in animals at the limiting exposure. Even if a limiting dose is identified in volunteer subjects, a greater dose may be tolerated in patients (5). Sramek and colleagues (1997) (6) have defined this transition from dosing in healthy volunteers to patients to be "bridging." For instance, the difference in tolerated dose for schizophrenic patients compared with normal volunteers is often greater than 25-fold (5). Interestingly, before pharmacokinetic (PK) data were routinely used to limit exposure, one explored an MTD relatively early and concluded that if response was not achieved by that dose, one did not have a viable clinical candidate.

Most marketed and late in development psychopharmacologic agents do show limiting side effects above the recommended dose range, especially if such doses are given initially. Starting with three or more times the lowest standard dose of a selective serotonin reuptake inhibitor (SSRI) will produce far more marked nausea (and even vomiting) than after building up to the same dose over several weeks, as is done for the treatment of obsessive–compulsive disorder (OCD). This may seem simply common sense but the perceived market advantage of the starting and ultimate therapeutic dose being the same discourages exploration of higher doses that require some sort of titration over time in order to be well tolerated. For instance, even though it was appreciated early on that an antidepressant, venlafaxine, might be more efficacious at higher doses that were associated with

TABLE 34.2. MEASURES THAT SERVE AS SURROGATES FOR PREDICTED BIOCHEMICAL EFFECT OF DRUGS TARGETED TO MONOAMINE NEUROTRANSMITTERS, TRANSPORTERS, RECEPTORS, AND DEGRADATIVE ENZYMES[a]

	Neurotransmitters and Metabolites[b]			Plasma Hormones			Physiologic	
Drug Class	**NE and MHPG**	**5HT, 5HIAA**	**HVA**	**PRL**	**GH**	**ACTH/Cortisol**	**Temperature**	**SWS**
Serotonergic								
SSRIs		↓5HT, ↓5HIAA		±↑				
Indirect-Ag				↑	↑			
$5HT_{1A}$-Ag				±↑	↑	↑	↓	
$5HT_{1B/D}$-Ag				↓	↑			
$5HT_{2C}$-Ag				↑		↑	↑	↑↓[c]
Noradrenergic								
NERI	↑NE, ↓MHPG				↑			
α2-Ag	↓NE				↑			
α2-An	↑NE							
Dopaminergic								
D2-An			↑↓[c]					
DA-Ag			↓					
Mixed								
Tricyclic antidepressants	↓MHPG	↓5HIAA						
MAOIs	↓MHPG	↓5HIAA	↓					
Atypical antipsychotics	±↑NE			=[d]				

5HIAA, 5-hydroxy indoleacetic acid; 5HT, serotonin; ACTH, adrenocorticotropic hormone; Ag, agonist; An, antagonist; GH, growth hormone; HVA, homovanillic acid; MAOI, monoamine oxidase inhibitor; MHPG, 3-methoxy-4-hydroxyphenylglycol; NE, norepinephrine; NERI, norepinephrine reuptake inhibitor; PRL, prolactin; SSRI, selective serotonin reuptake inhibitor; SWS, slow wave sleep.

[a]This table indicates the utilization of neurotransmitters and their metabolites, plasma hormones, and physiologic phenomena as surrogate markers for the biochemical effects produced by drugs on the indicated classes. Although the specified drug classes may affect other measures than those indicated (e.g. SSRIs also decrease MHPG), only those hypothesized to reflect a primary action are shown.

[b]In plasma, platelets, urine, and/or cerebrospinal fluid.

[c]Acute increase followed by decrease in responders.

[d]Equal sign (=) indicates no change.

more marked side effects (7), it was marketed at target lower doses (37.5 mg b.i.d.), which only produce effects consistent with serotonin reuptake inhibitory activity. In the over 225 mg per day range (which requires some degree of titration to avoid unacceptable side effects), venlafaxine produces effects consistent with norepinephrine (NE) as well as serotonin (5HT) uptake inhibition (8,9) and is reported to be superior to SSRIs (10,11). Obviously, it would be beneficial if the relevant dose ranges of such compounds could be better understood earlier rather than later in the life of a drug.

In this particular example, if valid markers of 5HT and NE uptake inhibition in the brain were investigated as early in development as possible, then the doses necessary to achieve both effects and those achieving only one could have been systematically pursued from the outset. Such markers would be "surrogate" markers for clinical doses, allowing one to test the widely held hypothesis that in a big enough population of patients with depression, one will find those who respond better to combined NE/5HT uptake inhibitors than to SSRIs alone. Table 34.2 shows examples of biochemical markers that may serve as surrogates.

In the section that follows we briefly review how traditional surrogate markers (in the broadest sense), which antedate the application of brain imaging technology, are used.

PHARMACOKINETICS

Analytical technology, especially that provided by much simpler to operate equipment, allows for precise quantification of very low concentrations in blood so that rapid and accurate determination of a new compound's pharmacokinetics (PK) is possible after much lower doses than required formerly. If a half-life is too short or long or if nonlinear pharmacokinetics are marked (e.g., half-life increases with doses in the expected therapeutic range), then development may go no further. Similarly, if a compound shows unacceptably wide variations in clearance because its metabolism is dependent on a highly polymorphic isozyme of cytochrome P-450 (e.g., CYP2D6), then it will be seen as of lower potential commercial value. Usually, *in vitro* tests using human hepatic microsomal preparations or P-450 isozyme specific cloned systems are used to screen for this possibility prior to human use, but these are not always

fully predictive. Thus, concentrations measured over time in Phase I studies serve, at this most basic level, as a surrogate of whether a compound has the potential to become a successful drug (12).

Drug interactions sometimes can constitute a serious safety risk (e.g., inhibition of terfenadine metabolism by CYP3A4 inhibitors leading to fatal QTc prolongation in genetically susceptible individuals) and, almost always, a marketing disadvantage. The same preclinical approaches referred to in the preceding are used to screen out compounds that depend for their metabolism on a problematic pathway. *In vivo* PK (and sometimes drug interaction data) are also obtained in animals, but more to set dose and frequency of administration to guarantee adequate concentrations for expensive chronic toxicology studies than to predict what the PK characterization will be in humans.

Low bioavailability (i.e., concentration after oral versus intravenous doses) is a special problem at this stage of preclinical development. First, for most compounds an oral form is seen as the ideal (if not only) option and low bioavailability almost always means very high variability in exposure to parent compound for a given dose. For instance, 2% to 10% bioavailability means an automatic fivefold range before individual variations seen in metabolic clearance after intravenous (IV) administration are taken into account versus 60% to 80% bioavailability, which only entails a $1\frac{1}{3}$-fold variation. High variability in exposure per unit dose makes clinical studies more difficult. Second, to reach the exposures required to define the concentration at which chronic toxicity is observed, low bioavailability means that much more compound will be necessary. This is actually a far more significant issue than is generally appreciated because amounts of material are limited early in development. It can be very costly to develop efficient synthetic schemes; one does not want to invest too much in syntheses until one is fairly sure that a compound is safe at pharmacologically active concentrations.

TOXICOKINETICS

This introduces the critical role of toxicokinetics, the goal of which is to establish in animals (usually rats and dogs) the ratio between the concentration that produces either unacceptable physiologic effects (e.g., QTc prolongation, seizures) or organ damage (e.g., liver, bone marrow) and the one that produces the targeted biochemical or behavioral effect, a ratio of at least 2 to 1. As the ratio approaches 1.0 it is increasingly unlikely that a compound will be taken into humans unless the observed unwanted effects are readily reversible and one is dealing with a condition for which existing therapies are poor or nonexistent (Alzheimer's disease [AD] or amyotrophic lateral sclerosis [ALS]). There are instances in which humans ultimately prove tolerant to drugs much more than would be predicted by these ratios, but this is only established after exposure to large numbers of subjects.

Although the ratios established in preclinical studies are interpreted as providing a reasonable estimate of any ultimate therapeutic index in humans, they are specific for both species and mechanism of action. In other words, the concentration necessary to achieve a behavioral or biochemical effect and produce toxicity can vary more than an order of magnitude across species. The relationship among *in vitro* biochemical effects, activity in some *in vivo* model, and undesired effects is poorly understood even at the preclinical level, particularly when one is investigating novel targets. Because efficacious *in vivo* concentrations in animals are based either on activity in an animal model (e.g., anticonflict activity to identify a potential anxiolytic) or biochemical changes (e.g., changes in brain serotonin) that are not measured in humans, we do not even have validation for existing drugs relating preclinical and clinical data (13). Not surprisingly, given the weakness of the link between an efficacious dose in rats and a therapeutic dose in humans, the clinical development of central nervous system (CNS) drugs is notoriously inefficient to identify therapeutic concentrations.

Measures of receptor occupancy or specific degree of enzyme inhibition do not drive the selection of an efficacious concentration. In other words, the basic preclinical work that underlies any test of a specific hypothesis such as "X% of inhibition produces effect Y" is rarely, if ever, done. Therefore, in toxicokinetic studies one hopes for very high ratios that provide a great deal of flexibility to study a extremely wide range of concentrations in humans, especially with novel compounds.

Phase I

Select starting doses for healthy volunteers based on findings from the preceding toxicokinetic studies, which is often one-tenth of the dose that produces a no-adverse-event level in the most sensitive species studied (4). Most marketed central nervous system (CNS) drugs produce some sufficiently unpleasant (but not dangerous) side effect that sets the dose range for subsequent studies, the so-called maximum tolerated dose (MTD). Multiple dose studies, administering various fixed doses over a 2- to 4-week period are also part of the Phase I protocols to get a general idea of safety and tolerability under likely clinical conditions (14). The observed steady-state concentration during repeated dosing, broadly speaking, is the first surrogate for the desired effect, the obvious but often incorrect assumption being that a certain value assures a certain biochemical effect in the brain based on extrapolations from preclinical data.

Once the initial safety studies are completed, there may be additional studies in healthy volunteers to assess efficacy. In the case of sedatives and hypnotics, healthy volunteers can be used reliably with a combination of subjective reports, objective measures of performance (looking for decrements), and the electroencephalogram (EEG) (15–19). These measures, however, are direct ones, of a desired or undesired effect, not surrogates. On the other hand, the so-

called *pharmaco*-EEG (P-EEG) is sometimes introduced as a surrogate measure of the therapeutic action a compound is likely to produce (20,21), but is probably more usefully viewed as a direct measure that a compound has produced some change in surface brain electrical activity. The P-EEG provides neither evidence of a direct effect in the brain nor proof of a hypothesized causative role of specific pharmacologic activity of the molecule (22).

In the majority of CNS Phase I studies, aside from measures of drug concentration, the only usual objective measures are targeted to the heart and other vital organs. Side effects are often simply recorded as spontaneous reports on a grid of prespecified subjective descriptors; sometimes a formal checklist is used. Thus, quantitative surrogate measures of drug effect are sparse.

Phase 1B/2A (Proof of Concept) Studies

There is wide variation in practice as to what is done, both with healthy volunteers and patient populations, before going on to full Phase II studies. These latter studies are powered to detect a clinically significant improvement, usually over placebo, often utilizing multiple dose arms (each with at least 50 or more patients) and multiple clinical sites. In the case of a compound acting on a novel target, the period before full Phase II trials is argued to be the phase during which exploratory work should be done (23). For instance, if one had an ampakine such as CX-516 that improved learning in rats, then one might show enhancement of some aspects of cognition in healthy volunteers after safe doses were established in Phase I studies (24). The improved performance in healthy subjects would then be a surrogate for improvement in AD to identify the appropriate dose.

In the more usual case of a variation on an existing mechanism (e.g., SSRIs), this is the period to verify which dose produces the desired biochemical effect so as to move as quickly as possible into Phase II trials with doses that one is confident will work (25,26). In this latter instance, the direct accessible biochemical measure becomes a surrogate for an effect in brain that is hypothesized to produce the clinical benefit.

We briefly review a range of specific measures that have been used in enough instances over the last two decades to qualify as surrogate markers. There is no available literature as to the extent to which these measures actually drove decisions about what doses to use in Phase II trials, but one can assume that they must have been influential in some cases. What is certain is that at least in the United States, outside of polysomnography (for sleep-related disorders) and EEG (for seizures), the measures have had no acknowledged regulatory impact. Assessments of decrements in performance (especially ability to concentrate, recall, and carry out motor tasks) and measures of cognitive enhancement all seem to be better classified as direct measures of an intended or unintended drug effect rather than surrogates. This leaves mainly biochemical measures, which can be broadly classified as ''neuroendocrine.''

Cerebrospinal Fluid

The monoamine hypotheses of depression and schizophrenia, which are, in turn, based on the pharmacology of antidepressants and antipsychotics, generated an interest in measures of norepinephrine (NE), serotonin (5HT), and dopamine (DA) in humans. In most instances these were initially studied in an attempt to distinguish patients from controls but came to be applied to validating predicted effects of psychotropic agents (27).

Thus, NE reuptake inhibitors (NERIs) decrease NE turnover as measured by NE and its metabolites in urine or cerebrospinal fluid (CSF) (28–31) and by increasing plasma NE (32) or, more precisely, its spillover rate (33, 34). Another marker of NE reuptake inhibition is based on the need for exogenously infused tyramine to be taken up into NE nerve endings to exert its effects, the so-called tyramine pressor test (35) or, more recently, the dorsal hand vein constrictor test (36). These latter two procedures have been recently employed to establish the dose at which venlafaxine produces NE reuptake inhibition (8,9,36). Because NE metabolism varies according to whether it occurs inside or outside neurons (37), a more definitive and perhaps more sensitive measure would be the ratio of extraneuronal to intraneuronal metabolites before and after treatment in an integrated pool (e.g., a 24-hour urine) (38) as shown for desipramine (30). However, all of these measures reflect functional changes in the peripheral sympathetic nervous system; therefore, they are at best surrogates for effects in the brain.

Some of these same measures have been applied to study other compounds, which are predicted to affect NE function either following acute or chronic administration. These include bupropion, clozapine, alpha 2 antagonists, and monoamine oxidase inhibitors (MAOIs) (39–46). Interestingly, such studies are not yet available to support or refute claims that the relatively new antidepressant mirtazapine produces alpha 2 antagonism in humans.

Most, if not all, drugs classified as SSRIs have been shown to inhibit platelet uptake of 5HT, most simply determined by investigating 5HT depletion in platelets following chronic administration (25,47–50). Some of these studies were clearly done prior to Phase II trials so they can be inferred to have influenced the selection of dose. Doses of SSRIs known to inhibit platelet uptake in humans have also been shown to reduce the turnover of 5HT in the CNS as reflected by reductions of its major metabolite 5-hydroxyindoleacetic acid (5HIAA) in CSF (44,51). Similarly, MAOIs reduce both platelet MAO and 5-HIAA in CSF (51,52). There are not, however, systematic dose response studies to compare the sensitivity of the peripheral and central measures. Moreover, 5HIAA in CSF obtained by a lumbar puncture reflects a complicated process of all sources of formation and clearance of the metabolite.

DA has been studied almost exclusively in terms of its metabolite, homovanillic acid (HVA) in blood and CSF. There is a complex relationship among administration of a

dopamine type 2 (D_2) antagonist, duration of treatment, clinical state, and HVA in either compartment (53–58). Although changes usually can be explained as consistent with altered turnover as a function of receptor antagonism, time, and presence or lack of clinical response, measures of HVA are not really useful as a surrogate measure of D_2 antagonism. Decreased HVA in CSF can be expected after mixed Types A and B MAO inhibition in the brain (52) and might be relevant to assessing DA uptake inhibition. There is, however, no available positive control for the latter. Bupropion, the one compound studied in regard to possible DA uptake inhibition, did not decrease HVA in the CSF (40,44).

Prolactin

Prolactin has the potential to be used as a marker for drugs affecting these systems, because either DA or 5HT can affect this circulating hormone. In the simplest and most widespread instance, it has been used as an index of D_2 antagonism. Unlike typical neuroleptics, atypical neuroleptics produce no elevations of prolactin at therapeutic dosages (59, 60). By extension, absence of prolactin elevation after antipsychotics could be considered a surrogate of low D_2 receptor occupancy in the striatum and, hence, imply low to absent motor side effects (61,62).

Prolactin is also elevated following various pharmacologic challenges such as: (a) those that are predicted to increase extracellular 5HT in the brain including fenfluramine, clomipramine, l-tryptophan, and 5-hydroxytrytophan; and (b) those that stimulate various types of 5HT receptors, including meta-chlorophenylpiperazine (mCPP) and some, but not all, putatively selective $5HT_{1A}$ agonists (63–66). In all of these instances, prolactin increase becomes a surrogate of increased serotonergic transmission in one or more regions of the brain. Blockade of this effect by appropriate 5HT receptor specific antagonists could serve, in turn, as a surrogate of a compound's ability to functionally antagonize the specified receptor in human brain (67–70).

Growth Hormone

There was a period in which plasma growth hormone (GH) was used as a surrogate for increased noradrenergic transmission in human brain (presumably at the level of the hypothalamus) after, for instance, the $\alpha 2$ agonist, clonidine, or an NE uptake inhibitor (71,72). One could, in turn, infer $\alpha 2$ antagonism by a test compound if it blocked the effect.

More recently, stimulation of plasma GH has been considered evidence of activation of both $5HT_{1A}$ and $5HT_{1B/1D}$ receptors (see Table 34.2 on page 459) (63). Sumatriptan increases GH, apparently through activation of the $5HT_{1B/1D}$ receptors (73,74), with the most recent evidence using the more brain penetrant, zolmitriptan, implicating $5HT_{1D}$ postsynaptic receptors (75). It has been suggested that stimulation of the 1B/1D receptors inhibits somatostatin release (76); however, an increasing volume of experimental research indicates that 5HT can act directly on the adrenal gland and possibly on the anterior pituitary as well (77). This provides another example of the same measure serving as a surrogate for very different CNS effects, the interpretation of which depends on knowing the potential target for a compound in advance. It is doubtful that any substantial decisions concerning doses of compounds affecting either noradrenergic or GABAergic or serotonergic systems have ever been made based on GH release stimulation or inhibition.

ACTH/Cortisol

Stimulation of the hypothalamic–pituitary–adrenal axis as reflected by increases of ACTH and/or cortisol has also been used as a marker of drug action in the CNS. This approach has been most extensively applied to evaluation of putative 5HT agonists, sometimes coupled with pretreatment with whatever antagonists were available (e.g., ritanserin and pindolol) (63). Such studies can generate evidence of apparent selectivity in postsynaptic receptor responses to indirect and direct agonists. ACTH release induced by 5-hydroxy-L-tryptophan (5HTP) is argued to occur through indirect activation of 5HT2 receptors because it is antagonized by ritanserin (78), whereas the cortisol response is not affected by doses of pindolol expected to produce $5HT_{1A}$ antagonism (67). Pindolol does, however, antagonize ACTH responses to a variety of agents classified as partial to full $5HT_{1A}$ agonists (64,79,80). Again, given the complexities of the ACTH/cortical response and the imperfect selectivity of the pharmacologic agents, quantitative conclusions as to degree of specific receptor activation or antagonism are not possible.

Other Physiologic Measures

Sleep EEGs have already been referred to and may have utility as surrogates of, for instance, activation or antagonism of 5HT2C receptors as reflected by decreases or increases of slow wave sleep, respectively (81,82).

Temperature decreases are consistently observed following $5HT_{1A}$ agonists (63,83), and hence can serve as a surrogate marker of $5HT_{1A}$ agonist effects in the CNS. Evaluating the ability of a novel compound to antagonize the hypothermia produced by a $5HT_{1A}$ agonist may be the easiest way to see if it antagonizes $5HT_{1A}$ receptors in the human brain.

LIMITATIONS OF CURRENT SURROGATE MARKERS

As described, there is no validated link between the concentration of drug in blood (or even CSF) and a specific biochemical effect in human brain (not to mention a specific brain region). Thus, even in the case of SSRIs, the most

widely prescribed class of drugs in neuropsychopharmacology, we do not know how closely platelet 5HT depletion reflects 5HT uptake inhibition in brain. Although known therapeutic doses of SSRIs invariably have been shown to deplete platelet 5HT, the converse is not clear; that is, any dose that is effective in platelets will be therapeutic. Matters are even less certain when it comes to using available surrogates of NE uptake inhibition to establish the dose of a drug. And, as already noted, primary dosing decisions are not made on the basis of whether compounds affect prolactin, growth hormone, or ACTH/cortisol responses.

One could use the CSF concentration of a drug as a reasonable estimate of the free drug concentration in brain under true steady-state conditions (84–86), and infer from one's preclinical *in vitro* and *in vivo* studies that this will produce a specific effect (87). The problem is that even in the most refined *in vitro* system, as represented by cloned human receptors expressed in some vector, the relationship among receptor occupancy, drug effect, and free drug concentration may be extremely different from that observed *in vivo* in humans thanks to multiple uncontrollable differences among these systems (13). Furthermore, *in vivo* animal studies raise questions about species differences; therefore, how does one select the dose for clinical studies when testing a new compound that is well tolerated with a wide range of safe concentrations that are predicted to be pharmacologically active by one or more preclinical models? How does one know that the target in question has been blocked or stimulated so as be sure that one is testing the hypothesis that such an effect produces therapeutic benefit? The answer is, one does not know with the surrogates (discussed in the preceding). This brings us to a discussion of the promise of direct measures of drug effect in the brains of living humans.

IMAGING STUDIES

Measures of Substrate Metabolism

Regional cerebral blood flow (rCBF) is a well-established surrogate marker widely used for both clinical diagnostic procedures and new drug development. rCBF is commonly assessed utilizing estimates of cerebral perfusion, such as single photon emission computed tomography (SPECT) with radioligands labeled with ^{123}I or ^{99m}Tc; for example, [^{123}I]iodoamphetamine, [^{99m}Tc]ethylenediylbis-L-cystein diethylester (ECD), and [^{99m}Tc]hexamethylpropyleneamine oxime (HMPAO). Although repeated assessments with multiple radiotracer injections are usually impractical because of the long half-lives of both ^{123}I and ^{99m}Tc, linearization techniques have been developed to estimate sequential measurements (88). Furthermore, ^{99m}Tc is relatively available in practically every hospital with a radiology or nuclear medicine department worldwide; therefore, SPECT procedures can be performed widely on a clinical basis.

By contrast, positron emission tomography (PET), another procedure to estimate rCBF, requires an onsite cyclotron, so it is not available in many areas. Recent third-party camera reimbursement of some PET procedures (primarily [^{18}F]fluorodeoxyglucose (FDG)) are making PET cameras more available, but cyclotrons are still relatively scarce. The two most widely used radioisotopes used in PET are ^{11}C with a half-life of 20 minutes and ^{18}F with a half-life of 110 minutes. Additionally, ^{15}O, with a half-life of 2 minutes is primarily employed in brain perfusion studies. The most available PET radioligand is FDG, a tool to measure glucose metabolism. Its advantages include ease of use, availability from commercial cyclotrons throughout much of the world, and sensitivity to a number of studies to facilitate drug development and assess mechanism of action (89). Its disadvantage is lack of specificity.

An example of the value of PET studies to obtain potential surrogate markers for development of drugs for substance abuse is the dysfunction of regional glucose metabolism in the limbic system and areas of working memory in cocaine euphoria and cocaine craving (90). [^{15}O] PET studies demonstrate increases in rCBF in the limbic system and decreases in the basal ganglia as manifestations of craving for cocaine following exposure to videotapes suggesting cocaine use (91).

ESTIMATION OF DOPAMINE RECEPTOR OCCUPANCY BY ANTIPSYCHOTIC DRUGS

It has been hypothesized that positive symptoms of schizophrenia, such as hallucinations and delusions, result from increased stimulation of postsynaptic dopamine (DA) receptors by DA (92). This DA hypothesis of schizophrenia is substantiated by the observation that positive symptoms of schizophrenia abate when DA receptor blocking drugs, such as neuroleptics, occupy the postsynaptic DA receptors. In 1988, Farde and colleagues demonstrated the concept that effective neuroleptic dosages for schizophrenia correspond to 80% to 90% occupancy of DA type-2 receptors (D_2Rs) by the drug (95). Thus, occupancy of 80% to 90% of D_2Rs may constitute a surrogate marker for the dosage of a D_2 antagonist producing maximal beneficial effects with minimal adverse and toxic effects. Furthermore, clinically equivalent doses of neuroleptics are estimated by comparing proportions of receptors occupied by various psychotherapeutic agents (93,94). Several examples of this approach are given in the following.

Receptor Occupancy by Typical Neuroleptics

The occupancy of D_2Rs by neuroleptics has been extensively evaluated by PET and SPECT. Historically, this began with the study of typical neuroleptics, including haloperidol and

fluphenazine, in healthy normal control subjects and patients with schizophrenia utilizing [^{11}C]raclopride (95,96), spiperone derivatives, including [^{11}C]N-methyl-spiperone (NMSP) (97), [^{18}F], or ^{76}Br (98). This research has established that a therapeutic response corresponds to occupancy of 65% to 90% of the D_2Rs by the drug; however, occupancy of greater than 90% of the D_2Rs is not associated with a greater therapeutic effect. Therefore, occupancy of 65% to 90% of D_2R_2 is correlated with a therapeutic dose of typical neuroleptics as well as other clinical manifestations of pharmacologic efficacy. For example, patients with acute extrapyramidal side effects were found to have higher DA receptor occupancies (82%) than those without (74%) (99, 100). Wolkin and colleagues (1989) found a comparable occupancy of haloperidol in responders versus nonresponders indicating that treatment nonresponse is not a function of insufficient CNS binding of the antipsychotic (100).

Kapur and colleagues recently confirmed that the D_2 occupancy is an important mediator of beneficial and adverse effects (101) in a study of first episode schizophrenia and haloperidol. Although the patients showed a wide range of D_2R occupancy (38% to 87%), the degree of D_2R occupancy predicted clinical improvement, hyperprolactinemia, and extrapyramidal side effects. Also, Daskalakis and associates (1998) (102) found a relationship between D_2R occupancy and hyperprolactinemia.

Receptor Occupancy by Atypical Neuroleptics

D_2R occupancy has been determined for humans treated with atypical neuroleptics. For example, receptors of people treated with clozapine exhibit low (20% to 65%) D_2R occupancy and high serotonin type 2A ($5HT_{2A}$) receptor occupancy (94,99,103). In studies directly comparing clozapine (450 mg/day) and haloperidol (5 mg/day), there was a reversal of receptor occupancy producing high D_2 blockade with haloperidol and high $5HT_{2A}$ blockade with clozapine. Indeed PET imaging has been an important approach for screening a number of new antipsychotics with an atypical profile. Risperidone has been shown to block 40% to 60% of D_2 receptors at only 1 mg (104) with even higher $5HT_{2A}$ receptor occupancy. Indeed, in patients with schizophrenia, only 4 mg/day is needed for 70% to 80% occupancy with minimal risk of extrapyramidal side effects (105). After an oral dose of 6 mg risperidone, 75% to 80% of D_2Rs and 78% to 88% of $5HT_{2A}$ receptors are occupied (106). Plasma levels of risperidone correlate with D_2R occupancy (107). The proportion of D_2R occupancy has been estimated for other atypical neuroleptics; for example, olanzapine, has been shown to have D_2 occupancy similar to risperidone and greater than clozapine (108). The usual clinical doses of olanzapine (10 to 20 mg/kg) produce 71% to 80% D_2R occupancy. These doses usually do not result in the adverse effects of dyskinesias and prolactin elevation. On the other hand, doses of greater than 30 mg/day olanzapine are associated with greater than 80% D_2R occupancy as well as dyskinesias and prolactin elevation. Olanzapine exhibits 59% to 63% D_2Rs occupancy after single 10-mg dosing. Furthermore, olanzapine shows a greater (68% to 84%) occupancy of D_2Rs than clozapine (20% to 67%) after 10 to 20 mg daily dosing in patients (60). These characteristics suggest that olanzapine differs from clozapine. Additionally, quetiapine is characterized by abatement of psychotic symptoms in association with a transient increase in D_2R occupancy (62).

Receptor Occupancy as a Surrogate Marker of Clinical Efficacy?

One of the most important questions about D_2R and $5HT_{2A}$ receptor occupancy is the prediction of doses yielding clinical efficacy. D_2R occupancy predicted the clinical improvement of 22 first episode schizophrenic patients randomly assigned to 1 or 2.5 mg/day of haloperidol for 2 weeks (101). Thus, D_2R occupancy is related to the clinical response to antipsychotics (99,109,110).

PET studies have helped demonstrate that high levels of D_2R occupancy occur at very low haloperidol doses (111). Kapur (1996) showed D_2R occupancy of 53% to 74% at only 2 mg haloperidol in first-episode patients (114). Kapur and colleagues (1997) also showed D_2 occupancies between of 53% to 88% for haloperidol doses of 1 to 5 mg. Thus, the conventional therapeutic practice of haloperidol doses of greater than 10 mg/day is too high for many schizophrenic patients because there is no increase in beneficial effect, whereas the risk of adverse effects increases in proportion to the dose (112). Nevertheless, Volavka and colleagues (113) (1995) showed that antipsychotic efficacy of haloperidol increases with plasma levels up to 12 ng/mL, plasma levels that would predict almost completely saturated D_2 receptors according to the Kapur and associates (1997) data (112). Similarly, Wolkin found D_2 receptor occupancy increasing with haloperidol plasma levels up to 15 ng/mL and almost complete D_2R occupancy saturation with haloperidol plasma levels above 15 to 20 ng/mL (100). Thus, Wolkin's (100) and Volavka's (113) findings confirm each other and contradict Kapur's (112,114) studies. These differences could be explained partly by the differences in radioligands and patient populations. Further research by other investigators with various populations is needed to resolve the controversy.

Another important implication of receptor occupancy with neuroleptics is the prediction of extrapyramidal side effects. The probability of acute dyskinesias is directly proportional to the proportion of D_2Rs occupied by the drug (99,110,115). Furthermore, receptor imaging demonstrates

a lower degree of D_2 receptor occupancy during treatment with atypical neuroleptics. For example, PET has been used to obtain the minimal effective dose of risperidone. The high D_2R occupancy associated with 6 mg or more risperidone daily suggests a high risk of acute dyskinesias. On the other hand, 4 mg risperidone daily results in 70% to 80% D_2R occupancy and a lesser risk of acute dyskinesias (116). An additional role for occupancy studies in drug development takes the form of the interpretation of the time course of receptor occupancy following a single drug dose. Such studies help determine the appropriate dosing regimen for future trials, such as once or twice a day dosing. For example, 70% to 90% of $5HT_{2A}$ receptors are occupied during the 24 hours after a single oral 20-mg dose of MDL100,907 (MDL), a selective serotonergic agent, whereas only 20% are occupied 24 hours after a 10-mg dose (117). These results suggest that a 20-mg dose may be administered once daily, whereas a 10-mg dose requires administration twice a day. Thus, occupancy studies constitute surrogate markers for the outcome variable and frequency of drug administration.

Another use of occupancy studies is the correlation of D_2R occupancy with plasma levels of neuroleptics. This approach has been successfully applied to estimate D_2R occupancy by haloperidol in patients with low doses of haloperidol (118). These preliminary results can be refined in future research with larger sample sizes.

In summary, receptor occupancies have helped establish the optimal dosage range of antipsychotic medications. These imaging methods also have a role to determine *in vivo* occupancy of new neuroleptics with multiple sites for D_2 and $5HT_{2A}$ binding. The studies probably have their greatest role in giving approximate dosage estimates for future clinical trials.

Other Roles for Neuroreceptor Imaging in Drug Development

Four major areas in which neuroreceptor imaging can assist in drug development are listed in Table 34.3. The first and most well developed area is in helping target rational drug dosing as in the neuroleptic studies described in the preceding. The second is the elucidation of the biodistribution of the drug by radiolabeling the drug or a derivative of the drug. Examples of this application include the characterization of MDL, which is the first selective $5HT_{2A}$ antagonist developed primarily for schizophrenia. MDL has been radiolabeled with [^{11}C] in an isotopic form such that a stable carbon atom (atomic number ^{12}C) is substituted with a radioactive atom (^{11}C) without a change in the pharmacology or chemistry. This procedure facilitates characterization of the biodistribution and washout characteristics of the agent (117).

The third application of neuroreceptor imaging to drug development is to better understand the mechanism of action of drugs. One example of this is in the development of drugs for cocaine abuse. Unlike neuroleptics and antidepressants, drugs developed to inhibit the action of cocaine have failed clinical trials. Although cocaine has been shown to affect multiple neurotransmitter systems, current research efforts to develop effective treatment for cocaine dependence focus on the dopamine system. Cocaine is hypothesized to produce euphoria by increasing the intrasynaptic concentration of dopamine. Cocaine has high affinity for the dopamine transporter (DAT); therefore, contemporary research to treat cocaine dependence includes the development of noncompetitive inhibitors of cocaine at this site without affecting dopamine transport. One example of potential treatments for cocaine dependence is the development of GBR12909 (GBR), a potent DAT inhibitor. This pharmaceutical, originally developed in Europe as an antidepressant, has found a potential new application as a prototypical drug for cocaine abuse. Prior studies have shown that IV infusion of GBR to Rhesus monkeys selectively reduced (1 mg/kg) and eliminated (3 mg/kg) cocaine self-administration (119). Villemagne and colleagues (120) tested the hypothesis that doses of GBR, which reduce self-administration, also produce significant occupation of DAT. Doses of 1, 3, and 10 mg/kg demonstrated occupancy of 26%, 53%, and 72%, respectively, in *Papio anubis* baboons (Figs. 34.1 and 34.2). These data suggest that doses that suppress cocaine administration also provide high occupancy of the DAT. Preclinical research supports the hypothesis that elevations of mesolimbic DA mediate the addictive and reinforcing effects of methamphetamine and amphetamine. *In vivo* rodent microdialysis has demonstrated that GBR attenuates cocaine and amphetamine induced increases in mesolimbic DA. Utilizing PET scans of a continuous infusion of [^{11}C]raclopride in baboons, Villemagne and colleagues (120a) also showed that GBR attenuates amphetamine induced striatal DA release by 74% (Figs. 34.3 and 34.4). Thus, GBR is a potentially effective agent to treat cocaine and methamphetamine dependence. This experi-

TABLE 34.3. COMPONENTS OF THE DRUG DEVELOPMENT PROCESS ACCOMPLISHED BY NEURORECEPTOR IMAGING

Rational drug dosing
Biodistribution of drug bound to radiolabels
^{11}C and ^{18}F for PET
^{123}I and ^{99m}Tc for SPECT
Therapeutic rationale for drug utilization
Mechanism of drug action

PET, positron emission tomography; SPECT, single photon emission computed tomography.

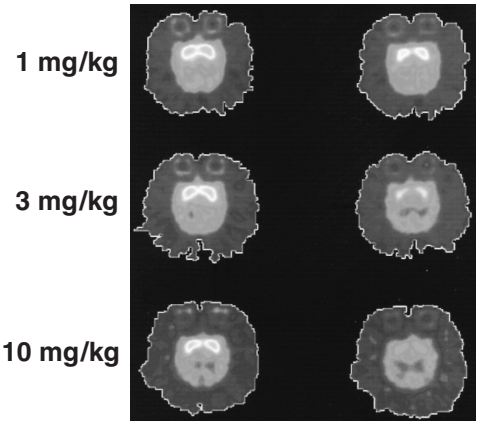

FIGURE 34.1. Reductions in dopamine transporter occupancy are shown in transaxial [^{11}C]WIN35,428 images in *Papio anubis* baboons before *(left)* and after *(right)* administration of three different doses of GBR. Each dose is given 90 minutes before [^{11}C]WIN35, 428 injection. The illustrations represent average PET images at midstriatal level between 70 and 90 minutes after the injection of the radiotracer normalized to the injected radioactivity. Modified from Villemagne V, Rothman RB, Yokoi F, et al. Doses of GBR12909 that suppress cocaine self-administration in non-human primates substantially occupy dopamine transporters as measured by [^{11}C]WIN35, 428 PET scans. *Synapse* 1999;32: 44–50. Copyright © 1999, Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

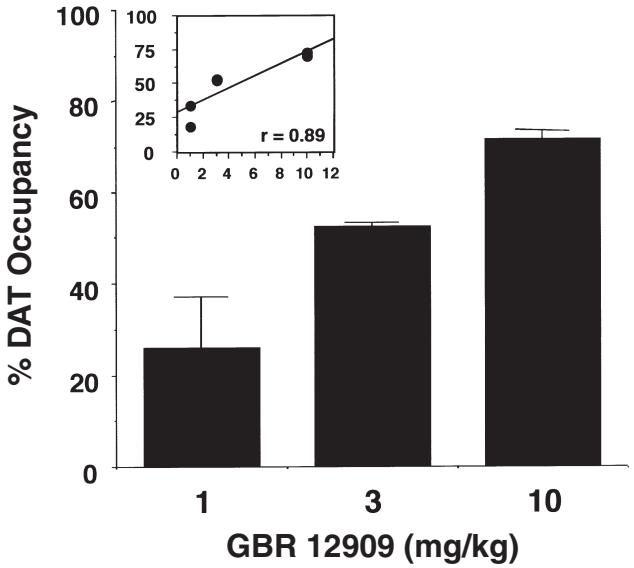

FIGURE 34.2. This histogram illustrates the percentage dopamine transporter (DAT) occupancy by GBR 12909 (GBR) as measured by positron emission tomography imaging with [^{11}C]WIN35, 428. DAT occupancy is represented as the percentage mean ± standard error of the mean differences between binding potentials at baseline and after GBR administration. Percentage occupancy is calculated by the formula as follows: [(Baseline binding potential − GBR binding potential)/Baseline binding potential] × 100. *Inset:* Relation between DAT occupancy and GBR dose (120). Modified from Villemagne V, Rothman RB, Yokoi F, Rice KC, Matecka D, Dannals RF, Wong DF. Doses of GBR12909 that suppress cocaine self-administration in nonhuman primates substantially occupy dopamine transporters as measured by [^{11}C]WIN35, 428 PET scans. *Synapse* 1999;32:44–50. Copyright © 1999, Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

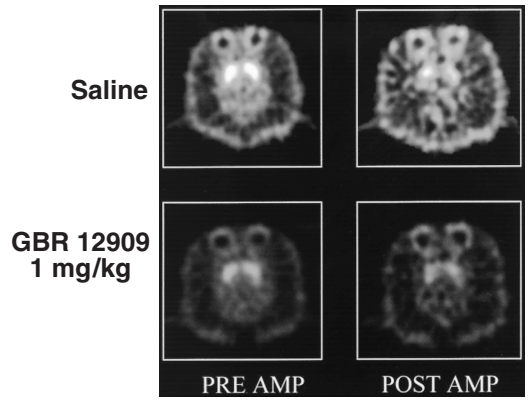

FIGURE 34.3. These images illustrate the binding of [^{11}C]raclopride to the basal ganglia of *Papio anubis* baboons treated with saline *(top row)* and GBR (1 mg/kg) *(bottom row)* after the administration of saline (3 mL/kg) (PRE AMP) *(left column)* or amphetamine (1 mg/kg) (POST AMP) *(right column).* After the administration of saline (3 mg/kg) *(top row)* there is prominent binding of [^{11}C]raclopride to the basal ganglia at baseline (PRE AMP) *(upper left)* and significant reduction after the administration of amphetamine (1 mg/kg) (POST AMP) *(upper right).* After the administration of GBR (1 mg/kg) *(bottom row)* there is reduced binding of [^{11}C]raclopride to the basal ganglia at baseline (PRE AMP) *(lower left)* and minimal reduction after the administration of amphetamine (1 mg/kg) (POST AMP) *(lower right)*. Modified from Villemagne VL, Wong DF, Yokoi F, Stephane M, Rice KC, Matecka D, Clough DJ, Dannals RF, Rothman RB. GBR12909 attenuates amphetamine-induced striatal dopamine release as measured by continuous infusion PET scans. *Synapse 1999;33:268–273.* Copyright © 1999, Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

mental model is also being utilized to evaluate other forms of GBR such as the long-acting decanoate derivative.

The fourth method in which neuroreceptor imaging can assist in drug development is the empirical evaluation of theories of disease, such as the DA hypothesis for schizophrenia. For example, Grace (1991) (121) proposed that schizophrenia is characterized by intrasynaptic concentrations of DA that are abnormally low in the basal tonic state and abnormally high in the simulated phasic state. This has been supported by numerous findings of elevated dopa decarboxylase measurements using [^{18}F]fluorodopa (122), elevated amphetamine induced dopamine release (123–125), and elevated D_2Rs (97,126). There is also some potential evidence that elevated intrasynaptic dopamine release is also found at baseline (126–128). In this example of the DA system, the combined strength of measuring presynaptic, postsynaptic, and intrasynaptic DA, for example, provides converging evidence to test this hypothesis. Development of additional ligands such as those for glutamate, glycine, and second messengers, will further expand the potential to evaluate the complex pathophysiology of schizophrenia.

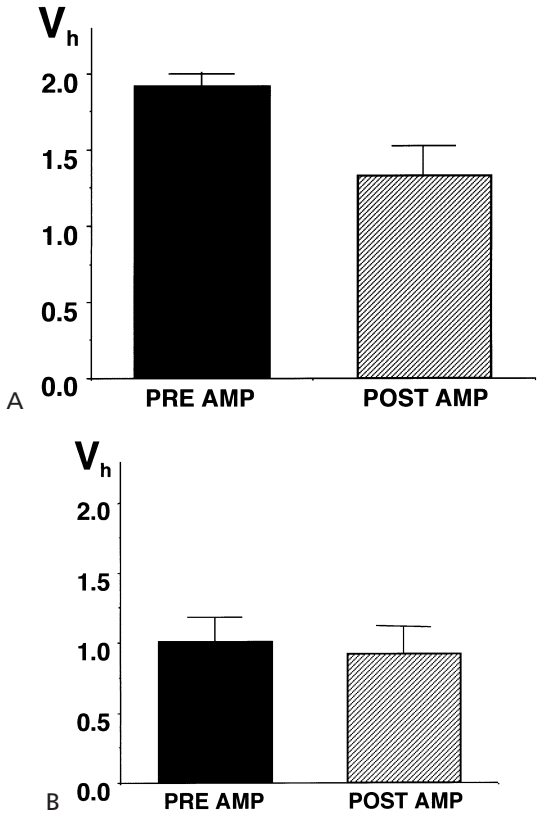

FIGURE 34.4. These histograms contrast the release of DA after administration of amphetamine (AMP) in *Papio anubis* baboons treated with saline *(left)* and GBR (1 mg/kg) *(right).* For each histogram, the abscissa indicates the intravenous administration of saline (3 mL/kg) (PRE AMP) or amphetamine (1 mg/kg) (POST AMP) and the ordinate is the average volume of distribution (V_h) and the standard error of the mean. The left histogram illustrates a significant reduction in V_h consistent with the release of DA induced by amphetamine. The right histogram demonstrates a reduced baseline (PRE AMP) V_h after administration of GBR consistent with an increase in baseline extracellular intrasynaptic DA concentration (PRE AMP) and the absence of a significant change in V_h after the administration of amphetamine (POST AMP). Modified from Villemagne VL, Wong DF, Yokoi F, Stephane M, Rice KC, Matecka D, Clough DJ, Dannals RF, Rothman RB. GBR12909 attenuates amphetamine-induced striatal dopamine release as measured by [^{11}C]raclopride continuous infusion PET scans. *Synapse* 1999;33:268–273. Copyright © 1999, Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

MAGNETIC RESONANCE SPECTROSCOPY

Magnetic resonance spectroscopy (MRS) provides surrogate markers to determine clinical endpoints to evaluate new therapeutic interventions for specific diseases. For example, choline metabolites estimated through photon MRS function as surrogate markers of cognitive motor symptom severity in HIV-1 (129). Additionally, [^{19}F]MRS has been explicitly employed to examine the pharmacokinetics of psychotropic medications containing a fluorine group such as fluvoxamine (130). This allows direct comparison of brain and plasma concentrations, and brain elimination half-lives (131), although the sensitivity allows only micromolar measures in contrast to the nanomolar measures attained with PET. These methods have also been employed in animal models using [^{19}F]nuclear magnetic resonance (NMR) chemical shift imaging to study the cerebral distribution of general anesthetics *in vivo* (132).

MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging (MRI) provides surrogate markers for disease progression to facilitate drug development. For example, T2-weighted cerebral MRI functions as a surrogate marker in early stages of demyelinating disease to predict disease progression and disability over the subsequent 10 years (132a–132e).

Another example of the use of MRI as a surrogate marker for drug development is in the diagnosis and treatment of subjects with AD. Atrophy of the hippocampal formation has been correlated with memory and cognitive impairments. Reductions in the volume of the hippocampus have been predictive for the individuals who later develop memory impairments consistent with AD (133). Another marker of the vulnerability to develop AD is the measure of the apolipoprotein E (APOE) genotype. The relative risk for AD is increased for people with the gene (134). The APOE gene is associated with loss of hippocampal volume (135). Cross-sectional studies in 116 healthy volunteers, 59 to 85 years old, demonstrated significantly larger ventricular volumes and smaller gray and white matter volumes in older compared to younger individuals and in men compared to women over a period of only 1 year. An increase of over 1,500 mm^3 in ventricular volume was demonstrated during this time but no detectable change in the total or regional brain volumes. This suggests that determination of the pattern and rate of these changes longitudinally could be a future predictor of cognitive declines and dementia (136).

Functional MRI (fMRI), a technique in which subjects are asked to perform particular mental or physical tasks while the MRI is obtained, may provide biomarkers useful for drug development. For example, the rCBF of cocaine-dependent subjects administered intravenous cocaine exhibited increases in the nucleus accumbens, subcallosal cortex, and hippocampus and decreases in rCBF in the amygdala, temporal pole, and medial frontal cortex (137). Future studies utilizing this paradigm could additionally assess whether another compound administered before cocaine can antagonize or amplify its affects.

BIOMARKERS OF ALCOHOL ABUSE

The synergistic combination of carbohydrate-deficient transferrin (CDT), gamma-glutamyl transferase (GGT),

and mean red cell volume (MCV) functions as a possible biomarker for alcohol abuse (138).

NEWER MARKERS FOR DRUG DEVELOPMENT

Several techniques recently have been developed to provide the means to assess the efficacy of newly developed potential drugs. These procedures assist clinicians in both the (a) diagnosis of neuropsychiatric disorders, and (b) monitoring of the course of disease progression and the response to drug treatments and other therapeutic interventions. The application of the following novel procedures to drug development is the result of a collaborative effort jointly of the National Institutes of Health (NIH), the United States Food and Drug Administration (FDA), the Health Care Financing Administration (HCFA), and private industry (139). The protocols described in this section are likely to be instrumental in drug development for neuropsychiatric disorders in the future.

External Imaging of Internal Bioluminescent and Fluorescent Signals

Biological processes, such as the propagation of cancer cells, are studied in animal models by the detection of bioluminescent (140) and fluorescent signals recorded externally by means of sensitive photon detection systems (141). For example, optical imaging systems visualize near-infrared fluorescent molecular targets triggered by enzymes released by cancer in experimental animals (142). Other examples include the evaluation of potential agents to treat human cancer and infection by assessing the behavior of cells from patients in animals utilizing bioluminescent markers. The technique utilizes bioluminescent compounds bound to specific enzymes that signal the proliferation of cancers and bacteria. Human subjects with cancer or infection are treated with agents to alter the expression of genes that produce the enzymes needed for the growth of the cancer or bacteria. Then animals are injected with (a) cells from the previously cancerous or infected tissue of treated humans, and (b) bioluminescent markers that respond to the presence of the enzyme that was hopefully blocked in the humans. Detection of the bioluminescent markers in the animals indicates the proliferation of the enzyme to be blocked in the treated human and, therefore, the failure of the therapeutic attempt. This technique, which provides a surrogate for the clinical effects, is being applied to a variety of human diseases including cancer and infection *(http://www.xenogen.com).* By analogy, this procedure could be applied to assess drugs developed to treat malignancy and infection of the CNS. For example, novel agents to alter the expression of the genes that code for the control of the production of enzymes required by cancer and infection of the CNS could be administered as therapeutic agents to suitable patients with malignancy and infection of the CNS. CSF from treated patients and bioluminescent compounds responsive to the presence of the enzyme that should be absent could then be injected into experimental animals. Detection of the bioluminescence in the experimental animal would then indicate failure of the new drug to prevent the growth of the tumor or infection. Thus, this technique offers the potential of surrogate markers to monitor the presence of CNS tumors and infections.

Simultaneous Optical and Magnetic Resonance Microscopy

Simultaneous optical and magnetic resonance imaging (MRI) is being developed in experimental animals. MRI contrast agents (143), such as fluorescently detectable magnetic resonance imaging agents, are utilized to permit light and magnetic resonance imaging microscopy at the same time (144). These compounds can be detected deep in tissue, not merely at the surface as with simple optical detection systems (e.g., for fluorescent dyes) (145). Although light microscopy can penetrate only 100 to 300 μm beneath the surface of organisms, MRI microscopy can penetrate 1 to 6 mm into an organism (144). For example, agents that can be simultaneously recognized by MRI microscopy and by fluorescent optical microscopy permit visualization of structures 1 to 6 mm below the surface of an organism approaching cellular resolution (i.e., 10 μm) (143,144).

Diffusion-Based Optical Imaging

Procedures to measure light emitted into opaque structures have been termed medical optical imaging (MOI), medical optical spectroscopy (MOS), near-infrared imaging (NIRI), and near-infrared optical spectroscopy (NIOS) (146). For example, three-dimensional optical coherence tomography (OCT) is a technique to image nontransparent biologic tissue by recording and analyzing light emitted into scattering media. OCT has been employed to visualize nerve fascicles in experimental animals for the microsurgical anastomoses of vessels and nerves (147). An example of diffusion-based optical imaging is the use of optical tomography to detect intraventricular hemorrhage in premature infants by external transmission of light emitted through the skull (146). These techniques may be utilized to develop treatments for human disease, including infections and malignancies (146). Hopefully, in the future they will be applied to CNS tumors and malignancies.

Magnetic Resonance Microscopy

Microscopic visualization of magnetic resonance images has detected transgene expression in experimental animals.

Identification of pathologic processes, including the proliferation of tumor cells in clinical settings (148), may be facilitated by this procedure. This technique offers the means to both detect the occurrence of malignancies and to monitor their growth (148). The application of this procedure to human CNS malignancy is a goal to be attained in the future.

Electron Paramagnetic Resonance

Electron paramagnetic resonance (EPR) (149) imaging and spectroscopy are procedures to spatially map parameters of physiologic importance by incorporating paramagnetic spin labels into the system of interest (150). This technique has been utilized to visualize oxygen concentration in the tissues of experimental animals (150) and to measure oxygen free radical generation in human endothelial cells exposed to anoxia and reoxygenation (151). EPR has been employed to estimate the production of nitric oxide in biological systems (152), a process vital to the measurement of the progression of pathologic processes (153), including cerebral ischemia (154) and malignancies.

CONCLUSION

Biomarkers and surrogate markers are tools currently utilized to develop new drugs. They provide evidence of the proof of concept required for successful Phase 1B/2A studies submitted to the FDA. Currently drugs are being developed by the use of neuroendocrine markers including CSF, prolactin, GH, ACTH, and cortisol. Imaging studies provide the means to estimate therapeutic dosages of new drugs. Surrogate markers include a variety of neuroimaging techniques including MRI, MRS, PET, and SPECT. Newer techniques for drug development are likely to include external imaging of internal bioluminescent and fluorescent signals, simultaneous optical and MRM, diffusion-based optical imaging, and EPR.

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