Annu. Rev. Pharmacol. Toxicol. 2005. 45:227–46
doi: 10.1146/annurev.pharmtox.45.120403.095758

First published online as a Review in Advance on September 7, 2004

BIOMARKERS IN PSYCHOTROPIC DRUG DEVELOPMENT: Integration of Data across Multiple Domains

Peter R. Bieck[1] and William Z. Potter
[1]*Eli Lilly & Company, Neuroscience Therapeutic Area, Lilly Corporate Center, Indianapolis, Indiana 46285; email: bieck@lilly.com*

Key Words data integration, CNS access, neuroimaging, CSF, proteomics, psychotropic drug biomarkers

■ **Abstract** This review focuses on the current status of biomarkers and/or approaches critical to assessing novel neuroscience targets with an emphasis on new paradigms and challenges in this field of research. The importance of biomarker data integration for psychotropic drug development is illustrated with examples for clinically used medications and investigational drugs. The question remains how to verify access to the brain. Early imaging studies including micro-PET can help to overcome this. However, in case of delayed tracer development or because of no feasible application of brain imaging effects of the molecule, using CSF as a matrix could fill this gap. Proteomic research using CSF will hopefully have a major impact on the development of treatments for psychiatric disorders.

INTRODUCTION

The drug development process has multiple phases and decision points that require development of stage-specific biomarkers (1, 2). Biomarker assays have to be validated for criteria such as reference range, accuracy (sensitivity, selectivity, specificity), and stability (3, 4).

There is general agreement that the main utilities of biomarkers in drug development are the following:

- Discovery and selection of lead compounds
- Generation of pharmacokinetic (PK) and pharmacodynamic (PD) models
- Aid in clinical trial design and expedite drug development
- Serving as surrogates for clinical or mortality endpoints
- Optimizing drug therapy based on genotypic or phenotypic factors
- Definition of patient enrollment in studies and help with stratification

Psychotropic Drug Development

A principal question in psychotropic drug development is whether there are better ways to predict therapeutic and unwanted effects of novel compounds targeting the central nervous system (CNS).

In the absence of clear answers to this question or better means of prediction, it is still possible to find better ways of testing novel compounds, especially if one views them as "reagents" for evaluating a hypothesis. In other words, application of multiple technologies may allow us to show that a molecule is acting on specific biochemical processes in humans. That, in turn, allows one to formally test hypotheses on whether a particular biochemical effect in patients is or is not associated with clinical change and/or physiological side effects. This approach goes beyond the generally recognized need to conduct early evaluations of drugs in humans more effectively and more rapidly with clinical pharmacological assessment of CNS using batteries of objective and subjective measures that must be valid and reliable. Independent of drug development, there has been great interest in finding biological markers of psychiatric disorders not only for elucidating underlying pathophysiology but also to serve as diagnostic tools or predicting treatment responses. The majority of biologic and laboratory markers and surrogate endpoints that have demonstrated an association between the marker and the underlying condition come from other therapeutic fields. Biomarkers currently being investigated in psychiatry and neurology encompass a wide variety of procedures (Table 1) (5, 6). None of these markers are useful for routine clinical practice. As cited in a textbook, "biological marker research in psychiatry often takes on the character of a fishing expedition with better fishing spots suggested by earlier encouraging findings or intriguing hypotheses" (7).

In what follows, we review the factors that determine the relationship between drug dosage and effect in light of the application of potential biomarkers of the latter. We provide a number of examples of how these domains of investigation can be integrated from discovery to the clinic.

To integrate the knowledge on biomarkers, a biomarker database is urgently needed that can accept data from the scientific community at large. Very recently, an information technology site for life sciences (http://www.integromics.com/) has started offering help to pharmaceutical companies and academics for integrating biomarker data using SpotFire® (http://spotfire.com/).

This need for integration of data is similar in other areas of research. At a recent conference on Data Integration for the Pharmaceutical Industry, the term integromics in drug discovery was used. Weinstein questioned the biological and pharmacological meaning of the results of genomics and proteomics, but showed how one can integrate them by using bioinformatics and chemoinformatics (http://discover.nci.nih.gov/). His group is using so-called micro array tools, such as MedMiner, MatchMiner, GoMiner, and CIMMiner for integration of literature, gene identifiers, gene visualizations, and expression maps (8).

TABLE 1 Examples for biomarkers in psychotropic drug development

Biomarker procedures	
Brain imaging technique	Computed tomography (CT), regional cerebral blood flow (rCBF), magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance spectroscopy (MRS), magnetoencephalography (MEG)
Cell-based imaging	Fluorescent resonant energy transfer,[a] confocal imaging in brain slices[b]
Electrophysiological marker	Electroencephalogram (EEG), pupillometry, saccadic eye movements
Laboratory-based marker[c]	Concentrations of catecholamines, hormones, enzymes, proteins, drugs, and drug metabolites
Psycho-immunological marker	Immunoglobulin, lymphocyte responses, lymphokine, cytokine, interleukin, interferon; viral serology; Alz-50; anticardiolipin antibodies (ACA)
Neuroendocrine marker	Dexamethasone-suppression test (DST), thyrotropin-releasing hormone stimulation test (TRHST), growth hormone (GH) challenge test
Provocative anxiety tests	Lactate infusion, carbon dioxide (CO_2) challenge, cholecystokinin (CCK) challenge
Genetic markers	DNA banking, genotyping, restriction fragment length polymorphisms (RFLPs)
Proteomic identification	Nuclear magnetic resonance (NMR), lipoprotein fractions and subfractions, matrix assisted laser desorption/ionization-mass spectrometry (MALDI-MS)

[a]Reference 5.

[b]Reference 6.

[c]Matrices mostly from plasma, urine, CSF, tissue, saliva, and hair.

Altman has reviewed the challenges regarding the integration and analysis of genomic, molecular, cellular, and clinical data and has merged at Stanford University what was called Clinical Informatics and Bioinformatics to Biomedical Informatics (9).

The publicly available internet research tool Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB at http://www.pharmgkb.org/)

demonstrates how a database can help researchers in understanding the contribution of genetic variation among individuals to differences in reactions to drugs. It is an integrated resource and can be researched for drugs, diseases, clinical outcome, pharmacodynamics, pharmacokinetics, molecular/cellular functional assays, and for genotype (10). As more and more measures of CNS during drug action become available, integration of genetic, proteomic, metabolic, and pharmacokinetic data can utilize these tools that are being developed.

CNS ACCESS TO THE SITE OF ACTION

Medications that affect the CNS have to be transported to the site of action. The blood brain barrier (BBB) often prevents sufficient exposure to this site: 98% of small-molecule drugs do not cross the BBB (11). With the recent exception of application of PET ligands, human studies do not specifically measure the extent of CNS penetration. A multitude of biological factors underlie what Spector calls the phenomenology of CNS transport: drug concentrations in plasma, CSF, brain, and extracellular fluid (Table 2). This author has stressed the importance and lack of systematic analysis of CNS transport in preclinical and clinical studies (12). To

TABLE 2 Systematic analysis of CNS transport[a]

Type of study	Example
Phenomenology	Concentrations in plasma, CSF, brain, and ECF
Physiology/Pharmacology	In vivo: Ventriculocisternal perfusion Brain uptake index (BUI) In situ intra-arterial brain perfusion Brain efflux index (BEI) Intravenous injection Intraventricular injection In vitro: Chorioid plexus (CP) preparation Brain capillary preparation
Biochemical Pharmacology, Anatomy, Histology	Purification of enzymes and receptors Specificity of transport Receptor analysis Affinity of ligands (ex vivo binding) Monolayer of cerebral capillary cells CP epithelial cells in vitro Histological localization of receptors
Molecular biology	Cloning and expressing genes "knockout" mice
Analogy	Kidney, gut, and liver systems

[a]Adapted from Reference 12.

date, the underlying physiological processes regulating a drug's access to brain are generally ignored in a still empirical approach with focus on the phenomenological level.

Cerebrospinal fluid (CSF) sampling is a tool that allows access to the central compartment, and ideally it would provide a better matrix than plasma to assess drug concentration close to the site of action. Early studies showed that sporadic simultaneous measurement of the CSF to plasma concentration ratio usually is inadequate to describe CSF penetration. CSF concentration-time curves lag behind those in plasma. The areas under the concentration-time curves in CSF and plasma at steady state or after a short-term infusion are accepted as measures of CSF passage (13). Continuous CSF collections for 12 h or longer provide concentration-time curves for drug and or biomarkers and serve as "dynabridge study" (14). This term was introduced to describe a type of exploratory study in which drug concentrations and activities in the central compartment of patients are measured. The techniques have been described as safe and well tolerated (15). The question remains concerning the extent to which CSF concentration is representative of that at the site of action.

One approach to systematically categorize intercellular communication in the brain is based on the concepts of wiring transmission (WT) and volume transmission (VT) (16). Morphological and functional observations suggest that CSF might represent an important vector for convection of VT signals, especially to peri- and paraventricular areas. Any brain cell can participate in VT, and any kind of substance, such as ions, drugs, classical transmitters, peptides, and neurosteroids, can be a signal (17, 18). MRI studies have indicated the existence of fluid movements from the CSF via the paravascular space and the extracellular space into the brain capillaries (19). Despite these supportive factors, it still remains to be shown when and if CSF concentrations reflect target drug concentrations.

Direct measures of drug concentrations in human brain tissue can be obtained in vivo using imaging techniques (20) or measured in postmortem brain (21, 22). Neurosurgeons apply microdialysis and voltammetry/spectrophotometry for continuous monitoring of substrates, metabolites, or neurotransmitters in the human brain with the disadvantage that these probing methods are invasive and focal (23).

Ahmed et al. have previously summarized the advantages and limitations of selected biomarker technologies for assessing CNS access (Table 3) (24). The main difference between the use of CSF and imaging consists in the ability for continuous monitoring versus the intermittent snap-shot imaging. We later discuss examples comparing studies utilizing these different methods.

TARGET DRUG-RECEPTOR INTERACTIONS

The most obvious measure of a drug interacting with its target is via receptor occupancy, a measure that is now feasible for a limited number of targets for which validated PET or SPECT ligands are available (reviewed in 24a).

TABLE 3 Advantages and limitations of selected biomarker technologies[a]

Technology	Advantages	Limitations
CSF[b,c]	Measurement of drug PK in central compartment Several surrogate markers available Possible to combine PK and PD measures in one protocol	Invasive Sensitive assay required
EEG[c]	Unequivocal positive results provide evidence of CNS effects of intervention Convenient for repeated measures within subjects	Observed effects generally not easily linked to specific mechanism of interaction Prone to artifacts and not fully standardized
MRI [b,c]MRS [c]fMRI [c]sMRI	Pharmacological doses of certain drugs can be accurately measured Structural and functional modalities can be combined to enhance overall signal detection	Motion artifacts in agitated patients More validation necessary for surrogate marker use Expensive
PET/SPECT [b]Tracer techniques [c]Functional methods [c]Occupancy studies	Highly sensitive detection of drugs that can be radiolabeled Direct evidence of effect of drug at site of action	Exposure to ionizing radiation Tracer not available for every application Expensive

[a]Reproduced with permission from the *American Journal of Geriatric Psychiatry* (Copyright 2002). American Psychiatric Publishing, Inc. Reference 24.

[b]Modality can demonstrate central penetration of a drug.

[c]Method can show central PD effect of a drug and/or serve as a surrogate marker in selected situations.

PK: pharmacokinetic(s); PD: pharmacodynamic(s); CSF: cerebrospinal fluid; EEG: electroencephalography; MRI: magnetic resonance imaging; MRS: magnetic resonance spectroscopy; fMRI: functional MRI; sMRI: structural MRI; PET: positron emission tomography; SPECT: single photon emission computed tomography.

Because this technology is generally limited to robust blockade and displacement of antagonist binding, other measures are required to establish the interaction of a drug with a target. These fall into the general class of functional measures, the most generalizable and promising of which may prove to be proteomics.

Proteomics is a research field aiming to characterize molecular and cellular dynamics in protein expression and function on a global level (25, 26). Clinical proteomics is a new subdiscipline that involves the application of these technologies at the bedside. Most advanced is the analysis of serum proteomic patterns

to provide diagnostic end points for cancer detection (27). Proteomic approaches to CNS disorders are progressing with the hope of establishing a CNS proteome database derived from primary human tissues (28). Areas of research are encompassing proteomes of nerve cells (29, 30), proteomic profiling of autopsy brain tissue from patients with Alzheimer's and Parkinson's disease (compared with control specimen from healthy subjects) (31, 32), and proteomic analysis of the CSF of patients with schizophrenia (33) or Alzheimer's disease (34). Multinational pharmaceutical and smaller private biotechnology-based companies show a huge interest in using proteomic techniques for new discoveries in psychotropic drug development (antipsychotics, anxiolytics, depression, schizophrenia) (25, 35).

Application of proteomics as a "read-out" of target-drug interactions is just beginning to be explored. Its success depends on whether there really are discretely identifiable patterns of changes in proteins associated with specific drug-target molecular interactions.

From a practical experimental viewpoint, the combination of CSF sampling with proteomics enables access to a body fluid in close contact with brain cells. CSF has only minimal protein content, which makes analyses less complicated compared with serum proteomic patterns. We are currently exploring the best means of achieving standardization of CSF collection, which is mandatory for its use in proteomic studies. In a parallel effort, because blood contamination cannot be entirely avoided during lumbar puncture, methods to correct for the variable contamination-associated changes in the CSF proteomic profile are being developed (36). A large survey in Europe confirmed that CSF/serum quotients of proteins represent method-independent values approaching the quality of reference values (37, 38).

DOWNSTREAM PHARMACOLOGICAL EFFECTS

Here we make a distinction between biochemical effects detectable in a matrix accessible to the site of action, which are specific to a particular molecular event (e.g., specific protein pattern changes in CSF), and biochemical or physiological effects, which are consistent with, but not necessarily specific to, a particular pharmacologic action. There is a four-decade history of measuring biochemical changes in CSF, blood, and urine as indices of such drug effects (24; reviewed in 24a). But, for instance, there are no established neuroimaging techniques available for determination of norepinephrine transporter (NET) inhibition in humans, which could be used in drug development, although promising first results in humans studying NET receptor occupancy following treatment with clinical doses of reboxetine have been reported (39). This latter study has utilized the specific PET ligand, (S, S)-[11C] MeNER, an O-methyl analog of the selective and potent NET inhibitor, (S, S)-reboxetine. For decades, however, peripheral measures that show a decrease in NE turnover after NET inhibition have been used to and criticized as reflecting changes in the peripheral sympathetic nervous system, which might

not be surrogates for effects in the brain. Nonetheless, because there is only one type of NET in the nervous system (peripheral and central), and metabolites of NE formed in the brain are excreted, some of the peripheral biomarkers might reflect changes in the CNS. Past experience has shown that peripheral biomarkers obtained early during clinical pharmacological evaluation of MAO inhibitors (40) can be validated at a later stage with PET imaging in peripheral organs (41) and in brain (42).

Interestingly, investigations of clinical syndromes can also provide support for an array of measures as indices of drug action. Recently, a functional polymorphism in the human NET has been discovered (43) in patients with orthostatic intolerance taking the form of a NET deficiency that can be assessed simultaneously with a multitude of measures (44). The same type of measures should also be applicable for testing drug-induced NET deficiency. In a study with duloxetine, a 5-HT and NE reuptake inhibitor, the following measures were applied: vital signs, tyramine pressor test, posture test, NE and its metabolite DHPG in plasma and urine, plasma melatonin, plasma inhibition of [3H]-nisoxetine binding (ex vivo ligand), and plasma duloxetine. Selected results, consistent with a dose response on measures related to NET inhibition, are shown in Figure 1*a–d*. The findings of this study suggest that a "portfolio" of biomarkers is useful for the assessment of NET inhibition because there were substantial differences in the sensitivity with which the different downstream biomarkers were affected: ex vivo binding = DHPG: NE ratio > tyramine pressor test > heart rate (45). Assessment of such biomarkers in CSF, plasma, and urine during treatment with the potent NET reuptake inhibitor atomoxetine (46) is presently ongoing. In the future, it will be possible to validate such peripheral NET reuptake biomarkers with the highly sensitive (but expensive!) PET imaging as well as by comparing them with proteomic patterns in the CSF.

CLINICAL RESPONSE

Evidence of drug effect in a model experimental paradigm can also be considered as a type of biomarker. Historically, provocative anxiety tests have been given to diagnose patients with panic disorders, but they also have been used in studies on healthy subjects for psychotropic drug development. The scope in drug development has been to delineate the mechanism of action of potential antianxiety agents and to determine a minimum effective dose and the duration of effect. However, these tests have not proved predictive of efficacy and are therefore limited to providing evidence of some drug effect (47). Pharmacological challenges with lactate, carbon dioxide, or cholecystokinin (CCK) can produce anxiety in healthy human subjects (48–52). They are usually regarded as models of unconditioned anxiety, comparable to panic disorder. Their use requires careful consideration of the experimental setting and the physiological changes. For example, Na-lactate requires a 20-min infusion and CO_2 needs several inhalations for 20 min each (53). CCK can be given as i.v. bolus.

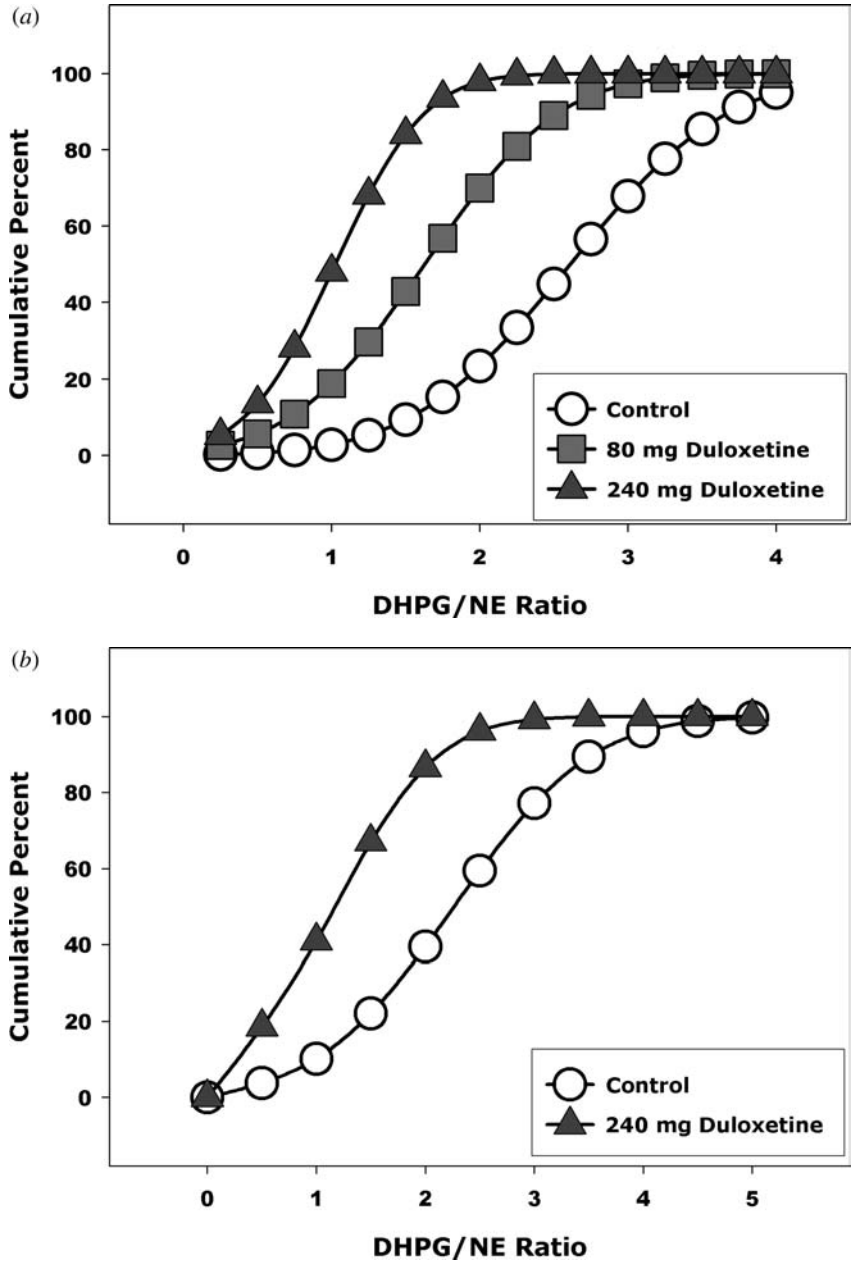

Figure 1 (*a*) Quantal dose-response curves of DHPG/NE ratio in plasma before and during duloxetine treatment. (*b*) Quantal dose-response curves of DHPG/NE ratio in urine before and during duloxetine treatment. (*c*) Effect of duloxetine on ex vivo [^{3}H] nisoxetine binding to NE transporters. (*d*) Effect of duloxetine on tyramine pressor dose to raise systolic blood pressure by 30 mm Hg (PD_{30}). From Reference 45.

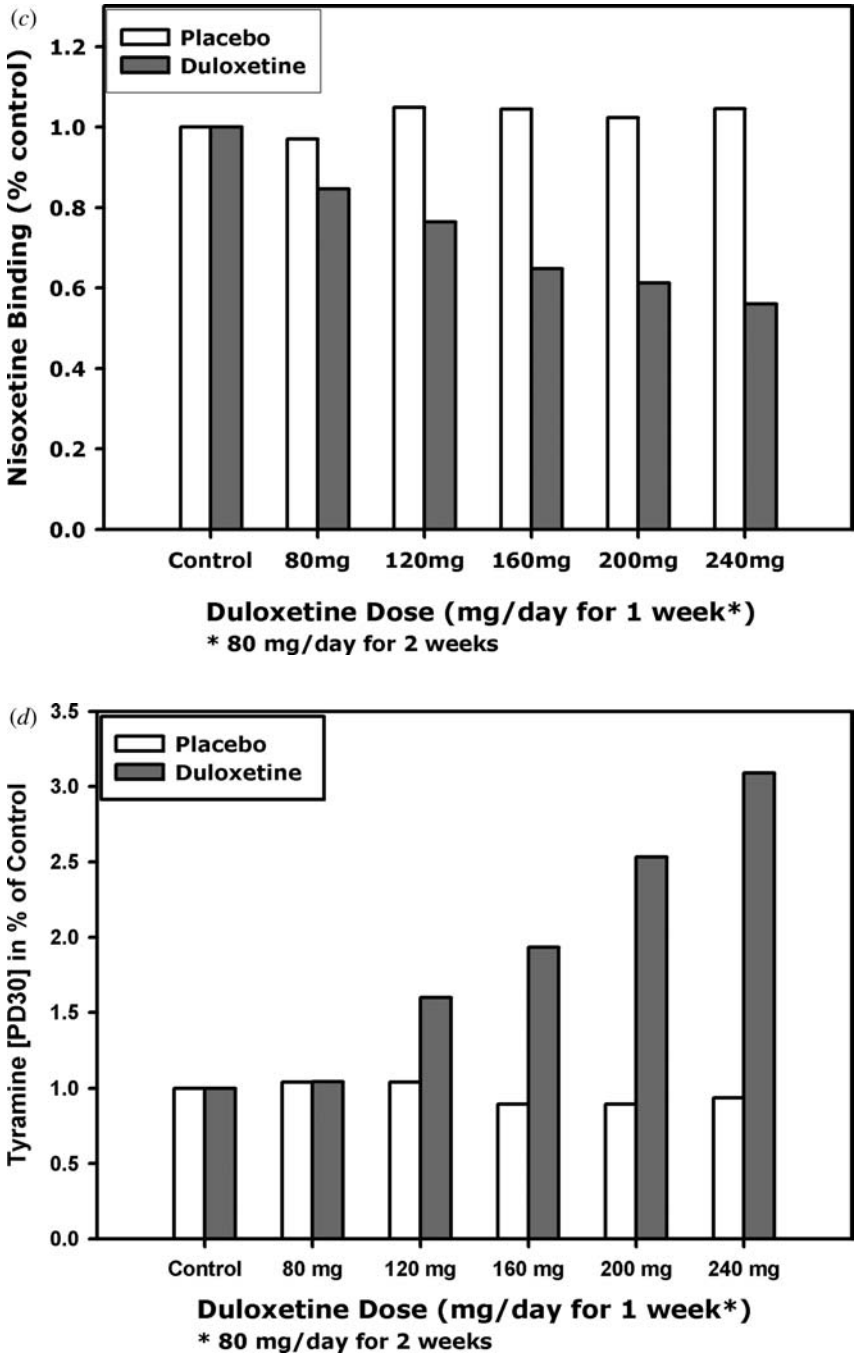

Figure 1 (*Continued*)

There are major differences between challenges with the endogenous occurring CCK, Na-lactate, and CO_2. Patients with panic disorders show a left-shifted dose response (i.e., are more sensitive) to CCK in comparison to healthy subjects (54). It has been argued that CCK fulfills the criteria of an ideal anxiety-challenging model (55). CCK not only elicits symptoms of anxiety but also produces physiological and hormonal changes through activation of the HPA axis (48, 51, 52). This is not the case during lactate or CO_2 challenge. Thus, there is a wider range of acute CCK effects that can be affected by drugs of multiple classes, such as imipramine (56), benzodiazepines (57), and vigabatrine (58), all of which antagonize CCK effects on panic symptoms.

EXAMPLES OF VALIDATED VERSUS EXPLORATORY BIOMARKERS

Retrospective Biomarker Collection

Few biomarkers assessing CNS drug effects have been validated, most are of the types already presented, and many are highly exploratory when a novel target is in question. Perhaps the most widely utilized biomarker in neuropsychiatric drug development is striatal dopamine-2 (D2) receptor binding, usually determined by assessing displacement of the PET ligand [11C] raclopride. High occupancy is usually associated with Parkinson-like side effects, whereas efficacy with so-called atypical antipsychotics can be achieved at lower levels of binding (59). An associated biomarker for atypical antipsychotics is to look for high 5-HT2 receptor occupancy in cortical areas assessed by displacement of spiperone or N-methyl spiperone (60). The same approach has recently been applied to find clinical trial doses for a selective 5-HT2A antagonist (61).

A particularly strong case for a validated marker depends on a retrospective analysis of cumulative data on fluoxetine. Table 4 (upper part) lists studies spanning more than a decade, which bridge from the in vitro 5-HT transporter K_i of fluoxetine to blood, CSF, brain (MRS) concentrations, receptor occupancy (PET), and biochemical effects (decreased platelet 5-HT uptake and CSF concentrations of 5-HIAA, the major metabolite of 5-HT). These measures, in turn, can be related to clinical effects (62–68). Prospective use of biomarkers to expedite CNS drug development is our goal and is beginning to have an impact (69–73). Perhaps the most striking current example of application of a biomarker to establish doses for large trials involves a NK-1 antagonist.

Recently, the FDA approved the NK-1 antagonist aprepitant based on the results of two well-controlled studies that included more than 1000 cancer patients receiving chemotherapy that induced severe nausea and vomiting (CINV) (74, 75). In these studies, fewer patients had symptoms of nausea and vomiting when aprepitant was part of their treatment (combination with ondansetron and dexamethasone) compared to patients who received standard antiemetic medicines. Human PET studies had shown that aprepitant crosses the BBB and occupies brain NK-1 receptors (76, 77). The relationship of dose and plasma concentration of aprepitant to

TABLE 4 Time course of biomarker collection

Drug	Publication	Biomarker	Reference
	Retrospective biomarker collection		
Fluoxetine SSRI	1978	Platelet [^{3}H] serotonin uptake	(62)
	1989	R_x scale: MADRS	(64)
		PD: 5-HIAA	
		PK: plasma and CSF	
	1993	^{19}F MRS brain	(65)
	2000	PET	(66)
	2002	5-HT transporter K_i	(63, 67)
	2003	^{19}F MRS brain	(68)
Prospective biomarker collection			
LY354740 Metabotropic glutamate receptor agonist			
	1998	mGlu2/3 receptor K_i	(69)
	2002	PK: plasma and CSF	(70)
	2003	PK: plasma and CSF	(71)
		CSF proteomics	
	2003	Anxiety model: CO_2	(72)
	2004	Anxiety model: CCK	(73)
	2003	Clinical studies	

R_x scale: treatment scale; MADRS: Montgomery and Åsberg Depression Rating Scale (Reference Montgomery & Åsberg: Brit. J. Psychiat. (1979), 134, 382-9). 5-HIAA: 5-hydroxy indole acetic acid; PD: pharmacodynamic(s); PK: pharmacokinetic(s); ^{19}F MRS: Fluorine 19 magnetic resonance spectroscopy; PET: positron emission tomography; 5-HT: 5-hydroxytryptamine; K_i: inhibitory constant; mGlu: metabotropic glutamate; CCK: cholecystokinin.

CNS receptor occupancy was defined in healthy subjects to predict the occupancy of central NK-1 receptors. The effective doses of aprepitant in patients with CINV were 125 mg and 375 mg (78), doses that lead to a receptor occupancy of >90% in healthy subjects. In a Phase II trial, therapy with aprepitant was associated with improvements in depression and anxiety symptoms that were quantitatively comparable with those seen with selective serotonin reuptake inhibitors (SSRIs) and significantly greater than those seen with placebo (79). But, in 2003 the Phase III clinical program was halted because the compound failed to demonstrate efficacy for the treatment of depression despite being used at doses producing >90% NK-1 receptor occupancy in the brain. Thus, the hypothesis that antagonism of NK-1 receptors produces antidepressant effects was properly tested and not supported. If trials with other NK-1 antagonists also fail to show sustained antidepressant effects, this will stand as the first example in antidepressant research of using a biomarker to show that a drug really did engage its target and thereby reject a hypothesis, not just a compound.

Prospective Biomarker Collection

The more common situation in this era of novel targets of unknown function in humans is not having any convincing method for showing that effects of a new compound result from engaging the stated biochemical target. Take the current case of LY354740, an analog of glutamate (Table 4, lower part). It is nanomolar potent, highly selective, and orally active at Group II cAMP-coupled metabotropic glutamate receptors (mGlu). Preclinical studies showed significant anxiolytic activity, comparable to diazepam. However, anxiolytic doses did not cause any of the unwanted secondary pharmacology associated with diazepam (sedation, neuromuscular coordination deficit, interaction with CNS depressants, memory impairment, or changing convulsive thresholds) (80). The affinity for recombinant human brain mGlu2/3 receptors, measured as displacement of 3H-LY341495 binding, shows K_i values of 85 and 125 nM. Agonist activity on human cloned metabotropic glutamate receptors, measured as decreases of forskolin-stimulated cAMP, shows EC_{50} values of 5 and 24 nM (81). Preclinical studies with this agonist have not, however, been able to relate the degree of mGlu2/3 receptor occupancy to behavioral or functional changes.

In the absence of any means of assessing receptor occupancy or any measurable physiologic effects in humans of doses up to 200 mg twice daily (Eli Lilly, unpublished results), we investigated the penetration of LY354740 into the CSF at steady state following BID dosing for two weeks to see if drug was present in the CNS compartment. The exposure of LY354740 in the CSF was approximately 5% of that measured in plasma, and the median CSF concentrations over 12 h at steady state were in the range of the in vitro potency for human mGlu2/3 receptors (70, 71). In a highly exploratory approach, the CSF proteome is being assessed for LY354740 treatment–related changes as evidence of a functional effect. Even if positive, this still cannot provide direct evidence that the predictions from in vitro models translate into a functional effect in living human brain without an analogous preclinical in vivo proteomic study. In addition to looking for direct biomarkers of functional effects, human anxiety models were applied in two studies (panic provocation by CO2 and CCK challenge) of LY354740. Ten of 12 subjects reported significantly fewer CCK-4-induced panic symptoms, had lower subjective anxiety ratings, and had lower CCK-4-elicited ACTH release following one week of treatment with a dose known to be in the range of the in vitro receptor potency (73). Collectively, the data support the utility of a multimodal biomarker development strategy with the mGlu2/3 receptor agonist for identifying biologically active doses of the compound to be used in large trials in anxiety-related conditions (72). The question remains open whether it will ever be technically feasible for many receptor agonists and potentiators to relate occupancy to effect leaving one dependent on an array of functional biomarkers.

In Table 5, selected CNS medications are summarized in context with assessed biomarkers. It becomes clear that access to the brain and the fluid surrounding it are least well known. Imaging studies usually become available later after tracer development has been successful.

TABLE 5 Accessible biomarkers in humans for selected drugs

Drug	Plasma PK[a]	CSF PK[a]	Proteomics CSF	Brain access	Receptor $[K_i]$[b]	PD[c] effects	R_x[d] effects
Atomoxetine Strattera® ADHD[e]	+				+	+	+
Fluoxetine Prozac® Depression	+	+		+ (I)[g]	+	+	+
Duloxetine Cymbalta® Depression	+	+	+		+	+	+
LY354740 Anxiety	+	+	+		+	+	(+)[h]
Aprepitant Emend® CINV[f]	+			+ (I)[g]		+	+

[a]PK: Pharmacokinetic(s).

[b]K_i: Inhibitory constant.

[c]PD: Pharmacodynamic(s).

[d]R_x: Treatment.

[e]ADHD: Attention-deficit/hyperactivity disorder.

[f]CINV: Chemotherapy induced nausea and vomiting.

[g](I): Brain image.

[h](+): Unpublished results.

CONCLUSION

Many extensive reviews on biomarkers in drug development have been published (24, 82, 83). This review focuses on the current status of biomarkers and/or approaches critical to assessing novel neuroscience targets with an emphasis on new paradigms and challenges in this field of research. Nevertheless, some old questions remain, such as how to verify access to the brain. Early imaging studies including micro-PET (84, 85) can help to overcome this, at least for those compounds that can be labeled and/or shown to affect another ligand. However, in case of delayed tracer development or because of no feasible application of brain imaging effects of the molecule, using CSF as a matrix could fill this gap. It is hoped that proteomic research using CSF will have a major impact on the development of treatments for psychiatric disorders.

In this review, the importance of biomarker data integration for psychotropic drug development has been illustrated with examples for both clinically used medications and investigational drugs. The combination of biomarker development with

current biomedical technologies applied to drug discovery can improve the level of innovation and efficiency of drug discovery and developmental programs because whether or not a drug engages its prestated target can be formally tested.

ACKNOWLEDGMENTS

The authors would like to acknowledge Robert A. Padich, Ph.D. of Lilly Global Scientific Information and Communications for assisting with the preparation of this manuscript. The authors are full-time employees of Eli Lilly and Company, but the views expressed in this review are those of the authors.

APPENDIX

Definitions

Generally accepted definitions defined by working groups (86):

- Biological marker (Biomarker): A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.
- Pharmacologic marker (effect or response): A change representing a molecular interaction between drug and body constituent or the observable output.
- Clinical end point: A characteristic or variable that reflects how a patient feels, functions, or survives.
- Surrogate end point: A biomarker intended to substitute for a clinical end point. A surrogate end point is expected to predict clinical benefit (or harm or lack of benefit) based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence.
- Proteomics: Proteomics represents the effort to establish the identities, quantities, structures, and biochemical and cellular functions of all proteins in an organism, organ, or organelle, and how these properties vary in space, time, or physiological state (87).

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Volume 45, 2005

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