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Review

Applications of Quantitative Pharmacodynamic Effect Markers in Drug Target Identification and Therapy Development

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Abstract. *Genome-wide transcriptional profiling is now feasible, and profiling of the proteome, although technically challenging, is advancing rapidly. Expression profiling provides a tool to accelerate discovery in a broad range of sciences, but its greatest impact on human health may be on the process of drug discovery and therapy development, and investigation of the functional networks underlying drug responses of diseased and normal tissue. For anticancer agents in particular, antitumor effects and toxicities to critical normal tissues may rest in a delicate balance that is governed by complex pharmacokinetic (PK) and pharmacodynamic (PD) inter-relationships. Recent advances in the development of mechanistic computational PD models promise to promote an understanding of these inter-relationships, provided suitable quantitative PD effect markers will be identified. Here we describe both advances toward the unsupervised application of PD models to complex expression profiling datasets, as well as approaches to address the technical requirement of these models for quantitative assessment of protein expression levels. Together, these models and analytical approaches may contribute to the rational design of more effective pharmacotherapies.*

The development of optimal therapeutic regimens can be challenging for many classes of drugs. The challenge is particularly acute for antineoplastic agents, given their frequently narrow therapeutic index and complex spectrum of cellular effects, which may not be fully understood in a comprehensive and mechanistic fashion. One example is paclitaxel (taxol), the first of the taxanes to receive clinical approval. Although its primary mechanism of action, stabilization of cellular microtubules, was described a quarter of a century ago (1), a more complete understanding of its action at a cellular level continues to evolve (2). Most recent is the finding that effects of the taxanes on non-tumor cell targets, such as tumor vascular endothelium, may contribute to their therapeutic efficacy, and that intriguing cellular responses can be elicited at ultra-low concentrations or protracted exposure times (3-6). Clearly, a detailed understanding of the complex direct and indirect responses of cells and tissues to antineoplastic agents, such as the taxanes, could provide insight that might enable the design of more effective and less toxic therapies.

Gene and protein expression profiling has the potential to reveal the complex response patterns of cells and tissues to pharmacophores. Expression profiling at the transcriptional level is feasible using numerous approaches, and proteome-wide profiling is advancing rapidly (7). For those drugs that act at the transcriptional level, the regulation of gene expression constitutes the basis for their pharmacological action. For the majority of drugs however, transcriptional changes may represent indirect responses to the pharmacophore, reflecting cellular adaptation to the cascading effects of the drug biosignal through various signal transduction pathways, or compensatory responses as cells seek to reestablish homeostasis in reaction to the drug's primary mechanism of action. Thus the interpretation of expression profiling data, as it relates to understanding the cellular mechanisms of pharmacophore action, can present challenges.

Here we discuss progress in utilizing pharmacodynamic (PD) models to mine expression-profiling data sets. The approach was to develop generic, semi-mechanistic pharmacodynamic models that are intended to reveal PD relationships, thereby assisting in the identification of drug responses at the transcriptional and translational level. We also describe progress in the development of analytical

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strategies that should enable highly sensitive and selective quantification of protein-level pharmacodynamic effect markers, which is essential for the type of PD analysis envisioned. The results provide proof-of-concept for quantifying expression changes of key effector proteins in pharmacological response networks.

Semi-mechanistic pharmacodynamic models for mining pharmacogenomic data. The analysis of expression profiling data to reveal pharmacodynamic mechanisms presents several challenges. Typical datasets are highly dimensional; they capture complex patterns of responses in large numbers of genes or gene products, and response patterns are not only drug-, tissue- and disease-specific, but also temporally inter-dependent. One recent transcriptional profiling study found approximately 1500 corticosteroid-responsive genes in rat liver out of nearly 9000 probed genes (8). Furthermore, the magnitude, time-course and pattern of pharmacodynamic responses can vary with the drug concentration and exposure profile (*i.e.*, pharmacokinetics). Biological and pharmacological variability are also fundamental features of such datasets and cells can develop pharmacodynamic tolerance. These factors complicate the discovery and validation of drug-responsive gene or protein level expression changes in genome-wide data sets. Relating the observed temporal responses in tissues to drug concentration and/or exposure profile represents an even greater challenge.

Various approaches have been applied to the analysis of expression data, particularly for transcriptional profiling (9-11). Frequently used methods of analysis can yield variable performance with noisy or sparse data and few approaches are designed specifically to reveal systems-level pharmacological relationships that represent a fundamental objective of PD analysis.

Quantitative pharmacodynamic models have been developed to assist in the understanding of drug concentration-time profiles (PK) and the magnitude and time-course of the pharmacological effects observed (12). Some PD models are mechanistic in nature, incorporating and in some cases inter-relating the multiple temporally-separated responses that may be observed in complex biological systems (8, 13-16). Statistical engines and methods for the comparison of PD models comprise an intrinsic component of the analysis.

For these reasons, certain types of models are well suited for the extraction of pharmacodynamic relationships from expression profiling data sets. However, PD model development, testing and selection is often an iterative and time-consuming task and generally neither the models nor the software in which they are implemented are easily applied to large data sets. Furthermore, expression profiling on genomic or proteomic scales may reveal unanticipated responses for which the mechanistic connections between drug and response are not yet understood in detail. This lack of information can complicate the development of PD models that are mechanistically realistic.

Our approach was to develop PD models that utilize generic response elements; these elements capture commonly-observed characteristics of different types of pharmacological responses and are applied wherever more explicit mechanistic details are not available. The use of generic response elements enables the models to be used as tools for seeking features in expression profile data sets that resemble characteristic responses to pharmacological agents. To enable the application of multiple PD models to large data sets without supervision, a flexible software framework was prototyped, with the results of each iterative model fitting captured in a relational database. By exploiting both statistical descriptors and model comparison descriptors to identify those types of model that best fit a particular gene or protein level response, a range of models representing different types of PD responses can be applied to a large number of individual expression responses. The overall hypothesis is that the models that best fit a particular response pattern contain generic response elements that may underlie the observed response.

Three types of pharmacological response elements were used to construct the generic PD model described here: direct effect, indirect response and signal transduction. An excellent recent review provides a more thorough description of these individual elements (16).

Direct effect models assume that drug concentration at the affected site directly controls the magnitude of the response. A Hill-type E_{max} model of the form

$$E = E_0 \pm \frac{E_{max}^{\gamma} * C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}}$$

often fits the concentration-effect (E) relationships observed in many biological systems (17). Characteristics of responses that are captured well by this model element include a sigmoidal, log-linear concentration-effect relationship, drug potency (EC_{50}), a baseline activation threshold (E_0) and a maximum achievable response (E_{max}). In the equation, γ determines the steepness of the concentration-effect relationship. For those cases in which the time required for drug distribution to the affected site causes a delay between the observed peak plasma concentration (C_p) and the pharmacological response, an *E_{max} link model* has been proposed (18). In this model, the response is driven by drug concentration at the affected site (C_e), and the relationship between C_p and C_e is described

by first-order processes of distribution and elimination from the affected site:

$$\frac{dC_e}{dt} = k_{1e}C_p - k_{e0}C_e$$

where k_{1e} and k_{e0} are first-order rate constants for distribution and elimination, respectively.

Indirect response models (19-25) capture mechanisms of action in which the drug alters a turnover process and were inspired by the observation of cases in which the kinetics of the turnover process temporally displace the time of peak activity from the time of peak drug concentration. Drugs that alter gene expression as a primary mechanism of action can be well-characterized by indirect response models (13). Such a model is shown in Figure 1, along with simulations of its behavior. Turnover of the response variable (R) is controlled by a zero-order production or synthesis rate (k_{in}) and a first-order elimination or degradation rate (k_{out}). At homeostasis, these are balanced. The action of the drug is to alter one of these processes, either by inhibition or stimulation. The result is a temporal decrease or increase in the response. The Hill E_{max} function is used to describe the drug concentration-effect relationship. Because a drug can affect either production or elimination of the response, and the effect can be inhibition or stimulation, the combination of two possible states for two rate constants results in four possible models; these represent prototypes of four different mechanisms of drug action, two of which are shown in Figure 1. In the indirect response model, the time of peak response is delayed as dose increases (24); this behavior contrasts with that of the direct effect model, thus the presence of an indirect response element in an observed pharmacological response can be tested experimentally.

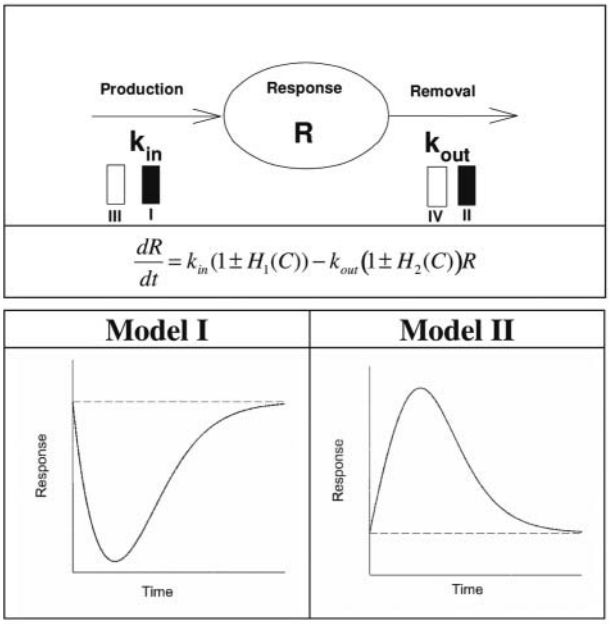

Figure 1. *Indirect response models. Top panel shows model in schematic format. The magnitude of the response (R) is determined by k_{in}, the zero order rate constant responsible for production of the response, and k_{out}, the first order rate constant responsible for removal of the response. A drug affects the rate constants either by inhibition (filled bars designated I and II) or stimulation (open bars designated III and IV); thus 4 distinct response patterns are possible. Middle panel shows the equations describing the model: R, magnitude of the response; C, drug concentration in the effect compartment; H(C), the Hill E_{max} function described in the text. Lower panels show simulations for 2 of 4 possible response patterns; Model I, k_{in} is inhibited, $H_1(C)$ is subtracted, $H_2(C)=0$; Model II, k_{out} is inhibited, $H_2(C)$ is subtracted, $H_1(C)=0$. Not shown: Model III temporal behavior is similar to Model II; $H_1(C)$ is added, $H_2(C)=0$. Model IV temporal behavior is similar to similar to Model I; $H_2(C)$ is added, $H_1(C)=0$.*

Signal transduction models were developed to describe situations in which a series of intervening processes are interposed between the initial action of the drug and the observed response, as in a signal transduction network (26, 27). Such models have considerable potential to account for delay between drug/receptor interaction and pharmacological response and to interrelate pharmacological responses that are linked but temporally separated.

A basic signal transduction or 'transit compartment' model, along with simulations that illustrate model behavior are shown in Figure 2. In this example, the transduction model consists of 3 elements in the response cascade, with τ representing the average signal transduction time through each step in the cascade. Unless other mechanistic detail is available, τ is considered constant for all steps. As additional information about a pathway is obtained, models describing those components can be substituted for the appropriate intermediate theoretical component (8, 13-15, 19, 28-30). Because the time-course and intensity of the biosignal is calculated explicitly for each compartment, this type of model could be used to search for gene expression events that are linked pair-wise but separated temporally. The response magnitude, shape and peak time vary in a characteristic manner, dependent upon input drug concentration (27) and (Figure 2), and thus the existence of this type of response element can be tested experimentally.

In Figure 2, the simulations show model behavior with the drug concentration at steady-state. In Model A, the response continues to increase until a maximal value is achieved. This type of model would be PK-driven, in the sense that it requires elimination of the drug to terminate the pharmacological response. Implementation of this model would require the incorporation of an accurate pharmacokinetic model that describes the drug concentration-time profile at the target tissue site. Model B

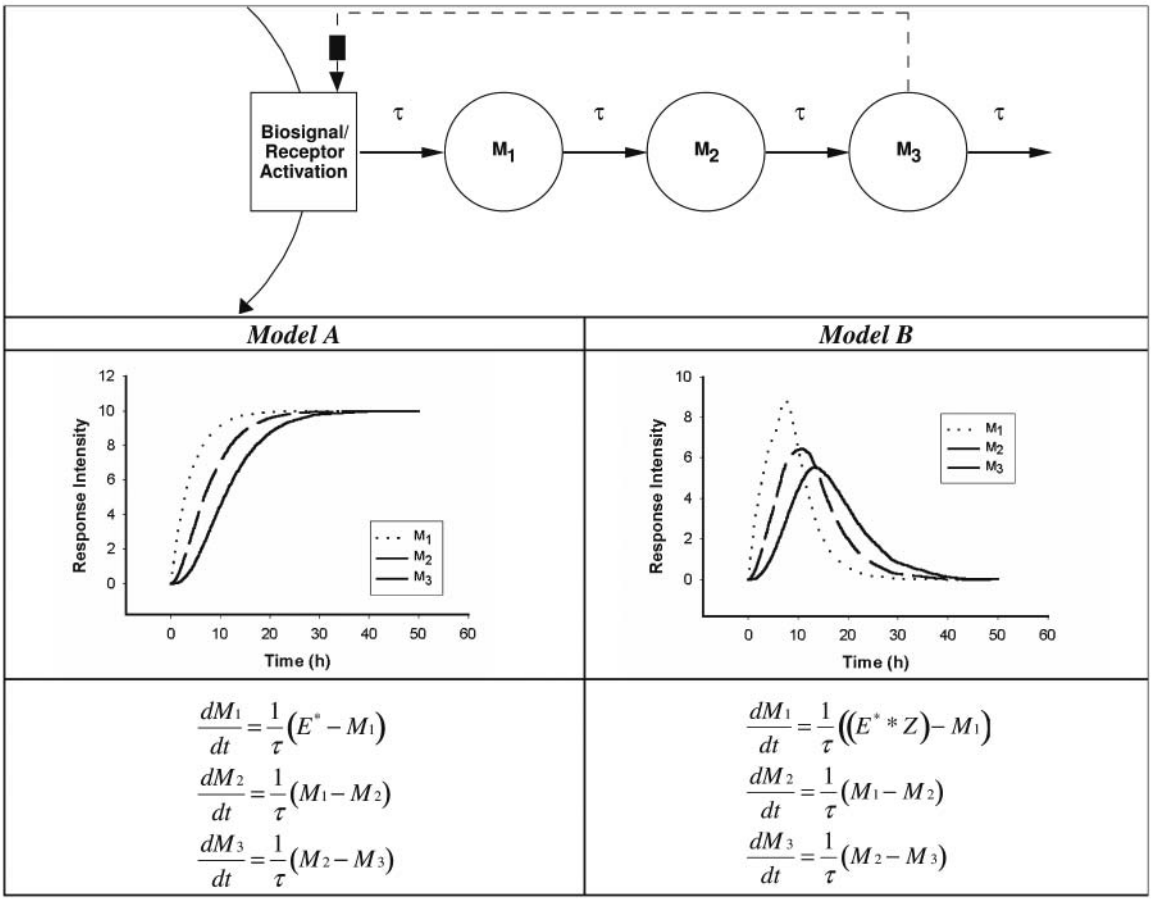

Figure 2. *Signal transduction models. Top panel shows model in schematic form; drug-receptor interaction elicits biosignal that transits with average time τ through several steps or secondary messengers (labeled $M_{1\text{-}3}$). Middle, bottom panels: equations and simulations for two similar models (A, B). E* represents the response model (e.g. the Hill E_{max} function described in text). For simplicity, τ=4 h in the simulations, the drug concentration is assumed constant and E*=10. Thus the response in each of the compartments $M_{1\text{-}3}$ plateaus to a maximum (Model A). Model B: same as Model A, except a feedback loop (dashed line, top panel) inhibits response to drug when a threshold is reached (variable Z transitions from 1 to 0).*

(Figure 2) incorporates a simple regulatory process in which a feedback loop terminates propagation of the biosignal through the cascade. An example would be the case in which down-regulation of a drug receptor is the result of drug action, as in the corticosteroid system (13). Overall, signal transduction models can be simple yet flexible enough to capture a variety of biological responses and can yield explicit hypotheses that are testable by experimentation.

Generic semi-mechanistic PD models. Because the mechanistic details linking drug concentration and an observed change in abundance of a specific mRNA or protein often are unknown, we developed a PD model (Figure 3) that represents a simplified hybrid of two approaches. It includes: i) the indirect-response element of Figure 1 as the engine that models the observed response (R) (29), and ii) parallel signal transduction cascades (designated *A* and *I* in Figure 3) (26, 27) that modulate the indirect response element. In this model, the drug triggers a biosignal that is propagated through the signal transduction pathways and impinges on the indirect response element, altering the rates of production and loss (k_{syn} and k_{deg}) of response R. The modulation may be negative (down-regulation) or positive (up-regulation). As noted above, the combination of two possible states for two rate constants yields four possible temporal response patterns.

Figure 4 shows the equations and simulations of the four response patterns that can be derived from the model shown in Figure 3. Although it appears complex, the model contains only 6 independent parameters and, in practice, only 5 parameters need to be estimated. In the absence of any drug, the gene or protein abundance is at baseline level R_0. The biosignal is triggered simultaneously (for simplicity) by the initial drug/receptor interaction and propagates with mean

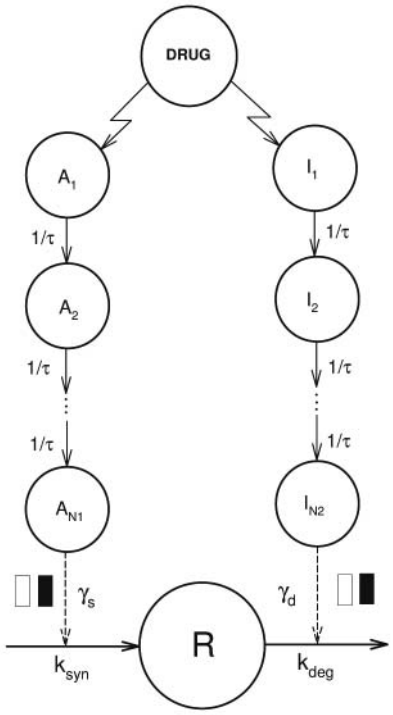

Figure 3. *Pharmacodynamic models for regulation of expression. Model combines the Indirect Response (Figure 1) and Signal Transduction models (Figure 2). A change in R (abundance of a specific mRNA or protein) is the indirect response elicited by a drug. The model consists of two signal transduction cascades, having $A_{1\text{-}N}$ and $I_{1\text{-}N}$ intermediate elements. These cascades impinge upon k_{syn} and k_{deg} the rate constants in the indirect response model that regulate mRNA/protein synthesis or degradation rates (see Figure 1 legend). The effect can be to increase (open bar) or decrease (filled bar) the rate constants.*

transit time τ through N compartments in the two independent transduction pathways, A and I. Differing propagation times in pathways A and I can be accomplished by independently varying N_1 and N_2, respectively. S_{syn} is the biosignal that impinges on k_{syn}, the zero-order process that controls the production of the response R (*i.e.* the rate of mRNA or protein synthesis). Similarly, S_{deg} is the biosignal that modulates k_{deg}, the first-order process that describes mRNA or protein degradation (13, 29, 31, 32). Baseline values for k_{syn} and k_{deg} are taken from literature when specific information is not available. The peak response occurs at time (N-1) * τ, with peak height of $((N-1)/e)^{N-1}/(N-1)!$. The significance of this feature of the model is that the signal is delayed and weakened as N, the number of intermediate compartments, increases (data not shown), thereby providing an experimentally-testable signature for this model.

Our approach to applying this model to large datasets is to vary N_1 and N_2 systematically as independent variables, searching for combinations providing the best fits of the model to the data. This reduces the number of fitted parameters. If average transit time τ is initially assumed to be identical for each individual step in the transduction cascade, a simplification that has worked well with data (26, 27), the number of simultaneously fitted parameters is reduced to 5.

The flexibility of this type of model in producing output behavior that fits remarkably well to complex temporal gene expression responses observed in a publicly-available data set (33) and appears to deal well with biological variability is shown in Figure 4. An attribute of this approach is that the model seeks to identify, within a temporal expression profile data set, a pharmacologically plausible response pattern that may underlie the data. Each of the mechanistic elements of the model exhibits a characteristic response as conditions (such as drug concentration and exposure time) are altered. Thus the approach holds promise for mining genomic and proteomic data sets to identify response patterns that resemble combinations of underlying pharmacological mechanisms. The application of these models to large-scale expression data sets (34, 35) is currently under way.

Accurate and sensitive quantification of low-copy pharmacodynamic effect marker proteins. An essential requirement for the PD analysis discussed above is to identify PD effect markers that are both relevant to the therapeutic response and amenable to accurate quantification. Data sets must be sufficiently rich to define both temporal responses and the concentration/exposure-effect relationship for the drug. Analytical sensitivity and selectivity are often issues in quantifying PD effect markers, because key signaling or effector molecules may exist in low abundance against a background of higher-abundance, irrelevant molecules. Transcriptional profiling, as performed using a number of current techniques, meets these requirements. However, important pharmacological responses to many drugs may not be transcriptionally regulated. For example, HIF-1 is a key signaling protein that regulates angiogenesis and also plays a role in the toxicity of microtubule-acting drugs such as the taxanes. It has been shown that changes in HIF-1 abundance signal the shift between norm-oxic and hypoxic conditions, and this change occurs without significant alteration in HIF-1 mRNA levels (36). Other signaling pathways are regulated by post-translational modifications such as phosphorylation (2) that are not reflected in transcriptional changes Therefore, direct quantification of PD markers at the protein level is necessary.

Because of the diversity of their chemical and physical properties, proteins are more difficult to analyze than nucleic acids. Methods that are high in both sensitivity and selectivity are required, given the large number of high-abundance proteins in cells and tissues, and the generally low abundance

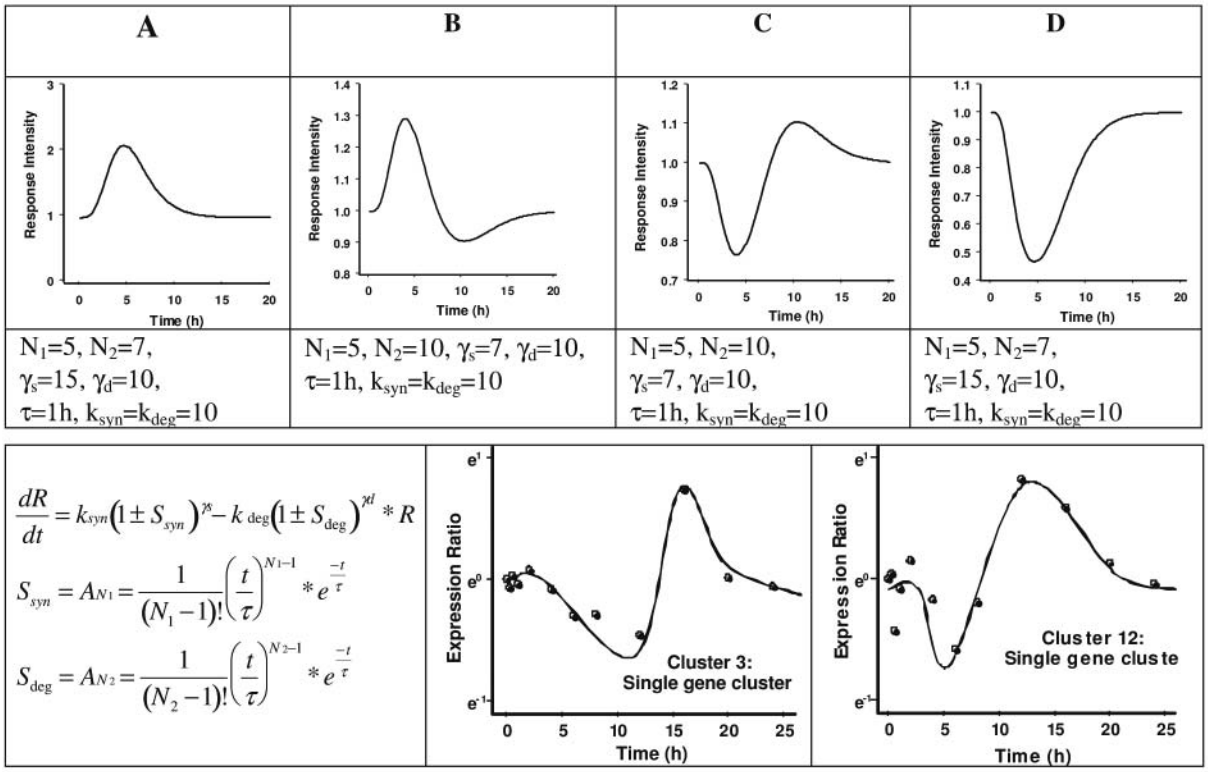

Figure 4. *Simulations of expression regulation models. Figure shows equations and simulations of the models based on the scheme shown in Figure 3. Response R (mRNA or protein level for specific gene) is the indirect response elicited by the drug; γ_s and γ_d denote signal amplification factors. The biosignals traveling through the signal transduction pathways (S_{syn} and S_{deg}) exert up- or down- regulation on the level of a specific mRNA or protein. In the absence of drug stimulus (S_{syn}= S_{deg}= 0), the baseline expression is $R_0 = k_{syn}/k_{deg}$. The selection of + or – signs corresponds to stimulation or inhibition of k_{syn} and k_{deg}. Other symbols are as described in previous figures. Top row: four expression profiles predicted by the model based on the balance of the second messenger systems modulating the response. Bottom row: Model fitted to two complex time profiles for single gene responses resembling simulations B and C (top). Data taken from a publicly-available data set (33) in which serum-starved cells were stimulated with serum at time=0, eliciting numerous transcriptional changes.*

of many effectors in signaling cascades. Numerous approaches have been employed for protein quantification, including electrophoretic, chromatographic (37-39), immune- (40, 41), and mass spectrometry (MS)-based methods (42-46). Compared with more traditional techniques, liquid chromatography (LC) coupled to MS improves the selectivity and quantitative accuracy (47) of the electrophoretic techniques, but does not completely overcome the tendency to discriminate against lower abundance proteins.

LC/MS-based approaches are among the most promising for quantification of PD effect markers, given their sensitivity, selectivity and accuracy, but quantifying low-abundance regulatory proteins against a background of higher abundance proteins and peptides in tissues nonetheless constitutes a major challenge. A series of isotope-coded techniques has been developed for protein expression profiling and quantification (47-49). Among these, the isotope coded affinity tag (ICAT) approach is the most prevalent. The ICAT reagent consists of an affinity retrieval tag such as biotin, a hydrocarbon linker region that functions as a heavy (^{13}C) or light (^{12}C) mass tag, and a protein-reactive group specific for an amino acid such as Cys (Figure 5). A chemical cleavage site may be incorporated in the tag to permit removal of the retrieval ligand after isolation of the tagged peptides. An advantage of the ICAT approach is that the mass tags permit physical mixing of an experimental and reference sample following initial separate ICAT labeling. In this way, the reference sample permits correction for sample-to-sample variations that occur during processing steps such as protease digestion and affinity retrieval. In principle, peptides labeled with either mass tag have identical chromatographic behavior and detection by MS consists of searching for paired isotopic peaks that differ by 9 Da (or 4.5 for doubly charged MS peaks, *etc.*). Differences in the abundance ratio of the paired ICAT peaks between the reference and experimental samples indicate relative differences in the concentration of the target protein.

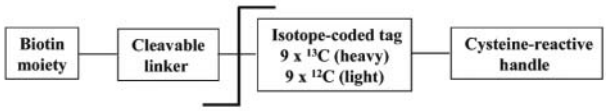

Figure 5. *Schematic representation of ICAT reagent. A functional group reacting covalently with peptide side chains (such as Cys) links a tag to a subset of peptides in a complex mixture. The affinity ligand (biotin) permits retrieval of tagged peptides from mixture. The 9-carbon linker region consists of heavy (^{13}C) or light (^{12}C) atoms. The cleavage site permits removal of the affinity tag after retrieval of tagged peptides, reducing perturbation of chromatographic behavior.*

Hundreds of thousands of peptides may be generated as a result of trypsin digestion of tissue homogenates. By retrieving only the Cys-containing peptides that are tagged by the ICAT reagent, this approach reduces the complexity of biological matrices and thereby reduces the bias against low-abundance target proteins. Nonetheless, a large number of ICAT peptides are retrieved from tissue samples. This hampers automatic LC-MS/MS strategies, which analyze only a limited number of the highest intensity ions entering the MS analyzer at each time point, and therefore are biased against low-abundance peptides.

Our aim was to develop an approach that would permit quantification of lower-copy protein targets of pharmacological significance that were predicted by transcriptional profiling. In this case, targets are known but numerous, and are buried in a sea of peptides derived from higher-abundance proteins. We employed an ICAT strategy (50) and chose the well-characterized corticosteroid induction system as proof-of-principle, given the large number of drug-responsive transcriptional changes already established (8, 14, 15).

Following cleanup of rat liver homogenates using strong cation exchange (SCX) chromatography, a long, shallow capillary LC gradient was used to reduce the number of co-eluting peptides entering the MS detector (50). The chosen targets were anticipated to be low in abundance; therefore automatic LC-MS/MS strategies would likely fail. Furthermore, pure protein was not available for most targets. Our approach was to use published genomic data to predict the m/z (mass-to-charge ratio) for all possible tryptic ICAT peptides derived from each protein of interest, and screen the entire ICAT-derivatized peptide mixture from tissue homogenates for those target peptides.

In the first stage of the assay, an ion trap MS was used to search for any detectable ICAT peptide having an m/z matching one that could be derived from a target protein of interest. All hits were sequenced to confirm identity, thus ruling out a substantial number of isomeric peptides (data not shown; (50)) that could be expected from the large number of tryptic peptides that would be generated from liver. Any positively identified target peptides that were selected for quantification were then synthesized, in order to verify the identification using LC retention time, verify parent and product m/z, and also to permit optimization of MS assay conditions for quantifying that peptide.

In the second stage of the assay, an identical LC gradient was run into a triple quadrupole MS/MS instrument in order to quantify target peptides. The intent was to take advantage of the wide dynamic range, very high sensitivity and selectivity of the triple quadrupole instrument when operating in Multiple Reactions Monitoring (MRM) mode (51, 52).

In this proof-of-principle study, two peptides derived from the target tyrosine aminotransferase (TAT), a corticosteroid-induced enzyme, were successfully identified. One, designated T7 (Figure 6), was selected for quantification because of its significantly more intense MS signal (data not shown). An assay for the *T7* peptide was developed. It showed good linearity over the range of 5-500 fmol on column with a detection limit of approximately 0.1 fmol (data not shown). Using the *T7* peptide to quantify TAT induction (Figure 6, top) a 5-fold increase in TAT protein was observed within 4 h of corticosteroid treatment of rats (50). TAT enzymatic activity was also assayed, and it peaked 6 h after treatment (Figure 6, middle). An apparent delay between the induction of TAT protein and TAT activity was observed, suggesting that additional post-translational processing may occur. In Figure 6 (bottom), TAT activity was normalized by the amount of TAT protein quantified in the same sample. It can be seen that the variability of the data decreases considerably, indicating that biological variability (animal to animal) is greater than analytical variability (sample to sample).

The results with this ICAT-based approach suggests the feasibility of identifying and quantifying low-abundance, pharmacologically-relevant PD effect markers in tissues or cells. Based on the results obtained, TAT abundance at peak expression appeared to be only approximately 50 fmol per 100 μg of total liver protein.

This approach also is amenable to quantification of multiple targets simultaneously, and also permits re-interrogation of processed samples in the event that additional target proteins become of interest (50). The processed ICAT samples used for TAT quantification were stored frozen (–80˚C) for 3 months and then re-analyzed to quantify the induction of ornithine decarboxylase, another corticosteroid-induced protein that was detected in the MS data because of the change in relative abundance of ICAT labels in liver samples from corticosteroid-treated rats (data not shown). The current status of this research is to improve the ability to discover quantifiable peptides from target proteins of interest, thereby streamlining the process for the quantification of large numbers of target protein proteins.

Conclusion

Quantitative, mechanistic, computational pharmacodynamic models have progressed to the point where they can integrate pharmacological responses in complex biological systems. However, their use in the analysis of PD response data often has been *post-hoc*, aiding in the mechanistic understanding of response data and in the development of experimentally testable hypotheses regarding mechanisms underlying the observed responses. Generic PD models, based on distinct types of observed pharmacological responses, along with improvements in the ability to apply models in an unsupervised manner to large data sets, suggest the feasibility of employing PD models for the primary analysis of temporal expression data. An advantage of these models is that each is 'prejudiced' to discovering plausible pharmacological response profiles within a data set. These models should also be capable of integrating higher-dimensional data, such as temporal responses observed with different drug dosages or pharmacokinetic profiles. Few other approaches for the analysis of expression profiling data offer a capability grounded in pharmacological principles.

The application of PD models to profiling data is most successful when rich data sets are available that capture temporal responses under multiple conditions of drug concentration and exposure. Although transcriptional profiling is routine, quantification of multiple targets at the protein level remains a challenge. The approaches described here suggest the promise of using quantitative liquid chromatography/mass spectrometry techniques to provide the data necessary for successful PD analysis of pharmacological responses in biological systems.

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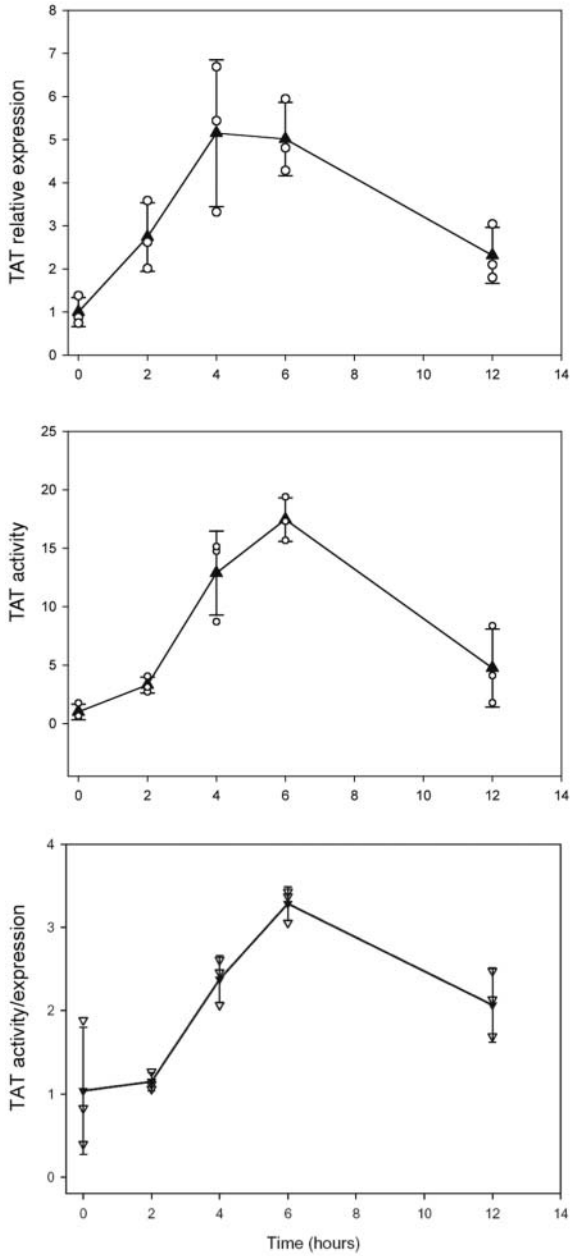

Figure 6. *Quantification of TAT induction by MPL in rat liver. Peptide T7 (R)VVITVPEVMMLEACSR(Q), derived from tyrosine amino transferase (TAT), was identified in liver homogenates of rats treated i.v. with 50 mg/kg methylprednisolone (MPL) and was used to quantify TAT induction by MPL. Liver homogenates were prepared at each time point indicated and 100 μg total protein was derivatized with ^{13}C-ICAT (heavy reagent). The reference homogenate, taken 4 h after MPL dosing, was labeled with ^{12}C-ICAT (light reagent) and spiked into each time point sample. Samples were digested with trypsin, ICAT peptides were retrieved by affinity chromatography, separated by reverse phase chromatography using a long, shallow LC gradient, and analyzed in MRM mode using a triple quadrupole MS/MS instrument. Top panel: induction of TAT as determined by quantification of T7 peptide; each point represents a single animal. Middle panel: induction of TAT as determined from enzymatic activity; bottom panel: TAT activity normalized by the amount of detected TAT protein. Top and middle panel redrawn with permission from (50).*

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