

Inherent gene expression variabilities and the need for personalized drug dosage

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Abstract

Simulations of the effects of DNA polymorphisms, transcriptional noise, and epigenetic differences within and among populations suggest highly divergent responses to drug dosage, resulting in considerable risk of adverse effects. These findings highlight the need for personalized calibration of drug dosage.

Key words:

personalized medicine, dosage selection, genetic polymorphism, gene expression variability, gene regulatory networks, systems biology.

Suggested Running Header: dose-response variability

Introduction

Drug dosage selection must minimize adverse effects while maximizing efficacy. Here, using simulations of the effects of an antagonist drug on downstream gene expression levels, we show that inherent differences among individuals, and within the cells of individuals, lead to large differences in dosage requirements.

The effects of genetic polymorphisms on drug uptake and clearance rates are well known, and in cases such as warfarin [1], dramatic. The simulations presented here focus on drug-action at the cellular level and assume optimal drug targeting, delivery and clearance rates. Surprisingly, we find that even below-average inter-individual variations in gene expression levels lead to considerable variations in response to drugs. Our results suggest a need for personalized drug dosage selection.

Gene expression variation is known to arise from at least three different mechanisms. First, non-genetic factors such as age, gender, and health history are well known to affect gene expression. Metabolic status [2], smoking [3], exercise [4], and emotional state [5] have also been shown to modulate the expression of large numbers of genes by 2-fold or more. Second, inherent transcriptional noise in individual cells arises from random variations in cellular content, and the fact that transcription is a sequential process involving a small number of copies of each gene [6]. Measurements in single mammalian cells suggest a protein abundance coefficient-of-variation of 15-30%, and long-lasting (>24 hours) concentration changes [7]. Third, widespread DNA-sequence variations cause several-fold differences in gene expression both within and across populations [8-10]. These variations are observed in multiple cell types and affect a large proportion of genes in both healthy and diseased individuals. Genes involved in the immune system – a target of many drugs – appear to be particularly variable, both within and among populations [11].

Here, using a simple kinetic model we show that – even under very conservative assumptions – unavoidable variations in gene expression result in highly divergent responses to the same drug dose, and lead to several-fold differences in optimum dosage.

Even individuals who appear to respond well to treatment are shown to harbor many non-responsive cells. These effects highlight the need for personalized calibration of drug dosage.

Results

Fig. (1A) shows a schematic of our illustrative model: inappropriate activation of a signaling pathway is inhibited by an antagonist drug that binds the pertinent receptor with much greater affinity than the activating ligand. Gene1 and Pathway1 represent processes downstream of signal transduction, which are activated in the diseased state. Gene2 and Pathway2 represent downstream processes inhibited in the diseased state. Gene2 is repressed by Gene1, so that any variability in Gene1 expression feeds into Gene2 and compounds its variability. For simplicity, the response of an individual to drug treatment is modeled as a linear function (here the average) of the responses of the relevant cells within the individual.

In the simplest version of our model, cellular behavior is noise-free (deterministic), and all individuals in a population are identical. In that case, cell, individual and population responses are identical. More interesting outcomes arise when variability is introduced and the model is simulated stochastically. In the following, we sequentially add sources of gene expression variability to the model and evaluate the resulting variability to drug treatment.

Fig. (1B) shows example response distributions of 1000 individuals. Non-genetic differences among individuals were mimicked by varying all model kinetic parameters randomly by up to 10% (resulting in <2-fold variation in Gene1 expression at zero dosage, see Supplementary Fig. (S1)). The stochastic gene expression model and nominal parameter values (see Methods in Supplementary Materials) are based on our earlier model of gene expression in macrophages [12].

To translate the above dose-response distributions into illustrative measures of drug efficacy, Gene1 is defined to be adequately suppressed when its expression is less than

10% of its average expression in the diseased state. Similarly, Gene2 is defined to be adequately expressed when its expression exceeds half of its mean in healthy cells. At each drug dosage, we can now count the number of individuals in whom genes 1 and 2 have responded adequately to treatment. Fig. (1C) shows the resulting dose-response curve. Note that Gene2 – whose expression is noisier than Gene1 – has a more graded response and requires a higher drug dose.

Fig. (1C) can be used to define a therapeutic window for our hypothetical drug. At lower drug doses too few people benefit from treatment (<95% of cells responding). At very high doses (gray box), the drug may have adverse effects. The area between these regions defines the therapeutic window for the drug.

Next, inherent gene expression noise within cells was mimicked by randomly varying all kinetic parameters of the network in Fig. (1A) by an amount not exceeding 10%. This noise level results in an mRNA1 variance to mean ratio well below that measured [13] (2.7 versus 4.1). Fig. (2A) shows example distributions of cellular responses to drug treatment for the *average* individual from the population in Fig. (1B) (see also Supplementary Fig. (S2)). At the doses deemed acceptable in Fig. (1C), many cells in this individual do not respond to treatment, even though the individual is well within the therapeutic window. While these cells do not contribute significantly to the *average* mRNA2 level of an individual, they can result in poor treatment outcomes if they are virulently contagious or proliferative.

The dose-response distributions of Fig. (2B) add the effects of allelic variations to the above scenarios by modifying the expression level of Gene1 to (i) double and (ii) half nominal values (see also Supplementary Figs. (S3) and (S4)). These particular values were selected to mimic gene duplication or deletion of a gene copy. Similar effects can arise from other mutations within the network. At the dosage shown in Fig. (2B), all but one of the 1000 individuals in Fig. (1) responded to treatment. Using the same treatment success criteria as Fig. (1), over 12% (128/1000) of population (i) fail to respond, while population (ii) receives a 2.5 – 4 fold overdose (with potential adverse effects). In fact, no

single dosage is satisfactory for both populations, as illustrated by the dose-response curves of Fig. (2C).

Finally, Fig. (2D) shows that over 17% (177/1000) of the cells of the average individual in population (i) do not respond to treatment, though the individual is deemed to have responded. In other words, at the dosage optimum for the population in Fig. (1), less than half of population (i) is treated successfully.

Discussion

The simulation studies presented here suggest dramatic differences in drug dosage requirements among individuals, even within genetic populations. The model presented here is intended for illustrative purposes. The two downstream genes modeled represent two common archetypes: genes directly downstream of a signaling pathway, and genes regulated by signaling-activated transcription factors.

In this study, the complexity of the model and number of free parameters were kept to a minimum in order to highlight the fundamental nature of the observations reported. Because there are no feedbacks and no cross-talk paths to other pathways, our model is monotonic in its behavior, and not sensitive to its parameter values. Any set of parameter values that allow the two genes in the model to switch on and off according to the availability of ligand and/or drug molecules, result in model behaviors similar to those reported here.

Real biological pathways have many cross-connections and feedbacks. Such linkages can create both sensitivity and robustness. In particular, we and others have shown that some genetic regulatory networks are capable of filtering out gene expression variability (see for example [14, 15]). To date, mechanisms that provide robustness to gene expression variations have only been discovered in a limited number of cases. Whether such “noise-filtering” occurs downstream of common drug targets is not known. We hope that our findings will motivate urgent experimental evaluation of the extent of gene expression variability downstream of known drug targets. A related challenge will be the

development of quantitative biomarkers that will allow cheap, fast, and accurate measurement of patient response to drugs.

Conclusions

Personal genomics is widely expected to revolutionize drug development by enabling the design of customized drugs for population-specific susceptibilities. The simulation studies presented in this paper suggest that even for population-specific drugs, and even after correcting for differences in drug uptake and clearance rates, dosage levels may need to be optimized on a person-by-person basis.

The recent sequencing of the diploid genome of a single individual [16] has revealed a surprisingly large number of genomic polymorphisms, with multiple variants affecting individual pathways [17]. Nonlinear interactions among variants can lead to still greater gene expression variations than modeled here. Thus, the need for personalized drug dosage selection may be even more pressing than indicated by the conservative estimates presented here.

Conflict of interest

The author declares he has no competing financial interests.

Acknowledgements

The author's research is supported in part by NHLBI grant number HL089102.

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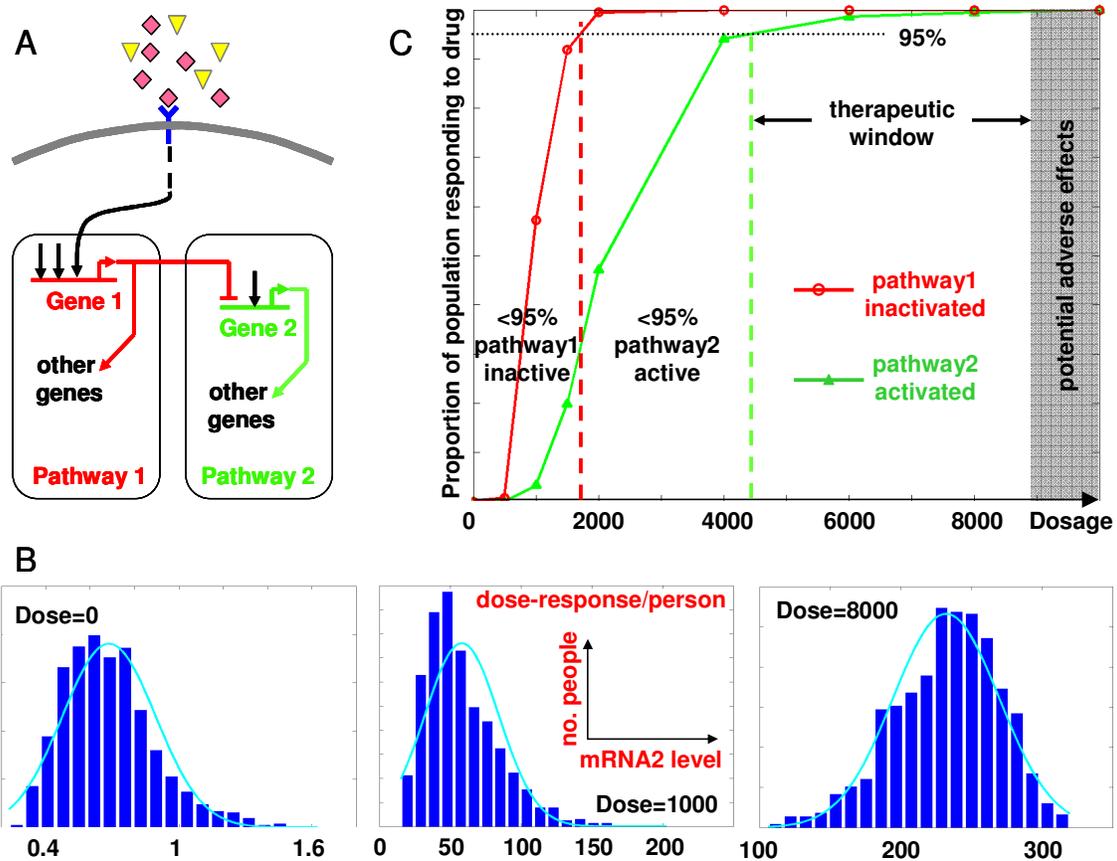


Figure 1, the model and its behavior. **A**. Model schematic: a hypothetical signaling pathway is inappropriately activated (triangles represent ligands) in a hypothetical disease state. Drug molecules (diamonds) bind the receptor with much greater affinity; suppressing inappropriate activation of Pathway1, and permitting activity of Pathway2. **B**. Sample Gene2 response distributions in 1000 simulated individuals. Gene2 is off without treatment (leftmost panel). Its expression increases nonlinearly with increasing drug dose (middle and right panels). Superimposed curves show Normal distributions with the same mean and variance as the histograms. mRNA2 levels are per person averages. **C**. Population dose-response curves showing different response rates by Gene1 and Gene2.

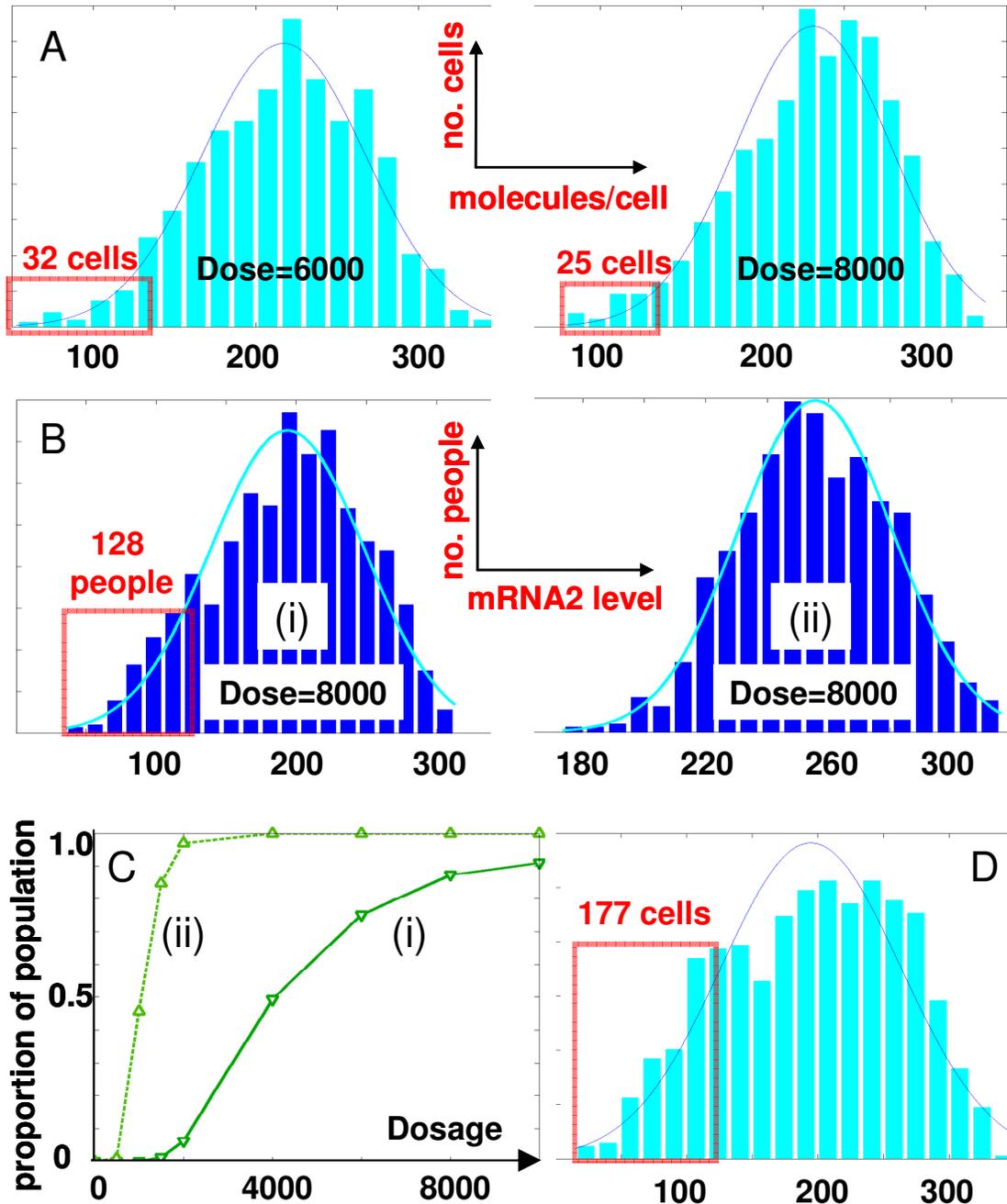


Figure 2, cellular and organismic variability in response to drugs. **A.** mRNA2 cell-cell variability within a single individual (the average person in Figure 1). Two dosage levels within the therapeutic window of Figure 1 are shown. **B.** Effect of allelic variation on dose-response in individuals: (i) doubled Gene1 expression, (ii) halved Gene1 expression. mRNA2 levels are per person averages. Population (i) has 128 non-responding individuals. **C.** Proportion of people with adequate mRNA2/person dose-response for populations (i) and (ii). **D.** Distribution of mRNA2 molecules/cell in

the *average* individual in B(i). Although considered to have responded well to treatment, the individual harbors 177 non-responsive cells (out of 1000 modeled).