Network-based discovery through systems biology

Systems Pharmacology: An opinion on how to turn the impossible into grand challenges

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A pharmacology that hits single disease-causing molecules with a single drug passively distributing to the target tissue, was almost ready. Such a pharmacology is not (going to be) effective however: a great many diseases are systems biology diseases; complex networks of some hundred thousand types of molecule, determine the functions that constitute human health, through nonlinear interactions. Malfunctions are caused by a variety of molecular failures at the same time; rarely the same variety in different individuals; in complex constellations of OR and AND logics. Few molecules cause disease single-handedly and few drugs will cure the disease all by themselves when dosed for a limited amount of time.

We here discuss the implications that this discovery of the network nature of disease should have for pharmacology. We suggest ways in which pharmacokinetics, pharmacodynamics, but also systems biology and genomics may have to change so as better to deal with systems-biology diseases.

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Introduction

Thanks to mathematical modeling, *pharmacokinetics* (PK) has made great strides: nowadays much less drug attrition is due to drug failing to reach its target [1]: PK, or 'phase 0' of drug development appears to be in good shape. Although this may seem to speak for the utility of mathematical modeling in the drug development process, it also highlights that the effectiveness of the other phases of drug development, in particular phases II and III, that is, of the prediction of effectiveness against actual disease in human, and of the prediction of toxicity, have not improved in parallel [1]. For, over the same time period the rate at which drugs reached the market has decreased whilst the investment in drug research has increased [2,3].

Indeed, failures in phase II (trial in tens of patients asking whether the drug has some therapeutic effect and if it is safe enough for a trial in a large enough group for the result to be statistically significant) and phase III (clinical trial with some 1000 patients testing whether the drug performs better than standard therapy, and is safe) may have increased, certainly per dollar invested in drug development [1]. And some drugs have failed in phase IV (i.e. in the market itself), often due to

idiosyncratic toxicity, that is, being toxic to a fraction of the individuals that is smaller than was noticeable in the phase III clinical trial [4]. The consequence is that we should not be content with the current overall success rate of pharmacology.

In addition to all this, the number of drugs even entering the drug development pipeline has not increased as much as what one would have hoped for, given the expected empowerment by genomics. This is however less attributable to pharmacology than to the phase -II (i.e. phase minus II) preceding it, that is, drug target discovery.

How about disease control itself?

Is there any reason to be complacent? The answer has to be in the negative. Since Nixon announced the War on Cancer and the USA began to invest in research in a disease as hidden (then) as breast cancer, research has grown exponentially up to annual spending exceeding 15 billion US$ per year [5]. Yet, the incidence from cancer has only decreased slightly in men, most probably because many of them could be convinced to quit smoking. Diseases such as obesity are greatly on the increase all over the world, threatening to cripple its economies [6]. The assessment *is* a bit unfair; the incidence of cancer has increased because people live longer in most countries. Also for obesity and for type-2 diabetes, the assessment is unfair because of an increased incidence, which is due to commercialization of nutrition and an increase in the world's food supply. Regardless, we need to do better than this.

And the scientific base?

Has not that improved in recent years? Yes, it has. In fact tremendously so. After a long and steady increase of scientific knowledge through biochemistry, molecular biology and cell biology, the genomics revolution and the consequent functional genomics revolution have been much more powerful than anticipated. Where previously the life sciences could study individual objects reasonably well, both at the physiological level and at the molecular level and preferably *in vitro*, now relatively small samples of cells, tissue or even ecosystems, can be used to reveal the complete genome sequence, the identity and concentration of all mRNA, miRNA and of most proteins and of a great many metabolites. All of these can be studied at the same time, enabling the integrative analysis of (virtually) all molecules that together establish function. Fluorescent imaging techniques enable the inspection of the dynamic behavior of macromolecules in living cells, whilst molecular genetic techniques enable one to modulate gene expression and capture physical interactions of macromolecules. It would seem that hereby we are able to identify and measure the activity of every catalytic, regulatory and memory molecule in the human or in tissue culture models thereof. Knowledge-wise we therefore seem to be

close to understanding. Yet we understand neither health nor disease other than in incomplete and vague descriptions. Given that the human body only consists of molecules, what is keeping us from this understanding of health and disease? What is keeping us from an operational understanding that would enable us to cure most diseases definitively? It is precisely such understanding that is required for pharmacology to live up to its potential.

Animal models for what is missing?

One obvious aspect that is missing from the straightforward genomics paradigm is the issue of biological organization: a living organism is more than a bag of enzymes and regulatory proteins. Health is an issue of many tissues working together in the right ways, that is, proteins have to be active in the appropriate compartment and at the right time. The Virtual Physiological Human (VPH) initiative is promoting this line of thought for very good reasons: Most of the functioning of the human may be determined at the physiological rather than the molecular level [7].

At this physiological level, the human is much more homologous of course with other intact mammals than with yeast. Hence, the experimental foot of this line of thought seems to suggest experiments with mammals. Such experiments come with ethical issues strongly limiting the types of experiments that can be done. They are also limited in high-throughput capacity. And then animal experiments as such, do not seem to represent the reality in the human sufficiently well. All too often drugs that pass all animal tests fail in Phase II or Phase III. This may not be too surprising: drugs that we consider effective in human may only work for one third of the human population [8]. This means that a test of the drug in that third would not predict the drug's effect in the other two thirds of the human population. The explanation for this phenomenon is sought in genetic, nutritional and behavioral differences between the individuals. If this is the explanation, why should one expect a drug that works in mouse, to work even for 33% of all humans? Surely the difference between mouse and man exceeds the genetic difference between two human individuals.

The empirical strategy

An historically effective way out of this dilemma is not to wait for complete understanding. After all, who understands all the details of the car one is driving to work, or of the chips in one's computer? Still, we get by fairly well. The usual procedure is to learn a bit how things work, then experiment around the experience one has, and share experiences with colleagues. This is also how our clinical practice overcomes the issue of incomplete knowledge. Pharmacokinetics (PK) has become successful by developing a method that makes sense but neglects much detail and complexity. Calibrated with a test set of drugs, it is used for other drugs after