DDT Vol. 7, No. 20 (Suppl.), 2002

Small- and large-scale biosimulation applied to drug discovery and development

C.J. Musante, Annette K. Lewis and Kevin Hall

Biosimulation uses mathematics to quantitatively represent the dynamics of biological systems and thereby analyze and predict system behavior. Biosimulations can be classified into two general categories: small-scale models designed to address a specific problem, and large-scale models of detailed regulatory mechanisms used to address a broad scope of questions. Both classes of biosimulations have been applied to problems important for drug discovery and development. Small-scale biosimulations have been particularly useful for interpreting clinical data and developing novel biomarkers. Large-scale biosimulations typically integrate a wide variety of data and can provide insights into how complex biological systems are regulated in both health and disease. Because large-scale biosimulations represent detailed regulatory mechanisms and their interactions, they can predict the overall clinical effect of modulating individual pathways or targets. In this mini-review, we describe several examples of how small- and large-scale biosimulations have been applied to problems important for drug development in diabetes, HIV, heart disease and asthma.

C.J. Musante
Annette K. Lewis
and Kevin Hall
Entelos, Inc.
4040 Campbell Ave.
Ste. 200, Menlo Park
CA 94025, USA
tel: +1 650 330 5200
fax: +1 650 330 5201
e-mail:
musante@entelos.com

▼ Analysts predict that *in silico* technologies could reduce average development times by two to three years and overall costs by more than $200M per approved drug [1]. Although some envision this capability as being unattainable until perhaps 2015 [2], biosimulations are already solving real-world problems [3,4].

Biosimulation uses mathematics to quantitatively represent the dynamics of biological systems and thereby analyze and predict system behavior [5]. Biosimulations have been used to identify and validate novel drug targets, assess their pertinence to human physiology, design clinical trials, identify biomarkers, explain data variability, identify optimal therapeutic strategies and predict drug safety [4,6,7]. Given the rising cost of developing and marketing new drug therapies, pharmaceutical companies need new ways to increase productivity. Biosimulation directly addresses this challenge.

Biosimulation can be categorized into two general classes: (1) small-scale biosimulations, consisting of a few equations and parameters that are designed to address a specific, well-defined problem, and (2) large-scale biosimulations, consisting of tens to hundreds of equations and parameters that mechanistically represent complex biological processes and their interactions. Although there is a long history of both classes of biosimulation, this mini-review will highlight a few illustrative examples applied to drug discovery and development in diabetes, HIV, heart disease and asthma. We will discuss the advantages and disadvantages of both small- and large-scale biosimulation approaches.

Small-scale biosimulations

Small-scale biosimulations are directed towards specific problems and have been useful tools in the drug development process and clinical management of disease. Here, we review two example small-scale biosimulations that have been used to help collect and interpret clinical data.

Minimal-model glucose biosimulation

Insulin is a pancreatic hormone involved in the regulation of many physiologic processes, including hepatic glucose production and skeletal muscle glucose uptake. Decreased sensitivity of various organs to insulin is associated with

 PII: S1359-6446(02)02442-X

obesity, cardiovascular disease, hypertension and diabetes. Because many present and future therapies are aimed at improving insulin sensitivity, it is important to have a clinical measure of insulin sensitivity to assess therapeutic efficacy. However, the 'gold standard' for measuring insulin sensitivity, the euglycaemic hyperinsulinaemic clamp, is labor-intensive, time-consuming and expensive [8,9], making it inappropriate for use in large-scale clinical trials.

Bergman *et al.* developed an alternative clinical measure of insulin sensitivity using a biosimulation of the plasma glucose response to an intravenous bolus infusion of glucose [10,11]. Their 'minimal-model' consisted of a pair of nonlinear ordinary differential equations describing the plasma glucose kinetics and the kinetics of insulin in an interstitial fluid compartment. By using the measured plasma insulin profile as a forcing function, Bergman *et al.* determined a unique set of model parameters for each patient, such that the simulated plasma glucose response best matched the measured glucose data. The insulin sensitivity index for each patient was then determined as a simple function of the optimal parameters.

The minimal-model insulin sensitivity index was found to be repeatable and in good agreement with the index obtained using the euglycaemic hyperinsulinaemic clamp [12–14]. Because the minimal-model analysis is straightforward and economical [15], it has been widely used to assess insulin sensitivity in large-scale clinical trials [16]. The minimal-model methodology continues to be improved and extended to provide novel clinical measures of glucose and insulin dynamics [17–19].

HIV-1 replication biosimulation

Acquired immunodeficiency syndrome (AIDS) is a chronic disease that begins with HIV infection and in adults progresses over a median period of ten years. Measurement of the plasma concentration of HIV RNA, known as the viral load, is considered the best predictor of disease progression in untreated HIV-infection [20].

To investigate HIV replication dynamics, Perelson *et al.* developed a biosimulation of HIV and T-cell dynamics following administration of a potent HIV protease inhibitor [21]. By assuming that the system was in a quasi-steady state prior to therapy, and that the number of uninfected T cells would not appreciably change over the first week of therapy, the authors were able to analytically solve the three ordinary differential equations of their model and express the viral load as a function of time. Clinical measurements of viral load were made several times over the course of one week of therapy, and model parameter values were adjusted to best match these data. This provided *in vivo* parameter estimates of HIV clearance and the rate of loss of HIV-producing T cells for each patient. From these parameter estimates, Perelson *et al*. computed the infected T-cell lifespan, the HIV production rate prior to therapy, and the average viral generation time.

The numerical parameter estimates suggested that HIV replication and turnover were much larger than previous estimates. Based on these new estimates, Perelson *et al.* made three conclusions that are important for the development of HIV treatments. First, effective anti-viral therapies will act within a few days to detectably lower the plasma viral load. Thus, clinical efficacy of an anti-viral compound can be determined rapidly. Second, the risk of developing drug-resistant viruses is high given the estimated replication rate and the previously measured rapid mutation rate of HIV. Thus, treatments should consist of a combination of anti-retroviral agents, requiring the virus to mutate simultaneously at multiple positions before acquiring drug resistance. Third, although only two to three weeks of anti-viral treatment is necessary to decrease viral load by approximately 99%, treatment regimens must be continued for a sufficient time to deplete other viral compartments, such as latently infected cell populations and sanctuary sites, which may spark a high rate of viral replication if therapy is withdrawn. These insights have 'transformed thinking about HIV disease and have had a major impact on clinical management' [20]. Further development and use of HIV biosimulations continue to provide a better understanding of disease processes [22].

Large-scale biosimulations

Large-scale biosimulations are designed to comprehensively represent physiologic mechanisms responsible for health and disease. Such models are designed to address a wide variety of problems, predict overall system behavior and help design experiments and interpret their results. These models are generally relatively large, consisting of tens to hundreds of equations and parameters.

Cardiac electrophysiology biosimulation

Detailed, quantitative biosimulations of cardiac cell electrophysiology have been used to better understand the pathophysiology of heart disease [23]. For example, Winslow *et al.* investigated whether the observed altered gene expression of ion channels, pumps and exchangers could account for the known electrophysiological properties of congestive heart failure [24]. Beginning with a detailed biosimulation of a normal canine ventricular cell, the authors adjusted model parameters to simulate altered protein levels based on observed gene expression from failing ventricular cells. They found that simulations of the diseased action potentials and calcium dynamics accurately matched experimental measurements. With this model, Winslow *et al.* determined the relative contribution of each of the altered protein levels to the observed cellular electrophysiological behavior. Although these results gave insight

into the cellular defects associated with heart failure, it is necessary to understand how spatial propagation of the cardiac action potential is altered to predict clinical outcome.

By incorporating the cellular electrophysiology biosimulation into a realistic, three-dimensional model of the canine ventricle, Kohl *et al.* were able to predict disrupted spatial propagation of cardiac action potentials in heart failure [25]. They showed that the altered protein levels caused the electrical activation pattern to change from a normal, coordinated pattern to a dangerous, abnormal pattern of irregular circulating waves of electrical activity. To predict how pharmacological modulation of cellular electrophysiology affects clinical outcome, the authors simulated the effect of adding an ATP-sensitive potassium channel opener and showed that such an intervention returns the heart to a more normal activation pattern. These cardiac biosimulations have led to a better electrophysiological understanding of the clinical manifestations of heart failure and have predicted potentially effective targets for therapeutic development.

Asthma biosimulation

Asthma is a chronic inflammatory disease of the lower airways. To better understand asthma pathophysiology in the context of the complex interactions between airway tissues and the allergen-induced immune response, Stokes *et al.* created an asthma biosimulation that encompasses airway physiology and the inflammatory effector system [26]. This model accurately simulated the acute and chronic characteristics of asthma, including both early- and late-phase airway obstruction following allergen challenge, airway hyper-responsiveness and chronic eosinophilic inflammation [27]. The asthma biosimulation also exhibited characteristic responses to known therapeutics.

To evaluate the predictive capability of the asthma biosimulation at the clinical level, Stokes *et al.* investigated the ability of a leukotriene receptor antagonist and a long-acting β_2-agonist to reduce the severity of exercise-induced asthma (EIA) [27]. The biosimulation accurately predicted the efficacy of each therapy when subsequently compared with clinical data from Merck and Co., Inc (Whitehouse Station, NJ, USA). Both the biosimulation and the clinical results showed greater protection in EIA with the leukotriene receptor antagonist than with the long-acting β_2-agonist. In addition, administration of a short-acting β_2-agonist, an acute rescue therapy for EIA, was more effective in combination with the leukotriene receptor antagonist than when combined with the long-acting β_2-agonist. The biosimulation was further used to elucidate the mechanisms underlying these clinical observations.

Perhaps the most compelling result of the asthma biosimulation was the prediction that a therapeutic in clinical trials, an interleukin-5 (IL-5) antagonist, would not be effective for treating acute airway obstruction in asthma [28]. IL-5 increases eosinophil number in the airways – a hallmark characteristic of asthma. Based on animal studies, anti-IL-5 therapy should decrease airway eosinophil number and thereby reduce airway obstruction [29–31]. Surprisingly, although anti-IL-5 effectively reduced eosinophil number, the asthma biosimulation predicted that this therapy would have little effect on improving airflow obstruction. This prediction was confirmed by the results from an anti-IL-5 clinical trial [32]. The biosimulation further showed that ongoing airway obstruction was because of the continued presence of other resident and infiltrating cells in the airway, highlighting the significant redundancy in the system.

Advantages and disadvantages of small- and large-scale biosimulation

Although the examples described above demonstrate the use of biosimulation in drug development and the interpretation of laboratory and clinical data, there are advantages and disadvantages of both small- and large-scale biosimulations.

Small-scale biosimulations

Advantages: By design, small-scale biosimulations have a clear, focused application, and modeling assumptions are naturally guided by the intended application. Model parameters can often be uniquely determined and, as illustrated above, experimental parameter estimation is often the goal of such models [21,10]. Because of their small-scale, these models are relatively easy to understand and analyze. When model equations cannot be solved explicitly, computers can numerically solve the equations using only modest computing power.

Disadvantages: Small-scale biosimulations are typically limited to the application for which they were designed. However, extensions in their scope can be implemented by including representation of additional biological processes. For example, the HIV replication model of Perelson *et al.* [21] was expanded to include the dynamics of latent cell populations and sanctuary sites to investigate the time required for anti-viral therapy to eliminate the virus from these compartments [33]. Another disadvantage of small-scale models is that their parameters are typically highly aggregated representations of complex underlying biological mechanisms. Because they do not attempt to represent these mechanisms, they are unable to predict the consequences of manipulating the biological pathways contributing to these aggregate parameters.

Large-scale biosimulations

Advantages: Large-scale biosimulations are designed to comprehensively represent physiological mechanisms responsible for health and disease. Therefore, large-scale models can be used to investigate a variety of questions and predict the clinical effects of modulating individual pathways and drug targets. Because

of the nature of such biosimulations, the use of diverse data sets is required to quantitatively represent the physiological processes responsible for overall system behavior. By incorporating multiple data sets into a single comprehensive biosimulation, critical knowledge gaps in the disease area can often be identified.

Disadvantages: Although large-scale biosimulations can address a wide variety of questions, it can sometimes be difficult to determine when they have been stretched beyond their scope of applicability. This difficulty can arise from the use of diverse data sets, often from multiple species; modeling assumptions not guided by a pre-defined use; and uncertain parameter estimates. (Of course, the problem of uncertain scope of applicability is also shared by *in vitro* and *in vivo* experimental models). The typical complexity of large-scale biosimulations, often consisting of hundreds of equations and parameters, makes them challenging to analyze. Nevertheless, they can be extensively interrogated to better understand how the modeled physiological mechanisms generated the simulation results, and whether or not these mechanisms are biologically reasonable. Finally, development of comprehensive models requires extensive resources, including high-performance computing and sophisticated modeling and analysis software.

Summary

As illustrated in this mini-review, small-scale biosimulations can be used to identify clinical biomarkers and improve understanding of disease and the effects of therapy. Large-scale biosimulation can additionally predict the clinical efficacy of modulating drug targets. This makes it possible to eliminate ineffective targets early in the development process and to focus on those with a high probability of success. By combining traditional experimental research with iterative development of large-scale biosimulations, researchers can design more efficient and focused experiments and provide a larger context for interpreting experimental results. Thus, biosimulation has the potential to enhance the pace of drug discovery and development.

Acknowledgements

The authors would like to acknowledge N. Defranoux, M. French, J. Fujisaki, S. Michelson, C. Stokes and B. Sudbeck for their thoughtful review of the manuscript.

References

1 *Pharma 2005 Silicon Rally: The Race to eR&D* (1999) Pricewaterhouse Coopers Report. http://www.pwcglobal.com

2 *The Fruits of Genomics* (2001) Lehman Brothers and McKinsey & Company Report 30, 34

3 Fikes, B.J. (2000) *In silico* biology prediction: fortune telling gets sophisticated. *Biopeople* Winter, 17–23

4 Ho, R.L. (2000) Biosystems modelling for *in silico* target validation: challenges to implementation. *Emerg. Ther. Targets* 4, 699–714

5 Hall, K. *et al.* (2002) Biosimulation: dynamic modeling of biological systems. *Annu. Rep. Med. Chem.* (in press)

6 Noble, D. *et al.* (1999) Biological simulations in drug discovery. *Drug Discov. Today* 4, 10–16

7 Noble, D. and Colatsky, T.J. (2000) A return to rational drug discovery: computer-based models of cells, organs and systems in drug target identification. *Emerging Therapeutic Targets* 4, 39–49

8 DeFronzo, R.A. *et al.* (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am. J. Physiol.* 237, E214–E223

9 Scheen, A.J. *et al.* (1995) How to explore insulin sensitivity in man? *Ann. Endocrinol. (Paris)* 56, 523–530

10 Bergman, R.N. *et al.* (1979) Quantitative estimation of insulin sensitivity. *Am. J. Physiol.* 236, E667–E677

11 Bergman, R.N. (1989) Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. *Diabetes* 38, 1512–1527

12 Krempf, M. *et al.* (1994) Minimal model for determination of insulin sensitivity: repeatability in control and obese subjects. *Diabetes Res. Clin. Pract.* 26, 145–148

13 Bergman, R.N. *et al.* (1987) Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. *J. Clin. Invest.* 79, 790–800

14 Beard, J.C. *et al.* (1986) The insulin sensitivity index in nondiabetic man. Correlation between clamp-derived and IVGTT-derived values. *Diabetes* 35, 362–369

15 Swan, J.W. *et al.* (1994) Assessment of insulin sensitivity in man: a comparison of minimal model- and euglycaemic clamp-derived measures in health and heart failure. *Clin. Sci. (Lond.)* 86, 317–322

16 Haffner, S.M. *et al.* (1999) Insulin sensitivity in subjects with type 2 diabetes. Relationship to cardiovascular risk factors: the Insulin Resistance Atherosclerosis Study. *Diabetes Care* 22, 562–568

17 Dalla Man, C. *et al.* (2002) The oral glucose minimal model: estimation of insulin sensitivity from a meal test. *IEEE Trans. Biomed. Eng.* 49, 419–429

18 Caumo, A. *et al.* (2000) Insulin sensitivity from meal tolerance tests in normal subjects: a minimal model index. *J. Clin. Endocrinol. Metab.* 85, 4396–4402

19 Vicini, P. *et al.* (1997) The hot IVGTT two-compartment minimal model: indexes of glucose effectiveness and insulin sensitivity. *Am. J. Physiol.* 273, E1024–E1032

20 Cohen, P.T. (1998) Clinical overview of HIV disease: HIV InSite Knowledge Base Chapter. http://www.ucsf.edu

21 Perelson, A.S. *et al.* (1996) HIV-1 dynamics *in vivo*: virion clearance rate, infected cell life-span, and viral generation time. *Science* 271, 1582–1586

22 Perelson, A.S. (2002) Modelling viral and immune system dynamics. *Nat. Rev. Immunol.* 2, 28–36

23 Noble, D. (2002) Modeling the heart – from genes to cells to the whole organ. *Science* 295, 1678–1682

24 Winslow, R.L. *et al.* (2001) Computational models of the failing myocyte: relating altered gene expression to cellular function. *Philos. Trans. R. Soc. Lond. A* 359, 1187–1200

25 Kohl, P. *et al.* (2000) Computational modeling of biological systems: tools and visions. *Philos. Trans. R. Soc. Lond. A* 358, 579–610

26 Stokes, C.L. *et al.* (1999) Computer-based mathematical model of asthma. *J. Allergy Clin. Immunol.* 103, A980

27 Stokes, C.L. *et al.* (2001) A computer model of chronic asthma with application to clinical studies: example of treatment of exercise-induced asthma. *J. Allergy Clin. Immunol.* 107, A933

28 Lewis, A.K. *et al.* (2001) The roles of cells and mediators in a computer model of chronic asthma. *Int. Arch. Allergy Immunol.* 124, 282–286

29 Kung, T.T. *et al.* (1995) Involvement of IL-5 in a murine model of allergic pulmonary inflammation: prophylactic and therapeutic effect of an anti-IL-5 antibody. *Am. J. Respir. Cell. Mol. Biol.* 13, 360–365

30 Mauser, P.J. *et al.* (1995) Effects of an antibody to interleukin-5 in a monkey model of asthma. *Am. J. Respir. Crit. Care Med.* 152, 467–472

31 Mauser, P.J. *et al.* (1993) Inhibitory effect of the TRFK-5 anti-IL-5 antibody in a guinea pig model of asthma. *Am. Rev. Respir. Dis.* 148, 1623–1627

32 Leckie, M.J. *et al.* (2000) Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 356, 2144–2148

33 Perelson, A.S. *et al.* (1997) Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature* 387, 188–191