Arch Toxicol (2014) 88:2071–2075
DOI 10.1007/s00204-014-1384-6

EDITORIAL

The virtual liver: state of the art and future perspectives

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Published online: 21 October 2014
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Developments over the past two decades have improved our ability to obtain comprehensive and quantitative data, for example, by genome-wide analysis of gene expression, proteomics, lipidomics and metabolomics. Moreover, both imaging and image analysis have been improved which offers new possibilities to quantify the three-dimensional organization of cells and tissues. However, research in disease pathogenesis is often hampered by the difficulty to understand the complex, time-resolved interplay among numerous components. Here, mathematical modelling helps clarifying the underlying principles. The mathematical models formalize the relationship between individual components, test their interactions in a virtual setting and may even simulate influences that are (still) difficult to analyse experimentally. In recent years, model simulations have been instrumental to elucidate mechanisms and principles that were not accessible by traditional approaches. To promote systems biology research in the field of the liver with the aim to gain a better understanding of the basic mechanisms of liver function as well as key principles of liver

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diseases, the *Virtual Liver Network* has been formed; this interdisciplinary research network currently comprises more than 60 partners from different disciplines, such as biology, chemistry, biochemistry, pharmacy, toxicology, mathematics/bioinformatics, physics and hepatology. A specific feature of the *Virtual Liver Network* is that it models liver functions at different scales, from subcellular organization, cellular functions, for example, metabolic homeostasis and drug metabolism, up to functions that require entire liver lobules or even the whole organ. A unique achievement of the '*Virtual Liver*' is that these scales have been successfully bridged by integrated models which now allow simulations how processes at the subcellular or cellular level influence lobular or even whole organ functions and vice versa. The possibilities and perspectives of '*Virtual Liver Approaches*' will be illustrated by some selected examples:

- *The subcellular level: self-organizing principles of organelles*. An important outstanding question is what are the molecular principles underlying the biogenesis of cellular organelles, and hence, the organization of the cytoplasm. Many molecules have been identified and demonstrated to function in intracellular trafficking. In the particular case of endosomes, the small GTPase Rab5 was proposed to be a key factor in endocytosis but also in the biogenesis of early endosomes. The importance of Rab5 in endosome biogenesis was tested using a combination of mathematical model and experimental validation in the mouse livers as model system. To study the dependency of the endosome number on the levels of Rab5, a mathematical model was formulated to predict the consequences of the loss of Rab5 for the number of early endosomes (Zeigerer et al. 2012). Different scenarios were considered. For example, the endosome could change in number but keep a constant level of molecular machinery (Rab5 and its effectors) per endosome or, alternatively, keep a constant number of endosomes but decrease the density of molecular machinery. Experimental validation was performed by silencing Rab5 by RNAi in the mouse liver in vivo. Titration of Rab5 showed that the endocytic system was resilient against depletion of Rab5 and collapsed only when depletion exceeded approximately 80 % of control levels. Comparison of the experimental data with the model simulations supported the scenario whereby the endosomes are depleted, but those remaining maintain a constant level of molecular machinery (Zeigerer et al. 2012). The mathematical model also explains why the relatively wide variations in Rab5 expression have only a minor influence on the numbers of endosomes, but the system collapses as soon as critical thresholds are exceeded.
- *The cellular scale: enzyme networks guarantee metabolic stability*. The contribution of liver metabolism to the homeostasis of the plasma glucose level was investigated on the basis of a comprehensive kinetic model of hepatic glucose metabolism (König et al. 2012; König and Holzhütter 2012). The model comprises the pathways glycolysis, gluconeogenesis and glycogen turnover. The phosphorylation state of key regulatory enzymes is controlled by the plasma levels of insulin and glucagon. Model simulations revealed that the set point of glucose homeostasis, defined by a lack of hepatic net uptake or release of glucose, is shifted from 5.5 mM (normal) to about 8 mM in case of diabetes type 2 (T2DM). Intriguingly, the model also revealed that a strict insulin therapy, dropping the glucose level in T2DM subjects down to the normal level, critically increases the risk of severe hypoglycaemic episodes. The model also clearly demonstrates that a more efficient T2DM therapy can be achieved by lowering the impact of glucagon.
- *The lobular level: intercellular communication guarantees maintenance and regeneration of tissue architecture*. One of the outstanding features of the liver is its enormous regenerative capacity. Upon damage of liver tissue, not only the liver mass but also the architecture is restored within a relatively short period of time. However, until recently, little was known about the principles coordinating this architectural restoration. One popular theory was that the release of cytokines from damaged tissue attracts the regenerating cells. Another theory was that an oxygen gradient established after damage guides the regenerating cells. Alternatively, it was proposed that the border between healthy, regenerating hepatocytes and a neighbouring necrotic region is simply pushed in response to pressure arising from cell proliferation. However, a mathematical approach based on a spatio-temporal model demonstrated that another mechanism, named 'hepatocyte sinusoid alignment' (HSA), plays a key role in orchestrating regeneration of the liver microarchitecture (Höhme et al. 2007; Hoehme et al. 2010; Hammad et al. 2014). HSA means that regenerating hepatocytes align towards the direction of

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the closest sinusoidal endothelial cells (LSEC). In other words, the liver microvasculature formed by LSECs acts as a 'guide rail' for regenerating hepatocytes; but also in healthy liver, the 3D network of LSECs determines the spatial organization of parenchymal cells and organizes, for example, the hepatocyte sheets. Recently, possible molecular mechanisms have been reported that could contribute to HSA: LSECs secrete angiocrine factors, such as Wnt2 and HGF, which induce hepatocyte proliferation (Ding et al. 2010, 2014). Also, expression of angiopoietin-2 (Ang2) by LSECs plays a critical role during regeneration (Hu et al. 2014). Ang2 downregulation in response to liver damage leads to reduced TGF-β release from LSECs which enables proliferation (Hu et al. 2014). Moreover, spatio-temporal analysis facilitates even more far-reaching predictions, for instance, that a lesion where only hepatocytes are destroyed can easily be regenerated. However, as soon as LSECs are destroyed, liver regeneration is predicted to be severely compromised. This leads to the pathophysiologically critical question whether destruction of LSECs represents the critical turning point from perfect regeneration to fibrosis. Continued work in this field will show whether this model prediction can also be validated.

- *Integrating the cellular, lobular and whole organ scales: Tissue damage may switch the orientation of enzymatic reactions*. Ammonia is a product of amino acid metabolism. Because of its toxicity, ammonia concentrations in blood and cells are tightly controlled. Intensive research in this field has shown that enzymes of the urea cycle detoxify ammonia in the periportal compartment of the liver lobule, whereas glutamine synthetase removes ammonia leaking to the pericentral region. When these well-documented mechanisms were included into a metabolic/spatio-temporal model, it became evident that they are not sufficient to quantitatively explain ammonia detoxification (Schliess et al. 2014). The model predicted too high blood ammonia concentrations. Further experiments showed that damaged hepatocytes release glutamate dehydrogenase (GDH) into the blood, where it detoxifies ammonia by forming glutamate, thereby catalysing a reaction with opposite orientation than in periportal hepatocytes, where GDH generates ammonia for urea cycle enzymes. This so far unknown 'switched' reaction of GDH in blood explains the aforementioned discrepancy between the model and the experimental data. Finally, it could be shown that infusion of GDH together with the cofactors α-ketoglutarate and NADPH into hyperammonemic mice rapidly and efficiently reduced ammonia blood concentrations; whether this result from mice can be used to treat patients with severe hyperammonemia is subject of further studies.
- *Integrating pharmacokinetic and spatio-temporal models: communication of the liver with other organs*. The liver is also the key detoxification organ for xenobiotics in the human body. A mechanistic understanding of liver physiology is hence of immediate relevance for drug pharmacokinetics. Within the Virtual Liver Network, a cocktail of six marketed drugs is used to quantify detoxification activity of the liver in mice and humans (Kuepfer et al. 2014). Recently, physiologically based pharmacokinetic (PBPK) modelling has been used to identify cohorts of patients with a specific phenotype in a hepatic uptake transporter (Krauss et al. 2013) or to predict occurrence rates of adverse events in high-risk subgroups of patients (Lippert et al. 2012). Vertical integration of computational models across different levels of biological organization has been used, for example, to simulate the effect of acetaminophen at the cellular scale (Krauss et al. 2012; Diaz Ochoa et al. 2013) or to describe the impact of steatosis on drug pharmacokinetics (Schwen et al. 2014). In future, integrated computational models are generally expected to play an increasingly important role for integration and analysis of experimental data in drug development and toxicology (Magdy et al. 2013; Godoy et al. 2013; Zanger and Schwab 2013; Thomas et al. 2013; Mielke et al. 2011; Schug et al. 2013; Heise et al. 2012).

When first introduced years ago, systems biology was expected to integrate the wealth of data acquired in the post-genome era into a mathematical model, or better into a series of integrated models linked across scales to generate a 'virtual liver' and eventually even a 'virtual human'. However, the examples illustrated above and further cases of successful research in the field of systems biology show that mathematical modelling has been used for a different purposes: Typically, certain hypotheses of mechanisms are expressed as either mathematical equations (or algorithmic instructions), and the parameters of these equations (or instructions) are determined based on experimental data (Drasdo et al. 2014). A frequent result of such a set of model simulations and experiments is that hypothesized mechanisms have to be rejected, leading to iterative cycles of modelling and experiment, which leave only those hypotheses mathematically compatible with the experimental findings, fully in the spirit of Karl Popper's "The logic of scientific discovery" (1934). A further advantage of mathematical models is that they may guide researchers towards the most informative experiments.

Currently, the use of model simulations or 'virtual approaches' in liver research is growing significantly. Such simulations are used in the field of metabolism (Gille et al. 2010; Schleicher et al. 2014; Bucher et al. 2011; Casanovas et al. 2014; Pelz et al. 2012; Thiele et al. 2013;

Mleczko-Sanecka et al. 2010; Zellmer et al. 2010), apoptosis research (Geissler et al. 2013; El Maadidi et al. 2014; Walter et al. 2008) and cell signalling (Huard et al. 2012; Blüthgen et al. 2009; Ehlting et al. 2011; Dooley et al. 2008; Stewart et al. 2012; Vlaic et al. 2012). In the future, modelling will be particularly helpful to dissect complex pathophysiologies, for example, liver steatosis, inflammation, fibrosis and cirrhosis, to predict drug toxicity, to integrate PBPK and spatial–temporal models and to understand key principles of regeneration after hepatectomy, during chronic liver diseases and after liver and stem cell transplantation. We expect that the 'virtual liver' approach will have a strong impact on the further progress in these fields of research.

Acknowledgments The authors thank the German Ministry of Education and Research (BMBF) that initiated and funded the *Virtual Liver Network*.

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