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Virtual Cell Based Assay simulations of intra-mitochondrial concentrations in hepatocytes and cardiomyocytes

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ABSTRACT

In order to replace the use of animals in toxicity testing, there is a need to predict human in vivo toxic doses from concentrations that cause adverse effects in in vitro test systems. The virtual cell based assay (VCBA) has been developed to simulate intracellular concentrations as a function of time, and can be used to interpret in vitro concentration-response curves. In this study we refine and extend the VCBA model by including additional target-organ cell models and by simulating the fate and effects of chemicals at the organelle level. In particular, we describe the extension of the original VCBA to simulate chemical fate in liver (HepaRG) cells and cardiomyocytes (ICell cardiomyocytes), and we explore the effects of chemicals at the mitochondrial level. This includes a comparison of: a) in vitro results on cell viability and mitochondrial membrane potential (mmp) from two cell models (HepaRG cells and ICell cardiomyocytes); and b) VCBA simulations, including the cell and mitochondrial compartment, simulating the mmp for both cell types. This proof of concept study illustrates how the relationship between intra cellular, intra mitochondrial concentration, mmp and cell toxicity can be obtained by using the VCBA.

1. Introduction

Numerous research initiatives are focusing on developing methods and approaches for reducing, refining, or even replacing tests on animals (Paini et al., 2010; Niklas et al., 2013; Worth et al., 2014). Among the different strategies, combinations of targeted high-throughput in vitro and in silico tools are considered to represent promising strategies for improved toxicity testing without the use of animals (Basketter et al., 2012). These new approaches could ultimately be important in evaluating the human health risk of thousands of chemicals.

In vitro models offer a high-throughput approach for assessing chemical-induced molecular and cellular changes. However, extrapolating these perturbations to an in vivo effect across chemicals, dose, time, and species is a challenge (Shah and Wambaugh, 2010). The use of silico models to describe the cellular system increases our understanding of the (adverse) effects observed in in vitro systems, and should also improve the translation of in vitro data to the in vivo situation. Since the mitochondrion is an important organelle in many toxicity pathways, the prediction of chemical effects on mitochondria is of high interest. Mitochondria perform two critical functions in the cell, namely the production of more than 90% of the cell's energy, and the control of cell survival as an integral part of programmed cell death (apoptosis) (Passarella et al., 2003).

There are three potentially adverse effects that result from mitochondrial disruption: 1. disrupted energy metabolism; 2. increased free radical generation; and 3. altered apoptosis. Here we address the disruption of mitochondrial energy metabolism as measured by changes in the mitochondrial membrane potential (mmp). The measurement of mmp provides information on the ability to couple electron transfer with ATP synthesis, as well as the ability of the organelle to take up and release ions and substrates across the inner mitochondrial membrane. It is well known that chemicals that act as inhibitors of mitochondrial ETC complexes or as uncouplers of oxidative phosphorylation can induce cell toxicity and death. Mitochondrial dysfunction triggered by inhibition of mitochondrial respiration or uncoupling of oxidative phosphorylation results in decreased ATP levels that are linked in a causative manner to the following events observed at the cellular level: (a) the loss of mitochondrial membrane potential; (b) the loss of mitochondrial protein import and protein biosynthesis; (c) reduced activities of enzymes of the mitochondrial respiratory chain and the Krebs cycle; (d) elevated levels of reactive oxygen species (ROS); (e) the loss of mitochondrial motility, causing a failure of mitochondria to re-

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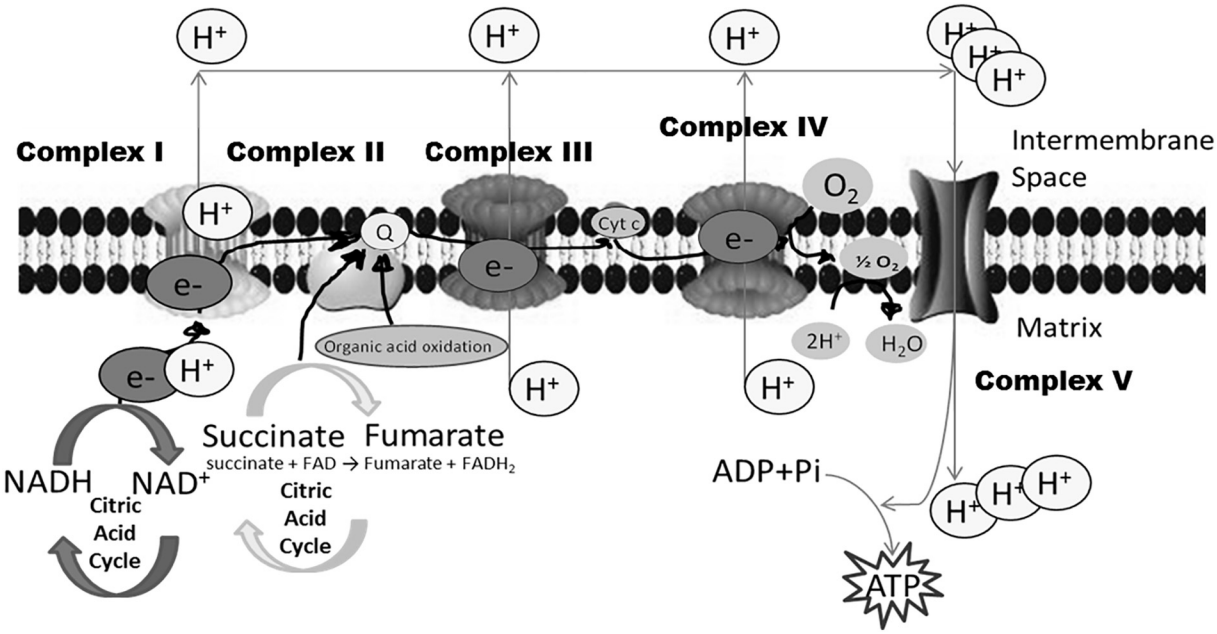

Fig. 1. Schematic representation of the mitochondrial electron transport chain (ETC). Mitochondria generate ATP by using the energy produced through the transfer of electrons (e-) in the ETC in a mechanism that pumps protons (H +) from the matrix into the intermembrane space, creating a transmembrane potential. The NADH and succinate generated in the citric acid cycle are oxidized, providing energy to power ATP synthase (Complex V). Complex I accepts electrons from the electron carrier nicotinamide adenine dinucleotide (NADH), and passes them to coenzyme ubiquinone (Q), which also receives electrons from complex II. Q passes electrons to complex III, which passes them to cytochrome *c* (cyt c). Cyt c passes electrons to Complex IV, which uses the electrons and hydrogen ions to reduce molecular oxygen to water (picture made using Protein Lounge, www.proteinlounge.com, and adapted version features also in EFSA, 2017).

localize to sites of increased energy demands, such as synapses; (f) destruction of the mitochondrial network; (g) increased mitochondrial uptake of Ca, causing Ca overload (Graier et al., 2007); and (h) rupture of the inner and outer mitochondrial membranes, leading to release of mitochondrial pro-death factors, including cytochrome *c*, apoptosis-inducing factor and endonuclease (Braun, 2012; Martin, 2011; Correia et al., 2012; Cozzolino et al., 2013).

Mitochondria generate ATP by utilizing the energy produced through the transfer of electrons down the electron transport chain (ETC, Fig. 1) in a mechanism that pumps protons from the mitochondrial matrix into the intermembrane space, creating an electrochemical proton gradient (or membrane potential) across the inner mitochondrial membrane (IMM) denoted $\Delta\Psi$. The reductive transfer of electrons through ETC protein complexes I–IV in the inner mitochondria membrane provides the energy to drive protons against their concentration gradient across the inner mitochondrial membrane (out of the mitochondrial cytoplasm). This results in a net accumulation of protons (H^+ ions) outside the membrane, which then flow back into the mitochondria through the ATP-generating F_1/F_0 ATP-synthase (Complex V), thus producing ATP and completing the ETC. The total force driving protons into the mitochondria (i.e., Δp), is a combination of both the mitochondrial membrane potential ($\Delta\psi_m$, a charge or electrical gradient) and the mitochondrial pH gradient (ΔpH_m, an H^+ chemical or concentration gradient), (Perry et al., 2011). In the presence of an exogenous chemical, this membrane potential may be altered (Nicholls and Ward, 2000; Mitchell and Moyle, 1969).

The Virtual Cell Based Assay (VCBA; Zaldívar et al., 2010; Zaldivar Comenges et al., 2011) was developed as an in silico predictive tool to simulate the fate and effects of chemicals within the well of a multi-well plate, as a function of time and under defined experimental conditions. The VCBA model (Zaldívar Comenges et al., 2016 present issue) is a mathematical model which takes into account the fate of a compound in the in vitro system, that is the partitioning between (i) the plastic wall, (ii) headspace, (iii) serum proteins, (iv) lipids, and potentially the compound dynamics within the cell. The VCBA consists also of a growth model with the cell growth phases (G1, S, G2, M phases). An additional feature takes into account the partitioning of compounds within the cell, and a toxicity model. The latter part of the model is based on two parameters: the no-effect concentration (NEC) and the killing rate (kr), linked to experimental cell viability. The main simulated property is the intracellular concentration of a specific chemical within the cell, and its corresponding effect on cell viability (Zaldivar Comenges et al., 2011; Zaldívar et al., 2010).

In the present study a mathematical description of the mitochondrion was added to the original VCBA model following the Horobin approach (Horobin et al., 2013; Horobin, 2015). By extending the VCBA to include the mitochondrial compartment, the model allows prediction of the concentration in the mitochondria, and to fit mmp experimental results.

In this paper we describe the following extensions to the original VCBA (Zaldívar Comenges et al., 2016 in press):

1. Extension to two cell models, one representing the liver (HepaRG) and one the heart (ICell cardiomyocytes). Adding these cell lines to the established VCBA is a step toward the characterization of chemical toxicity in multiple cell lines, representing different target organs and different toxic effects.
2. Addition of a mitochondrial compartment. This was done to simulate the intra-mitochondrial concentration. To predict the passage from the cell into the mitochondria, the Horobin et al., method was applied (Horobin et al., 2013; Horobin, 2015). The extent to which a molecule interacts with subcellular components, such as mitochondria, is based on the physicochemical properties (pKa, z, LogP) of the molecule (Horobin et al., 2007). Horobin and coworkers published a workflow on how to apply their approach for drug design: a physicochemical classification, a quantitative structure-activity relation (QSAR) model for low molecular weight compounds known to selectively accumulate in mitochondria, and the Fick – Nernst –Planck physicochemical model (Trapp and Horobin, 2005; Trapp et al., 2008).

These VCBA extensions are illustrated for three chemicals: carbonyl