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Research paper

A model of flux regulation in the cholesterol biosynthesis pathway: Immune mediated graduated flux reduction versus statin-like led stepped flux reduction

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ABSTRACT

The cholesterol biosynthesis pathway has recently been shown to play an important role in the innate immune response to viral infection with host protection occurring through a coordinate down regulation of the enzymes catalysing each metabolic step. In contrast, statin based drugs, which form the principle pharmaceutical agents for decreasing the activity of this pathway, target a single enzyme. Here, we build an ordinary differential equation model of the cholesterol biosynthesis pathway in order to investigate how the two regulatory strategies impact upon the behaviour of the pathway. We employ a modest set of assumptions: that the pathway operates away from saturation, that each metabolite is involved in multiple cellular interactions and that mRNA levels reflect enzyme concentrations. Using data taken from primary bone marrow derived macrophage cells infected with murine cytomegalovirus or treated with IFNγ, we show that, under these assumptions, coordinate down-regulation of enzyme activity imparts a graduated reduction in flux along the pathway. In contrast, modelling a statin-like treatment that achieves the same degree of down-regulation in cholesterol production, we show that this delivers a step change in flux along the pathway. The graduated reduction mediated by physiological coordinate regulation of multiple enzymes supports a mechanism that allows a greater level of specificity, altering cholesterol levels with less impact upon interactions branching from the pathway, than pharmacological step reductions. We argue that coordinate regulation is likely to show a long-term evolutionary advantage over single enzyme regulation. Finally, the results from our models have implications for future pharmaceutical therapies intended to target cholesterol production with greater specificity and fewer off target effects, suggesting that this can be achieved by mimicking the coordinated down-regulation observed in immunological responses.

1. Introduction

Cholesterol is central to a diverse range of cellular functions, including membrane development and maintenance [1], lipid raft formation and vesicular transport [2], steroid hormone synthesis [3], neurological development [4], and oxysterol [5] and vitamin D synthesis [6]. Recently, the cholesterol metabolism has been shown to have an important role in host–pathogen interactions. It has been documented to be perturbed in response to infection [7,8] and, conversely, cholesterol and its associated metabolites have been shown to alter inflammatory mediators [9,10].

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Cholesterol synthesis is one step in a pathway of metabolic interactions that is subject to catalytic regulation [11] and evidence suggests that this pathway is critical to the optimal growth of a range of viruses and microbes including cytomegalovirus (CMV), Hepatitis C (HCV), HIV, Japanese Encephalitis (JEV), West Nile (WNV), Dengue (DENV), Measles viruses (MV), African Swine Fever Virus (ASFV), Mycobacteria and Salmonella [7,8,12–19].

The cholesterol biosynthesis pathway itself comprises a sequence of metabolic interactions that occur across several organelles, starting with the processing of Acetyl-Coenzyme A (henceforth denoted ACoA – see Supplementary section 1 for a list of all metabolite abbreviations) in the mitochondria and ending with cholesterol synthesis in the endoplasmic reticulum [20,21]. This pathway branches in the peroxisome and endoplasmic reticulum into the sterol arm and the non-sterol arms (prenylation and dolichylation), the latter arms carrying flux away from the main sterol arm.

Coordinate transcriptional control of the enzymes of the cholesterol biosynthesis pathway is mediated by SREBP2 and feedback control occurs through regulation of SREBP2 transport. The SCAP:SREBP2 complex is ordinarily chaperoned to the Golgi complex where SREBP2 is cleaved, before it migrates to the nucleus to activate the suite of enzymes associated with the pathway. However, in the presence of relatively high concentrations of intracellular cholesterol or side-chain hydroxylated cholesterol, in particular 25-hydroxycholesterol, SCAP:SREBP2 is retained instead in the endoplasmic reticulum. Retention of SCAP:SREBP2 acts to down-regulate transcription of the enzymes acting on the pathway until ordinary levels of cholesterol and its derivatives have been restored. Hence, the pathway undergoes transcriptionally mediated regulation through changes to enzyme concentrations [20].

Recently, we reported a modest, but statistically significant, decrement in the concentrations of enzymes associated with the cholesterol biosynthesis pathway, in response to both infection and interferon treatment in macrophages. This was observed at the transcriptional level and was shown to correlate with reduced protein concentrations [12]. This decrement was found to be part of the innate immune response, intended to suppress viral growth. However, the mechanism through which such changes decrease the activity of the cholesterol biosynthesis pathway is something that has yet to be fully elucidated in the published literature.

We have sought to investigate this mechanism of regulation, exploring the impact on flux that results from such enzyme decrements. This problem is experimentally challenging, but tractable with computational methods.

Flux is the natural quantity to consider when studying metabolic pathway function and flux studies have been employed both theoretically [22,23] and experimentally [24,25]. The flux through the pathway describes the stoichiometrically adjusted rate of production of each metabolite and so captures whether and how the production rate of the metabolites affect each other. Ultimately, the final flux value in the pathway describes the rate of cholesterol synthesis. Metabolic Control Analysis (MCA) and Flux Balance Analysis (FBA) are two typical approaches to studying flux in a pathway system. However, MCA approaches focus on the effects of individual infinitesimal changes in enzyme activity rather than the compound effects of multiple finite changes and FBA, in its standard form, does not relate flux changes to substrate concentration changes. As a result, they are inappropriate for our study in which we validate the pathway model at the level of the substrate, implement multiple finite enzyme decrements and model the effects of chemical inhibition. We build an ordinary differential equation (ODE), dynamical model of the sterol pathway using Michaelis–Menten and mass action kinetics that incorporates additional interactions to represent the consumption of metabolites in non-sterol related processes. We demonstrate that multiple small decreases in enzyme activity can suppress the flux through the main cholesterol biosynthesis pathway. This suppression presents itself as a graduated reduction when the profile of flux is considered.

Cholesterol levels have also been demonstrated to be an important risk factor in cardiovascular disease [26,27] and their control is an active area of research [28]. Current therapies involve the use of statins to competitively inhibit the enzyme HMGCR which is responsible for catalysis of the interaction transforming 3-hydroxy-3-methyl-glutaryl Coenzyme A (HCoA) to Mevalonate (M). However, the efficacy of such therapies is limited by drug toxicity and off target effects [29–31]. Here, we show that statin treatment regulates the flux through the pathway in a manner that is markedly different to that following infection. The metabolic interaction catalysed by HMGCR is significantly upstream of cholesterol biosynthesis and we show that the impact of a statin-like treatment is to suppress flux throughout most of the pathway. This impacts significantly upon many of the metabolites upstream of cholesterol and upon the non-sterol arms, thereby incurring off-target effects. In contrast, because coordinate enzyme regulation leads to a graduated reduction in flux along the pathway, it has a less dramatic impact upon the branches upstream of cholesterol production.

This manuscript is organized as follows. In Section 2, we describe the experimental and mathematical methods employed to determine enzyme and metabolite levels in response to infection and IFNγ treatment and to model the pathway. In Section 2.1, we describe the experimental method and in Sections 2.2 and 2.3, we describe how the model was built, how the initial conditions were defined and how the model was used to simulate pathway activity. In Section 3, we present the results of using the model to study the flux through the pathway, with Sections 3.1–3.4 describing the validation of the model and the impact on the flux of the response to IFNγ treatment, to CMV infection and to statin intervention, respectively. In Section 4, we discuss these results, their relationship, the off-target effects and their implications for specific, targeted regulatory strategies. In Section 5, we summarize our results. Supplementary material in support of the results presented here is available online.

2. Materials & methods

2.1. Experimental measurements

Enzyme levels were inferred from gene expression measurements of bone-marrow derived macrophage cells in two time course experiments, one in which cells were infected with murine cytomegalovirus (mCMV) and one in which cells were treated with IFNγ. Measurements were taken at half hour intervals for 12 h using Agilent microarray platforms and at 24 h for select members using QPCR. Agreement between mRNA expression and protein concentrations was validated by quantitative western blotting for selected members of the pathway [12].

Intracellular cholesterol concentration was determined enzymatically using the Amplex-Red cholesterol assay kit (Molecular Probes) according to manufacturer recommendations. Briefly, cells were washed with 1 ml ice cold PBS and then lyzed in 200 μl cold Lipid buffer containing 0.5 M of potassium phosphate, pH 7.4, 0.25 mM cholic acid, and 0.5% triton X-100. Cell lysates were sonicated on ice with three 10-s pulses at high intensity. 20 μl were then used to determine protein concentration using a standard BSA assay to normalize the protein concentration. For cholesterol measurement, 20 μl of each sample were added to 80 μl assay solution, which contained 300 μM Amplex Red reagent, 2 U per ml HRP and 2 U per ml cholesterol oxidase, 0.1 M of potassium phosphate, pH 7.4, 0.05 mM cholic acid, and 0.1% triton X-100. After preincubation for 30 min at 37 °C under light exclusion conditions,