PHYSIOLOGY BASED MODEL OF CHOLESTEROL METABOLISM AND ITS INTERACTIONS WITH XENOBIOTICS

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Abstract

In 2000, nearly half of all death causes in EU were related to problems with cardiovascular system, while 10-15% of population has elevated levels of cholesterol, which is know to be a risk factor for cardiovascular diseases. Statins have been developed to lower cholesterol levels, however, in some people they are known to produce serious adverse effect, especially in combination with some other drugs that are usually administered in parallel with statins. In order to investigate the cholesterol metabolism and its interactions with xenobiotics, a mathematical model of cholesterol has been designed. Since it is a very complicated system, model development is still under way, but the model is showing similar characteristics as have been observed in human and mouse hepatocites. The modelling started with cholesterol pathway that can be found in KEGG database. Next, using Pathway Studio, the literature items in PubMed were searched to extract latest knowledge on the cholesterol metabolism, thus the model structure was updated with the latest findings. Next, the model was reduced to show only basic characteristics of the system. Model simulations show good agreement with experimental data and introduce some new structural elements to the system.

Keywords: Modelling, Simulation, Cholesterol, Systems Biology, Functional Genomics

Presenting Author's Biography

Aleš Belič received B.Sc and Ph.D. degrees in electrical engineering from the University of Ljubljana, Slovenia in 1994, and 2000 respectively. He is currently Associate Professor at the Faculty of Electrical Engineering, University of Ljubljana. Main areas of his professional interest are artificial intelligence modelling techniques in bio-medical areas. Currently he is involved in modelling of cholesterol pathways in human in the frame of 6th European Framework project STEROLTALK, and in functional analysis of EEG signals.

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1 Introduction

In 2000, nearly half of all death causes in EU were related to problems with cardiovascular system, while 10-15% of population has elevated levels of cholesterol, which is know to be a risk factor for cardiovascular diseases [1]. Cholesterol is the basic building stone for cell membranes and has, in general, two origins, one is exogenous and other is endogenous. Since either too low or too high levels of cholesterol are potentially dangerous for the organism, the cholesterol level is strictly regulated with complex mechanisms. Exogenous cholesterol comes directly from food, whereas endogenous cholesterol is produced from acetyl co-enzyme A in a series of several enzyme reactions. When sufficient amounts of cholesterol are obtained from food the endogenous pathway is shut-down. However, when there is not enough exogenous cholesterol, endogenous pathway becomes active. The cause for too high levels of cholesterol can, therefore, be either in too much fat-rich food or in damaged internal cholesterol control. First cause can be relatively easily cured by applying a diet that is in balance with actual needs of the organism. Second reason, however, has more complex causes that are currently not entirely understood and cannot be solved with diet. Therefore, statins were developed, to reduce the endogenous production of cholesterol. Statins should attach themselves on the HMGCR enzyme, which is the first enzyme specific for the cholesterol production, and thus reduce its activity. However, the statins may attach themselves to more than just HMGCR enzymes in the cholesterol production pathway and interfere also with processes in post-cholesterol metabolism. When designing a hypercholesteremia drugs that lower cholesterol levels by interfering with cholesterol production on enzyme level, cholesterol intermediates must be carefully monitored. Elevated levels of cholesterol intermediates as a direct or indirect effect of drugs must be avoided, since all the cholesterol intermediates are highly toxic. Because the control of cholesterol levels is a very complex system that is highly integrated with other control systems in the cell, statin interactions sometimes disturb other pathways as well. Disturbance of other pathways by statins is the main cause for its severe adverse effects. When other drugs are being administered in parallel with statins and are metabolized by the same enzymes as statins, the adverse effects are worse. In spite of the fact, that statins are widely used, many of their effects are not understood or even known. In order to better understand the effects of statins, mathematical model is being designed. The model will have to explain all known statin effects, as well as indicate some yet unknown modes of action.

2 Modelling

Modelling started with cholesterol pathway that could be found in the Internet [2] (see Figure 1). The backbone of the model represent equations that describe enzyme reactions (eq. 1).

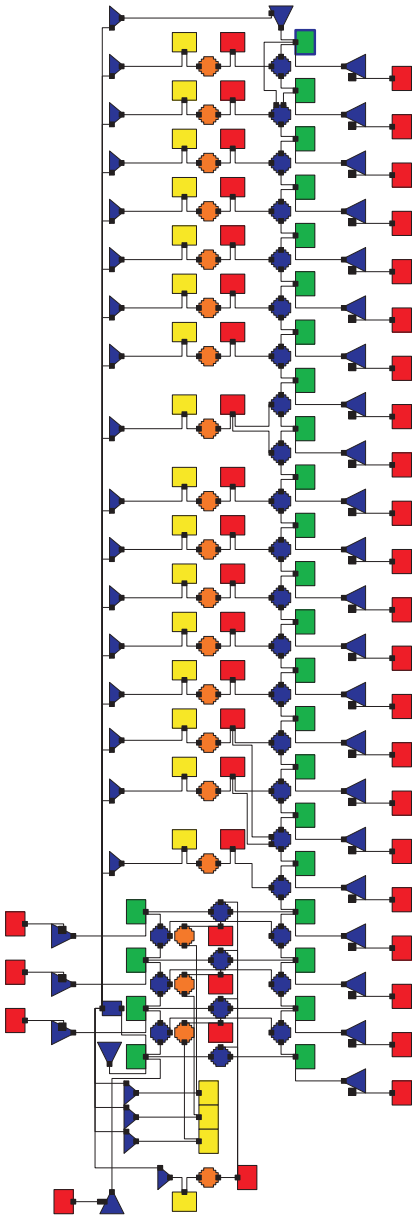

Fig. 1 First version of cholesterol pathway (round objects - reactions, square objects - substances, larger triangular objects - sources and sinks of metabolites, smaller triangular objects - mRNA control)

$$\begin{aligned} \frac{dP}{dt} &= k_P \cdot C \\ \frac{dC}{dt} &= k_C \cdot E \cdot S - k_P \cdot C \\ \frac{dS}{dt} &= -k_C \cdot E \cdot S \\ \frac{dE}{dt} &= -k_C \cdot E \cdot S + k_P \cdot C \end{aligned} \tag{1}$$

In eq. 1 the symbols have the following meaning: P - levels of product, C - levels of complex enzyme-substrate, E - levels of enzyme, S - levels of substrate, k_P - rate constant for product formation, and k_C - rate constant for complex formation. In special case, when

levels of enzyme are constant, and steady state for C is achieved, these equations are reduced to Michaelis-Menten equation (eq. 2).

$$\frac{dP}{dt} = \frac{k_m}{V_m + S}S \qquad (2)$$
$$\frac{dS}{dt} = -\frac{k_m}{V_m + S}S$$
$$k_m = E_0$$
$$V_m = \frac{k_P}{k_C}$$

In eq 2 the symbols have the same meaning as in eq. 1, E_0 - total quantity of enzyme (free and bound to complex). Linear kinetics is even simpler, as can be seen in eq. 3.

$$\frac{dP}{dt} = k \cdot S \qquad (3)$$
$$\frac{dS}{dt} = -k \cdot S$$

Where k is linear rate constant. The eqs. 1, 2, and 3 are used to describe the level of metabolites, the level of proteins (enzymes), mRNA level and their interactions. The structure in Figure 1 was composed in Dymola 5.3 [3], and represents a systemic view of cholesterol production pathway. For modelling, a library of objects was created. The objects were grouped in two groups: substances and reactions. Thus, object-oriented approach could be used. In the system the levels of mRNA are controlled with the level of cholesterol such that low levels of cholesterol produce raised levels of mRNA and high levels of cholesterol reduce mRNA levels. Thus the cholesterol controls its levels. Exogenous cholesterol thus reduces the levels of mRNA and reduces the production of endogenous cholesterol. Each intermediate of cholesterol metabolism has also some alternative pathway of metabolism that does not end in cholesterol. However, the intensity of these alternative pathways is very low, as can be expected in normal conditions of cholesterol production. Next, the model was updated according to KEGG database [4] and latest publications that were found in PubMed and searched with assistance of Pathway Studio [5]. The updated model can be found in Figure 2. The pathway in the second version of the model was extended with some additional metabolites and corresponding proteins and mRNA, at the same time the mechanism was added where, high levels of cholesterol also result in faster elimination of all enzymes in the pathway. However, the complexity of the model makes the experimenting with the model extremely difficult and non-transparent, therefore, the model was simplified (see Figure 3. The simplified version of cholesterol model has similar characteristics as the original model, however it includes only a few key metabolites and proteins for endogenous production of cholesterol.

3 Biological experiments

Biological experiments were performed in parallel with modelling on immortal human hepatocites cell-lines.

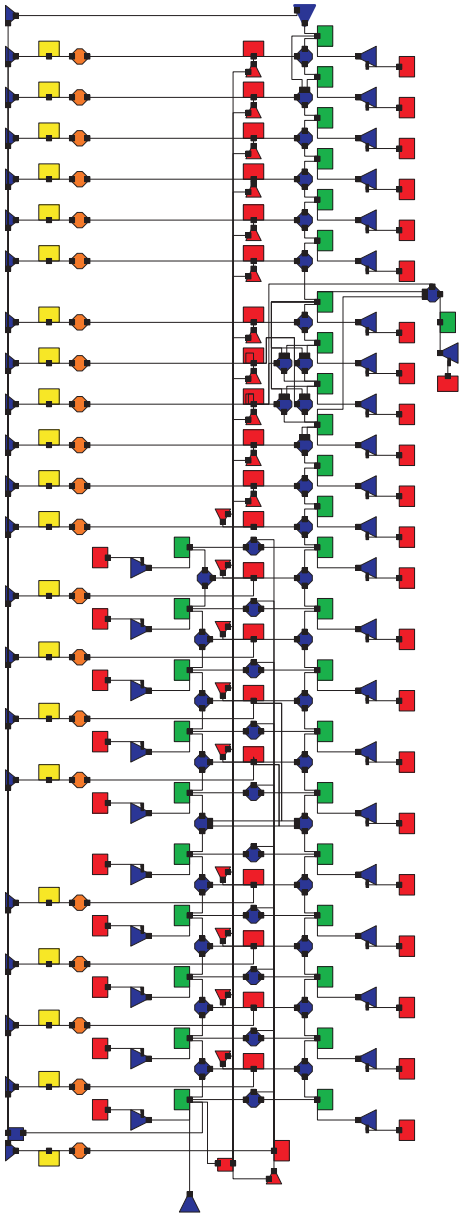

Fig. 2 Second version of cholesterol pathway (round objects - reactions, square objects - substances, larger triangular objects - sources and sinks of metabolites, smaller triangular objects - mRNA control and controlled enzyme elimination)

The cell-lines were treated with 6 compounds, atorva- and rosuvastatin, as representatives of commercially available statins, and LK-935, LK-980, LK-9104B, and LK-9109B research compounds from LEK pharmaceuticals d.d.. Six metabolites were measured on cell-lines: lanosterol, FF-MAS, lathosterol, 7-dehydro cholesterol + zymosterol, desmosterol, and cholesterol.

4 Modelling and simulation results

The model simulations showed, that inhibition or elimination of HMGCR enzyme resulted in highly reduced endogenous production of cholesterol, as was also ob-

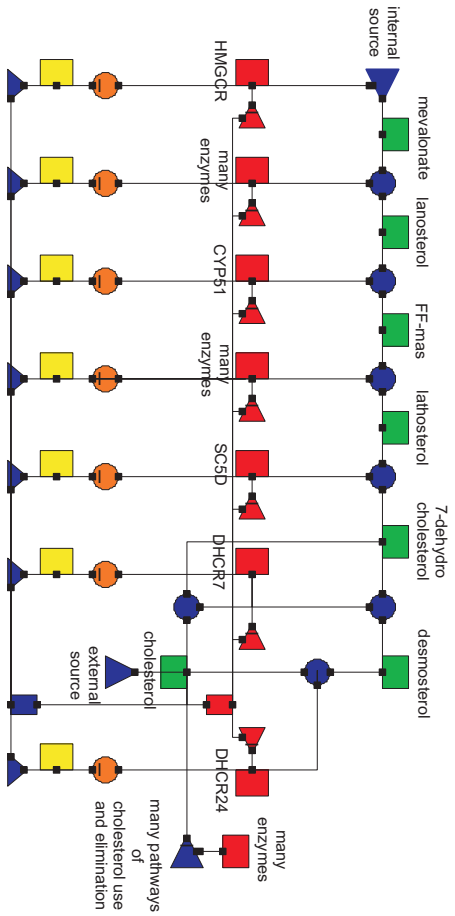

Fig. 3 Simplified version of cholesterol pathway (small dark (blue) triangular object to the left - mRNA control, light (yellow) square objects on the left - mRNA, light (orange) round objects on the left - enzyme production according to mRNA levels, dark (red) square objects - enzymes, dark (blue) round objects - enzyme reactions, dark (blue) large triangular objects - metabolite source or sink, small light (red) triangular objects - controlled enzyme elimination, light (green) objects on the right - metabolites, small dark (blue) square object - mechanism of mRNA control with respect to cholesterol levels, small light (red) square object - mechanism of enzyme elimination control with respect to cholesterol levels)

served in human hepatocites. The model also showed, that in order to reduce the cholesterol by inhibiting the enzymes, the substrate to the reaction that was controlled with inhibited enzyme must have some strong alternative pathways of metabolism or the only result is raised level of substrate with no effect to the end product. Alternatively, the raised levels of substrate either activate the chain of inhibitions of enzymes that control previous steps all the way to HMGCR or inhibit directly HMGCR. After comparing simulation results with experimental data for rosuvastatin it was therefore clear that rosuvastatin inhibits CYP51 as well as HMGCR, or lanosterol is inhibiting HMGCR. In case that lanosterol is inhibiting HMGCR, the rosuvastatin may only inhibit CYP51. The effect of lanosterol on HMGCR was later confirmed in the literature [6]. The model suggests the following:

- The reduction of mRNA levels in case of high cholesterol levels is much faster than its elevation in case of too low cholesterol levels.
- The elimination of enzymes in case of high cholesterol levels is more intense for the enzymes prior to CYP51, including CYP51 than for the enzymes that appear later in the pathway.
- The speed of metabolite production is much larger (approx. 10000 times) than the production of enzymes in normal conditions. However, when disturbed, the production of enzymes can be raised even several 10 times, whereas the metabolite production cannot change as much.

If the above findings are not incorporated into the model, the model simulation shows large oscillations of levels of all involved substances to any disturbance of the model. Current version of the model is presented in Figure 4. Similar can be observed when simulating the complex model with all known substances included. In Table 1, measured and simulated values of relative changes in cholesterol and all of measured intermediates are shown. The following was observed during simulations of effects of used xenobiotics:

ast: To simulate atorvastatin effects, the levels of HMGCR and CYP51 enzymes levels had to be lowered. In the model, the constant elimination rate for HMGCR was set 0.1 and to 0.04 for CYP51, whereas in normal situation the elimination rates for all enzymes were 0.00001.

rst: Effect of rosuvastatin can be simulated by inhibiting HMGCR enzyme. Its constant elimination rate in the model was set to 8 times higher value than in situation where no xenobiotic was present.

lk1: Simulation results for LK-935 can be obtained by inhibiting CYP51, if high levels of lanosterol are assumed to inhibit HMGCR, otherwise, HMGCR must be blocked as well, in latter case, blocking of HMGCR by the LK-935 is less effective than blocking of CYP51.

lk2: Simulation results for LK-980 can be obtained by inhibiting DHCR7, TM7SF2, SC5D, and CYP51 enzymes in the model (assuming, that high levels of lanosterol inhibit HMGCR). However, since levels of 7-dehydro cholesterol + zymosterol remain on normal levels while all other either severely drop or are raised, it can be expected that high levels of both or one of them inhibit one or more enzymes that act on intermediates before 7-dehydro cholesterol and zymosterol. Considering the model, it is most likely that they affect CYP51, since this would reduce the number of enzymes that are affected directly by the drug most and still explain the levels of intermediates.

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Tab. 1 Measured(M) and simulated(S) relative values of cholesterol and its intermediates

intermediate metabolite	lanosterol		FF-MAS		lathosterol		7-dehydro cholesterol +zymosterol		desmosterol		cholesterol	
xenobiotic	M	S	M	S	M	S	M	S	M	S	M	S
none	1	1.0	1	1.0	1	1.0	1	1.0	1	1.0	1	1.0
atorvastatin (ast)	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
rosuvastatin (rst)	0.0	0.1	0.4	0.3	0.4	0.3	0.5	0.4	0.6	0.4	0.3	0.3
LK-935 (lk1)	6.2	6.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LK-980 (lk2)	1.0	1.1	2.5	2.4	0.5	0.5	1.0	1.1	0.0	0.1	0.1	0.1
LK-9104B (lk3)	1.3	1.5	3.7	3.6	0.9	0.7	2.0	1.7	0.1	0.0	0.2	0.0
LK-9109B (lk4)	1.0	1.1	6.5	5.9	0.1	0.1	1.0	1.1	0.0	0.1	0.0	0.1

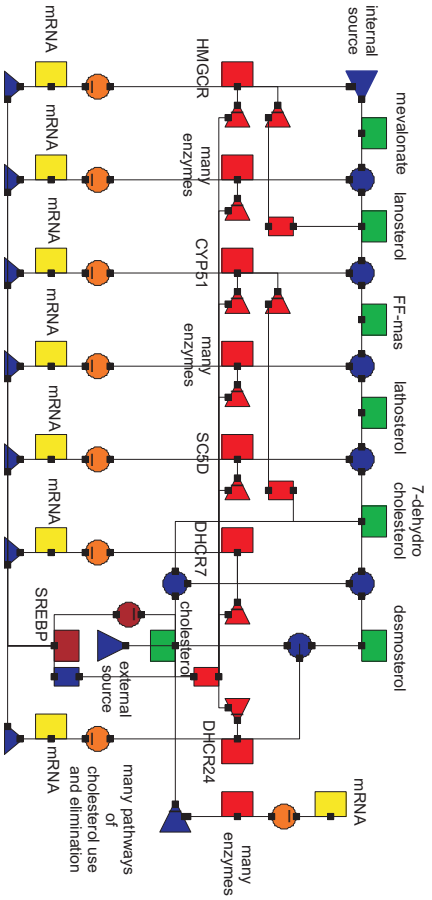

Fig. 4 Current version of simplified cholesterol pathway model (small dark (blue) triangular object to the left - mRNA control, light (yellow) square objects on the left - mRNA, light (orange) round objects on the left - enzyme production according to mRNA levels, dark (red) square objects - enzymes, dark (blue) round objects - enzyme reactions, dark (blue) large triangular objects - metabolite source or sink, small light (red) triangular objects - controlled enzyme elimination, light (green) objects on the right - metabolites, small dark (blue) square object - mechanism of mRNA control with respect to cholesterol levels, small light (red) square object - mechanism of enzyme elimination control with respect to cholesterol levels)

lk3: To obtain simulation results for LK-9104B the same enzymes must be inhibited as for LK-980, however, the LK9104B is 5-times more effective (reduction of normal enzyme elimination rates had to be 5-times higher as for LK-980). Again, inhibition of one of enzymes that act on intermediates before 7-dehydro cholesterol and zymosterol by 7-dehydro cholesterol or zymosterol is suggested by the model (again most likely CYP51, since that reduces the number of directly affected enzymes by the LK-9104B most).

lk4: Simulation results can be obtained by inhibiting DHCR7, TM7SF2, and CYP51 enzymes. Again, inhibition of one of enzymes that act on intermediates before 7-dehydro cholesterol and zymosterol by high levels of 7-dehydro cholesterol or zymosterol is suggested by the model (again most likely CYP51, since that reduces the number of directly affected enzymes by LK9109B most).

5 Conclusions

Although the model is very complex and non-linear, its structure is sensitive to some changes of parameter that do not correspond to reality. Therefore, the model has the potential to help in understanding the complex system of cholesterol regulation in the cell and how it reacts to certain class of xenobiotics. At this stage it is not possible to identify, if the effects on enzymes are directly produced by the xenobiotic, or the xenobiotic has simply triggered some auto-regulation mechanism, however, the model shows what enzymes were affected in the test. The model also suggests that high levels of lanosterol and 7-dehydro cholesterol and/or zymosterol inhibit one or more enzymes that act on intermediates before them. The effect description of lanosterol on HMGCR was later found in the literature, whereas no support for the effect of 7-dehydro cholesterol and/or zymosterol on CYP51 could be found yet.

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