1 Neuroprotective effects of tibolone during astrocytic metabolic inflammation: a network based approach

Abstract:

1.1. Introduction

Astrocytes are the most common cells in mammal brains, their main associated function is act as neuron activity support, energy metabolism and neurotransmitter syntesis regulators.

1.2. Material and Methods

1.2.1. Tissue Specific Model Construction

The tissue specific model construction process started with the identification of all enzyme-coding genes expressed over the mean in at least 50 % of samples for healthy human astrocytes indexed in the GEO database [4] as GSE73721 [16]. Gene identificators convertion from GeneCards[13] to ENTREZ [7] was performed throught 'UniProt.ws' R Package [3]. Reactions associated with the identified genes were mapped from the Human Genome Scale Metabolic Reconstruction RECON 2.04 downloaded from the VMH Lab (https://vmh.uni.lu) [15]. The R package 'g2f' [2] was used to identify and fill the gaps using all no gene associated reactions included in RECON 2.04, as well as to identify and remove all blocked reactions from the reconstruction. All reactions involved in the conversion of extracellular glutamate, glycine, cysteine and glucose to extracellular glutamine, glycine, serine-D, reduced glutathione, lactate and ATP respectively were added. Exchange reactions were limited to components of the Dulbecco's Modified Eagle Medium (DMEM) as input and gliotransmitters (glutamine, D-serine, ATP, glutamate), reduced glutathione, lactate, glucose, nitric oxide, prostaglandins and leukotrienes as output. Finally, syntax, mass-charge validation and creation of SBML files were carried out through the 'minval' R Package [10]. Reaction limits

(upper and lower bounds) were constrained proportional to the mean gene expression reported for genes included in Gene-Protein-Reaction (GPR) [14] associated to each reaction in samples of 47 to 63 years old using 'exp2flux' R package [9]. All analysis were done by the 'sybil' [5] R Package running under R 3.3.1 [11].

1.2.2. Flux Balance Analysis

Flux Balance Analysis (FBA) is a linear optimization method for simulating metabolism that allows to identify the set of reactions involved in the production of a biological response within a metabolic model [8]. The metabolic reactions are represented internally as a stoichiometric matrix (S), of size $m * n$, where m represents the compounds and n the reactions; the entries in the matrix are the stoichiometric coefficients of the metabolites participating in a reaction [12]. The flux through all of the reactions in a network is represented by the vector v, which has a length of n. The concentrations of all metabolites are represented by the vector x, with length m. The systems of mass balance equations at steady state, $\frac{d_x}{d_t} = 0$ or $S * v = 0$. FBA seeks to maximize or minimize an objective function which can be any linear combination fluxes, to obtain a flux for each reaction, indicating how much each reaction contributes to the objective function [8]. FBA for healthy, inflammated and medicated scenarios was resolved using GLPK 4.60, setting the generic human biomass reaction included in RECON 2.04 and each one of reactions described in table **1-1** as objective functions. Models were analyzed by comparing fluxes between scenarios, metabolites production rate and sensitivity analysis.

Table 1-1: Objective functions used to evaluate astrocytes metabolic capabilities [1]

ID	FORMULA REACTION	DESCRIPTION
Glu2Gln	1 glu_L[e] ⇒ 1 gln_L[e]	Glutamate - Glutamine Cycle
Gly2SerD	1 gly[e] ⇒ 1 ser_D[e]	Glycine to D-serine conversion
Glc2Lac	1 glc_D[e] ⇒ 2 lac_L[e]	Lactate production from Glucose
Glc2ATP	1 glc_D[e] ⇒ 36 atp[e]	ATP production from Glucose
Cys2GTHRD	1 cys_L[e] + 1 glu_L[c] + 1 gly[c] ⇒ 1 gthrd[e]	Catch of Cysteine to produce reduced Glutathione

1.2.3. Metabolic Scenarios

To test neuroprotective effects of tibolone during astrocytic metabolic inflammation we define three different metabolic scenarios. A 'healthy' scenario, where palmitate uptake rate was freely set by optimizer; an 'inflammated' scenario, where uptake rate of palmitate was forced to be stable in the mean of the half maximal inhibitory concentration (IC50) value for all objective functions included in table **1-1**. IC50 values were calculated through a robutness

analysis performed using uptake of palmitate ('EX_hdca(e)' in RECON 2.04) as control reaction and a 1000 points in the range from 0 to 1 $mMgDW^{-1}h^{-1}$ for each objective function. Uptake value where each objective function reached IC50 was selected and subsequently averaged. Finally, a medicated scenario, defined as an inflammated scenario that include 279 reactions associated with tibolone and estradiol-derivated compounds metabolism. Ten specific reactions described in table **1-2** associated to specific Tibolone action mechanism non included in RECON 2.04 were added to medicated scenario.

Table 1-2: Reactions associated to Tibolone specific action mechanism in brain [6]

ID	FORMULA REACTION	DESCRIPTION
T1	tibolone[e] $\Leftrightarrow$	Tibolone exchange reaction
T2	tibolone[e] $\Leftrightarrow$ a3OHtibolone[e]	3αhidroxytibolone interconvertion
T3	tibolone[e] $\Leftrightarrow$ b3OHtibolone[e]	3βhidroxytibolone interconvertion
T4	tibolone[e] $\Rightarrow$ d4tibolone[e]	Tibolone Δ^4 isomer formation
T5	b3OHtibolone[e] $\Rightarrow$ d4tibolone[e]	Tibolone Δ^4 isomer formation from 3β-hidroxytibolone
T6	a3OHtibolone[e] $\Rightarrow$ estradiol[c]	Estradiol receptor agonist action mechanism of 3α-hidroxytibolone
T7	b3OHtibolone[e] $\Rightarrow$ estradiol[c]	Estradiol receptor agonist action mechanism of 3β-hidroxytibolone
T8	d4tibolone[e] $\Rightarrow$ prgstrn[c] + tststerone[c]	Progesterone and androgen receptor activation by tibolone Δ^4 isomer
T9	a3OHtibolone[e] $\Leftrightarrow$ a3SOtibolone[e]	3αhidroxytibolone interconvertion to sulfated inactive compounds
T10	a3SOtibolone[e] $\Rightarrow$	Tibolone inactive form in blood

1.2.4. Metabolic Changes

Metabolic changes across metabolic scenarios were measured through two different approximations. Flux differences for each reaction between optimized scenarios were measured using the fold change calculated as described in equation 1-1.

$$foldChange = \frac{valueModel2 - valueModel1}{|valueModel1|} \tag{1-1}$$

Additionally, to obtain a full perspective about inflammation effects in metabolites production, the production of each metabolite was set as objective function in each metabolic scenario and differences were evaluated as well as flux differences.

1.2.5. Proinflammatory, Antiinflammatory and Tibolone Action Mechanism Associated Enzymes

Identification of enzymes involved in proinflammatory and antiinflammatory responses as well as in the tibolone action mechanism were identified through several sensitivity analysis as follows: Proinflammatory enzymes, are those that catalyze reactions that being knocked out allows an increase of objective function value. Antiinflammatory enzymes, are those associated to reactions that being knocked out reduce even more the objective function value. Tibolone action mechanism associated enzymes are those that catalyze reactions that being knocked out inhibit entirely the metabolic effect of tibolone.

1.3. Results

1.4. Conclusion

1.5. Bibliography

Bibliografía

[1] Igor Allaman, Mireille Bélanger, and Pierre J. Magistretti. Astrocyte–neuron metabolic relationships: for better and for worse. *Trends in Neurosciences*, 34(2):76–87, feb 2011.

[2] Kelly Botero, Daniel Osorio, Janneth Gonzalez, and Andres Pinzon. *g2f: Find and Fill Gaps in Metabolic Networks*, 2016.

[3] Marc Carlson. *UniProt.ws: R Interface to UniProt Web Services*, 2016.

[4] Ron Edgar, Michael Domrachev, and Alex E Lash. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res*, 30(1):207–210, 2002.

[5] Gabriel Gelius-Dietrich, Abdelmoneim Desouki, Claus Fritzemeier, and Martin J Lercher. sybil – Efficient constraint-based modelling in R. *BMC Systems Biology*, 7(1):125, 2013.

[6] Helenius J. Kloosterboer. Tissue-selectivity: the mechanism of action of tibolone. *Maturitas*, 48(SUPPL. 1):30–40, aug 2004.

[7] Donna Maglott, Jim Ostell, Kim D Pruitt, and Tatiana Tatusova. Entrez Gene: gene-centered information at NCBI. *Nucleic acids research*, 33(suppl 1):D54—-D58, 2005.

[8] Jeffrey D Orth, Ines Thiele, and B O Palsson. What is flux balance analysis? *Nature Biotechnology*, 28(3):245–248, 2010.

[9] Daniel Osorio, Kelly Botero, Janneth Gonzalez, and Andres Pinzon. *exp2flux: Convert Gene EXPression Data to FBA FLUXes*, 2016.

[10] Daniel Osorio, Janneth Gonzalez, and Andres Pinzon. minval: MINimal VALidation for Stoichiometric Reactions, 2016.

[11] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, 2016.

[12] Karthik Raman and Nagasuma Chandra. Flux balance analysis of biological systems: Applications and challenges. *Briefings in Bioinformatics*, 10(4):435–449, 2009.

[13] Michael Rebhan, Vered Chalifa-Caspi, Jaime Prilusky, and Doron Lancet. GeneCards: integrating information about genes, proteins and diseases. *Trends in Genetics*, 13(4):163, 1997.

[14] Ines Thiele and Bernhard Ø Palsson. A protocol for generating a high-quality genome-scale metabolic reconstruction. *Nature Protocols*, 5(1):93–121, jan 2010.

[15] Ines Thiele, Neil Swainston, Ronan M T Fleming, Andreas Hoppe, Swagatika Sahoo, Maike K Aurich, Hulda Haraldsdottir, Monica L Mo, Ottar Rolfsson, Miranda D Stobbe, and Others. A community-driven global reconstruction of human metabolism. *Nature biotechnology*, 31(5):419–425, 2013.

[16] Ye Zhang, Steven A. Sloan, Laura E. Clarke, Christine Caneda, Colton A. Plaza, Paul D. Blumenthal, Hannes Vogel, Gary K. Steinberg, Michael S B Edwards, Gordon Li, John A. Duncan, Samuel H. Cheshier, Lawrence M. Shuer, Edward F. Chang, Gerald A. Grant, Melanie G Hayden Gephart, and Ben A. Barres. Purification and Characterization of Progenitor and Mature Human Astrocytes Reveals Transcriptional and Functional Differences with Mouse. *Neuron*, 89(1):37–53, 2016.