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

Abstract:

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1.1. Introduction

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 [3] as GSE73721 [14]. Gene identificators convertion from GeneCards[11] to ENTREZ [5] was performed throught 'UniProt.ws' R Package [2]. 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) [13]. The R package 'g2f' [1] 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 () as output. Finally, syntax, mass-charge validation and creation of SBML files were carried out through the 'minval' R Package [8]. Reaction limits (upper and lower bounds) were constrained proportional to the mean gene expression reported for genes included in Gene-Protein-Reaction (GPR) [12] associated to each reaction in samples of 47 to 63 years

old using 'exp2flux' R package [7]. All analysis were done by the 'sybil' [4] R Package running under R 3.3.1 [9].

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 [6]. 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 [10]. 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 [6]. 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 sensibility analysis.

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

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 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. Inflammated Scenario

Inflammated Scenario was defined as an optimized model where uptake 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 robustness 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 $\mathrm{mMgDW^{-1}h^{-1}}$ for each objective function. Uptake value were each objective function reached IC50 was selected and subsequently

averaged.

1.2.4. Medicated Scenario

1.3. Results

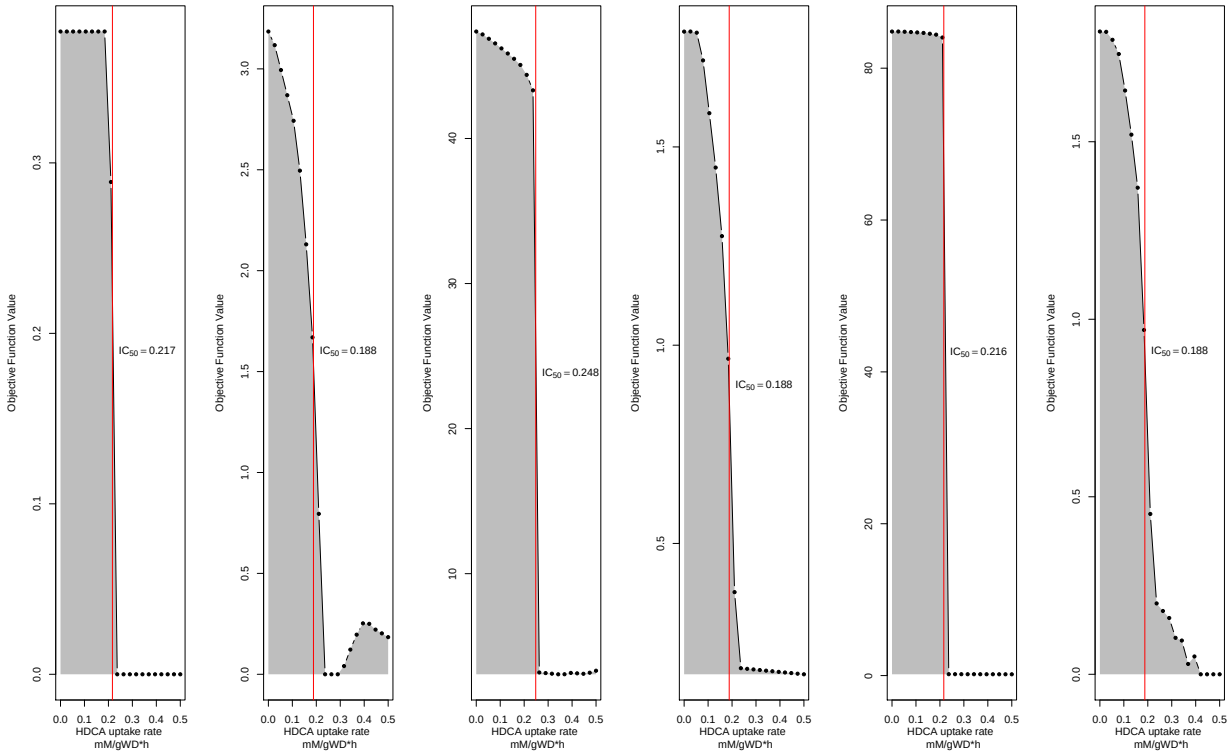

Figure 1-1: •

1.4. Conclusion

1.5. Bibliography

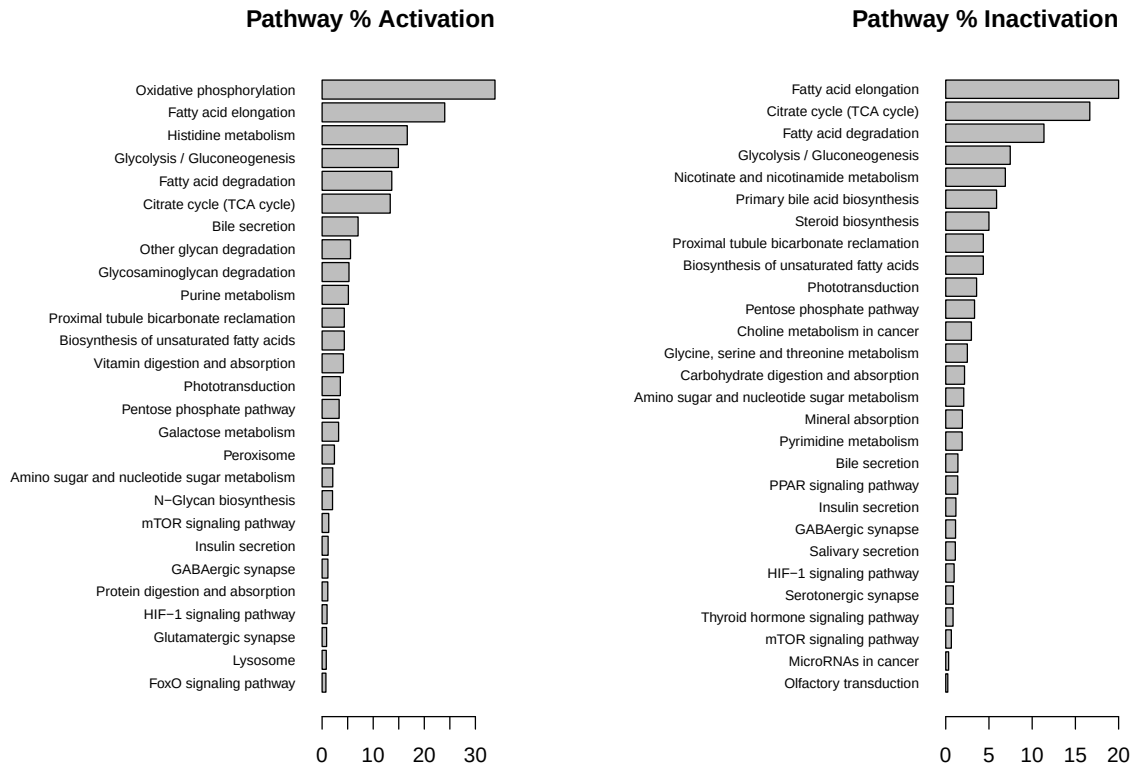
Pathway % Activation
Oxidative phosphorylation
Fatty acid elongation
Histidine metabolism
Glycolysis / Gluconeogenesis
Fatty acid degradation
Citrate cycle (TCA cycle)
Bile secretion
Other glycan degradation
Glycosaminoglycan degradation
Purine metabolism
Proximal tubule bicarbonate reclamation
Biosynthesis of unsaturated fatty acids
Vitamin digestion and absorption
Phototransduction
Pentose phosphate pathway
Galactose metabolism
Peroxisome
Amino sugar and nucleotide sugar metabolism
N−Glycan biosynthesis
mTOR signaling pathway
Insulin secretion
GABAergic synapse
Protein digestion and absorption
HIF−1 signaling pathway
Glutamatergic synapse
Lysosome
FoxO signaling pathway
0
10
20
30
Pathway % Inactivation
Fatty acid elongation
Citrate cycle (TCA cycle)
Fatty acid degradation
Glycolysis / Gluconeogenesis
Nicotinate and nicotinamide metabolism
Primary bile acid biosynthesis
Steroid biosynthesis
Proximal tubule bicarbonate reclamation
Biosynthesis of unsaturated fatty acids
Phototransduction
Pentose phosphate pathway
Choline metabolism in cancer
Glycine, serine and threonine metabolism
Carbohydrate digestion and absorption
Amino sugar and nucleotide sugar metabolism
Mineral absorption
Pyrimidine metabolism
Bile secretion
PPAR signaling pathway
Insulin secretion
GABAergic synapse
Salivary secretion
HIF−1 signaling pathway
Serotonergic synapse
Thyroid hormone signaling pathway
mTOR signaling pathway
MicroRNAs in cancer
Olfactory transduction
0
5
10
15
20

Figure 1-2: •

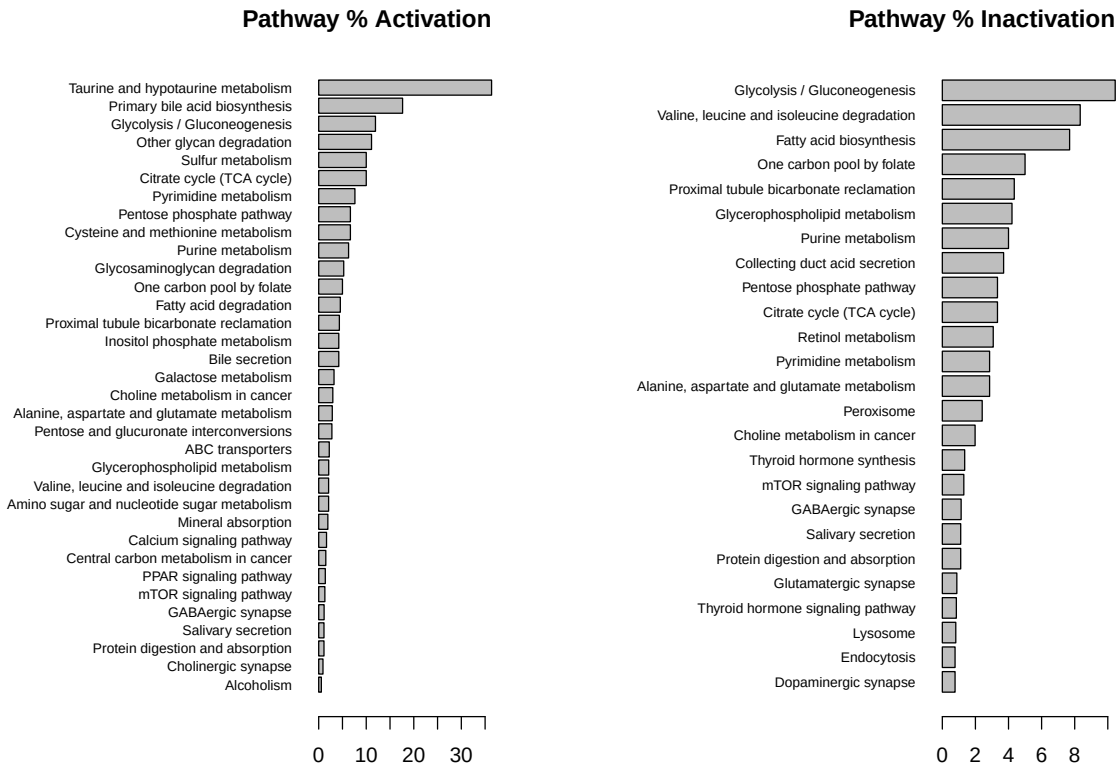
Pathway % Activation
Taurine and hypotaurine metabolism
Primary bile acid biosynthesis
Glycolysis / Gluconeogenesis
Other glycan degradation
Sulfur metabolism
Citrate cycle (TCA cycle)
Pyrimidine metabolism
Pentose phosphate pathway
Cysteine and methionine metabolism
Purine metabolism
Glycosaminoglycan degradation
One carbon pool by folate
Fatty acid degradation
Proximal tubule bicarbonate reclamation
Inositol phosphate metabolism
Bile secretion
Galactose metabolism
Choline metabolism in cancer
Alanine, aspartate and glutamate metabolism
Pentose and glucuronate interconversions
ABC transporters
Glycerophospholipid metabolism
Valine, leucine and isoleucine degradation
Amino sugar and nucleotide sugar metabolism
Mineral absorption
Calcium signaling pathway
Central carbon metabolism in cancer
PPAR signaling pathway
mTOR signaling pathway
GABAergic synapse
Salivary secretion
Protein digestion and absorption
Cholinergic synapse
Alcoholism
0 10 20 30
Pathway % Inactivation
Glycolysis / Gluconeogenesis
Valine, leucine and isoleucine degradation
Fatty acid biosynthesis
One carbon pool by folate
Proximal tubule bicarbonate reclamation
Glycerophospholipid metabolism
Purine metabolism
Collecting duct acid secretion
Pentose phosphate pathway
Citrate cycle (TCA cycle)
Retinol metabolism
Pyrimidine metabolism
Alanine, aspartate and glutamate metabolism
Peroxisome
Choline metabolism in cancer
Thyroid hormone synthesis
mTOR signaling pathway
GABAergic synapse
Salivary secretion
Protein digestion and absorption
Glutamatergic synapse
Thyroid hormone signaling pathway
Lysosome
Endocytosis
Dopaminergic synapse
0 2 4 6 8

Figure 1-3: •

Bibliografía

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

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

[3] 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.

[4] 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.

[5] 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.

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

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

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

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

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

[11] 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.

[12] 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.

[13] 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.

[14] 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.