Kynurenergic Treatments of Huntington's Disease - Mathematical Modeling Insights

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Introduction

In my research as a part of PRUV, I developed mathematical models of tryptophan metabolism in healthy subjects and Huntington's Disease patients, using the work of Stavrum et al.[1], and applied these models to explore possible treatments of Huntington's disease. The models are a set of differential equations, describing relationship between the rate of change of a metabolite along a metabolic pathway and linear combinations of velocities of reactions where this metabolite participates. The latter were approximated using equations from Michaelis-Menten kinetics and some similar expressions. I introduce multiple modifications such as different maximum velocities of certain reactions, separation of the branches of the pathway in different cell types, and excretion o fthe neuroactive metabolite kynurenic acid, to make sure the models fit the purpose, and represent the experimental data well. I proceed to test different treatment strategies that are based on curbing or increasing the activity of enzymes that play a role in tryptophan metabolism, and find the extent of alteration that could maximize the therapeutic benefits. I find that from multiple perspectives, blocking of the enzymes IDO or TDO would be the best therapeutic approach, although blocking of KMO, the most popular of the explored approaches, could fit the purpose as well. Manipulating TPH and KYNU, although unlikely to be effective by themselves, could achieve specific goals and be used in combination with the aforementioned treatments. 3HAA, as well as KAT, are likely to be less attractive therapeutic targets.

Background and rationale

Huntington's Disease (HD), a debilitating progressive neurodegenerative disorder, is caused by a genetic mutation. Evidence suggests that abnormalities in the metabolism of tryptophan via the kynurenine pathway, observed in HD patients, play a causal role in the disorder's pathology. In particular, the neurodegeneration could be caused by the shift of balance from neuroprotective kynurenic acid (KA) to the neurotoxic metabolites quinolinic acid (QA) and 3-hydroxykynurenine (3HK). Multiple scientists have proposed treatment strategies based on interfering with the activities of the enzymes in the metabolic pathway to shift the balance from neurotoxic to neuroprotective metabolites. Experiments with animal models have shown some promising results for some treatment following this strategy.

There are, however, a couple of challenges associated with the suggested treatments via animal experiments. Animal models of HD, have their own, often unknown peculiarities which make their physiology different from human HD patients. Also, since decreasing neurotoxic and increasing neuroprotective metabolites *excessively* leads to a number of new adverse effects, the researchers want to estimate how much inhibition or activation is needed to restore healthy metabolite levels without going too far.

I aimed to address these challenges by constructing a mathematical model of tryptophan metabolism in healthy persons and HD patients that fits the empirical data for human subjects. This allows us to avoid the consequences of the unforeseen mechanisms present in animal models, and to make sure that the output matches the measurements in humans.

It also allows us to easily and quickly construct an empirical function which maps enzyme activity to metabolite concentrations, and, from observing its behavior, estimate the degree to which the enzyme needs to be activated or inhibited in an HD patient to restore healthy metabolite concentrations. I thus aimed to use the model to explore different treatment strategies suggested or tried on animal models in the previous research, making conclusions about which enzymes would be good targets for inhibition or activation, and to what extent their activity should be altered to yield the best therapeutic outcome with the greatest chances for patient benefits.

Procedure

The main software tool I used for the work was MATLAB application for biological and pharmacological modelling, SimBiology. The model of healthy subjects is largely based on the model of tryptophan metabolism in the brain and the liver developed by Stavrum et al. [1]. Initially, I attempted to change the parameters in the model in such a way that the output fits the measurements of Stoy et al.[2] in blood plasma of late-stage HD patients, measured on tryptophan loading. I have thus added modifications to the model of Stavrum et al. that allowed me to reproduce the results of Stoy et al. For most of these modifications, Rios-Avila et al.[3] was used as a guide. A single blood compartment was replaced with portal circulation (exchanges with the liver) and peripheral circulation (exchanges with the brain), which communicated with each other, to reproduce more closely how metabolite concentrations change in the human tissues dynamically. A gutcompartment was added, from which tryptophan is transported to the portal circulation. The scaling factor that was necessary for obtaining accurate concentrations of the products of non-enzymatic reactions was calculated, based on the reaction velocities measured in the study by Pearson et al.[4]. Kynurenic acid (KA) and xanthurenic acid (XA), metabolites significant in Stoy's experiment. However, since the information about brain/liver excretion and transport was lacking, these were only present as end-point metabolites with constant concentrations in Stavrum et al.[1]. I set these to be non-constant. Excretion with a rate proportional to the concentrations were added, and the constants that defined the proportion were chosen to produce experimentally measured steady-state values.

As I proceeded with the study of the existing literature on HD and kynurenine pathway, and became familiar with more experimental results, I changed my approach. The levels of metabolites in the brain, both in terms of quantity and the qualitative nature of the differences between the control group and the experimental group, were quite different from those in blood plasma. Moreover, the totality of evidence suggested that even though many studies did not specify the extent of neurodegeneration in the HD subjects, the patterns of abnormalities in KP metabolism were different in early-grade and later-grade HD patients, and it was in the early stage when the abnormalities were the starkest. At that point, the bulk of damage to neurons was yet to have occurred, and thus the therapeutic intervention would

be the most effective. I thus intended to create a model of *early-stage* HD specifically, based on the data from Guidetti et al.[5] on concentrations of metabolites in post-mortem brains of healthy subjects and HD brains of different grades.

The gut compartment and the separation between portal and peripheral circulation were thus removed, as these were only significant for simulations that involve changes of metabolite concentrations in dynamics. The excretion term for XA was removed as well. The excretion term for KA remained in place, since it was necessary to observe the changes in KA, a key neuroactive metabolite relevant to data in Guidetti[5]. The scaling factor remained as calculated previously as well. Additionally, a crucial new modification was implemented. Based on the information supporting the fact that two branches of the kynurenine pathway – one leading to KA formation, another to 3HK/QA formation - were separated and localized in different types of brain cells, the brain compartment was broken into two compartments where the corresponding reactions take place. The model of healthy subjects was then tailored to the control group of the study by Guidetti et al.[5] in such a way that the weighted average across the two brain compartments were within the margin of error from the results of the experiments measured in frontal cortex.

The initial version of my model of HD brain was constructed using experimentally measured enzyme activities in the brains of HD patients. For KMO and TPH, for which no human data was found, measurements in transgenic mouse models of HD were used. It was still evident that the values of the kynurenine pathway metabolites were low, which strongly indicated activation of the IDO or TDO enzymes. There was not sufficient evidence to indicate which of the two enzymes contributes to this more besides the suggestion of Stoy et al.[2]. I did not agree their suggestion and explained why using the enzyme kinetics equations used by Stavrum et al. to construct their model. I thus made an assumption that these enzymes were inhibited proportionally, proposing that future experimental studies could determine the contributions and adjust the conclusions accordingly. When the model was ready and all experimental values from Guidetty et al. were reproduced, I proceeded to explore the following treatment strategies: inhibition of KMO (directly in the brain, in the liver, in the whole body), inhibition of IDO/TDO (in the brain, the liver and the whole body), activation of TPH (in the brain), inhibition of 3HAO and activation of KYNU (in the brain).

To accomplish this, I used the parameter scan program in the SimBiology model analyzer. It generated a sample of model variants differing by the values of a single or multiple parameters. Each subsequent variant had the parameter value that differed by a fixed number from the corresponding parameter value of the preceding variant. The program then calculated the steady state concentrations for each, and presented a dataset with the concentrations for each variant as an output.

Results and conclusions

For KMO inhibition, the basic findings observed in experimental animals, healthy and models of HD, were reproduced in our model as well. The QA levels and the 3HK levels dropped to zero. The levels of KA increased several-fold. These effects seen in various models with the gene encoding KMO deleted were also observed in our model in the case when the KMO in

the liver and the brain were inactive. A compound designed by Toledo-Sherman et al.[6] that inhibits KMO in the periphery, and at least to some degree, in the brain of healthy rats, produced elevated KA, and KYN levels in the striatum. 3HK levels, were also elevated, but not as much as when only the liver enzyme was inhibited. All of this agrees with the inhibition of KMO in the liver and in both tissues in my HD model. Finally, inhibiting KMO led to the decrease the 3HK:KA ratio, elevation of KA, decrease and even restoration to the levels of control of 3HK and QA, under certain conditions. All of these justify the potential use of KMO inhibitioon in HD treatments.

However, I have also noted some challenges when it comes to inhibiting KMO as a therapeutic strategy. The range of inhibition that shifts the balance away from neurotoxic metabolites sufficiently but does not deplete QA and 3HK excessively is quite narrow. Another negative effect of the KMO inhibition in the liver (and in both tissues) is the two-fold decrease in serotonin, which might exacerbate the depressive symptoms of HD, especially prevalent in the earlier stages of the disease.

The inhibition of IDO/TDO in the brain was shown to restore all the downstream KP metabolites that were elevated in HD, and decrease the 3HK:KA ratio. Moreover, the extent to which the enzyme(s) need to be inhibited in order to restore the normal levels of KP metabolites is less than that for KMO. Additionally, the decline of metabolite concentration as Vmax of IDO/TDO is decreased is far more steady and smooth than that for KMO, which means that the inhibition need not be as precise as for KMO in order to deplete QA and 3HK levels sufficiently, but not excessively. Additionally, the two-fold increase in serotonin concentration, observed in IDO/TDO inhibition, could help curb depression and anxiety in HD patients. These offer noticeable advantages of this approach compared to KMO inhibition.

Inhibition or activation of a number of other enzymes could produce therapies that are more "targeted". Activation of TPH (an enzyme at the start of the serotonin pathway, a route of tryptophan metabolism alternative to KP) produced only modest, although noticeable, decreases in KP metabolites and significant elevation of serotonin levels. It would thus not suffice to normalize the KP abnormalities seen in HD, but unlike the inhibition of other enzymes, including IDO, TDO and KMO, it could restore normal levels of serotonin, and possibly ameliorate, to an extent, the depressive symptoms and anxiety in HD patients.

KYNU activation changes 3HK levels and the 3HK:KA ratio significantly, while having little effect on the concentrations of other metabolites, thus it could be used in case the intended therapeutic approach is to lower 3HK levels and the 3HK:KA ratio.

Inhibition of 3HAA, even as it is capable of bringing down neurotoxic QA, has lower chances of proving itself as an effective a therapeutic strategy. The enzyme activity sufficiently low to decrease QA levels necessitates an elevation of 3HAA 100-fold, which, considering possible carcinogenic properties of this metabolite, could be highly undesirable. There is also only about 1.5% difference in the enzyme activity between barely decreasing QA, and dropping the QA levels to zero, so this this approach demands an extraordinary precision from the designers of the compound.

Activation of KAT several-fold could decrease L-Kyn significantly but has a modest to small effect on other metabolites, including KA, which rises up to 30% at a 10-fold increase in the enzyme Vmax. Considering the negative side effects of increased KA levels, and the fact that these are as high or higher in the frontal cortex and the neostriatum of early-grade HD patients as compared to controls, activation of KAT is less than likely to achieve the therapeutic goals of ameliorating neurodegeneration in the early stages of HD.

Possible future studies proposed

The results of our modeling showed how the options differ with respect to metabolite concentrations, but not with respect to the extent of neurodegeneration and the severity of the side effects. This could only be addressed by conducting further experiments on animal models of HD that investigate the relationship between the levels of neuroactive metabolites, the extent of the neuronal death, and the severity of each side effect. If enough data points are gathered in such studies, a new mathematical model could be created that describes the aforementioned relationship, and it would need to be quite complex and feature multiple mechanisms of neurodegeneration such as cell damage via excitotoxicity and via oxidative stress. The researchers could also investigate experimentally the inhibition of multiple enzymes, for example, IDO or TDO to restore the KP metabolite levels, and TPH to restore serotonin levels.

Table 1: Comparison between calculated steady-state concentrations in the model of a healthy subject and experimentally measured concentrations of tryptophan metabolites in healthy humans.

External tryptophan in the blood was chosen to be 30 μM, which falls within the normal physiological range.

Metabolite	Units	Caclulated	Measured	References
3HAA				
Brain	nM	297	223—353	[7]
Liver	μM	5.1	4.5—5.3	[8]
L-Kynunerine				
Brain	μM	1.5	1.08—1.86	[5]
Liver	μM	5.4	5.1—5.7	[8]
3HK				
Brain	nM	277	178—344	[5]
Liver	μM	4.7	4.5—5.1	[8]
QA	nM	235	190—310	[5]
Serotonin	nM	31	25.6—52.8	[7]
KynA				
Brain	nM	454	354—556	[5]
Liver	μM	4.4	4.1—4.5	[8]
Tryptophan				
Brain	μM	20.6	18.3—21.6	[5]
Liver	μM	21.2	19.6—23	[8]

Table 3: Comparison between calculated steady-state concentrations in the model of an HD patient and experimentally measured concentrations of tryptophan metabolites in frontal cortex of post-mortem HD brain.

External tryptophan in the blood was the same as for table 1. Most of the measured values were taken from grade 1 HD values in Guidetti et al.[5]. The rest were taken from Beal et al.[7].

Metabolite	Units	Caclulated	Measured	References
3HAA	nM	463	319—477	[7]
L-Kynurenine	μM	3.82	3.47-4.63	[5]
3HK	nM	731	641—997	[5]
QA	nM	566	399—583	[5]
Serotonin	nM	658	15.3—22.2	[7]
KA	μM	1.1	0.77—1.49	[5]
Tryptophan	μM	9.8	18.8—22.3	[7]

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