Targeting the Kynurenine Pathway in Huntington’s Disease

Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder caused by expansion of a polyglutamine tract in the huntingtin (Htt) protein. This fatal disorder most severely affects the neostriatum of the brain, with symptomatic manifestations including chorea, cognitive deficits and psychiatric disturbances. Recent studies have found that perturbation of kynurenine pathway (KP) metabolism represents a signature for HD pathology, and that alteration of brain KP metabolite levels plays a causative role in this disease and may thus be targeted therapeutically. The KP is responsible for approximately 95% of tryptophan degradation in mammals, ultimately yielding NAD+. As three KP metabolites are neuroactive, this pathway is of particular interest for the neuroscience community. The enzyme kynurenine 3-monooxygenase (KMO) lies at a critical branching point in the pathway between the formation of the neurotoxic metabolites 3-hydroxykynurenine (3-HK) and quinolinic acid (QUIN), and the neuroprotective metabolite kynurenic acid (KYNA)—such that inhibition of KMO leads to neuroprotection via increased levels of KYNA relative to 3-HK/QUIN. In addition to HD, the metabolites in this pathway have been implicated in several brain diseases and are discussed at length below.

Neuroactive kynurenine pathway metabolites

QUIN, described as a central intermediate of the KP in 1964, was the first metabolite in the pathway to receive attention by neuroscientists when it was discovered that it was a potent excitotoxin in vivo via specific agonism of NMDA-sensitive glutamate receptors. Subsequent studies spearheaded by Robert Schwarz found that intrastriatal injection of QUIN in rodents produced several phenotypes reminiscent of HD. Furthermore, intrastriatal injections of QUIN spare a subclass of aspyr striatal neurons which are also retained in HD patient brains. In addition, QUIN generates free radicals and antioxidant treatments reduce QUIN-dependent toxicity in rats, suggesting a combinatorial action of QUIN. The KP metabolite 3-HK is also toxic in neuronal cell lines and primary neurons by production of free radicals such as Fenton reagent. 3-HK can potentiate QUIN phenotypes in striatal co-injection experiments—increasing lesion volume and further impairing behavioural phenotypes. KYNA, the third neuroactive KP metabolite, is a broad-spectrum antagonist of ionotropic excitatory amino acid receptors at supraphysiological concentrations. At endogenous concentrations KYNA competitively inhibits the glycine co-agonist site of NMDA receptors and is a strong non-competitive inhibitor of the α7 nicotinic acetylcholine receptor. KYNA is neuroprotective against QUIN-induced excitotoxicity and seizures in rats and results in behavioural changes in rodents, mimicking properties of NMDA receptor antagonists. Mice with a targeted deletion of the gene encoding the yeast orthologue of KMO leads to neuroprotection via increased levels of these metabolites are observed in the cerebellum. Exacerbating this increase in neurotoxic KP metabolites, levels of the neuroprotective metabolite KYNA are decreased in the cortex of HD patient brains. In total, these data suggest that in HD the KP is shifted away from the neuroprotective branch of the pathway. Perturbations in levels of KP metabolites have been documented in several brain diseases, including HD, Alzheimer’s disease (AD), stroke, cerebral malaria, and HIV dementia. Levels of QUIN and 3-HK in the neostriatum and neocortex of early stage HD patients (Grade 0/1) are elevated several-fold, but no significant changes in levels of these metabolites are observed in the cerebellum. In HD patient brains. Interestingly, the timing of the increases in 3-HK/QUIN levels mirrors the differences in onset of pathology observed in these models—suggesting a causative role for KP metabolites in disease progression.

KMO inhibition as a therapeutic strategy in Huntington’s Disease

Interest in the therapeutic potential of the KP was further stimulated by a study we published in 2005 using the baker’s yeast Saccharomyces cerevisiae. In a genome-wide screen, we found that deletion of the gene encoding the yeast orthologue of KMO reduced cellular toxicity caused by mutant htt. We proceeded to show that flux through the central KP is increased in HD yeast—mirroring the observations in HD patients and HD mice. Importantly, we found that 3-HK and QUIN were undetectable in the KMO deletion strain, and that levels of ROS returned to control levels, suggesting a role for ROS in KD dependent toxicity in yeast. Furthermore, manipulation of the KP via deletion of other KP genes showed a strong correlation between levels of 3-HK/QUIN and mutant htt toxicity.

Recently, in collaboration with Charalambo
Kyriacou, we performed a detailed mechanistic study of KMO and the KP in a fruit fly model of HD. Here we found that genetic inhibition of KMO was robustly neuroprotective. Furthermore, we discovered that several compounds which inhibit KMO activity also reduce neurodegeneration in HD flies. Complementing this, both genetic and pharmacological inhibition of KMO led to a reduction of 3HK relative to KYNA. By feeding KP metabolites to HD flies, we showed for the first time that KP metabolites are causative in mutant but dependent neuron loss. We observed that HD flies fed 3HK no longer displayed the neuroprotective benefits of KMO gene deletion, and that HD flies fed KYNA exhibited decreased neurodegeneration. Genetic inhibition of tryptophan 2,3-dioxygenase (TDO), which catalyses the first step in the KP, was similarly neuroprotective. As this is a step catalysed in the mammalian brain by both TDO and indoleamine 2,3-dioxygenase (IDO) — and inhibitors are available for both enzymes — this work suggests that additional points in the KP could be targeted therapeutically in HD, and perhaps other neurodegenerative disorders.

The therapeutic potential of KMO inhibitors was further underscored by a complementary collaborative study conducted by Paul Muchowski and Robert Schwarcz. In this work, a novel orally bioavailable prodrug (JM6) of the KMO inhibitor Ro 61-8048 was developed and tested in both HD and AD model mice. In both models, treatment with JM6 led to decreased synaptic loss. Furthermore, JM6 treatment increased the lifespan of HD model mice and rescued behavioural deficits in AD mice. Quite provocatively, it was found that neither JM6 nor Ro 61-8048 crosses the blood–brain barrier in rodents, indicating that the protective effects conferred upon these mouse models of neurodegeneration are due to inhibition of KMO in the periphery. Further analyses in this study suggest that the observed neuroprotection is due to increased blood levels of kynurenine, transport of this metabolite into the brain, and preferential conversion into KYNA. The peripheral action of JM6 may prove particularly useful in increasing clinical benefits and reducing possible toxic side-effects of combinational therapies with other neuroprotective brain-penetrant compounds.

In summary, these studies highlight the therapeutic potential of targeting the KP with small molecular inhibitors in HD and other neurodegenerative disorders. In all cases it appears that reduction of 3HK/QUIN relative to KYNA is central to the neuroprotective effects observed. While KMO inhibition is thus far the most robustly validated of the KP targeting approaches, other pathway enzymes such as IDO and TDO may also prove to be important therapeutic targets for these disorders, and are worthy of further exploration.

**REFERENCES**


