

Tryptophan 2,3-dioxygenase as a potential target for the treatment of neurodegenerative disorders

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The kynurenine pathway is crucial for the metabolism of tryptophan in mammals. It has been suggested that a dysregulation of the kynurenine pathway plays an important role in the development of neurodegenerative diseases. The first and rate-limiting step of the kynurenine pathway of tryptophan degradation is catalysed by the enzymes tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO), which leads to the formation of N-formylkynurenine, which in turn leads to the formation of kynurenine. Kynurenine-3-monooxygenase catalyses the synthesis of kynurenine's neuroactive metabolites: the neurotoxic 3-hydroxykynurenine and quinolinic acid and the neuroprotective kynurenic acid. There is evidence that an imbalance between these three metabolites could potentially lead to neurodegeneration, which could cause neurodegenerative diseases. Recently several studies have highlighted the therapeutic potential of the enzyme TDO. Therefore, TDO as a therapeutic target in Parkinson's disease, Huntington's disease and Alzheimer's disease is reviewed. Inhibition of the kynurenine pathway was found to be neuroprotective in several models of Parkinson's disease, Huntington's disease, and Alzheimer's disease. Particularly, inhibition of TDO was found to be neuroprotective in fruit fly and mice models of these neurodegenerative diseases. It is hypothesized that inhibition of TDO is neuroprotective by restoring the balance between the neuroactive metabolites of the kynurenine pathway. Furthermore, the therapeutic potential of IDO and kynurenine-3-monooxygenase is highlighted. Even though more research is needed to further validate TDO as a potential target, it shows great potential as a target for the treatment of neurodegenerative disorders.

The kynurenine pathway (KP) is the primary pathway for the metabolism of the essential amino acid tryptophan (TRP) in mammals, and it has been closely linked to the pathogenesis of several neurodegenerative diseases.¹⁻⁵ It plays an important modulatory role in the immune response, and it is often up-regulated when an immune response takes place.⁶ Tryptophan depletion can result in suppression of T cell proliferation and T cell apoptosis.⁷⁻⁹ The KP exists mainly in the liver, where most of the tryptophan degradation takes place.¹⁰ The first step of the KP is the formation of formylkynurenine, which is catalysed by either tryptophan 2,3-dioxygenase (TDO) or indoleamine 2,3-dioxygenase (IDO) (Fig. 1).⁴ TDO and IDO work as rate-limiting enzymes, TDO primarily in the liver, but it also exists in the brain, while IDO works, amongst others in the lungs, intestine, male and female reproductive systems and the brain.¹⁰ IDO is regulated by cytokines, and it is primarily activated by interferon gamma (IFN- γ). In contrast, TDO, the main enzyme

responsible for TRP catabolism and homeostasis, is activated by corticosteroids and TRP.⁶

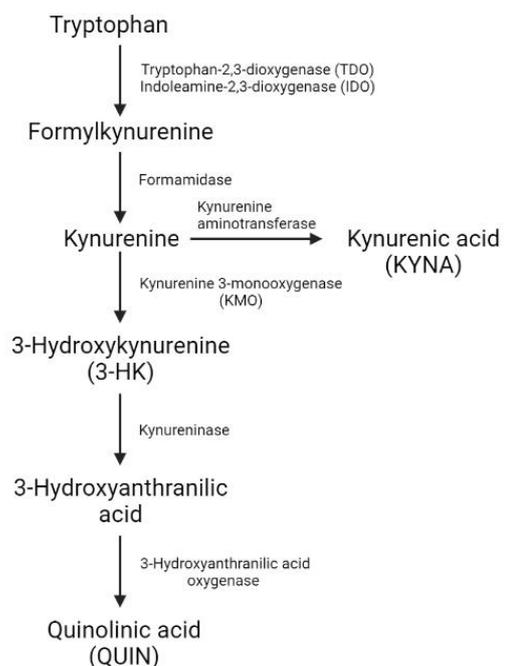


Figure 1. Schematic overview of the kynurenine pathway.

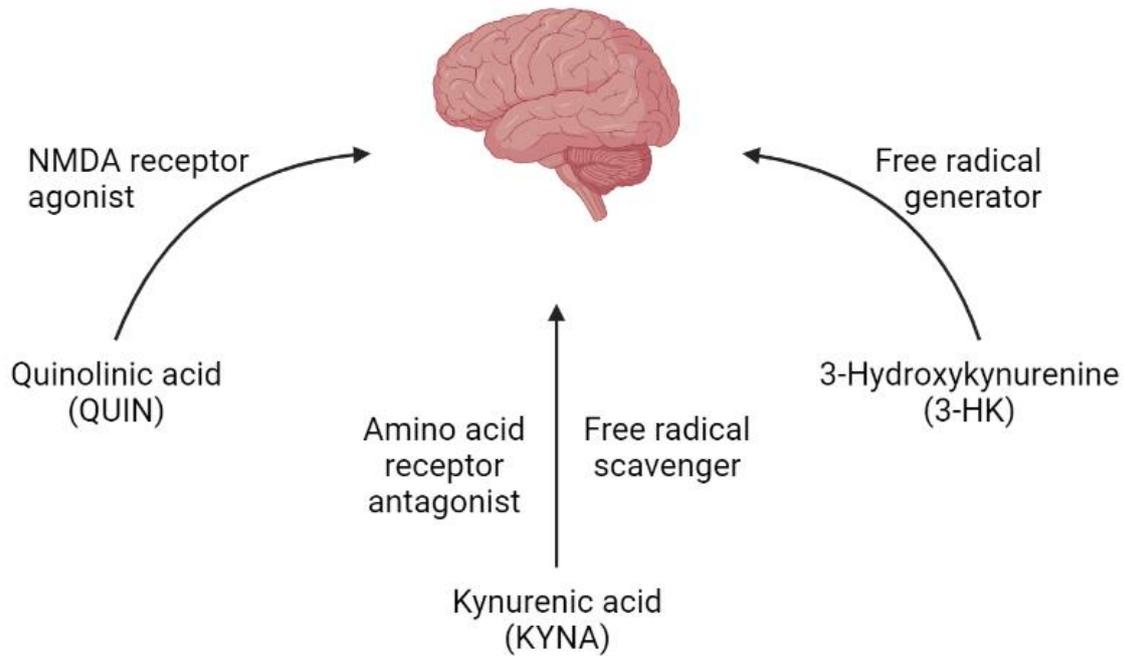


Figure 2. Schematic overview of the modes of action of the neuroactive metabolites of the KP.

The activation of TDO and IDO leads to the formation of kynurenine (KYN) and its metabolites (Fig. 1), which have several pathophysiological effects in both the brain and peripheral tissue.¹¹ Firstly, KYN is a ligand that has an immunosuppressive effect on the aryl hydrocarbon receptor, and KYN dampens inflammation.¹² In addition, there are three downstream metabolites of KYN that have shown to be neuroactive: kynurenic acid (KYNA), 3-hydroxykynurenine (3-HK), and quinolinic acid (QUIN).^{1,2,13} Whereas 3-HK and QUIN are neurotoxic, KYNA has neuroprotective properties.² The neurotoxic metabolites activate the formation of free radicals and heighten the oxidative stress level, which causes neuronal death.^{14,15} The neuroprotective metabolite provides protection against excitotoxic lesions.¹⁶ As mentioned before, 3-HK is mainly a potent free-radical generator^{13,17,18}, while QUIN has additional excitotoxic properties by acting as an *N*-methyl-D-aspartate (NMDA) receptor agonist¹⁹⁻²¹ and KYNA acts as an antagonist of excitatory amino acid receptors and a free-radical scavenger in the brain²²⁻²⁴ (Fig. 2). In addition, KYNA, 3-HK and QUIN possess anti- and pro-oxidant effects.^{11,25} Dysregulation of the KP leads to imbalances in neuroactive metabolites.^{2,26} A shift toward an increase of the neurotoxic

metabolites 3-HK and QUIN, relative to the neuroprotective KYNA, may contribute to the development of neurodegenerative diseases by causing an increase in neurodegeneration.^{3,5}

Previously the therapeutic potential of inhibiting TDO in neurodegenerative diseases was highlighted.^{2,3} At two points in the KP its metabolites are regulated: (1) the first step and rate-limiting conversion of TRP into *N*-formylkynurenine by TDO or IDO; and (2) the synthesis of 3-HK from KYN by kynurenine-3-monooxygenase (KMO).^{3,5} The mechanism by which TDO or IDO inhibition is protective is still unknown. However, it is known that KMO inhibition is protective by normalizing an imbalance between the neurotoxic and neuroprotective metabolites.³ TDO might be neuroprotective via a similar mechanism, by increasing KYNA relative to 3-HK and QUIN, or rather due to an increase in TRP levels.³

TDO inhibition was previously found to reduce neurodegeneration in a fruit fly model by increasing TRP³ and increasing the neuroprotective KYNA relative to the neurotoxic 3-HK.²⁷ Even though TDO deficient mice were found to develop normally, they did display changes in neurogenesis.²⁸ These previous findings underline the therapeutic potential of inhibiting the

TDO in the KP for the treatment of neurodegenerative diseases such as Parkinson's disease, Huntington's disease and Alzheimer's disease. Therefore, the purpose of this review is to further investigate TDO as a target for treatment of neurodegenerative diseases.

Age-related diseases

Age was found to have an influence on KP activity. These age-related changes in the KP could play a role in the onset and progression of age-related diseases.^{11,29-31} These age-related diseases, such as Alzheimer's disease, Huntington's disease, and Parkinson's disease, have some similarities that will be further elaborated on here, while the disease specifics will be discussed later. First of all, the metabolites of the KP act as cross-organ signalling molecules that regulate the pathophysiological events of age-related diseases, amongst others neuroinflammation, NMDA receptor activation and metabolic dysfunction.¹¹ In addition, age-related changes in KYN metabolism and changes in KYN uptake along large amino acid transporters contribute to these diseases.¹¹

Van der Goot et al. (2012) studied the enzyme TDO and the KP metabolites in relationship to age-related protein toxicity in a *C. elegans* model. TDO is suggested to play a role in protein homeostasis during aging by increasing tryptophan levels.²⁷ In the process of aging, the expression of TDO naturally increases.³² This may be of importance in an age-dependent decrease of protein homeostasis. Inhibition of TDO could delay this age-dependent process. The KP was found to regulate age-related protein toxicity, which plays a role in neurodegeneration.²⁷ In fact, in a *C. elegans* model, TDO was found to upregulate age-related α -synuclein toxicity, which is an aggregation-prone protein. Although TDO is suggested to regulate α -synuclein toxicity through TRP, *in vitro* TRP itself does not seem to have a direct effect on α -synuclein aggregation.²⁷ It is theorized that TRP or its derivatives activate signalling molecules that suppress α -synuclein toxicity.²⁷ In addition, inhibition of TDO results in the suppression of the toxicity of other proteins that are the sensors of a healthy homeostasis.²⁷ Finally, depletion of TDO causes an increase in TRP level, and an

increase in TRP suppresses toxicity, as has been demonstrated by feeding worms with additional L-TRP. This suggests that TRP is important in the regulation of protein toxicity by TDO.²⁷

On the contrary, Braidy et al (2011) found that in rats TRP levels and TDO activity in the brain, liver and kidney decreased with age.³³ IDO activity in the brain increased with age, while IDO activity in the liver and kidney decreased with age. The age-related changes in TRP metabolism could have an influence on several biological processes, including activation of the NMDA receptor. Age-related changes like these could contribute to neurodegeneration in elderly people.³³

In ageing humans, an increase in TRP degradation rate was found.^{34,35} In addition, an increase in inflammation and IDO activity was observed in elderly people.³⁶ This increase in inflammation causes an upregulation of KP activity, which results in overproduction of QUIN, which in turn might increase the chance of getting a neurodegenerative disease in elderly people.³⁷ There are no observations on TDO in elderly people, however as there are many similarities between IDO and TDO, TDO activity might also be increased in elderly people. However, further research is needed to confirm this.

Parkinson's Disease

Parkinson's Disease (PD) is the second most common neurodegenerative disease. PD is characterized by motor impairment, which is caused by dopaminergic neuron loss, and the formation of Lewy bodies, which are misfolded and aggregated α -synuclein in the neurons.³⁸⁻⁴⁰ The motor symptoms of PD include tremor, rigidity, postural instability and bradykinesia, which is when patients are slow to start voluntary movements and get progressively slower in their movements and the extent of repetitive actions.^{38,39} Neurodegeneration and chronic NMDA receptor activation contribute to the development of PD.¹¹ The overactivation of the NMDA receptor leads to an increase in glutamate levels and induces excitotoxicity.⁴¹

The KP is involved in the pathogenesis of PD, as it regulates neurodegeneration and NMDA receptor activation. An increase of the

neurotoxic metabolites of the KP can cause an increase in neurodegeneration. Furthermore, age-related changes in KYN metabolism and changes in cerebral KYN uptake could contribute to the development of PD.¹¹ Aging in a healthy population was associated with an increase in KYN, KYNA and QUIN in serum and cerebrospinal fluid (CSF). In patients with PD, reduced KYNA concentrations were found in the brain,^{11,42} while KYN and QUIN concentrations did not differ from age-matched controls.¹¹ Estimations of KYN brain uptake did not differ between patients and healthy controls.¹¹

The dopaminergic neuron loss associated with PD results in progressive locomotor dysfunction.⁴³ Breda et al. (2016) provided evidence that inhibition of TDO, or KMO, enhances locomotor function and alleviates the shortened life span caused by the disease in fly models of PD. They found that both genetic and pharmacological inhibition of TDO provides neuroprotection in fruit fly models of PD, genetic inhibition was done by downregulating the gene encoding for TDO using RNAi and pharmacological inhibition was done by using a TDO inhibitor.³ Furthermore, neurodegeneration in PD can be reduced by manipulating the levels of metabolites within the KP, e.g. through inhibition of 3-HK or promotion of KYNA.⁴³

These are important findings, however, these results were found in fly models of PD. More recently, Perez-Pardo et al. (2021) provided the first evidence that TDO inhibition in a PD mice model decreases motor and nonmotor symptoms. In addition, it reduced the loss of dopaminergic neurons.⁴⁴ In addition, after TDO inhibition an increase of KYN and TRP in the plasma and brain was found.⁴⁴ Furthermore, it was found that a dual TDO and IDO inhibitor penetrates the brain of PD mice, which alleviated neurodegeneration, reduced inflammatory cytokines and QUIN, increased KYNA production in the blood, and lowered IDO expression in those mice.⁴⁵ There were no significant changes in TDO expression.⁴⁵ However, analysis has shown that the compound inhibits both TDO and IDO.⁴⁵ The dual inhibitor did block TDO/IDO mediated *N*-formylkynurenine and QUIN production in the brain.⁴⁵ These discoveries indicate that inhibition of TDO might be

beneficial in PD treatment of humans. Even though the dual TDO and IDO inhibitor does not directly influence the TDO expression, it has promising properties in the treatment of PD.

Huntington's Disease

Huntington's disease (HD) is a neurodegenerative disease that is caused by a CAG trinucleotide repeat in the gene encoding the huntingtin (htt) protein.^{2,46} HD is characterized by movement disorders and a decline in cognition. Patients can suffer from both motor and psychiatric symptoms. Motor symptoms include chorea and loss of coordination, while the psychiatric symptoms include depression, psychosis and obsessive compulsive disorder.⁴⁶

Impairments in the KP metabolism have been linked to HD. In particular the neuroactive metabolites of the KP are involved in the pathophysiology of HD, as they can directly modulate neurodegeneration.^{2,3} Furthermore, modulation of the KP was shown to contribute to toxicity of the mutation in the htt gene.¹³ Previously, it was observed that inhibition of KMO is protective in a HD yeast model of mutant htt toxicity.^{47,48} This is supported by the more recent finding that both genetic and pharmacological inhibition of KMO decreases neurodegeneration in HD model flies, by respectively knocking down the gene encoding for KMO and using a KMO inhibitor.² They also found that there is a correlation between this neuroprotection and decreases in 3-HK relative to KYNA.² Similarly, genetic inhibition of TDO leads to a neuroprotective shift towards KYNA synthesis.² Finally, Campesan et al. (2011) demonstrated the relationship between KYNA and 3-HK by feeding these metabolites to HD flies, which showed to have a direct influence on the neurodegeneration.² Feeding KYNA to HD flies resulted in a neuroprotective ratio between KYNA and 3-HK.²

The levels of KYNA were found to be reduced in patients with HD, which likely contributes to neurodegeneration.⁴⁹ Consequently, increased levels of KYNA relative to 3-HK seem to be essential to the neuroprotection caused by TDO inhibition in HD fruit flies.³ Additionally, TRP treatment induces a reduction in neurodegeneration by causing a shift in KP toward KYNA synthesis.³ The mechanism behind this is not

entirely clear, however, it is suggested that TRP treatment is linked to an increase in the production of KYNA.³ Furthermore, TRP can block the toxicity provided by 3-HK by competing for the same amino acid transporters.¹⁴ Therefore, a decline in brain QUIN levels along with a decrease in 3-HK, relative to KYNA could be especially promising in the treatment of HD.^{3,50} The discovery that TDO inhibition is protective in the fruit fly, validates this protein as a novel therapeutic target for HD.²

To further investigate the neuroprotection of TDO in inhibition in fruit flies, research in an HD mouse model has been done. Research was focussed on the KP and its metabolites in HD animal models and in HD patients. For instance, it has been suggested that KMO plays a more significant role in HD than TDO or IDO, and that therefore it is more relevant to inhibit KMO activity to affect the KP metabolism.⁵¹ This is supported by the observations they made in HD mice, which showed an increase in cerebral KMO activity.⁵¹ In addition, an increase in 3-HK and QUIN levels has been measured in the striatum and cortex of patients with early stage HD.⁵² These increases are associated with up-regulation of IDO mRNA expression⁵³ and a reduction in kynurenine aminotransferases (KAT) activity, which is crucial for KYNA synthesis.⁵⁴ These observations indicate that the approach of targeting the KP and TDO and IDO or KMO may have therapeutic relevance in HD.

Alzheimer's disease

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease, and is characterized by neuronal cell loss and progressive accumulation of misfolded β -amyloid peptide ($A\beta$) senile plaques in the brain and neurofibrillary tangles.^{41,55} AD causes a decline in brain functioning, which results in deficits in memory, spatial orientation, language and behaviour.⁵⁶

The KP has links with inflammation, insulin resistance, and neuroactive metabolites. Therefore, it is of major interest in AD.⁴ Wu et al. (2013) assessed the role of the KP in the pathology of AD.¹ They assessed the expression and localization of key components of the KP, including TDO, in different brain areas of an AD mice model.¹ For instance, the production of

QUIN is progressively and age-dependently increased in the hippocampus of AD mice.¹ It was found that TDO colocalizes with QUIN, neurofibrillary tangles and amyloid deposits in the hippocampus of AD patients.¹ In addition, significantly higher TDO and IDO immunoreactivity was found in the hippocampus compared with age-matched controls.¹ Although this is no evidence for causality, it suggests that the KP could play a role in the neurodegeneration in AD. This is supported by immunohistochemical studies that provide evidence that the KP is up-regulated in AD patient brain tissue, leading to overproduction of the neurotoxic QUIN.⁵⁷ Besides, the data of Guillemain et al. (2005), implies that QUIN may be a neurotoxic and inflammatory factor in the process that leads to neurodegeneration in AD.⁵⁷ AD is characterized by an intense inflammatory response around $A\beta$ senile plaques.⁵⁷ Furthermore, it is established that QUIN immunoreactivity is present in $A\beta$ senile plaques and neurons that are sensitive to tangling.⁵⁷

There is evidence that TRP is involved in the pathogenesis of AD, as TRP is involved in neurotransmission, immune function and KYN synthesis and is a serotonin precursor.⁵⁸ TRP concentrations in the blood were found to correlate inversely with the degree of cognitive deficit, but not with the length of the disease.⁵⁷ Inhibition of TDO is correlated with an increase of TRP.³ This suggests that high TDO activity might cause a decrease of TRP.

TDO is over-expressed in both AD mouse brains and in the brains of AD patients. This was found through mRNA expression analysis in mice and immunohistochemistry in the brains of AD patients.¹ In addition, IDO was also found to be highly expressed in the brains of AD patients.¹ This suggests that activation of the KP by TDO and IDO could be involved in neurofibrillary tangles formation and could be associated with senile plaques. This further underlines that the KP may play an important role in the neurodegenerative processes of AD. In addition, they showed in both mouse and human brains that TDO is mainly expressed in neurons and in some astrocytes.¹ The study provides further evidence that high TDO and IDO over-expression leads to excessive formation of KP

metabolites such as QUIN, which are neurotoxic and are likely involved in the neurodegenerative processes in AD.¹ QUIN is not the only endogenous NMDA receptor agonist, however it is more sensitive to NMDA receptor antagonists than the other agonists.⁵⁹ Therefore, reducing NMDA-induced excitotoxicity by inhibition of IDO or TDO activity might be a potential therapeutic target. Due to its additional neuroprotective properties, such as reducing the neurotoxic QUIN and 3-HK, this might be more effective than the use of an NMDA receptor antagonist, which is a commonly used treatment against AD nowadays.

It has been hypothesized that blocking IDO may slow the progression of AD, this was further researched in a mice model.⁵⁸ The overactivity of IDO may activate several mechanisms that are involved in the pathogenesis of AD, leading to neuronal loss and deficits in behaviour.³ Inhibition of TDO or KMO improves locomotor performance and improves shortened life span in AD model flies. Both genetic and pharmacological inhibition of TDO provides neuroprotection in *Drosophila melanogaster* models of AD.³ These fruit flies are a useful model for the KP, as in these flies TDO is the main enzyme that catalyses the first step in the KP, which makes it a convenient model to examine this step of the KP.³ Genetic inhibition is done by downregulating the gene encoding for TDO, and pharmacological inhibition is done by a chemical TDO inhibitor.³ After treatment with an IDO inhibitor, AD mice showed improvements in cognition as well as anxiety- and depression-related behaviours, which shows the therapeutic potential of targeting the KP and TRP metabolism in AD.⁵⁸ Moreover, there is evidence that reducing KP activity can relieve some of the AD symptoms in mouse models.^{49,60-62} In an AD mice model TDO inhibition can improve behavioural and recognition memory.⁶³ As assessed in behavioural experiments, such as novel object recognition and Morris water maze.⁶³ It is suggested that hippocampal damage in patients with AD can be, up to a certain degree, halted or reversed by blocking the KP by IDO.⁵⁸ These findings suggest that the KP, and inhibition of either or both TDO and IDO, seems a promising target for the treatment of AD. As inhibition of either of these enzymes showed improvement in behaviour and provided neuroprotection.

However, inhibition of KMO also has therapeutic relevance in AD.

Discussion

Research shows that the KP plays a large role in the process of neurodegeneration in several age-related neurodegenerative diseases, such as PD, AD and HD. Age was found to have an influence on KP activity, such as an increase in TRP degradation rate and this could potentially lead to the development of age-related neurodegenerative disease and the progression of these diseases due to overproduction of QUIN. A combination of age- and disease-related upregulation of KP activity could contribute to a decrease in neurogenesis and an increase in excitotoxicity leading to neurodegeneration.¹¹

TDO and IDO were found to be over-expressed in the brains of AD patients and there was an increase in TDO/IDO activity.¹ This leads to excessive formation of the metabolites of the KP, such as QUIN, which are likely involved in the neurodegenerative processes of AD. For example, by, together with TDO, colocalizing with neurofibrillary tangles and senile β amyloid deposits in the brain. Inhibition of TDO, and/or IDO, is often associated with a shift towards the neuroprotective KYNA, relative to either one or both of the two neurotoxic metabolites, QUIN, or 3-HK. The neuroprotective KYNA is reduced in patients with neurodegenerative diseases. In addition, KAT activity is reduced in HD patients.⁵ This suggests that in patients with HD, the KP goes more through the neurotoxic branch of the KP,⁵ resulting in an increase in KMO and an increase in the neurotoxic metabolites 3-HK and QUIN. This also suggests that inhibition of KMO or promotion of KAT might be a relevant target. It is unclear why inhibition of TDO or IDO results in this shift toward neurotoxic branch of the KP. Future research is needed to further unravel this.

Inhibition of the KP is promising in the treatment of neurodegenerative diseases due to its overactivity playing a role in neuroinflammation and neuronal excitotoxicity.¹¹ This is supported by Breda et al. (2016) who suggested that alterations in the level of neuroactive KP metabolites could underlie several therapeutic

benefits.³ An overview of the inhibitors mentioned in this review can be found in Table 1. Inhibition of TDO provides neuroprotection in models of PD, HD and AD. In PD it was already shown in insects, but more recently it was also found that TDO provides neuroprotection in a PD mouse model.^{44,45} Furthermore, a dual TDO and IDO inhibitor was found to be neuroprotective in a PD mouse model.⁴⁵ Even though this study does not show the individual contributions of TDO and IDO, it does show a new potential treatment for PD. In AD it was found in a fruit fly model, and recently Sorgdrager et al. (2020) showed that inhibition of TDO, by TDO inhibitor 680C91, restored recognition deficits in AD mice.⁶³ However, whether inhibition of TDO in AD mice is neuroprotective should be further validated. In HD it was found in a fruit fly model that genetic inhibition of TDO provides neuroprotection. These findings, however, need to be further validated in animal models.

In fact, it was suggested that inhibition of TDO might not be the ideal method to target the KP.⁵⁸ It may be more beneficial for developing treatments for AD to target the individual metabolites in the KP, as the neuroactive metabolites of the KP have opposing roles in neurodegenerative diseases and an imbalance between those

neuroactive metabolites could contribute to neurodegeneration.⁵⁸ For instance by inhibiting the production of neurotoxic metabolites 3-HK or QUIN, or by promoting the production of the neuroprotective metabolite KYNA. For instance, QUIN was found to be overproduced in the brains of AD patients. The enzymes KAT and KMO are responsible for the production of respectively KYNA, and 3-HK and QUIN. Inhibition of KMO or promotion of KAT could be relevant here. This is supported by a study that suggested that in HD KMO was of more importance to the pathophysiology than TDO or IDO.⁵¹ Therefore it was also suggested that it might have therapeutic relevance to inhibit KMO rather than TDO or IDO.

There is more research needed to unravel the underlying mechanisms and to provide more evidence that inhibition of TDO could be beneficial in the treatment of the neurodegenerative diseases. However, it was found that TDO inhibition is neuroprotective, and that it seems to restore the balance between the neuroprotective and neurotoxic metabolites of the KP. Therefore, inhibition of the KP and its enzymes, especially TDO, but also IDO and KMO show great potential as a target for the treatment of neurodegenerative diseases.

Table 1 Overview of inhibitors mentioned in this review

| Model | Inhibitors | Results | Ref. |
|---|--|---|-------------|
| <i>Parkinson's disease</i> | | | |
| Intrastratial rotenone in mice | TDO inhibitors: NTRC 3531-0, oral LM10, oral | <ul style="list-style-type: none"> • NTRC-3531-0 increased plasma & brain TRP • NTRC-3531-0 & LM dose dependently inhibit CNS & gut phenotype | 44 |
| MPTP induced mouse model | Dual IDO/TDO inhibitor: Compound 23, oral (1 <i>H</i> -indazole-4-amine) | <ul style="list-style-type: none"> • Decrease in IDO expression and QUINA • Increase in KYNA | 45 |
| Human α -Synuclein expressing <i>Drosophila melanogaster</i> | TDO inhibitor: 680C91, oral | <ul style="list-style-type: none"> • Provides neuroprotection in PD | 3 |
| <i>Huntington's Disease</i> | | | |
| HD model flies (<i>Drosophila melanogaster</i>) | KMO inhibitor: UPF 648 | <ul style="list-style-type: none"> • Provides neuroprotection in HD • Increase in KYNA relative to 3-HK | 2 |
| R6/2 mouse model | KMO inhibitor: JM6, oral | <ul style="list-style-type: none"> • Extends life span • Prevents synaptic loss • Decreases microglial activation | 62 |
| <i>Alzheimer's Disease</i> | | | |
| 3xTg-AD mouse model | IDO inhibitor: DWG-1036, oral | <ul style="list-style-type: none"> • Improvements in AD behavioural symptoms and depression-like behaviour | 58 |
| APP23 mouse model | TDO inhibitor: 680C91, oral | <ul style="list-style-type: none"> • Improvements in recognition memory • No measurable changes in cerebral kynurenine metabolites | 63 |
| Human $A\beta_{42}$ peptide expressing <i>Drosophila melanogaster</i> | TDO inhibitor: 680C91, oral | <ul style="list-style-type: none"> • Provides neuroprotection in AD | 3 |
| APPtg mouse model | KMO inhibitor: JM6, oral | <ul style="list-style-type: none"> • Prevents spatial memory deficits, anxiety-related behaviour, and synaptic loss | 62 |

References

1. Wu, W. *et al.* Expression of Tryptophan 2,3-Dioxygenase and Production of Kynurenine Pathway Metabolites in Triple Transgenic Mice and Human Alzheimer's Disease Brain. *PLoS One* **8**, (2013).
2. Campesan, S. *et al.* The kynurenine pathway modulates neurodegeneration in a drosophila model of Huntington's disease. *Curr. Biol.* **21**, 961–966 (2011).
3. Breda, C. *et al.* Tryptophan-2,3-dioxygenase (TDO) inhibition ameliorates neurodegeneration by modulation of kynurenine pathway metabolites. *Proc. Natl. Acad. Sci. U. S. A.* **113**, 5435–5440 (2016).
4. Giil, L. M. *et al.* Kynurenine Pathway Metabolites in Alzheimer's Disease. *J. Alzheimer's Dis.* **60**, 495–504 (2017).
5. Amaral, M., Outeiro, T. F., Scrutton, N. S. & Giorgini, F. The causative role and therapeutic potential of the kynurenine pathway in neurodegenerative disease. *J. Mol. Med.* **91**, 705–713 (2013).
6. Chen, Y. & Guillemin, G. J. Kynurenine pathway metabolites in humans: Disease and healthy states. *Int. J. Tryptophan Res.* **2**, 1–19 (2009).
7. Lee, G. K. *et al.* Tryptophan deprivation sensitizes activated T cells to apoptosis prior to cell division. *Immunology* **107**, 452–460 (2002).
8. Munn, D. H. *et al.* Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* **281**, 1191–1193 (1998).
9. Munn, D. H. *et al.* Inhibition of T cell proliferation by macrophage tryptophan catabolism. *J. Exp. Med.* **189**, 1363–1372 (1999).
10. Badawy, A. Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects. *Int. J. Tryptophan Res.* **10**, (2017).
11. Sorgdrager, F. J. H. *et al.* Age- and disease-specific changes of the kynurenine pathway in Parkinson's and Alzheimer's disease. *J. Neurochem.* **151**, 656–668 (2019).
12. Munn, D. H. & Mellor, A. L. Indoleamine 2,3 dioxygenase and metabolic control of immune responses. *Trends Immunol.* **34**, 137–143 (2013).
13. Thevandavakkam, M., Schwarcz, R., Muchowski, P. J. & Giorgini, F. Targeting kynurenine 3-monooxygenase (KMO): implications for therapy in Huntington's disease. *CNS Neurol. Disord. Drug Targets* **9**, 791–800 (2010).
14. Okuda, S., Nishiyama, N., Saito, H. & Katsuki, H. 3-Hydroxykynurenine, an endogenous oxidative stress generator, causes neuronal cell death with apoptotic features and region selectivity. *J. Neurochem.* **70**, 299–307 (1998).
15. Okuda, S., Nishiyama, N., Saito, H. & Katsuki, H. Hydrogen peroxide-mediated neuronal cell death induced by an endogenous neurotoxin, 3-hydroxykynurenine. *Proc. Natl. Acad. Sci. U. S. A.* **93**, 12553–12558 (1996).
16. Sapko, M. T. *et al.* Endogenous kynurenate controls the vulnerability of striatal neurons to quinolinate: Implications for Huntington's disease. *Exp. Neurol.* **197**, 31–40 (2006).
17. Hiraku, Y. *et al.* Metal-mediated oxidative damage to cellular and isolated DNA by certain tryptophan metabolites. *Carcinogenesis* **16**, 349–356 (1995).
18. Ishii, T., Iwahashi, H., Sugata, R. & Kido, R. Formation of hydroxanthommatin-derived radical in the oxidation of 3-hydroxykynurenine. *Arch. Biochem. Biophys.* **294**, 616–622 (1992).

19. Schwarcz, R., Whetsell, W. O. & Mangano, R. M. Quinolinic acid: An endogenous metabolite that produces axon-sparing lesions in rat brain. *Science*. **219**, 316–318 (1983).
20. Stone, T. W. & Perkins, M. N. Quinolinic acid: A potent endogenous excitant at amino acid receptors in CNS. *Eur. J. Pharmacol.* **72**, 411–412 (1981).
21. Stone, T. W., Stoy, N. & Darlington, L. G. An expanding range of targets for kynurenine metabolites of tryptophan. *Trends Pharmacol. Sci.* **34**, 136–143 (2013).
22. Carpenedo, R. *et al.* Presynaptic kynurenate-sensitive receptors inhibit glutamate release. *Eur. J. Neurosci.* **13**, 2141–2147 (2001).
23. Foster, A. C., Vezzani, A., French, E. D. & Schwarcz, R. Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid. *Neurosci. Lett.* **48**, 273–278 (1984).
24. Goda, K., Hamane, Y., Kishimoto, R. & Ogishi, Y. Radical scavenging properties of tryptophan metabolites. Estimation of their radical reactivity. *Adv. Exp. Med. Biol.* **467**, 397–402 (1999).
25. González Esquivel, D. *et al.* Kynurenine pathway metabolites and enzymes involved in redox reactions. *Neuropharmacology* **112**, 331–345 (2017).
26. Zhang, S. *et al.* A brain-permeable inhibitor of the neurodegenerative disease target kynurenine 3-monooxygenase prevents accumulation of neurotoxic metabolites. *Commun. Biol.* **2**, (2019).
27. Van Der Goot, A. T. *et al.* Delaying aging and the aging-associated decline in protein homeostasis by inhibition of tryptophan degradation. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 14912–14917 (2012).
28. Lanz, T. V. *et al.* Tryptophan-2,3-Dioxygenase (TDO) deficiency is associated with subclinical neuroprotection in a mouse model of multiple sclerosis. *Sci. Rep.* **7**, 1–13 (2017).
29. Cervenka, I., Agudelo, L. Z. & Ruas, J. L. Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. *Science*. **357**, (2017).
30. Lim, C. K. *et al.* Involvement of the kynurenine pathway in the pathogenesis of Parkinson's disease. *Prog. Neurobiol.* **155**, 76–95 (2017).
31. Schwarcz, R. & Stone, T. W. The kynurenine pathway and the brain: Challenges, controversies and promises. *Neuropharmacology* **112**, 237–247 (2017).
32. Dupuy, D. *et al.* Genome-scale analysis of in vivo spatiotemporal promoter activity in *Caenorhabditis elegans*. *Nat. Biotechnol.* **25**, 663–668 (2007).
33. Braidy, N., Guillemin, G. J., Mansour, H., Chan-Ling, T. & Grant, R. Changes in kynurenine pathway metabolism in the brain, liver and kidney of aged female Wistar rats. *FEBS J.* **278**, 4425–4434 (2011).
34. Frick, B., Schroecksadel, K., Neurauter, G., Leblhuber, F. & Fuchs, D. Increasing production of homocysteine and neopterin and degradation of tryptophan with older age. *Clin. Biochem.* **37**, 684–687 (2004).
35. Pertovaara, M. *et al.* Indoleamine 2,3-dioxygenase activity in nonagenarians is markedly increased and predicts mortality. *Mech. Ageing Dev.* **127**, 497–499 (2006).
36. Sas, K., Szabó, E. & Vécsei, L. Mitochondria, oxidative stress and the kynurenine system, with a focus on ageing and neuroprotection. *Molecules* vol. 23 191 (2018).
37. Pérez De-La Cruz, V., Carrillo-Mora, P. & Santamaría, A. Quinolinic acid, an endogenous molecule combining excitotoxicity, oxidative stress and other toxic mechanisms. *Int. J.*

- Tryptophan Res.* **5**, 1–8 (2013).
38. Venkatesan, D., Iyer, M., Narayanasamy, A., Siva, K. & Vellingiri, B. Kynurenine pathway in Parkinson's disease—An update. *eNeurologicalSci* vol. 21 100270 (2020).
 39. Sveinbjornsdottir, S. The clinical symptoms of Parkinson's disease. *J. Neurochem.* **139**, 318–324 (2016).
 40. Beach, T. G. *et al.* Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. *Acta Neuropathol.* **119**, 689–702 (2010).
 41. Maddison, D. C. & Giorgini, F. The kynurenine pathway and neurodegenerative disease. *Semin. Cell Dev. Biol.* **40**, 134–141 (2015).
 42. Ogawa, T. *et al.* Kynurenine pathway abnormalities in Parkinson's disease. *Neurology* **42**, 1702–1706 (1992).
 43. Cunningham, P. C., Waldeck, K., Ganetzky, B. & Babcock, D. T. Neurodegeneration and locomotor dysfunction in *Drosophila* scarlet mutants. *J. Cell Sci.* **131**, (2018).
 44. Perez-Pardo, P. *et al.* Pharmacological validation of TDO as a target for Parkinson's disease. *FEBS J.* **288**, 4311–4331 (2021).
 45. Ning, X.-L. *et al.* X-ray Structure-Guided Discovery of a Potent, Orally Bioavailable, Dual Human Indoleamine/Tryptophan 2,3-Dioxygenase (hIDO/hTDO) Inhibitor That Shows Activity in a Mouse Model of Parkinson's Disease. *J. Med. Chem.* **64**, 8303–8332 (2021).
 46. Jimenez-Sanchez, M., Licitra, F., Underwood, B. R. & Rubinsztein, D. C. Huntington's Disease: Mechanisms of Pathogenesis and Therapeutic Strategies. *Cold Spring Harb. Perspect. Med.* **7**, 1–22 (2017).
 47. Giorgini, F., Guidetti, P., Nguyen, Q. V., Bennett, S. C. & Muchowski, P. J. A genomic screen in yeast implicates kynurenine 3-monooxygenase as a therapeutic target for Huntington disease. *Nat. Genet.* **37**, 526–531 (2005).
 48. Giorgini, F. *et al.* Histone deacetylase inhibition modulates kynurenine pathway activation in yeast, microglia, and mice expressing a mutant huntingtin fragment. *J. Biol. Chem.* **283**, 7390–7400 (2008).
 49. Vamos, E., Pardutz, A., Klivenyi, P., Toldi, J. & Vecsei, L. The role of kynurenines in disorders of the central nervous system: Possibilities for neuroprotection. *J. Neurol. Sci.* **283**, 21–27 (2009).
 50. Guidetti, P. & Schwarcz, R. 3-Hydroxykynurenine potentiates quinolinate but not NMDA toxicity in the rat striatum. *Eur. J. Neurosci.* **11**, 3857–3863 (1999).
 51. Sathyaikumar, K. V. *et al.* Dysfunctional kynurenine pathway metabolism in the R6/2 mouse model of Huntington's disease. *J. Neurochem.* **113**, 1416–1425 (2010).
 52. Guidetti, P., Luthi-Carter, R., Augood, S. & Schwarcz, R. Neostriatal and cortical quinolinate levels are increased in early grade Huntington's disease. *Neurobiol. Dis.* **17**, 455–461 (2004).
 53. Mazarei, G. *et al.* Expression analysis of novel striatal-enriched genes in Huntington disease. *Hum. Mol. Genet.* **19**, 609–622 (2009).
 54. Jauch, D. *et al.* Dysfunction of brain kynurenic acid metabolism in Huntington's disease: focus on kynurenine aminotransferases. *J. Neurol. Sci.* **130**, 39–47 (1995).
 55. Ting, K., Brew, B. & Guillemin, G. The involvement of astrocytes and kynurenine pathway in Alzheimer's disease. *Neurotox. Res.* **12**, 247–262 (2007).
 56. Garcez, M. L., Jacobs, K. R. & Guillemin, G. J. Microbiota Alterations in Alzheimer's Disease:

- Involvement of the Kynurenine Pathway and Inflammation. *Neurotoxicity Research* vol. 36 424–436 (2019).
57. Guillemin, G. J., Brew, B. J., Noonan, C. E., Takikawa, O. & Cullen, K. M. Indoleamine 2,3 dioxxygenase and quinolinic acid Immunoreactivity in Alzheimer's disease hippocampus. *Neuropathol. Appl. Neurobiol.* **31**, 395–404 (2005).
 58. Fertan, E. *et al.* Effects of the novel IDO inhibitor DWG-1036 on the behavior of Male and female 3xTg-AD mice. *Front. Pharmacol.* **10**, 1–16 (2019).
 59. Jhamandas, K. H., Boegman, R. J., Beninger, R. J., Miranda, A. F. & Lipic, K. A. Excitotoxicity of quinolinic acid: modulation by endogenous antagonists. *Neurotox. Res.* **2**, 139–155 (2000).
 60. Deora, G. S. *et al.* Multifunctional Analogs of Kynurenic Acid for the Treatment of Alzheimer's Disease: Synthesis, Pharmacology, and Molecular Modeling Studies. *ACS Chem. Neurosci.* **8**, 2667–2675 (2017).
 61. Yu, D. *et al.* The IDO inhibitor coptisine ameliorates cognitive impairment in a mouse model of Alzheimer's disease. *J. Alzheimer's Dis.* **43**, 291–302 (2014).
 62. Zwillig, D. *et al.* Kynurenine 3-monooxygenase inhibition in blood ameliorates neurodegeneration. *Cell* **145**, 863–874 (2011).
 63. Sorgdrager, F. J. H. *et al.* The Effect of Tryptophan 2,3-Dioxygenase Inhibition on Kynurenine Metabolism and Cognitive Function in the APP23 Mouse Model of Alzheimer's Disease. *Int. J. Tryptophan Res.* **13**, (2020).