Potential of kynurenine metabolites in drug development against neurodegenerative diseases

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Reactive oxygen species (ROS) and kynurenines: Kynurenines represent a relatively heterogeneous group of tryptophan metabolites (Figure 1A). The amino acid tryptophan is metabolized in the humans by the kynurenine or serotonin pathway. For a long time, the kynurenine pathway was assumed primarily to constitute the source for nicotinamide-adenine dinucleotide phosphate, one of the most utilized redox active enzyme cofactors. However, in the last years, various kynurenines were identified as important endogenous neuroactive agents of highly quinolinic (QUIN) and kynurenic acid (KYN) interact with the excitatory N-methyl-D-aspartate (NMDA) receptors. Additionally, KYN can bind to a7-nicotinic receptors in high concentrations. Furthermore, several kynurenines showed apparent effects on the immune system and inflammation that is often associated with degeneration processes (Schwarcz and Stone, 2017). Since kynurenine metabolites may influence ROS concentrations, they can affect redox signal cascades by interfering with redox homeodynamic equilibrium. Within the tissue, ROS can participate in various physiological and pathological processes, such as maturation, reproduction, inflammation, and, under specific conditions, programmed cell death by ferroptosis. The function of the ferroptosis in organisms is still far from being satisfactorily understood. In this context, the accumulation of iron can contribute to the development of neurodegenerative pathogenesis in damaged brains. The senile plaques of patients suffering from Alzheimer’s disease, accumulate iron up to a concentration of about 0.9 mM, three times more than in healthy controls (0.3 mM). Usually, iron is stored in complexes with storage proteins, such as transferrin and ferritin, or occurs as cofactors of various enzymes as heme and nonheme coordination complexes. If iron coordinates other molecules than the mentioned ones, it is called “poorly liganded” iron that can catalyze uncontrolled production of highly reactive hydroxyl (HO•) and radicals via the Fenton reaction (Kell, 2010). Again, hydroxyl radicals can increase levels of “poorly liganded” iron by oxidative destruction of the iron storage proteins.

Fe3+ + H2O2 → Fe2++ + OH• + O2 (Fenton reaction)

Additionally, the rate of the radical production can be enhanced by agents supporting iron redox cycling, for example ascorbic acid. The concentration of ascorbic acid in the brain is quite high (up to 1 mM).

Perspective of kynurenines for novel drug development: Antioxidants, such as kynurenines and their chemical analogues, can inhibit neurodegeneration. This aspect of KYN has been extensively investigated. This 4-hydroxyquinoline derivative (Figure 1A) was identified as an endogenous anticonvulsant and neuroprotectant, which behaves as an antagonist of excitatory NMDA receptors. Recently, the general view of KYN agonism on nicotine receptors became questionable (Stone, 2020). Furthermore, KYN showed antioxidant and iron chelation properties (Kubicova et al., 2019). However, all these properties were only apparent in relatively high concentrations of KYN. Accordingly, KYN cannot disturb signal functions of ROS under normal physiological conditions, and additionally, KYN does not decrease the bioavailability of iron ions for metalloenzymes. KYN penetrates only very poorly through the blood-brain barrier (BBB). The natural cerebral concentrations of KYN are low, about 150 nM. Therefore, its derivatives such as 7-chlorokynurenic acid or KYN amide derivatives with improved abilities to pass through BBB into the brain were developed in recent decades (Li et al., 2016; Feher et al., 2019). The substituted amides of KYN affected electrophysiological functions investigated on hippocampus slides (Feher et al., 2019). Furthermore, KYN and its amides such as N-(3-dimethylamino)propyl)-4-hydroxyquinoline-2-carboxamide (Figure 1B) or 4-hydroxy-N-(2-(1-pyrrolidinyl)ethyl)-quinoline-2-carboxamide (Figure 1C) prolonged longevity and increased reproduction as well as growth of bdelloid rotifers, microinvertebrates that recently are used in pharmacological research (Datki et al., 2019). However, elevated brain concentrations of KYN are usually associated with schizophrenia (Stone, 2020). Therefore, kynurenine aminotransferase II inhibitors (Figure 1A) were suggested as promising novel agents against cognitive impairments or psychoses (Rossi et al., 2019). As perspective kynurenine aminotransferase II inhibitors are investigated substitute heterocyclic hydroxamates, 3-amino-3,4-dihydro-1-hydroxy-7-methoxy-6-(benzyl)-2H-quinoline (Figure 1D) and a diazole analogue (Figure 1E) (Rossi et al., 2019). Additionally, the external KYN, a potato constituent, may affect brain functions via the gut-brain axis (Schwarz and Stone, 2017).

Xanthurenic acid (XA) represents a further, often investigated kynurenine metabolite with quinoline structure (Figure 1A). Contrary to KYN, XA can penetrate the BBB probably utilizing a transporter mechanism. Therefore, XA can be accumulated in the brain up to the concentrations of 1 µM.

Still, kynurenine concentrations depend on metabolic conditions (Schwarz and Stone, 2017). For example, pyridoxine (vitamin B6) hypovitaminosis can lead to elevated KNYA and XA concentrations. Although the cerebral concentrations of XA are higher than KYN, XA physiological functions are not fully known. However, in the brains of patients suffering from schizophrenia, XA concentrations are unusually low (Rossi et al., 2019). Additionally, XA shows more effective antioxidant and iron chelating abilities than KYN. Compared to KYN, the better chelating capabilities of XA are caused by the 8-hydroxy group in the XA molecule (Kubicova et al., 2019). 8-Hydroxyquinoline is well known as an excellent chelator of transition metals, included iron, and as very efficient ROS scavenger (Chobot et al., 2018). Therefore, 8-hydroxyquinoline derivatives are candidates for neurodegenerative diseases treatment.

Another extensively investigated kynurenine metabolite is QUIN that has been identified as an agonist of NMDA receptors, primarily in the neocortex and hippocampus (Figure 1A). Additionally, QUIN can affect inflammation that accompanies most degenerative diseases (Schwarz and Stone, 2017). Furthermore, QUIN is a potent iron chelator but has no re dopexy properties. It can influence hydroxyl radical production in the Fenton reaction by affecting iron catalytic activities. However, these effects depend on the acid concentration and the overall reaction milieu (Kubicova et al., 2013). The role of QUIN in pathological processes of psychiatric and neurodegenerative diseases is often discussed. QUIN and other kynurenines are involved in pre-natal brain development. Moreover, kynurenines can freely penetrate through the fetal BBB (Schwarz and Stone, 2017). In the adult brain, physiological concentrations of QUIN are lower than 100 nM but pathological levels can increase up to 10–40 µM.

The antioxidant and iron chelation treatment is often voiced as a promising cure approach of neurodegenerative diseases. Especially, plant polyphenols are extensively discussed in this context and their beneficial effects on human health are well proven (Dhakal et al., 2019). However, the plant polyphenol bioavailability from the human gut and further crossing through BBB into the brain increases. Contrary to plant polyphenols, kynurenines are endogenous metabolites of brain tissue. Moreover, kynurenines can affect the brain processes by interactions with receptors and by modulation of ROS (Kubicova et al., 2019). Additionally, XA shows more effective antioxidant and iron chelating abilities than KYN. Compared to KYN, the better chelating capabilities of XA are caused by the 8-hydroxy group in the XA molecule (Kubicova et al., 2019). 8-Hydroxyquinoline is well known as an excellent chelator of transition metals, included iron, and as very efficient ROS scavenger (Chobot et al., 2018). Therefore, 8-hydroxyquinoline derivatives are candidates for neurodegenerative diseases treatment.

Our investigations showed that a redox homeodynamic equilibrium, stabilization requires the appropriate rate of ROS and metal concentrations. An antioxidant can demonstrate the expected activity if it is present in the proper concentration, the
right time, and the correct compartment of the tissue. In case of lower or higher antioxidant agent concentration, the antioxidant effects can disappear or be even changed to a dangerous pro-oxidant activity (Chobot et al., 2014). Consequently, oxidative stress increases and tissue damages become more evident.

Furthermore, the presence of other redox active substances or competing chelators (e.g., ascorbic acid, other kynurenines, cysteine, tyrosine, tocopherols) merits attention. For example, the presence of ascorbic acid in combination with another chelator in reaction mixtures can modulate antioxidant activities of kynurenine and quinolinic acid substantially (Kubicova et al., 2013, 2019).

ROS concentration is a further factor that affects antioxidant activity. We use hydrogen peroxide to simulate oxidative stress in damaged tissue. In the range of tested concentrations, KYNA was antioxidant in presence of hydrogen peroxide. However, the antioxidant effects of QUIN depended on its concentration and the dose-response curve was U shaped. Compared to KYNA and QUIN, XA showed excellent antioxidant properties in all tested reaction milieus (Kubicova et al., 2019).

This antioxidant variability seems to depend on different factors, including the reversibility of the chemical reactions and the occurrence of other simultaneously running chemical reactions. Therefore, the predictability of the antioxidant effects is very difficult. Nevertheless, the in vitro assays and electrochemical experiments can offer some insights into possible pro- or antioxidant activities of the tested substances. The in vitro experiments, albeit limited in interpretation, may help to understand the results of in vivo experiments. The chemical complexity of the cell protoplast varies in

**Figure 1** | Simplified kynurenine pathway and examples of perspective currently investigated substances.

(A) Simplified kynurenine pathway. (B–E) Chemical structures of perspective compounds: (B) and (C) kynurenic acid amides showing effects on the longevity of bdelloid rotifers, (D) and (E) a chemical structure example of kynurenine aminotransferase II inhibitors.

**References**

Chobot V, Hadaček F, Kubicova L (2014) Effects of selected dietary secondary metabolites on reactive oxygen species production caused by iron(II) autoxidation. Molecules 19:20023-20033.

Chobot V, Hadaček F, Bachmann G, Weckwerth W, Kubicova L (2018) Antioxidant properties and the formation of iron coordination complexes of 8-hydroxyquinoline. Int J Mol Sci 19:3917.

Datki Z, Galik-Olah Z, Bohar Z, Zadori D, Fulop F, Szatmari I, Galik B, Kalman J, Vesei L (2019) Kynurenic acid and its analogs are beneficial physiologic attenuators in bdelloid rotifers. Molecules 24:2171.

Kubicova L, Kubicova E, Chobot V, Kubicova L, Hadacek F, Bachmann G, Weckwerth W (2013) Quinolinic acid: Neurotoxin or oxidative stress modulator? Int J Mol Sci 14:21328-21338.

Kubicova L, Hadaček F, Bachmann G, Weckwerth W, Chobot V (2019) Coordination complex formation and redox properties of kynurenic and xanthurenic acid can affect brain tissue homeodynamics. Antioxidants 8:476.

Li CF, Chen XM, Chen SM, Mu RH, Liu BB, Luo L, Liu XL, Geng D, Liu Q, Yi LT (2016) Activation of Hippocampal BDNF signaling is involved in the antidepressant-like effect of the NMDA receptor antagonist 7-chlorokynurenic acid. Brain Res 1630:73-82.

Rossi F, Migliano R, Ferraris DM, Rizzi M (2019) The synthesis of kynurenic acid in mammals: An updated kynurenine aminotransferase structural KATAlog. Front Mol Biosci 6:7.

Schwarz R, Stone TW (2017) The kynurenine pathway and the brain: Challenges, controversies and promises. Neuropharmacology 112:237-247.

Stone TW (2020) Does kynurenic acid act on nicotinic receptors? An assessment of the evidence. J Neurochem 152:627-649.