

Implications for the Kynurenine Pathway and Quinolinic Acid in Amyotrophic Lateral Sclerosis

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Key Words

Kynurenine pathway · Quinolinic acid · ALS, toxicity pathways

Abstract

The kynurenine pathway (KP) is a major route of *L*-tryptophan catabolism leading to production of several neurobiologically active molecules. Among them is the excitotoxin quinolinic acid (QUIN) that is known to be involved in the pathogenesis of several major inflammatory neurological diseases. In amyotrophic lateral sclerosis (ALS) degeneration of motor neurons is associated with a chronic and local inflammation (presence of activated microglia and astrocytes). There is emerging evidence that the KP is important in ALS. Recently, we demonstrated that QUIN is significantly increased in serum and CSF of ALS patients. Moreover, most of the factors associated with QUIN toxicity are found in ALS, implying that QUIN may play a substantial role in the neuropathogenesis of ALS. This review details the potential role the KP has in ALS and advances a testable hypothetical model.

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The Kynurenine Pathway

The kynurenine pathway (KP) is a major route of *L*-tryptophan (TRP) catabolism, resulting in the production of nicotinamide adenine dinucleotide and other neuroactive intermediates (fig. 1) [1–5]. These include kynurenine (KYN) [6], kynurenic acid (KYNA), 3-hydroxykynurenine (3-HK) [7, 8], picolinic acid [9] and quinolinic acid (QUIN) [1, 6, 10]. The KP also plays a role in certain physiological functions such as behavior, sleep, thermoregulation and pregnancy [1, 2]. Recently, the KP has been found to be critical in the development of immune tolerance [11]. Two theories have been proposed: (1) that TRP degradation suppresses T-cell proliferation by dramatically depleting the supply of this critical amino acid, and (2) that some downstream KP metabolites act to suppress certain immune cells possibly in addition to the effect of tryptophan depletion [11]. Additionally, there is evidence that metabolites derived from the KP are involved in the neurocytotoxic mechanisms associated with several inflammatory brain diseases [1, 12–14].

Among the KP metabolites, the *N*-methyl-*D*-aspartate (NMDA) receptor agonist and excitotoxin QUIN is perhaps the most important in terms of biological activity [15]. QUIN can use different mechanisms, direct or indirect, to exert its neurotoxicity [for review, see 14]. De-

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pending of its concentration, QUIN can lead to acute neuronal death or to chronic and progressive neuronal dysfunction by at least four mechanisms: (1) it can activate the NMDA receptor in pathophysiological concentrations leading to an increase of intracellular calcium [16]; (2) QUIN can increase glutamate release by neurons and inhibit its uptake into the synaptic vesicle by astrocytes leading to excessive microenvironment glutamate concentrations and neurotoxicity [17]; (3) QUIN can lead to significant lipid peroxidation [18–21], and lastly (4) QUIN can potentiate its own toxicity and that of other excitotoxins (e.g. NMDA and glutamate) in the context of energy depletion [22, 23]. We previously showed that chronic exposure to QUIN in pathophysiological appropriate concentrations causes ultrastructural changes in cultured human neurons [24]. QUIN contributes to several neurodegenerative diseases [1, 13, 14]. We demonstrated recently that it is probably involved in the neuropathogenesis of Alzheimer's disease [25, 26].

Another product of the KP, KYNA, is an antagonist of all ionotropic glutamate receptors especially for the glycine site of the NMDA receptor [1] and thus can antagonize some of the effects of QUIN and other excitotoxins [27]. However, it is not present in sufficient concentrations: the amounts of QUIN in the CSF, brain and systemic circulation always exceed KYNA by up to 15-fold [28–30].

KYN is an early product of the KP that can cause a marked increase in expression of nerve growth factor (NGF) transcripts in astrocytes [31]. KYN can be further metabolized to 3-HK, which is known to be able to generate highly reactive free radicals. However, 3-HK alone does not cause neural damage presumably because the normal brain has efficient free radical scavengers to neutralize its effects [31, 32]. But 3-HK can synergize with relatively low doses of QUIN to potentiate its excitotoxicity [33].

The cellular location of the KP is only partly understood. It is completely expressed in monocytic lineage cells, including macrophages, microglia [34–37] but only partly expressed in astrocytes and neurons [38, 39]. We have shown that astrocytes have a critical KP enzyme missing [38] and do not produce QUIN and in fact degrade it [39].

The KP is switched on when cells are activated or exposed to a variety of cytokines especially interferon (IFN)- γ . However, the degree to which the KP is activated may vary according to as yet unexplained differences between individuals perhaps rendering some more susceptible to neurological disability [40].

Some of the KP enzymes can be modulated especially the first and rate-limiting enzyme indoleamine dioxygenase (IDO). We and others have shown that IFN- γ and to a lesser extent IFN- β , IFN- α , tumor necrosis factor- α (TNF- α), interleukin (IL)-1 and platelet-activating factor can lead to up-regulation of the cellular expression of IDO with consequent increased QUIN production [36, 41, 42]. IDO can also be down-regulated by IL-4 [43] and nitric oxide (NO) [44]. However, it should be noted that NO does not inhibit IDO in microglial cells [45].

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is the most common form of the motor neuron diseases (MND) [46]. ALS is a fatal degenerative disease affecting adults usually in mid to late life. It is characterized by degeneration of motor neurons in the motor cortex, brainstem and spinal cord resulting in variable combinations of spastic paralysis, flaccid muscle weakness, wasting, and fasciculations [47]. The disease process affects initially a limb (spinal form) or the speech or swallowing (bulbar form). There is inevitable progression to involvement of all limbs and bulbar function. The disease process remains exclusively motor during the whole duration of the disease. Electromyography can often confirm the diagnosis and rule out other conditions such as multifocal motor neuropathy with conduction block. Radiological investigations eliminate other causes. Respiratory insufficiency becomes more common as the disease progresses. Patients usually die within 3–5 years from symptom onset [48].

Although the cause of ALS is unknown, there is evidence that the excitatory neurotransmitter glutamate plays an important role in neuronal cell death in the disease. Several risk factors, such as exposure to welding and soldering, inhalation of lead vapor, and exposure to other particular chemicals, as well as electrical trauma are postulated as contributing to the pathogenesis of ALS. About 90% of all ALS patients have the sporadic form. Among the 10% with the familial form, approximately 20% are associated with mutations of the copper/zinc superoxide dismutase-1 (Cu,Zn-SOD1) gene. However, because only a subset of cases can be attributed to one particular molecular defect (such as mutation of SOD1 or the genes encoding for VAPB, ALS2, ALS4 [49–51]), the etiology of ALS is likely to be multifactorial [52]. Indeed it is not clear what factors contribute to the causation of the more common sporadic cases [53].

Neuropathologically, ALS and the transgenic mouse model SOD1-G93A (carrying a high copy number of the mutated human SOD1 gene) are associated with extensive proliferation of activated microglia and astrocytes [54] suggesting that neuroinflammation is likely to play a role in the degenerative process [55, 56]. Activated microglia, through the release of several neurotoxins, are considered to be important in the pathogenesis of a wide variety of brain diseases [57]. This has led to our hypothesis that microgliosis plays a role in ALS.

What Are the Conditions for the KP and QUIN Being Associated with ALS?

For the KP and QUIN to be involved in the neuro-pathogenesis of ALS, at least two features must be present: inflammation with the presence of pro-inflammatory cytokines especially IFN- γ (for IDO activation) and activated monocytic cells (which produce QUIN).

Neuroinflammation and IFN- γ

As mentioned above, inflammation is certainly observed in both the brainstem and spinal cord of ALS patients as well as in SOD1 mouse models [55, 56]. An accumulation of large numbers of activated microglia and reactive astrocytes, as well as some T cells, infiltrating macrophages, mast cells and dendritic cells have been described in the ALS brain and spinal cord [58, 59]. These activated immune cells release numerous inflammatory and neurotoxic mediators, including cytokines, chemokines, NO, proteases, prostaglandins, complement fragments, and COX-2 [56]. Moreover, the concentrations of three inflammatory cytokines IFN- γ , TNF- α and IL-1 β known to activate IDO have been found to be significantly increased in the spinal cord of G93A mice in comparison with non-transgenic mice [60]. The entry of T cells, more particularly the Th2 subset, may be an important source of IFN- γ , the most potent inducer of IDO [41]. Interestingly, some studies showed that the damage motor neurons themselves release IFN- γ [61, 62].

Microglial Activation and Infiltrating Macrophages

Microglia are the resident immune cells of the CNS [63]. They are described as a major protagonist in the initiation and the propagation of motor neuron death in ALS [for review, see 57]. The number of activated microglia in postmortem spinal cord tissue of ALS patients is significantly higher than in controls [59, 64]. Activated microglia are implicated in the phagocytosis of degenerating

neurons [65]. Microglial activation is an early event in the progression of ALS appearing before astrocytosis and entry of peripheral leukocytes [66]. The association between rapid progression of ALS and activated microglia has been reported by Banati et al. [67].

In addition to activated microglia in the CNS, entry of peripheral macrophages and some dendritic cells into the brain or the spinal cord can lead to production of significant quantities of QUIN [58, 59]. It should be emphasized that macrophages are able to produce 20- to 30-fold more QUIN compare to microglia [34].

While microglial activation in its early stages may be associated with tissue repair in some diseases [68, 69] through the elaboration of neurotrophic factors [70], this does not seem to be dominant phenomenon in ALS. The switch between the neuroprotective and neurotoxic/phagocytic phenotype is regulated in large part by cross-talk with neighboring neuronal cells, though the precise mechanisms are not understood [69, 71].

What Is the Evidence for the KP and QUIN Being Associated with ALS?

There are several intriguing correlations linking TRP catabolism, the KP, and QUIN to the pathology of ALS.

Direct Evidence

Some recent studies have provided direct links between TRP metabolism and ALS.

KYNA, QUIN in CSF and Serum from ALS Patients Ilzecka et al. [72] showed that KYNA (fig. 1) concentrations in CSF were significantly higher in patients with bulbar onset of ALS compared to controls and to patients with limb onset. Levels of KYNA in CSF were also higher in patients with severe clinical status compared to controls. Serum KYNA was significantly lower in ALS patients with severe clinical status compared to controls and to patients with mild clinical status. However, there was no correlation between KYNA concentration with either the age of patients or the duration of the disease. Ilzecka et al. suggested that this increase of KYNA in CSF of patients with bulbar onset and in patients with severe clinical status may be associated with neuroprotective role of KYNA against excitotoxicity [73, 74]. Within the CNS, KYNA is mainly produced by activated astrocytes and is likely to be part of their neuroprotective functions [38].

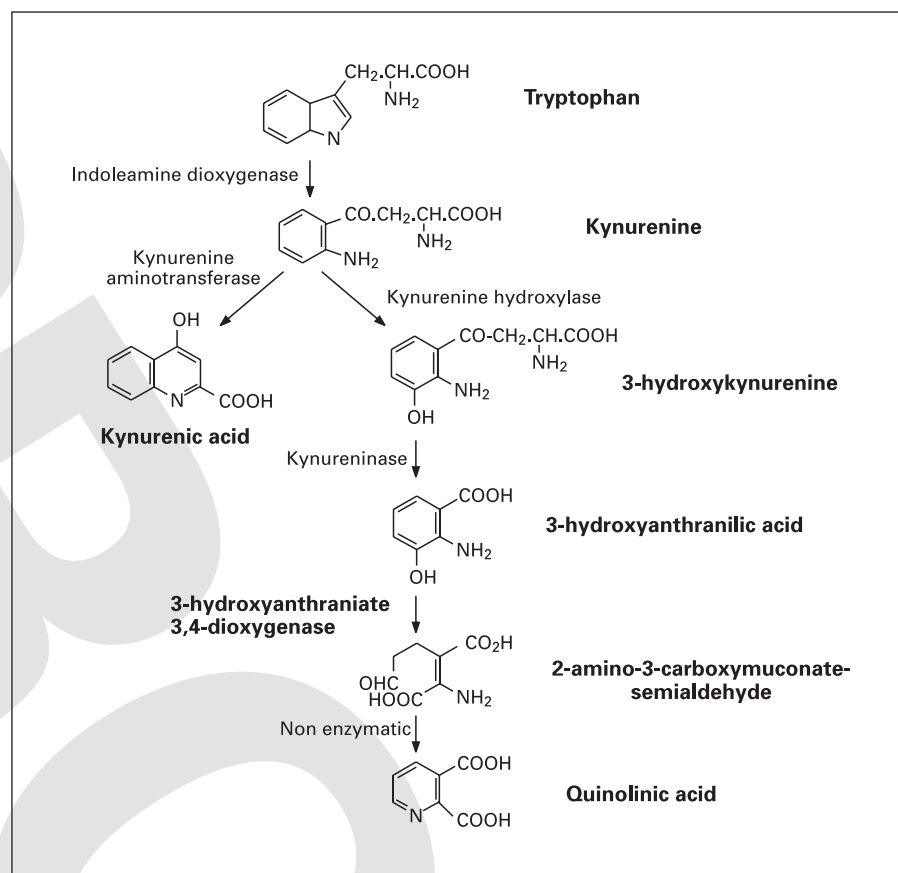


Fig. 1. The kynurenine pathway.

We have quantified levels of QUIN using gas chromatography mass spectrometry in matching serum and CSF from more than 150 ALS patients. Results showed that levels of QUIN in both CSF and serum from ALS patients are significantly increased in comparison with controls (unpubl. data).

Cu,Zn-SOD Aggregation and Tryptophan, KYN

Aggregated SOD1 is one of the main components of the inclusion bodies commonly found in motor neurons and astrocytes of ALS patients. Some studies suggest that SOD1 aggregation may play a key role in ALS neuropathology [52, 75, 76] and that ALS may be placed in the class of protein-misfolding diseases [77, 78].

Cu,Zn-SOD is a dimeric enzyme but there is no cooperative effect between the two subunits. When the quaternary structure is disrupted, the protein adopts a soluble monomeric form [79]. Oxidation of the tryptophan residue (TRP-32) to KYN on the surface of hSOD1^{WT} leads to inter-subunit covalent bond formation and subsequent aggregation of the protein [80, 81]. The presence of extra

tryptophan can totally inhibit covalent dimer formation. Indeed, tryptophan may scavenge the oxidants responsible for hSOD1^{WT} covalent aggregation [80]. Activation of the KP within the CNS and spinal cord of ALS patients would lead to significant decrease of TRP levels so that it may be hypothesized that this is involved in the SOD1 dimerization and aggregation. However, orally administered TRP does not seem able to change SOD activity at least in rat erythrocytes [82].

Indirect Evidence

QUIN Effect on Cu,Zn-SOD Expression

A study by Noack et al. [83] showed that Cu,Zn-SOD expression is found only in astrocytes in the rat brain. Following QUIN injection into the striatum of the rat with subsequent significant neuronal loss, the level of Cu,Zn-SOD was markedly increased in a time-dependent manner (fig. 2). Cu,Zn-SOD was not detected in neurons or microglia. This increase of QUIN induced-Cu,Zn-SOD expression in astrocytes is assumed to have a neuroprotective role by preventing or limiting oxidative

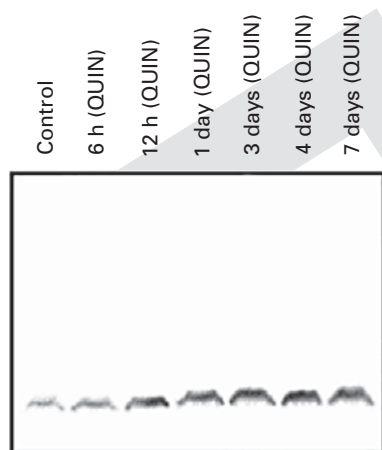


Fig. 2. Western blots of Cu/Zn-SOD from striatal tissue samples collected during the time course of QUIN-induced neurodegeneration from the lesion site [83].

damage. However, an increased expression of the mutated form of the SOD1 can lead to deleterious effects such as intracellular aggregates of SOD1 in motor neurons and astrocytes, and vacuolar pathology associated with damaged mitochondria [84].

ALS, QUIN, the KP and Oxidative Stress

Various neurodegenerative disorders including ALS have been causally linked to the generation of reactive oxygen species (ROS), oxidative stress and lipid peroxidation [85–89].

QUIN leads to the ROS formation in neurons [90, 91] and one of the major aspects of QUIN toxicity is lipid peroxidation [18–21]. In synaptosomes from rat brain, lipid peroxidation has been shown to be increased by 256% after injection of QUIN 100 μ M [92]. A recent *in vivo* study showed that QUIN infusion in sheep fetal brain leads to an increase of HNE (4-hydroxynonenal) formation [93]. HNE is the most prevalent toxic lipid peroxidation end-product formed during oxidative stress [94]. HNE is found increased in the cytoplasm of the residual motor neurons in the spinal cord and CSF of patients with sporadic ALS [95–97]. A recent study demonstrated that HNE in sublethal doses leads to a loss of spinal motor neurons [98]. This may be linked to microglial activation and contribute further to the oxidative stress in ALS [87, 99, 100], as HNE is also one of the most powerful compounds involved in microglial activation [69, 101].

Two other components of the KP have some neuroprotective and antioxidant roles. As described above, KYNA is increased in the CSF of ALS patients [72] although as previously mentioned this is insufficient to counter the effects of QUIN. Intracerebral administration of KYN by increasing endogenous KYNA concentrations might exert neuroprotective effects through blockade of the NMDA receptor through a NO and cGMP pathway in the cerebellum and hippocampus of rats [102]. 3-Hydroxyanthranilic acid (3-HAA) is a further KP product that is a potent free radical scavenger [103], which may have some neuroprotective effects. In IFN- γ -stimulated macrophages, 3-HAA inhibits peroxidation of LDL by acting in synergy with α -tocopherol [104]. It remains to be seen whether enough 3-HAA is produced *in vivo* to have these beneficial effects. This would seem unlikely given that 3-HAA is an intermediate in the KP and is metabolized further under normal conditions.

ALS, Glutamate, QUIN and Excitotoxicity

Excitotoxicity may play an important role in ALS pathology [84, 105–108]. The link between ALS excitotoxicity and QUIN is at two levels: the type of receptors used by QUIN and the effect of QUIN on glutamate levels. Motor neurons appear to be particularly susceptible to excitotoxicity mediated via AMPA-kainate receptors [109] via the excitatory amino acid transporter-2 (EAAT2/GLT-1) [110] and via the NMDA receptors. NR2A expression in ALS cases is significantly decreased in comparison to controls [111]. Interestingly, QUIN acts on the receptors NR1+NR2A or NR2B subunits [112–114] implying that this loss of NR2A-positive motor neurons may possibly be associated with an excitotoxic mechanism. There is a body of evidence implicating glutamatergic toxicity as a contributory factor in the selective neuronal injury occurring in ALS [110]. It has been hypothesized that motor neurons could express a specific type of glutamate receptor [106]. Limitation of glutamate-mediated toxicity is so far the only neuroprotective therapeutic strategy that has shown benefit in terms of slowing ALS progression [115]. Biochemical studies have shown increased glutamate levels in the CSF of ALS patients [116]. High glutamate concentrations were correlated with a spinal onset of the disease, more impaired limb function and a higher rate of muscle deterioration [116]. QUIN contributes to increase extracellular glutamate concentrations by at least four different mechanisms leading to excessive microenvironment glutamate concentrations and neurotoxicity: (1) QUIN stimulates synaptosomal glutamate release by neurons [117]; (2) QUIN can inhibit glu-

tamate uptake into the synaptic vesicle by astrocyte [17]; (3) QUIN decreases glutamate uptake by synaptic vesicles in the rat brain [17], and (4) QUIN can also limit glutamate recycling to glutamine in the astrocyte by decreasing glutamine synthetase activity [83, 118]. In terms of neuronal damage, QUIN can potentiate its own toxicity and that of other molecules involved in excitotoxicity such as glutamate in the context of energy depletion [22].

ALS, QUIN and Apoptosis

Apoptotic phenomena appear to be involved in the degenerative process of motor neurons in ALS [119–121]. Dying motor neurons with morphological features of apoptosis have been described in ALS [120, 122]. Increased expression of pro-apoptotic proto-oncogenes Bcl-2, c-jun, and also caspases 1 and 3 has been found in ALS [120, 123–125]. QUIN is able to induce neuronal apoptosis [126, 127]. We showed recently that QUIN in pathophysiological concentrations also induces selective astrocyte apoptosis with activation of caspase 3 [128]. Consequently, there would be loss of normal astrocyte-related neurotrophic factors, which in turn would be expected to be associated with neuronal apoptosis.

ALS, QUIN, Chemokines and Cytokines

Production of various cytokines and chemokines by microglia and astrocytes has been described in ALS [60, 125, 129]. Levels of chemokines such as MCP-1, MIP1 α , and RANTES are increased in spinal cords of G93A-SOD1 mice [60]. MCP-1 is increased in the CSF and serum of ALS patients [59, 130]. Levels of cytokines such as IL-1 to IL-12, TNF- α , IFN- γ are elevated in the spinal cords of G93A-SOD1 mice [60]. It has been reported that microglia may facilitate the death of motor neurons in vitro via the release of TNF- α [60, 131]. We demonstrated that QUIN is able to induce production of chemokines, particularly high levels of MCP-1, by human primary astrocytes [132, 133] and IL-1 β mRNA expression in human astrocytes and macrophages [25].

ALS, QUIN and Mitochondrial Dysfunction

Alteration of mitochondrial function is a prominent feature of ALS [134, 135]. Marked swelling of the mitochondria has been described in the motor neurons of SOD1-93A mice at different stages of the disease, which may lead to a progressive motor neuronal death. Mitochondrial dysfunction predisposes motor neurons to ionotropic glutamate receptor-mediated excitotoxicity [136]. Excitotoxicity may lead to activation of the mito-

chondrial permeability transition pore [137]. Injection of QUIN into the rat striatum produces progressive mitochondrial dysfunction, leading to time-dependent changes in energy metabolism, which may be a common and critical event in the cell death cascade found in ALS [23].

ALS, Astrocyte, KP and NGF

Reactive astrocytes are frequently found surrounding degenerating motor neurons in ALS patients and mice models [54]. In the ventral spinal cord of SOD1-G93A mice, reactive astrocytes express NGF [138]. In vitro stimulation of spinal cord astrocytes with lipopolysaccharide or peroxy-nitrite induces production of NGF [138]. Increase of NGF levels lead to the death of motor neurons expressing the p75 neurotrophin receptor by a mechanism involving NO and peroxy-nitrite formation [138]. KYN has a potent stimulatory effect on NGF production whereas QUIN and KYNA only slightly increase NGF synthesis [31, 139].

Hypothetical Model

Together with the literature, our preliminary results allow us to formulate a hypothetical model of the KP and QUIN involvement in neuropathogenesis of ALS (fig. 3). In general, inflammation-related production of QUIN leads to the death of motor neurons. However, QUIN amplifies inflammation through the increased production of several chemokines, cytokines and many other neuroactive metabolites, which can be paradoxically beneficial and/or deleterious for motor neuron integrity. This dynamic and complex interplay between QUIN, activated monocytic cells, and astrocytes may be directly and/or indirectly involved in the amplification of the inflammatory response and motor neuron survival or death.

In the context of the ALS, there also specific roles for QUIN and the KP metabolites. As noted above, QUIN increases SOD1 expression, tryptophan depletion through KP activation leads to SOD1 aggregation, and QUIN leads to excess glutamate.

While additional studies are still necessary to better understand the complex multifactorial involvement of the KP in the pathogenesis of ALS, the KP and its metabolites appear already as a new potential target for therapeutic intervention.

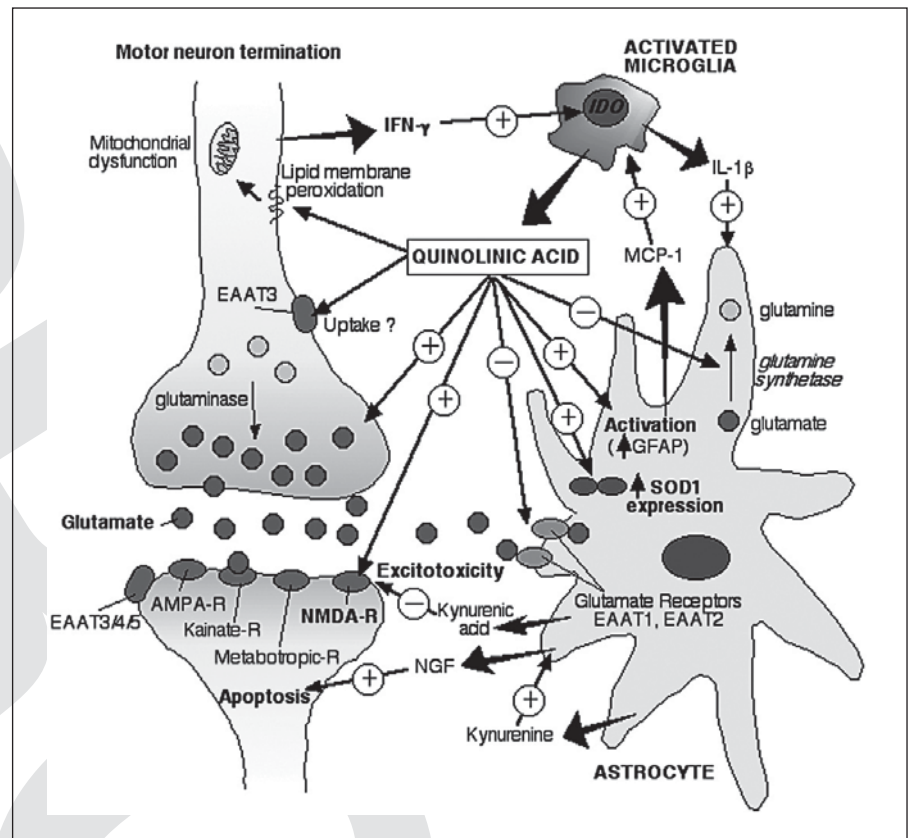


Fig. 3. Hypothetical model of the involvement of QUIN in the neuropathogenesis of ALS.

Concluding Remark: Relevance of the ALS Mouse Models

The failure of recent clinical trials based on very promising results from SOD1-93A mice [140], raises the possibility that current ALS mouse models may not be optimal. Some studies have described obvious differences between human and rodent models in the neurotoxic mechanisms involved in ALS. In the ALS model, SOD1-G93A, Migheli et al. [141] demonstrated that motor neurons are not dying by apoptosis but more likely by necrotic mechanisms. While apoptosis has been described in ALS patients [120], the SOD1-G93A mouse model lacks this apoptosis. As described above, oxidative stress and production of ROS are involved in the neuropathology of ALS. A study by Colton et al. [142] showed rodent and human microglia produce significantly different amounts of superoxide anion and also that activated murine microglia produce large quantities of NO while human microglia do not. In regard to the KP, murine and rat microglia do not seem able to produce detectable amounts of QUIN [143, 144]. This emphasizes the im-

portant heterogeneity of the KP between species [144] and highlights the importance of the choice of animal models to study neurodegenerative mechanisms in human diseases. Indeed, some authors have even gone so far as to say that human neurodegenerative diseases such as Alzheimer's disease, and probably ALS, are specifically 'primate diseases' [145, 146].

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