Kynurenine System and Multiple Sclerosis, Pathomechanism and Drug Targets with An Emphasis on Laquinimod

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Abstract: Multiple sclerosis is a common chronic, disabling autoimmune neurological disease affecting mainly young adults. In its pathomechanism, neurodegenerative and acute inflammatory characteristics are both involved. Disease-modifying therapies aim to reduce relapse-rate and slow down the deterioration in neurological functions. The currently available therapies fail to exert neuroprotective effects and most of them are associated with potentially toxic side-effects, therefore, ongoing research aims to develop novel drug candidates to cover these therapeutic gaps. The kynurenine pathway has been implicated in both the physiological processes of the central nervous system and in the pathomechanism of several neurological disorders as well. Alterations of the kynurenine pathway metabolites have been detected in multiple sclerosis and a number of potential therapeutic targets related to this metabolic route have been already identified. Laquinimod is a quinoline carboxamide showing structural similarities with kynurenic acid, which proved to have beneficial effects on reduction of brain atrophy and disability progression. The kynurenine pathway is therefore a promising target for the development of future drugs for the treatment of autoimmune diseases such as multiple sclerosis.

Keywords: Multiple sclerosis, laquinimod, kynurenine system, neuroprotection, neuroinflammation.

INTRODUCTION

Multiple sclerosis (MS) is a chronic, immune-mediated disease of the central nervous system (CNS) causing demyelination and axonal damage. It is the second most common cause of neurological disability in young adults [1]. The exact pathomechanism of MS is unknown, but both genetic and environmental factors are believed to contribute as trigger factors to the development of an autoimmune process. MS is not an inheritable disease, but several gene loci have been identified as risk factors, among them the importance of the major histocompatibility complex (MHC) HLA DR15/DQ6 allele is outstanding [2]. Epstein-Barr virus infection, cigarette smoking and vitamin D deficiency belong to environmental factors which have been linked to an increased risk of MS [2]. The diagnosis of MS is mainly clinical, based on the revised version of the McDonald criteria, the main concept of which is the verification of symptom dissemination in time and space [3]. The most prevalent form is relapsing-remitting MS (RRMS), in which recurring episodes of neurological symptoms occur. RRMS can later transform into a secondary progressive form, when a slow but continuous progression of disability is present without relapses. Approximately 10% of the cases have primary progressive MS [4]. For the time being, disease modifying therapy is only available to the relapsing-remitting form of the disease. The currently available treatments for MS are all anti-inflammatory which are able to reduce the duration and number of relapses. However, no proved neuroprotective molecules are available to facilitate remyelination. First line therapies include different forms of beta-interferon and glatiramer acetate, while intravenous second-line therapies include natalizumab, alemtuzumab and mitoxantrone [2]. Until 2010, only parenterally administered medications were given to patients. Since then, three oral medications, namely fingolimod, teriflunamide and dimethyl-fumarate have been approved for the treatment of RRMS. All these drug were shown to have potentially toxic side effects, for example cardiotoxicity of mitoxantrone, hepatotoxicity of natalizumab and teriflunomide, teratogenicity of teriflunomide or the development of progressive multifocal leukoencephalopathy (PML) in the case of natalizumab, fingolimod or dimethyl-fumarate [2, 5]. Because of the less favourable safety profile, these medications remain second line treatments, and a therapeutic challenge is to carefully choose the appropriate patients. Novel drugs are therefore needed with a more favorable safety profile, and also for the treatment of progressive cases of MS. Several other oral drugs are currently under clinical trials and are expected to add to the armamentarium of MS treatment soon.
The kynurenine pathway (KP) is the main metabolic route of the essential amino acid tryptophan (Trp). In this cascade of enzymatic steps, several neuroactive molecules are produced including both neurotoxic and neuroprotective ones. The KP and its metabolites have been implicated not only in the physiological functioning of the central nervous system (CNS) but also in the pathomechanism of several neurological disorders and in the immunoregulation as well [6-9]. The enzyme indoleamine-2,3-dioxygenase (IDO) is of outstanding importance in this process as it may take part in the immunoregulation through Trp depletion and the production of kynurenines. Alterations in the balance of neurotoxic and neuroprotective kynurenines have been revealed in all phases of MS, which is supposed to contribute to the pathomechanism and progression of the disease [10]. In recent years, several compounds related to the KP have been developed with the aim to treat MS: synthetic tryptophan analogs, endogenous tryptophan metabolites, structural analogs, IDO inhibitors and kynurenine-3-monooxygenase inhibitors have been investigated [11]. Among these, laquinimod is the most promising drug. This review aims to give an overview of experimental findings on the role of the KP in the pathomechanism of MS and also of clinical trials evaluating the mode of action, safety, tolerability and efficacy of laquinimod.

**THE KYNURENINE PATHWAY**

Trp is the precursor of several biologically active compounds such as serotonin, melatonin, nicotinamide adenine dinucleotide (NAD) and approximately 1% takes part in protein biosynthesis. However, more than 95% of the Trp is metabolized through the KP (Fig. 1) [12]. The first and pivotal product of Trp degradation through the KP is L-kynurenine (L-KYN), this rate-limiting step can be catalyzed by the enzymes tryptophan-2,3-dioxygenase (TDO) or IDO. TDO and IDO show differences in organ distribution and inducing factors as well. TDO is located almost exclusively in the liver, while IDO is present in most of the other tissues including the different cells of the CNS. L-KYN can be metabolized into different products of the KP, depending on the enzymatic machinery present in the different cell types. The main branch of the KP is a cascade of enzymatic steps leading to the production of NAD, this branch gives rise to several neurotoxic kynurenines as well. In this arm, L-KYN is transformed by kynurenine-3-monooxygenase (KMO) into 3-hydroxy-kynurenine (3HK), which consequently gives rise to 3-hydroxy-anthranilic acid and quinolinic acid (QUIN). Another possible transformation of L-KYN is the generation of kynurenic acid (KYNA) via the action of kynurenine-amidotransferases (KATs). At present, four different KAT subtypes have been described, each of them possessing different biochemical properties [13]. Another side-arm of the KP is the synthesis of anthranilic acid by kynureninase, which is further transformed into 3-hydroxy-anthranilic acid. The enzymatic machinery of the KP is differently distributed between the cells of the CNS, e.g. astrocytes produce mainly KYNA because they lack KMO, while microglial cells synthesize mainly the neurotoxic kynurenines [14, 15].

**Peripheral Kynurenine Metabolism**

Under physiological conditions, the enzymatic machinery of the KP has a much higher activity at the periphery than in the CNS, and the hepatic KP is responsible for the metabolism of more than 90% of Trp [16, 17]. Physiological concentrations of kynurenines have been measured in human plasma, but and hepatic concentrations have been also reported measured directly form rat liver homogenates. The physiological concentrations of different kynurenines in the rat liver are 2.86±0.86 for 3HK, 0.10±0.01 for 3-hydroxyanthranilic acid, 0.07±0.02 for KYNA and 0.16±0.06 for anthranilic acid [16]. The effect of intraperitoneal administration of different kynurenines on hepatic KP has been investigated, and the results demonstrated that the activity of TDO is elevated by KYNA and by 3-hydroxy-anthranilic acid, but not by 3HK. Trp in a dose of 50mg/kg was also able to temporary increase TDO activity and the serum levels of kynurenines. Interestingly, the kynureninase inhibitors benzerazine and carbidopa were both able to enhance TDO activity and reduce KAT activity in rat liver in this study [16]. However, previous in vitro investigations yielded confusing results, as they reported an inhibition of TDO by benzerazine [18].

**Neuroactive and Immunoregulatory Functions of Kynurenines**

The neurotoxic effect of QUIN is mainly due to its capacity to act as a potent agonist of N-methyl-d-aspartate (NMDA) receptors thereby inducing glutamatergic excitotoxicity but it may also cause lipid peroxidation and oxidative stress [19, 20]. The other important neuroactive metabolite of the KP, KYNA has been suggested to have neuroprotective properties because it is able to counteract glutamatergic excitotoxicity through the antagonism of ionotropic glutamate receptors [21]. KYNA acts as a competitive antagonist on NMDA receptors, in lower concentrations it binds to the strychnine-insensitive glycine binding site while at higher concentrations it is able to block the glutamate-binding site as well [22, 23]. On another ionotropic glutamate receptor, the α-amino-3-hydroxy-5-methyl-4-isoxazolinepropionic acid (AMPA) receptors KYNA exerts a concentration-dependent dual effect: in lower, nanomolar concentrations it is able to facilitate them, while in a higher concentration range it acts as an inhibitor [24, 25]. Another targets of KYNA are the presynaptic α7 nicotinic acetylcholine receptors, where it evokes inhibition, thereby reducing presynaptic glutamate release [26, 27].

The possible immunoregulatory function of the KP is mainly related to the activation of IDO. IDO can be induced by proinflammatory cytokines such as interferon-γ, interleukin-1 or tumor necrosis factor-α. IDO activation results in the elevated synthesis of kynurenines and Trp depletion. Trp depletion inhibits the proliferation of reactive lymphocytes [7]. Moreover, QUIN and 3-hydroxyanthranilic acid also directly lead to the selective apoptosis of TH1 lymphocytes [28]. IDO activation leads to the preferential inhibition of TH1 cells, but Trp depletion and the elevated level of kynurenines also increase the amount of regulatory T cells, which also inhibit TH2 cells. These effects are considered to contribute to the immunoregulation as a negative feedback [7, 29, 30].
THE ROLE OF KYNURENINES IN THE PATHOMECHANISM OF MS

Experimental autoimmune encephalitis (EAE) is the most widely used animal model for MS because it is histologically similar to human MS [31, 32]. The importance of the immune modulating effect of IDO in EAE has been confirmed by the fact that inhibition of the IDO led to a significantly decrease of the neuroinflammatory process and a decrease of the exacerbation [33]. Increased levels of the neurotoxic 3-HK and QUIN have been measured in the spinal cords of EAE rats [34, 35]. QUIN in pathological concentrations results in neuronal, astroglial, and oligodendroglial cell death [36-38]. The clinical evidence on the implication of KP in MS was confirmed by the finding that Trp levels are lower in the serum and cerebrospinal fluid (CSF) of MS patients [39-41]. On the other hand, the neurotoxic L-KYN was found to be elevated in IFN-β treated MS patients compared to untreated RRMS patients [42]. Levels of pro-inflammatory cytokines such as IFN-γ and TNF-α rise in MS patients, consequently resulting in the activation of IDO [43]. IFN-β has been proved to be able to activate IDO in human macrophage cultures [44]. Importantly, levels of KAT enzymes in the red blood cells and plasma KYNA concentrations have been measured to be significantly elevated of MS patients compared to controls [45]. KYNA levels have been described to be elevated also in the CSF of MS patients [46]. On the contrary, in postmortem MS brain sections reduced levels of KAT have been detected [47]. Interestingly, during remission, reduced KYNA serum levels while in acute relapses elevated concentrations were measured in the CSF of MS patients [48, 49]. The explanation of these results might be the possible preventing role of KYNA in the acute phase while in the progressive phase the reduced KYNA levels reflect a metabolic shift in the KP towards neurotoxicity.

THERAPEUTIC POSSIBILITIES RELATED TO THE KP

The KP produces both and NMDA antagonist and an NMDA agonist compound, e.g. KYNA and QUIN, respectively. An NMDA receptor antagonist has been described to be able to prevent blood-brain barrier breakdown in EAE [50]. KYNA and its pharmacological derivatives might therefore be a possible candidate for future drug development. Notably, KYNA itself cannot cross the blood-brain barrier, this is possible only by synthetic KYNA analogs [51, 52]. Several synthetic KYNA analogs have been synthesized in recent years, which proved to have better pharmacological properties than KYNA [53, 54]. Another alternative therapeutic intervention may be to achieve a shift in the KP towards the formation of KYNA to reduce the level of neurotoxic compounds. Specific inhibitors of KMO, kynureninase and 3-hydroxi-anthranilic acid dioxygenase have been developed [55-57]. The systematic use of KMO inhibitors leads to decrease of 3HK and QUIN in parallel with the elevation of the level of KYNA [34]. IDO-1 inhibitors result in significantly decreased QUIN concentration and prevention of oligodendrocyte apoptosis [58].

Several Trp metabolites have been shown to have beneficial effects in experimental models of MS. A synthetic Trp metabolite (N-[3,4-dimethoxyxynamoyl]-anthranilic acid (3,4-DAA), also known as Tranilas) resulted in decreased proliferation of myelin-specific T cells and inhibition of the proinflammatory cytokines produced by TH1 cells. The same analog, Tranilas in EAE animals significantly decreased to number and severity of relapses, and also the amount of inflammatory nodes in the brains and spinal cords of the treated animals. These data indicate the immunosuppressive effect of this molecule [59].

Type-4 metabotropic glutamate receptor (mGlu4) knock-out mice treated with the endogenous Trp metabolite cinabbarinic acid, which is the partial agonist of the mGluR4, the immune response was shifted toward T reg cell production [60].

The above mentioned therapeutic possibilities related to the KP are only in very preliminary phase of investigations. However, another molecule, laquinomod has already been widely investigated and is a potential immunomodulatory
oral drug for the management of RRMS. Laquinimod is a quinoline carboxamide, which shows structural similarities with the KP metabolites 3-HAA, 3-HKA and KYNA, and it is also able to cross the blood-brain barrier (Fig. 2) [61-63]. Interestingly, laquinimod is currently being investigated in Huntington’s disease (NCT02215616), where also significant alterations of the KP have been described [64, 65]. A synthetic KYNA analog proved to have beneficial effects in animal models of Huntington’s disease, which suggests that KYNA analogs and possibly laquinimod may be a future candidate for drug development in this disease [66].

**LAQUINIMOD**

Laquinimod was first synthetized by Jonsson et al. as a structural variant of roquinimex (Linomide®) [67]. Roquinimex showed promising results in phase II and III clinical trials in the treatment of MS, however phase III trials were stopped due to severe adverse events that included pericarditis, myocardial infarction and sepsis [68, 69]. Modifications were made to the quinolone ring and elongation of the amide methyl group of roquinimex was carried out to achieve the chemical structure of N-ethyl-N-phenyl-5-chloro-1,2-dihydro-4-hydroxy-1-methyl-2-oxo-3-quinoline-carboxamide (laquinimod) [67]. These alterations led to a significant reduction in severity of side effects and also to a marked increase in the efficacy of the drug.

**Pharmacokinetics**

Laquinimod is a small molecule with a molar mass of 356,803g/mol [67]. 98% of laquinimod is bound to proteins in the plasma [70]. Since it is a small compound, it is able to diffuse freely across the blood-brain barrier (BBB) [71]. Its estimated concentration in the CNS is 13 % of the blood concentration [72]. For the time being, there are no known receptors for active transport of laquinimod. After administration, the plasma concentration reaches maximum level within an hour [73]. It is metabolized in microsomes of liver cells, mainly by CYP450 3A4 isozyme of the CYP 450 (cytochrome P450) enzyme family [62]. 10 % of the drug is excreted without metabolism [74]. The half-life of laquinimod in humans is approximately 80 hours and it does not seem to accumulate in the body after prolonged administration.

**Modes of Action**

The exact mode of action of laquinimod has yet to be discovered, however several studies investigated its properties and proposed immunomodulatory and neuroprotective rather than immunosuppressive effects.

Among other quinoline-3-carboxamide compounds, laquinimod binds to S100A9 in EAE mice [75]. This protein is found on the surface of different monocyte populations and is involved in signaling pathways that lead to secretion of pro-inflammatory molecules. Binding of this surface protein caused inhibition of interaction between S100A9 and two other receptors, toll-like receptor 4 (TLR-4) and receptor of advanced glycation end products (RAGE), therefore hindering release of inflammatory cytokines (TNFα and IL-1).

Adhesion and migration of leukocytes in mice were inhibited by laquinimod by decreasing levels of matrix metalloproteinase 9 (MMP9) and very late antigen-4 (VLA-4) [76, 77].

Brück et al. studied the effects of laquinimod in rats and found decreased migration of T cells into the CNS, and a shift towards production of anti-inflammatory cytokines (TGFβ and IL-4) instead of pro-inflammatory molecules (TNFα and IL-12) [71]. These effects were found to be dose dependent.

Another study investigated the effects of laquinimod on microglia [78]. Decreased density of microglia in the spinal cord of laquinimod treated EAE mice was found, which corresponded with reduced axonal damage. Furthermore, decreased secretion of both pro- and anti-inflammatory cytokines was observed. Reduced production of TNFα was elicited by stimulation of TLR 2 and 4.

In laquinimod treated patients, decreased secretion of chemokines by mature dendritic cells was found after lipopolysaccharide stimulation [79]. Also, the number of CD1c+ and plasmacytoid CD303+ dendritic cells was reduced among peripheral blood mononuclear cells.

Gurevich at el. found in a high-throughput in vitro study that laquinimod reduced expression of MHC class II genes and, therefore hindered antigen presentation [80]. Also, altered expression of genes involved in the NFκB pathway and T cell activation of B cells was found.

It was reported that in vivo laquinimod treatment of mice resulted in reduction in CD4+ dendritic cells, which regulate differentiation of T-cells [81]. Consequently, reduced frequencies of Th1 and Th17 cells along with an increase in regulatory T-cells were found. Also, augmented development of type II monocytes and dendritic cells, which cause a shift towards anti-inflammatory cytokine production, was observed in the study.

Laquinimod was shown to inhibit T cell secretion of INFγ, IL-17, granulocyte-macrophage colony-stimulating factor (GM-CSF) and TNFα [79, 81]. In contrast, increased production of IL-4 by CD4+ T cells was observed, again pointing to a shift towards Th2 mediated immune response.

An in vitro study found an increase after laquinimod treatment in CD86+ CD25+ and IL10+ CD25+ subpopulations of B cells, which are involved in immunoregulatory functions [82]. Furthermore, these cells reduced T cell proliferation and also the percentage of INFγ+ T cells, thus implying beneficial effects in MS.

Zilkha-Falb and colleagues examined gene expression alterations in patient participating in the ALLEGRO study, one and six months after initiation of treatment with laquinimod (0,6 mg/day) [83]. They observed significantly decreased TGFβ and NFκB signaling, furthermore reduced expression
of molecules involved in leukocyte activation, adhesion and transmigration (P-selectin, integrin family members: ITGB1/3/5/6/8 and ITGAV, metalloproteinase family members: MMP16/24/26/28 and ADAM12/18/22, inflammatory chemokines: CCL19 and CXCR1/2).

The proposed neuroprotective effect of laquinimod might be exerted by increasing the level of brain derived neurotrophic factor (BDNF), which is produced not only by neurons but also monocytes, B and T cells [84]. BDNF is essential to the development of the CNS via regulating synaptic plasticity, neuronal and axonal growth [85]. A study found in situ overexpression of BDNF in the striatum, lateral septal nucleus, nucleus accumbens and cortex of EAE mice after laquinimod treatment [86]. Also, increased frequency of immunosuppressive Foxp3+ Treg cells among inflammatory cells was observed. These findings were associated with reduced astrogliosis, axonal and myelin damage.

Thöne et al. studied blood samples of 203 patients with MS treated with 0.6 mg/day dosage of laquinimod and found a significant increase in serum levels of BDNF in 76 % of patients compared to baseline and placebo-treated participants [87]. The same study group examined EAE mice after laquinimod treatment and found significantly reduced numbers of spleen-derived CD11b+ monocytes.

Another study showed reduced inducible nitric oxide synthase (iNOS) activation and therefore decreased levels of NO in the spinal cord of EAE mice [77]. NO is well known to have neurodegenerative effects by causing oxidative stress. Reducing the levels of this potent toxic agent might be another possible neuroprotective mechanism of laquinimod.

Synaptic alterations due to laquinimod treatment were also hypothesized. It was shown that slowed progression of EAE occurred after glutamatergic excitotoxicity decreased and GABAergic transmission was augmented by laquinimod treatment [88].

In summary, based on the findings mentioned above, we conclude that laquinimod exerts its beneficial effects directly in the CNS and via modulating the immune system peripherally. It has a widespread immunomodulatory effect. Laquinimod reduces activation and modulates the function of APCs (dendritic cells, macrophages, microglia in the CNS and even B-cells) thus hindering proliferation of T and B cells, which have a deleterious role in the pathogeneses of MS. In addition, a Th1/Th17 to Th2 shift has been put forward. Consequently, reduction in the production of proinflammatory cytokines and increase in anti-inflammatory molecules were observed. Migration of leukocytes into the CNS is also reduced by laquinimod. Furthermore, neuroprotective effects of laquinimod have been proposed. Elevated level of BDNF after laquinimod treatment, decreased activity of iNOS and reduced glutamatergic excitotoxicity has been reported. All these effects lead to reduced demyelination, axononeural damage and cell death (Fig. 1).

**CLINICAL STUDIES**

**Phase I Trials**

So far 8 phase I studies have been conducted to investigate the safety, tolerability and pharmacokinetic properties of laquinimod in healthy volunteers and MS patients [62, 89]. Doses between 0.1-2.4 mg/day were studied. Laquinimod was found to be well tolerated.

**Phase II Trials**

Polman et al. conducted a multicenter, double-blind, randomized, placebo controlled and three armed trial [63]. 209 RRMS patients (Expanded Disability Status Scale [EDSS] no greater than 5.5 and active disease according to MRI criteria) were divided into three groups receiving 0.3 mg/day; 0.1 mg/day of laquinimod or placebo for 24 weeks. Primary outcome measure was the mean cumulative number of active lesions on brain MRIs. A significant reduction (44%; p<0.0498) of the mean cumulative number of active lesions was observed in the 0.3 mg/day laquinimod group compared to the placebo group. A more pronounced effect was found in those patients who had at least one active lesion at baseline MRI (54% reduction; p=0.005). No significant difference was observed in the primary end point in the 0.1 mg/day laquinimod group. Although the study did not aim to assess clinical end points, EDSS and MSFC (Multiple Sclerosis Functional Composite) were measured, but no significant differences were found in these scores between groups over the 24 weeks. Laquinimod was found to be well tolerated during the study.

The second phase II study was a multicenter, double-blind, randomized and placebo controlled 36 weeks long trial [90]. 306 RRMS patients (EDSS between 1 and 5, with at least one relapse in the previous year and at least one gadolinium enhancing lesion on brain MRI) were randomly assigned to 0.3 mg/day; 0.6 mg/day of laquinimod or placebo. The primary objective was to assess changes in the number of gadolinium enhancing (Gd+) lesions at 24, 28, 32 and 36 weeks. The authors reported 40.4 % (p=0.0048) reduction in the cumulative number of Gd+ lesions in the 0.6 mg/day laquinimod group. Furthermore, 44 % (p=0.0013) decrease in the cumulative number of new T2 lesions and 51 % (p=0.0064) reduction in the number of new T1-hypointense lesions were found in the same group compared to the placebo arm. Strikingly, the 0.3 mg/day laquinimod group showed no significant difference compared to the placebo group, contradicting the findings of the previous phase II study. It was hypothesized, that the triple dose gadolinium used in the study by Polman et al. resulted in increased sensitivity, and also a possible slower onset of low-dose laquinimod compared to the higher dose could explain this observation. One case of Budd-Chiari syndrome occurred in a patient who was heterozygous for factor V Leiden mutation. Herpes simplex and herpes zoster infections were more common in the 0.3 mg/day laquinimod group. Mild arthralgia and transiently elevated liver enzymes were the most common adverse events.

A double-blind, 36 week-long extension of the study was conducted enrolling 257 out of 306 participants [91]. Patients previously on placebo were given either 0.3 mg/day or 0.6 mg/day laquinimod. Main outcome measures were the number of new Gd+ lesions and the number of new hypointense T1 lesions. Among the participants who were switched from placebo, a 52 % (p=0.0006) reduction in the mean number of Gd+ lesions was found. The effect on new
hypointense T1-lesions was statistically nonsignificant. Patients who were initially treated with laquinimod continued to have a sustained effect. Beneficial effect on clinical scores (EDSS and MSFC) were also nonsignificant. No deaths or serious adverse events were reported in the study.

An open-label extension of the core and double-blind study conducted by Comi et al. was carried out, in which all 209 participants received 0.6 mg/day of laquinimod, regardless of what their therapy in the previous trials was [91]. 155 patients reached 24 months of the study. The confirmed disability progression on EDSS decreased from 14.8% to 10.5% during the initial 18 months. 61% of patients remained free of Gd+ lesions on 42 months. The most common adverse events were nasopharyngitis, back pain and headache.

**Phase III Trials**

The ALLEGRO (Assessment of Oral Laquinimod in Preventing Multiple Sclerosis) study was a randomized, double-blind, placebo-controlled, phase III clinical trial involving 24 countries [92]. 1106 patients with RRMS (EDSS no greater than 5.5 and a disease duration of at least 6 months) were enrolled. They received 0.6 mg/day laquinimod or placebo for 24 months. The primary end point was the number of confirmed relapses. Secondary clinical end points were disability progression on EDSS and MSFC scores sustained for at least 3 months. Secondary imaging end points were the cumulative number of Gd+ lesions and cumulative number of new or enlarged lesions on T2-weighted images at 12 and 24 months. The annualized relapse rate showed a statistically significant, albeit modest reduction (0.30±0.02 vs. 0.39±0.03, p=0.002) in the laquinimod group. The secondary disability progression end point was significantly decreased in the laquinimod group (11.1% vs. 15.7%, p=0.01). However, little overall change was observed in the MSFC scores at 24 months. Laquinimod showed beneficial effects on both secondary MRI end points. The mean cumulative number of Gd+ lesions and new or enlarged lesions on T2-weighted images were reduced (p<0.001 in both cases). 112 serious adverse events were reported in 61 patients in the laquinimod group. Appendicitis occurred more often compared to the placebo group (5 vs. 1 respectively). 8 cases of neoplasms occurred in the laquinimod group compared to 6 in the placebo arm. The most frequent adverse events included abdominal pain, back pain, cough and elevated levels of alanine aminotransferase. This finding was reversible in all cases without discontinuation of laquinimod or after 2 months of discontinuation. No cases of liver failure occurred. An open-label extension is currently ongoing with 844 out of the 864 patients who finished 24 months with the study drug.

The BRAVO (Benefit-Risk Assessment of Avonex and Laquinimod) study was the second phase III clinical trial, with the aim to further assess the safety, tolerability and efficacy of laquinimod and to descriptively compare its effect with interferon-beta 1a (INFβ-1a, Avonex®) [93]. 18 countries were involved in the randomized, placebo-controlled 24-month trial. 1331 RMS patients (18-55 years of age; EDSS no greater than 5.5; at least one relapse in the last 12 months, two relapses in the past 24 months or one relapse in the past 12-24 months and one Gd+ MRI lesion in the previous 12 months) were enrolled, and received 0.6 mg/day laquinimod, matching placebo or INFβ-1a intramuscular injection 30 μg once a week. 1090 participants completed the trial. The primary end point was the annualized relapse rate over the 24 months of the trial. Secondary endpoints were the percent change in normalized brain volume, and disability progression measured by EDSS and MSFC scores. Exploratory MRI endpoints included the cumulative number of Gd+ lesions and cumulative number of new or enlarging T2 lesions at 12 and 24 months. Laquinimod showed a decrease in annualized relapse rate, which was statistically nonsignificant compared to placebo (0.28±0.03 vs. 0.34±0.03, p=0.075). However, after adjustment for imbalance between groups in baseline MRI disease activity, a statistically significant 21% (p = 0.0264) reduction in annualized relapse rate was demonstrated. Percent brain volume change was significantly reduced in the laquinimod group (treatment effect vs. placebo 0.28%, p<0.001). In contrast, INFβ-1a treatment did not show beneficial effects on brain volume compared to placebo (treatment effect -0.11%, P=0.14). The reduction in disability progression in the laquinimod group was 40.6% (p=0.042) at six months. INFβ-1a showed a nonsignificant reduction in disability worsening (28.3%, P=0.14) at six months compared to placebo. A nonsignificant reduction in the laquinimod group was observed regarding exploratory MRI endpoints, whereas INFβ-1a treatment showed significant reductions. Descriptive comparison of laquinimod and INFβ-1a therapy did not show significant differences in annualized relapse rate, EDSS and MSFC scores, however laquinimod had a more pronounced beneficial effect on brain volume loss compared to INFβ-1a. One death occurred in the laquinimod group due to sepsis after early termination. The most common adverse events with laquinimod treatment were headache, increased alanine transaminase levels, abdominal pain and nausea. No cases of liver failure were reported.

The clinical trials with laquinimod (Table 1) showed modest effects on focal inflammatory activity of MS, but demonstrated significant beneficial effects on reduction of brain atrophy and disability progression. Laquinimod was found to be safe and well tolerated. The most common adverse event was increase in liver enzymes, however no cases of liver failure were observed in any of the clinical trials.

**Ongoing Clinical Trials**

The third phase III clinical trial assessing the safety, tolerability and efficacy of a higher dose of laquinimod is the CONCERTO (The Efficacy and Safety and Tolerability of Laquinimod in Subjects With Relapsing Remitting Multiple Sclerosis) trial, which is currently in progress but not recruiting participants. It is a multinational, multicenter, randomized, double-blind, parallel-group, placebo-controlled study evaluating two different doses of laquinimod (0.6 mg/day vs. 1.2 mg/day) in patients with RRMS.

Previous clinical trials and animal studies suggest that laquinimod might exert neuroprotective effects. Based on these findings, two ongoing trials investigate the effect of laquinimod in diseases where neurodegeneration plays a prominent role in disease pathomechanism [88].

The ARPEGGIO (A Randomized Placebo-Controlled Trial Evaluating Laquinimod in Primary Progressive Multiple Sclerosis) study was the second phase III clinical trial with the aim to further assess the safety, tolerability and efficacy of laquinimod and to descriptively compare its effect with interferon-beta 1a (INFβ-1a, Avonex®) [93]. 18 countries were involved in the randomized, placebo-controlled 24-month trial. 1331 RMS patients (18-55 years of age; EDSS no greater than 5.5; at least one relapse in the last 12 months, two relapses in the past 24 months or one relapse in the past 12-24 months and one Gd+ MRI lesion in the previous 12 months) were enrolled, and received 0.6 mg/day laquinimod, matching placebo or INFβ-1a intramuscular injection 30 μg once a week. 1090 participants completed the trial. The primary end point was the annualized relapse rate over the 24 months of the trial. Secondary endpoints were the percent change in normalized brain volume, and disability progression measured by EDSS and MSFC scores. Exploratory MRI endpoints included the cumulative number of Gd+ lesions and cumulative number of new or enlarging T2 lesions at 12 and 24 months. Laquinimod showed a decrease in annualized relapse rate, which was statistically nonsignificant compared to placebo (0.28±0.03 vs. 0.34±0.03, p=0.075). However, after adjustment for imbalance between groups in baseline MRI disease activity, a statistically significant 21% (p = 0.0264) reduction in annualized relapse rate was demonstrated. Percent brain volume change was significantly reduced in the laquinimod group (treatment effect vs. placebo 0.28%, p<0.001). In contrast, INFβ-1a treatment did not show beneficial effects on brain volume compared to placebo (treatment effect -0.11%, P=0.14). The reduction in disability progression in the laquinimod group was 40.6% (p=0.042) at six months. INFβ-1a showed a nonsignificant reduction in disability worsening (28.3%, P=0.14) at six months compared to placebo. A nonsignificant reduction in the laquinimod group was observed regarding exploratory MRI endpoints, whereas INFβ-1a treatment showed significant reductions. Descriptive comparison of laquinimod and INFβ-1a therapy did not show significant differences in annualized relapse rate, EDSS and MSFC scores, however laquinimod had a more pronounced beneficial effect on brain volume loss compared to INFβ-1a. One death occurred in the laquinimod group due to sepsis after early termination. The most common adverse events with laquinimod treatment were headache, increased alanine transaminase levels, abdominal pain and nausea. No cases of liver failure were reported.

The clinical trials with laquinimod (Table 1) showed modest effects on focal inflammatory activity of MS, but demonstrated significant beneficial effects on reduction of brain atrophy and disability progression. Laquinimod was found to be safe and well tolerated. The most common adverse event was increase in liver enzymes, however no cases of liver failure were observed in any of the clinical trials.
### Table 1. Summary of clinical trials with laquinimod.

<table>
<thead>
<tr>
<th>Clinical Study</th>
<th>No. of Patients</th>
<th>Groups</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase IIa (Polman et al. 2005)</td>
<td>209</td>
<td>LAQ 0.3 mg/day; LAQ 0.1 mg/day; placebo</td>
<td>Reduction of mean cumulative no. of active lesions (44%; p=0.0498) in LAQ 0.6 mg/day group; No significant difference in LAQ 0.1 mg/day group vs. placebo group; No significant differences in EDSS and MSFC</td>
</tr>
<tr>
<td>Phase IIb (Comi et al. 2008)</td>
<td>306</td>
<td>LAQ 0.6 mg/day; LAQ 0.3 mg/day; placebo</td>
<td>Reduction in the cumulative no. of Gd+ lesions (40.4%; p=0.0048) in LAQ 0.6 mg/day group; Decrease in the cumulative no. of new T2 lesions (44%; p=0.0013) in LAQ 0.6 mg/day group; Reduction in the no. of new T1-hypointense lesions (51%; p=0.0064) in LAQ 0.6 mg/day group; LAQ 0.3 mg/day group showed no significant difference vs. placebo group</td>
</tr>
<tr>
<td>Phase IIb double-blind extension (Comi et al. 2010)</td>
<td>257</td>
<td>LAQ 0.6 mg/day; LAQ 0.3 mg/day</td>
<td>Reduction in the mean no. of Gd+ lesions in patients switched from placebo to LAQ (52%; p=0.0006); Sustained effect on patients continued on LAQ; Nonsignificant effect on EDSS and MSFC</td>
</tr>
<tr>
<td>Phase IIb open-label extension (Comi et al. 2010)</td>
<td>209</td>
<td>LAQ 0.6 mg/day</td>
<td>Confirmed disability progression on EDSS decreased from 14.8% to 10.5%; 61% of patients remained free of Gd+ lesions</td>
</tr>
<tr>
<td>Phase III ALLEGRO (Comi et al. 2012)</td>
<td>1106</td>
<td>LAQ 0.6 mg/day; placebo</td>
<td>Modest reduction in ARR (0.30±0.02 in LAQ vs. 0.39±0.03, p=0.002 in placebo group); Disability progression on EDSS and MSFC scores decreased in LAQ group (11.1% vs. 15.7%, p=0.01); Reduced mean cumulative no. of Gd+ lesions in LAQ group (p&lt;0.001); Reduced new or enlarged lesions on T2-weighted images in LAQ group (p&lt;0.001); Nonsignificant decrease in ARR in LAQ vs. placebo group (0.28±0.03 vs. 0.34±0.03, p=0.075); After adjusted analysis statistically significant 21% (p = 0.0264) reduction in ARR in LAQ vs. placebo group; Percent brain volume change reduced in LAQ vs. placebo group (treatment effect vs. placebo 0.28%, p&lt;0.001); 40.6% reduction in disability progression in LAQ vs. placebo group (p=0.042); Descriptive comparison of LAQ and INFβ-1a did not show significant differences in ARR, EDSS and MSFC scores</td>
</tr>
<tr>
<td>Phase III BRAVO (Vollmer et al. 2014)</td>
<td>1331</td>
<td>LAQ 0.6 mg/day; INFβ-1a 30 µg/week; placebo</td>
<td>(0.5, 1.0 and 1.5 mg/day) in patients with Huntington’s disease.</td>
</tr>
</tbody>
</table>

Sclerosis, Gauging Gradations in MRI and Clinical Outcomes) trial is a phase II trial aimed at assessing efficacy, safety and tolerability of two oral doses of laquinimod (0.6 mg/day or 1.5mg/day) as compared to placebo in primary progressive MS patients.

The third ongoing clinical trial with laquinimod is the phase II LEGATO-HD (Laquinimod Efficacy and Safety in a Global Trial Of Huntington’s Disease) trial, which evaluates the efficacy and safety of three oral doses of laquinimod (0.5, 1.0 and 1.5 mg/day) in patients with Huntington’s disease.

Despite the positive results of clinical trials, the Committee for Medicinal Products for Human Use (CHMP) rejected approval for laquinimod in 2014 [89]. According to the CHMP, higher occurrence of malignancy was observed after long-term exposure to laquinimod in animal studies. Despite that no treatment related cancer was observed in clinical trials, the CHMP stated that long-term cancer risk after
Laquinimod treatment could not be ruled out. Also, the CHMP noted that again, based on animal studies, there is a possible teratogenic effect of laquinimod. Since there was only a modest effect of laquinimod in clinical trials, the CHMP concluded that the potential risk of long-term laquinimod treatment currently outweighs its beneficial effect on reducing disability progression in RMS patients and rejected its approval in the European Community.

Another setback for laquinimod is that in the CONCERTO and ARPEGGIO trials 8 cardiovascular (possibly ischemic) events were reported with higher doses of laquinimod [90, 91]. Seven adverse events were observed in the CONCERTO trial with 1.2 mg/day laquinimod and one event in the ARPEGGIO trial in the 1.5 mg/day laquinimod group. Consequently, the higher dose arm of these two ongoing trials (CONCERTO, ARPEGGIO) and the highest dose of the LEGATO-HD trial were discontinued in early 2016 [92]. No cardiovascular adverse events were reported in the lower dose arm of the studies and all three trials continue with the 0.6 mg/day laquinimod arm and 1 mg/day arm in the LEGATO-HD study.

CONCLUSION

Based on the clinical and experimental studies, laquinimod might be a suitable medication for combination therapy in MS. Since it is an oral, once-a-day medication, patient compliance, adherence and satisfaction should improve compared to the parenteral medications currently in the first line armamentarium of MS treatment. Influencing the KP might offer a valuable therapeutic option in MS and other autoimmune diseases. KYNA analogs, KP enzyme inhibitors or structural analogs of kynurenines might offer promising candidates for future drug development.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.
Kynurenine System and Multiple Sclerosis, Pathomechanism and Drug Targets


