ORIGINAL CONTRIBUTION

Excitatory Amino Acids and Multiple Sclerosis

Evidence From Cerebrospinal Fluid

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Background: Recent evidence suggests an altered glutamate homeostasis in the brain of patients with multiple sclerosis (MS), as seen in experimental models of MS.

Objective: To test whether the excitotoxic insult contributes to the pathological process in MS by measuring glutamate and aspartate levels in the cerebrospinal fluid of MS patients and control individuals.

Participants: Twenty-five patients with the relapsing-remitting form of MS during a stable clinical phase, 30 patients with relapsing-remitting MS during relapse, and 25 patients with the secondary progressive form of MS were included in the study. Data were compared with those of 20 age-matched control subjects without diseases of the central and peripheral nervous systems.

Methods: Glutamate and aspartate levels in the cerebrospinal fluid were measured by high-performance liquid chromatography.

Results: Cerebrospinal fluid glutamate levels were significantly higher in patients assessed during relapse compared with those of the patients with relapsing-remitting MS examined during the stable clinical phase and the controls ($P<.001$). The levels of glutamate detected in patients with relapsing-remitting MS during the stable phase who had active lesions were significantly higher than in those without neuroradiological evidence of disease activity ($P<.001$). Significantly higher levels of glutamate were found in patients with secondary progressive MS with an increase of 1 or more points on the Expanded Disability Status Scale score compared with stable patients with secondary progressive MS and control subjects ($P<.001$).

Conclusions: Neurotoxic events occur in MS patients, and they can be responsible for oligodendrocyte and neuronal cell death in patients with this demyelinating disease. The manipulation of glutamate-altered homeostasis or antagonizing glutamate receptor–mediated excitotoxicity may have therapeutic implications in MS patients.

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THE PATHOGENETIC mechanisms underlying multiple sclerosis (MS), the prototypic disimmune disease of the central nervous system, involve inflammatory perivascular infiltrates, myelin loss, and oligodendrocyte and axonal damage.[1,2] The latter has been suggested to be strongly related to MS activity by in vitro and in vivo studies.[3,4]

The putative effectors of oligodendrocyte and axonal damage in MS patients include cell-mediated cytotoxicity; antibody- and/or complement-mediated proinflammatory cytokines, such as tumor necrosis factor α; matrix metalloproteinases; autoantibodies; and reactive oxygen and nitrogen species.[5-10]

Recent experimental evidence[11,12] showed that excitatory glutamatergic mechanisms, mediated by *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors, could be responsible for damage not only of oligodendrocytes but also of neurons. In the animal model of MS of experimental allergic encephalomyelitis (EAE), these excitotoxic mechanisms have been advocated to be further effectors of oligodendrocyte death and axonal damage and one of the major causes of neurological impairment.[13,14]

Potential sources of glutamate in EAE and MS are activated macrophages, microglial cells, and astrocytes, producing much of this excitotoxin via the up-regulation of the glutamate-synthesizing enzyme glutaminase.[11,15] This has been demonstrated in the experimental model of MS and in MS lesions in autopsic brain tissues.[16,17] Extracellular levels of glutamate and an increase in glutaminase expression can be affected

by an altered glutamate homeostasis in EAE and MS.[18]

A reduction in glutamate transport and/or the glutamate-metabolizing enzymes glutamate dehydrogenase (GDH) and glutamine synthetase (GS) has, in fact, been demonstrated in astrocytes in EAE.[19]

A recent study by Werner et al[20] confirmed the high level of glutaminase expression in macrophages and microglia in close proximity to dystrophic neurons within active MS lesions, as seen in animal models. In the same study,[20] the glutamate transporter 1, but not the glutamate/aspartate transporter nor the excitatory amino acid transporter, showed a low expression around active MS lesions, in which the enzymes GDH and GS were absent, and in chronic silent lesions, suggesting additional metabolic impediments.

Based on the hypothesis of the potential role played by excitotoxic mechanisms in autoimmune demyelination of MS, the present study investigated the cerebrospinal fluid (CSF) levels of the 2 excitatory amino acids, glutamate and aspartate, in patients affected by relapsing-remitting (R-R) MS, during the stable phase of the disease and within 72 hours of the onset of relapse. The same levels were also measured in the CSF of patients with secondary progressive (SP) MS, and the levels in both patient groups were compared with those of 20 control subjects. Finally, these levels were correlated with evidence of disease activity on magnetic resonance imaging (MRI) scans by the presence and number of gadolinium (Gd)-enhancing lesions.

METHODS

PATIENTS

Eighty patients with clinically defined MS for at least 2 years, according to the criteria of McDonald et al,[21] were enrolled in the study. They included 25 patients with R-R MS during the stable phase of the disease, 30 patients with R-R MS during relapse, and 25 patients with SP MS. The Expanded Disability Status Scale (EDSS) score was calculated according to Kurtzke.[22] None of the patients had undergone immunosuppressant therapy or adrenocorticotrophin hormone or corticosteroid treatment within 2 months before the study.

Patients with R-R MS during the stable phase of the disease (≥2 months after the last relapse) and those assessed within 72 hours of the onset of relapse (before beginning corticosteroid treatment) and patients with SP MS underwent lumbar puncture to determine CSF levels of glutamate and aspartate.

All patients underwent a clinical assessment and MRI 2 days before and after the lumbar puncture. Control CSF specimens were also obtained from 20 age-matched subjects who were admitted to the Neurologic Clinic of the Neuroscience Department, University of Perugia, and underwent lumbar puncture for diagnostic purposes. In all these subjects, CSF and blood examinations and, if necessary, instrumental investigations, including neuroimaging, excluded central nervous system diseases (MS, vasculitis, and other autoimmune diseases affecting the central nervous system). In the same control subjects, systemic diseases (diabetes mellitus, renal or hepatic dysfunction, and inflammatory diseases) were excluded by the appropriate laboratory examinations. Neurodegenerative diseases were also excluded. All control subjects were drug free for at least 2 months, and none of them was taking any medication at CSF sampling.

ANALYSIS

Clinical Examination

A neurological examination and the quantification of neurological impairment by the EDSS were performed. Relapse was defined by the appearance of new neurological symptoms or worsening of preexisting neurological symptoms lasting at least 48 hours, in the absence of febrile illness, in a patient who had been neurologically stable or improving for the previous 30 days, accompanied by objective changes on a neurological examination (worsening on the EDSS of 0.5 points or a worsening by ≥1.0 points on the pyramidal, cerebellar, brainstem, or visual functional system scores).

In patients with SP MS, the EDSS score was measured at lumbar puncture and compared with the score measured 6 months before. Two subgroups were defined based on the increase or on no increase of 1 or more points on the EDSS. Clinical progression was also confirmed 3 and 6 months after the clinical examination performed at lumbar puncture (sustained progression). None of the patients with SP MS experienced any clinical relapse during the study period (beginning 6 months before the lumbar puncture to 6 months after the lumbar puncture).

Magnetic Resonance Imaging

Brain and spinal cord MRI scans were performed in a single session with a clinical 1.5-T whole body MRI system (Signa Advantage; GE Medical Systems, Milwaukee, Wis). A standard head coil was used for imaging. Imaging was performed using a fast spin-echo sequence with an echo train of 8, a repetition time of 4000 milliseconds, echo times of 18 and 100 milliseconds, a slice thickness of 5 mm with a 1-mm gap between slices and complete coverage of the brain, a field of view of 24×24 cm^2 with an in-plane spatial resolution of 0.75 mm, and an acquisition matrix of 256×256 pixels. T1-weighted (repetition time, 650 milliseconds; and echo time, 15 milliseconds), proton density–weighted (repetition time, 2000 milliseconds; and echo time, 15 milliseconds), and T2-weighted (repetition time, 200 milliseconds; and echo time, 70 milliseconds) images were obtained in the axial plane. In the spinal cord, 9 contiguous 3-mm sagittal T1-, T2-, and proton density–weighted slices were obtained. In the brain and spinal cord, T1-weighted images were also obtained after the injection of 0.2 mL/kg body weight (0.1 mmol/kg) of Gd–diethylenetriamine pentaacetic acid (Schering, AG, Berlin, Germany). The number of Gd–diethylenetriamine pentaacetic acid–enhancing lesions in the brain and spinal cord was quantified.

CSF Sample Collection

Ten milliliters of CSF was collected in polypropylene tubes containing EDTA, 1 mg/mL, and kallikrein, 500 IU/mL. Cerebrospinal fluid samples were centrifuged at 4°C, and CSF aliquots were immediately frozen at –80°C. For glutamate determination, CSF samples were deproteinized with ice-cold trichloroacetic acid, 100 g/L (1:20 by volume); after centrifugation (3000*g* for 10 minutes at 4°C), the clear supernatant was immediately adjusted to pH 7.3 with sodium bicarbonate and stored at –80°C until determination.

Determination of Glutamate and Aspartate Levels in the CSF

Cerebrospinal fluid levels of glutamate and aspartate were measured by high-performance liquid chromatography, using *o*-phthalaldehyde precolumn derivatization and electrochemi-

Table 1. Details of Patients With MS and Control Subjects*

Variable	Patients With R-R MS: During a Stable Phase (n = 25)	Patients With R-R MS: During Relapse (n = 30)	Patients With SP MS (n = 25)	Control Subjects (n = 20)
Male-female ratio	9:16	12:18	8:17	8:12
Age, y	36.5 ± 5.8	32.7 ± 6.5	42.2 ± 4.6	37.3 ± 3.8
Disease duration, y	4.5 ± 2.1	3.0 ± 1.8	10.7 ± 5.4	NA
EDSS score†	2.5 ± 0.7 (1.5-3.5)	4.0 ± 2.0 (2.0-5.0)	4.8 ± 0.9 (3.5-6.0)	NA
Patients with Gd-enhancing lesions	13	30	0	NA
No. of Gd-enhancing lesions				
Brain	2.2 ± 1.4	3.1 ± 2.3	0	NA
Spinal cord	0	1.9 ± 0.8	0	NA

Abbreviations: EDSS, Expanded Disability Status Scale; Gd, gadolinium; MS, multiple sclerosis; NA, data not applicable; R-R, relapsing-remitting; SP, secondary progressive.
*Data are given as mean ± 2 SDs unless otherwise indicated.
†Data in parentheses are ranges.

Table 2. Glutamate and Aspartate Levels in the CSF of Control Subjects and Patients With R-R MS During a Stable Phase of the Disease and During Relapse

Group	Glutamate Level, Mean ± SEM, mg/dL	Statistical Significance	Aspartate Level, Mean ± SEM, mg/dL	Statistical Significance
Control subjects (n = 20)	0.050 ± 0.017	NA	0.035 ± 0.009	NA
Patients with R-R MS				
During a stable phase (n = 25)	0.080 ± 0.031	vs control subjects, $P<.007$ vs patients with SP MS, $P=.09$	0.051 ± 0.014	vs control subjects, $P<.007$ vs patients with SP MS, $P=.12$
With Gd-enhancing lesions (n = 14)	1.103 ± 0.024	vs control subjects, $P=.13$ vs patients with R-R MS without Gd-enhancing lesions, $P<.001$	0.054 ± 0.010	vs control subjects, $P=.07$ vs patients with R-R MS without Gd-enhancing lesions, $P<.001$
Without Gd-enhancing lesions (n = 11)	0.053 ± 0.017	vs control subjects, $P=.08$ vs patients with R-R MS assessed during relapse, $P<.001$	0.042 ± 0.013	vs control subjects, $P=.09$ vs patients with R-R MS assessed during relapse, $P<.001$
During relapse (n = 30)	0.103 ± 0.033	vs control subjects, $P<.001$ vs patients with R-R MS during a stable phase of the disease, $P<.001$	0.058 ± 0.017	vs control subjects, $P<.001$ vs patients with R-R MS during a stable phase of the disease, $P=.048$

Abbreviations: CSF, cerebrospinal fluid; Gd, gadolinium; MS, multiple sclerosis; NA, data not applicable; R-R, relapsing-remitting; SP, secondary progressive.
SI conversions: To convert glutamate from milligrams per deciliter to micromoles per liter, multiply by 67.97. To convert aspartate from milligrams per deciliter to micromoles per liter, multiply by 75.13.

cal plus fluorometric detection. A pump apparatus (Bio-Rad Laboratories, Milan, Italy) coupled to an electrochemical detector (Coulochem 5100A; ESA Coulochem Inc, Chelmsford, Mass) with a coulometric analytic cell (model 5011) and a fluorescence detector (Jasco FP-920; Jasco International, Como, Italy) was used with a 150 × 4.6-mm inside diameter reversed-phase column (C18 Rosil HL; Bio-Rad Laboratories). The phosphate buffer, prepared from 25-mm potassium phosphate and 150-mL/L acetonitrile, was applied as the mobile phase. The flow rate was 1.0 mL/min. The glutamate and aspartate contents were evaluated after comparison with external glutamate and aspartate calibration solutions. The data in the CSF specimens were expressed as micromoles per liter.

STATISTICAL ANALYSIS

An analysis of variance with a Tukey test was performed to compare the mean CSF levels of glutamate and aspartate in patients with R-R MS, during the stable phase and during relapse, with those of patients with SP MS and those of control subjects. The same analysis was used to compare the mean CSF levels of glutamate and aspartate in patients with R-R MS with and without Gd-enhancing lesions. The mean values of the 2 excitatory amino acids in the CSF of patients with SP MS with and without progression of 1 or more points of the EDSS score were also compared. The Fisher least significant difference was used to compare the main effect means in the analysis of variance. The minimum level of statistical significance was 5% for 2-sided tests.

The Pearson product moment correlation coefficient was calculated between the CSF levels of glutamate and aspartate and the number of Gd-enhancing lesions in patients with R-R MS with neuroradiological evidence of disease activity during relapse and during the stable clinical phase.

RESULTS

Details of the patient and control groups are reported in **Table 1**. The mean ± SEM levels of glutamate and aspartate of patients with R-R MS, during the stable phase of the disease and within 72 hours after relapse, and of patients with SP MS are shown in **Table 2** and **Table 3**, respectively. Significantly higher CSF levels of glutamate emerged in patients with R-R MS compared with those of control subjects, and this increase was more evi-

Table 3. Glutamate and Aspartate Levels in the CSF of Patients With SP MS

Patients With SP MS	Glutamate Level, Mean ± SEM, mg/dL	Statistical Significance	Aspartate Level, Mean ± SEM, mg/dL	Statistical Significance
Total (N = 25)	0.073 ± 0.024	vs control subjects, $P<.01$ vs patients with R-R MS during a stable phase of the disease, $P=.13$ vs patients with R-R MS during relapse, $P<.003$	0.046 ± 0.014	vs control subjects, $P<.01$ vs patients with R-R MS during a stable phase of the disease, $P=.08$ vs patients with R-R MS during relapse, $P<.004$
Those without changes in the EDSS score in the past 6 mo (n = 13)	0.062 ± 0.024	vs control subjects, $P=.16$ vs patients with SP MS with an increase of at least 1 point in the EDSS score in the past 6 mo, $P<.001$	0.035 ± 0.017	vs control subjects, $P=.21$ vs patients with SP MS with an increase of at least 1 point in the EDSS score in the past 6 mo, $P<.002$
Those with an increase of at least 1 point in the EDSS score in the past 6 mo (n = 12)	0.103 ± 0.014	vs control subjects, $P<.001$ vs patients with R-R MS during relapse, $P=.04$	0.061 ± 0.015	vs control subjects, $P<.003$ vs patients with R-R MS during relapse, $P<.04$

Abbreviations: CSF, cerebrospinal fluid; EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; R-R, relapsing-remitting; SP, secondary progressive.
SI conversions: To convert glutamate from milligrams per deciliter to micromoles per liter, multiply by 67.97. To convert aspartate from milligrams per deciliter to micromoles per liter, multiply by 75.13.

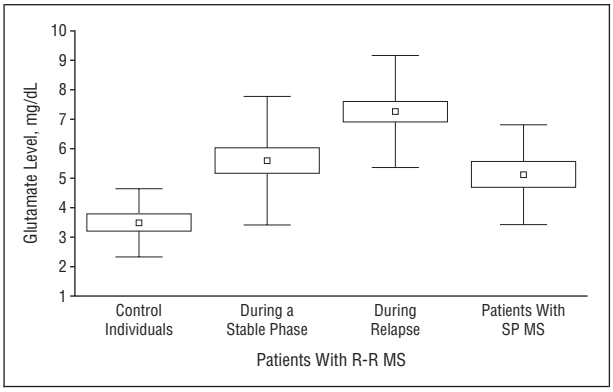

Figure 1. Glutamate levels in the cerebrospinal fluid of patients with relapsing-remitting (R-R) multiple sclerosis (MS), during relapse and during a stable clinical phase; of patients with secondary progressive (SP) MS; and of control individuals. Squares indicate the mean; rectangles, SEM; and bars, SD. To convert glutamate from milligrams per deciliter to micromoles per liter, multiply by 67.97.

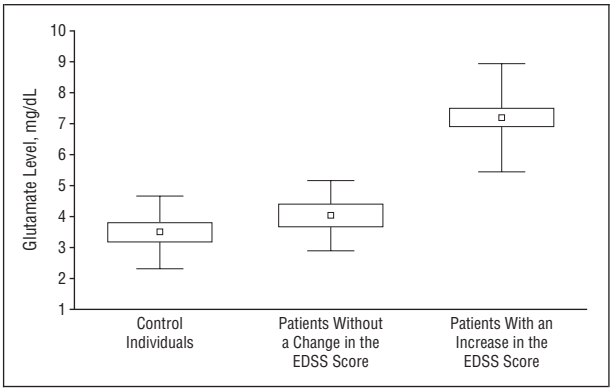

Figure 2. Glutamate levels in the cerebrospinal fluid of patients with secondary progressive multiple sclerosis with an increase of 1 or more points and without a change in the Expanded Disability Status Scale (EDSS) score in the last 6 months. Squares indicate the mean; rectangles, SEM; and bars, SD. To convert glutamate from milligrams per deciliter to micromoles per liter, multiply by 67.97.

dent in patients with R-R MS assessed during relapse ($P<.001$) (**Figure 1**). Among patients with R-R MS assessed during the stable phase of the disease, we found significantly higher levels of glutamate in those with 1 or more Gd-enhancing lesions on the MRI scan ($P<.001$) (Table 2). Glutamate levels in the CSF of patients with R-R MS without MRI evidence of disease activity did not differ from those of the control group. Patients with SP MS who had an increase of at least 1 point in the EDSS score in the last 6 months showed glutamate levels that were significantly greater than those measured in the CSF of patients with SP MS without significant changes in the EDSS score in the same period ($P<.001$). No significant difference emerged in the CSF levels of glutamate between the patients with SP MS without evidence of changes in disability and those determined in the CSF of the control group (**Figure 2**). A similar trend was observed for aspartate levels in the patient groups with R-R MS and SP MS (Tables 2 and 3, respectively).

In patients with R-R MS who were examined within 72 hours of the onset of relapse, the number of brain Gd-enhancing lesions was positively correlated with CSF glutamate levels ($r=0.49$, $P<.005$) and to a lesser extent with those of aspartate ($r=0.40$, $P<.02$) (**Figure 3** and **Figure 4**, respectively). No correlation was observed between the spinal cord Gd-enhancing lesions and the levels of the 2 excitatory amino acids.

The values of the r correlation coefficients between glutamate and aspartate levels in the CSF and the number of Gd-enhancing lesions in the patients with R-R MS who underwent lumbar puncture during a stable phase of the disease were 0.39 ($P<.04$) and 0.34 ($P<.05$), respectively.

COMMENT

The present research demonstrated an increase in glutamate and aspartate levels in the CSF of patients with R-R MS during relapse and even during a stable clinical phase (these patients had MRI evidence of disease activity). A significant correlation was found between the number of Gd-enhancing lesions and increased CSF levels of

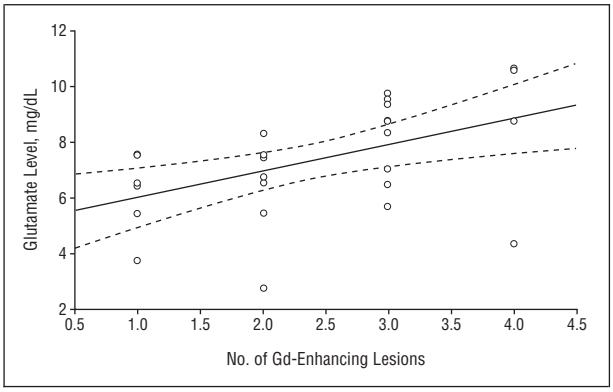

Figure 3. The number of brain gadolinium (Gd)-enhancing lesions and cerebrospinal fluid glutamate levels in patients with relapsing-remitting multiple sclerosis assessed during relapse were positively correlated (r=0.49, P<.005), using regression analysis. Circles indicate individual patients; unbroken line, the mean; and dotted lines, 95% confidence interval. To convert glutamate from milligrams per deciliter to micromoles per liter, multiply by 67.97.

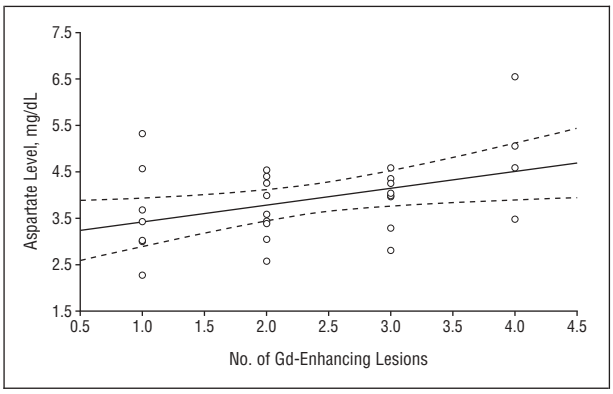

Figure 4. The number of brain gadolinium (Gd)-enhancing lesions and cerebrospinal fluid aspartate levels in patients with relapsing-remitting multiple sclerosis assessed during relapse were positively correlated (r=0.40, P<.02), using regression analysis. Circles indicate individual patients; unbroken line, the mean; and dotted lines, 95% confidence interval. To convert aspartate from milligrams per deciliter to micromoles per liter, multiply by 75.13.

glutamate, particularly in patients assessed during relapse. Even in the patients with SP MS, an increase in the CSF levels of glutamate and aspartate emerged compared with those in the control subjects, particularly in patients with a progression of neurological impairment revealed by the increase of 1 or more points in the EDSS score in the last 6 months.

These findings concur with the results obtained by Stover et al,[23] who demonstrated a 2- to 3-fold increase in the levels of the 2 excitatory amino acids in MS patients during clinical relapse but not in patients with silent MS. The researchers observed similarly high glutamate and aspartate levels in the CSF samples from patients with viral meningitis and myelopathy. They therefore suggested that the great amount of glutamate released, reflected by the high concentrations in the CSF, induces an excessive activation of NMDA receptors, which is fundamental for damage and neurodegeneration in patients with these diseases. Altered glutamate homeostasis is, therefore, not confined to MS, but can also be observed in central nervous system inflammation caused by viral infections, such as subacute sclerosing panencephalitis and tropical spastic paraparesis.[20] The increase in glutamate levels was also found in the CSF of patients with AIDS-associated dementia complex, although the relevance of this finding has not been well established.[24-26]

The finding by Garseth et al[27] of no significant changes in excitatory amino acids in patients with R-R MS and chronic progressive MS compared with patients with acute polyradiculoneuropathy is surprising in light of our findings and those of Stover et al,[23] and needs to be correctly considered, particularly regarding the definition of the active phase of disease.

Although the increase in excitatory amino acids does not seem to be specific for MS, the association with an altered glutamate homeostasis has been clearly demonstrated in autopsic specimens from MS patients in which oligodendrocyte and axonal damage was related to glutamate-producing macrophages and microglia, whereas the extent of glutamate of neuronal origin has not been emphasized. Similar results emerged from the model of EAE, in which an increase in the extracellular level of glutamate in the spinal cord reached a peak during the acute symptoms and persisted longer after their remission.[13]

Altered glutamate homeostasis in MS patients and in the EAE model consists of an increase in glutaminase in macrophages and microglial cells around dystrophic neurons and a low expression of glutamate transporter 1 in active lesions, accompanied by the lack of expression of the 2 enzymes GDH and GS involved in glutamate metabolism.[20] Immunoreactivity for both enzymes seemed to be lost by oligodendrocytes within active lesions and in the surrounding areas, and occurred instead in astrocytes. This was also true for silent lesions, in which only occasional GDH and GS immunoreactive astrocytes or microglial cells were observed. The reduction of glutamate-metabolizing enzymes seems to be peculiar for MS, and contrasts with the high level of expression of these enzymes in healthy individuals and in individuals with non–MS-related neurological diseases. In MS patients, long-term glutamate metabolic impairment (lack of glutamate transporter 1 and loss of the GDH and GS enzymes) could be responsible for the failure of oligodendrocytes to appropriately remyelinate the neuronal damage contributing to the neurological impairment of patients.

This in vitro finding concurs with our results suggesting a significant relationship between disease activity, clinically evident or substantiated by the presence of Gd-enhancing lesions, on the MRI scans of the brain of our patients with R-R MS.

The mechanisms underlying GDH and GS loss from oligodendrocytes in MS patients are not completely known, but proinflammatory cytokines are the major candidates. Moreover, activated macrophages and microglial cells produced many reactive oxygen species, which can also affect glutamate detoxification. In particular, GS is extremely sensitive to oxidative damage.[28]

A further mechanism that can explain the increase in glutamate and aspartate levels in the brain and CSF of MS patients is the increase in peroxynitrite formation. Nitric oxide production is, in fact, increased in MS

patients because of the overexpression of nitric oxide synthase, particularly the inducible forms, by microglial cells and astrocytes.[9,29] Nitric oxide, other than potentiating per se glutamatergic transmission, can interact with superoxide anions that are produced in great amount in MS patients, resulting in peroxynitrite formation.[30-34] Peroxynitrites avidly nitrate tyrosyl residues on neuronal proteins, profoundly affecting their functions.[35] Glutamate transport proteins are highly sensitive to peroxynitrites, because their function is strongly inhibited by tyrosyl nitration, with 50% inhibition by 50-μmol/L peroxynitrites.[36] The effect of increased production of peroxynitrites on the uptake of glutamate and aspartate should increase the CSF levels of excitatory amino acids. This relationship should be investigated in future research involving MS patients.

Although excitotoxic mechanisms were mainly attributed to NMDA activation in the first studies on this topic, other non-NMDA glutamate receptors seem to be involved. In the animal model of MS, the increase in glutamate seems to be followed by an activation of the AMPA receptor, and is accompanied by the down-regulation of glutamate transporter 1 and the glutamate/aspartate transporter.[13] Such dysregulation of glutamate metabolism is suppressed by treatment with the AMPA receptor antagonist NBQX (1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzo[f]quinoxaline-7-sulfonamide disodium), suggesting the putative role of strategies aimed to antagonize glutamate receptor activation, even in MS.[14,20]

The increased vulnerability of oligodendrocytes to AMPA/kainate receptor–mediated glutamate excitotoxicity should be emphasized, which could account for the dysfunction of oligodendrocytes and defective remyelination.[37,38] Oligodendrocytes lack an array of calcium-binding proteins that are instead present in neurons; therefore, they may be unable to buffer sustained or occasional calcium influx.[39]

In a study by Matute,[40] the potential involvement of kainate receptors in oligodendroglial injury of the optic nerve by glutamatergic overload was also suggested. The major component of kainate receptors, subunit 6, is edited to a lower extent in the optic nerve and in oligodendrocytes, and this confers on them a much larger conductance and a higher permeability to calcium.[41,42]

In the same study,[40] it was also emphasized that glutamate uptake can be less efficient in nonsynaptic than in synaptic regions, which are generally surrounded by astroglial processes that in normal conditions are endowed with transporters.

To explain potential glutamate excitotoxicity facilitated by the defect in glutamate detoxification, the role of tumor necrosis factor α released from inflammatory infiltrates should also be noted. This proinflammatory cytokine down-regulates high-affinity glutamate uptake in human astrocytes and antagonizes the up-regulating effect of corticosteroids on glutamate-metabolizing enzymes.[43,44] This mechanism could also account for the presence of lesional and normal-appearing white matter pathological alterations in MS patients.

Conversely, tumor necrosis factor α impairs astrocytes' response to glutamate by reducing the intracellular calcium increase, and this may indirectly affect neuronal synaptic transmission. This can be a further mechanism for reversible disability in MS patients.[45]

Inflammatory and excitotoxic injuries, in the presence of altered glutamate detoxification, can together account for the damage of neurons, which can be favored by oligodendrocyte dysfunction and loss. Myelinating axons display ionotropic glutamate receptors, and there are also putative gap junctional communications between axons and regenerating myelin-producing cells that render axons particularly vulnerable to the ion imbalance due to excitotoxic injury, resulting in neural loss.[46,47]

In general, NMDA and non-NMDA glutamate receptor–mediated excitotoxic injury results in oligodendrocyte and neuronal death in MS patients, which can occur as apoptosis, necrosis, or a hybrid form.[48]

Experimental evidence from animal models of MS furnishes promising results using AMPA/kainate receptor antagonists in ameliorating the neurological scores of animals, and supports the hypothesis that neuroprotection together with immunomodulation can be an achievable target. Relatively safe drugs with neuroprotective effects are also awaited for in MS, as for neurodegenerative diseases, and the modality of their administration (eg, the best time for administration) should be investigated.[16,48]

However, the activation of glutamate receptors does not always play a critical negative role in MS. Experimental data demonstrated that activation of group III metabotropic glutamate receptors is able to inhibit the production of the chemokine RANTES in glial cells. The potential usefulness of the pharmacological activation of this glutamate receptor in the treatment of neuroinflammatory diseases has also been supported by the finding that 1-2-amino-4-phosphonobutyrate, a specific agonist of the group III metabotropic glutamate receptors, significantly increased the rate of recovery from EAE.[49] These findings reveal the complexity of intervention in glutamate metabolism in MS.

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Author contributions: *Study concept and design* (Drs Sarchielli and Gallai); *acquisition of data* (Drs Greco and Alessandro Floridi); *analysis and interpretation of data* (Drs Sarchielli and Ardesio Floridi); *drafting of the manuscript* (Drs Sarchielli, Greco, Ardesio Floridi, and Alessandro Floridi); *critical revision of the manuscript for important intellectual content* (Drs Sarchielli and Gallai); *statistical expertise* (Dr Sarchielli); *administrative, technical, and material support* (Drs Gallai and Ardesio Floridi); *study supervision* (Drs Gallai and Ardesio Floridi).

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