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Changes of Glycine-Aspartate Metabolism at Glutamatergic Synapses of Multiple Sclerosis: Evidence from Cerebrospinal Fluid

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INTRODUCTION & AIM

Multiple sclerosis (MS) is a chronic immune-mediated disorder of the central nervous system characterized by inflammation, demyelination, and neurodegeneration. During myelin phagocytosis, activated macrophages and microglia generate reactive oxygen and nitrogen species, contributing to oxidative stress [1]. Metabolic disturbances and altered inhibitory/excitatory neurotransmission have been observed in both gray and white matter since the early stages of MS [2]. Astrocytes play a key role in neurotransmitter recycling [3] and neuronal energy metabolism [4, 5], while mitochondrial dysfunction [6] and reduced cerebral blood flow contribute to axonal degeneration [7, 8].

Oxidized phospholipids and synaptic damage have been identified in normal-appearing gray and white matter [9], often beyond the detection limits of conventional MRI.

Our study aims to identify combinations of cerebrospinal fluid (CSF) biomarkers that reflect these biochemical changes and may help monitor disease progression [10]. The investigated parameters include selected neurotransmitters (glutamate - Glu, aspartate - Asp, glycine - Gly, γ-aminobutyric acid - GABA), markers of lipid peroxidation (MDA, F_2-isoprostanes), total antioxidant status (TAS), and neuronal injury (NSE).

PATIENTS & METHODS

Study participants:
- 85 MS patients (76 RRMS, 9 SPMS), non-neurological controls (CG), and other neurological disorders (OND).
- Most MS patients (male, female) treated with interferon-β; glatiramer acetate was second most common.
- ONDs showed no clinical & radiological signs of MS or intrathecal inflammation (IgG index< 0.7, Table 1).

Clinical assessment:
- Neurological exams: sensory testing, evoked potentials, MRI confirmation of inflammatory lesions, CSF IgG detection.

CSF collection & storage:
- 5–10 mL via lumbar puncture, centrifuged (4000 rpm, 10 min, within 1 h), aliquoted, stored at –80 °C ≤1 month.

CSF/serum proteins analyzed at the clinic:
- total proteins after precipitation with 3% SSA (physiol. range: 150-430 mg/l CSF); albumin and IgG nephelometrically

Neurotransmitter analysis (RP-HPLC, [11]):
- Amino acids (Glu, Asp, Gly, GABA) derivatized with *o*-phthaldialdehyde (6 mM) and 2-mercaptoethanol (28 mM) in 50 mM borax buffer pH 10.
- Working solutions: 0.04–30 μmol/L; final injected concentration: 5–3750 pmol/mL.
- Separation: Purospher Star RP-18e column with LichroCART (5 μm; 250 x 4 mm) with pre/post-column; acetonitrile/ 10 mM phosphate buffer (pH 5.8) gradient elution (flow 1 mL/min, fluorescent detection at 330/440 nm).

Oxidative stress markers:
- MDA: The Ohkawa et al. TBARS method [12] modified by introducing an organic extraction (n-butanol) and quantification of the MDA–TBA adduct by HPLC with UV detection at 532 nm, improving sensitivity and specificity for CSF samples.
- 8-iso-$PGF_2\alpha$: competitive ELISA (8-Isoprostane EIA Kit, Cayman Chemical), samples 2–20× diluted.
- TAS: TEAC method [13], UV spectrophotometry.

Neuronal injury marker:
- NSE: classical sandwich ELISA (Quantikine ®Human NSE ELISA Kit, R&D Systems), undiluted CSF.

Instrumentation:
- HPLC: LaChrom system (Merck-Hitachi) which consisted of a L-7100 pumps, an UV and FL detector with variable wavelength (L-7400, L-7480), an injection valve with 20μl and 50μl dosing loop, type 7725i (Rheodyne, USA) and the Clarity Lite evaluation program for PC (AzetChrom s.r.o., SR)
- ELISA/TAS absorbance: EPOCH spectrophotometer (BioTek), data analysis via Gen5 2.01 and UV Probe.

Statistical analysis:
- Software: Microsoft Office Excel 2010, StatsDirect 2.3.7, IBM SPSS Statistics version 22.
- Parametric: unpaired t-test; non-parametric: Mann–Whitney test.
- Correlation strength assessed via r^2: <10% low, 10–50% medium, 50–80% high, ≥80% very high.

Ethics:
- Approved by Kramáre University Hospital with Policlinic, Bratislava; informed consent obtained.

Data presentation:
- Median with interquartile range (Q1–Q3, 25–75%).

RESULTS & DISCUSSION

Table 1: Demographic characteristics of patients

	ONDs	CG	MS	RR MS	SP MS
age (years)	37 (34.5-49)	48 (32-53)	35 (29-44)	34[1] (27-43)	52 (37-52)
man vs. woman	10 vs. 21	16 vs. 10	38 vs. 47	34 vs. 42	4 vs. 5
Qalb x 10^{-3}	4.14 (2.51-5.08)	4.19 (3.75-5.06)	5.52[2] (4.11-7.61)	5.49 (3.88-7.54)	9.32 (7.35-9.81)
IgG index	0.450 (0.43-0.48)	0.437 (0.43-0.45)	0.499 (0.44-0.67)	0.513 (0.44-0.67)	0.412 (0.40-0.43)
total proteins (mg/l)	360.0 (259-405)	388.0 (307-435)	415.5 (314-537)	408.0 (311-523)	565.0 (459-634)
EDSS	-	-	2.5 (2-3.5)	2 (2-3)	4[3] (4-4)
n	31	26	85	76	9

[1]/ [2]/ [3] statistically significant difference in comparison to CG / ONDs (p≤0.05) / SM and RR SM group (p≤0.0005). EDSS= Expanded Disability Status Scale

Table 2: Measured amino acid levels in CSF

	Asp (μmol/l)	Glu (μmol/l)	Gly (μmol/l)	GABA (μmol/l)
MS	0.160[2] (0.045-0.262)*	0.089 (0.032-0.154)	1.598[1] (1.239-1.878)**	0.152 (0.117-0.204)
RR MS	0.160 (0.044-0.281)	0.089 (0.034-0.150)	1.626[1] (1.262-1.877)	0.159 (0.117-0.204)
SP MS	0.208 (0.046-0.381)	0.139 (0.029-0.213)	1.541 (1.132-2.208)	-
CG	0.055 (0.029-0.262)	0.038 (0.026-0.062)	0.962 (0.611-1.465)	0.137 (0.114-0.216)
ONDs	0.026 (0.019-0.104)	0.071 (0.025-0.196)	1.447 (0.858-1.617)	0.076 (0.047-0.104)

[1]/ [2] statistically significant difference in comparison to CG / ONDs, p≤0.05. *CSF Asp levels were significantly increased in EDSS≤3 (0.281 [0.22-0.32] μM) compared to EDSS>3 (0.142 [0.06-0.21] μM). Data of Asp levels in patients with EDSS≤3 belonged mostly to patients who were in relapse at the time of withdrawal. **The Gly concentration in the relapse phase (1.65 μmol/l, n=15) showed a trend to increase in comparison to the remission phase (1.30 μmol/l, n=6), but this difference was not significant.

Table 3: Measured levels of lipid peroxidation, neuronal damage and antioxidant capacity in CSF

	8-iso-$PGF_2\alpha$ (pg/ml)	MDA (μmol/l)	NSE (ng/ml)	TAS (mmol/l)
MS	0.835 (0.24-2.36)	1.33 (0.59-2.21)	5.284 (4.22-6.86)	0.205 (0.13-0.27)
RR MS	0.830 (0.24-2.36)	1.13 (0.57-2.21)	5.284 (4.22-8.01)	0.206 (0.12-0.27)
SP MS	1.860 (0.21-2.29)	2.83 (1.50-3.70)	5.870 (4.38-5.93)	0.179 (0.14-0.23)
CG	0.690 (0.24-2.15)	0.80 (0.55-1.31)	4.820 (3.39-8.57)	0.164 (0.14-0.26)
ONDs	1.140 (0.31-7.93)	0.96 (0.39-1.53)	5.86 (3.81-7.12)	0.214 (0.19-0.25)

Table 4: Correlations (p≤0.05) of the measured parameters in the means of Spearman's rank correlation coefficient (ρ)

	8-iso-$PGF_2\alpha$			TAS	NSE	
	MS	RR MS	ONDs	CG	MS	RR MS
Asp	0.467	0.679	0.886*#	0	n.s.	0.133
Glu	0.546*	0.573*	0.771	0.429	0.137	0.264
Gly	-0.274	n.s.	0.262	0.786*	-0.495*	-0.559*

* statistically significant correlations with p≤0.05; # only 7 observations; n.s. < 7 observations.

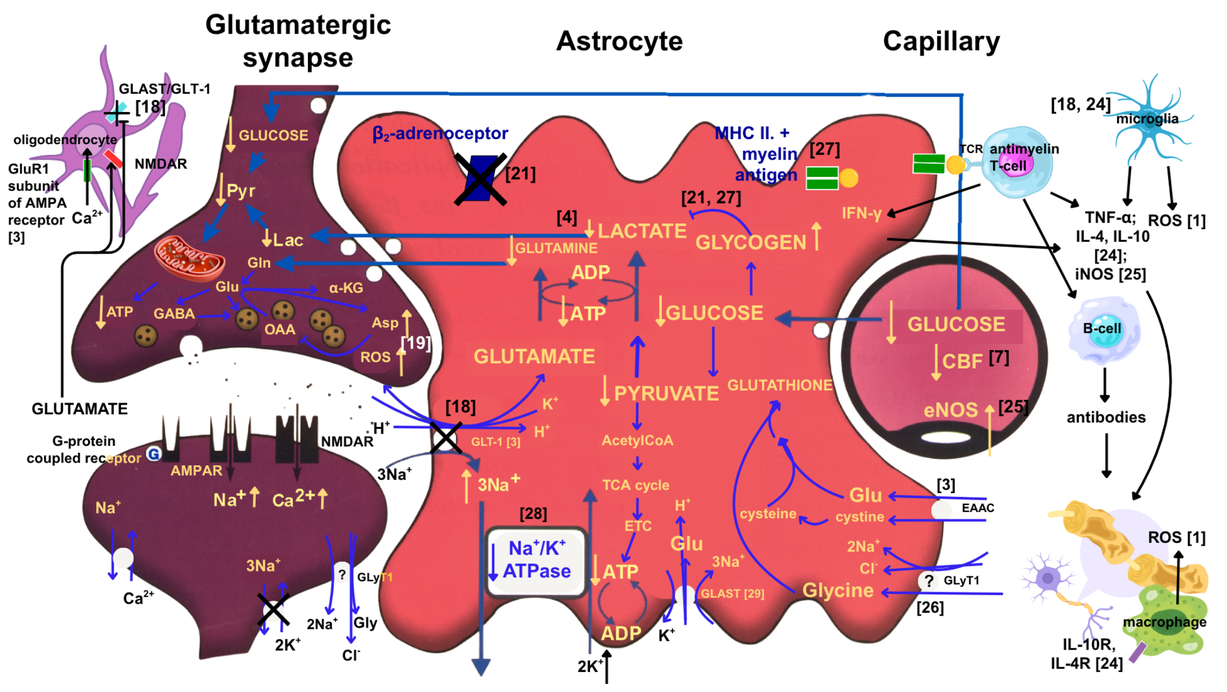
Although our MS patients showed no significant immune activation (Table 1), the presence of lipid peroxidation products in CSF (Table 3) indicates oxidative stress and lipid damage in brain tissue [14]. Our patients (~50% of RRMS group) were supplemented mostly by vitamin E (vitamin D, respectively) which act like a membrane antioxidant, thus could maintain membrane integrity in conditions of elevated oxidative stress. Recently, DMTs (interferons, glatiramer acetate, S1P modulators) were associated with increased TAS, decreased total oxidant status and lower oxidative index in RRMS [15].

CSF **Glu** levels in MS and RRMS patients correlated positively with a product of non-enzymatic free-radical mediated peroxidation of arachidonic acid *in vivo* **8-iso-$PGF_2\alpha$** (p = 0.029/0.041), but not with **MDA** (Table 4), supporting a mechanistic link between excitotoxicity and lipid peroxidation [16]. Lipid peroxidation may also alter neurotransmission by affecting membrane receptor distribution and fluidity [17]. The lack of correlation with MDA is also intriguing. This suggests that not all oxidative stress markers behave equivalently in relation to Glu: possibly 8-iso-$PGF_2\alpha$ is more tightly linked to lipid peroxidation and excitotoxic processes, whereas MDA may reflect a broader or different oxidative process.

Elevated **Asp** in MS (Table 2) may result from impaired transporter/enzyme activity modified by ROS [18,19], loss of GLAST/GLT-1 in cortical lesions [18], or reduced mitochondrial Acetyl-CoA supply due to impaired astrocyte metabolite delivery [20, 21].

Increased **Gly** in CSF (Table 2) is unlikely due to non-specific effects, as total protein and BBB integrity were unchanged (Qalb <10^{-2}, Table 1). Gly may reflect connective tissue remodeling during demyelination and showed a negative correlation with **NSE** (r^2 = 24–31%) and positive correlation with **TAS** (r^2 = 61.8%, Table 4), suggesting a neuroprotective role by modulating synaptic plasticity and limiting excitotoxicity. This aligns with studies showing that high synaptic Gly (use of GlyT1 inhibitor) reduces NMDAR-mediated injury [22].

Elevated **GABA** levels in CSF of our patients with MS and RR MS (14-52% higher than in CG and ONDs, Table 2) could be associated with structural reorganization in the sensorimotor cortex and elevated motoneuron activity as a possible compensatory mechanism for restoration of motor functions [23].



CONCLUSION

From our preliminary results we conclude that some studied neuromediators (**Gly, Asp**) have a potential to be used as a **biomarkers** monitoring the disease activity, an aspect with retaining clinical applicability from the newest revisions of McDonald diagnostic criteria [30]. **Aspartate** could be useful as an indicator of **oxidative stress-induced metabolic changes during acute exacerbation**; **Glycine** has a potential as a **cytoprotectant and immunosuppressant** playing a role in **remyelination (recovery after relapse)**, the support of which in the pathophysiology of MS is currently the subject of intensive research [31]. We hypothesize there is a possible direct link between **glutamate-mediated toxicity and a "specific" oxidative stress marker (8-iso-$PGF_2\alpha$)** which appears under-reported in the literature.

REFERENCES (DOI)

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