Imbalance Glutathione Biosynthesis in ASD: A kinetic patterns “in vivo”.

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Introduction

•Markers of oxidative stress are strongly associated with greater cellular lesions and manifest severe mitochondrial dysfunction in autism spectrum disorders (ASD) pathology. (1) Previous studies indicate that ASD is associated with deficits in the antioxidant defense of glutathione in selective regions of the brain, such as the cerebellum and the cortexes of the frontal, temporal, parietal and occipital lobes, however, the molecular mechanisms of oxidative stress continue being unclear.

•Glutathione (GSH; γ-*L*-glutamyl-*L*-cysteinyl-glycine) is the most abundant endogenous antioxidant present in mammalian cells (0.1 to 15 mM) and plays a protective role for exogenous toxins and endogenous, especially in the central nervous system. The biosynthesis pathway have two consecutive reactions that consume ATP and include two enzymes both; glutamate cysteine ligase (GCL), [E-6.3.2.2], formerly known as gamma-glutamylcysteine synthetase (GCS) and glutathione synthetase (GSS), [E-6.3 .2.3] to generate GSH (2).

•In our previous studies, we described the kinetic imbalance in tri-cellular metabolism of N-acetyl-aspartyl glutamate (NAAG), in anterior (ACC) and posterior (PCC) cingulated cortices relate to the executive control networks and the attention alert functions respectively, linked to ASD pathogenesis. (3)

Aim

To study the specie reduced of glutathione (GSH) biosynthesis in the cingulated cortices, as target of oxidative stress related to ASD, using resonance magnetic spectroscopy (^{1}H•MRS).

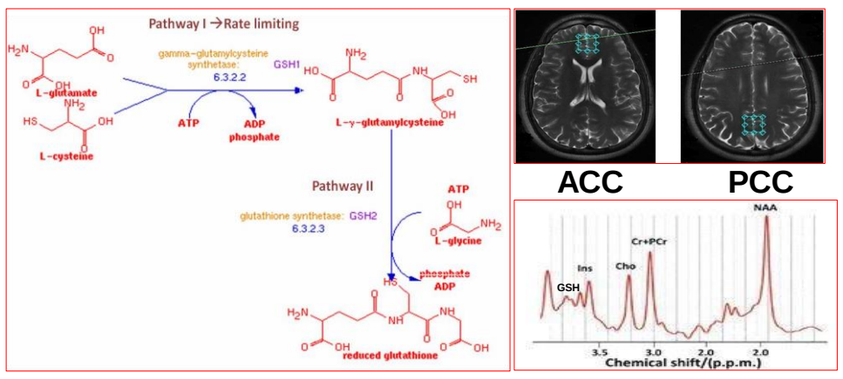

Figure 1. Biosynthesis of glutathione pathway. Localizations voxel ACC & PCC and Chemical shift example.

Materials and Methods

Single voxel (^{1}H•MRS) in bilateral anterior (ACC) and posterior (PCC) cingulated cortices, in ASD (n=21) and controls with typical development (TD, n=46), matched for age and gender and Autism Quotients (AQ) score. The metabolites concentration was measured and affinity between enzyme and substrate associated with glutathione biosynthesis (see Fig.1) was calculate by Michaelis Menten constant (Km). Although, Km isn't a direct measure of an enzyme's affinity for a substrate, however, it is indirectly related to affinity between substrate and enzyme reaction and is defined as the substrate concentration at which the reaction rate is half of the maximum (Vmax) (see Table 1). Statistic one-way ANOVA and Bonferroni correction were applied.

Table 1. Demographic metabolites concentration of biosynthesis of glutathione in ASD and TD group measures by 1H-MRS.

Brain areas/metabolites [mM]	ASD (n=19)	TD (n=46)	P value
ACC			
Glu	12.10 (3.92)	10.54 (5.64)	*p=.02
GSH	3.08 (0.48)	3.75 (1.21)	*p=.05
PCC			
Glu	10.22 (3.19)	10.71 (2.06)	n.s.
GSH	3.43 (1.33)	3.23 (0.80)	n.s.

Note: (mM), milimolar; Glu, glutamate; GSH, glutathione; *p < 0.05, Bonferroni correction

Conclusions

•Imbalance enzymatic kinetic in glutathione biosynthesis in the autism cingulated cortices is a novel finding indicative of a chronic neuroinflamatory state in these regions.

•We further conclude that a better understanding of the enzymatic activity in the synthesis of glutathione in the cingulated cortices can lead us to a new therapeutic pathway in the treatment of individuals with ASD.

Results

The glutathione (GSH) reduce species concentration was significantly decreased (3.08mM; P = 0.05) in ACC of AQ3 conversely, glutamate concentration (12.10 mM; P = 0.02) was increased (see Table 1) in ASD.

The Michalis Menten constant (Km) showing that glutathione biosynthesis decreased significant [1.1e-12 (mM); R2 = 0.001] in autism compared to the TD group (see Table 2), showing that the affinity between substrate and enzyme is significantly higher in individuals with autism. Furthermore, the dissociation constant (ki) was reduced by 67.22% in consequence.

Table 1. Demographic metabolites concentration of biosynthesis of glutathione in ASD and TD group measures by 1H-MRS.

Brain area	ASD (n=21)				TD (n=46)			
	Vmax [µM/min]	K*m* [mM]	Ki [mM]	R^2	Vmax [µM/min]	K*m* [mM]	Ki [mM]	R^2
ACC								
Glu<->GSH	12.60	$1.1e^{-12}$	72.42	0.001	11.32	0.26	220.9	0.01
PCC								
Glu<->GSH	15.12	1.50	~ $1.9e^{+18}$	0.51	~ 62.20	9.41	1.84	0.22

Note: Michaelis Menten constant (Km); Maximun enzime velocity (Vmax); Dissociation constant (ki); R square (R2); glutatihone (GSH); glutamate (Glu);

Maximum rate (Vmax) of the appearance of the product, which depends on the slowest pathway of the enzymatic reaction was significantly decreased (15.12 µM/min; R2 = 0.51) in PCC (see fig.2).

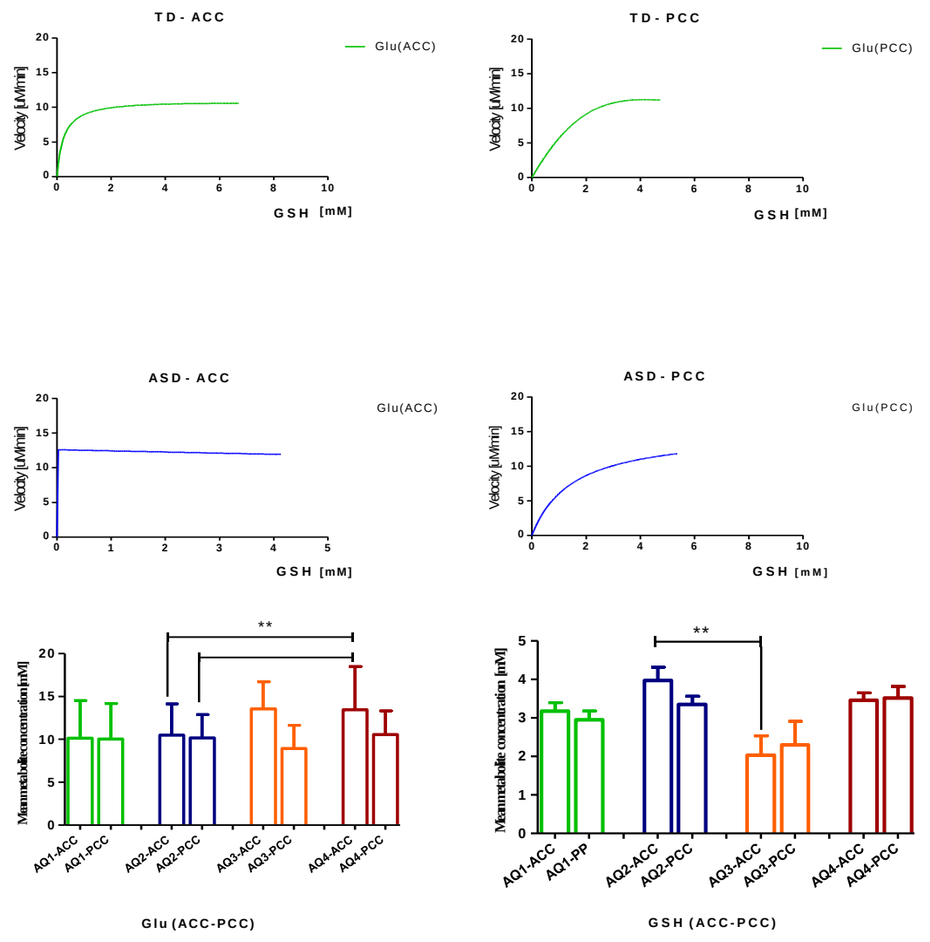

Figure 2. Enzymatic kinetics plot showing reaction rate as a function of substrate concentration. As well as, showing de differences in AQ1, AQ2, AQ3 y AQ4 of Glu and GSH concentration. *p < 0.05, Bonferroni correction.

Referencias

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