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Imbalance Glutathione Biosynthesis in ASD: A kinetic patterns "in vivo".

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Abstract:

Biomarkers of oxidative stress are strongly associated with severe mitochondrial dysfunction in Autism Spectrum disorders (ASD) neuropathology, associated with deficits in the antioxidant defense of glutathione in selective regions of the brain, however, the molecular mechanisms of oxidative stress continue being unclear. 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. In the present study, we use proton resonance magnetic spectroscopy (^{1}H-MRS) to study the specie reduced of glutathione (GSH) biosynthesis in the cingulated cortices, as target of oxidative stress in individuals with ASD. The single voxel of ^{1}H-MRS in bilateral anterior (ACC) and posterior (PCC) cingulated cortices, in adults with ASD and controls with (TD) typical development (n = 21 and n = 46 respectively) were assessed. Glutathione (GSH) concentration were significantly decreased in ACC (P = 0.05). Also, the affinity between enzyme and substrate associated with the biosynthesis of reduced species at glutathione was calculate by Michaelis Menten constant (Km) showing that glutathione biosynthesis decreased significant ($1.1e^{-12}$ mM; R^2 = 0.001) in anterior cingulated cortex in autism and, the dissociation constant (ki) was reduced by 67.22% in consequence. Comparatively, 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; R^2 = 0.51) in posterior cingulated cortices. Imbalance enzymatic kinetic in glutathione biosynthesis in the autism cingulated cortices is a novel finding indicative of a chronic neuroinflammatory state in these regions and, can lead us to a new therapeutic pathway in the treatment of individuals with ASD.

Keywords: Glutathione biosynthesis; Autism spectrum disorders; Proton Magnetic Resonance Spectroscopy; Kinetic chemistry.

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1. Introduction

The defense against the toxic effects of reactive oxygen species (ROS) is an essential task within the brain during a long human life, which indicates the presence of an effective antioxidant system (Mangia et al., 2007). However, the balance between ROS generation and antioxidant processes can be altered, causing neurological disorders such as Alzheimer's and Parkinson's (Antuono, Jones, Wang, & Li, 2001; Kickler et al., 2007; Rupsingh, Borrie, Smith, Wells, & Bartha, 2011). The same way, markers of oxidative stress are strongly associated with greater cellular lesions and manifest severe mitochondrial dysfunction in autism spectrum disorders (ASD) neuropathology (Frye et al., 2013). Despite, previous studies indicate that ASD is associated with deficits in the antioxidant defense of glutathione in selective regions of the brain (Rose et al., 2012), such as the cerebellum and the cortexes of the frontal, temporal, parietal and occipital lobes, 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. It biosynthesis pathway, have two consecutive reactions that consume ATP, including two enzymes: glutamate cysteine ligase (GCL), [E-6.3.2.2], formerly known as gamma-glutamyl cysteine synthetase (GCS) and glutathione synthetase (GSS), [E-6.3 .2.3] to generate GSH (Copeland, 2013). In addition, lower GSH levels (Rossignol & Frye, 2014) and markers of increased oxidative stress (Adams et al., 2009) have been correlated with ASD severity.

The proton magnetic resonance spectroscopy (^{1}H-MRS) is a non-invasive neuroimaging technique that estimates specific chemical metabolite measures *in vivo*, of different metabolites in specific cerebral regions (Aoki, Kasai, & Yamasue, 2012). One the most important contributions of ^{1}H-MRS to clinical neurology is its ability to quantify neuronal loss and to demonstrate reversible neuronal damage (Soares & Law, 2009). 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 neuropathogenesis (Jimenez-Espinoza, Marcano, & Gonzalez-Mora, 2017). This study extended prior work in anterior and posterior cingulated cortices area by establishing metabolic abnormalities, which have been identified by ^{1}H-MRS. We aim is to study the glutathione (GSH) biosynthesis in the cingulated cortices, as target of an enzymatic oxidative imbalance in individuals with ASD using ^{1}H-MRS.

2. Results and Discussion

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

The Michaelis Menten constant (Km) showing that glutathione biosynthesis decreased significant [$1.1e^{-12}$mM; R^2 = 0.001] in autism compared to the TD group (see Table 1), 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.

Conversely, 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; R^2 = 0.51) in PCC (see Fig.1). Decrease in ACC of Km and Ki in individuals with autism, does not mean that the enzyme is not present since these constants are independent of the concentration of enzymes and only depends on the K-Off / K-On rate constants for the union from the substrate to the enzyme.

Table 1. Measured of enzyme affinity for the substrate using the Michaelis Menten constant (Km). Pvalue < 0.05.

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Brain areas	ASD				TD			
	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**

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Note: (mM), milimolar; (µM/min), micromolar per minute; (Vmax), maximum velocity; (Km), Michaelis Menten constant; (Ki), dissociation coefficient; (R2), coefficient of determination; (Glu), glutamate; (GSH), glutathione.

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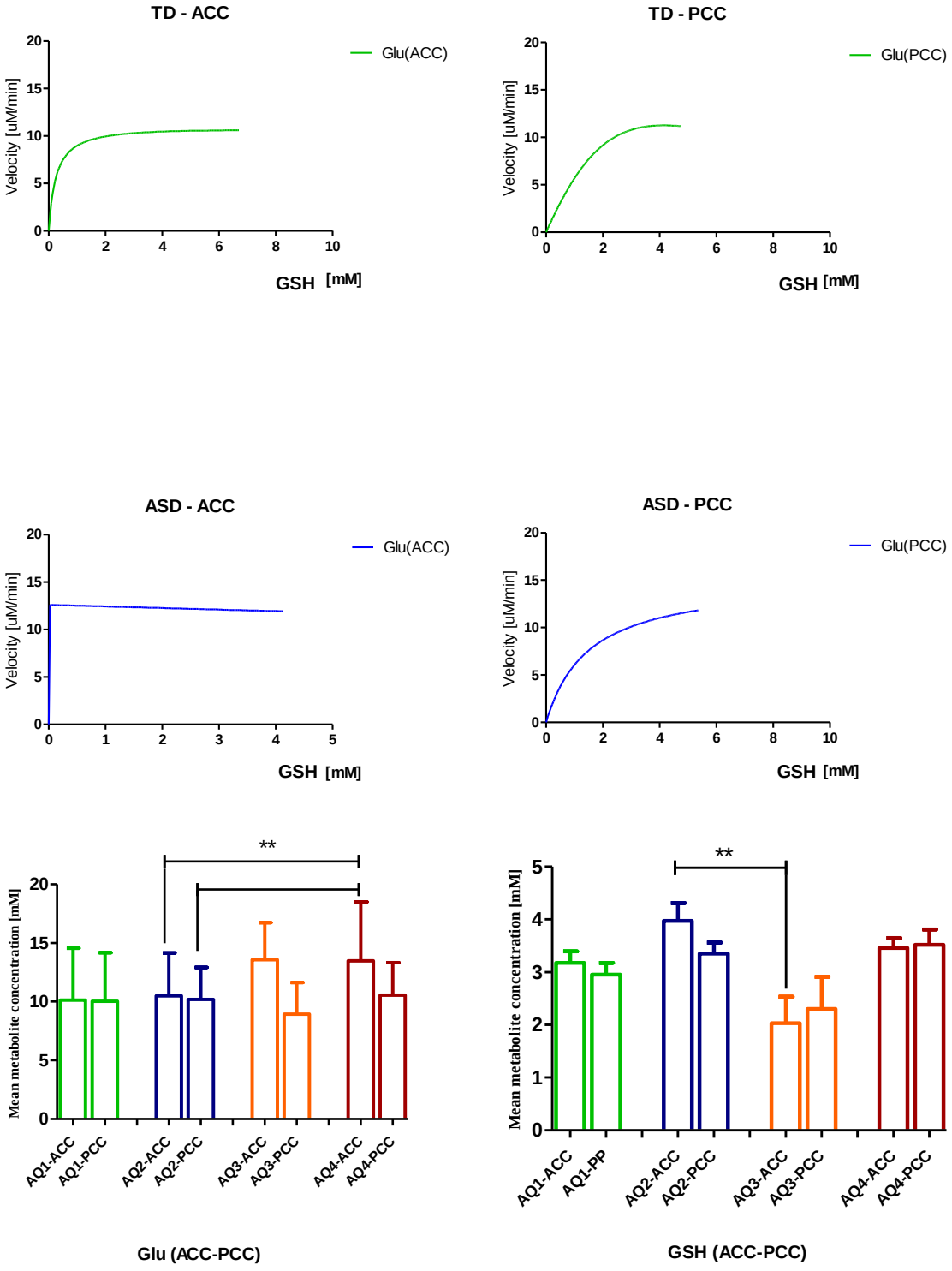

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Figure 1. Diagram of reaction rate and constant of Michaelis-Menten (Km), as function of substrate concentration in GSH Biosynthesis. Mean of metabolites Glu and GSH concentration correlated with ASD severity by AQ-score.

3. Materials and Methods

The Single voxel of resonance magnetic spectroscopy (^{1}H-MRS) in bilateral anterior (ACC) and posterior (PCC) cingulated cortices in adults with a clinical diagnosis of ASD (n=21) and controls with typical development (n=46), matched for age and gender and Autism Quotients (AQ) score were assessed.

The affinity between enzyme and substrate associated with GSH biosynthesis 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).

Statistic one-way ANOVA and Bonferroni correction were applied.

4. Conclusions

Our findings indicate that, at a small amount of substrate, the rate increases rapidly and linearly in ACC, suggesting that the active sites of the enzyme are saturated with the substrate, whereas the enzyme substrate complex is very tight and rarely dissociates without the substrate reacting to give the product. Imbalance enzymatic kinetic in glutathione biosynthesis in the autism cingulated cortices is a novel finding indicative of a chronic neuroinflammatory 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.

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Author Contributions

C.J-E, designed the experiment, oversaw its implementation, critical analysis of the results, and wrote the final manuscript; F.M.S. performs the spectroscopy analysis; J.L.G-M, assisted in the development paradigm.

Conflicts of Interest

The authors declare no conflict of interest.

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