Cortical metabolic alterations induced by genetic redox deregulation in GCLM KO mice and the protective effect of N-acetylcysteine treatment: Relevance for schizophrenia

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Introduction: Glutathione (GSH), a major redox regulator and antioxidant, is decreased in cerebrospinal fluid and prefrontal cortex of schizophrenia patients [1]. Genes of the key GSH-synthesizing enzyme, glutamate-cysteine ligase catalytic (GCLC) and modifier (GCLM) subunit, are associated with schizophrenia suggesting a genetic origin for impaired GSH synthesis [2]. This redox deregulation together with environmental oxidative stress may play a major role in schizophrenia. Using GCLM knock-out (KO) mice with 70% decreased brain GSH levels, we have shown that redox deregulation results in abnormal brain morphology and function and behavioral abnormalities [3]. As schizophrenia is likely a developmental disease, we investigated the impact of genetically deregulated redox system on the neurochemical profile of the developing cortex in GCLM KO mice. In addition, we now tested whether partially restoring the redox balance with the GSH precursor and antioxidant *N*-acetylcysteine (NAC) could normalize the neurochemical profile of GCLM KO mice.

Methods: Wild-type (WT) and GCLM KO mice received NAC (Fluimucil, Zambon, Switzerland) in drinking water at 2.4 g/L before and during mouse pregnancy and after birth throughout the entire experimental period. The neurochemical profile in the frontal cortex of KO and WT mice receiving either NAC treatment or only tap water (non-treated mice) was determined on postnatal days 10, 20, 30, 60 and 90 as previously described [4,5]. Briefly, ^{1}H NMR spectroscopy was performed on a 14.1 T, 26 cm VNMRS spectrometer (Varian, Magnex) using a home-built 8 mm diameter quadrature surface coil. Mice were anaesthetized under 1 to 2% isoflurane in oxygen gas. Field homogeneity was adjusted by FASTMAP [6], and ^{1}H NMR spectra were acquired from VOIs of 2.5 to 4 µL placed in the anterior cortex, using SPECIAL [5,7] with TE of 2.8 ms and TR of 4 s. Typically, spectra were acquired with 480 scans. Metabolite concentrations were estimated with LCModel [8], using corrections for water content variation during development. Data was analyzed with a combined statistical linear model accounting for several variables including genotype, age and NAC treatment.

Table 1. Sumary of metabolic alterations in the cortex of GCLM HZ and KO mice relative to WT and effect of NAC. Arrows indicate increase or reduction of metabolite concentration (↓/↑ P<0.05, ↓↓/↑↑ P<0.01). Orange squares indicate normalization by NAC treatment.

Metabolite(s)	P10		P20		P30		P60		P90	
	HZ	KO	HZ	KO	HZ	KO	HZ	KO	HZ	KO
Alanine			↓ (24%)							↑ (28%)
Glutamine			↑ (23%)	↑↑ (35%)		↑ (34%)				
Glutamate				↑ (12%)						
Glutathione		↓↓ (83%)		↓↓ (83%)		↓↓ (85%)	↓ (24%)	↓↓ (88%)		↓↓ (95%)
Myo -Inositol				↑ (24%)						
Lactate										↑↑ (68%)
N-acetylaspartate				↑ (14%)						
PCre/Cre			↓ (19%)							
Gln / Glu				↑ (23%)		↑ (21%)				

Results and discussion: As previously shown [4], GCLM KO mice displayed nearly undetectable GSH levels compared to WT. This GSH depletion triggered alterations of its metabolic precursor glutamate at P20 (+12%, P<0.05). Cortical glutamine, produced from glutamate, was also increased in KO animals (+35%, P<0.001). At this age, when compared to WT, KO mice also showed higher cortical *myo*-inositol (+24%, P<0.05) and *N*-acetylaspartate (NAA) (+14%, P<0.01) concentrations. Furthermore, the ratio glutamine/glutamate was increased at all measured ages (P<0.05 at P20/P30). Finally, adult KO mice (P90) displayed increased cortical levels of lactate (+68%, P<0.001) and alanine (+28%, P<0.05). Chronic supplementation with NAC did not affect cortical GSH levels. It was expected that NAC acting as GSH precursor could not restore GSH levels in KO animals since a surplus of precursor availability cannot overcome KO-induced enzyme dysfunction. However, via its direct antioxidant properties NAC was expected to boost the antioxidant system in KO animals. The neurochemical profile in the cortex of NAC-supplemented WT and KO animals was clearly distinct from non-NAC-treated mice and the concentrations of numerous metabolites notably modified at P10. Interestingly, concentrations of glutamate, glutamine, *myo*-inositol and *N*-acetylaspartate at P20 in the cortex of NAC-treated KO animals were normalized to the levels of non-treated controls (P>0.05 for all).

Compared to WT mice, GCML KO mice displayed altered concentrations of glutamate, glutamine and their ratio in the anterior cortex, suggesting impaired glutamatergic neurotransmission. Alterations in glutamatergic metabolism are frequently found in brains of schizophrenia patients, and the increased Glutamine/Glutamate ratio in particular translates well to patient studies [9]. Late accumulation of lactate and alanine at P90 points towards modified mitochondrial metabolism. NAA accumulation may reflect reduced deacetylation by oligodendrocytes, raising the possibility of impaired myelination processes in the anterior cortex of GCLM KO mice, which needs further investigation. The results highlight P20 as sensitive period during development for these alterations. Although not able to restore GSH levels, chronic NAC supplementation had protective qualities during this critical period and restored most metabolic alterations in the cortex of KO mice. This suggests that NAC can partially counteract the dysregulated redox status.

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