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Title

Cuprizone-treated mice, a possible model of schizophrenia, highlighting the simultaneous abnormalities of GABA, serine and glycine in hippocampus

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Main Manuscript (1000/1000 words)

Dear Editors

Abnormalities of neurotransmission via dopamine and glutamine in the brain have long been thought to be involved in the pathophysiology of schizophrenia (Howes and Kapur, 2009; Moghaddam and Javitt, 2012). In addition, involvement of even more factors such as D-serine, an allosteric modulator of N-methyl-D-aspartate (NMDA) receptor, γ-aminobutyric acid (GABA) and abnormal immune system have been independently proposed (Chiapponi et al., 2016; Jones et al., 2005; Nunes et al., 2012). These factors may contribute to the pathophysiology of schizophrenia interdependently.

Hippocampus is one of the brain regions that shows significant alteration in schizophrenia, such as volume change (Heckers and Konradi, 2002). Kraguljac et al. reported increased level of glutamate in unmedicated patients with schizophrenia by using magnetic resonance imaging (Kraguljac et al., 2013). Although altered levels of other neurotransmitters have been assumed, it is difficult to measure each neurotransmitter with high specificity and sensitivity.

Recently, a combination of liquid chromatography and mass spectrometry (LCMS) has enabled highly sensitive and high-throughput measurement of biological compounds. Especially, Multiple-Reaction Monitoring (MRM) is particularly sensitive and selective method for measuring small molecules. Using the MRM method, we herein measured various neurotransmitters at the same time in the hippocampus of cuprizone-treated mice, which is relevant for schizophrenia-related abnormalities (Xiao et al., 2008; Zhang et al., 2008), and we also evaluated the effect of quetiapine, a widely-used antipsychotic drug.

A detailed description of our method is shown in the supplemental materials. Briefly, C57BL/6 mice (male, 8 weeks old) were randomly allocated to four groups (CT, Control; CP, Cuprizone; CP+ VH, Cuprizone and, Vehicle; and CP+QP, Cuprizone and Quetiapine). For one week, mice were fed either a diet containing 0.2% cuprizone, or a control diet consisting standard mouse chow. Saline (VH group) or quetiapine (10 mg/kg, QP group) were administrated intraperitoneally 1, 2, 3, and 4 days prior to the experiment. The whole brain from each mouse was removed and each left part of brain was heat-stabilized to inactivate the enzymes. Hippocampus was carefully isolated from the heated brain tissue. The extracts of the homogenized hippocampi were used for MRM analysis. MRM data were processed to acquire peak area of target compounds and the signal levels were normalized to the internal standard (4-hydroxybenzophenone).

To examine the effect of cuprizone exposure on neurotransmitters in hippocampus, we measured 20 compounds by means of MRM using a triple quadrupole mass spectrometer. The majority of compounds did not show significant change in their intensity level (Bonferroni-corrected significance level: 0.05/20 = 2.5E-3). On the other hand, drastic changes were observed in serine, GABA and glycine (**Fig 1ABC**). The signal level of Serine in cuprizone-exposed group showed a 75 % decrease. GABA level showed a remarkable increase of 300 %.

Further, we examined the effect of quetiapine on these three compounds in cuprizone-treatment mice (**Fig. 1DEF**). The signal level of serine and GABA were significantly rescued by quetiapine treatment (**Fig. 1DE**). However, the signal level of glycine was not rescued by quetiapine treatment (**Fig. 2F**).

Glutamine hypothesis is one of the major models for the pathologic mechanism to explain schizophrenia, and a number of reports support the theory (Coyle, 1996; Moghaddam and Javitt, 2012). We performed systematic analysis of the effect of cuprizone on the neurotransmitters in hippocampus, however we could not detect significant change in the level of difference in glutamine, glutamic acid and glutamine/glutamic acid ratio. On the other hand, serine, glycine and GABA showed prominent changes in their signal levels in cuprizone-treated mice, both of which have been thought to be involved in the pathophysiology of schizophrenia (Tuominen et al., 2005). D-serine works as a neurotransmitter involved in glia-synapse interaction and D-serine diminishes NMDA neurotransmission. In addition, plasma serine levels were reported to be decreased in patients with schizophrenia (Sumiyoshi et al., 2004). Glycine is an obligatory co-agonist of NMDA receptors, and NMDA receptor-mediated glutamatergic neurotransmission is suggested to be one of the main causes of schizophrenia (Labrie and Roder, 2010). Decreased level of GABA in hippocampus and other brain regions in patients with schizophrenia has been reported (Heckers and Konradi, 2015; Steiner et al., 2016) . However, we observed increased level of GABA in the hippocampus of cuprizone-treated mice. In this study, we analyzed tissue extract, therefore the GABA level does not necessarily reflect the amount of GABA in the synaptic junction. Further investigations are required to illustrate the roles of GABA under the pathophysiology of schizophrenia.

Quetiapine is one of the major atypical antipsychotics used for schizophrenia, bipolar disorder and major depression (Riedel et al., 2007). The present result has suggested that quetiapine alleviate clinical symptoms by modulating the level of serine

and GABA in hippocampus. It is still unknown how quetiapine affects the synthesis or metabolism of serine and GABA in hippocampus. One-week treatment of cuprizone is known to activate glial cells including astrocytes and microglia before demyelination (Tezuka et al., 2013). On the other hand, microglial activation has been highlighted in schizophrenia including at high-risk stage (Bloomfield et al., 2016; Monji et al., 2009; Selvaraj et al., 2018). We previously reported that various antipsychotics including quetiapine have an effect to down-regulate the activation of microglia *in vitro* (Bian et al., 2008; Kato et al., 2007; Kato et al., 2011; Sato-Kasai et al., 2016; Seki et al., 2013), and *in vivo* studies have shown suppressing effects of quetiapine in cuprizone treatment model (Shao et al., 2015; Wang et al., 2015; Xiao et al., 2008; Zhang et al., 2008; Zhang et al., 2012). Thus, microglia modulating effects of quetiapine may be one of the possible underlying mechanisms in the present result. Other molecular mechanisms should also be considered using different schizophrenia-related models (Kraeuter et al., 2019).

Based on this study, we have proposed that cuprizone-treated mice may be an appropriate model showing the abnormalities of GABA, serine and glycine in the pathology of schizophrenia, but not suitable to examine the glutamine abnormalities of schizophrenia. Further translational studies including behavioral experiments are needed to understand the multi-factorial mechanisms of schizophrenia using the present model and clinical samples.

Appendix A. Supplementary data

Supplementary data to his article can be found online.

Conflict of interest

The authors declare no conflicts of interest in relation to the work described.

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Contributors

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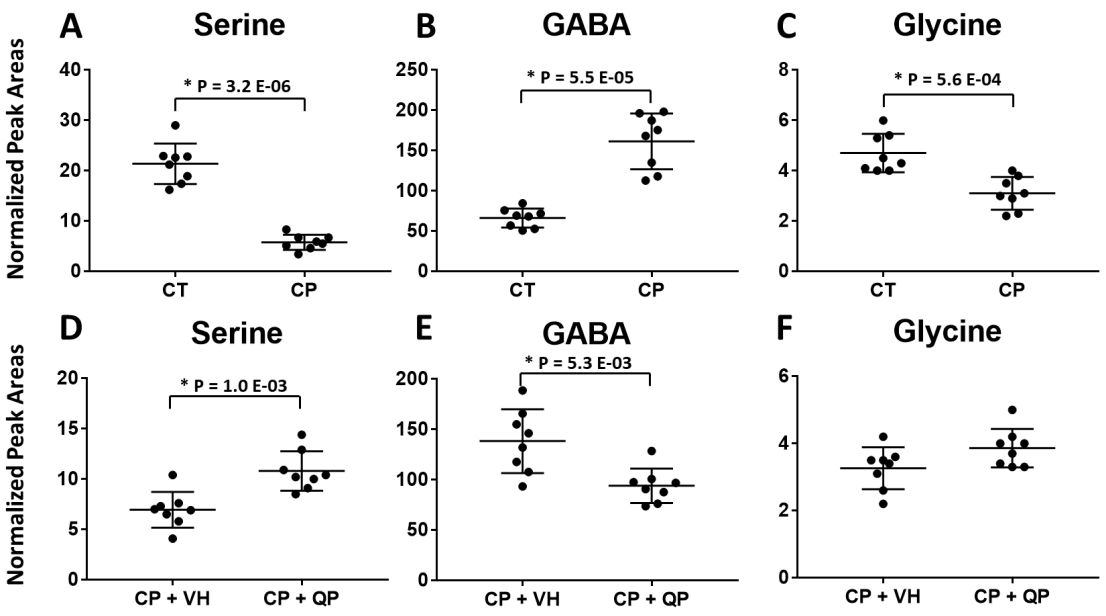

Fig. 1. Effects of cuprizone and quetiapine on serine, GABA and glycine. Data points of MRM signal of each samples, mean value and standard deviation are indicated (N = 8). CT, control; CP, cuprizone; VH, vehicle; QP, quetiapine. Statistical analysis was performed using the unpaired Student's t-test. A p-value <0.05 was considered statistically significant. We applied a conservative Bonferroni correction to control for false-positive deriving from multiple testing. P values ≤ 0.05 / (number of metabolites detected) were considered to be statistically different.

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