Schizophrenia Research 210 (2019) 326–328

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Letter to the editor

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

Keywords:
Neurotransmitter
Multiple-reaction monitoring
Metabolomics
Cuprizone
Quetiapine
Hippocampus

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. 1).

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).

https://doi.org/10.1016/j.schres.2019.06.010

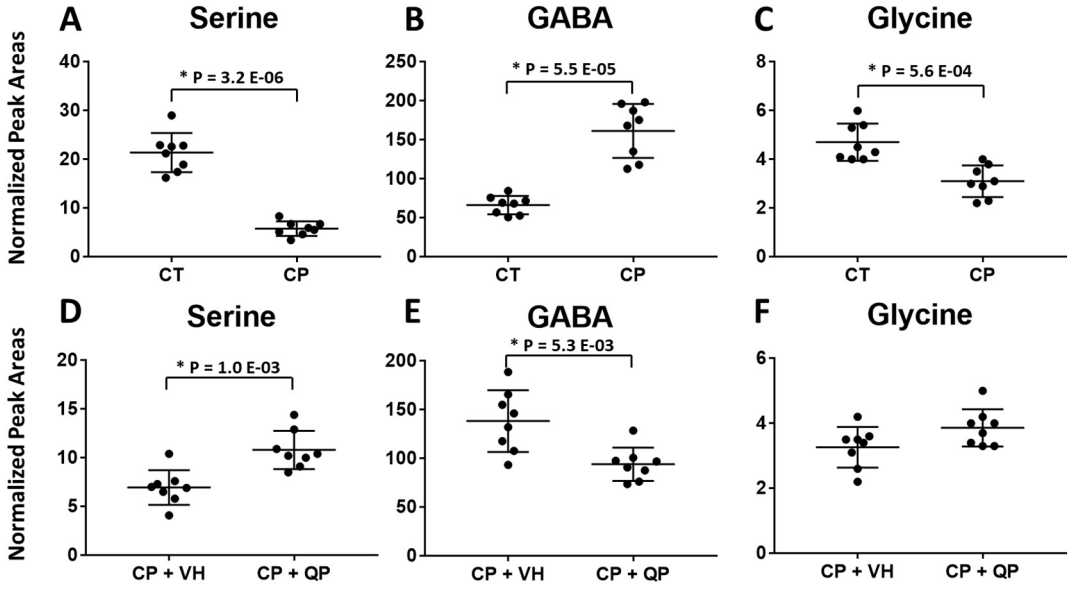

Fig. 1. Effects of cuprizone and quetiapine on serine, GABA and glycine. Data points of MRM signal of each samples, mean value and standard deviation are indicated ($N = 8$). CT, control; CP, cuprizone; VH, vehicle; QP, quetiapine. Statistical analysis was performed using the unpaired Student's *t*-test. 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.

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.

Role of the funding source

This work was supported by a Grant-in-Aid for Scientific Research on (1) KAKENHI - the Japan Society for the Promotion of Science (to E.H.: 17K14968; to D. M.: 26713020; to T.A.K., M.O. and S.K.: 24650227, 25293252, 16H02666, 26713039, 26860933, and 18H04042), (2) MEXT Funding-Project for Developing Innovation Systems Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program in Japan (D.M. and T. A. K.), (3) Innovative Areas "Glia Assembly" and "Will Dynamics" of The Ministry of Education, Culture, Sports, Science, and Technology, Japan (25117011 to S.K.; 16H06403 to T.A.K.), (4) The Japan Agency for Medical Research and Development (AMED) (*Yugo-Nou* JP18dm0107095 to T.A.K.: *Seishin-Syogai Taisaku-Jigyo* JP18dk0307075 to S.K. and T.A.K.), (5) Young Principal Investigators' Research Grant of Innovation Center for Medical Redox Navigation, Kyushu University (to T.A.K.), (6) Takeda Medical Research Foundation (to T.A.K.), and (7) the SENSHIN Medical Research Foundation (to T.A.K., M.O., and S.K.). The funders had no role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript.

Contributors

All authors contributed substantially to the scientific process leading up to the writing of the present manuscript. D.M. and T.A.K. the principal investigators of the present research, E.H. the first author, and M.O. created the conception and design of the project and wrote the protocol. The performance of experiments and data analyses/interpretation were performed by E.H., M.O., E.H., M.O., D.M., Y.F., T.A.K. and S.K.. E.H. wrote the first draft of the manuscript. Critical revisions of the manuscript were made by T.A.K., D.M., Y.F. and S.K., All authors approved this submission in its current form.

Declaration of Competing Interest

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

Acknowledgement

We acknowledge the excellent technical assistance provided by Ms. Ayaka Maeda, Ms. Yuka Matsushita, Mr. Shogo Inamine and Ms. Aya Yamada.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2019.06.010.

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4 May 2019