October 2018
No.2018-009-e

Journal of Integrated Creative Studies

Report on my visit to the Institute for Integrated Radiation and Nuclear Science, Kyoto University in 2018

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Abstract. The homochirality of the L-amino acids is fundamental for the development and maintenance of life. Therefore, in the past D-amino acids were considered only as unnatural molecules in life science and there has been little knowledge on the function of D-amino acids in mammals. However, these atypical amino acids were recently found in various higher organisms, including human in the form of free amino acids, peptides, and proteins. Free D-serine, D-alanine and D-aspartate may function as a synaptic modulator in the mammalian brain. In particular, D-aspartate may play a role as a novel neuronal messenger in the maturation and differentiation of the central nervous system. On the other hand, in peptides, D-amino acids are well established as essential amino acids conferring biological activities on opioid peptides and neuropeptides. Consistently with their emerging biological interest and biomedical translational value, very recent improvements in analytical chemistry, which detect amino acid enantiomers at the femtomole level, demonstrated that these atypical amino acids are no longer “unnatural” molecules in living organisms.

Keywords: D-Amino acid, Analytical Chemistry, aging, brain functioning

Subject of the Report

This report summarizes my scientific activities during my 2 months stay as Visiting Professor in the laboratory of Biochemical Gerontology group at the Institute for Integrated Radiation and Nuclear Science, Kyoto University. These activities were fully supported by the International Research Unit of Advanced Future Studies, Kyoto University, Japan.

Introduction

Besides D-serine, another D-amino acid with endogenous occurrence in the mammalian brain, D-aspartate, has been recently shown to influence NMDA receptor (NMDAR)-mediated transmission. D-aspartate is present at the extracellular level, binds to the agonist site of NMDARs and activates this subclass of glutamate receptors. Along with its direct effect on NMDARs, D-aspartate can also indirectly stimulate glutamatergic neurotransmission in specific brain areas by evoking L-glutamate release through the presynaptic activation of NMDA, AMPA/kainate and mGlu5 receptors. D-aspartate is enriched in the embryonic brain of rodents and humans and strongly decreases after birth, due to the post-natal expression of D-aspartate oxidase (DDO) enzyme, which selectively catabolizes this D-amino acid. Based on the hypothesis of NMDAR hypofunction in schizophrenia pathogenesis, recent studies suggested a relationship between perturbation of D-aspartate metabolism and this psychiatric disorder, in both animal models and humans. Consistently, neurophysiological and behavioural characterization of Ddo knockout (Ddo-/-) and D-aspartate-treated mice evidenced that abnormally higher endogenous levels of this D-amino acid enhance NMDAR-dependent synaptic transmission and plasticity, dendritic morphology and memory. Remarkably, increased D-aspartate levels influence schizophrenia-like phenotypes in mice, as shown by improved fronto-hippocampal connectivity, attenuated prepulse inhibition deficits and abnormal circuits activation induced by the psychotomimetic drug, phencyclidine. In healthy humans, genetic variation predicting reduced expression of DDO mRNA in the prefrontal cortex is associated with greater prefrontal grey matter and activity during working memory. Moreover, neurochemical detections in post-mortem patients affected by schizophrenia have shown significant reductions of D-aspartate content in prefrontal regions, associated with an increase in DDO mRNA expression or activity of DDO enzyme. Other recent results evidenced a pharmacological link between D-aspartate metabolism and the second-generation antipsychotics, olanzapine. In fact, it has been reported that olanzapine, differently from other typical and atypical antipsychotics, is able to inhibit the enzymatic activity of both human and murine DDO, and induces a significant extracellular release of D-aspartate and L-glutamate in the prefrontal cortex of freely moving mice. Overall, these results suggest a potential deregulation of D-aspartate metabolism in schizophrenia pathophysiology and highlight the existence of new potential therapeutic avenues for treating neuropsychiatric disorders associated with hypofunctional glutamatergic neurotransmission. Conversely, since D-Asp acts as an agonist on NMDARs at GluN2B site, we should take into account that the effects of this molecule reflect those produced by NMDAR stimulation, not only for better but also for worse. Indeed, it is well established that NMDARs promote synaptic strength and connectivity on one side but, on the other side, they can produce neuronal death if their stimulation is too intense or long (Hardingham and Bading, 2003). In line with detrimental NMDAR-related effects, persistent increase of D-Asp levels results in precocious decay of basal glutamatergic transmission, synaptic plasticity and hippocampal reference memory in 13/14-month-old Ddo-/- mice (Errico et al., 2011), mirrored by structural alterations of the excitatory glutamatergic inputs and reduced levels of synaptic GluN1 and GluN2B subunits (Cristino et al.,

2015). In addition, recent studies also revealed that the lack of DDO leads to severe neuroinflammation processes and cell death in an age-dependent manner (Punzo et al., 2016). Overall, these data point out that the Jekyll and Hyde behaviour of NMDARs (Hardingham and Bading, 2003) can be recapitulated in different stages of life by the lack of DDO activity in Ddo-/- mice.

1. Lectures

During my stay at Kyoto University, I have given 4 talks.

The first seminar, entitled “Free D-aspartate exerts an opposing role upon age-dependent NMDAR-related synaptic plasticity and memory decay”, was held on July 26, 2018, at the University of Kyoto and was intended for a general audience as part of an Advanced Future Studies seminar.

The second talk, entitled “Free D-Aspartate: from bacterial cell wall to potential involvement in schizophrenia”, was held at Kitasato University School of Pharmacy, Tokyo, on August 2, 2018. The seminar, attended by expert scientists in the field of D-Amino acids research, focused on the involvement of dysfunctional D-Aspartate metabolism in the pathophysiology of Schizophrenia.

The third talk entitled “Free D-Aspartate: an endogenous NMDA receptor agonist enriched in the developing brain with potential involvement in neuroendocrine functions”, was held at the Department of Chemo-Pharmaceutical Sciences at Kyushu University, on August 20, 2018. The seminar, attended by expert scientists in the field of D-Amino acids and Analytical Chemistry, focused on the role of D-Aspartate in mammalian brain metabolism and its influence in regulating neuroendocrine functions.

The fourth talk entitled “D-Aspartate Oxidase activity prevents D-Aspartate dependent NMDA neurotoxicity” was held at Nara Women's University, on August 30, 2018. The talk, attended by Professors and students in the field of animal physiology, focused on the role of D-Aspartate in cognition and its potential translational value in neurodegenerative diseases.

2. Research collaboration

My research encompasses different topics related to D-Amino acids metabolism and its potential application to human health and diseases. During my stay in Japan, I started 2 scientific collaborations between my research group, the lab of Biochemical Gerontology team at Kyoto University, directed by Prof Noriko Fujii and other Italian and Japanese Universities, including Kitasato University School of Pharmacy, Nara Women's University and Kyushu University.

In the following, I describe in some detail the project collaborations that were at the main focus of my scientific activity during my stay at Kyoto University.

1 Comparison of D-Amino acids content in Mediterranean Diet vs Japanese traditionally Foods

Foods contain large quantities of atypical substances of external origin, which influence their digestibility to a considerable degree. An example is the D-stereoisomer amino acids, which are formed from common L-stereoisomer amino acids, either in the course of the production process or as a consequence of changes in the microbiological quality of the foods. The presence of these D-stereoisomer amino acids results in a substantial reduction in the digestibility of dietary protein and the availability of the transformed amino acids. However, despite the fact that in the past D-amino acids in foods were considered undesirable, in light with our recent results (Errico et al., 2015) we hold the opinion that in certain cases D-amino acids, like D-serine and D-aspartate can nevertheless be beneficial to the human health including cognition and synaptic plasticity. Based on this idea, in close collaboration with Prof Noriko Fujii (Biochemical Gerontology Lab at the Division of Radiation Life Science Kyoto University) and Prof Francesco Errico (Department of Agricultural Sciences, University of Naples "Federico II", Portici, Italy), we intend to analyse by HPLC and MS the D-amino acid content in foodstuff belonging to Mediterranean diet in comparison with typical Japanese food of Kansai region. We aim to unveil the existence of a potential relationship between D-serine and D-aspartate content, specific food consumption and healthy ageing in humans.

2 Analysis of DDO activity and D-Amino acids content in the brain and peripheral organs of transgenic mice overexpressing DDO enzyme during postnatal life.

While the biosynthetic pathway of D-Aspartate is still unclear, the enzyme responsible for D-Aspartate degradation, D-aspartate oxidase (DDO), has been well known for a long time (Still et al., 1949). DDO is a peroxisomal flavoenzyme that oxidizes D-Aspartate in presence of H2O and O2, producing α-oxaloacetate, H2O2 and NH4+ (Katane and Homma, 2010). A specific tripeptide signal at the C-terminus of DDO targets this enzyme to peroxisomes (Setoyama and Miura, 1997; Amery et al., 1998) where the toxic H2O2, produced by D-Asp oxidation, can be safely removed by peroxisome-resident catalases (Beard, 1990). Consistent with a primary role for DDO activity in controlling its substrate concentration, it has been reported that spatial and temporal expression of this enzyme is reciprocal to D-Aspartate (Van Veldhoven et al., 1991; Punzo et al., 2016). During mouse lifespan, the postnatal decrease of cerebral D-Aspartate is mirrored by a time-dependent increase of Ddo gene expression, as Ddo mRNA levels are very low from E14 to one week of age and then strongly rise during the following weeks until adult phase (Punzo et al., 2016). Notably, the temporal pattern of Ddo mRNA expression reported in the mouse brain matches closely with the substantial increase in DDO enzymatic activity found in the rat brain during the first weeks of life (Van Veldhoven et al., 1991). Like D-Aspartate, also DDO is prominently localized in neurons, and only marginally expressed in glial population (Zaar et al., 2002). Overall, reciprocal spatiotemporal localization of DDO and D-Aspartate suggests that during adulthood this enzyme is necessary to remove its endogenous substrate from brain regions where D-Aspartate is no more required and, indeed, could be functionally detrimental and neurotoxic for neuronal activity and survival, as found in the brain of elderly constitutive knockout mice for Ddo gene (Ddo-/-)(Errico et al., 2011; Cristino et al., 2015; Punzo et al., 2016). The substantial and persistent accumulation of D-Aspartate in the brain of Ddo-/- mice further

suggests that DDO is the only enzyme that catalyses the endogenous degradation of D-Aspartate, throughout the entire animal lifespan (Errico et al., 2006; Huang et al., 2006; Errico et al., 2011). On the other hand, we speculate that dysfunctional D-Aspartate metabolism occurring during neurodevelopment may affect early critical processes dependent on NMDARs and, in turn, contribute to schizophrenia vulnerability. Our recent generation of a transgenic mouse model (DDO OV) with prenatal and postnatal depletion of D-Aspartate levels may aid to disclose the importance of the transient occurrence of D-Aspartate in developmental brain processes and, in turn, the potential involvement of dysregulated D-Aspartate metabolism in a neurodevelopmental psychiatric disorder like schizophrenia. Based on this consideration, in collaboration with Prof Noriko Fujii (Kyoto University), Prof Hiroshi Homma (Laboratory of Biomolecular Science, Graduate School of Pharmaceutical Sciences, Kitasato University), Prof Kenji Hamase (Laboratory Bio-Analytical Chemistry, Graduate School of Pharmaceutical Sciences, Kyushu University) and Prof Francesco Errico (Department of Agricultural Sciences, University of Naples "Federico II", Portici, Italy), we intend to explore the enzymatic DDO activity and the D-amino acids content in the peripheral organs, brain and testis obtained in the novel DDO overexpressing transgenic mice at different prenatal and postnatal stages to understand the still obscure role of D-Aspartate in mammalian brain development and its influence in regulating Testosterone synthesis and release.

3. Summary. During my stay, I had the great opportunity to closely interact with Prof Noriko Fujii, Head of the Biochemical Gerontology group at the Division of Radiation Life Science Institute for Integrated Radiation, and Nuclear Science, Kyoto University, and to start two international collaborations between Italian and Japanese groups in the emerging field of D-Amino acids.

Acknowledgements

I would like to extend my great gratitude to Professor Noriko Fujii for her warm hospitality, help and fruitful collaboration. I would also like to thank the entire Biochemical Gerontology group at the Division of Radiation Life Science for making enjoyable my stay in Japan. Finally, I wish to extend a special thank to Prof Murase for his brilliant scientific and philosophical suggestions and Ms. Ono for her kindness, and administrative support.

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