Form 2

Dissertation Abstract

Report no.	(Course-based) No. 917 ~~(Dissertation-based)~~	Name	KYAW HTET AUNG
Dissertation title	Effects of Environmental Chemical Exposure on Brain Development and Mechanism of Developmental Neurotoxicity 脳発達に対する環境化学物質曝露の影響と発達神経毒性機構に関する研究		

Abstract

It has been well demonstrated that exposure to environmental chemicals increase the risk of human health, including the prevalence of neurodevelopmental disorders in children. There are many kinds of chemicals found in our environment, some of which are suspected to have an adverse effect of the nervous system leading to the decrease in the quality of life. However, most of chemicals are remained to assess their neurotoxicity. There are a few substances that have been studied for their neurotoxicity by assessing the behavioral and neuropathological changes in *in vivo* animal models. Nevertheless, such research strategy using *in vivo* animal models could not be suitable for screening the neurotoxicity of many chemicals, because they need time consumption and expensive costs. Thus, it is required to develop appropriate testing methods including *in vitro* systems for more efficient and accurate evaluation of the neurotoxicity of chemicals. Therefore, the present study aimed (1) to develop a new *in vitro* neurotoxicology method using live imaging of cultured neurons that can effectively monitor both neuritogenesis and apoptosis, (2) to demonstrate the reliability and relevance of the method as a tool for assessing chemical neurotoxicities comparing the data of live imaging of cultured neurons with that obtained from neurons of animal models, and (3) to apply live imaging technique to elucidation of toxic mechanisms of chemicals.

Sodium arsenite ($NaAsO_2$) is known as a neurotoxicant to induce apoptosis of neurons. However, cytotoxic effects inducing apoptosis is not sufficient for accurate assessment of neurotoxicity. In the present study, live imaging analyses were performed to examine the effects of $NaAsO_2$ on both apoptosis and neuritogenesis in SCAT3-expressing Neuro-2a cells, a mouse neuroblastoma cell line. SCAT3 is a caspase-3 cleavage peptide sequence linking two fluorescent proteins; enhanced cyan fluorescence protein (ECFP) and Venus. The life and death of SCAT3-expressing cells can be monitored by calculating the ratio of the fluorescent intensities of ECFP to that of Venus. Furthermore, neurite outgrowth can also be monitored by calculating the ratio of the area of neurites to that of cell body that can be visualized with Venus. As the results of live imaging, the ratio of neurite area to cell body area was significantly decreased by 5 and 10 μM $NaAsO_2$, but not by 1 μM. On the other hand, the ECFP/Venus emission ratio was not changed. This finding suggests that neurite outgrowth is suppressed by $NaAsO_2$ without induction of apoptosis. In addition, the expression of several cytoskeletal genes involved in neuritogenesis

was measured to know the mechanism by which $NaAsO_2$ inhibits neurite outgrowth. As the results, $NaAsO_2$ exposure increased the mRNA levels of the light and medium subunits of neurofilament and decreased the mRNA levels of tau and tubulin in a dose-dependent manner, although no significant effect was found in the mRNA levels of the heavy subunit of neurofilament, microtubule-associated protein 2, or actin. These results suggest that alteration in the cytoskeletal gene expression is responsible for the inhibitory effects of $NaAsO_2$ on neurite outgrowth in Neuro-2a cells.

Next, to determine whether the adverse effects of $NaAsO_2$ on neuritogenesis in Neuro-2a cells are also found in neurons of the mouse brain, 85-ppm $NaAsO_2$-containing drinking water was given to pregnant mice from gestational day 8 to 18, and the derived pups were used to measure the length of neurite and the number of neuronal and glial cells in the prelimbic cortex (PrL) in adulthood. The morphometrical analyses showed that the length of neurite was significantly decreased by developmental exposure to $NaAsO_2$. The number of pyramidal and non-pyramidal neurons and glial cells was not changed in any layers of the PrL by $NaAsO_2$, except in the layer V and VI of the PrL where the number of pyramidal neurons was significantly increased by $NaAsO_2$. These results suggest that *in utero* $NaAsO_2$ exposure disrupts the structural formation of the PrL by impairing neuritogenesis. Thus, the adverse effects of $NaAsO_2$ on neuritogenesis were observed not only in *in vitro* cultured neuronal cells monitored by live imaging but also in neuronal cells in the brain of animal model, indicating that live imaging of cultured cells is a useful method for screening of the developmental neurotoxicity of chemicals.

For further demonstration of the use of live imaging for neurotoxicology, live imaging analysis was carried out to elucidate the mechanism underlying di(2-ethylhexyl) phthalate (DEHP)-induced apoptosis in Neuro-2a cells. DEHP, a widely used plasticizer, is known to induce apoptosis in a variety of cells. However, the mechanism by which DEHP induces apoptosis in neurons is not well explored yet. First it was confirmed by live imaging using SCAT3 that exposure to DEHP (100 μM) significantly induced apoptotic cell death in Neuro-2a cells. Next the mRNA levels of some genes, which is known to be involved in oxidative stress or inflammation, was measured after Neuro-2a cell were exposed to DEHP, resulting in that the mRNA levels of hemeoxygenase-1 (HO-1) were significantly increased by exposure to DEHP at 100 μM for 24 h. This result indicates the possibility that DEHP-induced apoptosis is mediated by HO-1. To clarify this possibility, live imaging of SCAT3-expresing Neuro-2a cells was performed to determine whether knockdown of the HO-1 gene by siRNA was able to cancel the cytotoxicity of DEHP. The analysis showed that apoptotic cell death of Neuro-2a cells induced by DEHP (100 μM) was completely attenuated by knocking down of the HO-1 gene. Taken together, DEHP induces apoptosis in Neuro-2a cell with presumably increasing in the expression of HO-1 gene as an oxidative response to DEHP exposure.

In conclusion, this study highlights the usefulness of *in vitro* live imaging technique, which can evaluate the effect of chemicals not only on cell viability but also on neurite outgrowth.

Moreover, live imaging technique is also available to clarify the mode of neurotoxic actions. This study shows future perspective of *in vitro* approach using live imaging to evaluation of toxicity of many chemicals, which can cover some of information gaps of traditional neurotoxicity testing.