The protective effects of levetiracetam on spinal muscular atrophy models

Shiori Ando\textsuperscript{1,2}, Kazuki Ohuchi\textsuperscript{1,2}, Michinori Funato\textsuperscript{2}, Shinsuke Nakamura\textsuperscript{1}, Masamitsu Shimazawa\textsuperscript{1}, Hideo Kaneko\textsuperscript{2}, Hideaki Hara\textsuperscript{1}

\textsuperscript{1}Molecular Pharmacology, Gifu Pharmaceutical University, Japan, \textsuperscript{2}Department of Clinical Research, National Hospital Organization, Nagara Medical Center, Jordan

Background
Spinal muscular atrophy (SMA) is an inherited disease characterized by loss of spinal motor neuron and severe skeletal muscle atrophy. SMA is caused by low expression level of survival motor neuron (SMN) protein subsequent to deletion or mutation of SMN 1 gene. Recently, an antisense oligonucleotide that increases SMN protein level has been approved for SMA treatment. This breakthrough gave great progress in the SMA therapy, but it does not completely fulfill the present clinical problems. In this study, we evaluated the therapeutic potential of levetiracetam, which is widely used for the therapy of epilepsy.

Methods
Induced pluripotent stem cells from an SMA patient (SMA-iPSCs) were differentiated into spinal motor neuron by the same steps described normal embryonic development. To evaluate the effect of levetiracetam, SMA-iPSCs-derived spinal motor neurons (SMA-iPSCs-MNs) were exposed to levetiracetam at 100 \( \mu \)M. To examine the effect of levetiracetam on cell death, we performed TdT-mediated dUTP nick end labeling (TUNEL) staining. The expression level of cleaved caspase 3 was determined by Western blots analysis. The neurite elongation was examined by immnostaining. We also evaluated the expression level of SMN protein by Western blots analysis.

Results
In the SMA-iPSCs-MNs, TUNEL-positive cells were increased compared to Control-iPSCs-MNs. Compared to vehicle-treated SMA-iPSCs-MNs, TUNEL-positive cells were decreased in levetiracetam-treated SMA-iPSCs-MNs. The expression level of cleaved caspase 3 was also decreased in levetiracetam-treated SMA-iPSCs-MNs. Furthermore, levetiracetam improved impaired neurite elongation of SMA-iPSCs-MNs. On the other hand, the expression level of SMN protein was not changed in levetiracetam-treated SMA-iPSCs-MNs compared to vehicle-treated SMA-iPSCs-MNs.

Conclusions
Levetiracetam suppressed the apoptotic cell death and improved the neurite elongation in SMA-iPSCs-MNs. These protective effect of levetiracetam seemed to be independent of SMN protein expression. These results may propose the potential effect of levetiracetam for the therapy of SMA.