# Tacrolimus-induced neurotoxicity and investigation of potential protective agents against tacrolimus-related neurotoxicity

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| 論　文　名 | Tacrolimus-induced neurotoxicity and investigation of potential protective agents against tacrolimus-related neurotoxicity<br>（タクロリムスによる神経毒性の発現メカニズムの解明と予防薬の探索） |
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## 論 文 審 査 の 結 果 の 要 旨

Calcineurin inhibitors (CNIs), including tacrolimus, are widely used as immunosuppressants in transplantation therapy and dramatically improve patient survival. However, apart from their benefits, neurological adverse events caused by CNIs are one of the major problems encountered in clinical practice. Common symptoms of CNI-related neurotoxicity include tremor or headache in mild cases and posterior reversible encephalopathy syndrome in severe cases. Although CNI dose reduction may relieve tremors, mild symptoms might still be experienced, negatively affecting patients' quality of life. Moreover, dose reduction also increases the risk of rejection of transplanted organs, and it is preferable to prevent tremor while maintaining the therapeutic intensity of CNIs.

Although the mechanism of CNI-induced neurotoxicity has not yet been elucidated, several studies have reported and discussed these adverse events. First, CNIs are usually restricted from entering the brain through the blood–brain barrier (BBB); however, previous studies have shown that CNIs disrupt BBB function by inducing nitric oxide synthesis and increasing transforming growth factor-β1 levels in brain endothelial cells and pericytes, respectively. Moreover, CNIs transported into the brain directly influence the neurons. CNIs suppress brain-derived neurotrophic factor and tyrosine kinase receptor B expression in the rat brain, which play vital roles in neurogenesis, cell survival, and regulation of synaptic plasticity. However, the part of the brain which is damaged after CNIs are transported to the brain remains unclear. Additionally, there are no potential protective agents against these adverse events in clinical settings.

Therefore, the aims of this study are to investigate the pathological mechanisms of tacrolimus-induced neurotoxicity and explore potential neuroprotective drugs for this neurotoxicity.

In order to investigate tacrolimus toxicity *in vitro* and seek for potential drugs that protect against tacrolimus-induced cell death, SH-SY5Y cells were cultured and treated with tacrolimus in different concentrations for 1-24 h. The results showed tacrolimus decreased the cell viability of SH-SY5Y cells in a concentration-dependent and time-dependent manner. To find out whether tacrolimus induces cell apoptosis, western blot analysis and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) /4',6-diamidino-2-phenylindole (DAPI) staining were performed. The increasing cleaved poly ADP-ribose polymerase (PARP)/PARP and cleaved caspase 3/caspase 3 expression levels at 3 h, and increasing TUNEL positive cells at 3 and 6 h showed that tacrolimus induced apoptosis in SH-SY5Y cells. Combined with the

result that SOD activity was considerably increased 3 h after tacrolimus treatment, suggesting that an imbalance of reactive oxygen species and antioxidant properties correlates with tacrolimus-related neurotoxicity. Five compounds (N-acetylcysteine, glutathione, MK-801, cilnidipine, and ibudilast) were used to investigate their possible protective effects against tacrolimus-induced cell death. Only 100 μM ibudilast significantly reduced the decrease in cell viability by tacrolimus and significantly suppressed the increase in the cleaved PARP/PARP expression level by tacrolimus. Hence, ibudilast had protective effects against tacrolimus-induced cell death *in vitro*.

Next, this study investigated whether tacrolimus induced neurotoxicity in rats and whether ibudilast could be a protective agent against this neurotoxicity. The animal experiments in this study consisted of two parts: the first part involved the administration of saline as a vehicle, tacrolimus 2.5 mg/kg/day, and 5.0 mg/kg/day; the second involved investigating the protective effect of ibudilast 7.5 mg/kg/day against tacrolimus 5.0 mg/kg/day. The results revealed that rats treated with tacrolimus (2.5 mg/kg) showed no chronic neurotoxic behavior until day 15, whereas those treated with tacrolimus (5.0 mg/kg) showed a significant increase in the neurotoxicity score on days 8 and 15. Moreover, tacrolimus concentrations in the cerebrum and cerebellum were correlated with the severity of the neurotoxic score (score 0 vs. score 1–3). Histopathological studies demonstrated that tacrolimus penetrated the brain and caused neurotoxic events by damaging the cerebral cortex and CA1 area of the hippocampus. Moreover, co-administration of ibudilast significantly ameliorated tacrolimus-induced neuron damage followed by neurotoxic behavior. On the other hand, the brain/blood tacrolimus concentration ratio in the tacrolimus + ibudilast group was not different from that in the tacrolimus group. These results imply that tacrolimus penetrated into the brain and caused neurotoxic events based on dose-dependent neuronal cell death in the cerebral cortex and hippocampal CA1 region. Ibudilast, a clinically available nonselective PDE inhibitor, showed a protective effect on both pathological neuronal death and neurotoxic behavior without affecting the transfer of tacrolimus into the brain. Since CNIs are essential drugs in immunosuppressive therapy, these findings regarding ibudilast protection against CNI-induced neurotoxicity lead us propose a novel strategy for patients who have undergone organ transplantation.

以上の結果より、本研究の意義は大きく、博士（臨床薬学）の学位に値すると認める。