Revelation of 2ccPA Function during Repairing of Traumatic Brain Injury

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Traumatic brain injury (TBI) is a type of neurological trauma caused by physical damage such as traffic accidents or a fall and disrupts the brain structure and function due to the biomechanical insult to the cranium. TBI can result in primary injury which is followed by axonal and vascular damage and induce blood brain barrier breakdown by secondary injury, which is followed by inflammation and neuronal death. As the damage leads to sequelae such as motor dysfunction and language impairment, more than 10 million patients are suffering from TBI sequelae annually, and TBI contributes to the deaths of nearly 50,000 people every year. However, TBI treatment is only symptomatic, and fundamental therapeutic agents required to inhibit brain inflammation and neuronal cell death.

One of the candidates for therapeutic agents is cyclic phosphatidic acid (cPA), which has a similar chemical structure and has antagonistic functions to lipid mediator, lysophosphatidic acid (LPA). LPA was found to be elevated in the cerebrospinal fluid of TBI patients and demonstrated that blocking LPA signaling suppresses inflammation in mouse models of TBI. These findings support the notion that cPA may diminish the inflammation after TBI and be effective as a therapeutic agent. In addition, 2-carba-cPA (2ccPA), a metabolically stabilized derivative of cPA in which the phosphate oxygen at the *sn*-2 position is replaced with a methylene group in anticipation of becoming a beneficial therapeutic agent in TBI, has been demonstrated to efficiently reproduce many biological functions of cPA.

In this study, I aimed to applying it as a therapeutic agent, elucidate the mechanism of the effect of 2ccPA on tissue bleeding, inflammation, and neuronal cell death in TBI to elucidate that it is an effective factor in the treatment of TBI. I performed the stab wound were made in murine cerebral cortices using needles as a TBI model and compared the mice that continued to administer 2ccPA and PBS as a vehicle control every other day. The mice were euthanized at 1 hour, days 1, 3, 5, and 7 after the injury, and the brains were removed for analysis.

First, I focused on the effect of 2ccPA on tissue bleeding in primary injury. Hemorrhage levels were assessed by measuring extravasation of serum immunoglobulin G (IgG) in the injured regions in the brains, and it was found that 2ccPA significantly suppressed the level of IgG extravasation after the stab wound injury in the

cerebral cortex.

Next, I focused on the effect of 2ccPA on inflammation in secondary injury. It is known that glial cells in the brain are deeply involved in the induction of inflammation during brain injury. Therefore, I focused on microglia and astrocyte, a type of glial cell, as an indicator of inflammation. To determine whether 2ccPA affects the activation of microglia cells and astrocytes in the cerebral cortex after TBI, I examined the expression levels of a microglial marker, ionized calcium binding adaptor molecule 1 (Iba1), and glial fibrillary acidic protein (GFAP) in the vicinity of the stab wounds using immunoenzymatically and immunofluorescent stained mouse brain sections. 2ccPA suppresses the microglial cell and astrocyte activation around the wound region and contributed to the repair of inflammation. Additionally, 2ccPA promoted microglial polarization towards a neuroprotective phenotype (M2) rather than a neurotoxic phenotype (M1) and thereby suppressed the activation of inflammation after the stab wound.

Finally, I focused on the effect of 2ccPA on neuronal cell death in secondary injury and the mechanism of how 2ccPA regulates neuronal cell death after TBI. I analyzed the percentage of cleaved caspase-3-positive cell numbers in NeuN-positive in the stab-wounded cortices. The administration of 2ccPA to injured mouse brains significantly suppressed the percentage of neuronal death. Furthermore, I found that 2ccPA contributes to neuroprotection via astrocytes after stab wound injury and as a detailed mechanism, tenascin C one of the extracellular matrix proteins expressed from astrocyte, is a major neuroprotective factor after TBI.

This study clarified the hemostatic, anti-inflammatory, and neuroprotective effects of 2ccPA in TBI, which may lead to the development of fundamental therapeutic agents for TBI patients by applying 2ccPA. In addition, this new finding that anti-inflammatory and neuroprotective effects of 2ccPA via glial cells in the brain will contribute to the advancement of research on glial function in the brain. These results of this study are expected to serve as basic research for the development of medical treatment for TBI by targeting glial cells in the brain.