Report on "Radiation Disaster Recovery Studies"

Course: Radiation Disaster Medicine

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Regarding"Radiation Disaster Recovery Studies"

Even now, one decade after the disaster, Fukushima prefecture has not been able to recover to its pre-disaster state. There are many things that are unlikely to go back. Recovery from radiation disasters is such a difficult path. It is so difficult to recover from a radiation disaster.

Radiation disaster is troublesome in that it continues linearly rather than dot on the time axis, unlike other local disaster. As a result, it is not possible to start immediate recovery after a disaster.

From survey of return rate for each difficult-to-return area that was gradually released, it is clear that the longer it takes to be able to return, the lower the return rate. Unless the population grows, it will be difficult to recover it to its pre-disaster state. This is because it is difficult to generate the demand and economic power to support the convenient services and businesses that were in pre-disaster state. Therefore, one of the keys to reconstruction is the return of people.

There are many factors involved in whether people return. It is difficult to show a definitive method of how people return. But, it can be said that one of the basic and important point of how people return is health of people, physically and mentally. It is difficult to evaluate the physical effects of radiation disaster because it can have deterministic and stochastic effects. However, there is no doubt that there is a mental effect of radiation disasters, such as depression, delirium, and progression of dementia. The neuroinflammation due to external stress is known to cause these. In previous studies, it was reported that microglial activation is involved in neuroinflammation due to external stress. However, the mechanism is unknown.

Microglia and neuroinflammation have also been suggested to be involved in many other diseases, sepsis-associated encephalopathy (SAE) is one of those that cause delirium and progression of dementia among the diseases that I specialize in as a doctor. SAE is a diffuse brain dysfunction associated with sepsis that is not attributed to a direct central nervous system (CNS) infection, structural abnormalities, or other types of encephalopathy. The mechanism of SAE is unknown, too. Interestingly, there is the same animal model used for both depression and delirium and SAE; murine endotoxemia model.

The mechanisms of these diseases are possible to be similar and that the results of research on SAE is possible to lead to the prevention and treatment of depression, delirium, and progression of dementia. Therefore, I decided to work on research on SAE.

Animal studies have shown that microglial cells activated by systemic inflammation cause the release of pro-inflammatory mediators such as TNF-α and IL-1β and play an important role in cognitive impairment as a model of sepsis-associated delirium. And translocator protein 18 kDa; TSPO is

upregulated in immune cells such as microglia, it has attracted a surge of interest as a biomarker of the inflammatory response in the CNS. TSPO is mitochondrial membrane proteins. Although there are many unclear points about its function, involved in inflammation such as cytokine release. I make a hypothesis that TSPO causes neuroinflammation in SAE, and we conducted experiments to investigate cytokine expression, microglial activity, and locomotor activity using murine endotoxemia model to reveal the involvement of TSPO in SAE.

Title of Doctoral Thesis

Pharmacological and genetic inhibition of translocator protein 18 kDa ameliorated neuroinflammation in murine endotoxemia model

Summary of Doctoral Thesis

Sepsis-associated encephalopathy (SAE) is a diffuse brain dysfunction associated with sepsis that is not attributed to a direct central nervous system (CNS) infection. SAE develops a wide variety of disorders in neural functions, ranging from delirium to long-term cognitive impairment. The mechanism of SAE is unknown.

Previous studies have shown that microglial cells activated by systemic inflammation cause the release of pro-inflammatory mediators such as TNF-α and IL-1ß and play an important role in cognitive impairment as a model of sepsis-associated delirium. Considering the significant role of microglia in the immune response in the CNS, elucidating the molecular mechanisms underlying microglial activation will provide deep insight into understanding SAE.

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I make a hypothesis that TSPO causes neuroinflammation in SAE, and we conducted experiments to investigate cytokine expression, microglial activity, and locomotor activity using murine endotoxemia model to reveal the involvement of TSPO in SAE.

C57BL/6J male mice administered a single intraperitoneal injection of Lipopolysaccharide (LPS) was chosen as endotoxemia model. We use pharmacological and genetic inhibition of TSPO. TSPO antagonist; ONO-2952 was for pharmacological, and the TSPO knockout was for genetic.

We make 4 groups of mice, first is vehicle-pretreated wildtype with saline injection for control group, and second is vehicle-pretreated wildtype with LPS injection. Third and fourth are ONO-pretreated wildtype with LPS injection for pharmacological inhibition and TSPOKO with LPS injection for genetic inhibition.

The ONO-2952 solution, or vehicle solution, was delivered ad libitum in the drinking bottles provided to the animals, beginning 4 days before injection. All mice administered a single intraperitoneal injection of LPS or saline after open field test (Pre). And, all mice euthanized after open field test (Post)

which was done 24 hours after injection. Brain tissue is Sampled for quantitative PCR (qPCR) or immunohistochemistry.

To investigate the relationship between typical inflammatory cytokines and *TSPO* expression, qPCR was done. LPS administration significantly increased the expression of *TNF-α* and *IL-1β* as well as *TSPO* in the hippocampus. ONO-2952 pretreatment significantly reduced the expression of *TNF-α* and *IL-1β*, which did not alter the transcriptional level of *TSPO*. No discernible level of *TSPO* expression in TSPOKO. Germ-line knockout of *TSPO* gene significantly reduced the expression of *TNF-α*.

To investigate changes in microglial activation, immunohistochemistry of ionized calcium-binding adapter molecule 1 (Iba1) in the hippocampus was performed, and fluorescence intensity was quantified as microglial activation. LPS administration significantly increased microglial activation. ONO-2952 pretreatment significantly reduced microglial activation of LPS. Microglial activation of LPS was significantly reduced in TSPOKO group.

To investigate the localization of TSPO expression, immunohistochemistry of TSPO and Iba1 in the hippocampus was performed, and fluorescence intensity was quantified. TSPO was predominantly localized in the blood vessels, whereas a minimally discernible level of TSPO was observed in Iba1-positive microglia in the intact brain. LPS administration significantly increased TSPO expression in activated microglia. ONO-2952 pretreatment significantly reduced TSPO expression in activated microglia. No TSPO expression in TSPOKO group.

To investigate the effects of contents so far on locomotor activity, open field test was done. Because, delirium is the mildest symptom of SAE, and human delirium has been shown to resemble the sickness behavior in animal models. The ratio of total distance and total movement duration of Post decreased compared to Pre was evaluated. LPS administration significantly reduced locomotor activity. ONO-2952 pretreatment significantly suppressed the reduction of locomotor activity. The reduction of locomotor activity was significantly suppressed in TSPOKO.

TSPO inhibition suppressed microglial activation and pro-inflammatory cytokine production induced by systemic inflammation, and reduction of locomotor activity was suppressed in behavioral test. It was suggested that TSPO plays an important role in the SAE mouse model. Monitoring of TSPO activity in other septic animal models and human sepsis in the future may provide an understanding of the molecular mechanism of SAE.

○Other theses published in academic research journals

Kazuya Kikutani, Hiroshi Giga, Koji Hosokawa, Nobuaki Shime, Hidenori Aizawa : Microglial translocator protein and stressor-related disorder. Neurochemistry International, Volume 140: 104855, 2020.