

論文の内容の要旨

論文題目 Vagus nerve stimulation even after injury ameliorates cisplatin-induced
 nephropathy via reducing macrophage infiltration
 (迷走神経刺激は障害後であっても腎保護効果を有する)

氏名 宇仁 理恵

There are several interactions between the kidney and other important organs. Interactions with the heart and liver are referred to as the cardio-renal syndrome and hepato-renal syndrome, respectively. An interaction between the nervous system and the kidney has also been recognized. Acute kidney injury (AKI) is estimated to affect approximately 15% of hospitalized patients and 60% of critically ill patients. Despite substantial advancements in healthcare technology and availability, the incidence of AKI is increasing, and its morbidity and mortality remain high. In addition, AKI is a risk factor of chronic kidney disease (CKD) and end-stage renal disease (ESRD). Therefore, it is very important to prevent AKI development and progression to CKD. Nevertheless, main treatment options for AKI are supportive care such as avoidance of nephrotoxic agents and hydration. Specific kidney-oriented therapy is yet to be developed. The nervous system processes the input from peripheral inflammation and issues output for effector cells in a circuit referred to as the cholinergic anti-inflammatory pathway (CAP), with the vagus nerve as an essential component. Novel therapies including ultrasound treatment and vagus nerve stimulation (VNS) aim to modulate this pathway and have been helpful in attenuating inflammatory diseases such as sepsis, inflammatory bowel disease and rheumatoid arthritis. VNS was approved by the Food and Drug Administration in 1997 for the treatment of refractory partial-onset epilepsy and in 2005 for chronic or recurring depression. Its safety is proven in that more than 100,000 VNS devices have been implanted in over 75,000 patients worldwide. Animal studies have suggested that these methods may also be effective for kidney protection in AKI, but the exact mechanism underlying this effect remains unclear.

Previously, it was reported that vagus nerve stimulation protected the kidney from ischemia-reperfusion injury (IRI) through activation of the CAP. Although there are many kinds of inflammatory cells such as B cells, T cells, and dendritic cells in the spleen, the anti-inflammatory effect of CAP stimulation is delivered through activation of $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR) on splenic macrophages. Considering its anti-inflammatory effect, VNS is a potent tool for treating inflammatory disorders including AKI. However, there have been no reports that have demonstrated the effectiveness of VNS after kidney injury. In this study, I hypothesized that VNS is renoprotective even after the development of AKI, and I investigated the renoprotective effect of VNS in IRI model and cisplatin-induced nephropathy model, which is one of the common animal aseptic AKI models. Cisplatin is a major tumoricidal drug that has long been used for the treatment of a number of cancers. Cisplatin is taken up in

renal tubular epithelial cells and induces AKI, an important dose-limiting toxicity that frequently leads to cessation of therapy, by damaging the DNA inside the cells.

First, I evaluated whether VNS was effective after injury in IRI model, but it was not renoprotective. Then, I switched to a cisplatin induced nephropathy model. C57BL/6 (7–10 weeks old, 20–25 g) mice were injected with cisplatin, and VNS was conducted 24 hours before injection, 24 hours and 48 hours after injection. I performed VNS by mid-cervical incision and placing bipolar silver electrodes on the left vagus nerve and applied electrical stimulation for 10 minutes. For the sham operation group, we simply exposed the vagus nerve using an identical incision. Surprisingly, pre-VNS did not show renoprotection in cisplatin-induced nephropathy model. Only VNS 24 hours after cisplatin administration, when plasma creatinine and blood urea nitrogen (BUN) levels were not elevated significantly but early tubular injury was already developed, was effective. Kidney function (plasma creatinine and BUN levels), histology, and a kidney injury marker (Kim-1), which is known to be upregulated early before the elevation of plasma creatinine in cisplatin-induced nephropathy, were evaluated 72 hours after cisplatin administration. VNS treatment significantly suppressed cisplatin-induced kidney injury in terms of plasma creatinine level and attenuated tubular injury supported by histology and Kim-1 expression. In order to further explore the role of the spleen and splenic macrophages, key players in the CAP, splenectomy and adoptive transfer of macrophages treated with the selective $\alpha 7$ nicotinic acetylcholine receptor agonist GTS-21 were conducted.

I conducted splenectomy or sham operation 5 days before cisplatin injection. The renoprotective effect was abolished by splenectomy. There were no significant differences between splenectomized mice and sham operated mice in kidney function and histology, suggesting the importance of spleen as a reservoir of immune cells and a place for immune cell interactions. To investigate the role of the specific component of the spleen, I conducted adoptive transfer of splenic macrophages. This is because the anti-inflammatory effect of CAP stimulation is delivered through activation of the $\alpha 7$ nAChR on splenic macrophages. We collected the splenic macrophages using the magnetic cell separation method (MACS) and incubated with vehicle or the selective $\alpha 7$ nAChR agonist GTS-21 for 1 hour, and then intravenously injected to recipient mice. Adoptive transfer of macrophages itself did not affect the kidney function of healthy control mice, but at 72 hours after cisplatin injection, previously increased plasma creatinine and BUN levels were significantly decreased in the mice that had received the GTS-21-treated macrophages after disease induction. Tubular injury was also ameliorated by GTS-21-treated macrophage transfer. The expression level of Kim-1 in the kidney was also decreased significantly in the GTS-21-treated macrophages-injected group. This experiment indicates that interaction between GTS-21-treated macrophages and tissue resident cells or circulating immune cells is essential for kidney protection. Next, with the aim of investigating the effect of CAP activation on systemic inflammation, I evaluated the expression of various cytokines in plasma. Among 23 cytokines tested, the levels of four cytokines—CCL2, CCL11 (Eotaxin), G-CSF, and IL-12(p40)—were significantly reduced by VNS. Followingly, I evaluated the expression level of those

cytokines in the kidney. Real-time PCR showed a significant decline in the expression levels of CCL2, IL-12b, and G-CSF in the mice treated with cisplatin followed by VNS. As macrophages have been proven to be another key player in the CAP and CCL2, one of the most potent chemokines promoting monocyte and macrophage chemotaxis, was downregulated both in the kidney and blood, I hypothesized that CAP activation prohibits macrophage migration to the injured kidneys. In vitro experiments demonstrated that GTS-21 significantly decreased the expression of CCL2 in cisplatin treated macrophages. Therefore, I evaluated the macrophage infiltration of the kidney in the VNS-treated or sham-treated mice after cisplatin administration by flow cytometry analysis and immunohistochemical staining of F4/80 positive macrophages. Flow cytometry analysis showed that, at 24 hours after cisplatin injection, the number of CD45-positive leukocytes, including macrophages and T cells, was slightly elevated, and the number of the cells in each leukocyte fraction was further increased 72 hours after cisplatin administration. In contrast, VNS significantly reduced the infiltration of macrophages by cisplatin. This reduction of macrophage infiltration into the kidney was confirmed by immunohistochemical staining of F4/80-positive macrophages.

In summary, VNS after injury protected the kidney in cisplatin-induced nephropathy model but not in IRI model. Pre-VNS, which was effective in IRI model, was not protective for cisplatin model. This discrepancy might stem from the difference of disease model or experimental conditions. Future studies will be needed for other kidney injury models. I also exhibited that activation of the CAP suppressed CCL2 expression and following macrophage infiltration into the kidney, thereby repressing the local inflammation in the kidney. What is interesting here is macrophage could be both pro- and anti-inflammatory. Once they are stimulated by CAP activation, they become protective. On the other hand, without the stimulation, they infiltrate in the injured kidney and promotes inflammation.

To the best of my knowledge, this is the first study to demonstrate the effectiveness of VNS after kidney injury. Considering the feasibility and anti-inflammatory effects of VNS, the findings suggest that VNS may be a promising therapeutic tool for acute kidney injury.