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Title	Study on the Pharmacological Effects of Organogermanium Compound THGP on RIG-I-Mediated Viral Sensing and Viral Replication during Influenza A Virus Infection [an abstract of dissertation and a summary of dissertation review]
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学位論文内容の要旨

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学位論文題名

Study on the Pharmacological Effects of Organogermanium Compound THGP on RIG-I-Mediated Viral Sensing and Viral Replication during Influenza a Virus Infection

(インフルエンザウイルス感染時における RIG-I を介したウイルス認識およびウイルス複製に対する有機ゲルマニウム化合物 THGP の薬理的効果に関する研究)

During microbial infections, microbes-associated molecular patterns (MAMPs) are mainly recognized by pattern recognition receptors (PRRs), including transmembrane-type Toll-like receptors (e.g., TLR3 and TLR9) and cytoplasmic sensors, such as RIG-I (retinoic acid-inducible gene I), MDA5 (melanoma differentiation-associated protein 5) and cGAS (cyclic GMP-AMP synthetase) to trigger antiviral immune response. In addition to these interactions, the recognition of viral nucleic acids by viral polymerase is a crucial event during viral replication. It has previously been reported that, a hydrolysate of Ge-132, 3-(trihydroxygermyl) propanoic acid (THGP) shows a modulatory effect on microbial infections, inflammation, and immune responses. However, the detailed mechanism by which THGP can modify these processes during viral infections remained unknown. In this dissertation, the effect of THGP on RIG-I-mediated viral sensing and viral replication during influenza virus (FluV) infection have been investigated. The results of this study demonstrated that THGP can specifically downregulate type I interferon (IFN) production in response to stimulation with a cytosolic RNA sensor RIG-I ligand 5'-triphosphate RNA (3pRNA). Consistently, treatment with THGP resulted in the dose-dependent suppression of IFN induction upon infections with FluV, which are known to be mainly sensed by RIG-I. Mechanistically, THGP directly binds to the 5'-triphosphate moiety of viral RNA and competes with RIG-I-mediated recognition. Furthermore, it has been found that THGP can directly counteract the replication of FluV by inhibiting the interaction of viral polymerase with RNA genome. Finally, FluV RNA levels were significantly reduced in the lung tissues of THGP-treated mice when compared with untreated mice. These results suggest a possible therapeutic implication of THGP showing a direct antiviral action, together with the suppressive

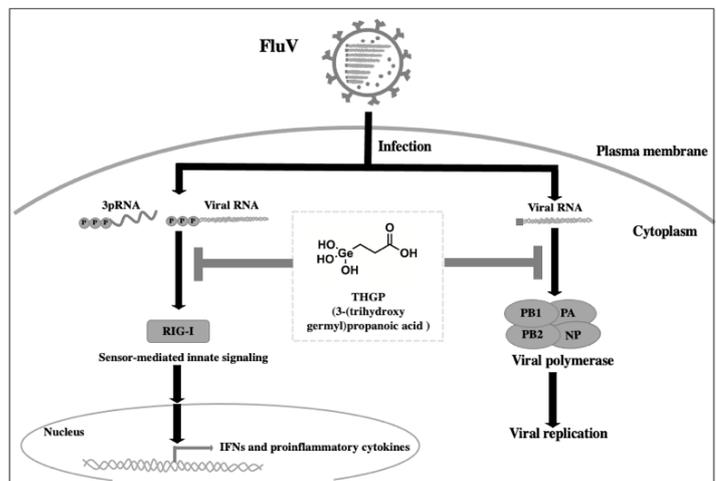


Figure 1: A schematic model showing the dual effect of THGP on RIG-I-mediated viral sensing and viral replication during influenza virus (FluV) infection

activity of innate inflammation (Figure 1).

The structure of this dissertation is as follows:

Chapter 1 is introduction. In this part, I briefly described about innate and adaptive immunity. Next, I systematically summarized the innate immune responses, mainly described different PRRs-mediated innate immune responses including RIG-I like receptors, cGAS, and TLR signaling pathways during viral infections. In addition, the properties of viruses such as genomic organization of different viruses, life cycle, replication mechanism, pathophysiology of FluV and the immune escape strategies adopted by different viruses have also been described. Furthermore, I introduced about THGP that is research subject in this study and its different therapeutic approaches.

In **Chapter 2**, the purpose of this study has been described.

In **Chapter 3**, the experimental details such as materials and methods are given.

In **Chapter 4**, the results of this study are presented. At first, by using *in vitro* and *in vivo* models, I found that THGP itself did not affect cell growth and did not show any toxic effect on the survival rate and the body weight curves of mice. Next, I examined the effect of this compound on RIG-I, MDA5, cGAS and TLR-4's ligand induced type I IFN responses and the results of this study demonstrated that THGP can specifically downregulate IFN- β production in response to stimulation with a cytosolic RNA sensor RIG-I ligand 5'-triphosphate RNA (3pRNA) but not double-stranded RNA, DNA or lipopolysaccharide, which are ligands for MDA5, cGAS and TLR-4 respectively. Consistently, treatment with THGP resulted in dose-dependent suppression of type I IFN induction upon infections with FluV and vesicular stomatitis virus that are known to be mainly sensed by RIG-I but not EMCV, which is recognized by MDA5. Next, I investigated THGP acts on which level of RIG-I signaling pathway and it has been found that, THGP did not affect the uptake of 3pRNA into cells. Mechanistically, the detailed molecular analyses displayed that, THGP directly binds to the 5'-triphosphate moiety of viral RNA and 3pRNA and competes with RIG-I-mediated recognition, resulting in decreased type I interferon production. Moreover, THGP treatment restored the body weight loss and improved the survival rate of FluV-infected mice. Furthermore, it has been observed that, THGP can directly counteract replication of FluV by inhibiting the interaction of viral polymerase with RNA genome. Finally, FluV nucleoprotein RNA levels were significantly reduced in the lung tissues of THGP-treated mice, as compared with untreated mice. These results suggest a possible therapeutic implication of THGP that shows direct antiviral action together with a suppressive activity of innate inflammation.

In **Chapter 5**, future perspectives and discussion have been described. The high mutation rate of the FluV genome makes this virus a continual and re-emerging threat to human health. To combat future FluV pandemics, we need therapeutics to supplement or replace current antiviral drugs against FluV. In this regard, THGP could be a novel antiviral agent that directly targets FluV genome interfering with the interaction between viral RNA and viral polymerase.