氏　　名	林　宏明
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学位論文題目	Pharmacological Characterization of TAS05567, a Potent and Selective Inhibitor of Spleen Tyrosine Kinase, in Animal Models of Inflammatory Diseases and B-cell Malignancies （炎症性疾患モデル及びB細胞性リンパ腫モデルを用いた選択的且つ強力なSyk阻害剤TAS05567の薬理学的特性に関する研究）

	（職名）	（学位）	（氏名）
主　査	筑波大学　教授（協働大学院）	博士（農学）	吉木　淳
副　査	筑波大学　教授（協働大学院）	博士（獣医学）	保富　康宏
副　査	筑波大学　教授（協働大学院）	農学博士	神谷　俊一
副　査	筑波大学　准教授	博士（農学）	宮前　友策

Abstract of thesis

Spleen tyrosine kinase (Syk) is a non-receptor cytoplasmic tyrosine kinase that is primarily expressed by cells of hematopoietic lineage. Syk influences diverse biological events, such as cytokine production, degranulation, differentiation and proliferation, suggesting that Syk is profoundly involved in the development of autoimmune diseases, allergic diseases and malignancies. Indeed, several ATP-competitive Syk inhibition, notably fostamatinib, have already been evaluated in clinical trials with patients of autoimmune diseases, including rheumatoid arthritis (RA) and immune thrombocytopenic purpura (ITP). Even though forstamatinib was efficacious against chronic refractory ITP, it is unclear whether inhibition of Syk activity specifically contributed to the clinical response and adverse events observed in the clinical trials, as this drug inhibits several kinases, including vascular endothelial growth factor receptor (VEGFR) 2 and Janus kinase (JAK) 2. Another Syk inhibitor, entospletinib, has been developed for the treatment of leukemia, but it inhibits multiple protein kinases within 100-fold of its inhibitory concentration 50 (IC50) value for Syk. These findings suggest that novel Syk inhibitors with different activity profiles from the above-mentioned inhibitors are required to provide new therapeutic options. On this background, the author tried to identify a novel, potent, and selective orally available Syk inhibitor. Investigation of structure-activity relationships led to identification of TAS05567 from chemically synthesized Syk inhibitory compounds. The profile of TAS05567 was characterized by using biochemical and cell-based assays. The author further assessed the therapeutic potential of the compound for autoimmune disease, transmission allergic disease and B-cell malignancies

using preclinical animal models. TAS05567 potently inhibited the activity of Syk, showing an average IC50 of 0.37 nM in enzyme assays. TAS05567 was more potent than R406 (an active metabolite of fostamatinib), exhibiting an average IC50 of 13 nM. The specificity of TAS05567 for Syk was determined at 50 nM (approximately 140 times the IC50 value) using a panel of 192 kinases. In these analyses, TAS05567 only showed >70% inhibition of 43 kinases. In addition, the IC50 values of TAS05567 and R406 were determined for VEGFR2 and JAK2. Whereas TAS05567 showed at least 10-fold selectivity for Syk over the 4 kinases, R406 showed equal or greater efficacy against these kinases. Next, the author evaluated the effectiveness of TAS05567 in blocking the B-cell receptor (BCR)-dependent signaling cascade using B-cell line. TAS05567 marked inhibition of the phosphorylation of BLNK (IC50=1.8 nM), PLCγ2 (IC50=23 nM) and Erk1/2 (IC50=9.8 nM) induced by anti-IgM. TAS05567 also showed stronger inhibition of these molecules in the B-cell receptor-dependent signaling cascade than R406. In addition, the author evaluated the inhibitory effects of TAS05567 on FcγR-mediated TNF-α release by macrophages. TAS05567 showed concentration-dependent inhibition of TNF-α production by IgG stimulation. Syk also contributes directly to bone resorption in RA via its kinase activity-mediated regulation of receptor activator of nuclear factor kappa-B (RANK) signaling pathway that is essential for differentiation of osteoclasts. TAS05567 strongly inhibited the formation of mature osteoclasts induced by M-CSF and RANKL in a concentration-dependent manner.

Next, the author evaluated effects of TAS05567 in autoimmune rodent models of arthritis, collagen antibody-induced arthritis (CAIA) and collagen-induced arthritis (CIA). TAS05567 inhibited the development of arthritis based on clinical scores of CAIA or hind paw volume in CIA. Additionally, serum biomarker (MMP-3, IgG and COMP) levels were significantly lower in the TAS05567 groups than in the controls. In the experiment of rat CIA, the author pathologically examined the left and right hind paws. TAS05567 significantly reduced inflammatory cell infiltration into the synovium and pannus formation and markedly ameliorated damage to cartilage and bone. The author monitored changes in body weight during the treatment as an index of general toxicity and assessed red blood cell count and hemoglobin levels because anemia was observed in patients during clinical trials of drugs with JAK2 inhibition. TAS05567 did not significantly reduce body weight, the red blood cell count or hemoglobin level compared with control rats. Furthermore, the author examined whether TAS05567 could attenuate platelet count decreases in mice with thrombocytopenia induced by administration of an antiplatelet antibody, because Syk is profoundly involved with the pathogenesis of ITP via the activation of FcγR. In mice injected with an anti-CD41 antibody, the platelet count decreased by more than 50% compared with sham mice. In this model, TAS05567 provided significant protection against anti-CD41 antibody-induced thrombocytopenia in a dose-dependent manner. The author evaluated the therapeutic effects of TAS05567 on other indications, including type 1 allergic diseases as well as B-cell malignancies, using *in vivo* assay as well as preclinical animal models. Syk reportedly plays a crucial role in FcεR-mediated production of chemical mediators by mast cells. Therefore, the author evaluated the inhibitory effects of TAS05567 on IgE-induced histamine release by RBL-2H3 cells. TAS05567 suppressed histamine release by IgE stimulation in a concentration-dependent manner. The author next examined whether TAS05567 is a potent drug for treatment of B-cell malignancies, and confirmed cytotoxic activity of TAS05567 toward diffuse large B-cell lymphoma (DLBCL) cells, which depend on chronic active BCR signaling for survival. TAS05567 reduced the cell viability of several DLBCL cell lines in a concentration-dependent manner. To examine whether TAS05567 could suppress the type 1 allergic reactions *in vivo*, the author used 2,4,6-trinitrophenol (TNP)-IgE transgenic mice. Administration of picryl chloride to the ears of the mice provoked swelling, which reached a

peak 2 h after antigen challenge. TAS05567 strongly suppressed ear swelling from 1 to 4 hrs after antigen challenge.

Finally, the *in vivo* antitumor activity of TAS05567 was evaluated in SCID mice bearing SU-DHL-10 xenograft. TAS05567 significantly inhibited the tumor growth and decreased the levels of EGR2 mRNA and phosphorylation of BLNK in the tumor tissues, as compared with vehicle control. The author determined the pharmacologic profile of a novel selective Syk inhibitor, TAS05567, which potently inhibited BCR signaling, FcR-dependent cellular functions, osteoclast differentiation and proliferation of DLBCL cells. After oral administration, TAS05567 not only ameliorated symptoms in animal models of several inflammatory diseases, but also suppressed tumor growth in the mouse xenograft model using DLBCL cells. Notably, TAS05567 showed less off-target activity, such as VEGFR2 and JAK2 inhibition, than R06, suggesting that the efficacy/toxicity profiles of the Syk inhibitor and fostamatinib differ. The data suggest that TAS05567 would be an efficacious novel treatment for inflammatory diseases as well as B-cell malignancies.

Abstract of assessment result

【Review】

Spleen tyrosine kinase (Syk) is a non-receptor cytoplasmic tyrosine kinase which influences diverse biological events and pathogenic conditions such as autoimmune diseases, allergic diseases and malignancy. Several Syk inhibitors have already been tested and evaluated in clinical trials with relevant patients. However, the results have so far been reported as unsatisfactory due to the lack of specificity and adverse effects. On this background, the applicant in this study tried to identify a novel, potent, and selective orally available Syk inhibitor and succeeded in identification of TAS05567 from chemically synthesized Syk inhibitory compounds. The applicant carefully used several experimental systems of *in vitro* biochemistry, cell-based assays and *in vivo* animal experiments, and pharmacologically characterized the novel compound TAS05567. The data clearly indicated the high potency and selectivity of the TAS05567 on Syk and the efficacy of TAS05567 on inflammatory conditions and B-cell malignancy in animals. The results were properly presented and interpreted followed by appropriate discussions and strategic future plans with well selected references to establish safe and efficacious therapies. The achievement of this study will contribute in the field of innovation and disease mechanism and facilitate the future development of new drugs and better therapies for unmet needs of the relevant disease patients.

【Result】

The final examination committee conducted a meeting as a final examination on January 7, 2019. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All the committee members reached a final decision that the applicant has passed the final examination.

【Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded with Doctor of Philosophy in Disease Mechanism.