氏名（本籍）	中村康次（長崎県）
学位の種類	博士（薬学）
学位記番号	乙第359号
学位授与の日付	2019年9月30日
学位授与の要件	学位規則第4条第2項該当
学位論文題目	**Pharmacological studies on immunosuppressive effects of the novel JAK inhibitor on allograft rejection in preclinical transplantation models** **（臓器移植拒絶反応に対する新規JAK阻害剤の免疫抑制効果に関する薬理学的研究）**
論文審査委員	（主査）教授　礒濵洋一郎 教授　西川　元也　　准教授　月本　光俊 教授　樋上　賀一　　教授　北村　大介

論文内容の要旨

Various immunosuppressive drugs have been developed to suppress rejections occurring at the time of organ transplantation and engrafting grafts. The current standard immunosuppressive protocol consists of three drug groups, calcineurin inhibitors (CNIs), corticosteroids, and mycophenolate mofetil (MMF). In particular, CNIs, tacrolimus and cyclosporin are the most important cornerstone on the basis of their significant effectiveness. However, the use of CNIs is limited due to their side effects, such as nephrotoxicity. In addition, the improvement of long-term allograft survival by control of chronic rejection is another unmet medical need in transplant medical treatments because current immunosuppressive drugs cannot control graft loss due to chronic rejection, such as interstitial fibrosis, tubular atrophy, glomerulosclerosis and intimal thickening. The nephrotoxicity by CNIs is considered as one of the causes of chronic rejection. Therefore, new drugs that can suppress chronic rejection is important to improve the long-term prognosis of transplanted graft.

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) families in mammals

consist of four JAK members, namely JAK1, 2, 3 and tyrosine kinase 2 (TYK2). These kinases are intimately involved in signal transduction from immune cell surface receptors, particularly cytokine receptors such as IL-2, IL-6, IL-12, IFN-γ and erythropoietin. Accordingly, JAK families have been intensely studied as promising targets for the treatment of rheumatoid arthritis, inflammatory bowel disease, transplant rejection and other immune-mediated disorders. Currently, tofacitinib, balicitinib and peficitinib have been approved as therapeutic drugs for rheumatoid arthritis. In transplantation, tofacitinib significantly improved allograft rejection in renal transplant patients, but has not been launched as a drug for allograft transplant rejection because of adverse outcomes by the overexposure. Several JAK inhibitors have demonstrated immunosuppressive efficacy against acute rejection but not chronic rejection in preclinical studies. Therefore, it is still unknown whether JAK inhibitors have the therapeutic potential to prevent chronic rejection.

We generated AS2553627 as a novel and potent JAK inhibitor. Here, the aims of this investigation are to evaluate its efficacy against not only acute rejection but also chronic rejection in rat and monkey transplantation models and to investigate the potential as a therapeutic agent to achieve CNI-sparing for transplantation.

Firstly, we compared the in vitro pharmacological profiles of AS2553627 with those of the existing JAK inhibitors tofacitinib and peficitinib to reveal the characteristics of AS2553627 as a JAK inhibitor. AS2553627 inhibited JAK kinases activity without inhibitory effects on any other kinases. The IC_{50} values for JAK kinases activity of AS2553627 were lower than those of tofacitinib and peficitinib. IL-2 is an important cytokine in acute rejection which activates JAK1 and 3 and regulates the growth and differentiation of T cells. AS2553627 potently inhibited rat and human T cell proliferation by IL-2 stimulation compared with tofacitinib and peficitinib. Next we evaluated the preventive effect on acute rejection in a rat cardiac transplant model. Oral administration for 14 days of AS2553627 alone or co-administration with a sub-therapeutic dose of tacrolimus effectively prolonged allograft survival times, suggesting the synergistic effect with tacrolimus on acute rejection.

To evaluate the effect on chronic rejection in cardiac transplantation, recipients were administered a therapeutic dose of tacrolimus for 90 days. In combination with tacrolimus, AS2553627 significantly reduced allograft vasculopathy and fibrosis that optimal dose of tacrolimus could not inhibit. Renal transplantation is the most common among all organ transplantation. We evaluated the effect of AS2553627 on chronic rejection in a rat renal transplantation model and conducted multilateral analysis to reveal the detailed mechanism of action of AS2553627 on chronic rejection. AS2553627

in combination with tacrolimus exhibited low plasma creatinine and a marked decrease in urinary protein and kidney injury markers. At 13 weeks after transplantation, AS2553627 also inhibited chronic allograft histopathological changes such as glomerulosclerosis, interstitial fibrosis and tubular atrophy. In addition, upregulation of cell surface markers, fibrosis and inflammation-related genes were reduced by AS2553672, particularly CD8 and IL-6 mRNAs, indicating that AS2553627 prevented cell infiltration and inflammation in renal allografts. These data in rodent transplantation models suggest the therapeutic potential of AS2553627 to prevent the development of acute and chronic rejection and improve long-term allograft survival after transplantation.

Organ transplantation in non-human primates (NHPs) has been extensively used to test new immunosuppressants because immune system and physiological features in NHPs closely resemble those in humans. MMF has been used as a standard immunosuppressive therapy as well as tacrolimus, but often causes gastrointestinal adverse effects. To improve long-term graft survival, new immunosuppressive drugs that can replace MMF in combination therapy with low-dose CNIs are needed. Thus, we investigated the efficacy of AS2553627 in combination with a sub-therapeutic dose of tacrolimus on allograft rejection in a monkey renal transplantation model. Furthermore, we investigated the possibility of replacing MMF with AS2553627 by comparing with the efficacy of a clinically relevant dose of MMF. In combination therapy with tacrolimus, pharmacokinetic analysis indicated that MMF 20 mg/kg/day achieved the clinical target exposure and prolonged allograft survival, with median survival time (MST) of 24 days. Oral administration of AS2553627 in combination with tacrolimus significantly prolonged allograft survival to MST of > 90 days with low plasma creatinine levels. Histopathological analysis indicated that acute T cell-mediated rejection events such as vasculitis and interstitial mononuclear cell infiltration were significantly inhibited by AS2553627 compared with MMF. All AS2553627-treated monkeys surviving > 90 days exhibited less interstitial fibrosis and tubular atrophy than MMF-treated monkeys. These results suggest that AS2553627 replacing MMF is an attractive CNI-sparing strategy to prevent renal allograft rejection.

In the present study, we generated a novel JAK inhibitor, AS2553627, which has potent inhibitory activities on JAK kinases and the suppressive effect on T cell activation compared with existing JAK inhibitors. AS2553627 monotherapy or combination with sub-optimal dose of tacrolimus strongly prevented acute rejection in rat cardiac and monkey renal transplantation models. These results suggest that AS2553627 can achieve CNI-sparing to reduce the CNI-toxicity which is currently a problem in the clinical settings. Furthermore, AS2553627 also inhibited chronic allograft rejection

such as vasculopathy, fibrosis, tubular atrophy and glomerulosclerosis which were found in long-term survived animals. Taken together, AS2553627, a novel JAK inhibitor has the therapeutic potential to meet current unmet medical needs in transplantation.

論文審査の結果の要旨

本論文の科学的妥当性、独創性、有用性等について審査を行った。
移植医療において免疫抑制剤薬は最も重要な役割を果たしている。特に、タクロリムスなどのカルシニューリン阻害剤（calcineurin inhibitor: CNI）をはじめとした免疫抑制薬により臓器移植後の急性拒絶反応の制御が可能となった現在では、移植医療の最大の課題は長期生着のための慢性拒絶のコントロールである。また、腎毒性などの副作用の面でも、CNI と併用することでこれを減量できる新規免疫抑制薬の開発が切望されている。申請者は、これらの課題を解決する新たな薬物として高い選択性を持つ Janus kinase (JAK) 阻害薬 AS2553627 に着目し、ラットおよびサルの移植モデルにおいて、AS2553627 が急性拒絶だけでなく慢性拒絶を抑制可能であるか、さらに CNI 減量が達成可能であるかを生化学的および薬理学的に評価している。

第 1 章では *in vitro* の実験系で酵素阻害薬としての生化学的な特性を調べ、AS2553627 が既存の JAK 阻害剤である tofacitinib および baricitinib を上回る強力な JAK1、2、3、TYK2 阻害活性と IL-2 刺激 T 細胞増殖抑制作用を示す一方、JAK 以外のキナーゼに対してはほとんど阻害作用を示さないことを示した。また、*in vivo* のラット心移植モデルでも、AS2553627 は単独で、または減量した CNI であるタクロリムスとの併用投与で有意な生着延長効果を示すことを確認し、AS2553627 が JAK 阻害薬としての高い選択性と急性拒絶反応に対する強力な抑制作用を持つことを示した。

第 2 章では、タクロリムス投与下に生着したラット心移植または腎移植モデルの個体において認められる慢性拒絶に対する AS2553627 の有効性を評価し、本薬物が血管内膜肥厚、線維化、尿細管萎縮、糸球体硬化などの慢性拒絶像を著明に抑制することを示した。特に、ラット腎移植モデルでは血漿中クレアチニン、尿蛋白、尿中バイオマーカーなどの腎機能も AS2553627 によって維持されることを示した。さらに、移植腎における遺伝子解析も実施し、血球細胞、線維化および炎症関連マーカーの上昇が AS2553627 投与により抑制されることを示した。

第 3 章では、より臨床予測性の高いサル腎移植モデルを用いて、AS2553627 の効果を評価した。本モデルでは、特に CNI とともに現在臓器移植の免疫抑制薬として用いられているミコフェノール酸モフェチル（MMF）との薬効を比較検討した。タクロリムスを臨

床用量まで減量し、これにMMFを併用した群の生存日数の中央値が24日であったのに対し、AS2553627併用群では90日以上と著明な延命効果を示した。またAS2553627の併用群ではMMF群と比較して、急性拒絶の所見である単核細胞浸潤や血管炎、慢性拒絶の所見である線維化や尿細管萎縮が著明に抑制されることを病理組織学的に示した。
以上の結果より、新規JAK阻害剤AS2553627は現在の移植医療の課題である慢性拒絶の抑制とCNI減量を達成可能な治療薬として、移植患者に新たな価値を提供できる可能性を示すことができた。

これらの成績はいずれも、薬理学的に高度な実験手法と多面的な解析により裏付けられており、新規JAK阻害薬であるAS2553627が、現行の移植医療の課題である慢性拒絶の抑制と副作用軽減のためのCNI減量を達成するために有益な治療薬として、移植医療に提供できる可能性を示すものとして高く評価できる。
以上のことから、本論文は本研究科の博士論文として相応しい学術的および社会的な意義を持ち、さらに当該研究分野の進展に貢献するものと判断でき、博士（薬学）の学位論文として十分に価値あるものと認められる。