The contribution of experimental microsurgery to medicine development: The history of world-first agonist medicine development with S1P receptor functions

Eiji Kobayashi M.D. Ph.D.
Keio University School of Medicine,
Department of Organ Fabrication

Prologue

The role of academia in terms of developing medicine in our country is highly advanced. However, the academia-industry infrastructural improvement for clinical development has not reached to some extent; world-class. I have been involved in the research for new and innovative immunosuppressive agent for a long time thanks to the fact that I have extended research method of preclinical models based on surgical technology. One of them is S1P agonist medicine.

Fingolimod (FTY720) is phosphorylated *in vivo* and acts as S1P receptor. S1P as physiologically active agent releases lymphocyte from lymph nodes by combining with S1P1 receptor on lymphocyte. Nevertheless, phosphorylated FTY720 merged into S1P1 receptor, the emerging rate of receptor with S1P decreases and results in the isolation of lymphocyte in the lymph nodes. Consequently the lymphocyte is not invasive in allograft inflammation and the immunoregulative strength is considered to be active.

Experimental microsurgery technique is indispensable for generating organ transplant models of small animals. The technique has been developed as a means of academic research method, on the contrary, at medicine development corporations they focus on the toxicity test at GLP facilities rather than generating models for the effectiveness experiments. The reason is that appropriate technical instructions for acquiring experimental microsurgery technique is not available at medicine development corporations. The technical development of experimental microsurgery should be disseminated in collaboration between academia and corporation. The summary describes the features of development so far. I hope it acts as a basic information for many researchers who take academia-industry collaboration into consideration.

The history of development

Tochukaso (*Cordyceps sinensis* (Berkeley) Saccardo); Ophiocordyceps sinensis is a fungus that parasitizes larvae of ghost moths and produces a fruiting body valued as a herbal remedy. The fungus germinates in the living larva, kills and mummifies it, and then the stalk-like fruiting body emerges from the corpse. It is known in English colloquially as caterpillar fungus.

(A Gift from Prof. Huifang Chen (University of Montreal))

Tochukaso is known to have various species and has been used as a crude drug with bioactivity in China from ancient days. In 1994 Prof. Tetsuro Fujita of Kyoto University succeeded in having solely separated ñMilliosinò from the culture medium of Tochukaso. FTY720 has been developed over the repeated structural conversions to decrease the strong toxicity of ñMilliosinò by Mr. Chiba of Yoshitomi Pharmaceuticals Co., Ltd. and the research group of Taito Pharmaceuticals Co., Ltd. In the beginning, Yoshitomi Pharmaceuticals with the hope that it would act as an immune suppressive agent, they have started to verify with various small animalsôtransplantation models in collaboration with the deceased director, Mr. Suzuki of National Center for Child Health and Development. I have joined in the stage of researches for applying FTY720 to organ transplantation and have proven of immunological rejection and the effectiveness of GVHD by using ratôs small bowel transplantation model.

By that time at Suzuki laboratory, they had been extending experiments of FTY720 as immunosuppressive agent with comparatively simple organ transplant model such as heart. On the other hand, the rat small bowel transplant model met clinical requirements at that time due to the fact that it had been accompanied with strong rejection developing GVHD (graft versus host disease) which was extremely interesting from immunology viewpoint. I have been improving the rat small bowel transplant model that requires comparatively high level of microsurgical technique and have converted it to high-in-reproducibility verification model through the improvement of vascular anastomosis model by the cuff.

A novel immunosuppressant, FTY720, with a unique mechanism of action, induces long-term graft acceptance in rat and dog allotransplantation.
Suzuki S, **Enosawa S**, Kakefuda T, Shinomiya T, Amari M, Naoe S, Hoshino Y, Chiba K.
Transplantation. 1996 Jan 27;61(2):200-5

Immunosuppressive effect of a new drug, FTY720, on lymphocyte responses in vitro and cardiac allograft survival in rats.
Suzuki S, **Enosawa S**, Kakefuda T, Li XK, Mitsusada M, Takahara S, Amemiya H.
Transpl Immunol. 1996 Sep;4(3):252-5.

Prevention of graft rejection and graft-versus-host reaction by a novel immunosuppressant, FTY720, in rat small bowel transplantation.
Mitsusada M, **Suzuki S**, Kobayashi E, **Enosawa S**, Kakefuda T, Miyata M.
Transpl Int. 1997;10(5):343-9.

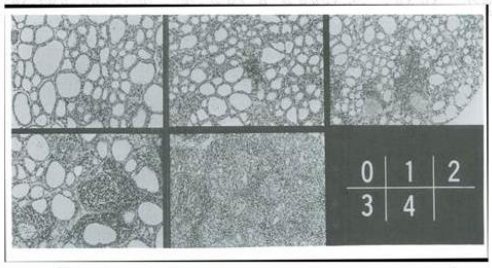
Organ transplantation to autoimmune disease

At that time the research results that lymphocyte almost disappeared from the periphery and no infection symptom was found even though 100 times of the suggested dosage was given have caught lots of attentions among researchers. The former symptom was once considered to be apoptosis induction but was found later that it resulted in the homing effect of lymphocyte in lymph nodes.

The product has been owned by Mitsubishi Tanabe Pharma and the technology was transferred to Novartis Pharma in 1997. Novartis started clinical trials in the area of transplantation but withdrew from the trial due to the fact that they had not been able to generate better results in the comparative experiments with MMF (Mycophenolate mophetil) in the clinical kidney transplantation (in the year 2005). Nevertheless, Novartis proceeded to the applied research of FTY720 for autoimmunity disease model such as MS (Multiple Sclerosis). MS is categorized as a rare disease and extremely incurable, the fact has led the effectiveness and safety of FTY720 to launch the medicine named ñGilenyaò which is globally used on clinical scenes. The point we need to pay attention is that no paper for basic research is published while clinical trials are undertaken to target patients by pharmaceutical corporations despite that the large number of papers published and caught by eminent researchersôeyes from the research results for organ transplantation models.

The applied research of FTY720 for autoimmune disease

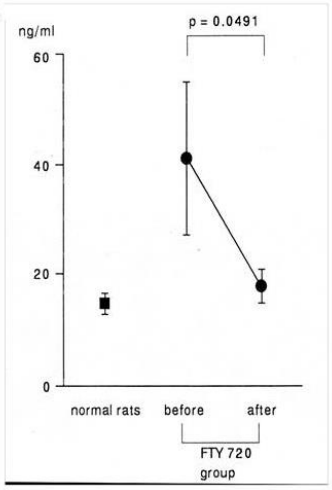
The reports of the FTY720 effectiveness for autoimmune have been recently proven as Experimental Allergic Encephalomyelitis(EAE) applications by corporations. However, before the clinical trials on the above corporations started, my team of researchers had verified the effectiveness by autoimmune thyroiditis rat model first time on global basis.



Comparison of histological gradings of EAT rats with and without FTY720.

Score	FTY720 treatment	Disease control
0	6	1
1+	4	4
2+	0	3
3+	0	2
Weighted score/N	0.4	1.6

p = 0.0051



(Hozumi Y, Kobayashi E, et al. Life Sciences 1999)

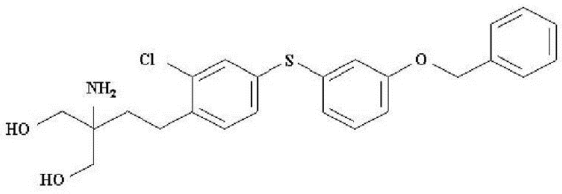
The thymectomy in neonatal period for this autoimmune thyroiditis rat model is a must. We have generated the model by maximizing the microsurgical technique.

Furthermore, it shows another better effect with mouse SLE (systemic lupus erythematosus) model compared to that of existing steroid treatment *(Okazaki H, Kobayashi E, et al. J Rheumatol 2002)*

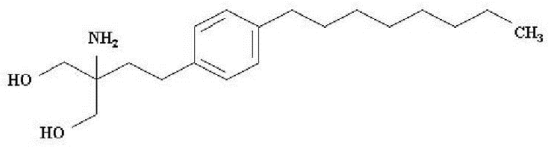
New S1P Agonist KRP-203

Around the same time, Kyorin Pharmaceutical Co., Ltd. started to test the immuneregulatory strength of KRP-203, a substance structurally resembling to FTY720 which had been developed from other field of research. As there was no experimental models rather than skin transplant within the corporation, my research department launched the experiments with small animals on consignment research basis.

KRP-203



FTY720



Below is the list of papers concerning KRP-203. At that time, while FT720 clinical trials had been ongoing, the verification experiments in line with clinical application were conducted.

Rat Heart Transplant Model

KRP-203, a novel synthetic immunosuppressant, prolongs graft survival and attenuates chronic rejection in rat skin and heart allografts.
Shimizu H, Takahashi M, Kaneko T, Murakami T, Hakamata Y, Kudou S, Kishi T, Fukuchi K, Iwanami S, Kuriyama K, Yasue T, Enosawa S, Matsumoto K, Takeyoshi I, Morishita Y, **Kobayashi E**.
Circulation. 2005 Jan 18;111(2):222-9. Epub 2005 Jan 10

Efficacy of mycophenolic acid combined with **KRP-203**, a novel immunomodulator, in a rat heart transplantation model.
Suzuki C, Takahashi M, Morimoto H, Izawa A, Ise H, Fujishiro J, Murakami T, Ishiyama J, Nakada A, Nakayama J, Shimada K, Ikeda U, **Kobayashi E**.
J Heart Lung Transplant. 2006 Mar;25(3):302-9. Epub 2006 Jan 18

A novel immunomodulator **KRP-203** combined with cyclosporine prolonged graft survival and abrogated transplant vasculopathy in rat heart allografts.
Takahashi M, Shimizu H, Murakami T, Enosawa S, Suzuki C, Takeno Y, Hakamata Y, Kudou S, Izawa S, Yasue T, **Kobayashi E**.
Transplant Proc. 2005 Jan-Feb;37(1):143-5

Rat Ao Transplant Model

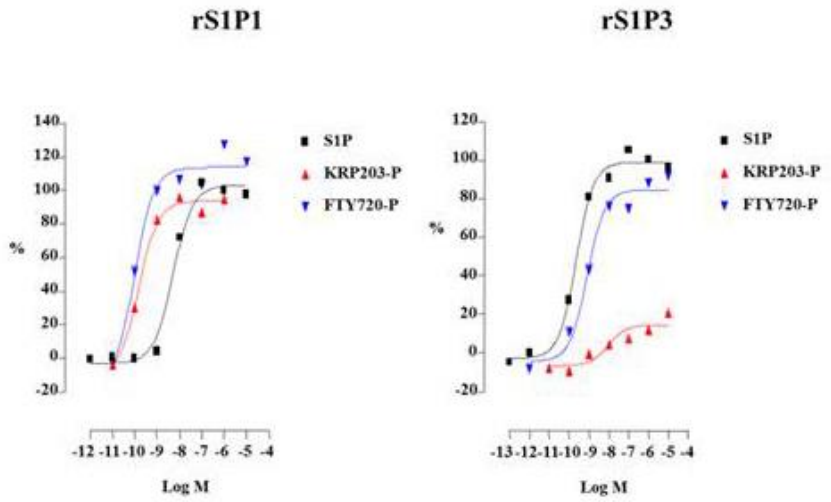
Change from cyclosporine to combination therapy of mycophenolic acid with the new sphingosine-1-phosphate receptor agonist, **KRP-203**, prevents host nephrotoxicity and transplant vasculopathy in rats.
Fujishiro J, Suzuki C, Kudou S, Yasue T, Hakamata Y, Takahashi M, Murakami T, Hashizume K, **Kobayashi E**.
J Heart Lung Transplant. 2006 Jul;25(7):825-33. Epub 2006 Jun 5

Rat Renal Transplant Model

Use of sphingosine-1-phosphate 1 receptor agonist, **KRP-203**, in combination with a subtherapeutic dose of cyclosporine A for rat renal transplantation.
Fujishiro J, Kudou S, Iwai S, Takahashi M, Hakamata Y, Kinoshita M, Iwanami S, Izawa S, Yasue T, Hashizume K, Murakami T, **Kobayashi E**.
Transplantation. 2006 Sep 27;82(6):804-12

In the beginning, we paid attention to the medicine with the expectation that it would be applied as a medicine with fewer side effect to cardio-related organs thanks to the weak affinity to S1P3. Currently, the clinical trials are being undertaken in order to confirm of the effectiveness and safety to human.

In the consequence of these researches, we have verified the effectiveness with mouse hepatitis model due to the fact that hepatitis catches attention in case of autoimmune and inflammation. It has been confirmed through the comparative test using the same amount of S1P, KRP-203 is superior to FTY720 in terms of effectiveness.



Calcium Mobilization Assay in the Rat $S1P_1$ or $S1P_3$-transduced CHO-K1 cells.

(Shimizu, et al. Circulation 111;222,2005)

With a series of research results the patent application was submitted in 2006 and it was approved 6 years after the application in 2012. The invention was elaborated between Dr. Kaneko and myself, on the other hand, the patent rights have been transferred to Kyorin Pharmaceutical Co., Ltd. on account of the academia-industry treaty. In regard to KRP-203, the co-research has been ongoing between Kyorin and Novartis Phama.

Sphingosine-1-phosphate receptor agonists suppress concanavalin A-induced hepatic injury in mice.
Kaneko T, Murakami T, Kawana H, Takahashi M, Yasue T, **Kobayashi E**.
Biochem Biophys Res Commun. 2006 Jun 23;345(1):85-92. Epub 2006 Apr 25

A novel sphingosine-1-phosphate receptor agonist **KRP-203** attenuates rat autoimmune myocarditis.
Ogawa R, Takahashi M, Hirose S, Morimoto H, Ise H, Murakami T, Yasue T, Kuriyama K, Hongo M, **Kobayashi E**, Ikeda U.
Biochem Biophys Res Commun. 2007 Sep 28;361(3):621-8. Epub 2007 Jul 23.

Closing remarks

The paper describes the course of academia-industry collaboration through the world-first S1P agonist medicine development. Corporation in line with its mission, they have advantages in the abundant funds for researches. On the other hand, academia has a laboratory where they can maximize surgical techniques together with the abilities of scientific verification based on experimental microsurgery for clinical trials. For a closing note, I really expect further pragmatic collaboration between academia and corporation through experimental microsurgery for numerous patients waiting for new medicines.

Finally, I thank my co-workers at the division of organ replacement research, Jichi Medical University. Especially, Prof. Takashi Murakami (Currently assigned to the Lab. of Tumor Biology, Takasaki University of Health and Welfare, Japan) has been working on Immunomodulation Therapy of the Control of Immune Cell Trafficking by use of S1P agonists (Nova book "Current Immunosuppressive Therapy in Organ Transplantation").