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Chinese Medicine and New G protein-Coupled Receptors for Lipid Mediators

Dong-Soon Im

Laboratory of Pharmacology, College of Pharmacy,

Pusan National University, Republic of Korea

Recently, two papers reported that the phosphorylated form of FTY720, an immunosuppressant, acts on sphingosine 1-phosphate (S1P) receptors, which are a subfamily of G protein-coupled receptors (GPCR), and alters lymphocyte trafficking. The structure of FTY720 is very similar to sphingosine and has two hydroxy groups that can be phosphorylated. The immune modulating action of FTY720 is mediated in the blood by phosphorylation and in the immune cells through activation of S1P receptors by its phosphoryl metabolite. Interestingly, the chemical origin of synthetic FTY720 was ISP-1 (also known as myriocin), a metabolite from fungi (*Isaria sinclairii*). Isaria sp. is one of the entomopathogenic fungi categorized as 'Vegetable Wasps and Plant Worms (VWPW)', which have been used in oriental medicine for thousands of years. In this article, I'd like to review recent findings with a research story linking Chinese medicine and a group of GPCRs.

What are vegetable wasps and plant worms

Vegetable Wasps and Plant Worms (冬蟲夏草, entomopathogenic fungi) have been known as a mysterious medicine in Asian countries, especially in China, Korea, and Japan. The Chinese characters (冬蟲夏草, Dong-Chung-Ha-Cho), meaning winter-insect and summer-plant, come from the fact that the fungus enters into the living insect, nurtures itself, feeds on insides of the host, and eventually grows out onto the surface of the host insect's cadaver in the summer. Even in China, where they have been used as a traditional medicine for thousands of years, they were described confusingly: "Vegetable wasps and plant worms (VWPW) are worms but not worms and are plants but not plants. They are just God's medicine." Therefore, they have been considered, along with the ginseng (*Panax ginseng*) and the young antlers of deer, as one of three oriental medicines that give eternal youth.

An immune modulator, FTY720

A nucleotide analogue, cordycepin (3'deoxyadenosine), was first purified from VWPW, *Cordyceps sinensis* and *C. militaris*. It blocks polyadenylation and inhibits tumor cell growth (leukemias, melanomas, etc). The second known material from VWPW was cyclosporin, the classical immunosuppressant. This was the impetus for searching for other immune suppressants from VWPW. ISP-1/myriocin, a sphingosine-

like structure, was found in the culture medium of *Isaria sinclairii*, an imperfect form of the genus *Cordyceps*. The same structure was first isolated from a thermophilic fungus (*Myriococcum Albomyces*) and named myriocin in 1972. ISP-1 suppressed the proliferation of lymphocytes in mouse allogeneic mixed lymphocyte reaction (MRL), but had no effect on the growth of human tumor cell lines. It was 10- to 100-fold more potent than cyclosporin A as an immunosuppressive agent of the immune response *in vitro* and *in vivo*. Later the target of ISP-1 was found to be serine palmitoyltransferase, an enzyme necessary for sphingolipid biosynthesis. ISP-1 inhibits the enzyme of an IL-2-dependent mouse cytotoxic T-cell line. A Japanese group did a medicinal chemistry study on ISP-1 and made a more potent immunosuppressant compound named FTY720. However, unlike ISP-1, FTY720 does not inhibit serine palmitoyltransferase and is not antiproliferative. FTY720 reduces the number of circulating lymphocytes (T cells and B cells) in peripheral blood. The immunosuppressiveness of FTY720 comes from the sequestration of circulating mature lymphocytes into peripheral lymph nodes, mesenteric lymph nodes and Peyer's patches by acceleration of lymphocyte homing. Thus, there are clear differences in FTY720 and ISP-1's mode of action.

Immunosuppressant vs Immunomodulator

In organ transplant patients, current therapeutic protocols for prevention of graft

rejection include inhibitors of the T-cell/B-cell activation cascade, that is, cyclosporin, rapamycin, FK506, and some others. However, generalized immunosuppression may weaken defense against infection and malignancies. FTY720's reduction of the specific effector T cells, which are re-circulating from the lymph nodes to inflamed peripheral tissues and graft sites, protect allografts without generalized immunosuppression, which results in the prolonged survival of the grafted. Furthermore, its synergism with other immunosuppressants like cyclosporin may allow combinational therapy and reduce the toxicity of classical immunosuppressants. Specipically, this regimen combination effectively suppressed graft-vessel disease in a rat carotid artery transplant model. Its unique action has potential applications for autoimmune diseases including type I diabetes and multiple sclerosis. FTY720 is currently being tested in several experimental models for autoimmune diseases like systemic lupus erytheromatosus and autoimmune diabetes.

Sphingosine 1-phosphate receptors as the molecular target of FTY720

Sphingosine 1-phosphate (S1P) is a bioactive lipid acting through its specific GPCRs. Five members of a subfamily of GPCR, named originally 'Edg', have been characterized as S1P receptors. Platelets and serum are thought to be its source in the body. It has been shown that sphingosine kinases are present in cytosol and make S1P

from sphingosine. The structure of FTY720 is very similar to sphingosine and has two hydroxy groups that can be phosphorylated. Two separate groups tested the hypothesis that FTY720 is converted to a phosphorylated molecule and that the metabolite acts on S1P receptors. Pharmacokinetic studies of FTY720 have observed the conversion of the immunosuppressant, FTY720, to the phosphoryl metabolite in the blood after its administration. One group also showed that mouse sphingosine kinase-1 *in vitro* phosphorylates FTY720, although FTY720 was a poorer substrate than sphingosine. Furthermore, both groups showed that the phosphorylated form of FTY720 acts on S1P receptors in broken cell membranes and intact cell preparations. Synthetic FTY720-phosphate alters lymphocyte trafficking, which is the main pharmacological effect of FTY720 and also S1P does. The original FTY720 has very weak intrinsic activity on S1P receptors as does sphingosine. The phosphorylated one however was active on all S1P receptors except $S1P_2$. Notably, $S1P_4$ receptor showed a 20-fold higher affinity to the FTY720-phosphate than the natural ligand, S1P. The exact mechanism behind the FTY720 phosphorylation in the blood is not known yet. Recently, two papers suggested possible routes of sphingosine kinase activity in the vessels. One is translocation of sphingosine kinase to the plasma membrane by PKC activation. The other is extracellular export of sphingosine kinase-1 enzyme in the vascular endothelial cells.

Concluding remarks

A human clinical trial of FTY720 in renal transplant patients may help determine its promise in future clinical applications. However, transient, asymptomatic bradycardia, observed in the clinical trial, is a bit worrysome. There are several S1P receptors expressed in the heart ($S1P_1$, $S1P_2$, and $S1P_3$). Development of more selective agonists for S1P receptors may reduce side effects. Several questions need to be answered for the development of more selective drugs and for fulfilling scientific curiosity. Which S1P receptor(s) accelerates the lymphocytes homing? What kind of mechanism is involved in the homing, especially in terms of signal transduction pathways? What are the physiological roles of S1P in lymphocytes as an endogenous immune modulator and/or activator of lymphocyte homing? Furthermore, medicinal chemistry study on S1P is absolutely necessary to develop not only better drugs than FTY720 with reduced side effects but also drugs for other applications like cancers and hypertension. FTY720 prevented tumor growth and metastasis in mouse breast cancer models. Studies on the signaling might lead us to a new molecular target for the development of a new immune modulator. Finally, studies on traditional medicines like VWPW (冬蟲夏草) might give us opportunities to find new medicines and the nature of life. ISP-1 and cyclosporin were made in the culture of VWPW. It is not known whether

sexual forms (stromata), that is, *Cordyceps* make the same compounds. Since the tonic effect of VWPW has been observed in Asian countries for thousands of years, the fruiting body (stromata) and/or the asexual form of the entomopathogenic fungi might be the source for new drug discoveries. Currently about 800 species of entomopathogenic fungi are found in the world. Furthermore, the site of action of ginsenosides, the active constituents of ginseng (*Panax ginseng* C.A. Meyer), may be G protein-coupled receptor(s) and waiting for another discovery.

Discovery of New G protein-Coupled Receptors for Lipid Mediators

Molecular targets of various drugs have been found, using recently developed biochemical, physiological, and pharmacological tools. For example, aspirin inhibits cyclooxygenase, and morphine activates the opioid receptors in the plasma membrane. Molecular identification of the targets at the DNA level has become possible due to the advancement of techniques in molecular biology, and G protein-coupled receptors (GPCRs) have been found to be the molecular targets of many drugs. To date, approximately 170 GPCRs have been found to be receptors for known intercellular mediators such as hormones and neurotransmitters, and 367 genes have been recognized as GPCRs in the human genome. When their ligands are not known, these G protein-coupled receptors are classified as "orphan" GPCRs. Discovery of endogenous ligands

could be a springboard for the development of new therapeutic agents targeting on the receptors and would be help define the biological significance of the ligands. Many potentially significant discoveries have been made in the last several years. The present review will focus mainly on the recent assignment of lipid mediators to GPCRs. These GPCRs include GPR3, GPR6, GPR12, GPR23, GPR40, GPR41, GPR43, GPR63, TG1019 (also known as R527), and BG37 (also known as TGR5) which have been identified as receptors for intercellular lipid messengers; i.e. sphingosine 1-phosphate (S1P), sphingosylphosphorylcholine (SPC), dioleoylphosphatidic acid (doPA), lysophosphatidic acid (LPA), free fatty acids, eicosatetraenoic acid, and bile acids.