

3 December 2013

The University of Tokyo

Tokyo Institute of Technology

MRC National Institute for Medical Research

SBI Pharmaceuticals Co., Ltd.

## **Findings of Mechanism of 5-aminolevulinic Acid (5-ALA) and Iron in Synergistically Inhibiting Growth of *Plasmodium Falciparum* Malaria Parasites**

The University of Tokyo, a national university (main campus: Bunkyo-ku, Tokyo; President: Junichi Hamada), Tokyo Institute of Technology, a national university (main campus: Meguro-ku, Tokyo; President: Yoshinao Mishima), MRC National Institute for Medical Research (London, U.K. Director: Jim Smith), and SBI Pharmaceuticals Co., Ltd. (head office: Minato-ku, Tokyo; Representative Director and CEO: Yoshitaka Kitao; “SBI Pharmaceuticals”), presented their findings in *The Journal of Biochemistry* (2 December 2013) regarding the working mechanism of 5-aminolevulinic acid (5-ALA)<sup>\*1</sup> and bivalent iron in inhibiting the growth of *Plasmodium falciparum* (*P. falciparum*) malaria parasites.

### **1. Key Points of the Findings**

- 1) The findings revealed a crucial element of the mechanism of inhibiting the growth of *P. falciparum* through a combination of 5-ALA and bivalent iron.
- 2) The findings are expected to lead to the development of new medical products to treat malaria, a disease that affects a large number of people around the world.

### **2. Outline of Announcement**

Malaria is one of the world’s three major infectious diseases and it reportedly infects hundreds of millions of people and kills over 1 million people each year. It has been known that the administration of 5-ALA accumulates porphyrins<sup>\*2</sup> in cells that are infected with *P. falciparum* and the accumulated porphyrins can be used as a guide to spot and kill *P. falciparum* by irradiating light on them. However, irradiating light on the blood is unrealistic and thus has remained a major deterrent to commercialization of 5-ALA. The development of 5-ALA-based agents that do not require the irradiation of light has been long awaited.

Mr. Kiyoshi Kita (Professor, The University of Tokyo, Graduate School of Medicine), Mr. Shun-Ichiro Ogura (Associate Professor, Tokyo Institute of Technology, Graduate School of Bioscience and Biotechnology), and SBI Pharmaceuticals presented the results of the study at an academic conference in 2011. The results showed that the concomitant administration of 5-ALA and bivalent iron can inhibit the growth of *P. falciparum* without the need to irradiate light. Subsequently, MRC National Institute for Medical Research has joined to further research. An analysis of porphyrins in each organelle<sup>\*3</sup> of *P. falciparum* uncovered a crucial element of the working mechanism, where the combined use of 5-ALA and bivalent iron causes an accumulation of

porphyrins in certain organelles and generates active oxygen which in turn inhibits the growth of *P. falciparum*.

These findings are expected to lead to the development of new medical products to treat malaria, a disease that affects a large number of people around the world. The safety of 5-ALA and bivalent iron has already been established and both 5-ALA and bivalent iron have been used for food products and medical purposes. In addition, clinical development is expected to take place shortly. With fewer side effects compared to any of the existing antimalarial drugs, 5-ALA and bivalent iron are expected to be revolutionary antimalarial drugs that can be administered for prophylactic purposes.

### 3. Details of Findings

#### Background to Studies

Malaria is one of the world's three major infectious diseases along with tuberculosis and AIDS that have not been conquered by human beings. While chloroquine and quinine have long been used to treat malaria, the existing antimalarial drugs cause serious side effects. In addition, malaria parasites with tolerance to these drugs have been found in recent years, thus further exacerbating the problem. Although a series of studies to develop a vaccine for malaria prevention has been conducted, no effective product has been developed as of yet.

#### Details of Studies

There have been reported studies regarding photodynamic therapy (PDT), a method to apply highly concentrated 5-ALA to accumulate porphyrins in cells that are infected with *P. falciparum*. The accumulated porphyrins are used as a guide to spot and kill *P. falciparum* by irradiating light on them. However, irradiating light on the blood is unrealistic and thus has remained a major deterrent to the commercialization of 5-ALA. The development of 5-ALA-based agents that do not require the irradiation of light has been long awaited.

A combination of 200  $\mu\text{M}$  (micromolar) of 5-ALA, which is equivalent to one-tenth of the dose necessary for PDT to function effectively, and cobalt, zinc, magnesium, nickel, lead, copper, bivalent iron, and trivalent iron salt was tested on *P. falciparum* 3D7 culture which is the most pathogenic *P. falciparum*. The study concluded that only when 5-ALA is combined with bivalent iron, do they act synergistically to inhibit the growth of *P. falciparum*. After adding types of bivalent iron salt to the studies, sodium ferrous citrate (SFC)<sup>\*4</sup> was found to be the most effective. With a combination of 200  $\mu\text{M}$  of 5-ALA and 100  $\mu\text{M}$  of SFC, the growth-inhibition rate against *P. falciparum* reached approximately 60%.

When porphyrins in *P. falciparum* were analysed after applying 5-ALA, coproporphyrin I (CPI), coproporphyrin III (CPIII), and protoporphyrin IX (PPIX) were detected, with CPIII having the highest density. Regarding the distribution of porphyrins in each organelle, porphyrins were found in apicoplasts in the ring stage and in food vacuole in the trophozoite and schizont stages<sup>\*5</sup>. The study revealed that an accumulation of porphyrins in certain organelles has an important link with the growth-inhibiting effect against *P. falciparum*.

In addition, the concomitant administration of “5-ALA + SFC” and vitamin C, which is an antioxidant, reduced the growth-inhibiting effect against *P. falciparum*, suggesting that active oxygen<sup>\*6</sup> produced by 5-ALA and SFC may be involved in inhibiting the growth of *P. falciparum*.

The early commercialization of “5-ALA + SFC” is expected, given that tests on animals have been started with positive results starting to come through already and that the safety has been established with a phase I study<sup>\*7</sup> completed in UK with healthy individuals.

### Social Significance

Malaria is a worldwide problem especially in tropical areas and the expansion of infected areas due to global warming is an alarming concern. The spread of malaria from tropical areas to other areas due to the development of transportation systems, which is often referred to as “travelers’ malaria,” is now becoming a major worry.

The existing drugs to treat malaria are said to have a high possibility of causing side effects including blindness. Although a series of studies to develop vaccine for malaria prevention has been conducted, no effective product has been developed as of yet.

The safety of both 5-ALA and SFC, which is bivalent iron, for which a growth-inhibiting effect against *P. falciparum* was found, has already been established. Both 5-ALA and SFC have been used for food products and medical purposes. A phase I study has been completed for compound drugs composed of 5-ALA and SFC as a treatment for anemia induced by anticancer drugs. With fewer side effects compared to the existing antimalarial drugs, the compound drugs composed of 5-ALA and SFC will be revolutionary antimalarial drugs that can be administered for prophylactic purposes. The compound drugs are expected to contribute to eradicating malaria in the future.

### 4. Name of Journal

Name of Journal: *The Journal of Biochemistry*

Title of Published Article: Synergy of ferrous ion on 5-aminolevulinic acid-mediated growth inhibition of *Plasmodium falciparum*

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DOI: 10.1093/jb/mvt096

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## 6. Glossary:

\*1: 5-aminolevulinic acid (5-ALA)

5-ALA, one type of natural amino acid, is an important substance that serves as the material of haem in the human body. 5-ALA is contained even in food products and used as materials for health foods. In addition, 5-ALA was approved as an interoperative diagnostic drug for brain tumors using the characteristics of 5-ALA where it is metabolised to porphyrins only in tumor cells.

\*2: Porphyrin

Porphyrins are cyclic compounds containing 8 molecules of 5ALA and they characteristically absorb blue light and emit red light. Porphyrins are used to diagnose and treat cancers.

\*3: Organelle

Organelles are small structures that perform very specific functions within cells. They include the nucleus, the mitochondrion, the Golgi body, the food vacuole, and the apicoplast.

\*4: Sodium ferrous citrate (SFC)

SFC is a chemical compound effective for treating and preventing anemia and has been used widely in medical products and health foods for many years.

\*5: Life cycle of malaria parasites

Malaria parasites have a complicated life cycle. They initially infect the human liver via mosquitoes, and then after destroying the liver cells, they are released to the blood stream. They invade red blood cells and feed on the haemoglobin inside them. The parasites inside blood cells develop through three different stages- the ring, trophozoite, and schizont stages- in this order. After destroying the blood cells, merozoites are released to the blood stream again. At this stage, common symptoms of malaria, such as high fever and chills, occur. Malaria parasites have a unique organelle\*3 called the apicoplast that is thought to derive from the chloroplast.

\*6: Active oxygen

Active oxygen is a collective term for oxygen compounds such as singlet oxygen, superoxide, hydrogen peroxide, and hydroxyl radical that easily undergo a chemical reaction compared to oxygen in the atmosphere.

\*7: Phase I study

A preliminary study conducted with healthy volunteers to determine the safety of a medical product which is under development. Regarding “5-ALA + SFC,” a phase I study with European, U.S., and Japanese individuals was completed in the U.K., confirming its safety.