5-Aminolevlinic acid asymptomatic malaria project

Double-blind, parallel, randomized, placebo-controlled research to evaluate safety and efficacy of the 5-aminolevulinic phosphate (5-ALA PO4), sodium ferrous citrate (SFC) and zinc (Zn) with asymptomatic malaria parasite carriers

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Background

Malaria morbidity and mortality have decreased in Lao PDR due to extensive efforts by the Lao Government and international organizations, such as World Health Organization (WHO), The Global Fund to Fight AIDS, Tuberculosis and Malaria. Recently, the Lao Ministry of Health and WHO have adopted a goal to achieve the elimination of malaria by 2030. However, several studies demonstrated that there were asymptomatic Plasmodium carriers in the malaria-endemic areas in Lao PDR. Most of them were adult population who had histories of malaria episodes and were engaged in forest-related occupations. Some studies also suggested that asymptomatic *Plasmodium* carriers can be a

reservoir for the transmission of malaria by *Anopheles* mosquitos. However, such people will never take any antimalarial medicines until they become symptomatic. In addition, most asymptomatic *Plasmodium* infections cannot be detected by standard diagnostic methods (microscopy and rapid diagnostic test: RDT) that are available in the endemic areas. Current malaria control and elimination strategy in Lao PDR is targeting for symptomatic malaria patients. Therefore, to accelerate the elimination of malaria in Lao PDR, a new effective strategy for targeting asymptomatic *Plasmodium* carriers is urgently needed in the endemic areas. 5-aminolevulinic acid (5-ALA), which is produced by neopharma Japan Co., Ltd., as a health food supplement commercially available in Japan, Philippines, Vietnam and UAE, is a natural precursor of heme in all animals. It is a non-protein amino acid synthesized in mitochondria and through the activity of cytochrome C oxidase is involved in the electron transport chain. It was found from pre-clinical studies that sodium ferrous citrate (SFC) enhanced *Plasmodium falciparum*-killing potency of 5-ALA and significantly inhibited the parasite growth both in vitro and in vivo [1, 2]. These novel findings may lead us to develop a new functional health supplement containing antimalarial activity using 5-ALA. Moreover, 5-ALA is being sold as a health food supplement, which has the functional claim “5-ALA supports to bring higher fasting blood glucose levels closer to normal” in Japan [3, 4]. In this study, we will evaluate the acceptability, safety and efficacy of 5-ALA phosphate (PO4) with SFC and Zn for asymptomatic Plasmodium carriers in malaria-endemic villages, Nong district, Savannakhet province,

Lao PDR for one year. Efficacy of 5-ALA PO4 to *Plasmodium* infection will be examined by reduction of *Plasmodium* DNA positivity rate by PCR, compering to that of the Placebo group (only SFC and Zn). Since Zn deficiency is also a serious health problem in Lao PDR, participants of this study will take Zn as well to enhance a benefit for the study participants. In addition, to evaluate efficacy of 5-ALA PO4 to *Plasmodium* infection, we will evaluate the level of HbA1c, which is one of the markers of type 2 diabetes. It is reported that type 2 diabetes increases the risk of malaria infection [5]. Expected outcomes will contribute to malaria elimination and type 2 diabetes control in Lao PDR.

Objectives

• To assess the influence of 5-aminolevulinic acid phosphate (5-ALA PO4), sodium ferrous citrate (SFC) and zinc (Zn) to *Plasmodium* (*Plasmodium* DNA detected by PCR) in asymptomatic *Plasmodium* carriers.
• To assess the acceptability and safety of 5-ALA PO4 among Lao villagers who carry malaria parasites without symptoms as detected by PCR.
• To investigate the HbA1c level in asymptomatic malaria parasite carriers after administrations of 5-ALA PO4, SFC and Zn for daily usage.

The study period of the project

Two years (October 2019- September 2021)

Ethical approval

This study proposal was reviewed and approved by the Ethic Committee (No. 187), University of Health Sciences, Ministry of Health, Lao PDR on 26th June 2019 and the Institutional Review Board for Clinical Research (No. NCGM-G-003300-00), National Center for Global Health and Medicine (NCGM), Japan

on 27th September 2019. Permission of importation of 5-ALA PO4 (No. 9330) was also obtained from the Department of Food and Drug, Ministry of Health, Lao PDR on 20th September 2019.

Methodology

After having provided informed consent, potential participants will be enrolled in a screening during which all inclusion/exclusion criteria, including laboratory assessments, will be checked for eligibility. If full eligibility is confirmed, the participants will be randomized to three arms: Arm 1: 5-ALA PO4 25 mg/ day + SFC 28.7 mg + Zinc 10 mg (12 months), Arm 2: Placebo + SFC 28.7 mg + Zinc 10 mg (3, 6 or 9 months*) and then 5-ALA PO4 25 mg/day + SFC 28.7 mg + Zinc 10 mg (3, 6 or 9 months) or Arm 3: SFC 28.7 mg + Zinc 10 mg (12 months). Interim Analysis will be carried out at 3, 6 or 9 months (Figure 1).

*When the statistical difference of Plasmodium DNA positivity rate between Arm 1 and Arm 2 is observed at 3 months, Arm 2 participants will take 5-ALA PO4 25 mg/day + SFC 28.7 mg + Zinc 10 mg from 3 months through the end of the study period. If no statistical difference between the 2 groups is observed at 3 months and the statistical difference is observed at 6 months, Arm 2 participants will take 5-ALA PO4 25 mg/day + SFC 28.7 mg + Zinc 10 mg from 6 months through the end of the study period. If no statistical difference between the 2 groups is observed at 6 months and the statistical difference is observed at 9 months, Arm 2 participants will take 5-ALA PO4 25 mg/day + SFC 28.7 mg + Zinc 10 mg from 9 months through the end of the study period. If no statistical difference between the 2 groups is observed at 9 months, Arm 2 participants will take only SFC 28.7 mg + Zinc 10 mg for 12 months (entire the study period).

Clearance of *Plasmodium* DNA [Time Frame: 1, 2, 3, 6, 9 and 12 months] Defined as the positive rate of blood-stage of Plasmodium among participants confirmed by PCR and HbA1c

defined as the actual value from blood confirmed by handy HbA1c monitoring device within all-time periods are provided in Table 1. Blood sample (maximum 800µL per sampling) will be collected using lancet, syringe, needle and preserved on filter paper.

Follow up surveys will be conducted at 2 months later at the end of the administration of 5-ALA or Placebo. In the follow-up survey, a blood sample will be collected and examined by PCR for checking *Plasmodium* DNA.

Current situation (as of 31st October 2019)

The first field survey for the screening of asymptomatic Plasmodium carriers started on 20th October 2019 (until 14th November 2019). More than 1,100 blood samples were collected from adult participants (age: 18-65 years old) in malaria-endemic villages, Nong district, Savannakhet province. Those who had any signs and symptoms of malaria and pregnant ladies were excluded from the screening. PCR screening for detecting Plasmodium infection will be performed at IPL and NCGM. Administration of 5-ALA PO4 to asymptomatic Plasmodium carriers will be performed by the team of the University of Health Sciences leaded by Dr. Mayfong Mayxay. The roles of IPL in this project is laboratory analyses (DNA detection by PCR or LAMP methods) and support the field works.

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Scientific communications

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Table 1. List of dried blood samples on filter paper collected from malaria patients and suspected patients in five southern provinces and one northernmost province

Sampling period	Sampling time	Location	Type of blood samples	No. of samples
2015 Oct - 2016 April	2016 April	5 Southern Provinces	Malaria Positive only	2,409
2016 May-Oct or Nov	2016 Oct-Nov	5 Southern Provinces	Both Malaria Positive and Negative	10,813
2016 Nov - 2017 May or June	2017 May-June	5 Southern Provinces	Both Malaria Positive and Negative	14,453
2017 Nov-Dec	2017 Dec	Phongsaly	Both Malaria Positive and Negative	98
2017 June-2018 August	2018 August	4 Southern Provinces*	Malaria Positive only	2459
2018 Jan-2019 Jan	2019 January	Phongsaly	Both Malaria Positive and Negative	398
2018 August-2019 February	2019 February	5 Southern Provinces	Malaria Positive only	2472
Total				**33,102**

Malaria diagnoses was performed by microscopy or RDT. *Blood samples were not collected from Attapeu because of flooding in July 2018.

Table 2. Summary of *Plasmodium* species in five Southern Provinces 2015-2017

Province	Year					
	2015		2016		2017	
	No.	%	No.	%	No.	%
Savannakhet						
Pf	258	63.1	155	66.5	263	83.0
Pf+Pv	13	3.2	25	10.7	31	9.8
Pv	136	33.3	50	21.5	21	6.6
Pm	1	0.2	3	1.3	2	0.6
Po	1	0.2	0	0.0	0	0.0
Sub-Total	**409**	**100.0**	**233**	**100.0**	**317**	**100.0**
Salavan						
Pf	211	56.7	50	58.1	74	70.5
Pf+Pv	17	4.6	2	2.3	1	1.0
Pv	132	35.5	34	39.5	30	28.6
Pm	3	0.8	0	0.0	0	0.0
Po	9	2.4	0	0.0	0	0.0
Sub-Total	**372**	**100.0**	**86**	**100.0**	**105**	**100.0**
Sekong						
Pf	127	28.7	17	6.5	10	9.3
Pf+Pv	35	7.9	20	7.7	0	0.0
Pv	280	63.3	223	85.8	98	90.7
Pm	0	0.0	0	0.0	0	0.0
Po	0	0.0	0	0.0	0	0.0
Sub-Total	**442**	**100.0**	**260**	**100.0**	**108**	**100.0**
Champasak						
Pf	391	53.2	85	26.7	58	45.7
Pf+Pv	34	4.6	51	16.0	5	3.9
Pv	309	42.0	182	57.2	64	50.4
Pm	0	0.0	0	0.0	0	0.0
Po	1	0.1	0	0.0	0	0.0
Sub-Total	**735**	**100.0**	**318**	**100.0**	**127**	**100.0**
Attapeu						
Pf	120	44.1	27	26.7	57	54.3
Pf+Pv	11	4.0	2	2.0	4	3.8
Pv	140	51.5	70	69.3	43	41.0
Pm	1	0.4	1	1.0	1	1.0
Po	0	0.0	0	0.0	0	0.0
Pk	0	0.0	1	1.0	0	0.0
Sub-Total	**272**	**100.0**	**101**	**100.0**	**105**	**100.0**
Grand-Total	**2,230**		**998**		**762**	

Pf: *P. falciparum*; Pv: *P. vivax*; Pm: *P. malariae*; Po: *P. ova*le; Pk: P. knowlesi

Sampling period:
2015: October 2015 – April 2016
2016: May – October or November 2016
2017: November 2016 – May or June 2017

Table 3. Prevalence of artemisinin-resistant mutation in *K13*

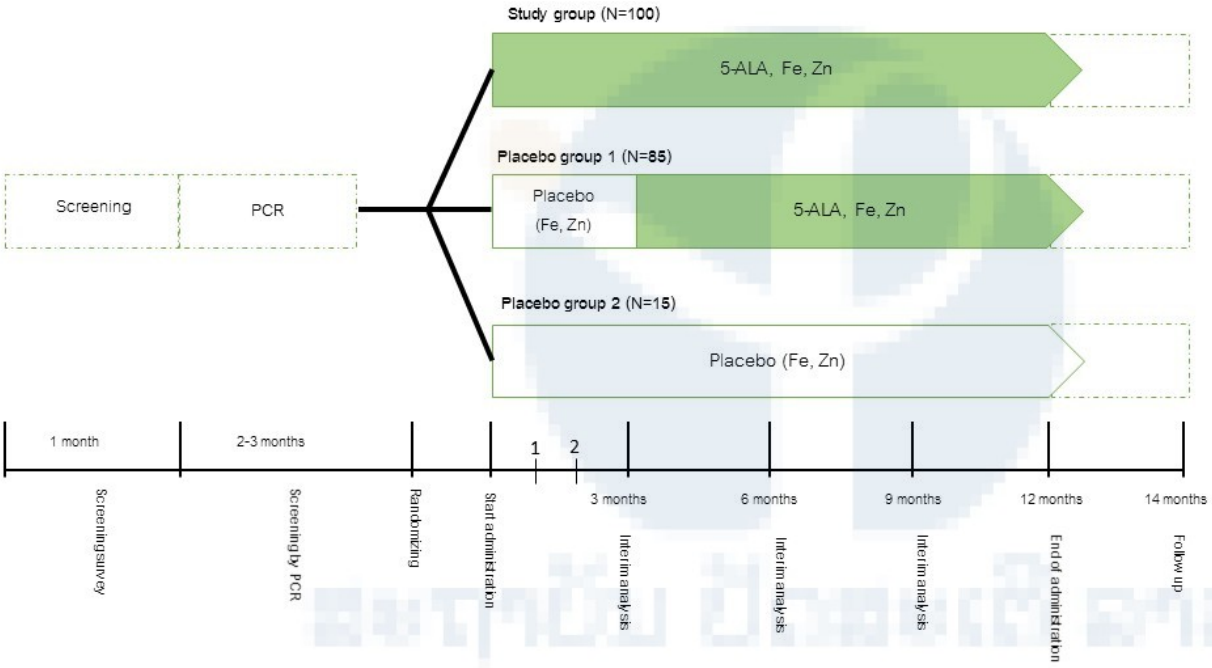
gene in *P. falciparum* in five Southern Provinces 2015-2017

Province	Year					
	2015		2016		2017	
	No.	%	No.	%	No.	%
Savannakhet						
Wild Type	184	72.2	131	78.4	248	94.3
Resistant Type	71	27.8	36	21.6	15	5.7
Sub-Total	**255**	**100.0**	**167**	**100.0**	**263**	**100.0**
Salavan						
Wild Type	90	41.1	26	52.0	50	70.4
Resistant Type	129	58.9	24	48.0	21	29.6
Sub-Total	**219**	**100.0**	**50**	**100.0**	**71**	**100.0**
Sekong						
Wild Type	97	60.6	13	43.3	4	44.4
Resistant Type	63	39.4	17	56.7	5	55.6
Sub-Total	**160**	**100.0**	**30**	**100.0**	**9**	**100.0**
Champasak						
Wild Type	115	27.3	48	37.2	14	23.0
Resistant Type	306	72.7	81	62.8	47	77.0
Sub-Total	**421**	**100.0**	**129**	**100.0**	**61**	**100.0**
Attapeu						
Wild Type	39	30.0	4	15.4	28	57.1
Resistant Type	91	70.0	22	84.6	21	42.9
Sub-Total	**130**	**100.0**	**26**	**100.0**	**49**	**100.0**
Grand-Total	**1,185**		**402**		**453**	

Sampling period
2015: October 2015 – April 2016
2016: May-October or November 2016
2017: November 2016 – May or June 2017

Table 4. Summary of blood sample testing by malaria RDT and PCR

Institute	Location	No. of malaria suspected	RDT Positive	PCR Positive
Institute of Preventative Medicine	Vientiane Capital	0	0	0
103 Hospital	Vientiane Capital	28	3	5
101 Hospital	Xiangkhouang Province	17	3	0
107 Hospital	Luang Prabang Province	27	3	0
108 Hospital	Vang Vieng, Vientiane Province	6	0	0
106 Hospital	Champasak Province	63	1	3
109 Hospital	Savannakhet Province	74	9	5
Total		**215**	**19**	**13**


Study group (N=100)
5-ALA, Fe, Zn
Placebo group 1 (N=85)
Placebo
(Fe, Zn)
5-ALA, Fe, Zn
Placebo group 2 (N=15)
Placebo (Fe, Zn)
Screening
PCR
1 month
2-3 months
1
2
3 months
6 months
9 months
12 months
14 months
Screening survey
Screening by PCR
Randomizing
Start administration
Interim analysis
Interim analysis
Interim analysis
End of administration
Follow up