Studies on antioxidant defense system in the liver stage of rodent malaria parasite

その他（別言語等）のタイトル	ローデントマラリア原虫肝臓型における抗酸化機構の研究
著者（英）	Usui Miho
学位名	博士（畜産衛生学）
学位授与機関	帯広畜産大学
学位授与年度	2013
学位授与番号	10105甲第52号
URL	http://id.nii.ac.jp/1588/00001368/

別記様式第５号（第４条，第７条，第９条関係）

学　位　論　文　要　旨

博士後期　課程　畜産衛生学　専攻
学籍番号　２３３３０
氏　　名　薄井　美帆　印

論文題目：Studies on antioxidant defense system in the liver stage of rodent malaria parasite
（ローデントマラリア原虫肝臓型における抗酸化機構の研究）

要旨

Malaria is a tropical disease being spread by *Plasmodium* parasite-infected female *Anopheles* mosquitoes. In 2010, an estimated 219 million cases of malaria occurred worldwide with 660,000 deaths. Five species are known to infect humans namely *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. Although most of the severe morbidities and mortalities associated with malaria are accounted with *P. falciparum*, the public health concern for *P. vivax* infection is still increasing. And until now, there are no effective vaccine available against malaria and thus the key interventions rely mostly on prompt and effective treatment with artemisinin-based combination therapies; use of insecticide-treated nets and indoor residual spraying of insecticide to control the vector mosquitoes. However, emergence of drug-resistant parasites and insecticide-resistant mosquitoes are seen in many parts of the world and therefore, development of new effective interventions to control the disease is highly needed.

Plasmodium is known to be sensitive to oxidative stresses, and its antioxidant defenses are considered to play a significant role in its survival and thus represent promising target for new chemotherapy against malaria. During their growth and proliferation, the parasite is subjected to the toxic effects of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that originate from the host cell as well as the parasite's own metabolism. For dealing with such ROS and RNS, malaria parasites depend critically on peroxiredoxins (Prxs) for maintaining redox homeostasis due to the absence of catalase and glutathione peroxidase in their genome. The enzymatic activity, physical property and localization of Prxs in several *Plasmodium* species have already been studied except in the liver stage of the parasites. Targeting the liver stage of the malaria parasite for chemotherapy will be a novel way of disease control as malaria infection begins with the invasion of the liver cells by the parasites. Thus, this study aims to understand more the role of the Prxs in the liver stage of malaria parasite using the rodent model *P. berghei*.

This study is consists of three chapters: 1) analysis of the localization and expression profile of three members of Prx namely, thioredoxin peroxidase-1 (TPx-1), 1-cystein peroxiredoxin (1-Cys Prx) and thioredoxin peroxidase-2 (TPx-2) in wild-type (WT) of *P. berghei* during the liver stage; 2) examination of the expression profiles of TPx-1, 1-Cys Prx and TPx-2 during the liver stage of *P. berghei*, in TPx-1 knock-out (KO) and TPx-2 KO parasite populations; and 3) examination and comparison of the phenotypes of three types of *P. berghei* (TPx-1 KO, TPx-2 KO and WT) in the liver stage.

In the first chapter, I examined the mRNA and protein expression profiles of TPx-1, 1-Cys Prx and TPx-2 in the liver stage of *P. berghei*. Salivary gland sporozoite of the parasite was prepared from the *Plasmodium*-infected *A. stephensi* mosquitoes, and used to

infect a human liver cell line (HepG2). The development of liver stage parasite was monitored by indirect fluorescent antibody assay (IFA) using anti-CSP (circum sporozoite protein)-1 antibody. Subcellular localization of Prx proteins were examined by IFA using protein-specific antibodies. Expressions of mRNA were examined simultaneously by quantitative reverse transcription-PCR (RT-qPCR). IFA studies confirmed the cytosolic localization of TPx-1 and 1-Cys Prx, and mitochondrial localization of TPx-2 in the liver stage parasite. The RT-qPCR experiments revealed that mRNA expression of the TPx-1 was detected shortly after the sporozoite infection, kept expressed until the schizont stage and decreased at the cytomere stage. This early up-regulation of TPx-1 mRNA suggested a role of this enzyme to protect the parasite from the intrahepatocyte environment. In contrast, 1-Cys Prx mRNA had begun increasing when the parasite developed into the schizont stage and was up-regulated toward the cytomere stage. This finding suggested the growth stage-specific expression of the cytosolic enzymes in the liver stage parasite. mRNA expression of the mitochondrial TPx-2 increased at trophozoite stage and peaked at schizont stage. Since the parasite develops exponentially from the schizont stage, ATP production required for the development may induce ROS in mitochondria. The cytosolic enzymes may also act on the ROS that leaked out from the organelle.

In the second chapter, I examined the expression profiles of TPx-1, 1-Cys Prx and TPx-2 during the liver stage of *P. berghei*, in TPx-1 KO and TPx-2 KO parasite populations, and compared them with those in the WT. RT-qPCR experiments revealed that mRNA expression levels of 1-Cys Prx mRNA at trophozoite stage and schizont stage were significantly higher in TPx-1 KO than those of WT. The mRNA expression of TPx-2 showed the same pattern as that of the WT. However, expression level of TPx-2 mRNA was significantly higher in TPx-1 KO than that of WT in schizont stage. In TPx-2 KO, the expression level of TPx-1 mRNA shortly after the sporozoite infection was significantly higher than that of WT. The expression levels of 1-Cys Prx mRNA shortly after the sporozoite infection, at trophozoite stage and at schizont stage were significantly higher in TPx-2 KO than those of WT. In conclusion, mRNAs coding for other Prx family in both TPx-1 KO and TPx-2 KO populations were up-regulated during their liver stage development.

In the third chapter, I examined and compared the phenotypes of TPx-1 KO, TPx-2 KO and WT in the liver stage. IFA assay with anti-CSP-1 antibody revealed that TPx-1 KO parasite cell was significantly smaller than that of WT in liver schizont stage. To further examine the parasite development, IFA with anti-merozoite surface protein (MSP)-1 antibody was performed, and the result indicated that TPx-1 KO liver stage parasite could develop as that of WT towards merozoite-forming stage. The size of merozoite in TPx-1 KO was also evaluated to assess the number of merozoite formed in each schizont and compared it to that of WT. The result showed that the mean merozoite size in TPx-1 KO was similar to that of WT, and suggested that the number of merozoite produced in TPx-1 KO was fewer than in WT.

Due to difficulties in studying the liver stages of *Plasmodium* parasite, its cellular and molecular properties in detail have not yet been fully understood. However, this stage of *Plasmodium* infection bears enormous potential for anti-malarial intervention. Investigating such redox system in malaria parasite for developing novel antimalarial drugs and vaccination strategies are interesting topics to be studied in the future.