

Brief communication (Original)

P-glycoprotein expression in human cerebral malaria: a preliminary study

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Background: P-glycoprotein is an efflux protein, which is expressed on several cell types, including vascular endothelial cells lining brain capillaries. The expression and activity of P-glycoprotein can be modulated under conditions, including inflammatory process. Cerebral malaria (CM), a complication caused by infections of *Plasmodium falciparum*, is considered an inflammatory disease.

Objective: We determined P-glycoprotein expression at protein levels in human CM brain capillaries.

Methods: Four brains, three CM and one control, were subjected to a postmortem study. The brain capillaries were isolated from cerebellum, cerebral cortex, striatum, and brainstem and analyzed for expression of P-glycoprotein using SDS-PAGE and western blot techniques. The expression levels in CM brain capillaries were assessed by comparing with that in control brain capillaries.

Results: P-glycoprotein decreased in all CM brain capillaries isolated from striatum, while two of three cerebellums showed the reduction, compared with the control. For cerebral cortex and brainstem brain capillaries, the expression decreased only in one of three CM subjects.

Conclusion: P-glycoprotein expression levels were modulated in CM brain capillaries, suggesting an involvement of P-glycoprotein in CM.

Keywords: Brainstem, cerebellum, cerebral cortex, cerebral malaria: P-glycoprotein, *Plasmodium falciparum*, striatum

P-glycoprotein is a member of the ATP-binding cassette (ABC) transporter superfamily, namely ABCB1 [1]. It was initially recognized in tumor and cancer cells that do not respond to chemotherapy [2]. Later studies revealed its role as an efflux transporter, expressed at the surface of several cell types, including epithelia of kidney, liver, lung, and intestine and vascular endothelial cells of the testis, ovary, and blood-brain barrier (BBB) [3]. At the BBB, P-glycoprotein is located at the luminal side of brain capillary endothelium, which has a vital role in protection of brain tissues from potentially neurotoxic agents. Expression and activity of P-glycoprotein are modulated by conditions, including inflammation, oxidative stress, heat shock, irradiation, inflammation, hypoxia, and glucose deprivation [4, 5].

Cerebral malaria (CM) is a form of severe malaria complication observed in some patients who are

infected with *Plasmodium falciparum* (Pf). The characteristics of CM are impaired consciousness, convulsions and unarousable coma. It is known that CM can lead to brain damage of the cerebellum, cortex, brainstem, and striatum [6-9]. This can result in mortality and morbidity, including neurological impairment [10]. There are several hypotheses proposed to explain the pathophysiology, involving cytoadherence and sequestration of Pf-infected erythrocytes in brain capillaries and venules, causing hypoxia and metabolic disturbance [11]. Induction of proinflammatory cytokines, including tumor-necrosis factor alpha (TNF- α) and interferon-gamma (IFN- γ) [12] are other factors in addition to disruptions of the BBB in the CM brain [13]. However, it is interesting that the malarial parasites do not enter brain parenchyma, yet they can induce neurological manifestations. It is possible that an efflux protein P-glycoprotein, with its neuroprotective role, is involved in CM pathophysiology. In the present study, we examined whether the expression of P-glycoprotein is changed in CM brains.

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Materials and methods

Sample collection

After obtaining informed consent from the subjects’ relatives and with permission of the Ethical Review Committee for Research in Human Subjects, Ministry of Public Health, Thailand, postmortem brains, cerebral cortex, cerebellum, brain stem and striatum, were collected from 4 subjects. Three died from CM and one died from other causes without brain involvement (Table 1).

Separation of brain capillaries

Brain capillaries were isolated according to a method reported earlier [14, 15]. Briefly, white matter was removed and tissues were homogenized. Then the homogenate was centrifuged for 10 min at 1,000 g at 4°C. The supernatant was removed, and the pellets were resuspended in DMEM containing 25% bovine serum albumin, followed by centrifugation at 1,500 g for 20 minutes and filtration through 180- and 41-mm nylon mesh to separate brain capillaries. The capillary filtrate was then washed twice with phosphate-buffered saline and kept at –80°C until analysis.

P-glycoprotein expression

Brain capillaries were resuspended with lysis buffer (10 mM Tris, pH 7.4, 5 mM EDTA, 126 mM NaCl, 1% Triton X-100, 0.1% SDS, and protease-inhibitor cocktails), and protein concentrations in the lysates were determined by using a bicinchoninic acid assay (Thermo Scientific Pierce, IL, USA) according to the supplier’s protocol. Twenty micrograms of proteins in the lysates were then separated by using SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

and transferred to nitrocellulose membranes for western blot analysis. After washing and blocking with 5% bovine serum albumin in Tris-buffered saline with Tween 20 (TTBS), the membrane was incubated with primary antibody against P-glycoprotein or actin, washed with TTBS and incubated with HRP-conjugated anti-mouse secondary antibody. Subsequently, chemiluminescence signals were developed by using a Pierce ECL SuperSignal Substrate kit. P-glycoprotein-band densities were quantitated, normalized to the corresponding actin band density and by setting the actin band density in the control as 100%.

Results

P-glycoprotein has an important role in protecting the brain from potential harm. We examined whether P-glycoprotein is involved in CM pathophysiology. Four postmortem brain samples (cerebellum, cerebral cortex, brain stem, and striatum) were collected from each subject and brain capillaries were isolated. The expression of P-glycoprotein in brain capillaries was then assessed using SDS-PAGE and western blotting. The results are shown in Figure 1. It was observed that P-glycoprotein was reduced in striatum brain capillaries isolated from CM-1, CM-2, and CM-3 (58.3%, 53.0%, and 56.0% of control, respectively). Moreover, cerebellum capillaries isolated from CM-1 and CM-2 also revealed reduction (47.0% and 48.8% of control, respectively), while that from CM-3 was slightly decreased (86.9% of control). Interestingly, only CM-1 showed decreases in P-glycoprotein in the cortex and brainstem brain capillaries (56.3% and 59.2% of control, respectively).

Table 1. Subject data

Subjects	Age (years)	Gender	Cause of death
Control	32	Male	Acute renal failure
CM-1	35	Male	CM
CM-2	0.5	Female	CM
CM-3	40	Male	CM

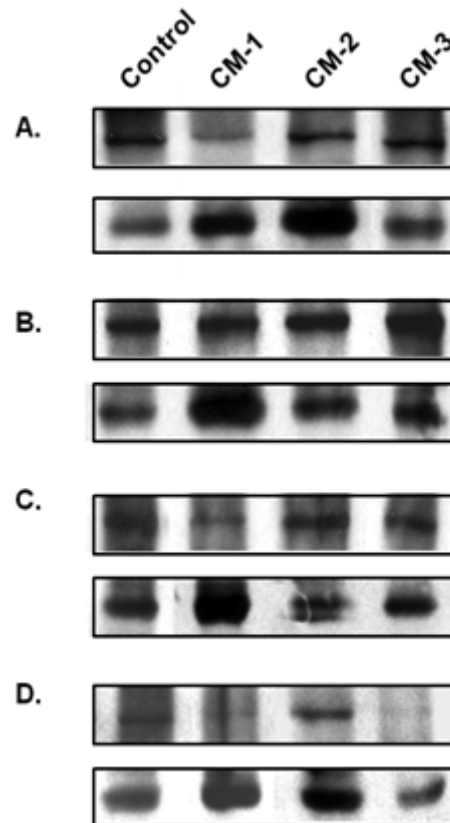


Figure 1. Expression of P-glycoprotein in CM. Brain capillaries were isolated from cerebellum (A), cerebral cortex (B), brainstem (C), and striatum (D) collected from 4 subjects (control, CM-1, CM-2, and CM-3). P-glycoprotein expression was assessed by using SDS-PAGE and western blotting techniques. The upper panels show P-glycoprotein bands and the lower panel show actin bands.

Discussion

P-glycoprotein is an important efflux protein and functions in neurological protection. However, its involvement in CM has not been studied. The present study is the first revealing expression change of P-glycoprotein in human CM. We conducted this preliminary study to determine if there are any changes in expression of P-glycoprotein in human brain capillaries isolated from CM subjects. We observed that P-glycoprotein decreased in striatum brain capillaries of all three CM subjects. However, P-glycoprotein in the cerebellum, cortex, and striatum brain capillaries did not change in any sample investigated. It is possible that CM pathophysiology does not necessary involve every part of the brain.

The BBB at brain capillaries has a vital function in neuroprotection. Vascular endothelial cells are important components of the BBB, allowing limited movement across BBB. In addition, these cells express

efflux proteins, including P-glycoprotein. In the pathophysiology of CM, BBB breakdown occurs at functional levels, while its morphology may be normal. Evidence reveals that sequestration of *Pf*-infected erythrocytes is an event preceding BBB breakdown [16]. It has been reported that neurological sequelae after CM vary because affected individuals might suffer from different areas of affected brain [17]. This implication supports our findings that reduction of P-glycoprotein was observed only in some brain areas of the CM patients and the change patterns were different. It is possible that sequestration of *Pf*-infected erythrocytes causes not only BBB breakdown, but also decreases in P-glycoprotein, which is expressed on vascular endothelial cells of the BBB.

CM is recognized as an inflammatory disease because several cytokines are released [12]. It has been reported that inflammation in the central nervous

system (CNS) leads to decreases in P-glycoprotein [18], and certain cytokines have effects on P-glycoprotein [19]. Other studies reported that P-glycoprotein decreases at an early stage of inflammation and returns to normal at a later stage [20, 21]. However, another study showed that P-glycoprotein can be upregulated in cells surviving from BBB damage [22]. Therefore, it might be proposed that modulation of P-glycoprotein expression during this inflammatory process is time dependent. This time-dependent modulation makes the pathophysiology of CM even more complex. If expression of P-glycoprotein is induced during inflammation, the time of sample collection will be critical for studying its change in CM. Moreover, other studies revealed that different cytokines can induce different effects on P-glycoprotein expression. TNF- α and IFN- γ induce downregulation of P-glycoprotein in rat brain capillaries [23] and human colon carcinoma cell lines [24], while TNF- α causes increases in P-glycoprotein in cerebral microvascular endothelial cells [25]. These studies imply that effects of cytokines on P-glycoprotein expression may vary, depending on inflammatory inducers and responding cells. Conversely, P-glycoprotein itself can modulate immunological response by regulating cytokine secretion [26]. Even though our results suggest an involvement of P-glycoprotein in CM, further studies are still needed to clarify the kinetic of change in P-glycoprotein and how P-glycoprotein is involved in CM. However, several elements remain to be elucidated to understand more fully how P-glycoprotein is involved in CM.

Acknowledgements

We thank Professor Francois Gimenez for his valuable help in isolation of brain capillaries. This work was financially supported by the Thailand Research Fund and the Office of the Higher Education Commission. The authors have no conflicts of interest to report.

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