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Possible role of heme oxygenase-1 and prostaglandins in pathogenesis of cerebral malaria: induction of heme oxygenase-1 by prostaglandin D2 and metabolite by human astrocyte CCF-STTG1 cells *in vitro*

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Abstract

Astrocytes are the most abundant cells in central nervous system that play role in maintaining the blood-brain-barrier and in neural injury, including cerebral malaria, a severe complication of *Plasmodium falciparum.* Prostaglandin D_2 (PGD_2) is abundantly produced in the brain and regulates the sleep response. Moreover, PGD_2 is a potential factor derived from *P.falciparum* within erythrocytes. Heme oxygenase-1 (HO-1) is catalyzing enzyme in heme breakdown process to release iron, carbon monoxide and biliverdin/bilirubin, and may influence iron supply to the *falciparum* parasites. Here, we showed that treatment of human astrocyte cell, CCF-STTG1, with PGD_2 and its metabolite 15d-PGJ_2 significantly increased the expression levels of HO-1 mRNA by RT-PCR. Western blot analysis showed that PGD_2 and 15d-PGJ_2 treatment increased the level of HO-1 protein, in a dose- and time-dependent manner. Thus, both prostaglandins may be involved in the pathogenesis of cerebral malaria by inducing HO-1 expression in malaria patients.

Keywords: astrocyte cell, heme oxygenase, iron, malaria, prostaglandin D_2

Introduction

Malaria is a worldwide protozoan infection, and most malignant malaria is caused by *Plasmodium falciparum*. Cerebral malaria is one of the most severe complications of *P. falciparum* infection (1). The parasite does not enter the brain parenchyma, but staying in the intravascular circulation, which is responsible for changes at the BBB. *In vitro* and *in vivo* studies support the role of astrocytes in controlling blood-brain-barrier (BBB) maintenance and regulation through their interaction with cerebral endothelial cells (2). The molecular basis underlying cerebral malaria nevertheless, remains unclear. Recently, the microsomal enzyme heme oxygenase-1 (HO-1) has been proposed as one of the factors that play significant role in pathogenesis of this malaria complication (3). It is the rate-limiting enzymes in heme catabolism to generate biliverdin IXα/bilirubin IXα, carbon monoxide, and ferrous iron (3). The expression levels of HO-1 is inducible or repressible, depending on cells types or cellular microenvironments, but expression levels of HO-2 are fairly constant (3). We have recently reported that the short $(GT)_n$ repeats ($n < 28$) in the HO-1 gene promoter are associated with higher incidence of cerebral malaria in the Karen ethnic minority group who live near the border between Myanmar and Thailand (4). The cell homogenates of *P. falciparum* have been shown to contain the activity that produces PGD_2 and PGE_2 after incubation with arachidonic acid (5). Moreover, PGD_2 was maintained at a higher level in serum of falciparum malaria patients than control serum (6). We also found that PGD_2 and 15d-PGJ_2, a minor species of PGD_2 metabolites, increased HO-1 gene promoter, mRNA levels and protein levels in retinal pigment epithelial cells that may associate with the pathogenesis of malarial retinopathy in malaria patients with cerebral complication (7). In the present study, we provide additional data to support the link between HO-1 and pathogenesis of cerebral malaria through the induction by PGD_2 in human astrocytes.

Methods

To examine the effects of PGD_2 and 15d-PGJ_2 (Cayman chemicals) on the expression levels of HO-1 protein and mRNA, CCF-STTG1 cells(ATCC®, # CRL-1718, USA) were grown to 70-80% confluence (RPMI 1640 medium, supplemented with 10% fetal bovine serum, 2 mM L-glutamine and 100 U/ml penicillin and 0.1 mg/ml streptomycin) before they were incubated with vehicle (ethanol), PGD_2 or 15d-PGJ_2. The Western blots were probed with HO-1 (SPA-895, StressGen Biotechnologies) and β-actin (Sigma) antibodies (7), followed by incubation with AP-goat anti-rabbit and AP-goat anti-mouse, respectively (ZyMax™, Invitrogen) for detection of HO-1 protein.

Total RNA was extracted from treated CCF-STTG1 cells using RNeasy® Mini Kit (Qiagen) and was transcribed to cDNA using Omniscript® RT Kit (Qiagen). Then HO-1 cDNA levels were determined by RT-PCR. Primers were designed according to the published cDNA sequences for human HO-1 (8).

Results

Effects of PGD_2 and 15d-PGJ_2 on the expression of HO-1 protein in CCF-STTG1 cells: Western blot analysis revealed that the expression of HO-1 protein was induced in a dose- and time-dependent manner by PGD_2 and 15d-PGJ_2 at final concentrations of 5 and 10 µM after 24-h and 6-h treatment, respectively (Figure 1A, 1B). The onset of the HO-1 induction with 15d-PGJ_2 was earlier than with PGD_2.

Increased expression of HO-1 mRNA in CCF-STTG1 cells treated with PGD_2 and 15d-PGJ_2: The expression of HO-1 mRNA was induced by PGD_2 or 15d-PGJ_2 in a dose-dependent manner (Figure 2A, B). The expression levels of HO-1 mRNA were increased after 6 h of the treatment with PGD_2 of 5 µM or 10 µM, and continuously increased at maximum level after 12 h (Figure 2A). For 15d-PGJ_2, the expression levels of HO-1 mRNA were detected at 3 h of treatment (Figure 2B). The maximum induction of HO-1 mRNA was achieved after 6 h of treatment, but it was decreased at 24 h (Figure 2B). The induction profiles of HO-1 mRNA were in good agreement with those of HO-1 protein (Figure 1A, B).

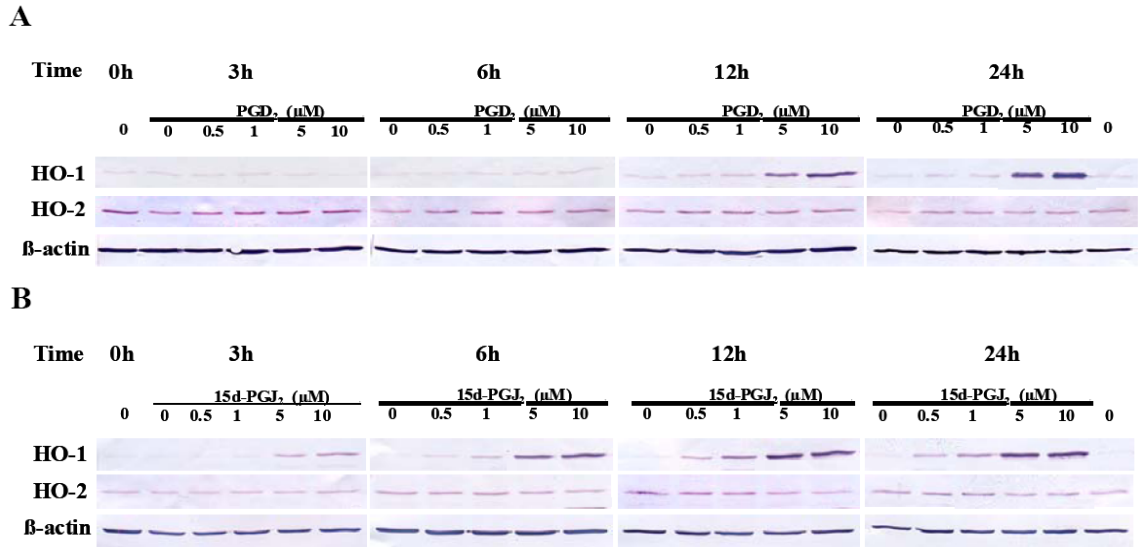

Figure 1. Effects of (A) PGD_2 and (B) 15dPGJ_2 on HO-1 protein in CCF-STTG1 cells. Data shown are from one of two independent experiments.

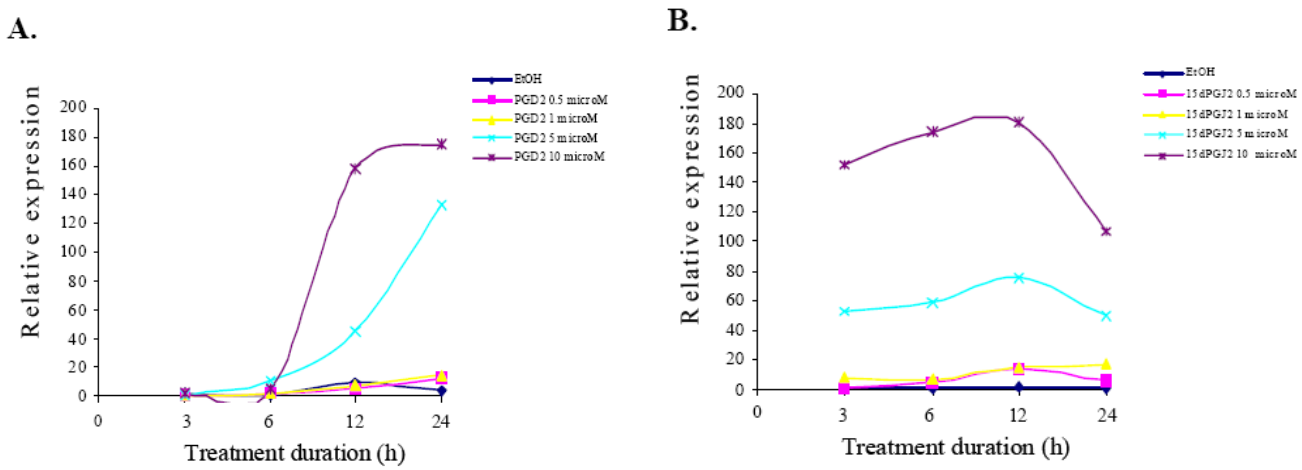

Figure 2. Expression of HO-1 mRNA by (A) PGD_2 and (B) $15dPGJ_2$ in CCF-STTG1 astrocyte cells. Data were obtained by dividing the intensity value for each sample with 0-h untreated control cells, which reflected basal expression level.

Discussion

The current result showing that treatment of the astrocyte cells CCF-STTG1 with exogenous PGD_2 consistently induced HO-1 expression, is in agreement with our recent report in retinal pigment epithelial cells (7). The induction profile of HO-1 expression with PGD_2 is essentially similar to that with 15d-PGJ_2, although the onset of the induction by PGD_2 is later than by 15d-PGJ_2. It remains to be explored whether PGD_2 by itself or a specific PGD_2 metabolite other than 15d-PGJ_2 is responsible for the induction of HO-1 expression. Apart from astrocyte cells themselves, falciparum parasites may also release PGD_2 that may hasten the expression of HO-1. This consequence additively results in the increase in HO-1 activity to catalyse heme to end products, especially, iron. Malaria growth and proliferation depend on iron supply from host cells, such as endothelial cells or neuron cells near the sequestration site. Therefore, PGD_2 might enhance the growth of parasites by modulating iron availability from host. The stimulation of HO-1 by PGD_2 and the metabolite 15d-PGJ_2 observed in this study is critical since excessive heme degradation may result in toxic levels of iron similar to that by carbonmonoxide, and bilirubin/biliverdin.

Conclusion

PGD_2 and 15d-PGJ_2 may be involved in the pathogenesis of cerebral malaria by inducing HO-1 expression in malaria patients. This information could be exploited for development of new antimalarial drugs acing as inhibitors of HO-1 to prevent the progression to severe cerebral malaria. Further studies are require to obtain supporting evidence in patients with malaria.

Acknowledgements

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