

# Cyclooxygenase-2 in the Pathogenesis of Murine Cerebral Malaria

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**Cerebral malaria (CM) is a severe complication of malaria, in which cytokine production can produce immunopathological consequences. Cytokines can up-regulate prostaglandin synthesis via an increase in cyclooxygenase (COX) enzyme activity. We investigated the expression of COX enzymes, COX-1 and COX-2, in the brain by use of murine models of CM and of malaria without cerebral involvement. Although COX-1 mRNA was induced in the brain in both models of malaria, COX-2 mRNA was induced specifically in CM. Inhibition of COX-2 with celecoxib resulted in an earlier onset of CM. Treatment with celecoxib did not alter the outcome of malaria infection without cerebral involvement. These data suggest that induction of COX-2 expression and prostaglandin synthesis may have a protective effect in CM.**

Cerebral malaria (CM) is a complication of *Plasmodium falciparum* infection and is responsible for more than a million deaths every year. Symptoms of the disease include seizures, loss of consciousness, and coma. Features observed at postmortem examination of the brain include hemorrhages, edema, and adherence of parasitized red blood cells (PRBCs) to the microvasculature.

Several rodent models of malaria exist. Infection with *Plasmodium berghei* ANKA causes neurological symptoms, such as seizures and coma, followed by death. The mouse model has proved to be invaluable in studies of the pathogenesis of CM [1]. Histopathological features are similar to those of human CM, except that, in the cerebral microvasculature, adherent monocytes predominate over PRBCs [2]. Infection with the same dose of another malaria strain, *P. berghei* K173, causes death ~14 days after inoculation; during this interval, the mice become severely anemic but do not experience

any neurological symptoms or brain histopathologic abnormalities.

The pathogenesis of human CM is not well understood. One theory suggests that “sludging” of the PRBCs in the cerebral microvasculature may cause local ischemic events [3]. Another idea is that the basis of the disease is immunopathological, involving the production of cytokines and other immune mediators [4]. It is likely that both processes contribute to the development of the disease, with the PRBCs “focusing” the immune response in the brain [5, 6].

We have investigated the roles played by some mediators of inflammation in murine CM. Prostaglandins are involved in many physiological and pathophysiological processes, including inflammation. Cyclooxygenases (COXs) catalyze the first step in the biosynthesis of prostaglandins from arachidonic acid. COX-1 is widely, and usually constitutively, expressed. Constitutive expression of COX-2 is found in the brain, kidneys, and gastrointestinal system and can also be induced by stimuli such as growth factors, bacterial endotoxin, and cytokines [7]. For this reason, COX-2 is thought to be responsible for much of the synthesis of prostaglandin that occurs during inflammation. Selective inhibitors of COX-2 that have fewer gastrointestinal adverse effects than non-selective COX inhibitors have been developed [8].

Plasma levels of bicyclo-prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and expression of COX-2 in peripheral blood mononuclear cells (PBMCs) of African children with *P. falciparum*

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malaria were highest in healthy children, followed by children with mild malaria, and were lowest in children with severe malaria [9]. Salicylates in the blood of Kenyan children correlated with poor outcomes in severe malaria [10]. These studies suggest that the synthesis of prostaglandins is beneficial in determining the outcome of malaria. Accumulation of COX-1 and COX-2 proteins was observed in the brains of patients with CM, suggesting that prostaglandins may also play a role in human CM [11].

In a murine model of CM, the administration of aspirin (a nonselective COX inhibitor) was found to decrease survival time in *P. berghei* ANKA infection [12]. We have investigated the expression of COX enzyme mRNA in the brain during malaria infection, using models both with and without cerebral involvement. The expression of mRNAs for some other enzymes involved in the biosynthesis of eicosanoid also was measured. We determined whether inhibition of COX-2 was deleterious in CM by use of celecoxib, an inhibitor of prostaglandin biosynthesis that is selective for the enzyme. Our results suggest that biosynthesis of eicosanoid via COX-2 is protective in murine CM.

## MATERIALS AND METHODS

CBA or C57Bl6 mice were intraperitoneally inoculated with 10<sup>6</sup> PRBCs from a *P. berghei* ANKA- or *P. berghei* K173-infected mouse. The *P. berghei* ANKA strain was obtained from Prof. G. Grau (Université de la Méditerranée, Marseille, France), and the *P. berghei* K173 strain was obtained from Prof. I. Clark (Australian National University, Canberra, Australia). The strains were independently isolated and have not been cloned subsequently. Celecoxib (Searle) was administered to the mice in their food (~800 mg/kg of food; Gordons Speciality Stockfeeds). Experiments were conducted in accordance with University of Sydney animal ethics guidelines.

**RNA extraction from mouse tissue.** Mouse brain tissue (250 mg) was placed in 1 mL of Tri reagent (Sigma) and was

homogenized by use of lysing matrix D and a Fastprep homogenizer (Qbiogene). Chloroform (0.2 mL) was added, and the lysate was mixed well. After microfuging at 12,000 relative centrifugal force (rcf) for 15 min, the aqueous layer was transferred to a new tube. RNA was precipitated with 500  $\mu$ L of isopropanol and pelleted by microfuging at 12,000 rcf for 15 min. The pellet was washed with 70% (vol/vol) ethanol (EtOH) and resuspended in water.

**RNA extraction from blood.** Cells were collected from ~500  $\mu$ L of blood by centrifugation at 9000 rcf for 2 min. The cells were then lysed, and RNA was extracted by use of an RNeasy kit (Qiagen), according to the manufacturer's instructions.

**cDNA synthesis and real-time polymerase chain reaction (PCR).** Any contaminating genomic DNA was removed by DNase treatment by use of the DNasefree kit (Ambion). cDNA was synthesized from up to 2  $\mu$ g of total RNA by use of 0.1  $\mu$ g of oligodT<sub>18</sub>, 0.6 mmol/L each nucleotide, 5 U of Prime RNase inhibitor (Eppendorf), and a Moloney murine leukemia virus reverse transcriptase kit (Invitrogen). Approximately 20 ng of cDNA was used in each 20- $\mu$ L PCR, which were performed in an ABI7700 PCR machine (Applied Biosystems) by use of Platinum Quantitative PCR SuperMix-UDG with added ROX reference dye (Invitrogen), 0.3 $\times$  SYBR Green nucleic acid stain (Molecular Probes), and 100 nmol/L each primer (listed in table 1). After a 10-min incubation at 95°C, amplification was achieved by 40 cycles of a 15-s incubation at 95°C, followed by a 60-s incubation at 60°C. The identity and purity of the PCR product was confirmed by melting-curve analysis. Expression levels in infected mice were compared with uninfected controls after adjustment according to levels of the reference housekeeping gene, hypoxanthine guanine phosphoribosyltransferase.

**Assessing the development of malaria and CM.** Mice were observed for the development of symptoms, including seizures and the Woolley-White sign, which is typically observed in murine CM [13]. When the Woolley-White sign is present, the mice, when picked up by the tail, display spasticity of the legs,

**Table 1. Oligonucleotide sequences used for reverse-transcriptase polymerase chain reaction in the present study.**

Gene or mRNA	Primer, 5'→3'	
	Forward	Reverse
HPRT	GCTTCCCTGGTTAAGCAGTACA	CAAACCTGTCTGGAATTTCAAATC
COX-1	GCCTGGTACTCACAGTGCGGT	ACCTGGTGGGTAGCGCAT
COX-2	AAGCGAGGACCTGGGTTCA	AAGGCGCAGTTTATGTTGTCTGT
5-lipoxygenase	TGCCATCCAGCTCAACCAA	GCCAGTCGTACTTTGAATCCGT
FLAP	TCATCAGCGTGGTCCAGAATG	CCTTGCTTTCATGCTCCACCT
IFN- $\gamma$	CAGCAACAGCAAGGCGAAA	GCTGGATCCGGAACAG
TNF	AATGGCCTCCCTCTCATCAGTT	CCACTTGGTGGTTTGCTACGA
IL-1 $\beta$	GTGGTTCGAGGCCTAATAGGCT	AGCTGCTTCAGACACCTTGCA

**NOTE.** COX, cyclooxygenase; FLAP, 5-lipoxygenase activating protein; HPRT, hypoxanthine guanine phosphoribosyltransferase; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

stiffness, or even convulsions. This causes them to spin, rather than climb into an upright position. Parasite load also was determined from day 4 after inoculation onward. A novel, objective assessment of mouse activity was developed. As some mice showed signs of becoming ill (e.g., ruffled fur), their activity was monitored for 40-min periods by use of video cameras. Four mice were monitored simultaneously. Each activity box contained a video camera (Monochrome, Infra-Red Sensitive CCTV Video Surveillance Camera; 480 H-Lines SONY HAD CCD and SONY Chip set), which was mounted on the top and center of the box and was directed down to provide a bird's-eye view. A quad processor subsampled each input image at a 2:1 ratio and combined 4 images together at the corners of a single synchronized output image. These combined images were passed at 25 Hz to a monochrome video acquisition card (PCI NI-IMAQ 1407; National Instruments) installed in an IBM-compatible personal computer and were digitized. The software for assessing activity was written in house, by use of LabView G and a library of image-analysis functions called NI-IMAQ Vision (both from National Instruments).

Each video image was converted to a bitmap (1 bit/pixel image depth) by use of an adjustable threshold. Particle-analysis routines were then used to search for all the dark objects in the image. Because the image of the test mouse was the largest particle in each quadrant, these were further analyzed to calculate the mouse's position by use of a center-of-mass algorithm. The measure of activity was calculated by dividing the change in mouse position (or distance moved) between successive images by the time between images.

The water content of the brain was determined by weighing the brain soon after death, drying it for 2 weeks at 37°C, and reweighing the dried brain. Some of the brain was also formalin-fixed for hisopathological examination. Sections were stained with hemotoxylin-eosin for this purpose.

**Bicyclo-PGE<sub>2</sub> assay.** Measurements of prostaglandin were performed early on day 5 of infection, before mice were showing symptoms of CM. Brain tissue was homogenized in 0.05 mol/L Tris buffer (pH 7.4). After the addition of 4 volumes of EtOH, the protein was precipitated by centrifugation for 10 min at 3000 rcf. The EtOH was evaporated, and the pH was adjusted with the addition of 2 volumes of 1× acetate buffer (0.041 mol/L acetic acid and 0.009 mol/L sodium acetate [pH4.0]). The extract was further purified through a SepPak column (Millipore) and was eluted with methanol. After evaporation, the samples were resuspended in buffer provided with a commercial EIA kit (Cayman Chemicals). PGE<sub>2</sub> was converted to bicyclo-PGE<sub>2</sub> and measured according to the manufacturer's instructions.

**Statistical analysis.** Data are expressed as mean ± SD. Differences between >2 groups were compared by use of the Kruskal-Wallis test, followed by multiple Dunn's post test by use of the

Prism program (version 3.0; GraphPad). Differences between 2 groups were compared with the Mann-Whitney *U* test.

## RESULTS

**Expression of genes in the brain and blood during malaria infection.** Levels of mRNA for a number of genes were measured in groups of 6 mice, with the exception of the time-course experiment that included only 3 mice/group. At day 6 after inoculation, there was a small but significant induction of COX-1 mRNA in the brain during *P. berghei* ANKA infection, and similar increases were observed during *P. berghei* K173 infection (figure 1A); and there was a significant induction of COX-2 mRNA in the brain in *P. berghei* ANKA infection, compared with no infection and *P. berghei* K173 infection (figure 1B). In comparison, at either day 6 or day 14 after inoculation, there was no significant induction during *P. berghei* K173 infection. A time-course analysis of induction found that expression of COX-2 mRNA began to increase at day 3 (figure 1C). Expression of COX-2 mRNA was decreased significantly in the blood later during *P. berghei* K173 infection, compared with no infection (figure 1D).

Expression of COX-2 is regulated by some cytokines, so the expression of these cytokines in the brain during malaria was investigated. At day 6 after inoculation, interferon (IFN)-γ, tumor necrosis factor (TNF), and interleukin (IL)-1β mRNA levels were significantly higher in the brains of mice with CM than in the brains of uninfected mice (figure 2A, 2B, and 2C, respectively). An increase in serum leukotriene B<sub>4</sub> (LTB<sub>4</sub>) levels has been observed in murine CM and has been found to be further increased by treatment with aspirin [12]. We thus investigated the expression during malaria of 2 key proteins involved in the synthesis of LTB<sub>4</sub>: 5-lipoxygenase activating protein (FLAP) mRNA was significantly up-regulated during CM and the later stages of *P. berghei* K173 infection (figure 3B), whereas 5-lipoxygenase was up-regulated only during the late stages of *P. berghei* K173 infection (figure 3A).

**Inhibition of COX-2 during CM.** An increase in the expression of COX-2 and COX-1 mRNA corresponded to an increase in bicyclo-PGE<sub>2</sub> levels in the brains of infected mice (figure 4). The increase in bicyclo-PGE<sub>2</sub> levels was significantly reduced in the celecoxib-treated mice, compared with untreated mice, although the levels did not return to baseline, indicating that the treatment was only partially successful in inhibiting biosynthesis of prostaglandin.

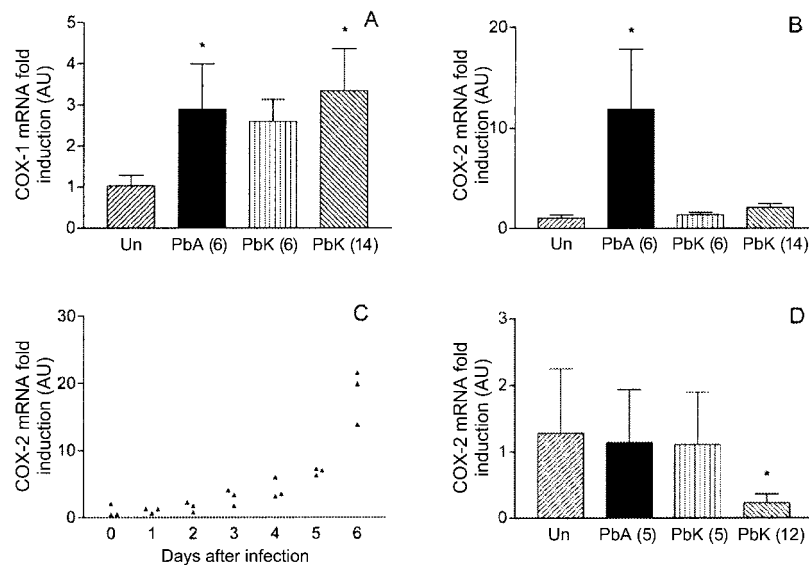
Allowing mice to progress to death is not in accordance with animal ethics guidelines, so a survival curve was not appropriate. Mice were killed when they became ill. We used 2 objective measures (activity and water content of the brain) to quantify the differences between celecoxib-treated mice and untreated mice. We conducted these measurements at a time when the

celecoxib-treated mice were showing signs of CM (e.g., ruffled fur, hunched appearance, and occasional seizures), but the untreated mice still appeared relatively well (i.e., early at day 6 of *P. berghei* ANKA infection). We used video cameras and the Labview program to track the movement of mice in a 50-cm<sup>2</sup> box for ~40 min. Four mice, 2 from each treatment group, were measured concurrently. We found that uninfected mice were more active than *P. berghei* ANKA-infected mice, who, in turn, were more active than *P. berghei* ANKA-infected, celecoxib-treated mice (table 2). In addition to being less active, celecoxib-treated mice were also observed to have seizures (as early as day 5 after inoculation) to a greater extent than untreated mice (data not shown). After the activity monitoring, the mice were killed. Part of the brain was taken for histopathological analysis to confirm that the mice were exhibiting the hallmarks of CM (small hemorrhages and patches of edema) [14]. The rest of the brain was weighed, dried, and reweighed, to determine the water content of the brain as an indicator of edema. We found that the celecoxib-treated mice had a significantly higher incidence of edema at that time point than did the untreated mice (table 2). The water content of the brain in the uninfected, celecoxib-treated mice did not differ from that in untreated control mice (data not shown). The water content of the brain of *P. berghei* ANKA-infected, untreated mice did not differ significantly from that in the uninfected control mice; however, from previous experiments [15], we know that edema will be present at a later time point than

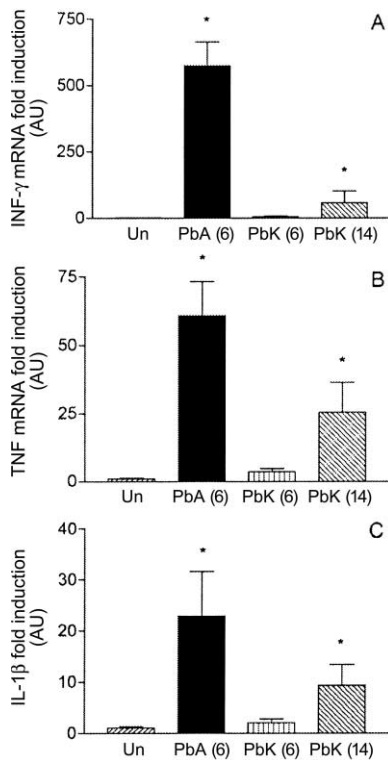
was used in this experiment. The parasitemias of the untreated and treated mice did not differ significantly, indicating that a more rapid increase in parasitemia was not the reason for the earlier onset of disease (table 2).

#### **Inhibition of COX-2 during *P. berghei* K173 infection.**

We investigated whether administration of celecoxib during *P. berghei* K173 infection was sufficient to induce CM in a model in which it does not normally occur. We used CBA mice, as in the *P. berghei* ANKA experiments, but we also performed the experiment in C57Bl/6 mice, which are susceptible to developing CM with low doses of *P. berghei* K173K173. A high inoculum (10<sup>6</sup> PRBCs) of *P. berghei* K173 that does not generally cause CM was used, because we wished to see whether inhibition of COX-2 would induce CM in a model in which it would otherwise not occur. No neurological symptoms (e.g., seizures and the Woolley-White sign) were observed during the infection. Mice appeared ill on days 13–14 after inoculation and were killed. No differences in the water content of the brain were observed (table 3), and there were no histopathological changes normally associated with CM (data not shown). Although there were differences in the parasitemias and hematocrit levels between the strains of mice, there were no significant differences between untreated and treated mice (table 3). Therefore, we conclude that administration of celecoxib had no detectable effect on the outcome of *P. berghei* K173 infection and that there was no evidence of CM.



**Figure 1.** Expression of cyclooxygenase (COX)-1 mRNA (A) and COX-2 mRNA (B) in mouse brains during malaria. C, Time course of the induction of COX-2 mRNA expression in mouse brains during *Plasmodium berghei* ANKA (PbA) infection (cerebral malaria). D, Expression of COX-2 mRNA in the blood during malaria. The mean  $\pm$  SD values of 6 CBA mice are shown, with the exception of the time course (C), in which the values for each of 3 CBA mice/day after infection are shown. The time points for panels A and B (shown in parentheses) are day 6 after inoculation (PbA and *Plasmodium berghei* K173 [PbK]) and day 14 after inoculation (PbK). The time points for panel D (shown in parentheses) are day 5 after inoculation (PbA and PbK) and day 12 after inoculation (PbK). \* $P < .05$ , vs. uninfected (Un) mice. AU, arbitrary units.



**Figure 2.** Expression of interferon (IFN)- $\gamma$  mRNA (A), tumor necrosis factor (TNF) mRNA (B), and interleukin (IL)-1 $\beta$  mRNA (C) in mouse brains during malaria. The mean  $\pm$  SD values for 6 CBA mice are shown. The mRNA levels were determined at day 6 after inoculation (shown in parentheses) with *Plasmodium berghei* ANKA (PbA) and *Plasmodium berghei* K173 (PbK) and at day 14 after inoculation with PbK. \* $P < .05$ , vs. uninfected (Un) mice. AU, arbitrary units.

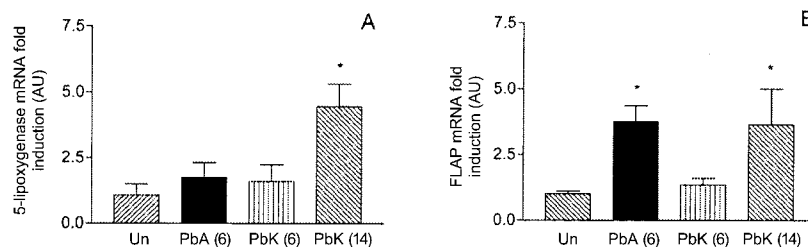
## DISCUSSION

In areas where malaria is endemic, aspirin and other COX inhibitors are commonly taken for fever and headache, so understanding the effects of these drugs on the outcome of malaria infection is important. Our results, like those of Xiao et al. [12], show that expression of COX-2 mRNA in the brain is highly induced in CM. Levels begin to increase on day 3 of infection, well before the onset of symptoms. In addition, we have found

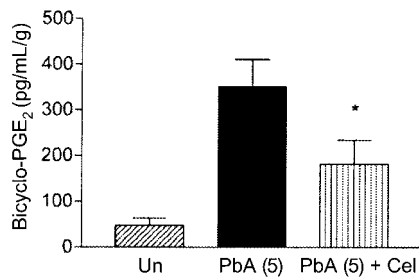
that this induction is much greater in CM than in malaria without cerebral involvement. An increase in prostaglandin levels, as determined by use of the bicyclo-PGE<sub>2</sub> assay, indicates that the induction of mRNA correlates with an increase in COX activity. The greater induction of COX-2 in CM could be due to the higher levels of cytokines (IFN- $\gamma$ , TNF, and IL-1 $\beta$ ) that are found in the brain and circulation during CM, compared with during *P. berghei* K173 infection. These cytokines have been demonstrated to induce COX-2 via NF- $\kappa$ B (TNF and IL-1 $\beta$ ) or IFN- $\gamma$ -stimulated response elements in other systems [16–18].

The inhibition of COX activity with aspirin reduces survival time in CM [12]. Our results with celecoxib suggest that inhibition of COX-2 alone causes mice to become moribund earlier. The increase in the water content of the brain and the presence of the hallmarks of CM histopathologic abnormalities indicate an earlier onset of CM, rather than toxicity of the drug. Administration of celecoxib did not alter the outcome of *P. berghei* K173 infection, indicating that, although inhibition of COX-2 worsens CM, it does not induce CM in mice with severe malaria.

COX-2 is generally considered to be proinflammatory, and, therefore, a COX-2 inhibitor might be expected to be beneficial in a disease such as CM, in which inflammatory mediators play an important role in pathologic processes. Other neurological diseases (e.g., Alzheimer's disease, amyotrophic lateral sclerosis, and ischemia) share features with CM, such as compromise of the blood-brain barrier. The use of COX inhibitors in experimental models of these diseases has been found to be protective in some cases [19–21]. In addition, seizures are a common feature of CM. Overexpressing human COX-2 in neuronal cells of transgenic mice leads to a greater intensity and lethality of kainic acid-induced seizures [22]. Some studies also report that COX-2 inhibitors reduce neuronal cell death in the kainic-acid model, although there is conflicting evidence with regard to this phenomenon [23, 24]. We have seen an earlier onset of seizures in the celecoxib-treated mice (data not shown), rather than a protective effect. Thus, it would seem that the administration of COX-2 inhibitors in murine CM does not have the beneficial effect seen in most other experimental models of



**Figure 3.** Expression of 5-lipoxygenase mRNA (A) and 5-lipoxygenase activating protein (FLAP) mRNA (B) in mouse brains during malaria. The mean  $\pm$  SD values of 6 CBA mice are shown. The mRNA levels were determined at day 6 after inoculation (shown in parentheses) with *Plasmodium berghei* ANKA (PbA) and *Plasmodium berghei* K173 (PbK) and at day 14 after inoculation with PbK. \* $P < .05$ , vs. uninfected (Un) mice. AU, arbitrary units.



**Figure 4.** Effect of treatment with celecoxib (Cel) on bicyclo-prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) levels in mouse brains during cerebral malaria. The mean ± SD values of 6 CBA mice are shown. The bicyclo-PGE<sub>2</sub> levels were determined at day 5 after inoculation (shown in parentheses). \**P* < .05, induction of bicyclo-PGE<sub>2</sub> levels in untreated *Plasmodium berghei* ANKA (PbA)-infected mice vs. Cel-treated, PbA-infected mice (PbA + Cel).

neurological disease, in which inflammatory processes play a role. However, our results are in agreement with those of a study of the effects of aspirin on murine CM [12]. A study in which administration of a stable analogue of prostacyclin was used to prevent the onset of murine CM [25] also supports a protective role for prostaglandins. In addition, treatment with prostacyclin was efficacious in the treatment of a patient with CM [26]. This suggests that CM may differ from the neurological diseases mentioned above in that the products of COX-2 activity could reduce immunopathologic abnormalities. An example of this is a model of carrageenin-induced pleurisy, in which it was postulated that the role played by COX-2 switched from proinflammatory to anti-inflammatory because of the subset of prostaglandins produced over the time course of the experiment [27]. Some potential beneficial effects of prostaglandins in CM may include increasing cerebral blood flow or inhibiting platelet aggregation [28, 29]. Another mechanism may be induction by PGE<sub>2</sub> of expression of the immunosuppressive cytokine IL-10 [30]. We found that levels of IL-10 mRNA in the brain were higher in *P. berghei* ANKA-infected mice; however, there was no significant difference between untreated and celecoxib-treated mice, at least at the end stage of the infection (data not shown).

A protective role for COX-2 in malaria is also suggested by the finding that disease severity in African children with *P. falciparum* malaria was inversely related to plasma bicyclo-PGE<sub>2</sub> levels and COX-2 expression in PBMCs [9]. We have found that COX-2 mRNA is down-regulated in the blood by day 12 (but not at day 5) of *P. berghei* K173 infection, suggesting that it is a later response to malaria infection. It should be noted that, at day 5 of *P. berghei* K173 infection, the mice are asymptomatic, and the day-12 group would more closely represent the patients with severe malaria in the human study. In addition, the induction of COX-2 mRNA in the brains of mice with CM is analogous to the overexpression of the protein in the brains of patients with CM [11]. This suggests that the

mouse experimental models provide a good approximation of prostaglandin metabolism in human malaria.

Higher levels of LTB<sub>4</sub> were measured in mice with murine CM treated with aspirin, compared with levels in untreated mice [12]. It was suggested that COX inhibitors may be deleterious in CM by shunting arachidonic acid into the lipoxygenase pathway, resulting in the production of leukotrienes. We did not test leukotriene levels in this study, but it is interesting to note that levels of 5-lipoxygenase mRNA were highest during late stages of *P. berghei* K173 infection and that the induction of FLAP was approximately the same in CM and late stages of *P. berghei* K173 infection. In addition, treatment with celecoxib made no difference to the mRNA levels of FLAP and 5-lipoxygenase during CM (data not shown). It is possible that increased biosynthesis of leukotriene may contribute to a poorer outcome in CM; however, it is also likely that the highest levels of leukotrienes occur late in *P. berghei* K173 infection, when pathological abnormalities are not evident in the brain.

COX inhibitors can have activities in addition to their effect on COXs. For example, high concentrations of aspirin can activate the mitogen-activated protein kinase (MAPK) cascade [31]. It has been postulated that induction of inducible nitric oxide synthase (iNOS) by p38 MAPK may contribute to the poorer outcomes (including cerebral symptoms) observed in malaria-infected children with high levels of salicylates in their blood [32]. Although iNOS mRNA is induced in the brain in murine CM (authors' unpublished data) and increased protein expression is observed in the brains of patients with CM [33, 34], the role of the enzyme in CM is unclear. The use of nitric oxide synthase inhibitors and iNOS gene knockout mice has shown that the lack of nitric oxide production does not have a significant protective effect on CM in mice [35, 36]. Celecoxib has not been reported to activate p38 MAPK and, because its structure is very different from that of aspirin, it does not share many of the nonspecific effects of aspirin [37]. One particularly relevant effect of celecoxib, other than inhibition of COX-2, is its ability to activate NF-κB, a transcription factor that regulates the expression of some important proinflammatory cytokines (e.g., IL-1 and TNF) [38]. This is postulated to result in the

**Table 2.** *Plasmodium berghei* ANKA infection in celecoxib-treated and untreated mice.

Measure of disease	Untreated	Celecoxib-treated
Parasitemia, %	13.8 ± 6.2	13.7 ± 6.8
Water content of brain, %	78.21 ± 0.53	79.52 ± 0.77a
Activity, AU	22.6 ± 14.0	3.8 ± 1.8a

**NOTE.** Data are mean ± SD results for 12 CBA mice at day 6 after inoculation. AU, arbitrary units.

a Denotes differences between untreated and celecoxib-treated mice that are statistically significant (*P* < .05). For reference, in untreated, uninfected mice, activity levels were 38.2 ± 6.7 AU, and the water content of the brain was 78.05% ± 0.26% (*n* = 6).

**Table 3. *Plasmodium berghei* K173 infection in celecoxib-treated and untreated mice.**

Measure of disease	CBA		C57Bl/6	
	Untreated	Celecoxib treated	Untreated	Celecoxib-treated
Parasitemia	36.6 ± 9.2	41.2 ± 11.0	59.1 ± 4.4	64.1 ± 2.2
Hematocrit	20.7 ± 2.6	22.5 ± 3.8	13.9 ± 2.4	12.4 ± 3.2
Water content of brain	78.40 ± 0.21	78.25 ± 0.31	78.18 ± 0.26	78.29 ± 0.44

**NOTE.** Data are mean ± SD percentages for 6 CBA or C57Bl/6 mice at day 12 after inoculation. For uninfected CBA and C57Bl/6 mice, respectively ( $n = 6$  in each group), hematocrit values were  $52.3\% \pm 5.4\%$  and  $45.3\% \pm 6.9\%$ , and the water content of the brain was  $78.05\% \pm 0.26\%$  and  $77.95\% \pm 0.19\%$ .

reduced anti-inflammatory action of celecoxib observed with higher concentrations of the drug. We estimate that the mice in our experiments did not ingest doses in this high concentration range. In addition, we measured the expression of some NF- $\kappa$ B-regulated genes, such as COX-2, and found that their mRNA expression did not differ significantly between treated and untreated mice with CM. This suggests that the deleterious effect of the drug is more likely to be due to the inhibition of COXs.

We found a small but significant increase in expression of COX-1 mRNA in the brain during CM, which is in agreement with the results of Xiao et al. [12]. In addition, we have demonstrated that an increase of the same magnitude is observed in malaria without cerebral involvement. Therefore, it is the increase in COX-2 expression that is specific and potentially important to CM. This finding, along with the selective COX-2 inhibitor producing a similar effect to that reported to be produced by a nonselective COX inhibitor, suggests that COX-2 is mostly responsible for production of protective prostaglandins. The use of celecoxib reduced levels of bicyclo-PGE<sub>2</sub> in the *P. berghei* ANKA-infected mice but not to levels observed in the uninfected mice. This phenomenon may be due to incomplete penetrance or effectiveness of the drug or, possibly, to the contribution of the increased COX-1 expression to the biosynthesis of prostaglandins.

Of the factors involved in biosynthesis of eicosanoid tested in this study, the induction of COX-2 is the only one that is strikingly specific to CM. Administration of a selective COX-2 inhibitor had a measurable and deleterious effect on the outcome of CM. The pattern of expression of COX-2 in the murine malaria models has similarities with that in human studies. This suggests that murine CM provides a good model to investigate the therapeutic potential of prostaglandin analogues or leukotriene biosynthesis inhibitors in CM.

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