RESEARCH ARTICLE

Investigating the Possible Interfering Effect of COX-2 Inhibitors on Antimalarial Activity of Mefloquine and Artesunate

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Abstract

Cyclooxygenase (COX) is the key enzyme responsible for the production of prostanoids. It plays important roles in the inflammatory process and pathogenesis of several diseases, including malaria. However, there has been no information of the inhibitory effects of COX inhibitors on inflammatory mediators and standard antimalarial drugs. Therefore, in this study, both selective and non-selective COX-2 inhibitors (aspirin, ibuprofen, piroxicam, and naproxen), alone or in combination, were investigated for their antimalarial activities *in vitro*. The antimalarial activity was assessed using the SYBR Green I fluorescent-based technique. For mefloquine-aspirin combination, the test wells consisted of mefloquine and aspirin at the ratios of 200:0, 140:30000, 100:50000, 60:70000, and 0:100000 nM. The concentration ratios for artesunate-aspirin were 50:0, 35:30000, 25:50000, 15:70000, and 0:100000 nM. The median (range) concentrations that inhibited parasite growth by 50% (IC_{50}) of aspirin for K1 and 3D7 clones were 1,889 (1,600-2,792) and 2,417 (912-2,630) nM, respectively. The corresponding values of mefloquine were 10.1 (8.1-13.9) and 23.4 (22.9-24.7) nM, respectively. The corresponding values of artesunate were 2.5 (1.6-3.4) *vs.* 2.2 (1.2-3.2) nM, respectively. The corresponding values for mefloquine were 10 (8-14) and 23 (23-25) nM, respectively. The corresponding values for artesunate were 2.5 (2-3) *vs.* 2 (1-3) nM, respectively. The IC_{50} values of ibuprofen, piroxicam and naproxen were higher than 100,000 nM for both clones. The median (range) sum fractional inhibitory concentrations (FIC) of mefloquine-aspirin interaction for K1 and 3D7 *P. falciparum* clones were 0.82 (0.79-1.0) and 0.97 (0.83-1.1), respectively. The corresponding sum FICs of artesunate-aspirin were 0.94 (0.88-0.95) and 0.95 (0.92-0.97), respectively. Results indicate indifferent antimalarial interaction between these two drugs when used in combination.

Keywords: Malaria, COX inhibitor, aspirin, drug combination

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Introduction

Inflammation is involved in pathological processes of several diseases, including malaria and cancer. Prostaglandins (PGs) are metabolic products of arachidonic acid (AA) *via* the cyclooxygenase (COX) pathway. COX-1 and COX-2 are two distinct enzyme isoforms encoded by separate genes.[1] COX-1 is constitutively expressed in most tissues and generates PGs for physiologic homeostasis. COX-2 is inducible by both inflammatory and mitogenic stimuli resulting in increased PG synthesis in neoplastic and inflamed tissues. PGE_2 and PGD_2 are the two PGs which play an essential role as mediators of fever[2] and immunosuppression.[3] Both mediate inflammation and initiate physiological responses similar to the symptoms observed during malaria infection. So far, there has been no direct evidence of the existence of the *COX* gene in *Plasmodium falciparum*. Kilunga et al.[4] reported a piece of indirect evidence for the involvement of PGs in the inflammatory process in *P. falciparum*. The ability of the malarial parasite to produce PGD_2, PGE_2, and PGF_2 was shown following exposure to 1 mM AA. The production of these PGs in the parasite homogenate was not affected by the non-steroidal anti-inflammatory drugs aspirin and indomethacin and was partially heat-resistant. On the other hand, PG biosynthesis by mammalian COX was completely inhibited by these chemicals including heat.[4]

The aim of the present study was to investigate antimalarial activities of selective (naproxen) and non-selective (aspirin, ibuprofen, and piroxicam) COX inhibitors, including the combination of aspirin with artemisinin-mefloquine combination in the *in vitro* model.

Materials and Methods

Chemicals and reagents

Aspirin, piroxicam, ibuprofen, naproxen, mefloquine and artesunate were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). The culture medium and chemical reagents were purchased from different sources; RPMI and gentamicin from Gibco BRL Life Technologies (Grand Island, NY, USA), commercial-grade ethanol from Labscan Co. Ltd., and SYBR Green I from Sigma-Aldrich Inc. (St. Louis, MO, USA).

In vitro antimalarial activities of aspirin, ibuprofen, piroxicam and naproxen

The 3D7 (chloroquine-sensitive) and K1 (chloroquine-resistant) *P. falciparum* clones were used in the study. The parasites were cultured according to the methods of Trager and Jensen with modifications.[5] The antimalarial activities of piroxicam, ibuprofen and naproxen were investigated in comparison with standard antimalarial drugs, mefloquine and artesunate, using SYBR Green I assay.[6,7] The highly synchronous ring-stage parasite was used in each assay. An aliquot of parasite inoculum (50 μL) with 2% parasitemia and 1% hematocrit was added into each well of the microtiter plate. The 96-well plates were added with tested drugs at a total of eight final concentrations as follows: 781, 1,562, 3,125, 6,250, 12,500, 25,000, 50,000, and 100,000 nM for aspirin, piroxicam, ibuprofen and naproxen;

1.56, 3.13, 6.25, 12.5, 25, 50, 100, and 200 nM for mefloquine; and 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, and 50 nM for artesunate.

The experiments were repeated three times and in triplicates for each experiment. The antimalarial activity of each compound represented by IC_{50} value (drug concentration that inhibits the parasite growth by 50%) was determined from a log dose-response curve plotted using the CalcuSyn™ version 1.1 (BioSoft, USA).

In vitro antimalarial activities of aspirin in combination with mefloquine or artesunate

The antimalarial activities of aspirin when used in combination with mefloquine or artesunate were investigated *in vitro* using the method described by Fivelman et al.[8] For mefloquine-aspirin combination, the test wells consisted of mefloquine and aspirin at the ratios of 200:0, 140:30000, 100:50000, 60:70000, and 0:100000 nM. The concentration ratios for artesunate-aspirin were 50:0, 35:30000, 25:50000, 15:70000, and 0:100000 nM. The control wells consisted of drug-free parasitized erythrocytes. The experiments were repeated three times (in triplicates for each experiment), and the IC_{50} values were analyzed as described above. Two IC_{50} values of the partner drugs for each of the five combination curves were calculated separately using the known concentration ratios of mefloquine, artesunate and aspirin. The fractional inhibitory concentration (FIC) of mefloquine, artesunate and aspirin were calculated for each point, and the isobolograms were plotted. To obtain numeric values for the type of interaction, results were expressed as sum FIC at the given IC (inhibitory concentration) using the formula: (IC*x* of agent A in the mixture/IC*x* of agent A alone)+(IC*x* of agent B in the mixture/IC*x* of agent B alone).[9] Sum FIC value indicates the type of antimalarial interaction as follows: 'synergism' if sum FIC<1; 'indifference' if sum FIC=1; and 'antagonism' if sum FIC>1.

Results

In vitro antimalarial activities of aspirin, naproxen, ibuprofen, piroxicam

The antimalarial activities of aspirin, naproxen, ibuprofen, and piroxicam, including the antimalarial drugs mefloquine and artesunate against K1 chloroquine-resistant and 3D7 chloroquine-sensitive *P. falciparum* clones are presented in Table 1. The IC_{50} values of ibuprofen, naproxen and piroxicam for both clones were higher than 100,000 nM. The median (range) IC_{50}s of aspirin for K1 and 3D7 clones were 1,889 (1,600-2,792) and 2,417 (912-2,630) nM, respectively. The corresponding values of mefloquine were 10.1 (8.1-13.9) and 23.4 (22.9-24.7) nM, respectively. The corresponding values of artesunate were 2.5 (1.6-3.4) *vs.* 2.2 (1.2-3.2) nM, respectively.

Antimalarial activities of aspirin in combination with mefloquine or artesunate

The antimalarial activities of mefloquine-aspirin and artesunate-aspirin combinations were investigated in K1 and 3D7 *P. falciparum* clones. The median (range) sum FICs for mefloquine-aspirin interaction for both *P. falciparum* clones were 0.82 (0.79-1.0) and 0.97 (0.83-1.1), respectively. The sum FICs for artesunate-aspirin interaction for both clones were 0.94 (0.88-0.95) and 0.95 (0.92-0.97),

respectively. The sum FIC values close to 1.0 observed for both clones indicated indifferent interactions for both combinations. The isobolograms representing the interactions between the two combinations in both parasite clones are shown in Figure 1 and 2.

Table 1. The median (range) IC_{50} values of aspirin, ibuprofen, naproxen, and piroxicam, in comparison with mefloquine and artesunate against K1 and 3D7 *P. falciparum* clones.

Drugs	The median (range) IC_{50} values (nM)	
	K1	3D7
Aspirin	1,889 (1,600-2,792)	2,417 (912-2,630)
Ibuprofen	> 100,000	> 100,000
Naproxen	> 100,000	> 100,000
Piroxicam	> 100,000	> 100,000
Mefloquine	10.1 (8.1-13.9)	23.4 (22.9-24.7)
Artesunate	2.5 (1.6-3.4)	2.2 (1.2-3.2)

Results were obtained from three independent experiments, in triplicates each.

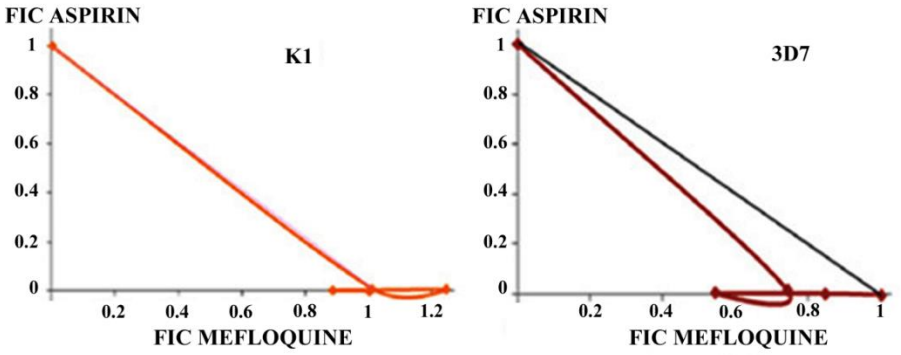

Figure 1. Isobolograms representing the antimalarial interactions between mefloquine and aspirin.

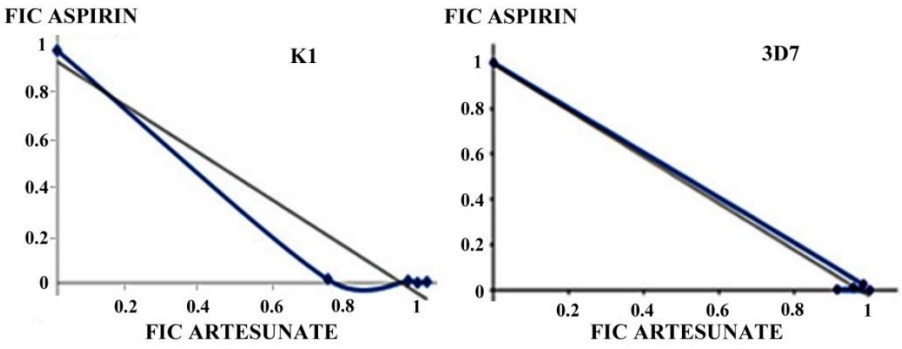

Figure 2. Isobolograms representing the antimalarial interactions between artesunate and aspirin.

Discussion

P. falciparum has been reported to produce PGD_2, PGE_2 and $PGF_{2\alpha}$ through a distinguishable pathway from that in human.[4] A large amount of hydroxy derivatives of a diverse group of polyenoic fatty acids were separated from parasite extracts.[10] However, there has been no direct support for the existence of either the gene(s) that encodes COX or lipooxygenase enzyme. Our results confirmed previous reports that conventional COX inhibitors, both selective and non-selective COX-2 inhibitors, have no intrinsic antimalarial activities.[4] The indifferent interaction was observed when the non-selective COX-inhibitor aspirin was used in combination with the standard antimalarial drug mefloquine or artesunate. This suggested that despite the lack of intrinsic antimalarial activities, COX-inhibitors at least did not interfere with antimalarial activities of antimalarial drugs, implying distinct PGs synthesis pathway from the mammalian host.

The PGs are important mediators of several host physiological processes including macrophage activity, vascular permeability, fever, erythropoiesis and proinflammatory responses to infection. Association between host PGE_2 and severity of malaria pathogenesis especially cerebral malaria and severe anemia has been reported in previous studies.[11-15] $PGF_{2\alpha}$ has been demonstrated to be the major PG produced by the malarial parasite, but its function remains unclear. Inhibition of host monocyte function by PGs and hydroxyl fatty acids produced by malarial parasite has previously been reported.[10] This could modulate the host defense mechanism by lowering the production of tumor necrosis factor-α (TNF-α).[16,17] Investigation of the link between the production of PGs by the malarial parasite and the clinical manifestation of malaria should further provide supportive information on the potential of COX-mediated PG synthetic pathway as a new target for anti-malarial drug development.

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