World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Fenofibrate inhibit the development of malaria in *Plasmodium berghei*-infected mice

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Received: 29-08-2014 / Revised: 21-09-2014 / Accepted: 25-09-2014

ABSTRACT

Malaria, an infectious disease begins with the introduction of protists into blood circulation and may cause death in severe cases. Development of resistance in Plasmodium parasites is major obstacle and emphasizes the need of novel strategy to combat the prevalent of disease. Fenofibrate, an agonist of peroxisome proliferator-activated receptor alpha (PPAR-α) is well known to treat hypertriglyceridaemia and mixed dyslipidaemia from decades. Recent studies reports possibility of its activity against the malarial parasite as well. Here we explore the Fenofibrate for activity against malaria in *Plasmodium berghei* infected mice The infected erythrocytes (IE) from control and treated mice was subjected to microscopic examination for analyse the mean percent parasitemia on day 4th, 7th, 10th, 14th and 21st after infection. The results of present study illustrate the activity of fenofibrate against *Plasmodium berghei* malaria parasite *in-vivo*. Significant distinction was observed in percent parasitemia of fenofibrate and vehicle treated mice. Treatment with 320mg/kg was found to be most suppressing amongst the entire treatments of fenofibrate.

Keywords- Fenofibrate, malaria, *Plasmodium berghei*, fibrates.

INTRODUCTION

Malaria infectious is one of the dangerous and oldest recorded diseases in the world. It is originated with the introduction of protists into circulation by a bite from an infected female Anopheles mosquito. After reaching the liver they get matured, reproduce and cause symptoms that normally include headache, fever, vomiting, etc. which may progress to death in severe cases. Tropical and subtropical regions around the equator including Sub-Saharan Africa, Asia, and the Americas are more prevalent to disease [1]. The stagnant waters from rainfall and warm temperatures of these regions provide ideal habitats for mosquito larvae [2]. Malaria is commonly linked with poverty and a major hindrance to economic development as well [3]. Increasing resistance towards anti-malarial drugs is one of the main barriers and emphasizes the need for novel effective agents. One of the potential research sources is the compounds that are approved for other complications and found to be effective in malaria infection. Reported safety, tolerability and Pharmacokinetic profile of such compounds make them more selective in the usual drug development process. Fenofibrate is peroxisome proliferator-activated receptor alpha (PPAR-alpha) agonist has been used clinically to treat hypertriglyceridaemia, mixed dyslipidaemia and insulin resistance. In recent studies about fenofibrate it has been shown that it is effective in tumor [4], neural and endothelial damage [5], liver damage and fibrosis [6,7] cardiac hypertrophy [8] besides it also accounts the possibility of fiberates to have anti-malarial activity.Wong and Davis [9] reports anti-malarial activity of fenofibric acid, a fenofibrate metabolite against *Plasmodium berghei in-vitro.* In contrast to these findings, no literature is available on *in-vivo* anti-malarial activity of fenofibrate, for that reason in the present study fenofibrate was explored for the blood-schizonticidal activity in *P. berghei*-infected mice.

MATERIALS AND METHODS

Animals and Parasites: Swiss albino mice (*Mus musculus)* of either sex (20±2g), supplied by the Central Animal Facility, National Institute of Pharmaceutical Education and Research were used in all the experiments. Animals were kept in temperature (22–24°C) and light (12 h on/off)

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controlled rooms and provided with standard animal feed and clean water. All experiments were carried out in accordance with the Guidelines for Care and Use of Animals in Scientific Research, Indian National Science Academy, and New Delhi, India, as adapted and promulgated by the Institutional Animal Ethics Committee. Rodent malaria parasite *P.berghei* used for infecting the mice was obtained from the Central Drug Research Institute, Lucknow.

Drugs and reagents: Fenofibrate was purchased from Sigma Aldrich, Bangalore, Wright's stain was obtained from HIMEDIA, isopropyl alcohol, Tri-sodium citrate, disodium hydrogen phosphate and potassium dihydrogen phosphate was obtained from Merck Ltd., Mumbai, India.

Preparation of Wright's stain: 1 g of Wright's stain was dissolved in 500 ml of methanol and kept undisturbed for two months in dark for maturation.

Cryopreservation of malaria parasites: Infected blood from Animals with parasitemia between 5–30% was collected in tubes containing citrate buffer and centrifuged at 2000 rpm for 7 minutes. Supernatant was collected and stored in liquid nitrogen in cryo-vials containing sterile solution of glycerol (28%) and mannitol or sorbitol (4.2%) in normal saline [10]. The cryo-vials were taken out from the liquid nitrogen, brought to 37°C and was diluted with 3.2% w/v sodium citrate saline solution and injected i.p. into each mouse in order to deliver a counted inoculum of 10^6 Infected Erythrocytes (IE) per mouse.

Blood schizonticidal activity: Anti-malarial activity of test compounds was explored by 4 day suppression test as described by Fidock et al. [11]. Five different groups (n=6) were made viz. vehicle control (ethanol 3% v/v), chloroquine (CQ) (8 mg/kg/day) fenofibrate lowest dose 16 mg/kg (LD_{50}†/100), medium dose 160 mg/kg (LD_{50}/10) and highest dose of 320 mg/kg (LD_{50}/5). On day 0, two hours after infection drug treatments were given to respective groups. All the drug treatments were given intraperitonially (*i.p.*). Same drug treatments were repeated for 3 more days (*i.e* day 1, 2 and 3) and from day 4 onwards (96 h post infection) parasitaemia was measured.

Enumeration of parasitaemia: A thin blood smear was made from a small drop of cut tail-blood of mouse and dried in air. The dried slides were placed on horizontal and then covered with Wright's stain for 4–5 min followed by covering with staining buffer for 12 minutes. Slides were then washed with more staining buffer, air dried and an appropriate area of a stained blood film (about 200 cells/field) was selected by monitoring under light microscope. Erythrocytes were examined for the presence of parasite in the selected area with the intention that fields are not counted for more than once. A total of 50 fields were observed (50 x 200 = 10,000). The observation of 10^4 erythrocytes per slide was usually found to be adequate. The parasitaemia was expressed as percent IE after microscopic examination of 10^4 erythrocytes.

Data and statistical analysis: Values expressed as mean ± S.E.M. Parasitaemia was calculated as describe by Muregi et al. [12]. Different treatments was compared by one way analysis of variance (ANOVA) followed by post hoc analysis by Tukey's test using Sigma Stat v. 3.5. $p< 0.01$ was considered significant.

RESULTS

Vehicle control group show the mean % parasitaemia of 2.78%, 7.21%, 15.85%, 26.75% and 47.58% on day 4, 7, 10, 14 and 21 respectively. Fenofibrate at the dose 16 and 160 mg/kg displayed significant suppressive action as compared to the vehicle control from day 4 to day 21 ($p<0.01$). Dose 320 mg/kg were found to be most suppressive with mean % parasitaemia of 0.00%, 0.12%, 0.86%, 2.32%, 7.49% and 16.38% on day 4, 7, 10, 14, 21 and 28 respectively (Fig. 1). Vehicle and fenofibrate (16 mg/kg) treated all the mice were dead within 28 day post infection while 160 and 320 mg/kg treated group show 60 and 80% survival respectively till day 28th. The percent survival in group treated with chloroquine (CQ) (8 mg/kg) was found to be 100% (Fig. 2).

DISCUSSION

The findings of the present study confirm the *in-vivo* anti-malarial activity of fenofibrate against *P. berghei*. The fenofibrate and vehicle treated mice show distinction in percent parasitemia among the groups ($p< 0.01$). A rapid increase in paracitemia was observed in vehicle control group post-infection with 10^6 IE. Fenofibrate treatments diminish the development of parasites in mice dose dependently and effect was found to be significant at all the three doses ($p< 0.01$). The dose of 320mg/kg was found to be most effective and show less mortality among the treatments. Chloroquine (CQ) completely eradicates the development of paracitemia in infected mice with 0% mortality. Fenofibric acid, a fenofibrate metabolite was reported to alter the lipid components of cells membrane in rodent and human by interfering with

the expression of ABC-1 [13, 14] Therefore, in the same way, similar mechanisms may involve in cidal action of Fenofibrate. Fenofibrate might also reverse the resistance by reducing efflux of chloroquine (CQ) and mefloquine through inhibition of the P-glycoprotein homologue 1 (Pgh1) mediated transport in parasites [15, 16]. *In-vitro* and *in-vivo* anti-malarial activity, well known pharmacokinetic, safety and tolerability profile [17, 18] make the fenofibrate a strong candidate for future clinical application.

Acknowledgements: The authors are gratefully acknowledged the management of National institute of Pharmaceutical Education and Research (NIPER), Mohali, (Punjab), India for providing the research facility and financial support.

Conflict of Interest: We declare that we have no conflict of interest.

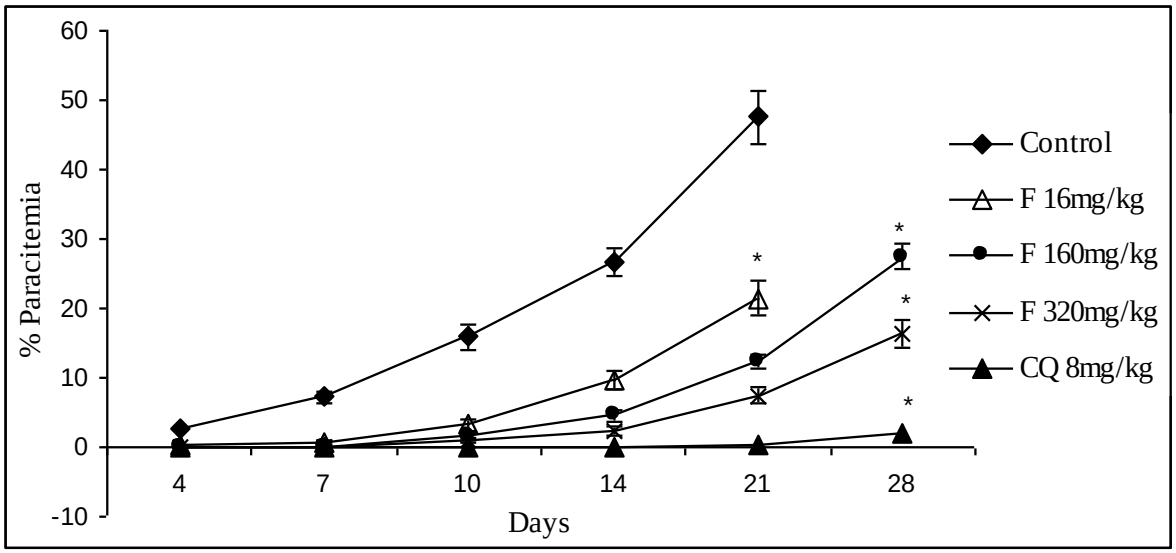

Figure 1. Effect of Fenofibrate (16, 160 and 320 mg/kg/day×4) and CQ (8mg/kg/day×4) on percent Parasitaemia in *P. berghei* infected mice. F- fenofibrate, CQ- chloroquine. All values are expressed in mean ± S.E.M, *p< 0.01 as compare to vehicle control.

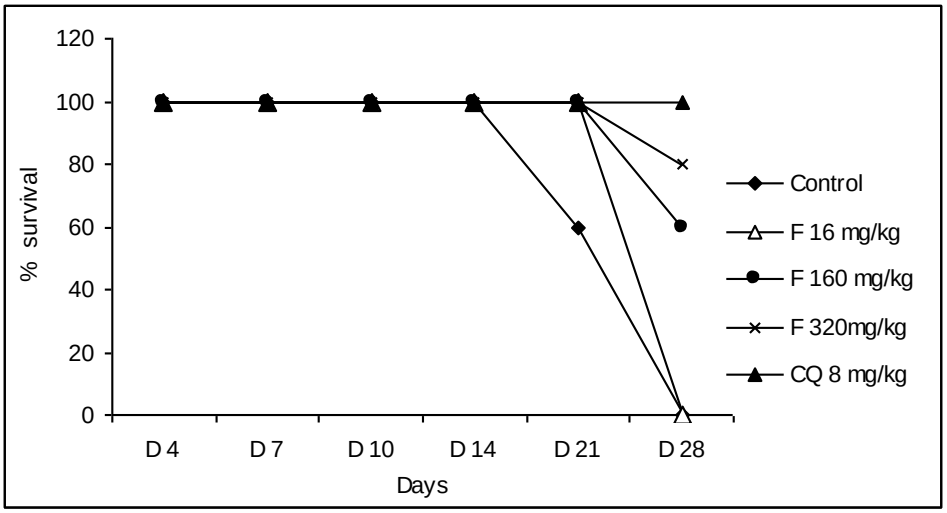

Figure 2. Effect of Fenofibrate (16, 160 and 320 mg/kg/day×4) and CQ (8mg/kg/day×4) on mortality of *P. berghei* infected mice. Results are expressed as percent survival. F- fenofebrate, CQ- chloroquine.

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