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SHORT COMMUNICATION

Assessment of in vivo antimalarial activity of rifampicin, isoniazide, and ethambutol combination therapy

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Abstract The existing armament of drugs for the treatment and prevention of malaria is inadequate due to development of resistance. In addition to this due to lack of economic enticement the rate of new drug development and new drug discovery in the segment of parasitic diseases is very low as compared to the other segments. This has necessitated the better deployment and usage of existing antimalarial drugs as well as discovery of antimalarial activity of drugs which are well characterized for other diseases; these approaches help to reduce the time and cost required for new drug discovery. The present study evaluated the antimalarial activity of antituberculosis drugs rifampicin, isoniazide, and ethambutol in monotherapy and combination in *Plasmodium berghei*-infected mice. Animals were observed for mortality, parasite progression, and toxicity for a period of 1 month. Rifampicin +isoniazide and rifampicin+isoniazide+ethambutol treatment resulted in an overall survival rate of 60% compared to 0% in vehicle-fed animals by 4 weeks after post-infection without showing any toxicity.

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Development of drug resistance to the available antimalarial drugs, worldwide presence of the parasite, high cost of new antimalarial drugs, and lack of enticement in this field has warranted the identification of new antimalarial drugs which act synergistically or additively in combination (Butler 2004). The objective of combining antimalarial drugs is to improve efficacy thus, parasite clearance will be faster in combination therapy compared to monotherapy. Additionally, combination therapy can delay the development and subsequent selection of drug-resistant parasites which prolongs the useful therapeutic life of drugs in the combination.

In this report, we combine rifampicin, isoniazide, and ethambutol for antimalarial therapy. The rationale to use this combination is based on animal and human experiments. Rifampicin (RIF) is an antituberculosis drug, a known RNA polymerase inhibitor of bacterial transcription and has antimalarial activity (Strath et al. 1993). The antimalarial activity of rifampicin was confirmed with *Plasmodium falciparum* in vitro and *Plasmodium chabaudi* in vivo. A rifampicin-tolerant sub-line of *P. falciparum* was selected in vitro. Rifampicin was effective against chloroquine resistance *P. falciparum* and the rifampicin-tolerant line had increased chloroquine sensitivity. However, recrudescence occurred on release of drug pressure (Strath et al. 1993). In a field study, rifampicin has been found to be partially effective and it has been suggested that it may be of value in combination therapy.

Isoniazide (INH) is another known antituberculosis drug which inhibits β-ketoacyl ACP synthase (FabH)—target of thiolactomycin, and enoyl ACP reductase (Fab I)—target of triclosan in *Mycobacterium tuberculosis*. Isoniazid has no clear antiplasmodial activity but delays malaria mortality in mice and reduces overall parasite load when given in combination with rifampicin (Alger et al. 1970).

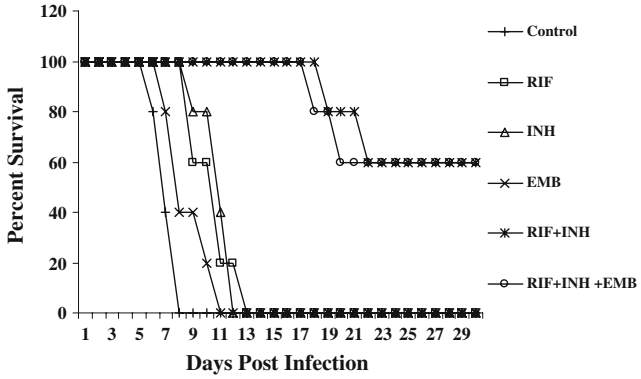

Fig. 1 Antimalarial activity of RIF, INH, and EMB in *P. berghei*-infected mice. Animals were treated orally by administration of drug doses every 24 h on days3–7 post inoculation (n=5 repeated three times; n=15 for each treatment)

Ethambutol (EMB) is bacteriostatic against actively growing TB bacilli; it works by obstructing the formation of the bacterial cell wall. The antimalarial activity of this drug is still not tested. However, addition of ethambutol to rifampicin and isoniazide combination therapy has shown promising results in tuberculosis treatment (Lenaerts et al. 2000). Due to the potential promise of ethambutol in combination therapy, in this report, we studied the antimalarial activity of EMB alone and the effect of addition of ethambutol to rifampicin and isoniazide combination antimalarial therapy (Genton et al. 2006).

Rifampicin, isoniazide, and ethambutol were a kind gift from Taj Laboratories Ltd. (Mumbai India). Dimethyl sulfoxide (DMSO) was obtained from E-MERC (Mumbai, India).

The animal study protocol was approved by the Kuvempu University Animal Ethical Committee. *Plasmodium berghei*-infected mice brought from IISc Bangalore were used as source and the parasites maintained in Albino mice (25±3 g) through blood transfusion in uninfected animals for every 5 days. Blood was freshly withdrawn through heart puncture of infected mice (parasitemia >70%) and 100 μl was injected intraperitonially to the test animals. Animals were divided into six treatment groups of five animals each. The mice were maintained according to the endorsed procedure.

The solubilized RIF (in DMSO), INH (in water), and EMB (in water) of volume 10 μl administered orally on days3, 4, 5, 6, and 7. The mice were observed for external symptoms and mortality, when the drugs were given alone and in combination. Blood from the tail vein was analyzed on different days for parasitemia using field stained smears under microscope. The animals were divided as per the treatment mentioned. Group I (positive control), Group II (RIF 15 mg/kg/day), Group III (INH 25 mg/kg/day), Group IV (EMB 25 mg/kg/day), Group V (RIF 15+INH 25 mg/kg/day), and Group VI (RIF 15+INH 25+EMB 25 mg/kg/day). Parasitemia level on the day of treatment was 1–3%. Animals were kept under observation to record the parasitemia progression and mortality. The observation was recorded daily until all the animals died from malaria. Drug concentration was decided as described earlier with slight modification (Lenaerts et al. 2000)

In vivo toxicity studies were conducted for RIF, INH, and EMB alone and in combination. The in vivo toxicity was determined in Swiss albino mice (n=6 for each treatment). RIF, INH, and EMB were given orally at the concentration tested for their antimalarialal activity, either alone or in combination. The animals were divided as per the treatment mentioned. Group I (control, no treatment), Group II (RIF 15 mg/kg/day), Group III (INH 25 mg/kg/day), Group IV (EMB 25 mg/kg/day), and Group V (RIF 15+INH 25+EMB 25 mg/kg/day). Serum level of enzymes alkaline phosphatase (ALP), serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and biochemical markers for nephrotoxicity like creatinine and urea were estimated on the eighth day following administration.

The efficacy of the antimalarial activity of antitubercular drugs has been examined in vivo. Here *P. berghei*-infected Swiss mice (~25–30 g body weight) were administered orally with rifampicin, isoniazide, and ethambutol alone and together on day3, 4, 5, 6, and 7 with respective controls. The mice were observed for parasitemia and mortality. We found that INH (25 mg/kg/day) treatment resulted in death of mice from 9 to 11 days and treatment with EMB (25 mg/kg/day) resulted in death of mice from 7 to 10 days. RIF (15 mg/kg/day) treatment resulted in death of mice from 9 to 12 days.

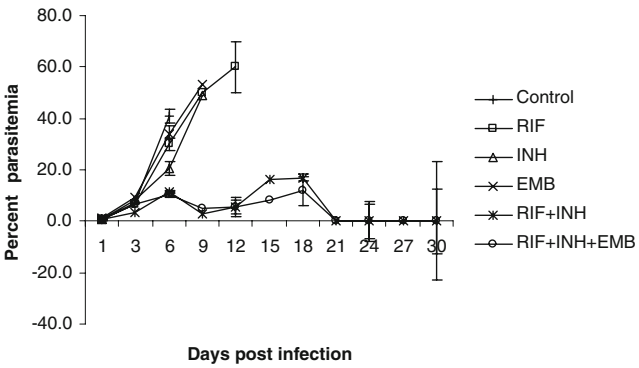

Fig. 2 Effect of RIF, INH, and EMB on parasite progression of *P. berghei*-infected mice. Animals were treated orally by administration of drug doses every 24 h on days3–7 post inoculation. Results are expressed as mean±SEM of two separate experiments. In an individual experiment five animals were taken for each group (n=5 repeated two times; n=10 for each treatment)

Table 1 Serum data of BALB/c mice that received rifampicin, isoniazide, and ethambutol alone and in combination

Sample	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Creatinine (mg/dl)	Urea (mg/dl)
Control	68.5±3.12	36±1.41	124±14.14	0.54±0.04	59.09±6.00
RIF	76±2.82*	43.5±2.12*	135±4.24	0.8±0.11*	62.56±1.87
INH	58.5±12.02	28.5±6.36	113±8.48	0.57±0.13	45.75±6.34
EMB	59.5±4.94	29.5±2.12	133±12.72	0.52±0.09	62.98±6.40
RIF+INH+EMB	85.3±8.02*	42.66±1.52*	106±7.93	0.78±0.04*	46.84±8.87

Animals were treated orally by administration of drug doses every 24 h for five consecutive days. The serum data shown are taken on day6. All parameters were expressed as mean values±SD (n=6 for each treatment)

SGPT serum glutamic pyruvate transaminase, *SGOT* serum glutamic oxaloactetatic transaminase, *ALP* alkaline phosphatase, *RIF* rifampicin, *INH* isoniazide, *EMB* ethambutol

*P<0.01, significant, all groups compared with control

Oral feeding of rifampicin+isoniazide and rifampicin+isoniazide+ethambutol combination to *P. berghei*-infected mice decreased blood parasitemia by 40–50% on day7 and enhanced their survival significantly compared to controls (P<0.001; Figs. 1 and 2). Rifampicin+isoniazide and rifampicin+isoniazide+ethambutol treatment resulted in an overall survival rate of 60% compared to 0% in vehicle-fed animals by 4 weeks after post-infection.

Toxicity studies were conducted to assess whether the tested combination therapy is safe or not. The enzyme levels of SGOT, SGPT, ALP, creatinine, and urea in serum of BALB/c mice treated with rifampicin (15 mg/kg/day), isoniazide (25 mg/kg/day), and ethambutol (25 mg/kg/day) alone and in combination. Significant increase (P<0.01) was observed in SGPT, SGOT, in RIF alone treated group and RIF+INH+ETB combination treated group. Creatinine levels in RIF and RIF+INH+ETB alone treated group (Table 1) also showed a significant increase.

The results obtained in the present investigation indicated that drugs given in combination were promising compared to a regimen of monotherapy. The parasitemia was cleared in the majority of mice treated in combination using two different trials including (a) rifampicin (25 mg/kg/day)+isoniazide (15 mg/kg/day) and (b) rifampicin (25 mg/kg/day)+isoniazide (15 mg/kg/day)+ethambutol (25 mg/kg/day) treatments for 5-day via oral route, compared to individual drug treatments (Fig. 2). Consistent with the clearance of parasitemia, the survival rate of animals in combination therapy was found to be 60% (Fig. 1).

In case of *P. berghei* parasitemias detected by smear observation, it was seen that in the first 2 days after combination drug treatment, very little effect on parasite progression could be noted but at later time points the therapy started clearing the parasite load and gave protection (Fig. 2).

Even though the exact mechanism by which this drugs acts as an antimalarial agent is not known, it can be hypothesized that, recently identified potential selective targets for components of type II fatty acid biosynthesis (Waller et al. 2003) and mevalonate-independent isoprenoid synthesis (Jomaa et al. 1999) are the site of action because both the pathways are also targets for these antituberculosis drugs in tuberculosis bacteria.

In vivo toxicity study was done to assess the effect of these drugs on normal biochemical parameters of liver function. RIF, EMB, and INH have been reported to cause disturbance in many normal biochemical parameters (Steele et al. 1991). Even though treatment with rifampicin alone and in combination of rifampicin+isoniazide+ethambutol elevate the clinically important enzyme levels like SGOT, SGPT, and creatinine in comparison to control, only >35% of the total mice treated (two out of six mice) have shown such significant increase. This data is in correlation with the data obtained from the earlier clinical trials (Steele et al. 1991).

The addition of EMB did not significantly improve the treatment outcome in our experimental setting. However, perhaps the three-drug therapy might be superior to the two-drug regimen with regard to decreasing the emergence of drug resistance.

The present study shows that TRIP, a combination of rifampicin, isoniazide, and ethambutol is safe and efficacious for treating malaria in a murine model, but is insufficient to clear parasites when each drug is used individually. Even though this combination appears to be effective in treating malaria care should be taken before implying this combination for malaria therapy due to the chances of development of drug resistance to tuberculosis in case of malaria and tuberculosis co-infection which is one among the major problems in African countries. However, this study provides a starting point for further development of these drugs for antimalarial treatment.

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