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Prophylactic activity of Chitosan against *Plasmodium berghei* infections in BALB/c mice

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ABSTRACT

The prophylactic effect of crustacean chitosan (CC) against malarial infection was examined in BALB/c mice. CC dissolved in 0.5% acetic acid at different concentrations was given either intraperitoneally (i.p.) or orally from day 0 to day 5. Mice which received 0.5% acetic acid were used as the negative control whereas mice treated with chloroquine diphosphate were served as the positive control. All mice were infected with parasitized red blood cells on day 4. Our results showed that CC treatment, either administered intraperitoneally or orally, possessed prophylactic effects on blood induced infection of *P. berghei*. All CC-treated mice exhibited longer life-span when compared to control mice received only vehicle. However, CC treatment intraperitoneally gave stronger protection where a dosage at 250 mg/kg showed significant parasite suppression as well as longer survival time of the infected mice. CC treatment through i.p. route also caused significantly higher neutrophil counts in the peripheral blood. We suggest that the activation of neutrophils in peripheral blood through prophylactic activity of CC may play a crucial role in the antimalarial activity of the host.

Key words: Chitosan, *Plasmodium berghei*, prophylactic activity, BALB/c mice.

INTRODUCTION

Chitosan is a polysaccharide comprising copolymers of glucosamine and *N*-acetyl glucosamine. It is usually prepared by deacetylation from chitin, the structural component in the exoskeleton of crustaceans and insects[1]. Chitosan is a multifunctional polymer with three functional groups (amino, primary and secondary hydroxyl groups) in the glucosamine residue[2]. The presence of a number of amino groups permit chitosan to chemically react with anionic systems and it is easily soluble in aqueous solutions of many inorganic and organic acids[3]. Due to its excellent properties such as biocompatibility, biodegradability, non-toxicity and adsorption properties, chitosan is receiving worldwide interest for its uses as antimicrobials, biomedical materials, cosmetics, food additives, agricultural material, and so on[1,4].

Chitosan suspensions or microparticles have been reported to possess immune stimulating activity such as activation of macrophage, inducing immunologic adjuvant effects, stimulating the production of cytokines, suppressing tumor growth and promoting resistance to infection by microorganisms[5-8]. Our previous work[9] showed that treatment of chitosan simultaneously with *Plasmodium berghei* inoculation was able to reduce the early parasitaemia in mice. In the present study we evaluated the prophylactic effect of chitosan on malarial infection in mice to explore its potential as a new prophylactic agent.

EXPERIMENTAL

Crustacean chitosan (CC) was purchased from ICN Biomedicals, USA. Chitosan powder was suspended in 0.5% acetic acid at desired concentrations.

Experimental animals

Eight-week-old inbred male BALB/c mice (18-23 g) were obtained from the Animal House, Universiti Kebangsaan Malaysia. The animals were housed in standard cages and acclimatized for a period of two weeks before the experiment. The mice were maintained on standard commercial pellet diet and drinking water *ad libitum*. The handling and experimental usage of the animals were according to the Universiti Kebangsaan Malaysia Animal Ethical Committee (UKMAEC) Guidelines 2003[10].

Parasite inoculation

Plasmodium berghei (pzz 1/00) obtained from the School of Bioscience and Biotechnology, Universiti Kebangsaan Malaysia was serially maintained in BALC/c mice by intraperitoneal (i.p.) injection of infected blood every 6-7 days. Blood collected from donor mouse having about 20% parasitaemia was diluted with Alsever's solution to prepare inoculum that consists of 10 x 10^6-*P.berghei* parasitized red blood cells per ml. Each mouse was then inoculated intraperitoneally with 0.1ml of infected blood containing about 1 x 10^6-*P. berghei* parasitized red blood cells.

Chitosan and drug administration

Chitosan and drug used in this study were intraperitoneally or orally administered. Oral treatment was given with the aid of a stainless metallic feeding cannula.

Evaluation of the prophylactic activity

The prophylactic activity was assessed by modifying the method described earlier by Singh & Puri and Okokon et al.[11,12] The mice were divided into groups of five animals each and treatments of chitosan (dissolved in 0.5% acetic acid) were given for six consecutive days (D0 to D5). Mice which received 0.5% acetic acid were used as negative control whereas mice treated with chloroquine diphosphate (CQ) at 10mg/kg body weight were served as positive control. On day five (D4), all mice were inoculated intraperitoneally with 1x10^6-*P. berghei* parasitized red blood cells. Ninety-six hours later, the parasitaemia level, degree of suppression and WBC differential counts were evaluated using thin blood smears obtained from tail bleeding and stained with Giemsa stain. Experiments were performed for both the intraperitoneal and oral treatments of CC.

Analysis of results

The percentage parasitaemia was determined microscopically by counting the number of parasitized red blood cells out of 1000 red blood cells in random fields of the stained slides. The mean percentage suppression[13] was calculated as:

$$\frac{\text{Mean parasitaemia (\%) in control - Mean parasitaemia (\%) in treated group}}{\text{Mean parasitaemia (\%) in control}} \times 100$$

The percentage of white blood cells (WBC) differential count was determined after counting at least a total of 200 white blood cells in random fields under a microscope.

Statistical analysis

The results were expressed as mean ± SEM. Student *t*-test was used to analyze data between groups and one-way ANOVA among groups. Values of $p<0.05$ were considered as significant.

RESULTS

Prophylactic activity of intraperitoneal treatments of crustacean chitosan

The results showed a significant ($p<0.05$) and dose-dependent reduction in the mean parasitaemias in mice which received CC treatment intraperitoneally (Table 1). The lowest dose of 50 mg/kg per day gave the highest suppression (99.12%) compared to 100 and 250 mg/kg per day doses (84.52% and 93.98% suppression, respectively). However, mice treated with 100 and 250 mg/kg per day survived much longer (15.40 ± 1.75 and 15.20 ± 0.37 days, respectively) as compared to mean survival period of 12.60 ± 0.81 days for dose at 50 mg/kg per day. Control mice treated with 0.5% acetic acid exhibited 11.80 ± 1.88 mean survival days. Chloroquine diphosphate (CQ), a standard antimalarial drug, caused a 100%-suppression but was unable to cure all mice of which only 20% of the mice recovered fully. WBC

differential counts obtained from tail blood samples demonstrated that neutrophil counts were significantly ($p<0.01$) higher in all CC-treated mice as compared to control and CQ groups. Fig. 1 shows the percentages of neutrophil counts at 66.82% ± 5.93, 65.09% ± 2.50 and 70.02% ± 2.98, respectively for all the tested doses of 50, 100 and 250 mg/kg per day as compared to lower values for control group (43.41% ± 3.03) and CQ group (37.21% ± 2.93).

Prophylactic activity of oral treatments of crustacean chitosan

Chitosan treatment when given orally apparently reduced the mean parasitaemia of the infected mice but the effects were less significant (Table 2). The CQ treatment caused a considerably higher suppression (93.30%) than the highest dose of the chitosan-treated groups which produced 77.21% mean of suppression. Although chitosan treatment at 250 mg/kg per day caused a higher degree of suppression than the lower dose of 100 mg/kg per day, it failed to generate a longer life span in the former. Moreover, the highest dose appeared to be toxic because 40% of the mice died before the development of patent malarial infection (Figure 2). WBC differential counts showed that all groups of mice shared a similar trend in their neutrophil, lymphocyte and monocyte counts. Although treatments at 100 and 250 mg/kg per day gave a relatively higher neutrophil counts (56.57% ± 3.36 and 63.52% ± 8.50 respectively), they were not significant when compared to control group (52.44% ± 3.57).

DISCUSSION

This is the first report on the prophylactic activities of crustacean chitosan against blood induced-*P. berghei* infection in mice. Our results indicated that the experimental agent CC, either administered intraperitoneally or orally, exhibited prophylactic activity against *P. berghei* infection in BALB/c mice. However, CC treatment through i.p. route showed stronger parasite suppression as evidence from the higher suppression rate at

Table 1: Protective effects of chitosan (CC) treatments administered intraperitoneally against *P. berghei* infection in mice.

Treatment	Dose (mg/kg, i.p.)	Mean parasitaemia (%)	Mean suppression (%)	Mean survival period (days)
CC	50	0.024 ± 0.017 *	99.12	12.60 ± 0.81
CC	100	0.422 ± 0.156	84.52	15.40 ± 1.75
CC	250	0.164 ± 0.065 *	93.98	15.20 ± 0.37
CQ	10	0.000 ± 0.000 *	100.00	23.00 ± 1.37 **
aa (Control)	10 ml/kg	2.726 ± 1.020	-	11.80 ± 1.88

Significantly different from the 0.5% acetic acid (aa) control group * ($P<0.05$), ** ($P<0.01$).

Table 2: Protective effects of chitosan (CC) treatments administered orally against *P. berghei* infection in mice.

Treatment	Dose(mg/kg, oral)	Mean parasitaemia (%)	Mean suppression (%)	Mean survival period (days)
CC	100	0.268 ± 0.196	64.08	19.40 ± 0.81
CC	250	0.168 ± 0.087	77.21	18.30 ± 1.20
CQ	10	0.052 ± 0.052 *	93.30	20.60 ± 1.08
aa (Control)	10 ml/kg	0.746 ± 0.381	—	13.60 ± 1.36

Significantly different from the 0.5% acetic acid (aa) control group * ($P<0.05$).

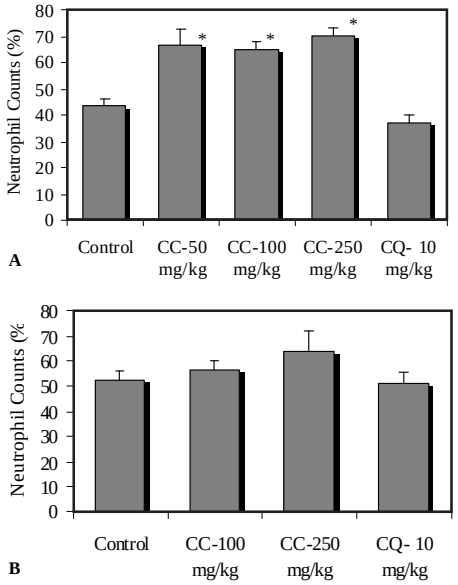

Fig. 1: Effect of intraperitroneal (A) and oral (B) chitosan treatments on neutrophil counts from tail blood samples. Percentages of neutrophils were significantly higher in all CC-treated mice (i.p. route) as compared to control and CQ group. Values are expressed as the mean ± SEM. * = p < 0.01

93.98% compared to oral treatment at 77.21% when given the same dose of 250 mg/kg per day.

Our results also showed that CC treatments through i.p. route caused a higher level of neutrophil counts in peripheral blood than the oral treatments. It is well known that chitosan dissolves in acidic digestive fluid of the stomach and coagulates in the alkaline fluid of the intest[14]. Thus the lower activity of chitosan to activate neutrophils in peripheral blood when administered orally may due to the physical changes of chitosan in the gastrointestinal tract. Previous studies have shown that neutrophils act as effector cells for host immunity in malaria[15]. In the present study, we found that the neutrophil counts were correlated with the parasite suppression. We suggest that the activation of neutrophils by chitosan may play a crucial role in the enhancement of antimalarial activity of the host.

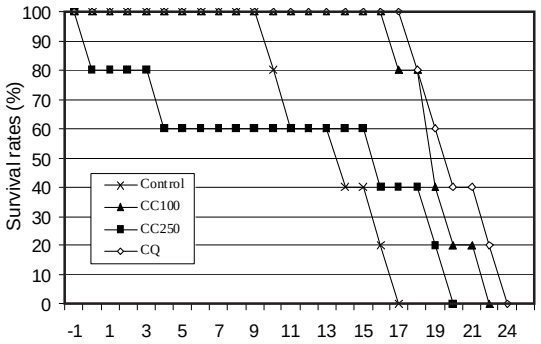

Fig. 2: Cumulative survival rates of mice infected with *P. berghei*, oral treatments with chitosan and chloroquine.

On the other hand, there was a noteworthy observation that the oral administration of CC at highest dose caused 40% of toxic deaths in treated mice. Koide[16] reviewed that chitosan treatment reduced the absorption of minerals and fat-soluble vitamins. Prolonged ingestion of chitosan may alter the normal flora of the intestinal tract which may result in the growth of resistant pathogens. Furthermore, ingestion of fine chitosan particles as a supplement reported to cause growth retardation. Therefore precautions should be taken for any long-term treatments of high doses of chitosan to avoid potential adverse metabolic consequences or perhaps a lower dose should be preferred.

In conclusion, the results of the present study have shown that crustacean chitosan possesses a considerable degree of prophylactic activities against blood induced-*P. berghei* infection in mice. The antimalarial activity was correlated with the activation of neutrophils in peripheral blood so as the suppression of parasitaemias by CC at the highest dose was similar to that of the standard drug. Although oral administration of CC at high dose was found to be toxic, all these results have provided strong foundation for further studies on CC as a potential biopolymer.

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