

Nonspecific Immunological Effect of Haelan 851 in Antimalaria Treatment

ABSTRACT: This paper describes the observed effects of nutritional supplementation with Haelan 851, Platinum Formula, oral nutritional beverage, on the nonspecific immunological effects on rats infected with malaria and later treated with conventional antimalaria treatments.

The materials used in this study involve the following:

1. Malaria parasite: ANKA strain of *Plasmodium berghei*, supplied by the Institute of Parasitological Research, China Academy of Preventative Medicine. The species was preserved through blood infection in mice.

2. Medicines and Nutritional Products:

- a) Haelan 851, Platinum Formula, liquid oral nutritional beverage
- b) Tragacanth powder
- c) Kang Yao Tong tablets, (K.Y.T. hereinafter)
 Note: K.Y.T. is a new antimalaria drug furnished by the Institute of Parasitological Research, China Academy of Preventative Medicine.
- d) Chloroquine and K.Y.T. suspensions made with tragacanth
- e) Indian ink, diluted with distilled water

3. Animals: 130 healthy male mice weighing between 18-22 grams were divided randomly into two large groups. These two groups were subdivided into six and seven subgroups containing 10 mice each. The mice were fed in separate cages following the standard routine feeding practice.

4. Preparation of chicken erythrocyte suspension: Blood was drawn from the wing vein of a healthy Leghorn chicken with a sterilized syringe and mixed into fivefold Asever solution and stored under refrigeration at a temperature of 4 degrees centigrade. Before use, it was washed centrifugally with sterilized normal saline three times and prepared into a 5% normal saline chicken erythrocyte suspension.

II. Method and result:

The 130 male mice, with the exception of those in the normal groups, were inoculated peritoneally with 2×10^7 malaria parasite infected erythrocytes and were given respectively the following medicines and/or supplemental nutrition.

- (1) Haelan 851, Platinum Formula, liquid oral nutritional beverage at 10 ml/kg/d x 5
- (2) Chloroquine at 100 mg/kg/d x 5 by means of perfusion of stomach

On the second day after the above procedure was completed, blood was taken from the tails of the mice for smearing, staining, microscopic examination and counting the infection rate percent and inhibition rate percent for the mice in each of the groups. Phagocytic tests of the abdominal macrophages and mononuclear macrophage system (MPS) were performed respectively.

RESULTS:

(1) Effect of Haelan 851, Platinum Formula, liquid oral nutritional beverage on the phagocytic function of the macrophages in the mice's abdominal cavities:

Twenty four hours after the finish of the treatment course, 60 mice in the six groups were injected peritoneally with 0.5ml of 5% chicken erythrocyte suspension. After an additional 16 hours, the abdominal fluid was collected. The animals were killed by dislocation of the cervical vertebrae, the abdominal skin was incised after disinfecting. 2.5 ml of Nank's solution was injected in through the peritoneum. The bellies were kneaded gently and a small hole was pierced on the peritoneum to withdraw 2 ml of abdominal fluid which was put into a small test tube and was well shaken. Then, the abdominal fluid was dripped on the slides to be incubated for 30 minutes in the incubator at a temperature of 37° centigrade. Then the floating chicken erythrocytes and other cells within were washed away with normal saline and blown dry to be fixed with methanol for five minutes and dyed with Giemsa-Wright stain and let dry. Slides were observed under oil immersion lens and phagocytes were counted. About 100 macrophages on each slide were observed continuously. The phagocytic percentage and indices were calculated according to the formulae listed below and the significant tests were performed.

$$\text{Phagocytic percentage (\%)} = \frac{\text{Number of macrophages that engulf chicken erythrocytes}}{\text{Total number of macrophages observed}} \times 100$$

$$\text{Phagocytic index} = \frac{\text{Total number of chicken erythrocytes engulfed}}{\text{Total number of phagocytes observed}}$$

The results (Table I) shows that the phagocytic function of the abdominal macrophages in mice decreased lower than the normal group ($P < 0.05$) after they had been infected with the malaria parasites. This observed level was lower than that of the normal group ($P < 0.05$).

Groups 1, 2 and 3 were respectively given Haelan 851, Platinum Formula, oral nutritional beverage plus K.Y. T. malaria medication, Haelan 851, Platinum Formula, oral nutritional beverage solely, and K.Y.T. malaria medication solely. The phagocytic percentages of the abdominal macrophages for those groups receiving the Haelan 851, Platinum Formula nutritional supplementation were significantly higher than the percentage in the control group ($P < 0.01$, $P < 0.01$, $P < 0.05$). After the Haelan 851, Platinum Formula, oral nutritional beverage was given to the mice of the normal group, the phagocytic index was evidently higher than that in the normal mice not given the Haelan 851, Platinum Formula nutritional supplementation ($P < 0.05$).

The parasite inhibiting rates in groups 1, 2, and 3 were 100%, 61%, and 96% respectively. The infection rate in the control group was 260%.

Table I
Effect of Haelan 851 and Haelan 851 + Chemotherapy on
Phagocytic Function of Mice's Macrophages in Abdominal Cavity

Groups	No. of Mice	Phagocytic Percentage % X+SD	Phagocytic Index X+SD
1. Haelan 851 + K.Y.T.	10	41.30 ± 2.312	1.040 ± 0.119
2. Haelan 851 alone	10	37.10 ± 1.912	0.670 ± 0.157
3. K.Y.T.	10	29.62 ± 1.740	0.397 ± 0.048
4. Haelan 851 (normal)	10	29.10 ± 1.595	0.840 ± 0.097
5. Normal Control Group	10	29.81 ± 1.005	0.380 ± 1.081
6. Infected Control Group	10	20.03 ± 1.567	0.300 ± 0.082

(2) Effect of Haelan 851, Platinum Formula, oral nutritional beverage on the phagocytic function of mononuclear-macrophage system (MPS) in mice with malaria:

The infection of malarial parasites, dose of nutrition and medicine, therapeutic course and medication through perfusion of the stomach in the seven groups of mice were just the same as those described before. On the second day after the finish of the treating course, blood was taken from the tails of the mice of the test group for smearing, Giemsa-staining and microscopic examination. 1,000 erythrocytes of each mouse were examined to count the infection rate percentage (%). Indian ink was diluted with distilled water at the ration of 2:1. the diluted solution was injected through the tail vein of the mouse at the dose of 0.5 ml/10g body weight based on Biozzi's method. Two and fifteen minutes after the injection, 0.02 ml of blood was taken from the right and left orbital veins respectively, and was dissolved in 0.1% NaCo₂ solution to test the concentration of the blood carbon (D) of the different times at the distance of 675 mm by means of a Type 721 spectrophotometer. This is to count the disappearing extent of carbon particles in blood. The K value of the phagocytic index was calculated according to the formula listed below:

$$K = \frac{\log D_1 - \log D_2}{t_1 - t_2}$$

The larger the K value, the stronger the phagocytic activity. For the results of this study, please see Table II below.

Haelan 851, Platinum Formula, oral nutritional beverage is capable of promoting the phagocytic activity in the peripheral blood, thereby enhancing the clearance rate of the mice's mononuclear-macrophage system (MPS) against carbon particles. After giving the mice nutritional supplementation with Haelan 851, Platinum Formula, oral nutritional beverage (Group 3), Haelan 851, Platinum Formula, oral nutritional beverage plus K.Y.T. (Group 1) and Haelan 851, Platinum Formula, oral nutritional beverage plus chloroquine (Group 2), the K values were 5.24 ± 0.369 , 6.03 ± 2.14 and 5.68 ± 0.322 respectively. They were significantly higher than the values of the infected control group (Group 7), the normal group (Group 6) and the chloroquine group (Group 5). The P values were all $P < 0.01$.

Although chloroquine is capable of resisting malarial parasites, it also inhibits the phagocytic activity of the mononuclear-macrophage system (MPS) in mice with malaria. When it was used combined with Haelan 851 nutritional supplementation, the phagocytic activity of the mice's mononuclear-macrophage system (MPS) increased markedly ($P < 0.01$). The new drug, K.Y.T., yielded a good antimalaria effect. When used combined with Haelan 851 nutritional supplementation, not only could they enhance the phagocytic activity of the mononuclear-macrophage system of the mice but also gave rise to a better therapeutic effect. The parasite inhibiting rates in groups 1,2,3,4, and 5 were 100%, 99.2%, 61.5%, 96.5% and 94.2% respectively. The infection rate in the infected group was 260%.

Table II
Changes of MPS's Phagocytic Function After Using Haelan 851, Platinum Formula, Liquid Oral Nutrition and Its Combined Medicine

Group	Number of Mice	K Value X + SD
1. Haelan 851 + K.Y.T.	10	6.03 ± 2.14
2. Haelan 851 + chloroquine	10	5.68 ± 0.322
3. Haelan 851	10	5.24 ± 0.360
4. K.Y.T.	10	3.77 ± 0.275
5. Chloroquine	10	3.32 ± 0.378
6. Normal	10	3.39 ± 0.269
7. Infected Control	10	3.10 ± 0.206

DISCUSSION

The phagocytic phenomenon of the macrophages has long been observed. Zhong Muilan pointed out in 1973 that the nonspecific phagocytic effect of the mononuclear-macrophage system (MPS) played a very important role in the clinical course and prognosis of malaria. In the experiments, it has been found that the appearing duration of the phagocytic activity of the mononuclear - macrophage system is very short, which goes down very quickly, after the mice are infected with *Plasmodium berghei*, hence, acute lethal infections in the mice will be induced.

In this experiment, the use of Haelan 851, Platinum Formula, oral nutritional beverage shows an immunologically stimulating effect both in experiments and clinics. It can stimulate the function of the MPS to increase the immunity of the malaria infected mice. Particularly, the combined use of Haelan 851, Platinum Formula, oral nutritional beverage with the new antimalaria drug K.Y.T. may improve the abdominal macrophages. It has a positive correlation between the enhancement of the therapeutic effect and the parasite vanishing rate as well as the parasite inhibiting rate. Chloroquine is a commonly used antimalaria drug at present, and when used combined with Haelan 851, Platinum Formula, oral nutritional supplementation, not only can it achieve the effect against malaria, enhance the nonspecific immune function of the mice, but also improve their health level. In the preliminary test, it was also found that Haelan 851, Platinum Formula, oral nutritional supplementation might delay significantly the blood affection of the protozoa, and prolong the survival period of the mice.