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Lack of Schizontocidal activity of three herbal decoctions on *Plasmodium berghei berghei* in mice

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Summary

This work reports the effects of three herbal decoctions on *Plasmodium berghei berghei* in mice. The schizontocidal activity of each decoction was determined on an established infection using chloroquine as a standard and distilled water as control. Also a repository study of the decoction was carried out in another group of mice. The three decoctions neither reduced parasitaemia nor prolonged the life of infected mice that received them.

Résumé

Cet étude rapport les actions de trois décoctions herbal sur la *Plasmodium berghei berghei* dans les souris. L'activité schizontocidal de chaque décoction était déterminé sur l'infection établi en utilisant la chloroquine comme étalon et de l'eau distillé comme le contrôle. Aussi l'étude répositoire du décoction est fait dans un autre groupe de souris. Les trois décoctions ni reduirent la parasitaemie ni prolongent la vie des souris infectés qui les ont reçus.

Introduction

In most developing countries herbal preparations in form of decoctions and infusions have been used to treat malaria. The dococtions usually include barks and leaves of different plants. The relevant morphological parts of the plants are boiled with water for a few hours or soaked in water or alcohol. This is taken according to the needs and desire of the patients. Do these decoctions affect the malaria parasites in any way or are their effects only psychological? We are in an era where there is a clarion call for development of new antimalarials to replace drugs against which resistance has developed. This work reports the effect of three of such decoctions on *Plasmodium berghei berghei* in mice.

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Materials and methods

Animals

The animals used were albino Swiss mice weighing between 18 and 25 g each and bred locally in the departmental animal house. They were fed on standard livestock cubes manufactured by Pfizer Products Nigeria Ltd., and they had free access to water.

Inoculation of the animals

0.2 ml diluted blood containing 1×10^7 parasitized red blood cells obtained from a donor animal was injected intraperitoneally into clean mouse. During each experiment a single donor mouse was used to infect all the animals to minimize variability in parasitaemia of test animals. Day of infection was termed D_0, and subsequent days D_1, D_2 etc.

Evaluation of parasitaemia

Thin blood films were made from the cut tail vein of animals infected with *Plasmodium berghei berghei*. These are stained with Giemsa stain after fixing in methanol. Parasite count

was made under oil immersion with the × 100 eye piece and × 6 objective using a tally counter. Ten microscopic fields were counted, the total number of red cells was first counted and then the total number of parasitized red blood cells. Percentage parasitaemia was assessed for each field and the mean percentage parasitaemia for each mouse was then calculated:

$$\% \text{ Parasitaemia} = \frac{\text{No. of parasitized red blood cells}}{\text{Total No. of red blood cells}} \times 100.$$

Preparation of the decoction

The components of the 3 decoctions are shown in Table 1. The components were collected from the University Botanical Garden. Ten g of each component was weighed, placed in a big pot and an amount of water equivalent to twice the total weight of all the components was added (this usually covered the herbs). This was then boiled for 1 h and left overnight. The contents was boiled for 1 h the following day to concentrate it. The water extract was then drained off the herbs and this served as the stock solution. This methodology was aimed, as much as possible, at mimicking that of the herbalists in accordance with the information obtained from them. The stock solution was stored in the refrigerator at 4°C.

Evaluation of schizontocidal activity in vivo using an established infection

The method used is similar to that of Ryley and Peters (1970). Parasitized mice were randomly distributed into groups of 5 or 10 mice every 72 h after parasite innoculation. The mice were given oral doses of chloroquine (5, 10, 20 mg/kg base), the decoction, or distilled water (control) daily for 5 days. A constant volume of 0.5 ml/20 g mouse was given in each case. Thin blood films stained with Giemsa were made daily from each mouse and the degree of parasitaemia determined. Also the time of death after inoculation was recorded for each mouse to obtain the mean survival time.

Repository activity in vivo

This method is similar to that described by Peters (1965). The animals were first treated

Table 1. Composition of the different decoctions used

Components	Decoctions		
	A	B	C
Citrus aurantifolia leaves	+	+	+
Dried *Carica papya* root	+	+	+
Dried *Carica papaya* leaves	+	+	+
Mangifera indica leaves	+	−	+
Mangifera indica bark	+	−	+
Alstonia boonei leaves	+	−	−
Alstonia boonei bark	+	−	−
Psidium guajava leaves	+	+	+
Morinda lucida leaves and bark	−	+	−
Khaya grandifoliola bark	−	+	+
Azadirachta indica leaves	−	−	+

+ = present
− = absent

with the decoction, pyrimethamine (1.2 mg/kg) or distilled water (control) for 3 days before they were infected with the malaria parasites on the 4th day. Seventy-two after infection, blood smears were made and the percentage parasitaemia determined. The average percentage chemosuppression of parasitaemia was calculated as follows:

$$\left(\frac{\text{Average \% parasitaemia in controls} - \text{Average \% parasitaemia test}}{\text{Average \% parasitaemia in control}}\right) \times 100.$$

Administration of the drug or decoction

All drugs were administered to the mice orally using a metal cannula. In administering the drugs orally, the animals were held by the scruff of the neck with the left hand and the tail was tucked into the hollow of the left hand. The cannula was held in the right hand and a clear passage into the oesophagus was sought before the right amount of drug was given.

Results

Blood schizontocidal activity of orally administered herbal decoction on an established infection in vivo

The results obtained showed that an inoculum containing 1×10^7 *Plasmodium berghei berghei* parasites was viable enough to cause infection in mice 72 h after passage producing between 10–15% parasitaemia.

The results of the blood schizontocidal activity of the three herbal decoctions, those of three different dose levels of chloroquine and that of control are all shown in Fig. 1.

There was a gradual daily reduction of the parasitaemia with all the three doses of chloroquine used. The degree of parasitaemia was drastically reduced on the second day of treatment with chloroquine. On subsequent days there was a gradual reduction in the level of parasitaemia. The highest dose of chloroquine (20 mg/kg) completely cleared the parasites from the blood of all mice by the 5th day of observation.

The results of the control animals and those receiving the herbal decoctions showed a general increase in the level of parasitaemia everyday throughout the 5 days of observation.

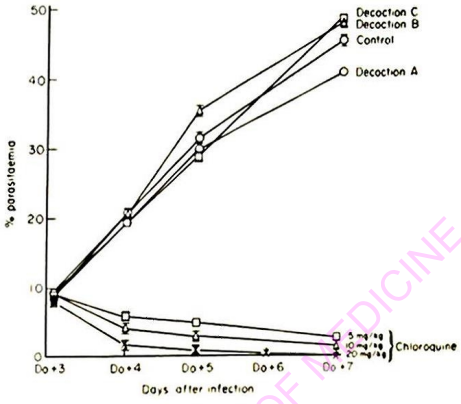

Fig. 1. Percentage parasitaemia in each group v. time after infection. Do = day of passaging.

The mean survival times (days) for each of the three decoctions were 7.8, 8.2 and 8.5. The mean survival time for the control was 8 days. The group that received 20 mg/kg chloroquine survived throughout the 21-day observation period.

Repository action

Decoctions A, B & C produced 11.6% 6.2% and 5.4% chemosuppression respectively. By contrast 1.2 mg/kg pyrimethamine produced 80.5% chemosuppression.

Discussion

In this study, three decoctions used in the traditional treatment of malaria have been screened for antimalarial action using *Plasmodium berghei berghei* in mice. None of the three herbal decoctions was observed to demonstrate any schizontocidal activity on *P. berghei berghei*. On the other hand the chloroquine treated mice recovered from infection, and survived throughout the period of observation. The control and those that received the herbal decoctions died with approximately the same survival time. The recovery of infection of animals treated with chloroquine is a clear

indication that the malaria parasite (*P. berghei berghei*) used is sensitive to this drug.

Despite the fact that all the procedures for preparation of these decoctions were in conformity with locally prepared ones, the results were inconsistent with the general belief by the people of the area. Personal testimonies of people who use these herbal decoctions are that the herbs relieve fever, but the work reported above showed that these herbs neither reduced parasitaemia nor prolonged the life of infected animals that received them. It could be that these herbs have antipyretic activity in man or that the differences in the pharmacokinetics of the drug in man and mice have contributed to lack of activity of the herbs in mice infected with *P. berghei berghei* as used in this work. However, this lack of schizontocidal activity observed to these decoctions in this work does not completely rule out their effectiveness in the treatment of malaria in Man, because care has to be taken in extrapolating animal experimentation to what happens in Man.

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