

# Effects of dihydroartemisinin on fine structure of erythrocytic stages of *Plasmodium berghei* ANKA strain

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**KEY WORDS** dihydroartemisinin; *Plasmodium berghei* ANKA strain; electron microscopy

## ABSTRACT

**AIM:** The fine structural changes of *Plasmodium berghei* ANKA strain after treatment with the dihydroartemisinin ( DATM ) were observed. **METHODS:** DATM 180 mg·kg<sup>-1</sup>·d<sup>-1</sup> was given ig to outbreed NIH mice infected with *P. berghei* ANKA strain. Blood samples were collected, embedded and examined by electron microscopy. **RESULTS:** In *P. berghei* ANKA strain, 1 h after drug administration, the parasites food vacuole membranes were destroyed, and the pigment grains showed some changes. The nucleus membrane, cytomembrane, and food vacuole membranes were stratified 2 h after administration. At the same time, swelling and separation of the outer and inner membrane or shrinking of the mitochondria were seen. Stripped nuclear and cytoplasm membrane were developed and vacuolizations were seen 4 h later. Eight hours after administration, a large number of parasite structures were destroyed except for a few parasite autophagic vacuoles. **CONCLUSION:** DATM was a fast-acting and effective antimalarial drug. Its primary target is the membrane system. No obvious resistant characteristics were found upto 24 generations after resistant induction test for 4 months.

## INTRODUCTION

Dihydroartemisinin ( DATM ) is one of the derivatives of artemisinin that is an antimalarial with unique component isolated from the wormwood *Artemisia annua* L ,

a traditional Chinese herb. It has a marked activity against chloroquine-resistant strains of *Plasmodium falciparum*<sup>[1]</sup>. It can quickly kill asexual forms of *P. falciparum*<sup>[2]</sup> and has a remarkable effect on *P. falciparum* gametocytes<sup>[3]</sup>. For further study on the mechanism of DATM on erythrocytic malaria parasites, the fine structure changes of *P. berghei* ANKA strain were observed.

## MATERIALS AND METHODS

**Materials** *P. berghei* ANKA line was introduced from National Institute of Health ( NIH ), USA, and its DATM " resistant line " was induced with DATM for 24 generations during a 4-month period study in our laboratory. Its resistant index ( RI<sub>50</sub> ) was 12.9-fold and its inducing dose was 109.4 MKD × 3 d. NIH mice weighing 18 - 22 g were provided by the Laboratory Animal Center of Guangzhou University of Traditional Chinese Medicine ( Guangdong Provincial Medical Animal License : 26-97009 ). DATM ( Batch No 970701 ) was the product of Beijing 6th Pharmaceutical Factory.

**Methods** Referring to a previous report<sup>[4]</sup>, mice were inoculated with 1 × 10<sup>7</sup> *P. berghei* ANKA or its " resistant line " infected erythrocytes. The mice infected with *P. berghei* ANKA strain were given DATM in a single dose of 180 MKD × 1 d ( as the total dose of ED<sub>50</sub> × 4 d ) when the parasitaemia reached about 20 % , 3 - 4 drops of blood were taken at 0 h ( before treatment ), 1 , 2 , 4 , and 8 h after treatment. In its resistant line, the blood was taken 7 d later with 20 % parasitaemia. The blood was dropped into a small glass bottle. After blood coagulation, the samples were fixed with 3 % glutaraldehyde for 5 - 10 min, then cut into squares of 1 mm<sup>3</sup> and fixed for about 1 h continuously, and washed with buffer 3 times ( Each time for 15 min ). After 1 h the samples were fixed by 1.5 % osmic acid, dehydrated with acetone, embedded in epoxy resin, and then polymerized

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in constant temperature desiccator ( 24 h at 35 °C ; then 48 h at 60 °C ). After ultra-thin sectioning , the samples were dyed with uranium acetate and plumbum citrate . It was examined with JEM-1200EX electron microscopy .

## RESULTS

The effects of DATM on the fine structural changes of the erythrocytic stage of *P. berghei* ANKA strain were as follows :

One hour after medication , the food vacuole membranes were destroyed and the form of pigment grains were sharply defined . The distribution of ribosome was uneven and vacuolations were seen ( Fig 1 B ).

Two hours after medication , the nuclear membranes , cytomembranes and food vacuole membranes were still clear . Swelling and separation of the outer and inner membranes or shrinking mitochondria were seen . The forms of pigment grain were short and diffused . Multilaminated cytomembrane and broadened space of the outer and inner cytomembrane were obvious ( Fig 1 C - E ).

Four hours after medication , stripped nucleomembrane and cytomembrane , stratified nucleomembrane , thickened mitochondria membrane , uneven ribosomes and cytoplasmic vacuolization were seen ( Fig 1 F , G ).

Eight hours after medication , a large number of parasite appeared disintegrated and degenerated , forming a number of parasitic autophagic vacuoles of varying sizes within the red blood cells . Valgus nuclearpores were occasionally seen in the parasites ( Fig 1 H , I ).

In short , the main effects of DATM on fine structure of erythrocytic stage of *P. berghei* ANKA strain were as follows : ( 1 ) the food vacuole membranes , mitochondria membranes , and cytomembranes were progressively changed ; ( 2 ) enlarged mitochondria , broadened space of the outer and inner food vacuole membranes and cytomembranes or whorled formation were seen ; ( 3 ) malaria parasites were destroyed . The destroyed structure and several autophic vacuoles were found in the slide .

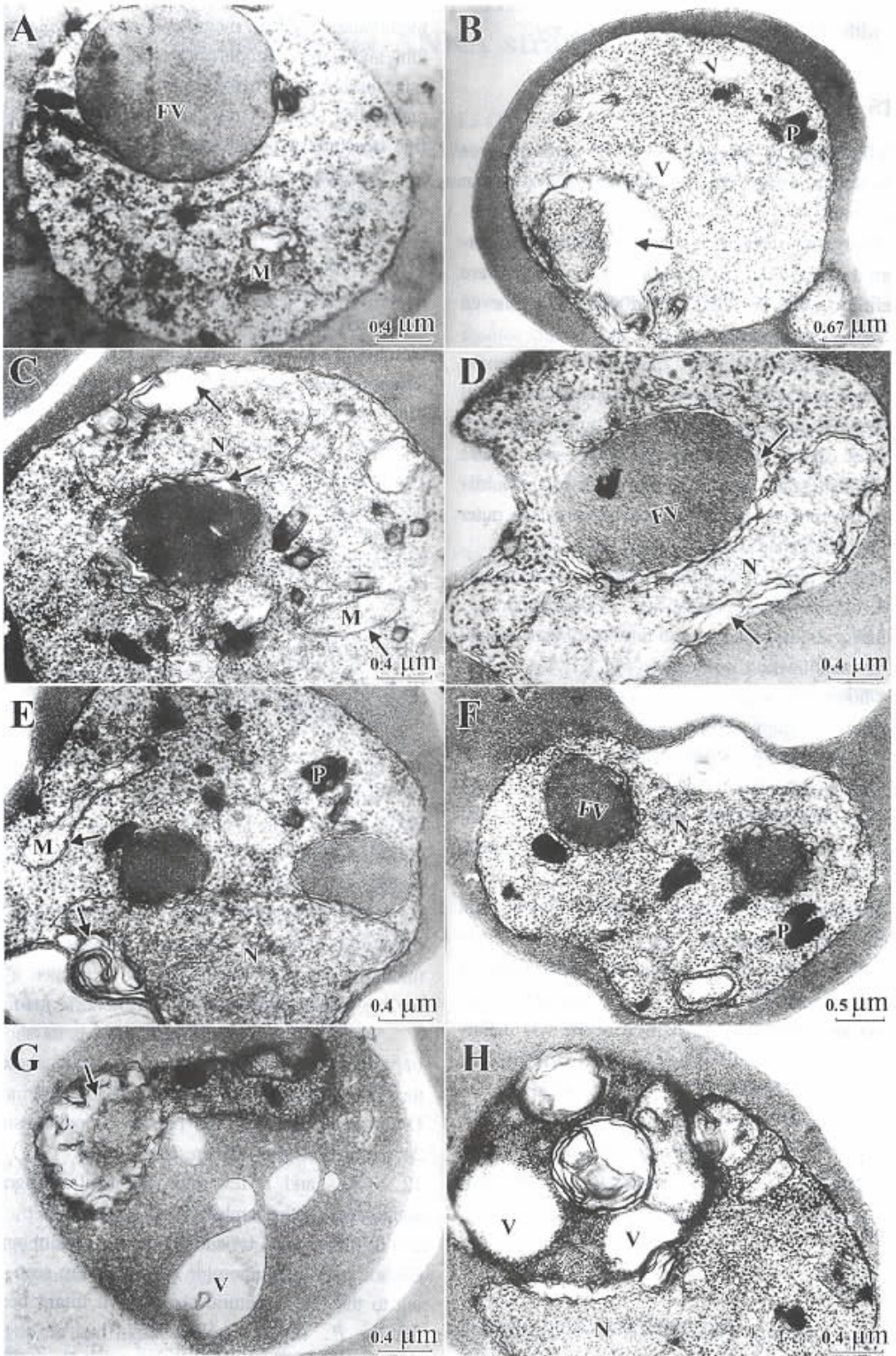
It was observed that the “ resistant line ” only contained stratified food vacuole membranes and cytomembranes , broadened space of membranes , uneven ribosomes and metamorphic pigments ( Fig 1 J - L ).

## DISCUSSION

Chinese Qinghaosu Antimalarial Coordinating

Research Group ( 1979 )<sup>5)</sup> has reports that artemisinin primarily causes membranous changes which take place at the food membrane , limiting membrane , mitochondria membrane , nuclear membrane and endoplasmic reticulum , and finally autophagic vacuoles are formed , the parasite loses a lot of cytoplasm , then degenerates and eventually dies . This study indicates that DATM destroyed fine structure of parasite rapidly and its destructive ability was stronger than that of artemisinin . The activities were very significant from 1 h after treatment . A large number of parasites were destroyed after 8 h , at that time only a few trophozoites autophagic vacuoles were observed . The experiments showed that : ( 1 ) the prominent pathological changes induced by DATM were injuries to the membrane structure of the parasite such as swelling of intermembranous spaces of limiting membranes , mitochondrial membranes , nuclear membranes , and the membranes of food vacuoles . Similar morphological changes were also induced by artemisinin<sup>5)</sup> . Since the food vacuole membranes were affected , the nutrition of parasites was blocked and the autophagic vacuoles formed quickly as a result of amino-acid hunger of parasites . The parasites died because of lost cytoplasm ; ( 2 ) DATM could affect the mitochondria of *P. berghei* ANKA strain , and on this point the results were different from those obtained by using *P. falciparum* . As we knew no appreciable damage appeared in the mitochondrial membranes of *P. falciparum* . It is recognized that the mitochondrion of *P. falciparum* is morphologically different from that of *P. berghei* in that the former contains microtubular cristae while the latter does not . The morphological difference between the two species of parasite could account for their difference in mitochondrial sensitivity to DATM . As Howell ( 1973 )<sup>6)</sup> reported that mitochondria of rodent malaria parasite has cytochrome oxidases , hence , the antimalarial action of DATM can be explained by its interference with the function of mitochondria membrane .

Another characteristic of DATM is its short half-life (  $t_{1/2}$  only 1.5 - 2 h ). So , this study shows that although *P. berghei* ANKA strain had been induced with DATM for 4 months and passing blood transmission for 24 generations but the resistant index ( RI ) was only 12.9-fold , and a few morphological changes such as some thickened membrane of food vacuole , cytoplasm and mitochondria , broadened space of membrane , uneven ribosome and metamorphic pigment were seen . According to the fine structural changes , it might be concluded that the *P. berghei* ANKA strain had no obvious resistance to DATM .





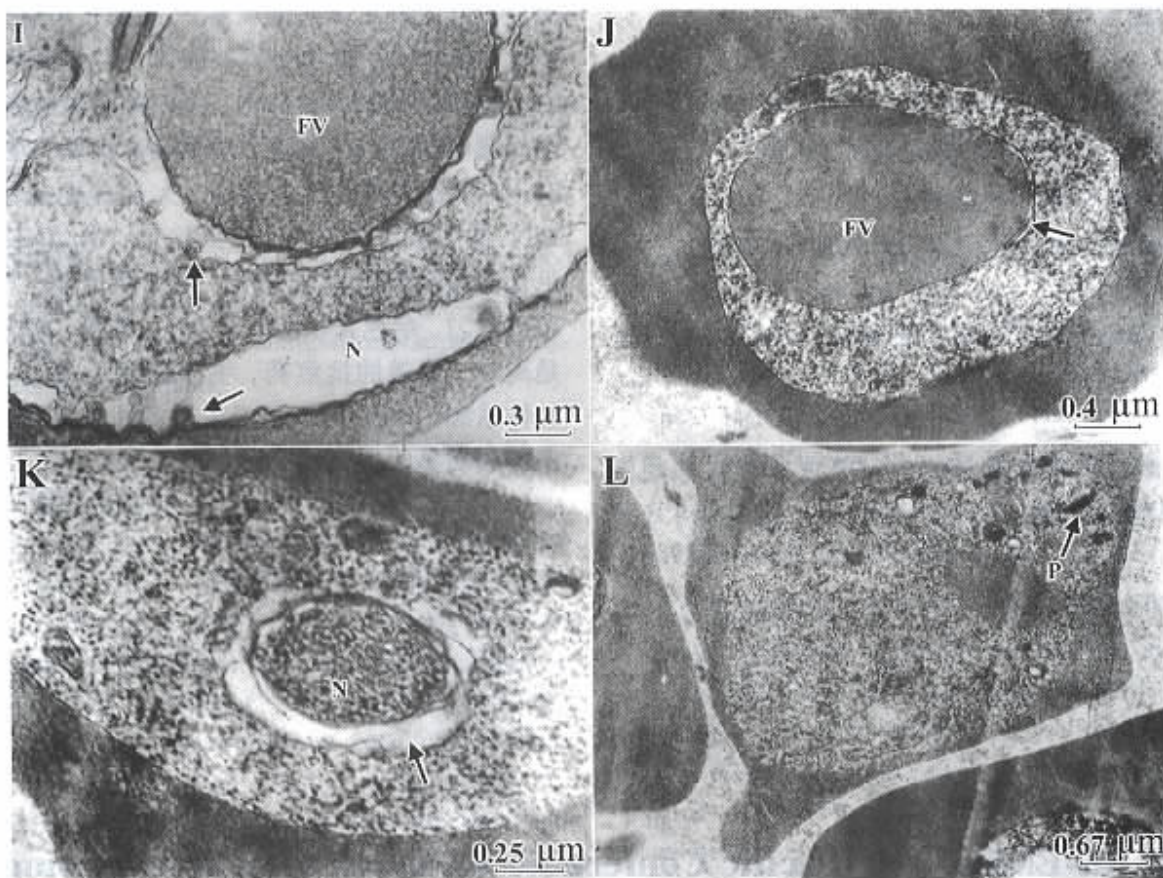


Fig 1. Electron micrographs of trophozoites of *P. berghei* ANKA strain from blood of mice treated with oral dihydroartemisinin 180 MKD $\times$ 1 (B-I) or trophozoites of its resistant line (J-K) and untreated trophozoite (A). B) Trophozoite after 1 h : the stripped nucleomembrane (→) and cytoplasmic vacuolization (V). C, D) Trophozoites after 2 h : the stratified cytomembranes , nucleomembranes , food vacuole membranes , and mitochondria membranes. (→). E) Trophozoite after 2 h : the change of membrane systems and the forms of pigment grains. (P , →). F, G) Trophozoites after 4 h : the stratified and stripped nucleomembranes , the uneven ribosomes and the cytoplasmic vacuolization (V). H, I) Trophozoites after 8 h : the nucleomembrane and cytomembrane stripped the cytoplasmic vacuolization and the valgus nuclearpores formed (→). J) The ring form of *P. berghei* ANKA strain that was induced with DATM for 24 passages : The food vacuole membrane was stratified. (→). K) The trophozoite after inducing with DATM for 24 passages. The broadened space membranes were seen (→). L) The trophozoite after inducing for 24 passages with DATM containing metamorphic pigments (P). (A, C, D, E, G, H, J $\times$  25 000 ; B, L $\times$  15 000 ; F $\times$  20 000 ; I $\times$  30 000 ; K $\times$  40 000)

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## 双氢青蒿素对伯氏疟原虫 ANKA 株超微结构的影响

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**关键词** 双氢青蒿素; 伯氏疟原虫 ANKA 株; 电子显微镜检查

**目的:** 观察双氢青蒿素对伯氏疟原虫 ANKA 株超微结构的影响. **方法:** 双氢青蒿素 180 mg/kg × 1 d 和

109.4 mg/kg × 3 d 分别给受染小白鼠灌胃, 定时取血进行红内期虫体电镜观察. **结果:** 在伯氏疟原虫 ANKA 株: 1 h, 食物泡膜被破坏, 疟色素有些变形; 2 h, 核膜、质膜和食物泡, 线粒体明显肿胀或皱缩, 内外膜剥离; 4 h, 核膜、质膜剥离加剧, 空泡形成; 8 h 少数滋养体仅存自噬泡, 大部分原虫结构被破坏. 试图培育的伯氏疟原虫抗 DATM/ANKA 株, 仅见食泡膜和质膜明显分层, 膜间隙增厚, 核糖体分布不均匀, 疟色素稍有变形等. **结论:** 双氢青蒿素是高效、速效的抗疟药, 主要作用于原虫表膜—线粒体系统. 抗性培育虽经 4 个多月血传 24 代, 但并未形成明显的抗药性的形态特征.

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