Effects of Low Dose of Ketotifen and Chloroquine Combination on the Ultrastructure of Chloroquine Resistant Strain of *Plasmodium Yoelii*

**YOU Li-qun**, **NI Bing**, **CAO Han-min**

1. Department of Journal of East China Normal University, Shanghai 200062, China
2. Department of Biology, East China Normal University, Shanghai 200062, China

**Abstract** 46 inbred NIH mice were infected by chloroquine resistant strain of *Plasmodium yoelii*. The ultrastructure changes were observed under the administration of ketotifen (10 mg·kg⁻¹·d⁻¹) and chloroquine (10 mg·kg⁻¹·d⁻¹) combination and one after another respectively. The effect of taking ketotifen and chloroquine combination showed that parasites died rapidly with a few of intermittent membranes and vacuoles. The effect of taking two kinds of drugs one after another showed that there were exceedingly rich membranes, concentric arrangement structures similar to rough endo-reticulum and conspicuously blocking of the formation of food vacuoles.

**Key words** ketotifen, chloroquine, *plasmodium yoelii*, chloroquine resistant strain, ultrastructure

**1 Introduction**

The widespread development of chloroquine resistant (CR) strain of malaria has resulted in searching for new effective anti-malaria drugs[1-4]. An anti-asthma and anti-malaria drug ketotifen (K) has been proved to show strong anti-malaria action by Pan et al[5-7]. Furthermore K had stronger reverse effect on *P. yoelii* (CR) than Verapmil[8]. The ultrastructure study has revealed that the effect of K (10 mg·kg⁻¹·d⁻¹) on chloroquine-sensitive (CS) strain of *P. yoelii* and K (40 mg/kg·d) on *P. yoelii* (CR) is different from other anti-malaria drugs with the characteristics of multilamellate pellicular complex resembling a medullary sheath[9,10]. The effect of K (10 mg·kg⁻¹·d⁻¹) and chloroquine (CQ, 250 mg·kg⁻¹·d⁻¹) combination has shown amount of concentric arrangement structures similar to rough endo-reticulum[10]. The present work examined the ultrastructure changes in *P. yoelii* (CR) exposed to K (10 mg·kg⁻¹·d⁻¹) and CQ (10 mg·kg⁻¹·d⁻¹) combination and one after another to further elucidate the mechanism of action of the medicine.

**2 Materials and Methods**

Animals: 46 male inbred NIH mice (22 ~ 24 g) were used in the work.

Parasites: The mice were inoculated intraperitoneally with 1 × 10⁷ *P. yoelii* (CR) infected red blood cell. After seven to ten days, the parasitemia reached approximately 15%. This highly chloroquine resistant strain of *P. yoelii* was introduced from the Department of Parasite Biology, Institute of Shanghai Parasitic Diseases.

Drug administration: A group of mice were given K (10 mg·kg⁻¹·d⁻¹) and CQ (10 mg·kg⁻¹·d⁻¹) combination orally (K + CQ group). Blood samples were taken at 10 h, 20 h and 30 h. A group of mice were given K (10 mg/kg) once, after 24 h given CQ (10 mg/kg) once orally (K-CQ group). Blood samples were taken at 10 h, 24 h after giving K, and at 2 h, 5 h, 10 h, 24 h after giving CQ. A group of mice were given CQ (10 mg/kg) once, after 24 h given K (10 mg/kg) once orally (CQ-K group). Blood samples were taken at 10 h, 24 h after giving CQ, and at 2 h, 5 h, 10 h, 24 h after giving K.

Chloroquine diphosphatesalt was bought from Shanghai Fourteen Pharmaceutical Factory and ketotifen succinate was gifted by Shanghai Sixteen Pharmaceutical Factory.

Sample collection and examination: Blood samples were taken from the tails of mice and fixed in 2.5% glutaraldehyde phosphate buffer of 4°C, pH 7.4. They were recollected by centrifugation at low speed and
resuspended in 3% melted agar. After the agar gelled at room temperature, the samples were cut into blocks approximately 1 mm³. Fixation and preparation of specimens for electron microscopic examination were performed routinely. Fixation and preparation of specimens for electron microscopic examination were performed routinely. The specimens were examined with a JEM-100CX II electron microscope.

3 Results

3.1 The morphology of the parasites in the pretreated samples

The morphology of the parasites in the pretreated samples was normal (Fig. 1).

3.2 The ultrastructural changes of P. yoelii (CR) treated with K+CQ

At 10 h after the administration, the structure inside the parasites was seriously damaged. Organelles were indistinct. Amount of large and small vacuoles were seen. The formation of schizonts was hindered (Fig. 2). At 20 h after the administration, the parasites were disintegrated gradually (Fig. 3). At 30 h after the administration, the remains of parasites shrank, in which granulated pigments could be observed. There were many vacuoles inside the red blood cells, and pellets outside the red blood cells might be produced by exocytosis (Fig. 4).

3.3 The ultrastructural changes of P. yoelii (CR) treated with CQ-K

At 10 h after giving CQ, the mitochondria increased and swelled (Fig. 5). At 20 h after giving CQ, the inside structures of parasites were still integrated (Fig. 6). At 2 h after giving K, the mitochondria swelled and broke, double membranes of parasites were injured, some rough endo-reticulum arranged in circle (Fig. 7). At 5 h after giving K, some parasites began to decompose and die (Fig. 8). At 10 h after giving K, the plasm of red blood cell was deeply trapped in the parasites, but the food vacuoles could not be formed (Fig. 9). At 24 h after giving K, there were remnants of decomposed parasites inside the red blood cell (Fig. 10).

3.4 The ultrastructural changes of P. yoelii (CR) treated with K-CQ

At 10 h after giving K, the mitochondria increased, dilated with two ends, the rough endo-reticulum were rich and some vacuoles could be found inside the parasites (Fig. 11). At 24 h after giving K, the normal schizonts could not be formed. There were layers of membranous structure resembling medullary sheath and high electronic density granules (Fig. 12). At 2 h after giving CQ, food vacuoles could not be formed. The nuclei were closely attached to the trapped red blood cell (Fig. 13). At 5 h after giving CQ, some severely damaged structures such as food vacuoles, vacuoles between two membranes of the parasites and parallelly arranged rough endo-reticulums could be observed (Fig. 14). At 10 h, 24 h after giving CQ, the remnant of parasites decomposed gradually. (Fig. 15, 16).

4 Discussion

(1) K can alone kill P. yoelii (CR) with dose of 40 mg·kg⁻¹·d⁻¹[5]. CQ can alone kill P. yoelii (CS) with dose of 10 mg·kg⁻¹·d⁻¹, but shows no effect on P. yoelii (CR) even with dose of 250 mg·kg⁻¹·d⁻¹[10]. The combination of K (10 mg·kg⁻¹·d⁻¹) and CQ (10 mg·kg⁻¹·d⁻¹) has strong and rapid anti-malaria effect on erythrocytic stages of P. yoelii (CR). The result is consistent with the chemotherapeutic and ultrastructure study of P. yoelii (CR) treated with K (10 mg·kg⁻¹·d⁻¹) and CQ (250 mg·kg⁻¹·d⁻¹) combination. These indicate that K can reverse parasite’s resistance to CQ.

(2) Jacobs G.H. stated in his research that CQ could not hinder the formation of food vacuoles of P. yoelii (CR)[12]. We observed deeply trapped plasm of red blood cell, but no food vacuole formation (Fig. 9, 13), which indicates that CQ may block the food vacuole’s formation under the effect of K.

(3) The results of electron microscopic observation treated with K first show two characteristics. One is rich rough endo-reticulum, which suggests that by exposing to low dose of K, the parasites may have ability to increase the reaction of compensatory protein synthesis. The other is extremely rich membranes and mutilamellate pellicular complex resembling medullary sheath. These are the same as our early research results[9,10].

(4) K has quick anti-malaria effect, but is not a quick acting anti-asthma drug. We infer they have different mechanism of action. K has group \( \text{CH}_3 \) It can combine with RNA polymerase of monoplast or-