可能在疟疾感染病理条件下，红血球对抗疟药的亲和性会增加，这种特殊富集作用可能是生物体一种自身保护反应措施。

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REFERENCES

1 Li Y, Yu PL, Chen YX, *et al*. Synthesis of Some derivatives of artemisinine. *Kexue Tongbao* 1979; 24 : 667

2 Jiang JR, Yan HY, Zhuang YH, Xu GY, Zhen YL. Absorption, distribution and excretion of artemether in mice and rats. *Acta Pharmacol Sin* 1983; 4 : 197

3 Prescott LF, Adjepon Y, Elizabeth R. Rapid gas-liquid chromatographic estimation of antipyrine in plasma. *J Pharm Pharmacol* 1973; 25 : 205

4 Niu XY, Ho LY, Ren ZH, Song ZY. Metabolic fate of Qinghaosu in rats; A new TLC densitometric method for its determination in biological material. *Eur J Drug Metab Pharmacokinet* 1985; 10 : 55

5 Li GQ, Guo XB, Jin R, Wang ZC, Jiag HX, Li ZY. Clinical studies on treatment of cerebral malaria with Qinghaosu and its derivatives. *J Trad Chin Med* 1982; 2 : 125

6 Fitch CD, Yunis NG, Chevli R, Gonzalez Y. High-affinity accumulation of chloroquine by mouse erythrocytes infected with *Plasmodium berghei*. *J Clin Invest* 1974; 54 : 24

7 Gu HM, Warhurst DC, Peters W. Uptake of [^{3}H]dihydroartemisinine by erythrocytes infected with *Plasmodium falciparum in vitro* *Trans R Soc Trop Med Hyg* 1984; 78 : 265.

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五种抗疟药对鸡疟原虫孢子增殖期作用的电镜观察[1]

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Electron microscopic observation of the sporogonic stage of *Plasmodium gallinaceum* after five antimalarials

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ABSTRACT Ultrastructural changes in *Plasmodium gallinaceum* oocysts and sporozoites were studies after 5 antimalarials (pyrimethamine, primaquine, artemisinine, 5-p-fluorobenzoxyl-primaquine citrate and nitroquine) were administered to *Aedes albopictus*. Obvious disfigurement, such as abnormal vacuoles of various sizes in the cytoplasma, thickened oocyst capsules and damaged sporozoite pellical membranes were found in many oocysts and sporozoites in the mosquitoes. When the grade of infection of sporozoites in the salivary glands of the 5 different groups of mosquitoes were compared with the control, the rank test ($H_c = 271$) showed a very significant variance ($P<0.01$).

KEY WORDS *Aedes*; *Plasmodium gallinaceum*; electron microscopy; primaquine; pyrimethamine; artemisinine; nitroquine

提要 用伯氨喹、乙胺嘧啶、青蒿素、5-对氟苯氧基伯喹柠檬酸盐及硝喹喂感染鸡疟原虫的阳性蚊。另外，以硝喹喂鸡后，再供蚊血餐。感染后d 8及d 12分别解剖蚊，电镜观察显示蚊体内部分或大部分卵囊及子孢子的胞质有空泡形成，卵囊被膜变厚，子孢子受损，胞质溶解或胞核浓缩变形，各喂药组蚊涎腺子孢子的感染度与对照组相比 $Hc = 271$，$P<0.01$。

关键词 伊蚊；鸡疟原虫；电子显微镜检查；伯氨

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喹；乙胺嘧啶；青蒿素；硝喹

自 Terzain 1947 年报道以蚊活体培养系统作为筛选抗疟药的方法以来，国内外许多学者在这方面做了许多探讨[1,2]。但其中仅少数曾以电镜观察[3,4]。我们以 5 种抗疟药，即伯氨喹(primaquine)、乙胺嘧啶(pyrimethamine)、青蒿素(artemisinin)、5-对氟苯氧基伯喹柠檬酸盐（5-*p*-fluobenzoxyl-primaquine, MP-90）和硝喹(nitroquine)，利用鸡疟原虫-白纹伊蚊系统进行试验，以透射电镜观察各药对卵囊及子孢子的作用.

MATERIALS AND METHODS

鸡疟原虫与白蚊伊蚊系 1982 年引自中国预防医学科学院寄生虫病研究所，前者血传保种于来亨鸡体内；后者饲养条件为 26±1℃，相对湿度 70-80%，每日光照 12 h。来亨鸡购自华中农业大学鸡场，体重 250 g，无自然感染.

伯氨喹和乙胺嘧啶由上海十一制药厂生产。青蒿素结晶由中医研究院中药研究所提取，由该院疟疾研究室提供。MP-90 由第二军医大学疟疾研究室合成，寄生虫学教研室提供。硝喹粉剂由上海医药工业研究院合成。上述各种抗疟药均以适量吐温 80 研磨，蒸馏水稀释，除乙胺嘧啶稀释为 0.1 mg/L 和硝喹稀释为 5 mg/L 外，其余药物均稀释为 1 mg/L，之后加葡萄糖成为 10% 糖液。

鸡疟原虫感染蚊的实验 当小鸡血中疟原虫数达 40/100 RBC，配子体数 1-10/100 RBC 时，以羽化后 2-3 d 并饥饿 24 h 的白纹伊蚊叮咬小鸡 30 min 血餐后的蚊虫分装 6 笼，每笼蚊数 80-100，在蚊吸血后，以清洁海棉块分别吸取上述各种药液置于第 1－5 笼喂蚊。第 6 笼对照组仅喂 10%葡萄糖液，感染小鸡被以上 6 笼蚊吸血后即给病鸡 ig 硝喹剂量 0.2 mg/kg，4 h 后，供第 7 笼蚊血餐，以后仅以 10%葡萄糖喂蚊。上述各笼蚊每日换药液或糖水 1 次，并于血餐后 3 d 各补充喂小鼠血 1 次。

阳性蚊涎腺的观察 吸血蚊饲养 12 d 解剖取涎腺在高倍镜下检查感染度：每个视野子孢子平均数在 10 为(+)，10-100 为(++)，101-1000 为(+++)，1000 为(++++)。

电镜标本制备与观察 将吸血蚊置于以二甲砷酸钠 0.2 mol/L 缓冲液配制的 2% 戊二醛中解剖，取出蚊胃，移至 4%戊二醛中固定 2-3 h(4℃)。再以二甲砷酸钠液洗 5 次，每次时间 0.5 h，以后移至缓冲液中，保存于 4℃ 冰箱，每天换 1 次缓冲液。1 wk 内以 1%锇酸后固定，系列丙酮脱水，618 树脂包埋，LKB 超薄切片，双染色，用 1260 EX 型透射电镜观察。

RESULTS

对照组的正常卵囊与子孢子 卵囊圆形，早期囊内胞质饱满，卵囊被膜 (cm) 内层形成空泡(v)有的空泡开始伸入胞质内(Plate 1 A)，随着空泡的延伸使胞质再分裂为成孢子细胞(s)，并可从其表面长出锥形子孢子芽(Plate 1 B)，至 d 8-12 形成大量游离子孢子，子孢子内含棒状体(rp)、微线体(m)、核(n) 及微孔(↑)结构 (Plate 2 A, B)。

不同抗疟药对卵囊或子孢子的作用

1 吸血蚊喂药组

1.1 伯氨喹组 卵囊被膜变厚，囊内有空泡及颗粒状物，晚期卵囊内子孢子发育不良(Plate 1 D, E)，胞质与核溶解 (Plate 2 E)。

1.2 MP-90 组 卵囊发育不良，卵囊被膜部分变厚，囊内胞质停止分化，有大小异常空泡(Plate 1 F)。子孢子的胞质溶解，核质浓缩，棒状体收缩成团(Plate 2 F)。

1.3 青蒿素组 卵囊发育不良，较正常卵囊小，胞质收缩或出现空泡，囊壁变厚 (Plate 1 G)。

1.4 硝喹组 卵囊被膜明显变厚，囊内出现大小不均的空泡，胞质结构模糊不清 (Plate 1 H)。子孢子部分被膜破损，棒状体融合，线粒体(mi)结构不完整，胞质出现大空泡 (Plate 2 I, J)。

1.5 乙胺嘧啶 子孢子内线粒体(mi)膨胀

(Plate 2 C)，胞质不均，出现溶解，部分被膜破损(Plate 2 D)。

2 鸡 ig 硝喹后供蚊吸血组　受染鸡 ig 硝喹后供蚊吸血，蚊内卵囊小，卵囊被膜厚，囊内出现大小不等空泡(Plate 1 I)。子孢子胞质受破坏，虫体变形，出现空泡，核消失 (Plate 2 G, H)。

吸血蚊 d 12 解剖涎腺，使用 4 种不同抗疟药的 5 组蚊，其涎腺子孢子感染度与对照组相比有显著性差异 Hc = 271，$P<0.01$(Tab 1)。

Tab 1.　The gland infection rate and sporozoite density of *Plasmodium gallinaceum* in *Aedes albopictus* fed on anti-malarials. *n* = Number of mosquitoes dissected 12 d after the infection of *P gallinaceum*.

Drug	Dose mg/L	*n*	Gland infection (%)	Grade of sporozoite infection				
				−	+	卄	卅	卌
Control		17	65(11/17)	6	1	3	3	4
Primaquine	1	16	38(6/16)	10	4	2		
MP-90	1	7	86(6/7)	1	6			
Artemisinine	1	14	29(4/14)	10	4			
Nitroquine	5	7	57(4/7)	3	4			
Nitroquine	0.2*	12	75(9/12)	3	7	1	1	

MP-90, 5-*p*-fluobenzoxyl-primaquine citrate
* Chicken oral dose of 0.2 mg/kg

DISCUSSION

以伯氨喹对鸡疟原虫作试验，证实对埃及伊蚊体内的卵囊及子孢子有杀死作用[3]，主要作用在子孢子[4]。伯氨喹能使卵囊变性并阻止子孢子形成与成熟[5,6]。本文也证实伯氨喹对鸡疟原虫的卵囊和子孢子有明显的破坏作用。

乙胺嘧啶对蚊胃壁上的 *Plasmodium falciparum* 及 *Plasmodium vivax* 幼囊核分裂有明显影响[7]，可以抑制卵囊的 DNA 合成和阻止 *Plasmodium yoelii* 孢子增殖的发育[8]。本文电镜观察到，乙胺嘧啶不仅干扰卵囊核的分裂，而且对新形成的子孢子有较严重的破坏作用。

青蒿素治疗间日疟现症患者后，人工感染中华按蚊，卵囊数明显减少，个别卵囊有不规则空泡形成及胞浆收缩[9]。据报道青蒿素系作用于疟原虫滋养体的膜结构，干扰表膜及线粒体的功能[10]。本研究证实青蒿素致囊壁及胞质发生类似变化，并对卵囊发育有明显影响。

硝喹对鸡疟原虫孢子增殖期有抑制作用[11]。本实验表明，不论以硝喹直接喂蚊或鸡 ig 硝喹后让蚊吸血，均损坏卵囊和子孢子。

本文 5 种药对鸡疟原虫孢子增殖期的发育抑制和破坏程度基本一致，可能由于给药量较大，使卵囊和子孢子均出现较严重的损伤和变形，但仍有部分卵囊发育正常，并有成熟子孢子进腺。这可能与以下因素有关：1) 蚊吸药液最早在血餐 30 min 以后，这时部分大、小配子已形成合子，而饱餐后的蚊可能延至 8 h 后才吸药液，这时部分动合子已钻进蚊胃壁到达胃弹性纤维膜下，不再与药液直接接触；2) 鸡 ig 硝喹 4 h 后供蚊吸血，蚊体内的药物可能尚未对大、小配子充分发生作用，致使部分疟原虫完成孢子增殖的发生。

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REFERENCES

1 Shao BR, Ye XY, Zheng H. Effect of pyronaridine and three other antimalarials on gametocytes and sporogony of *Plasmodium yoelii*. *J Parasitol Parasitic Dis* 1984; 2 : 28
2 Teklehaimanot A, Phuc ND, Collins WE, Barber AM, Campbell CC. Evalution of sporontocidal compounds using *Plasmodium falciparum* gametocytes produced *in vitro*. *Am J Trop Med Hyg* 1985; 34 : 429
3 Terzian LA, Stahler N, Weathersby AB. The action of antimalarial drugs in mosqitoes infected with *Plasmodium gallinaceum*. *J Infect Dis* 1949; 84 : 47
4 Terzian LA, Stahler N, Dawkins AT Jr. The sporogonous cycle of *Plasmodium vivax* in *Anopheles* mosquitos as a system for evaluating the prophylactic and curative capabilities of potential antimalarial compounds. *Exp*

Parasitol 1968; 23 : 56
5 Davies EE, Howells RE, Peters W. The chemotherapy of rodent malaria. XV. Fine structural studies on the sporogonic stages of *Plasmodium berghei* following exposure to primaquine. *Ann Trop Med Parasitol* 1971; 65 : 461
6 Omar MS, Collins WE, Contacos PG. Gametocytocidal and sporontocidal effects of antimalrial drugs on malaria parasites 1, Effect of single and multiple doses of primaquine on *Plasmodium cynomolgi*. *Exp Parasitol* 1973; 34 : 229
7 Shute PG, Maryon M. The effect of pyrimethamine on the gametocytes and oocysts of *Plasmodium falciparum* and *Plasmodium vivax*. *Trans R Soc Trop Med Hyg* 1954; 48 : 50
8 Zhu XP, Kong DF, Wang RF. Effect of pyrimethamine on the oocyst formation of *Plasmodium yoelii yoelii*. *J Parasitol Parasitic Dis* 1988; 6 : 111
9 胡昌仁、肖少玉、陈建明、湛德纯、李昌美、兰飞. 青蒿素对间日疟原虫孢子增殖的作用. 武汉冶医学报 1984; 1 : 9
10 中医研究院中药研究所药理研究室. 青蒿的药理研究. 新医药学杂志 1979; 1 : 23
11 Wang XX, Hu YM, Dong LJ, Deng BZ. Studies on the mechanism of sporontocidal effect of nitroquine (CL-679) on *Plasmodium gallinaceum*. *Acta Pharm Sin* 1981; 16 : 335

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Characteristics of the interaction of lycobetaine with DNA

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ABSTRACT The characteristics of the interaction of lycobetaine (LBT) with DNA were examined by fluorescence spectrometer, disc electrophoresis and restriction enzyme analysis. The apparent binding constant of LBT with calf thymus DNA has been determined as 1.67×10^6 L/mol by ethidium bromide displacement method. Based on electrophoresis titration, the mode of DNA binding was found to be through intercalation. Fluorescence quenching assay showed that the intrinsic association constant and the binding site size of LBT to calf thymus DNA were 0.26×10^6 L/mol and 2.6 base pairs, respectively. Selective inhibition of LBT on action of some restriction enzymes showed that LBT intercalate preferentially into GC base pairs. Neither DNA strand break nor interstrand cross-link was produced by LBT. LBT did not bind to DNA covalently and did not cause DNA alkylation.

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KEY WORDS lycobetaine; DNA; ethidium; disc electrophoresis; fluorescence spectrometry

Lycobetaine (LBT) is a new anticancer agent derived from lycorine, a main alkaloid from *Lycoris radiata* Herb. It can inhibit the growth of many experimental tumors including P 388, leukemia L1210, Lewis lung carcinoma, ascites hepatoma and Ehrlich ascites carcinoma[1]. It has been reported that LBT altered the circular dichroism of calf thymus DNA in solution[2], inhibited DNA and RNA syntheses[3], and blocked the expression of actively transcriptional genes[4]. Extensive evidence showed that nucleic acids were probably the principal cell target site. It prompted the present study of interaction of LBT with DNA.