中国药理学报 *Acta Pharmacologica Sinica* 1990 May; 11 (3) : 272-274

氯胍诱导猪尾猴疟原虫红外期受阻裂殖体的形成和首次虫血症的推迟出现[1]

江静波、廖家遒、黄建成、梁东升、李道生、伦照荣
(中山大学生物学系寄生虫学研究室，广州 510275，中国)

Induction of retarded exoerythrocytic schizonts by chloroquanide resulting in delayed parasitaemia of *Plasmodium inui* in *Macaca mulatta*

JIANG Jing-Bo, LIAO Jia-Yi, HUANG Jian-Cheng, LIANG Dong-Sheng, LI Dao- Sheng, LUN Zhao-Rong (*Parasitology Laboratory, Department of Biology, Zhongshan University, Guangzhou 510275, China*)

ABSTRACT Three Rhesus monkeys inoculated with a large number of sporozoites of *Plasmodium inui* dissected from the salivary glands of infected mosquitoes, *Anopheles dirus*. Two of the monkeys (inoculated with 8.06×10^5 and 1.3×10^7 sporozoites, respectively) were treated with chloroguanide base 6 mg / (kg · d) × 4 d starting from 24 h after the inoculations and the other one (inoculated with 5.93×10^6 sporozoites) was not treated with chloroguanide as control. The primary parasitaemia attacks occurred in the former 2 monkeys were 31 and 25 d respectively after inoculation, while in the control was 7.5 d.

Liver biopsies were done in all of the 3 monkeys, normal schizonts were seen in the control monkey on d 8 after inoculation, while none were detected in the 2 monkeys treated with chloroguanide. However, the retarded exoerythrocytic schizonts were found by indirect fluorescent antibody test (IFAT) in liver sections of the monkey inoculated with 1.3×10^7 sporozoites. Therefore, it is evident that moderate doses of chloroguanide retarded the formation of schizonts and thus delayed the primary parasitaemia.

KEY WORDS *Plasmodium inui*; chloroguanide / therapeutic use; *Anopheles dirus; Macaca mulatta*

Received 1989 Aug 07 Accepted 1990 Jan 3
[1]Project supported by the National Natural Science Foundation of China, №. 3860433; the National Education Commission Foundation of China, №. 32840419 and the Science Foundation of Zhongshan University.

提要 用抗疟原虫红外期药物—氯胍(基质剂量 6 mg / (kg · d) × 4 d)治疗感染猪尾猴疟原虫子孢子 24 h 后的两只猕猴. 两猴的首次虫血症分别比对照猴迟 17 和 23 d 出现，在治疗猴肝切片中发现受阻裂殖体，表明氯胍虽能有效地杀灭疟原虫红外期裂殖体，但当剂量不足时却能诱导受阻裂殖体的形成，导致首次虫血症推迟出现.

关键词 猪尾猴疟原虫；氯胍；抗疟药 / 药物疗法；大劣按蚊；猕猴

感染食蟹猴疟原虫子孢子后经氯胍处理的猴肝出现受阻裂殖体(retarded schizont)，其首次虫血症较未治疗猴推迟出现[1]，这似乎支持氯胍可使该疟原虫子孢子在猴肝形成受阻裂殖体，而引起首次虫血症推迟的假说[2,3]. 但因休眠体也可发育成裂殖体而产生复发虫血症[4]，所以，有可能所谓受阻裂殖体实际上是休眠体向裂殖体发育的一个阶段，而所谓的首次虫血症可能实际是首次复发. 因此，有必要用氯胍对无复发特性的猪尾猴疟原虫(*Plasmodium inui*)进行药物治疗试验，以证实它是否确能诱导形成受阻裂殖体,并引起首次虫血症推迟出现.

MATERIALS AND METHODS

虫种 猪尾猴疟原虫，1975 年在中国的熊猴 (*Macaca assenensis*) 中分离到，猴体或液氮中保存.

子孢子分离 大劣按蚊 (*Anopheles dirus*) 叮咬感染了猪尾猴疟原虫的猕猴，吸血后放在 27℃，湿度为 85% 的环境中饲养 12-14 d 后，当大量子孢子进到唾液腺时，收集子孢子并计数[1].

猴子感染 猕猴 (*Macaca mulatta*). 实

验前 2 wk,每天作血片检查,未发现疟原虫. 对照猴(#A, 2.5 kg)由股静脉接种子孢子 5.93×10^6 个. 另两猴为治疗猴(#B, 1.55 kg. #C, 2.5 kg), 分别接种子孢子 8.06×10^5 和 1.3×10^7 个.

药物治疗 治疗猴均在接种子孢子后 24 h ig 盐酸氯胍 (proguanil hydrochloride, 英国 ICI 产品), 基质剂量为 6 mg／(kg・d) × 4 d. 两猴在首次虫血症出现后的 d 10 和 d 14, 分别 ig 磷酸氯喹(上海第十一制药厂), 基质剂量为 100 mg／(kg・d) × 5–7 d. A 猴在首次原虫出现后 d 10 用氯喹治疗, 方法和剂量同上.

肝检查 B 猴在接种子孢子后的 d 7.5 和 17 分别进行第 1 次和第 2 次肝活检. C 猴在接种子孢子后的 d 9 和 d 28 各进行一次肝活检. A 猴于接种子孢子后的 7.5 d 作肝活检. 肝块用 Carnoy's solution 固定, 石腊包埋, 4 μm 切片, 用间接荧光抗体试验(IFAT)及吉氏–松香染色检查[5].

血液检查 三猴均在接种子孢子后 d 5 开始每天涂厚、薄血片作吉氏染色血液检查, 至首次虫血症出现.氯喹治疗后 2–3 d 血检 1 次、至少观察 4 个月.

RESULTS

A 猴在接种子孢子后 d 8 出现首次虫血症, 肝切片(4.2 cm^2)只查到 14 个发育正常的裂殖体(Fig 1,A), 大小为 15–23 × 17–28 μm, 未发现受阻的裂殖体. C 猴 d 9 的肝切片(4.5 cm^2)中找到了 5 个受阻的裂殖体, 其直径为 7–9 μm, 圆或卵圆形, 有两个核以上(Fig 1, B, C). 子孢子接种后 d 25 出现首次虫血症, d 28 肝切片(3.6 cm^2)中未再发现受阻裂殖体. B 猴 d 7.5 和 d 17 的肝切片(分别为 3.0 和 4.0 cm^2)中未发现任何裂殖体, 子孢子接种后 d 31 出现首次虫血症. 3 只猴在首次虫血症出现后, 经氯喹治疗, 检查 4 个月均未发现虫血症再出现.

DISCUSSION

A 猴在用氯喹治疗消除首次虫血症后一直未再发现红内期疟原虫, 进一步表明本实验所用的国内发现的猪尾猴疟原虫是无复发特性的. 所以治疗猴出现于接种子孢子后 d 25 和 31 的虫血症属推迟的首次虫血症, 而非首次复发虫血症当属无疑. 然而, 如何引起此虫血症的呢? 从 C 猴在接种后 d 9 (即氯胍停用后 d 5)的肝切片中发现有受阻裂殖体, 而 d 28 (即推迟的虫血症出现后 3 d)该猴的肝切片未再发现有受阻裂殖体, 我们认为应是由这种受阻裂殖体后来进一步发育为正常的裂殖体而产生的. 至于 B 猴有推迟的虫血症出现, 而肝内未发现受阻裂殖体的原因尚难确定, 可能与接种子孢子量较 C 猴少有关. 由于我们在本实验中所看到的受阻裂殖体形态和在食蟹猴疟原虫研究中所看到的受阻裂殖体形态[1]是一样的, 因此食蟹猴疟原虫在氯胍的作用下形成受阻裂殖体并引起推迟的首次虫血症是完全可能的. 形成受阻裂殖体的原因, 我们认为可能和药量不足有关. 因为氯胍是抑制疟原虫的二氢叶酸还原酶的[6,7], 当剂量不足时, 这种酶的作用仅受到部分抑制,疟原虫的发育因而减慢, 形成受阻裂殖体. 当药的作用过后, 酶的作用不再受抑制, 虫体又恢复正常生长, 形成正常裂殖体, 最终产生推迟的的首次虫血症. 但也可能和疟原虫种的特性有关. 江静波等氯胍的用量(基质剂量 10 mg／(kg・d) × 5 d) 比本实验大, 食蟹猴疟原虫子孢子同样在猴肝形成受阻裂殖体[1],而恶性疟原虫(*P. falciparum*)子孢子感染却可以由氯胍根治[2]. 所以尚需加大氯胍的用量来探讨受阻裂殖体形成的原因.

ACKNOWLEGMENTS 李逸明和江水同志参加荧光抗体染色检查, 刘元同志协助切片, 卢力心、肖晓梅、江水、陈茵茵、胡超群、何建国和胡永红协助解

剖蚊子.

REFERENCES

1 Jiang JB, Bray RS, Krotoski WA, *et al*. Observations on early and late post-sporozoite tissue stages in primate malaria. V. The effect of pyrimethamine and proguanil upon tissue hypnozoites and schizonts of *Plasmodium cynomolgi bastianellii*. *Trans R Soc Trop Med Hyg* 1988; **82** : 56

2 Bray RS. Studies on the exoerythrocytic cycle in the genus *Plasmodium*. In : *London School of Tropical Medicine Memoir no. 12*, London : Lewis, 1957; 1-117

3 Bray RS. The response of *Plasmodium vivax* to antifols. *Trans R Soc Trop Med Hyg* 1984; **78** : 420

4 Krotoski WA. Discovery of the hyponozoite and a new theory of malarial relapse. *Ibid* 1985; **79** : 1

5 Krotoski WA, Collins WE, Broderson JR, Warren McW, Krotoski DM. The 48-hour exoerythrocytic stage of *Plasmodium cynomolgi bastianellii*. *Am J Trop Med Hyg* 1981; **30** : 31

6 Rollo IM. The mode of action of sulphonamides, proguanil and pyrimethamine on *Plasmodium gallinaceum*. *Br J Pharmacol* 1955; **10** : 208

7 Peters W. *Chemotherapy and drug resistance in malaria*. London: Academic Press, 1970 : 579-82

中国药理学报 *Acta Pharmacologica Sinica* 1990 May; **11** (3) : 274-278

甲苯达唑对小鼠细粒棘球蚴的疗效与宿主免疫水平的关系

冯建军、肖树华 (中国预防医学科学院寄生虫病研究所[1], 上海 200025, 中国)

Relationship between the efficacy of mebendazole and immune level of mice infected with secondary cysts of *Echinococcus granulosus*

FENG Jian-Jun, XIAO Shu-Hua (*Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine*[1], *Shanghai 200025, China*)

ABSTRACT The proliferative response of lymphocytes to Con A and enzyme-linked absorbent assay were used to determine the levels of cellular and humoral immunity of mice infected with ip 2000 protoscolices of *Echinococcus granulosus* for 2, 4 or 8 months and treated twice with ip BCG 0.2 mg / mouse at an interval of 10 d, or with ip cyclophosphamide (Cy) 10 mg / (kg · d) × 5 d. The results showed that the immune levels of the host were stimulated by BCG, but depressed by Cy significantly. When the mice were treated with ip mebendazole (Meb) 25 mg / (kg · d) × 10 d in combination with ip BCG 0.2 mg / mouse on d 3 before Meb treatment and on d 7 after the beginning of Meb treatment, or with ip Cy 10 mg / (kg · d) × 5 d before Meb treatment, the inhibition rates of cyst weight and the alterations of germinal layers induced by the drugs were similar to those of corresponding groups treated with Meb alone. Cy also exhibited an apparent effect on mice infected with protoscolices for 2 months. Even so, no apparent synergetic effect was seen after combined treatment with Cy and Meb. The results suggest that the effect of Meb on secondary cysts of *E granulosus* was not affected by the host immune level

KEY WORDS echinococcosis / drug therapy; mebendazole / therapeutic use; BCG vaccine / therapeutic use; cyclophosphamide / therapeutic use; cellular immunity; antibody formation

提要 感染细粒棘球蚴原头节 2-8 个月的小鼠, ig 甲苯达唑 25 mg / (kg · d) × 10 d, 并于疗程前 3 d 及疗程 d 7 各 ip 1 次卡介苗 0.2 mg / 鼠, 或在疗程前 ip 环磷酰胺 10 mg / (kg · d), 连给 5 d, 其疗效与单用甲苯达唑治疗的相仿, 结果表明, 宿主免疫水平不影响甲苯达唑抗细粒棘蚴的作用.

关键词 棘球蚴病 / 药物疗法; 甲苯达唑 / 治疗; 卡介苗 / 治疗; 环磷酰胺 / 治疗; 细胞免疫; 抗体形成

Received 1989 Jul 28 Accepted 1989 Dec 16

[1] WHO Collaborating Center for Malaria, Schistosomiasis and Filariasis.