responses by impairing the release of interleukin 1 and interleukin 2. Eur J Immunol 1981; 11: 657 – 61.
11 Wu WR, Li Y, Zhang KR, Wang GL, Xie L, Bai HQ. Effects of dihydroetorphine on immune functions in rats treated by automatical intravenous injection. Chin Bull Drug Depend 1998; 7: in press.
12 Wu WR, Zheng JW, Li FY, Li Y, Zhang KR, Bai HQ. Involvement of μ-opioid receptors and α-adrenoceptors in the immunomodulatory effects of dihydroetorphine. Eur J Pharmacol 1998; in press.

二氢埃托啡自身给药对大鼠淋巴细胞功能的免疫抑制作用[1]

吴蔚然[2], 郑继旺[3], 李凤源, 李 颖[4]
(西安医科大学药理教研室, 西安 710061; [3] 北京医科大学中国药物依赖性研究所; [4] 北京医科大学免疫教研室, 北京 100083, 中国)

关键词 二氢埃托啡; 自身给药; 依赖(心理学); 淋巴细胞; 白细胞介素-2; 免疫抑制

目的: 研究二氢埃托啡(DHE)对大鼠淋巴细胞功能的影响及其精神依赖性潜力. **方法**: 用大鼠静脉自身给药模型评价精神依赖性; 用淋巴细胞增殖反应和白细胞介素-2 活性检测免疫功能. **结果**: DHE(总剂量 178 ± 13 μg)自身给药使大鼠形成稳定的精神依赖性, 且显著抑制由刀豆球蛋白刺激的淋巴细胞增殖反应(DHE 组 129 ± 11 Bq; 对照组 620 ± 36 Bq)和白细胞介素-2 活性(DHE 组: A_{570} = 0.28 ± 0.06; 对照组: A_{570} = 0.51 ± 0.03). **结论**: DHE 具有高度精神依赖性潜力, 且能明显抑制大鼠淋巴细胞增殖和白细胞介素-2 活性.

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1998 Jul; 19 (4): 390 – 393

Effects of nitroquine on ultrastructures and cytochrome oxidase of exoerythrocytic *Plasmodium yoelii* in rat liver[1]

CHEN Xiao-Hong[2], HU You-Mei, LIAO Ya-Qing, KE Jin- Xing[3], ZHANG Wen-Jun[3]
(*Department of Pharmacology*; [3]*Department of Instrumental Center*, *The Third Military Medical College*, *Chongqing 400038*, *China*)

KEY WORDS antimalarials; nitroquinolines; *Plasmodium yoelii*; electron microscopy; cytochrome-c oxidase; liver

AIM: To study the effects of nitroquine acetate (NA) on the ultrastructures and cytochrome-c oxidase (CCO) of exoerythrocytic forms (EEF) of *Plasmodium yoelii*. **METHODS**: Rats were inoculated with sporozoites directly into the liver. After 48 h rats were killed. Rat liver thin sections were incubated in histochemical reaction medium, then examined by transmission electron microscopy. NA (2 mg·kg^{-1}) was fed to rats 3.5 h and 14 h before killing the rats. **RESULTS**: At 3.5 h, in the parasites there appeared swelling and proliferation of mitochondria, dilation of endoplasmic reticulum, and reduction of the electron density of parasites' nuclei. The structures of the parasites disintegrated to form many autophagocytes 14 h after exposure to NA. The reaction products of CCO still existed until 14 h after using NA. **CONCLUSION**: CCO was not the starting point of NA action. NA interferes with the structure and function of the cytoplasm and nucleus of malaria parasites and exerts its antimalarial effects in many aspects.

Exoerythrocytic forms (EEF) of malarial parasites result in relapses of malaria. Nitroquine {2, 4-diamino-6-[(3, 4-dichlorobenzyl) nitros-amino] quinazoline} acetate (NA, CI-679) is an antimalarial drug, which is effective on the erythrocytic, exoerythrocytic, and sporogonic stages of many malaria parasites including plasmodia in human. Its mechanism of action is related to inhibiting DNA and protein synthesis of *Plasmodium*[1,2]. Ultrastructural

[1] Project supported by the National Natural Science Foundation of China, No 39270792.
[2] Correspondce to Prof HU You-Mei. Phn 86-23-6875-2292. Fax 86-23-6532-0699.
Received 1997-11-27 Accepted 1998-03-04

observations of the erythrocytic *Plasmodium yoelii in mice 30 min after feeding with NA revealed that the parasites mitochondria were swelled, and matrix was cavitated*[3]. These suggested that interfering with mitochondrial respiration of *Plasmodium* might be one of NA antimalarial mechanisms. On the other hand, the ultrastructural changes of EEF of *Plasmodium* after using NA have not been reported. Therefore, the present study was carried out to observe the effects of NA on the ultrastructures and cytochrome-c oxidase (CCO) of EEF of *P yoelii*.

MATERIALS AND METHODS

P yoelii BY265 strain was supplied by Department of Parasitology, The Third Military Medical College.

Rats Wistar rats, $n = 20$, weighing 100 $\pm$ s 20 g, were provided by Experimental Animal Center, The Third Military Medical College (Certificate № 24301050).

Drugs and chemicals NA produced by Shanghai Institute of Pharmaceutical Industry was dissolved in Me_2SO. Cytochrome-c and catalase were the products of Sigma. 3, 3'-Diaminobenzidine tetrahydrochloride (DAB 4HCl) was purchased from Fluka. All other chemicals were AR.

Preparation of infected livers *Anopheles stephensi* mosquitoes (♀, $n = 200 - 300$) infected with *P yoelii* 15 d previously were used. Procedures of separating sporozoites were described earlier[4]. Just before inoculation the sporozoites suspension was warmed to room temperature (20 ℃). Rats were anesthetized, then inoculated with $(1 - 1.5) \times 10^7$ sporozoites directly into the median lobe of liver.

Enzyme histochemistry At 48 h after injection rat was anesthetized and the liver was perfused with 100 mL cold Hanks' solution then 50 mL 2.5 % glutaraldehyde in PB (pH 7.2) within 5 min. NA (2 $mg \cdot kg^{-1}$) was fed to rats 3.5 h and 14 h before killing them. Rats in control group were fed with Me_2SO. The perfusion-fixed liver was cut into slices (30 μm – 50 μm) in ice bath, subsequently incubated in CCO incubation medium[5] at 37 ℃ for 60 min.

Electron microscopy The specimens were washed in cold PB for 30 min, postfixed for 60 min in 1 % OsO_4 solution, dehydrated in graded acetone, and embedded in Epon 618. Ultrathin sections (70 nm) were examined under a GEM-2000 EX electron microscope. The enzyme reaction products were electron-dense granules.

RESULTS

Ultrastructures of schizonts of *P yoelii* EEF in control group After 48 h of the inoculation EEF of *P yoelii* became mature, appearing mainly as schizonts. The parasite enlarged the host hepatocytes and pushed the nuclei to one side. The host cell had vacuoles in cytoplasm, and the electron-density of the host cell was lower than that of the uninfected cell. The schizont had several nuclear (N) profiles scattering throughout its cytoplasm. Mitochondria (M) appeared as long slender or rounded double membraned organelles, containing filamentous material and spare cristae. There were electron-dense granules distributed in M evenly. The endoplasmic reticulum (ER) formed large aggregates mainly consisting of rough-surfaced elements (Fig 1).

Ultrastructures of EEF of *P yoelii* in NA-treated group At 3.5 h after exposure to NA, the number of M in schizonts increased. M swelled, and the electron density of their matrix decreased. But their doubled membranes were still intact. Electron-dense granules were also distributed in M. ER of the *Plasmodium* dilated, appearing disorderly. The nuclear electron-density decreased (Fig 1C).

After 14 h of NA treatment, the structure of parasites degenerated and was broken down. In the cytoplasm, many oval residual bodies (0.2 $\mu m \times 0.4$ μm – 5.4 $\mu m \times 8.1$ μm) and some dense cotton-like material were left (Fig 1D). Some bodies consisted of degenerated merozoite, whose inner structure had become unclear. M appeared in the cytoplasm, showing intact two-layered membrane and cristae. Electron-dense granules disappeared from M (Fig 1E). Some bodies included ER. Some bodies consisted of dense aggregates. Some bodies contained just some net (Fig 1F).

DISCUSSION

CCO appeared to be located in the exoerythrocytic M of *P berghei*[6]. By using

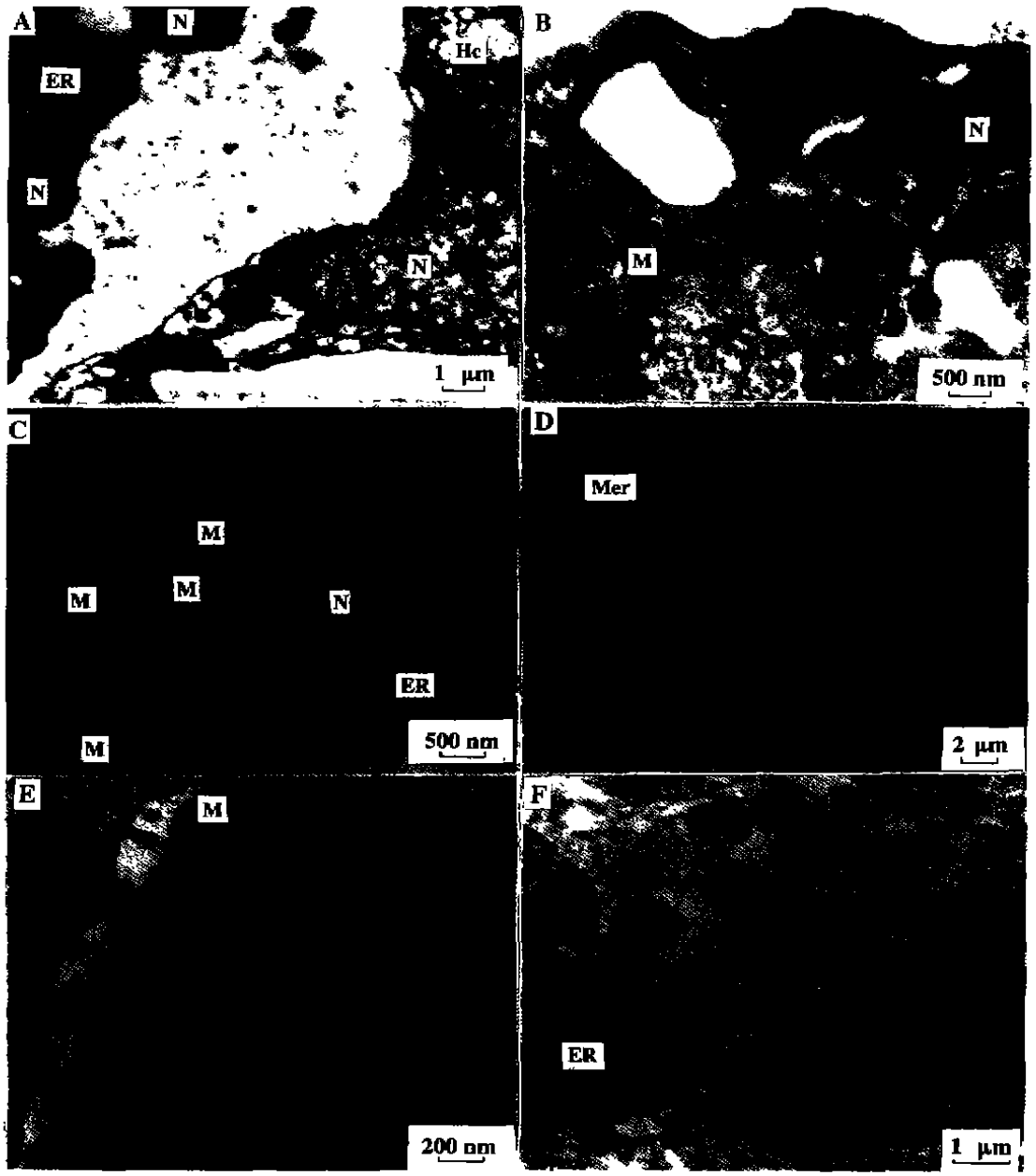

Fig 1. Rat liver cells and schizonts of *Plasmodium yoelii* exoerythrocytic forms. N = nuclei, ER = endoplasmic reticulum, Hc = hepatocytes, M = mitochondria, Mer = merozoite. A) Control, × 5000; B) control, × 15 000; C) 3.5 h after *po* NA 2 mg·kg^{-1}, × 15 000; D – F) 14 h after *po* NA 2 mg·kg^{-1} (D, × 2000; E, × 20 000; F, × 8000).

electron microscopic enzyme histochemistry, we found the CCO activity in the EEF of *P yoelii* and noted that the distribution of the enzyme reaction products followed closely the distribution of M. CCO is an important target of antimalarial drugs. In the present work, CCO reaction products still existed after 3.5 h of treatment with NA. This suggested that CCO was not the starting point of NA action. But in the 14 h specimens, parasites degenerated, and the parasite's cytoplasmic structures had been spoiled. That the electron-dense granules had disappeared from M may be the result of other NA actions such as inhibiting nucleic acid and protein synthesis of parasites[1-2], subsequently affecting M structures and functions.

In this paper, we studied the effects of NA on ultrastructures of EEF of *P yoelii* for the first time. Consistent with previous reports about ultrastructural changes of erythrocytic forms of *P yoelii* after using NA[3], our results showed that NA also changed many cellular apparatus of EEF of *P yoelii* including nuclei, ER, M, and so on. Dilatation of ER may be associated with inhibition of protein synthesis[2]. Decrease of parasites' nuclei electron-density may be related to the inhibition of nuclei acid synthesis[1]. Swelling of M affected M physiologic function. Therefore, increasing of M number might be a compensation reaction. Some authors suggested that it was a way to overcome drug's effects on parasites[7]. At 14 h after using NA, parasites degenerated. In order to clear the spoiled structures in cytoplasm, autophagocytoty happened in plasmodium. So residual bodies formed, and parasites disintegrated[3].

These findings indicated that NA interfered with the structure and function of the cytoplasm and nuclei of EEF of *P yoelii* and exerted its antimalarial effects in many aspects.

REFERENCES

1 Pang LH, Hu YM. Effect of nitroquine (Cl-679) *in vitro* on incorporation of [^{3}H]hypoxanthine into DNA and RNA of *Plasmodium yoelii*. Acta Pharmacol Sin 1988; 9: 349 – 52.

2 Zhou SW, Hu YM. Effect of nitroquine on the protein synthesis of intraerythrocytic stage of *Plasmodium yoelii in vitro*. Acta Pharmacol Sin 1991, 12: 372 – 5.

3 Zhou SW, Hu YM. The effects of nitroquine on the ultrastructures of intraerythrocytic stage of *Plasmodium yoelii* in mice. Acta Acad Med Mil Ter 1991; 13: 535 – 9.

4 Pacheco ND, Strome CPA, Mitchell F, Bawden MP, Beaudoin RL. Rapid, large-scale isolation of *Plasmodium berghei* sporozoites from infected mosquitoes. J Parasitol 1979; 65: 414 – 7.

5 Yang JS, editors. Cytochemical and cytobiological techniques. Beijing: Peking Union Med Coll and Beijing Med Univ Press; 1989. p 169 – 70.

6 Meis JFGM, Verhave JP, Wirtz P, Meuwissen JHETh. Histochemical observations on the exoerythrocytic malaria parasite *Plasmodium berghei* in rat liver. Histochemistry 1984; 81: 417 – 25.

7 Chen L, Qian YL, Li ZL, Zhang KH, Dai BQ, Liu ZF, *et al*. Effects of piperaquine on fine structure of erythrocytic stages of *P berghei* ANKA strain. Acta Pharmacol Sin 1986; 7: 351 – 3.

390 – 393

硝喹对大鼠肝红外期约氏疟原虫超微结构和细胞色素氧化酶的影响[1]

陈晓红、胡友梅[2]、廖雅琴、柯金星[3]、张文军[3]
(第三军医大学药理教研室, 重庆 400038, 中国)

R978

关键词 抗疟药; 硝基喹啉类; 约氏疟原虫; 电子显微镜检查; 细胞色素 c 氧化酶类; 肝

目的: 研究硝喹醋酸盐(NA)对红外期(EEF)约氏疟原虫超微结构和细胞色素氧化酶(CCO)的影响. **方法**: 大鼠肝脏接种子孢子, 48 h 后活杀大鼠. 将肝薄片进行组化孵育, 透射电镜观察. 分别在活杀大鼠前 3.5 h 和 14 h 给大鼠灌服一次 NA (2 mg·kg^{-1}). **结果**: NA 作用 3.5 h 引起原虫线粒体肿胀, 数量增多, 粗面内质网扩张, 胞核电子密度降低; NA 作用 14 h 原虫结构瓦解, 形成大量自噬泡. 原虫线粒体内 CCO 反应产物在给药后 3.5 h 仍然存在, 给药后 14 h 消失. **结论**: CCO 不是 NA 的起始作用点. NA 干扰了 EEF 原虫胞核及胞浆多个细胞器的结构和功能, 从多个环节发挥其抗疟效应.

感 谢

中国科学院成都地奥制药公司

资助《中国药理学报》