Ultrastructural study on effect of primaquine on sporogonic stage of Plasmodium yoelii nigeriensis

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Abstract Ultrastructural changes of oocysts and sporozoites of Plasmodium yoelii nigeriensis was observed. Anopheles stephensi were allowed to obtain blood meal from the mice which had been administered with primaquine diprophosphate at different doses and times. Mosquitoes were directed and prepared 6–13 d following infection.

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Electron microscopy showed that the development and morphology of a number of oocysts and sporozoites in infected mosquitoes after treatment became abnormal. The cytoplasm of the oocysts were partially or totally dissolved and formed vacuoles. The walls of oocysts were thicken. The nuclei and organelles of oocysts and sporozoites were destroyed or damaged. The extent of damage of the oocysts and sporozoites related to the dose of primaquine and the time after the drug administration.
Key words: primase; sporogony; oocyst; sporoblast; Plasmodium yoelii nigeriensis; electron microscopy.

Material and methods
约氏疟原虫 尼日利亚亚种 (Plasmodium yoelii nigeriensis) 引自英国伦敦热带病研究所 Peters 实验室，由实验室著名学者, 体重 20 ±
SD 1.8g，由完善实验用的动物饲养箱提供，疟原虫氏 (Anopheles stephensi) 为英国
雄, 饲养方法见文献 14)。

结果

实验小鼠给药及感染疟原虫 染色疟原虫率在 20—30%，每 100 个 WBC 现 93% 包涵体数在
30 个以上的标本 4:0 供实验 3—d 3 的 300
只小鼠按疟原虫血吸 2h, 吸血前驱虫 18h, 吸血前饲养 10% 葡萄糖, 为对照组, 每收
感染小鼠 100 只, 分别给 0.28, 1.25, 1.95 和 2.5 mg/kg 的伯氨喹液, 并分别
给予 2, 4, 8h 聚氯各取 4 尾小鼠一例对
比对疟原虫尾数等的异常的标本观察, 其间, 上述
g 均为 25% C 和相对温度 75—85% 的笼内
中保持。

伯氨喹液制备与观察 疟原虫感染后 0—4—6
解翼并以 0.2 mol/L, pH 7.0, 7.2 的二
甲基氯化铵缓冲液配制的 2% 二氯二胺为解翼液,
以氯仿处理中冷却 4 (4) 2—3h 后用上述
解翼液冲淋清洗, 直至无氯皿液为为止, 保
存于 4°C, 次日到 1 wk 内进行鉴定, 以光
线, 镜检, 切片, 用 H—600 型透射电镜进行观察。

Results

正常疟原虫的发育形态 观察 8 批不同龄
期 (7—11 d 龄) 正常发育的疟原虫, 在分化初期
由胞食胞自首先分裂, 再内含多个变分裂, 同
时膜壁内膜下层出现大小不等的空泡 (Plate 1—
A), 随后空泡逐渐增大并互相融合, 形成裂隙, 并
将胞质内膜分割 (Plate 1—B, C) 后将细胞质分
隔成大小不等的内泡, 每一内泡再分裂为多个胞
子细胞, 形态多样, 一个胞子内含有的 1,2
或多个成胞子细胞, 再多者成胞子细胞在多个胞
子细胞, 进行成胞子阶段 (Plate 1—D, E, F),
子胞子外被覆膜, 膜下可见长管状微管, 侧
端有一对长而微细的管体微管 (Plate 2—L)
伯氨喹作用下疟原虫及子胞子细胞超微结构变化
1 毫形 实验小鼠给 0.28mg/kg 伯氨喹后, 在不
同时间感染疟原虫的标本观察显示, 疟
虫 g 0.28mg/kg 伯氨喹后 2h 染色疟原虫的胞
胞外膜显著变大, 大部分发育成正常的胞子细胞, 一些胞
子细胞发育不良, 在囊泡膜中出现大小不等空泡, 而且胞体膜显著, 呈退化性变化 (Plate
1—O), 小鼠 g 0.28mg/kg 2h 胞子细胞染
色或胞子细胞, 大部分胞子细胞发育不良。此外, 4.8h 时疟原虫的胞子细胞形成
胞质溢出, 胞质溢出使胞质空泡, 子胞子
膜的胞质也出现空泡 (Plate 1—H), 小鼠 0.28mg/kg
Discussion

The experimental results indicate that the 2,4-dinitrophenylhydrazine (DNPH) and 2,4-dinitrophenol (2,4-DNP) are effective inhibitors of the activity of the key enzymes of the respiratory chain, leading to a decrease in the intensity of oxygen consumption and an increase in the concentration of end products of energy metabolism. These findings suggest that the inhibition of the respiratory chain enzymes by these chemicals is a potential mechanism for the toxicity of 2,4-DNP and DNPH.

References

Fig 1. A-D. Normal development of oocyst. A: Early differentiated oocyst showing the divided nuclei (N) and many vacuoles (V) appeared beneath the oocyst capsule, 7 d, ×1000. B: The vacuoles (V) penetrated ever deeper into cytoplasm of oocyst, 7 d, ×5000. C: The cytoplasm of oocyst subdivided and formed sporoblast (S), 7 d, ×2500. D: Early stage of sporoblast, buds (SB) emerged from the surface of the sporoblast body (B), 7 d, ×1500.

E-F: Late stage of sporoblast. E: The sporoblast body (B) was elongated shaped, 7 d, ×1500. F: The sporoblast body (B) was round shaped, 7 d, ×1500.

G-H. The change of oocyst in mosquito midgut infected at different time after mouse in different doses of primaquine. G: 0.25 mg/kg, 2 h, showing one normal oocyst (arrow) and two vacuolated oocysts, which appeared smaller, 7 d, ×2000. H: 1.25 mg/kg 8 h, one sporoblast in the oocyst, the sporoblast body and sporocyste buds contained many vacuoles, 7 d, ×1500. (See p 283)
Fig 2. I-N. The change of oocysts. I) 1.95 mg/kg, 2 h, part of the cytoplasm was dissolved while some were massing; vacuoles appeared in oocyst, the wall of oocyst was thickened. II d, <10000. J) 1.95 mg/kg, 8 h, the cytoplasm of oocyst appeared to contain many vacuoles, the wall of oocyst was thickened. J) 100000, K) 2.1 mg/kg, 2 h, the cytoplasm of oocyst filled with vacuoles, 11 d, 40000. L) Normal sporozoite showing the rosettes (R) and many granular micronemes (M), 11 d, <50000. M-N: 1.25 mg/kg, 2 h, showing the abnormal morphology of sporozoites, in which appeared vacuoles, the rosettes (R) and micronemes were injured, the rosettes clustered in lumps (arrow). (See p 266)