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# 血传、蚊传保种对约氏疟原虫毒力的影响及对其机制的探讨

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**提 要** 本实验以同一来源的约氏疟原虫经血传和蚊传两种方式在小鼠及斯氏按蚊体内传代，观察了其毒力的变化，并对毒力变化的机制进行了探讨。蚊传保种的疟原虫感染小鼠死亡率为13.89%～22.73%，而血传保种疟原虫感染的小鼠10天内全部死亡。长期血传的原虫经一代蚊传感染鼠死亡率降为0%～15%。长期蚊传的原虫连续3代血传感染鼠死亡率达100%。长期血传的疟原虫改嗜成熟红细胞，引起脑部病变。切除脾脏、破坏胸腺不能阻止脑病变的发生。脾脏对鼠疟原虫毒力株无选择作用。

**关键词** 约氏疟原虫；毒力；血传；蚊传；脑血管栓塞

原始的约氏疟原虫感染小鼠为温和型，主要偏嗜网织红细胞。近年来发现国内许多实验室保种的约氏疟原虫由温和型向致死型转变。低温保存及体外培养保种疟原虫，虽有许多优点，但保存的疟原虫毒力等一些生物学特性可发生改变，有的甚至不可逆[1]。因此保持鼠疟原虫毒力等生物特性稳定，是理想鼠疟模型建立必须解决的问题。

## 1 材料与方法

### 1.1 实验材料

小鼠昆明株用于常规保种，BALB/C株感染后置于24±1℃的室内用于测定毒力。蚊媒为斯氏按蚊(A.stephensi)Hor株。约氏疟原虫（P.yoelii）BY265株由首都医学院引进。血传保种的约氏疟原虫以Yn记录，蚊传保种的以Sn记录（n为所传代数）。

### 1.2 实验方法

感染小鼠死亡率为主要毒力指标，并测定转阴时间、转阴率、死亡时间、原虫率达峰值时间及原虫率峰值。

1.2.1 长期血传、蚊传：在$Y_7$、$Y_{36}$、$Y_{55}$和$S_6$、$S_{12}$、$S_{16}$分别测定上述毒力指标。

1.2.2 血传后改蚊传，蚊传后改血传：$Y_{48}$、$Y_{55}$、$Y_{56}$分别蚊传一代，$S_3$、$S_9$、$S_{16}$、$S_{20}$分别血传一代，$S_{16}$连续血传3代，测定各代毒力指标。

1.2.3 对红细胞偏嗜性：作$Y_{55}$、$S_{16}Y_1$感染小鼠红细胞煌焦油蓝染色，查网织红细胞率及网环率。

$$\text{网环率} = \frac{\text{网织红细胞中环状体总数}}{\text{网织和成熟红细胞中环状体总数}} \times 100\%$$

1.2.4. 切脾、抗胸腺血清处理：$Y_{36}$经切脾小鼠血传九代再传回正常小鼠，$Y_{36}$作对照，测定毒力指标。30只小鼠分3组：兔抗小鼠胸腺血清（ATS）处理组、正常兔血清处理组、非处理组。前两组从感染血传保种的疟原虫当天起，每天腹腔注射ATS或正常兔血清3ml至小鼠死亡。

1.2.5 脑组织及其它器官检查：取感染致死的小鼠大脑顶部少许脑膜和皮质作脑抹片，查脑血管栓塞，并作脑、肾、肝常规组织切片。

## 2 实验结果

### 2.1 长期血传、蚊传的毒力变化

表1可见血传保种的约氏疟原虫为致死型感染，毒力显著增加。$S_6$、$S_{12}$、$S_{16}$感染小鼠死亡率为22.73%、13.89%、15.00%，大部份小鼠均在2周～3周转阴，蚊传保种的疟原虫保持了原始毒力特征，为温和型感染。

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表1 长期血传P.yoelii毒力

Tab. 1 The virulence of P.yoelii transmitted by successive blood passage

| Generation of P.y | No. of mice | Peak of parasite rate (days) $\bar{x}\pm s_{\bar{x}}$ | Peak of parasite rate (%) $\bar{x}\pm s_{\bar{x}}$ | Time of death (days) $\pm s_{\bar{x}}$ | Mortality (%) |
|---|---|---|---|---|---|
| $Y_7$ | 20 | 7.25±0.56 | 55.20±1.92 | 9.11±0.70 | 100 |
| $Y_{36}$ | 18 | 6.44±0.43 | 58.11±3.41 | 8.00±0.57 | 100 |
| $Y_{55}$ | 28 | 5.05±0.18** | 54.29±2.00 | 7.05±0.34Δ | 100 |

Compared with $Y_7$ group; **P<0.01; ΔP<0.05

2.2 血传后改蚊传、蚊传后改血传的毒力变化

$Y_{48}$、$Y_{56}$、$Y_{56}$各蚊传一代，感染小鼠死亡率为0%～15%，小鼠多在两周左右转阴，说明长期血传后只经一代蚊传疟原虫毒力即可恢复。表2说明长期蚊传后血传一代的疟原虫毒力下降。表3可见连续血3代温和型感染即可转变为致死型。

表2 P.yoelii长期蚊传后血传一代毒力

Tab. 2 The virulence of P.yoelii by one blood passage after successive transmission through mosquitoes

| Generation of P.y | No.of mice | Peak of Parasite rate(days) $\bar{x}\pm s_{\bar{x}}$ | Peak of Parasite rate (%) $\bar{x}\pm s_{\bar{x}}$ | Time of clearance (days) $\bar{x}\pm s_{\bar{x}}$ | clearance rate (%) | Time of death (dayt) $x\pm S_{\bar{x}}$ | Mortality (%) |
|---|---|---|---|---|---|---|---|
| $S_3Y_1$ | 19 | 10.84±0.74 | 43.34±2.24 | — | 0 | 10.95±0.78 | 100 |
| $S_8Y_1$ | 18 | 9.00±0.53 | 41.53±2.32 | — | 0 | 14.56±0.87 | 100 |
| $S_{16}Y_1$ | 25 | 8.29±0.30 | 25.10±1.92** | 15.20±0.18 | 80 | 15.00±0.97 | 20 |
| $S_{18}Y_1$ | 20 | 10.96±0.68 | 21.03±1.79 | 17.43±0.31 | 70 | 16.00±0.55 | 30 |
| $S_{20}Y_1$ | 22 | 10.77±0.70 | 24.55±1.56 | 18.25±0.23 | 59.10 | 14.00±0.73 | 40.90 |

Compared with $S_3Y_1$ group; **P<0.01

表3 P.yoelii长期蚊传后连续血传3代的毒力

Tab. 3 The virulenece of P.yoelii by 3 successively blood passage after successive transmission through mosquitoes

| Generation ofP. y | No.of mice | Peak of Parasite sate (days) $\bar{x}\pm s_{\bar{x}}$ | Peak of Parasite rate (%) $x\pm s_{\bar{x}}$ | Time of clearance (days) $\bar{x}\pm s_{\bar{x}}$ | Clearance rate (%) | Time of death (days) | Mortality (%) |
|---|---|---|---|---|---|---|---|
| $S_{18}Y_1$ | 20 | 10.95±0.86 | 21.03±1.79 | 17.43±0.31 | 70.00 | 16.00±0.55 | 30.00 |
| $S_{18}Y_2$ | 21 | 11.57±0.41 | 25.24±0.91 | 20.25±0.30ΔΔ | 38.10 | 16.00±0.47 | 61.90 |
| $S_{18}Y_3$ | 20 | 10.26±0.61 | 41.35±2.64** | — | 0 | 13.16±0.78 | 100 |

Compared with $S_{18}Y_1$; **P<0.01; ΔΔ, P<0.01

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### 2.3 毒力改变的机制探讨

2.3.1 约氏疟原虫对红细胞的偏嗜性：$Y_{55}$原虫率达峰值的时间与网环率最低值时间均为第5天左右，原虫率达峰值时的网环率为4.82±0.59%。$S_{16}Y_1$原虫率达峰值的时间为第9天，而网环率最低值时间是第5天，原虫率达峰值时的网环率达98.04±0.37%，说明血传致死型约氏疟原虫改嗜成熟红细胞，蚊传温和型约氏疟原虫偏嗜网织红细胞，保留了原始特征。

2.3.2 脾脏的影响：$Y_{26}$经切脾小鼠血传后感染的20只正常小鼠8天左右均死亡，与$Y_{36}$毒力指标无差异。

2.3.3 致病力检查及病因探讨：血传感染的小鼠频死时有不同程度的中枢神经系统症状，如运动失调、抽搐、强直等，死亡后脑实质有出血斑（图1），脑血管被感染红细胞阻塞（图2）。215只血传感染致死

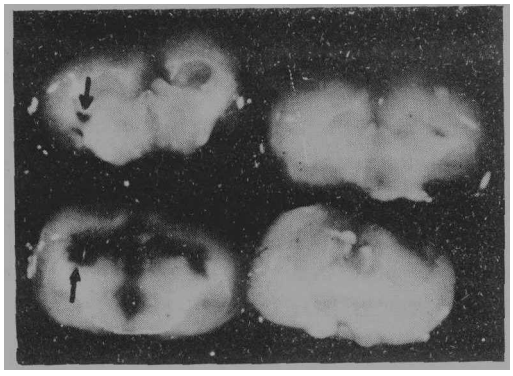


图1 血传P.yoelii感染致死小鼠大脑冠状剖面。示出血斑

Figl.1 The coronal section of the brain of mouse died of P. yoelii blood transmitted infection. Arrows indicate the hemorrhagic Patch.

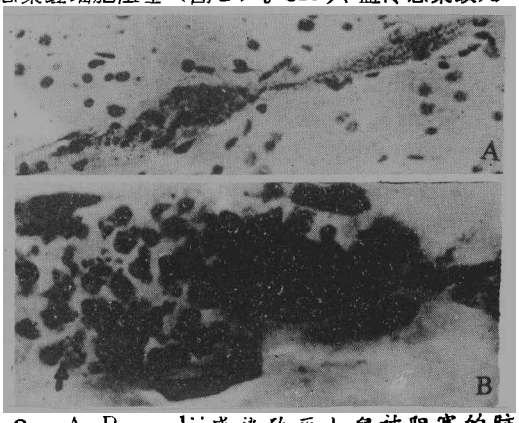



图2 A.P.yoelii感染致死小鼠被阻塞的脑血管呈气囊状。B.大量感染红细胞在脑血管内，示裂殖体。脑抹片，A×200；B×1 000

Fig.2 A. Balloon formation of a cerebral vessel caused by the blocking of the vessel by infected erythrocytes in mouse died of P. yoelii blood transmitted infection. B. Large mumbers of infected erythrocytes in occlusion of a brain vessel. Arrow indication the schizont. Brain smera, A×200, B×1000

的BALB/C小鼠中60.0%有脑血管栓塞，肾小血管及肝窦中有红细胞凝集。蚊传感染死亡的小鼠及活杀血传感染的小鼠均未发现脑出血及脑血管栓塞。切脾小鼠脑血管栓塞率与正常小鼠无差异。ATS处理组脑血管栓塞率为66.7%与正常兔血清处理组和非处理组间均无差异，表明去除脾脏及破坏胸腺不能阻止脑病变的发生。

**3 讨 论** 长期血传的约氏疟原虫毒力显著增加，改嗜成熟红细胞，引起脑型疟，这些特性与Yoelii等[1]经冷冻分离出的致死型约氏疟原虫相似，但血传的疟原虫经蚊传后毒力可逆转，不具遗传稳定性，推测其毒力变化机制与冷冻的致死型不同，未发生基因突变。血传的疟原虫感染的小鼠在频死时有中枢神经系统症状，经蚊传不能再诱发此症状及脑型疟，而冷冻的突变型约氏疟原虫蚊传仍可诱发脑型疟，但未见感染小鼠出现神经系统症状的报告。

长期血传的约氏疟原虫大量侵入成熟红细胞，使红细胞迅速消耗而不能补偿，因此贫血和脑型疟是引起血传感染小鼠死亡的重要原因。

有报告抗胸腺血清[2]、抗$L_3T_4$+T淋巴细胞单克隆抗体[3]、抗肿瘤坏死因子[4]及切除脾脏[5]等均可阻止伯氏疟原虫脑型疟和神经系统症状的发生。本实验提示T淋巴细胞可能对

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约氏疟原虫脑型疟的发生不起主导作用。

约氏疟原虫与恶性疟原虫脑型疟病理特征相似，最近有报道恶性疟感染的红细胞膜上有Thrombospondin受体或抗原，感染红细胞通过此受体与内皮细胞和未感染红细胞粘连，堵塞脑血管[6][7]。两种疟原虫致病机理是否相同值得探讨。夏氏疟原虫蚊传后能恢复原始抗原特性[8]，Jayawardena认为蚊体内子孢子在发育过程中鼠疟原虫抗原可"逆转"回原始型。本文提示子孢子发育过程中变化的毒力也可"逆转"。有报道经切脾猴血传的疟原虫毒力降低，鼠疟原虫有无此现象无人提及。本文表明脾脏对约氏疟原虫毒力株无选择作用，因此可排除脾脏对血传后毒力变化的影响。

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# A Study of Effects of Blood Passage and Mosquito Transmission on the Virulence of Plasmodium yoelii and the Mechanism

Qi Jie, Zhang Jun, Ren Huixian, et al (Department of Parasitology, Third Military Medical College, Chongqing 630038)

**Abstract** A strain of Plasmodium yoelii was propagated with serial blood passages through mice and with serial transmissions through mosquitoes to investigate the effects of the 2 kinds of propagation on the virulence of the malaria parasites. The experiments revealed that the mortality rate of the mice infected with P.yoelii after successive transmissions through mosquitoes was 13.89% to 22.73%, while those infected with P.yoelii after successive blood passages usually all died within 10 days. When mice were infected with P. yoelii of one generation mosquito transmission, after serial blood passages their

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mortality rate was 0 % to 15%. when mice were infected with P.yoelii of the third generation blood passages after successive transmissions through mosquitoes, then theirmortalitv rate was 100%.

The P.yoelii after serial blood passages mainly invaded the mature erythrocytes and could cause cerebral symptoms, which could not be prevented by splenectomy or antithymocyte serum therapy. The results of serial passages of the parasite torough splenectomized mice indicate that the spleen can not select the virulent strain of P.yoelii.

**Key words** Plasmodium yoelii; virulence; blood passage; mosquito transmission; cerebral ebolism.

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# Pathological Study of Transitional Mucosa Adjacent to Colorectal Carcinoma and Its Clinical Significance

Guo Shanchun, Liu Fengxuan, Cheng Yisheng, et al. (Department of Clinical Pathology, Southwest Hospital, Third Military Medical College, Chongqing 630038)

**Abstract** The specimens of 205 cases of colorectal carcinoma and its surrounding transitional mucosa (TM) and dysplastic tissue were studied with optical and electron microscopy, mucohistochemistry, immunohistochemistry, and microspectrophotometry. It was found that the distribution of TM was dependent on the Dukes' classification and histological types of colorectal carcinoma. The expression of the related antigens of monoclonal antibody $MC_3$, $MC_6$, CL-2, CL-4 in normal colorectal mucosa was negative, but the positive rate increased gradually in the order of TM, mild dysplastic tissue, moderate dysplastic tissue, severe dysplastic tissue, and cancer tissue. The amount of nuclear DNA of TM was intermediate between that of normal mucosa and that of dysplastic tissue, but much smaller than that of cancer. The findings suggest that the abnormal amount of DNA in TM surrounding colorectal carcinoma is responsible for the pathological changes in TM. The clinical significance of TM was also discussed.

**Key words** intestinal neoplasms; intestinal mucosa/PA; DNA; neoplasm; dysplasia; ultrastructure; immunohistochemistry

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