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苦参合剂对微小隐孢子虫感染小鼠肠黏膜的保护作用

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Protective Effect of Radix Sophorae Flavescentis Mixture on Intestinal Mucosa in Mice Infected with Cryptosporidium parvum

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摘要 目的 探讨苦参合剂对微小隐孢子虫（Cryptosporidium parvum）感染小鼠肠黏膜的保护作用。 方法 30只雄性BALB/c小鼠随机均分为对照组、感染组和苦参合剂治疗组。后2组小鼠经口灌服微小隐孢子虫卵囊1×105个，并在饮水中加入地塞米松（5 ?滋g/ml）和硫酸庆大霉素（40 ?滋g/ml）。治疗组各鼠于感染第8天起灌服苦参合剂0.2 ml，每周2次（间隔3 d），连续3周。感染组和治疗组小鼠自给药后每2天粪检微小隐孢子虫卵囊并计数；3组小鼠于感染后28 d剖杀，取各鼠空肠制备石蜡包埋切片，免疫组化法检测小鼠肠黏膜CD3+、CD4+和CD8+T细胞及IgA浆细胞数量的变化，另取感染组和治疗组各鼠的空肠进行透射电镜观察。 结果 苦参合剂治疗组与感染组相比，微小隐孢子虫卵囊排出量明显减少，持续排出卵囊的时间明显缩短。免疫组化检测结果显示，小鼠CD3+、CD4+ T细胞百分率和CD4+/CD8+比值治疗组（62.4%±1.4%、37.5%±3.1%和1.5±0.3）较感染组（49.7%±2.4%、25.7%±2.2%和1.1±0.3）明显升高（P<0.01）；感染组较对照组（66.5%±1.9%、40.1%±1.8%和1.5±0.2）明显降低（P<0.01）；各组间CD8+T细胞百分率差异无统计学意义（P>0.05）。治疗组小鼠IgA 浆细胞数量（52.7±3.5）明显高于对照组（8.3±2.3）和感染组（33.7±2.6）（P<0.01）。电镜观察结果显示，治疗组小鼠经苦参合剂治疗后，空肠超微结构明显改善，偶见结构已破坏的虫体，可见大量的溶酶体，线粒体结构正常，微绒毛已基本修复；感染组小鼠空肠可见结构完整的微小隐孢子虫卵囊和大量嵴断裂的线粒体，卵囊周围的微绒毛严重脱落，呈火山口状，卵囊壁与上皮细胞膜发生融合。 结论 苦参合剂具有抑杀微小隐孢子虫的作用，感染小鼠受治后，受损肠黏膜得到修复。

关键词： 微小隐孢子虫 BALB/c小鼠 苦参合剂 肠黏膜

Abstract： Objective To investigate the protective effect of radix sophorae flavescentis（RSF） mixture on intestinal mucosa in mice infected with Cryptosporidium parvum. Methods Thirty BALB/c male mice were randomly divided into control group, infection group and RSF mixture treatment group. Mice of the posterior two groups were inoculated intragastrically with 1×105 C. parvum oocysts, immunosuppressed with dexamethasone（5 ug/ml） and gentamycin sulfate（40 ug/ml） in drinking water. At the 8th day post-infection, mice in RSF mixture treatment group were treated with 0.2 ml dose of RSF mixture twice a week（three-day intervals）for three weeks. The mice in infection group and RSF mixture treatment group were monitored for oocyst shedding in fecal pellets every two days after treatment. At 28 days after infection, experimental mice were sacrificed, jejunal tissue was removed for preparation of paraffin-embedded sections. The changes of CD3+, CD4+, CD8+ T lymphocytes and IgA plasmocytes in intestinal mucosa were determined by immunohistochemistry. In addition, jejunums of infected mice and treated mice were collected, and ultrastructural changes were observed under electron microscopy. Results Compared with infection group, the level of oocyst shedding was obviously lower and the time of the oocyst discharging was significantly shorter in RSF mixture treatment group. The proportion of CD3+, CD4+ T lymphocyte and CD4+/CD8+ T cell ratio in infection group（49.7%±2.4%, 25.7%±2.2%, 1.1±0.3）were significantly lower than that of treatment group（62.4%±1.4%, 37.5%±3.1%, 1.5±0.3）and control group（66.5%±1.9%, 40.1%±1.8%, 1.5±0.2）（P<0.01）. CD8+ T lymphocytes showed no significant difference in each group（P>0.05）. The number of IgA plasmocytes in treatment group（52.7±3.5） was significantly higher than that of control group （8.3±2.3） and infection group （33.7±2.6）（P<0.01）. After administration for three weeks, the damaged C. parvum parasites were seldom seen in mouse jejunum, and lysosomes appeared in large number, RSF mixture treatment improved mitochondrial structure and repaired microvilli. In infection group, mitochondria ridges were significantly broken and microvilli surrounding C. parvum oocysts were shed, resulting in the appearance of crater-like lesions on the surface, the oocyst wall and host cell membrane fused together. Conclusion RSF mixture is effective against Cryptosporidium parvum. The damage of intestinal mucosa in infected mice can be repaired after treatment.

Keywords： Cryptosporidium parvum BALB/c mice Radix sophorae flavescentis mixture Intestinal mucosa

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