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刚地弓形虫感染对鼠胚胎神经干细胞的影响

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Effect of *Toxoplasma gondii* Infection on the Embryonic Neural Stem Cells in Rats

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摘要 目的 探讨大鼠孕早期感染刚地弓形虫对鼠胚胎神经干细胞增殖、分化和迁移的影响。 方法 12只SD孕鼠随机分为对照组和感染组，每组6只。感染组孕鼠于妊娠第1天（E1天）腹腔注射弓形虫RH株速殖子1×10^5/只，对照组注射等体积生理盐水。于E5天尾静脉取血，吉氏染色查找虫体。分别于E9、E10和E11天处死孕鼠，每组每次2只，逆转录PCR（RT-PCR）检测羊水中弓形虫B1基因，确认胚胎鼠是否感染弓形虫。体外原代培养鼠胚胎神经干细胞，噻唑蓝（MTT）法检测2组细胞增殖水平。分化培养神经干细胞，免疫荧光法检测巢蛋白（nestin）、微管相关蛋白2（MAP2）和胶质纤维酸性蛋白（GFAP）的表达，计算对照组和感染组分化为神经元和星形胶质细胞的百分率。取E9、E10和E11天的2组胚胎鼠神经组织作冰冻切片，免疫荧光法检测神经细胞黏附分子（NCAM）在胚胎不同发育时期神经组织中的表达情况。 结果 血涂片和RT-PCR结果证实，感染组孕鼠和胚胎鼠均感染弓形虫。体外培养神经干细胞，镜下见细胞形态符合神经干细胞特点，神经干细胞标志物nestin蛋白染色呈阳性。MTT结果显示，感染组细胞传代后增殖水平均低于对照组，其中传代后第3和第4天两组之间差异有统计学意义（$P<0.05$）。MAP2和GFAP荧光染色结果显示，对照组和感染组神经元分化百分率分别为15.15%（55/363）和8.73%（31/355），两者间差异有统计学意义（$P<0.05$）。对照组和感染组星形胶质细胞分化百分率分别为53.35%（199/374）和67.48%（249/369），两者间差异无统计学意义（$P>0.05$）。E9、E10和E11天2组胚胎神经组织均检测到NCAM蛋白表达，荧光随时间逐渐增强，对照组表达水平均显著高于感染组（$P<0.01$）。 结论 大鼠孕早期感染刚地弓形虫可抑制神经干细胞的增殖、分化和迁移。

关键词： 刚地弓形虫 神经干细胞 增殖 分化 迁移

Abstract： Objective To investigate the effect of *Toxoplasma gondii* infection on the proliferation， differentiation and migration of the embryonic neural stem cells （NSCs） in early pregnancy of rat. Methods Twelve pregnant Sprague-Dawley rats were randomly divided into control and infection groups. Rats in the infection group were each inoculated intraperitioneally with 1×10^5 *T. gondii* RH strain tachyzoites at day 1 （E1 day）. Same amount of physiological saline was intraperitioneally injected for rats in control group. At E5 day， blood samples were taken from caudal vein and Giemsa staining of blood cells was performed to find *T. gondii*. At E9， E10 and E11 day， two rats in each group per time point were sacrificed and reverse transcription PCR （RT-PCR） was performed to detect B1 gene expression of *T. gondii* in amniotic fluid to confirm *T. gondii* infection. NSCs were cultured *in vitro*. The proliferation level was detected by methyl thiazolyl tetrazolium （MTT） assay. After differentiation culture of NSCs， the immunofluorescence assay was conducted to detect the expression of nestin， microtubule-associated protein 2 （MAP2） and glial fibrillary acidic protein （GFAP） to calculate the ratio of NSCs which differentiated to neurons and astrocytes. The embryonic nerve tissues at E9， E10 and E11 day in each group were taken to make frozen sections. The immunofluorescence assay was carried out to detect the expression of neuronal cell adhesion molecule （NCAM） in the nerve tissues at different developmental stages. Results Both the results of blood smears and RT-PCR confirmed that the pregnant rats and embryos were all infected by *T. gondii* in infection group. The morphology of the cultured NSCs under microscope was consistent with the characteristics of the normal NSCs. In addition， the NSC biomarker nestin protein was stained positive. The MTT assay showed that the proliferation level was lower in infection group than that of the control， and statistical differences were found between the two groups at day 3 and 4 after passages （$P<0.05$）. The immunofluorescence staining of MAP2 and GFAP showed that the percentage of neuron differentiation was 15.15% （55/363） in control group and 8.73%（31/355） in infection group， respectively, with a statistical difference （$P<0.05$）, and the percentage of astrocyte differentiation was 53.35%（199/374） and 67.48% （249/369）, respectively （$P>0.05$）. In both groups， NCAM protein was found expressed at E9， E10 and E11 day in embryo nerve tissues. The fluorescence became stronger with time. The expression level in control group was significantly higher than that in infection group （$P<0.01$）. Conclusion *T. gondii* infection at early gestation may inhibit the proliferation， differentiation and migration of neural stem cells in rats.

Keywords： *Toxoplasma gondii* Neural stem cell Proliferation Differentiation Migration

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