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论著

基于胚胎干细胞实验模型评价黄芩苷的胚胎毒性

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摘要 目的 应用胚胎干细胞实验模型体系评价黄芩苷的胚胎毒性。方法 分别将胚胎干细胞D3和胚胎成纤维细胞(BALB/c 3T3)与黄芩苷20, 40, 60, 80和100 mg·L^{-1}共培养, MTT法检测细胞活性, 分别计算黄芩苷对胚胎干细胞D3和3T3细胞增殖半数抑制浓度IC_{50}(D3)和IC_{50}(3T3)。利用悬滴-悬浮-贴壁方法, 体外培养胚胎干细胞向心肌细胞分化, 实时定量PCR方法检测心肌细胞特异表达肌球蛋白重链(β-MHC)基因的表达, 计算胚胎干细胞D3定向分化半数抑制浓度, 即ID_{50}(D3)。利用胚胎毒性统计公式, 预测黄芩苷的胚胎毒性。结果 不同浓度黄芩苷作用10 d后, 胚胎干细胞D3和3T3细胞存活能力随着黄芩苷浓度增加缓慢下降, 黄芩苷对胚胎干细胞D3和3T3细胞增殖均有一定程度的抑制作用, 其IC_{50}(D3)和IC_{50}(3T3)分别为135.9和63.3 mg·L^{-1}。体外胚胎干细胞经悬滴-悬浮-贴壁培养可分化为能够表达β-MHC基因的心肌样细胞, 黄芩苷2, 5, 10, 20和40 mg·L^{-1}对胚胎干细胞定向分化为心肌细胞的抑制率分别为29.5%, 46.8%, 59.6%, 61.7%和69.0%, 黄芩苷对胚胎干细胞分化有一定程度的抑制作用, 其体外心肌细胞定向分化的ID_{50}(D3)为7.25 mg·L^{-1}。根据胚胎毒性计算公式, 计算得黄芩苷具有弱胚胎毒性。结论 黄芩苷具有弱胚胎毒性。

关键词 胚胎干细胞 黄芩苷 毒性作用
分类号 R99 R285.1

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Evaluation of embryotoxicity of baicalin based on embryonic stem cell test system

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Abstract

OBJECTIVE To assess embryotoxicity of baicalin using embryonic stem (ES) cell test *in vitro*. **METHODS** ES D3 cells and BALB/c 3T3 cells were cultured respectively with baicalin 20, 40, 60, 80 and 100 mg·L^{-1}. Cell morphology was observed with a phase contrast microscope and absorbance of the resulting colored solution at 570 nm was measured by microplate reader. According to the concentration-effect curve, 50% inhibition of cell growth (IC_{50}) and viability were obtained in ES cell line D3[IC_{50}(D3)] and in 3T3 cells [IC_{50}(3T3)]. ES cells were cultured in baicalin with using hanging drop-suspension-attachment method, then cardiac myoblasts specific genes myosin heavy chain (β-MHC) in differentiation of embryonic stem cell were detected by real time Q-PCR, the growth inhibitory rate was calculated with quantitative analysis, according to the concentration-effect curve, 50% inhibition of ES cells differentiation into cardiac myoblasts [ID_{50}(D3)] obtained. Baicalin embryotoxicity potential was predicted using statistics formula. **RESULTS** ES D3 cells and BALB/c 3T3 cells viability decreased slowly with the increase in baicalin concentration after they were cultured in different concentration of baicalin for ten days, which showed that baicalin had a certain degree of

inhibition on ES D3 cell and BALB/c 3T3 cell proliferation. The half-maximal proliferation-inhibition concentration(IC_{50}) of baicalin on ES cell line D3[[IC_{50}(D3)] and 3T3 cells [[IC_{50}(3T3)] was 135.9 mg • L^{-1} and 63.34 mg • L^{-1}. ES cells *in vitro* by hanging drop-suspension-adherent culture could differentiate to expression of β-MHC gene in myocardial cells. With baicalin 2, 5, 10, 20 and 40 mg • L^{-1}, the ability of ES cells differentiate into myocardial cells gradually decreased, the inhibitory rate was 29.5%, 46.8%, 59.6%, 61.7% and 69.0%, respectively, and this indicated that baicalin had a certain