实验研究

地塞米松与ATP联用在体外诱导细粒棘球绦虫原头节细胞凋亡的研究

康金凤1*,胡汉华1,袁武梅1,武贵臻1,陈蓉1,白山别克·吴汗2,艾赛提·卡力2

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目的 探讨地塞米松与三磷酸腺苷(ATP)联用在体外诱导细粒棘球绦虫原头节细胞凋亡的作用。 方法体外培养细粒棘球绦虫原头节,分别设地塞米松(5 mmol/L)组、 ATP(1.6 mmol/L)组、 地塞米松(5 mmol/L)+ATP(1.6 mmol/L)组和空白对照组,显微镜下观察原头节变化。药物诱导8 h后,选取原头节形态改变最明显的一组和空白对照组,透射电镜观察这两组原头节的超微结构,原位末端脱氧核糖核苷酸转移酶标记技术(TUNEL法)检测原头节中的凋亡细胞,半胱氨酸天冬氨酸蛋白酶-3(caspase-3)活性检测试剂盒检测该酶活性,琼脂糖凝胶电泳检测两组原头节的DNA片段。 结果 药物诱导8 h后观察,与对照组相比,地塞米松组和地塞米松+ATP组的原头节均出现团缩、顶突内凹和体积缩小,钙颗粒明显减少且模糊不清,未见原头节活动,其中地塞米松+ATP组原头节的形态改变更明显,故选择该组作为实验组,与空白对照组进行后续试验。透射电镜观察见实验组原头节中实质细胞体积缩小、细胞膜皱缩、细胞基质浓缩、核异染色质凝集呈团块状或新月形边集于核膜下,表现出凋亡细胞的特征。TUNEL法在实验组的原头节中检测到散在的凋亡细胞,对照组则未见凋亡细胞。实验组caspase-3活性约为对照组的12倍。电泳结果显示,实验组DNA中有约150 bp的核小体DNA片段。 结论 地塞米松与ATP联用可在体外诱导细粒棘球绦虫原头节细胞凋亡。

关键词 <u>细粒棘球绦虫</u> <u>原头节</u> <u>地塞米松</u> <u>三磷酸腺苷</u> <u>细胞凋亡</u> 分类号

Apoptosis Induced *in vitro* by Dexamethasone and ATP in the Protoscolex of *Echinococcus granulosus*

KANG Jin-feng^{1*}, HU Han-hua¹, YUAN Wu-mei¹, WU Gui-zhen¹, CHEN Rong¹, Baishanbieke WH². Aisaiti KL²

Abstract

Objective To explore the apoptosis induced by dexamethasone and adenosine triphosphate (ATP) in protoscolex of Echinococcus granulosus. Methods Protoscoleces were cultured in vitro and used for the experiment in 4 groups: dexamethasone (5 mmol/L) group, ATP (1.6 mmol/L) group, dexamethasone (5 mmol/L) +ATP (1.6 mmol/L) group, and RPMI 1640 medium as control group. The morphology of protoscolex was observed by light microscopy. After drug treatment for 8 h, the group with significant morphological changes in protoscolex was selected to observe the ultra-structure of protoscolex by transmission electron microscopy. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay (TUNEL) was employed to observe the apoptosis. Caspase-3 activity was detected with a kit, and DNA frag-ments were seperated by agarose gel electrophoresis. Results After induced for 8 h, the protoscoleces shrank in dexamethasone group and dexamethasone +ATP group, the rosellum was invaginated. Compared with the control, the calcareous corpuscles in the protoscolex significantly reduced and blurred in the two groups. The morphological changes in protoscolex of dexamethasone+ATP group was more obvious than that of dexamethasone group. Electron microscopy showed that dexame-thasone +ATP-treated protoscoleces possessed typical morphological features of apoptosis, including the cell volume reduction with densified cytoplasm, cell membrane blebbing, and nuclear heterochromatin peripheral aggregation below the nuclear membrane. A few apoptotic cells were found in protoscolex of dexamethasone+ATP group by TUNEL, while none in the control. Caspase-3 activity significantly increased 12-fold compared to the control. About 150 bp DNA fragment exhibited the typical DNA ladder formation

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characteristic for apoptosis in dexamethasone+ATP group. Conclusion Apoptosis in the protoscolex of *E. granulosus* may be induced by dexamethasone and ATP *in vitro*. Key words <u>Echinococcus granulosus</u> <u>Protoscolex</u> <u>Dexamethasone</u> <u>Adenosine</u> <u>triphosphate</u> <u>Apoptosis</u>

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通讯作者

作者个人主 页

康金凤1 *;胡汉华1;袁武梅1;武贵臻1;陈蓉1;白山别克·吴汗2;艾赛提·卡力2