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实验方法

应用Bhas 42细胞转化实验检测环磷酰胺、丝裂霉素C和氨苄西林钠致癌作用

宋征[1], 刘全海[2], 马璟[1]

1. 中国医药工业研究总院上海医药工业研究院 国家上海新药安全评价研究中心, 上海 201203;
2. 中国医药工业研究总院上海医药工业研究院 药理室, 上海 200083

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摘要 目的 采用Bhas 42细胞转化实验检测已知有遗传毒性的化学品在化学致癌中的引发和促生长作用,评价Bhas 42细胞转化实验检测化学品致癌作用的可靠性。方法 1 通过细胞毒性实验确定环磷酰胺、丝裂霉素C和氨苄西林钠进行Bhas 42细胞转化实验的浓度。 2 引发实验:细胞接种当日为第0天,第1天换成含有相应最终浓度的受试物或0.5% DMSO的DF5F培养基,培养72 h,第4天换成不含药物的DF5F培养基,培养至第21天。第22天将细胞固定、染色、并计数细胞数大于50的集落数。 3 促生长实验:接种当日为第0天,第4天换成含有相应受试物或0.5% DMSO的DF5F培养基并连续培养至第14天,期间第7天,第11天更换同样培养基,每15天换成不含药物的DF5F培养基,培养至第21天。第22天固定、染色细胞、计数细胞数大于50的集落数。结果 按照70%的细胞存活率以及前期文献结果确定最终浓度为:氨苄西林钠1750 mg·L^{-1};环磷酰胺1300 mg·L^{-1};丝裂霉素C 0.01 mg·L^{-1};3-甲基胆蒽1 mg·L^{-1},佛波酯0.05 mg·L^{-1}。引发实验结果显示,3-甲基胆蒽, 丝裂霉素C和环磷酰胺集落数显著多于空白对照、并且两者比值>2,判定为有引发作用的致癌化学品。促生长实验结果显示,佛波酯集落数显著多于空白对照、并且两者比值大于2,因此判定为有促生长作用的致癌化学品。结论 Bhas42细胞转化实验不仅可以检测出遗传实验可检测出的致癌阳性化学品和非致癌化学品,还可检测出遗传实验结果为假阴性的致癌阳性化学品,可以作为一种快速易操作的致癌物预测体外模型。

关键词 Bhas42细胞 引发实验 促生长实验

分类号 R979.1

Carcinogenesis activity detection of cyclophosphamide, mitomycin C and ampicillin Na by cell transformation assay in Bhas 42 cells

SONG Zheng[1], LIU Quan-hai[2], MA Jing[1]

1. National Shanghai Center for New Drug Safety Evaluation & Research, Shanghai Institute of Pharmaceutical Industry, China State Institute of Pharmaceutical Industry, Shanghai 201203, China;
2. Department of Pharmacology, Shanghai Institute of Pharmaceutical Industry, China State Institute of Pharmaceutical Industry, Shanghai 200083, China

Abstract

OBJECTIVE To detect the initiating and promoting carcinogenicity activity of some genotoxic chemicals, to evaluate the performance of Bhas 42 cell transformation assay for the detection of chemical carcinogenicity. **METHODS** 1 The dose of cyclophosphamide(CP), mitomycin C (MMC) and ampicillin Na applicable to the Bhas 42 cell transformation assay by cytotoxicity test was defined. 2 Initiation assay: the day of seeding cells was defined as the day before drug, on the 1st day, medium in each well was changed with the medium DF5F containing test chemical or 0.5%DMSO, and the treatment in the initiation phase was continued for 72 h. Following the exposure period, all treatment media were removed and the cells were refed with medium without the test chemical (the 4th day) and subsequently cultured in DF5F until the 21st day, receiving medium exchanges at the 7th day, the 11th day and the 14th day. The cells were fixed and stained on the 22nd day and counted the foci whose cells

number was more than 50. 3 Promotion assay: the day of seeding cells was defined as the day before drug. On the 4th day, medium in each well was changed with the medium DF5F containing test chemical or 0.5%DMSO, and the treatment in the promotion phase was continued to the 14th day. During the exposure period, all treatment media were replaced with medium containing the test chemical or 0.5% DMSO on the 7th day and the 11th day. The cells were then subsequently cultured in the DF5F without the test chemical from the 15th day to the 21st day. The cells were fixed and stained on the 22nd day and counted the foci whose cells number was more than 50. **RESULTS** According to the results of cytotoxicity test and previous results, the final concentration of chemicals were ampicillin Na: 1750, CP: 1300, MMC: 0.01, 3-methyl-cholanthrine (MCA): 1 and 12-O-tetradecamoylphorbol-13acetate (TPA): 0.05 mg • L^{-1}. The initiation assay results showed as the foci numbers of MCA, MMC and CP were significant increased in *t*-test and more than a 2-fold increase as compared with the