

复方中药注射液对人癌细胞体外杀伤效应 及其对细胞周期的影响

北京市肿瘤防治研究所药理组 张永健* 王耐勤 许树旭** 刘 彤

内容提要 复方中药注射液(ICHM)对人胃癌细胞BGC-823和人乳癌细胞MCF-7均有较强的杀伤作用。 IC_{50} 分别为0.02g生药/ml和0.018g生药/ml。流式细胞分析仪测定表明, ICHM使BGC-823细胞停止在 G_1 期, 导致S期细胞显著减少。通过 3H -TdR掺入试验观察到, ICHM能抑制BGC-823细胞的DNA合成。

复方中药注射液(ICHM)是由龙葵、白英、蛇莓、丹参、当归和郁金六味中草药制成的注射液。既往工作发现 ICHM 对多种实验性动物肿瘤有抑制作用和抗转移作用^[1,2], 其抗癌机理与几种因素有关^[3,4]。ICHM对人癌细胞的影响如何尚待观察, 本文旨在研究 ICHM对人癌细胞的杀伤效应以及对细胞周期的影响。

材料和方法

一、材料

1. 癌细胞: 人胃癌BGC-823细胞株由北京医科大学人民医院提供; 人乳癌MCF-7细胞株由美国AMC癌症研究中心细胞生物实验室提供。培养于含10%小牛血清的RPMI-1640培养液中。

2. 药物和试剂: ICHM制备方法同前^[5], 每ml含生药4g。 3H -亮氨酸(1mCi/ml, 比度130Ci/mmol)和 3H -胸腺嘧啶核苷酸(3H -TdR, 1mCi/ml, 比度20Ci/mmol)为上海原子核研究所产品。新生牛血清购自深圳光明生化制品厂。RPMI-1640为GIBCO产品。乙二胺四乙酸二钠(EDTA)为北京化工厂产品。

3. 仪器: 细胞周期使用FACS-420型流式细胞分析仪测定; 放射活性经Beckman LS-3810型液闪仪测定。

二、方法

1. 对人胃癌和乳癌细胞的杀伤效应(3H -亮氨酸释放试验): 将BGC-823或MCF-7细胞接种在96孔微孔培养板中, 每孔 2×10^4 细胞/0.2ml。37°C CO₂培养箱中孵育过夜。换以含 3H -亮氨酸的无亮氨酸RPMI-1640培养液, 孵育4h以标记细胞。弃上清液, 用培养液洗3次, 以洗掉未掺入的自由 3H -亮氨酸。每孔加入用10%小牛血清RPMI-1640稀释的ICHM, 稀释倍数为1:20、40、80、160、320和640, 每浓度药物三孔细

胞, 对照组不加药。孵育8h后撤药, 加培养液后继续孵育36h。洗掉死细胞, 用0.1N NaOH溶液0.2ml溶解贴壁细胞, 加到闪烁液中, 闪烁仪测cpm值。以对照组的cpm值为100%, 观察给药组各浓度的杀伤率, 求出 IC_{50} 。

2. 对BGC-823细胞株细胞周期的影响: 在BGC-823细胞的对数生长期, 加入含有相当0.04g生药/ml的ICHM, 对照组不加药。37°C孵育10h, 0.02% EDTA消化后制成细胞悬液, 离心, 磷酸缓冲液(PBS)洗涤3次, 70%乙醇固定。将细胞染色处理后输入FACS-420型流式细胞分析仪, 观察细胞周期。

3. 对BGC-823细胞DNA合成的影响: ICHM加药量及操作同细胞周期测定。PBS洗涤后, 用RPMI制成细胞悬液, 计数后分装于试管中, 对照组及给药组各9管, 每管 1×10^5 细胞/ml, 每管加入1 μ Ci 3H -TdR, 37°C孵育1h。0.45 μ 纤维滤膜收集细胞, 5ml生理盐水冲洗, 5%三氯醋酸沉淀蛋白, 无水乙醇固定。待干后, 放入闪烁液中, 测cpm值。

结 果

一、对人癌细胞的杀伤效应: 不同浓度的ICHM对BGC-823和MCF-7细胞都具有杀伤效应, 随药物剂量的增加, 杀伤作用也增强(见表1)。经线性回归处理, ICHM对BGC-823和MCF-7细胞的 IC_{50} 分别为0.02g生药/ml和0.018g生药/ml。表明ICHM对两株人癌细胞均有明显的杀伤作用, 且其杀伤作用强度相似。

二、对细胞周期的影响: 经ICHM处理的BGC-823细胞经流式分析仪测定发现, 与对照组比较 G_1 期细胞明显增多, 而S期和 G_2+M 期细胞相应减少, 表明ICHM能使癌细胞停滞在 G_1 期(见表2)。

三、对 3H -TdR掺入DNA的影响: 经0.04g生药/ml的ICHM处理的BGC-823细胞, 能明显抑制 3H -TdR的掺入, 提示BGC-823细胞的DNA合成受到抑制, 其结

表1 ICHM对BGC-823和MCF-7细胞的杀伤效应

药物剂量 (g/ml)	杀 伤 率 %	
	BGC-823	MCF-7
0.2	96	83
0.1	88	78
0.05	62	69
0.025	53	57
0.0125	53	49
0.0065	30	28

表2 ICHM对BGC-823细胞株细胞周期的影响 (M±SD)

组 别	实验 次数	各周期细胞%		
		G ₁	S	G ₂ +M
对 照	4	27.5±5.1	58.8±3.3	13.5±1.6
ICHM	6	49.8±5.2*	41.0±4.9*	9.5±1.5**

*P<0.001, **P<0.01

果与ICHM对细胞周期的影响一致。对照组细胞的cpm值为736.0±59.2(M±SD), ICHM组的cpm值为578.4±35.2, 掺入抑制率为21.3%。

讨 论

前文曾报道, ICHM对多种实验动物肿瘤具有明显的抑制作用, 抗癌谱较广^①, 但对人癌细胞是否具

有杀伤作用, 仍待证实。本实验结果表明, ICHM对体外培养的人胃癌细胞BGC-823和人乳腺癌细胞MCF-7均有显著的杀伤作用。两株人癌细胞的IC₅₀非常接近, 表明对两株细胞的作用不带有选择性。

通过流式细胞分析仪测定发现, 用药后G₁期细胞明显增多, S期和G₂+M期细胞相应减少, 表明ICHM作用于S期, 导致细胞停滞在G₁期。³H-TdR掺入实验结果也表明, ICHM使³H-TdR掺入率降低, 抑制DNA合成^②。因此, ICHM对癌细胞核酸合成的抑制作用是其抗癌作用的重要机制。

参 考 文 献

1. 梁亚云, 等。复方中药注射液抗肿瘤作用的初步研究。中西医结合杂志 1986; 6(9):549。
2. 王耐勤, 等。复方中药注射液对荷瘤小鼠的抗转移作用以及对巨噬细胞吞噬功能的影响。中西医结合杂志 1988; 8(特1):54。
3. 汪望仁, 等。复方中药和3', 5'-环腺苷酸(cAMP)对小鼠艾氏腹水癌细胞的抑制效应与癌细胞内cAMP含量及cAMP-PDE活性变化关系。北京医学 1980; 2(2):91。
4. 吕桂芝, 等。复方龙葵注射液对肝癌(H₂₂)细胞的影响。中西医结合杂志 1987; 7(2):97。
5. 吕桂芝, 等。复方中药注射液对小鼠艾氏腹水癌细胞膜表面的(Na⁺-K⁺)-ATP酶和微绒毛影响的电镜观察。解剖学报 1987; 18(2):187。

丹参注射液治疗陈旧性增生性瘢痕1例

武汉大学医院理疗科 杨 卫

笔者使用丹参注射液局部封闭法治疗陈旧性增生性瘢痕1例, 疗效满意。报告如下:

匡××, 女, 50岁, 工人。患者13岁时, 右肩外1/3处长一疖肿, 用手挤压排脓, 未经其他治疗而愈, 遂形成约1×2cm²椭圆形瘢痕。从19岁起, 此瘢痕开始逐渐隆起, 颜色变红, 奇痒难忍, 常被抓破出血, 继而向正常皮肤扩散、延长, 直至右臂上部三角肌处, 症状日益加重。曾多处求医, 病情未得到控制, 于1987年12月初前来就诊。检查: 一般情况好, 内脏无异常。皮损从右肩外1/3处至右臂上部三角肌处, 形状不规则, 约(0.5~3)×18cm², 颜色鲜红, 似新生的肉芽组织样, 约高出皮面0.1~0.2cm, 与正常皮肤界限分明, 质地较硬, 有抓痕血迹。采用丹参注射液(上海

第一制药厂生产)在皮损周围作封闭治疗; 停用其他一切药物。每次取2个部位, 每处用药2ml, 10天为1疗程, 每疗程间隔2~3天。治疗10天后, 皮损颜色转暗, 奇痒好转。第2疗程后, 上述症状又有所减轻, 质地稍平软。持续治疗6疗程后, 诸症基本消失, 三角肌处皮损下段有2~3cm转为正常肤色。随访半年, 未复发。

瘢痕疙瘩在临床上并不少见, 虽然治疗方法很多, 但一般收效不甚理想。笔者用丹参注射液治疗陈旧性增生性瘢痕1例, 收到预期疗效, 进一步证实了陈学忠等人提出的关于丹参能明显抑制成纤维细胞的生长和增生的理论。本疗法操作简便, 丹参无毒副作用, 易被患者接受, 为该症患者提供了新的治疗途径。

drial ATPase activity (μ mol pi/min/mg pr.) was lower in model group (0.280 ± 0.015) than in pretreated group (0.341 ± 0.018 , $P < 0.05$). Lipid peroxide (LPO) in liver homogenates and serum were 1.86 ± 0.43 n mol MDA/mg pr. and 12.26 ± 0.84 n mol MDA/ml respectively in model group, whereas in pretreated group they gave a much lower value (1.19 ± 0.12 and 6.55 ± 2.97) respectively. Those data showed very significant difference between two groups ($P < 0.01$). All the above indices of pretreated group yielded values close to those of normal control group. The results of experimental study in vitro were identical to those of experimental study in vivo. These experimental studies suggested that RDQ provide antagonistic effect on endotoxin induced damage of lysosomes and mitochondriae.

(Original article on page 412)

The Killing Action of Chinese Herbal Mixture on Human Tumor Cells in Vitro and Its Effect on Cell-Cycle

Zhang Yongjian(张永健), Wang Naiqin(王耐勤), et al

Dept. of Pharmacology, Beijing Institute for Cancer Research, Beijing

Previous studies had shown that injection of Chinese herbal mixture (ICHM) had antitumor and anti-metastatic actions on various transplantable animal tumors. In the present study, the authors observed the obvious killing actions of ICHM on human gastric cancer cell line BGC-823 and breast cancer cell line MCF-7 in vitro. The IC_{50} were 0.02 g crude drug/ml and 0.018 g crude drug/ml respectively. The study of the effect of ICHM on the cell-cycle of BGC-823 cell showed that ICHM decreased the number of cells in S phase and increased the number of cells in G_1 phase significantly. The results indicated that the BGC-823 cells was blocked on S phase by ICHM. Meanwhile, ICHM reduced 3H -TdR incorporation into DNA, the cpm value was much lower than that of the control.

(Original article on page 416)

Enhancement of Therapeutic Effect and Red Cell Immune Function by Radix Trichosanthis in Mice Bearing Ehrlich Ascites Carcinoma

Guo Feng(郭峰), et al

Laboratory of Immunology, Changhai Hospital, The Second Military Medical College, Shanghai

Various red cell immune functions were determined in 60 mice bearing Ehrlich ascites carcinoma treated and untreated with Radix Trichosanthis(RT), and compared with these of 30 normal mice. In mice bearing Ehrlich ascites carcinoma treated with RT, the rosette formation of red cell C_{3b} receptor, the rosette formation of red cell immune complex, the rosette formation rate of red cell round cancer, the rate of PMN phagocytosis were enhanced, and the activity of superoxide dismutase (SOD) was satisfactory higher than those in mice bearing Ehrlich ascites carcinoma untreated with RT, and almost same as those in normal mice, while the rosette inhibition rate of red cell C_{3b} receptor in serum was satisfactory lower than that in mice bearing Ehrlich ascites carcinoma untreated with RT, and almost same as that in normal mice.

(Original article on page 418)

Statistical Analysis of the Ethnopharmacologic Data Based on Chinese Medicinal Plants by Electronic Computer (II) Hamamelidae and Caryophyllidae

Xiao Peigen(肖培根), Wang Liwei(王立为) Qiu Guisheng*(仇桂生), et al

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences;

**Department of Mathematics, Peking University, Beijing*

Statistical analysis of Chinese medicinal plants, Hamamelidae and Carophyllidae, has been made to the clarification of the ethnopharmacologic data collected nationwide, and hence trend and regularity of these data have been abstracted and quantitatively presented. The main parameters of this study involved: family medicinal coefficient (α_f) i. e. the radio of medicinal/total genera within a certain family; genus medicinal coefficient (α_g) i. e. the radio of medicinal/ total species within a certain genus; traditional therapeutic usages coefficient (TRI) and extent of traditional therapeutic usages within a certain taxon (β). The results may be of value to the resources utilization, new drug searching, as well as systematization of traditional Chinese and herbal medicine.

(Original article on page 429)