

甘草锌对体外培养成纤维细胞的作用*

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内容提要 应用体外培养细胞方法观察了甘草锌对成纤维细胞分裂增生的作用。结果表明不同浓度的甘草锌使成纤维细胞贴壁生长率、细胞核分裂系数均高于对照组, 形态学发生显著变化。提示甘草锌抗溃疡作用同促进成纤维细胞合成纤维和基质有关。

关键词 甘草锌 成纤维细胞 贴壁生长率 细胞核分裂系数 胃溃疡

有资料表明, 从甘草中提取的有效成份甘草酸与微量元素锌结合而成的化合物——甘草锌, 对消化道溃疡具有一定的治疗效果, 并经动物实验和临床治疗所证实⁽¹⁾。为了进一步探讨甘草锌抗溃疡的作用机理, 我们用甘草锌对体外培养成纤维细胞的影响进行了初步观察, 报道如下。

材料与方法

一、培养液: Eagle 培养液 (MEM USA NO 410—1700), 使用前加 10% 小牛血清 (上海放射医学研究所提供)。

二、药物: 甘草锌 (棕色粉末), 系新疆制药厂供给, 批号 851203。配成相应浓度的水悬液, 高压灭菌消毒, 备用。

三、细胞与培养方法: 在无菌条件下取自 5 个月正常胎儿腹部真皮组织 (清除皮下脂肪), 经生理盐水和三种抗生素 (青霉素、链霉素、庆大霉素) 漂洗后剪成 0.5~1.0mm² 大小的组织块, 均匀接种于培养瓶中, 加入培养液, 置 37℃ 恒温培养箱中培养。生长铺满培养瓶后, 用 0.2% 的胰蛋白酶消化、计数, 分瓶扩大培养。3~4 代的细胞进行实验观察⁽²⁾。

四、实验步骤: 细胞在原代接种培养和分瓶扩大传代培养时, 均分实验组和对照组各 30 瓶。实验组在培养液中加一定浓度的甘草锌 (每毫升含甘草锌 50、75、100μg)。为了观察形态学变化, 培养瓶内放置小盖玻片。

1. 形态学观察: 细胞长满培养瓶, 取出盖玻片, 经甲醇固定 10 分钟, 用苏木素伊红染色 (HE 染色法), 二甲苯透明, 树脂封固, 在显微镜下观察形态特征并拍摄照片。

2. 细胞贴壁生长率: 原代接种观察其组织块贴壁生长数量。传代培养时, 以 5×10^4 个细胞数分瓶扩大培养。每次换培养液时收集、计数出漂浮在液体中未贴壁的细胞, 以下列公式计算出贴壁生长率。

$$\text{细胞贴壁生长率(\%)} = \frac{\text{传代时细胞数} - \text{未贴壁细胞数}}{\text{传代时细胞数}} \times 100\%$$

3. 细胞核分裂数: 每培养瓶取出一片盖玻片, 在面积约为 0.75cm² 范围内计数 500 个细胞中核分裂数, 除以细胞总数, 即得核分裂系数。

结 果

一、形态学观察: (1) 正常对照组 10 瓶, 培养 48 小时后, 组织块贴壁率为 45%。镜下: 组织块四周有微小细胞突起长出, 呈多角形。培养 10~15 天细胞铺展成片。经 HE 染色, 成纤维细胞与纤维细胞比为 5:3。(2) 实验用药组: 15 瓶, 组织块贴壁生长率为 80%, 36 小时后, 组织块四周有突起长出, 呈簇状、涡样生长。培养 7 天长满。经染色, 成纤维细胞与纤维细胞为 5:2。在固定玻片上, 成纤维细胞的胞体较大, 呈扁平星形, 胞质透明饱满, 具有细长的突起, 互相交错, 核大、卵圆形, 着色较浅, 核仁明显, 胞质弱嗜碱性, 表明细胞代谢处于旺盛时期。纤维细胞常贴附于胶原纤维上, 比成纤维细胞小, 细胞呈梭形, 突起较少, 核小略带长形, 染色较深, 核仁不明显, 胞质呈弱嗜酸性, 表明纤维细胞是功能不甚活跃的细胞。

二、细胞贴壁率: 经过 4 次传代扩大培养, 实验组与对照组各取 15 瓶, 收集各组培养液, 用血液计数板分别计算出未贴壁漂浮在培养液中的细胞数, 经统计学处理, 各组贴壁率为: 对照组平均为 63%; 实验组甘草锌浓度为 50、75、100μg 时, 各组贴壁率为 65%、78%、71%。其结果同形态学观察相吻合。

三、细胞核分裂系数：(1)正常对照组：成纤维细胞各期正常核分裂相均可见到，分裂系数为1.70~1.92%。(2)实验组：甘草锌浓度50、75、100 μ g时各期细胞分裂相为2.1%、3.6%、3.4%。每组取10张玻片计数。

讨 论

在细胞培养中，有些成纤维细胞很易贴壁于玻璃底物上，迅速生长增殖，是与细胞骨架中的微丝有关。Abee认为培养的细胞中，在充分展平和移动非常缓慢的成纤维细胞中有许多张力纤维，在细胞粘着上起很大的作用，是防止细胞从底物脱离的微丝成份⁽³⁾。实验中实验组的组织块及细胞贴壁生长率明显高于正常对照组，证明甘草锌具有促进成纤维细胞合成微丝的作用。形态学观察，成纤维细胞被染成紫蓝色，呈嗜碱性，说明细胞质内同蛋白质合成有关的粗面内质网和游离核蛋白体增多。实验组成纤维细胞明显多于纤维细胞。认为甘草锌能使处于静止状态的细胞激活成为成纤维细胞，使其核分裂相明显增多。用胰蛋白酶消化而脱落的细胞重新分装和扩大培养就是

利用微丝纤维解聚和聚合的功能。脱落的细胞如不能重新聚合微丝纤维就不能再次贴壁生长，而悬浮在培养液中2~3天即死亡⁽⁴⁾。推测，甘草锌具有促进微丝纤维聚合的作用；成纤维细胞还具有合成基质中氨基己糖多糖如透明质酸等蛋白多糖作用，从而构成结缔组织中的分子筛，使细胞间质呈胶样或粘稠状，显然亦能防止细菌扩散⁽⁵⁾。本实验表明，甘草锌抗消化道溃疡和抗炎作用同促进成纤维细胞分裂增生，合成纤维和基质有关。

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中西医结合治疗糖尿病肢端坏疽 20 例

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1984年3月~1989年5月，我们采用复方通脉液经患肢动脉注入，配合创面局部湿敷，治疗糖尿病肢端坏疽患者20例，疗效较满意，现报告如下。

临床资料 20例均确诊为糖尿病，男17例，女3例，年龄41~64岁，病程最长16年，最短8个月；肢端坏疽部位均在下肢，其中单下肢15例，双下肢5例。坏疽范围局限于足趾部11例，超越距趾关节7例，超越踝关节2例。均为湿性坏疽或干性转为湿性，且继发感染。5例并发高血压、冠心病，1例脑梗塞，1例糖尿病肾病、肾衰。

治疗方法 全组病例按糖尿病常规治疗。复方通脉液(654-2 10mg、复方丹参注射液 14ml、川芎嗪 80mg、维脑路通400mg，合并感染者加相应抗生素，用0.9%生理盐水 50ml稀释)经患侧股动脉注入，每天1次，连用10天，休息3天，30天为1个疗程。此外，配合654-2及相应抗生素创面局部湿敷，每天1次，30天为1个疗程。

结果 本组1例因广泛下肢坏死继发严重感染导致糖尿病昏迷，死于酮症酸中毒；1例糖尿病肾病，

死于肾衰。其余病例坏死局部组织血运好转，皮肤松软，黑褐色素沉着减退，肿痛消失，溃疡逐步愈合。其中经1个疗程获临床痊愈者9例，2个疗程痊愈6例，3个疗程痊愈1例，4~5个疗程痊愈1例，明显好转1例。

讨论 微循环障碍是糖尿病肢端坏疽的病理基础之一。因此改善患肢血液循环，增加肢体血液供应，是促使缺血性溃疡愈合的关键。复方通脉液具有缓解血管痉挛、扩张血管的作用，并能活跃和疏通微循环。654-2用于治疗末梢缺血性坏疽可提高血氧含量，改善组织缺氧，从而减轻代谢性酸中毒，并能促使水肿的吸收和减少炎症渗出。复方丹参、维脑路通、川芎嗪三者伍用，具有活血化瘀，消肿理气，消炎止痛，扩张小动脉，改善微循环，且有防栓和溶栓作用。临床应用无毒性反应。该药经患肢动脉注入，可于短时间内使药物在患肢动脉内发挥扩容作用，增加局部动脉内药物浓度和压力，并可起到机械冲击作用，使血流量骤增以抗血栓形成。

erythrocyte deformability, the blood viscosity, Hb, Ht, MCV, MCH levels indicate that there was a significant correlation between them and the fetal average birth weight respectively. This study indicates that blood stasis is one of the pathogenetic mechanisms of asymmetrical IUGR and that the PBCRS recipe could improve intrauterine growth environment of fetal which could treat and prevent IUGR in obstetrics. (Original article on page 157)

Effects of *Ligusticum wallichii* on the Plasma and CSF Levels of Dynorphin A1-13 in Rabbits under Acute Experimental Cerebral Ischemia

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By occluding the bilateral carotid arteries of rabbits to produce bilateral partial cerebral ischemia, and by using radioimmunoassays to measure the levels of dynorphin A1-13-like immunoreactivity (ir-Dyn A1-13) in plasma and cerebrospinal fluid (CSF), the authors find that the levels of ir-Dyn A1-13 in plasma and CSF have significantly increased ($P < 0.01$) after cerebral ischemia appears. The result of the *Ligusticum wallichii* Franch (*Ligusticum*) pretreatment to the test-group shows a definite improvement of the changes of ir-Dyn A1-13 levels in plasma and CSF. The severity of brain ischemic damage and neurologic dysfunction in *Ligusticum*-treated animals is lighter than that of saline-treated group, too. In this study, some new approaches are explored to explain the pathophysiology of cerebral ischemia and the mechanisms by which *Ligusticum* prevents and treats cerebral ischemia. (Original article on page 160)

The Role of Virus in Hemorrhagic Pancreatitis and the Therapeutic Effect of Rhubarb

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The living measles vaccine was injected into both of the main pancreatic duct and the ear vein of the rabbits in the model group. The serum amylase was significantly higher than that of the control saline group ($P < 0.001$ after 24h; $P < 0.05$ after 48h). The aggregation function of platelets were increased after 24h ($P < 0.01$, $P < 0.01$) and 48h ($P < 0.01$, $P < 0.05$) as compared with both two control groups. The blood stasis and hemorrhage of pancreases were observed in the model group. In the treatment group with Rhubarb the increase of serum amylase was much less marked than the control ($P < 0.001$ after 24h, $P < 0.05$ after 48h). The platelet aggregation reactivity was inhibited ($P < 0.01$ after 24h, $P < 0.05$ after 48h). The blood stasis and hemorrhage of pancreases were less evident. The results suggested that: (1) the virus may be one of pathogenetic factor of the hemorrhagic pancreatitis; (2) the rhubarb was effective in the treatment of hemorrhagic pancreatitis under our experimental conditions. (Original article on page 162)

Effect of Licorzine on Extracorporeal Fibroblast Culture

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This paper reports the experimental study of the effect of licorzine on the extracorporeal fibroblast culture. When each milliliter of the nutrient fluid contains 50 μ g, 75 μ g, 100 μ g of licorzine respectively, the rate of cellular growth and the coefficient of karyokinesis were much higher than those of the control group. There was a significant change in cell morphology. In the experimental group, the cells grew vigorously, and they appeared large, flat, branching, with fusiform or spindle shape in profile. The ratio of fibroblasts and fibrocytes was 5:2. In young fibroblasts processes interwove with each other, the cytoplasm became plump, and the cell nucleolus could be seen clearly. They were actively engaged in protein synthesis for the production of intercellular substance, the cytoplasm appeared relatively homogeneous and was basophilic because of high concentration of granular endoplasmic reticulum. Fibroblasts are considered to be responsible for the formation of the fibers and synthesize most of the amorphous components of the matrix. In the normal control group, the ratio of fibroblasts and fibrocytes was 5:3. In relatively static fibrocytes, the cytoplasm was sparse and slightly acidphilic since the endoplasmic reticulum was scanty. As licorzine has the function of increasing fibroblasts' synthesis of fibers and matrix, it can promote the healing of the gastric ulcer. (Original article on page 164)