

生血灵对原发性血小板减少性紫癜患者骨髓体外培养巨核系祖细胞的影响

上海中医学院附属岳阳医院血液科(上海 200031)

周永明 薛志忠 姚楚芳 张 艇 张琴明 黄振翹* 陆敬懿*

内容提要 采用改良的甲基纤维素-琼脂双层体外培养技术, 观察生血灵对原发性血小板减少性紫癜(ITP)患者骨髓巨核系祖细胞(CFU-Meg)的影响。结果表明: ITP患者CFU-Meg 平均产率明显低于正常对照组($P < 0.01$); 加入生血灵200mg/ml 时平均产率显著增加($P < 0.05$); 加入300 mg/ml 和500mg/ml 时增加均非常显著($P < 0.01$); 而正常骨髓细胞培养体系同时加入ITP患者血清和生血灵培养后, 平均产率显著高于患者组($P < 0.01$), 且接近正常对照组($P > 0.05$)。提示生血灵能抑制抗血小板抗体, 促进ITP患者CFU-Meg的增殖、分化和成熟。

关键词 生血灵 原发性血小板减少性紫癜 体外培养 巨核系祖细胞

生血灵是我院黄振翹教授根据中医理论、结合临床经验而研制的治疗原发性血小板减少性紫癜(ITP)的中药制剂, 临床治疗112例, 有效率达91.1%。为了探索生血灵的作用机理, 我们采用改良的甲基纤维素-琼脂双层体外培养技术, 观察了生血灵对ITP患者骨髓巨核系祖细胞(CFU-Meg)生长的影响, 现报告如下。

资料与方法

一、对象: 正常骨髓: 取自15例胸外科患者的肋骨, 男5例, 女10例; 年龄20~62岁, 平均35.1岁。这些患者无肿瘤细胞的骨髓转移, 未经化疗和放疗, 造血系统正常。ITP患者20例, 男6例, 女14例; 年龄18~58岁, 平均34.2岁, 均按首届全国血栓与止血学术会议修订的标准诊断^[1]。

二、方法

1. 骨髓细胞悬液制备: 取骨髓液2~3 ml, 以含有肝素10u/ml的RPMI1640培养液稀释, 经7号针头冲打骨髓小粒, 用细胞分离液(比重为1.077)密度梯度离心, 535G, 25分钟, 收集界面层单个核细胞, 洗涤3次, 最后用IMDM

培养液制成 2×10^6 /ml的细胞悬液。以台盼蓝试验鉴定细胞活力, 单个核细胞存活率在90%以上。

2. 植物血凝素-单个核细胞条件培养液(PHA-MNCCM)的制备: 参照Prival等的方法^[2]。取健康人肝素抗凝全血, 密度梯度离心, 分离出单个核细胞, 洗涤3次后, 用IMDM制成 1×10^6 /ml的细胞悬液, 加入1%PHA-P(v/v)和5%AB型血浆, 分装于培养瓶中, 置于37°C、5%CO₂、饱和湿度的CO₂培养箱中, 第6天收集上清液, 过滤后低温保存备用。

3. ITP患者血清的制备: 采血小板相关抗体(PAIGG)明显增高的ITP患者全血, 置无菌试管1小时(37°C), 离心(1487G)20分钟, 吸出上层血清, 低温保存备用。

4. 生血灵灭菌制剂: 生血灵由黄芪、党参、当归、地黄、旱莲草、大青叶和甘草等药物组成, 制剂由岳阳医院中药制剂室提供, 每毫升含生药5g。

三、实验步骤: 根据Messner等甲基纤维素法^[3]加以改良, 采用双层培养法, 直径为35mm的培养皿底层浇入1ml含有不同剂量生血灵的琼脂(终浓度为0.5%), 冷却后再加入上层培养体系1ml, 每ml培养体系中含5%

PHA-MNCCM, 30%去血小板AB型血浆、0.9%甲基纤维素、 5×10^{-4} M 2-巯基乙醇、 1×10^5 单个核细胞及适量IMDM。本实验分为五组:

(1)正常人骨髓细胞(正常)组。(2)正常人骨髓细胞加1%ITP血清(正常+S)组。(3)正常人骨髓细胞加1%ITP血清和生血灵(正常+S+Sh)组。(4)ITP患者骨髓细胞(患者)组。(5)ITP患者骨髓细胞加生血灵(生血灵)组。将培养皿置 37°C 、5% CO_2 、饱和湿度的 CO_2 培养箱中培养14天,每次实验至少一式三份,取其均值。

四、观察计数:于培养第7天用倒置显微镜观察细胞生长情况,第14天观察并进行集落计数,3个或3个以上细胞的细胞团作为1个集落,并用毛细玻管将单个集落取出涂片,瑞氏-姬姆萨染色、细胞组化染色进一步确定细胞成分。

结 果

培养第7天即可见多个细胞团,14天集落数达高峰,ITP患者CFU-Meg平均产率明显低于正常对照组;正常骨髓细胞加入PAIgG明显增高的ITP患者血清培养后,平均产率也显著减少;不同剂量的生血灵对患者CFU-Meg产率均有增加,其中以大于200mg/ml剂量的作用显著;而在正常骨髓细胞培养体系同时加

入ITP患者血清和生血灵培养后,其平均产率显著高于患者组,且接近正常组。参见附表。

讨 论

本组通过15例正常人和20例ITP患者的骨髓细胞体外半固体双层培养,观察了生血灵对CFU-Meg生长的影响,意欲探讨其作用机理。

巨核细胞表面与血小板有着相同的抗原成分,抗血小板抗体可分别作用于血小板及巨核细胞⁽⁴⁾。有人报告,在32例活动期的慢性ITP儿童中,膜上结合有IgG的巨核细胞数比对照组多10倍以上⁽⁵⁾。Hoffman等报道1例ITP病人的抗血小板抗体对CFU-Meg有细胞毒作用⁽⁶⁾。本组20例ITP患者骨髓细胞体外培养后,CFU-Meg平均产率明显低于正常对照组,且集落也较对照组小,正常骨髓细胞加入PA-IgG明显增高的ITP患者血清后,CFU-Meg产率也显著减少,表明ITP患者巨核系祖细胞造血有异常,即分化、成熟障碍。不同剂量的生血灵对患者骨髓CFU-Meg产率均有不同程度的增加,其中以大于200mg/ml剂量的作用明显,而正常骨髓细胞同时加入ITP患者血清和生血灵培养后,其平均产率明显高于患者组,且接近正常对照组,说明一定剂量的生血灵能抑制血小板抗体,并直接作用于患者巨核系祖细胞,促进其增殖、分化和成熟。

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附表 各组巨核祖细胞CFU-Meg
培养产率 ($\bar{x} \pm S$)

组 别	例数	CFU-Meg/ 10^5 MNC
正 常 组	15	$21.22 \pm 9.06 \Delta\Delta$
正常+S组	15	$12.24 \pm 5.77^{**}$
正常+S+Sh (300mg/ml)组	15	$20.25 \pm 4.76 \Delta\Delta$
患 者 组	20	$13.75 \pm 6.93^{**}$
生血灵组		
100mg/ml	20	$15.77 \pm 6.31^*$
200mg/ml	20	$18.72 \pm 5.54 \Delta$
300mg/ml	20	$21.60 \pm 7.35 \Delta\Delta$
500mg/ml	20	$21.84 \pm 7.15 \Delta\Delta$

注:与正常组比较, $^*P < 0.05$, $^{**}P < 0.01$; 与患者组比较, $\Delta P < 0.05$, $\Delta\Delta P < 0.01$

during the acute phase, which was reversed during the recovery phase and remained significantly elevated during the sequela stage compared with the controls, ($P < 0.001$), F test showed that both t-PA and PAI activities changed significantly during various stages (t-PA, $P < 0.01$; PAI, $P < 0.001$). Because of these changes, the ratio of PAI and t-PA fluctuated during different stages of the disease. This ratio decreased during the acute stage and came close to the normal levels during the recovery phase. The ratio, however, elevated abnormally during the sequela stage, compared with healthy controls ($P < 0.05$). In addition, the result of correlation test and linear regression analysis of serum t-PA and PAI activities in 54 cases showed a significant negative correlation ($P < 0.001$) existed between t-PA and PAI activities. The balance of plasma t-PA and PAI activities have the function in maintaining the normal bloodstream in human body. In the process of disease, the abnormality of plasma t-PA and PAI activities may be the different pathological characteristics involved in this stagnation process. If coordinated with differentiation of syndrome in TCM, it may have the important significance to follow-up, and determine prognosis and early diagnosis for certain potential clinical stagnation of blood.

Key Words ischemic stroke, stagnation of blood, tissue-type plasminogen-activator, plasminogen activator inhibitor

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Effect of Jiang-Zhi Zhong-Yao-Pian (降脂中药片) on TC, TG, TXB₂, 6-Keto-PGF_{1α} in Hyperlipemic Patients

Liang Xiao-chun (梁晓春), Guo Sai-shan (郭赛珊), et al

PUMC Hospital, Chinese Academy of Medical Sciences, Beijing (100730)

The purpose of this study was to verify the effect of a Chinese herbal medicine Jiang-Zhi Zhong-Yao-Pian to reduce serum lipoid. Efficacy was observed in 30 cases of hyperlipemia; 20 cases administered with evening primrose oil capsules were taken as controls. Each group took drugs for two or three months. The results were as follows: After treatment as compared with before treatment, the serum levels of TC, TG and TXB₂ dropped from 264.28 ± 70.52 mg%, 393.52 ± 250.42 mg% and 110.75 ± 43.52 pg/ml to 225.60 ± 50.93 mg%, 264.97 ± 252.81 mg% and 88.82 ± 46.50 pg/ml respectively ($P < 0.001$, < 0.01 , < 0.05). However, in the group taking evening primrose oil capsules, TC, TG and TXB₂ in comparing with the pre-treatment levels were changed from 251.33 ± 58.24 mg%, 316.35 ± 104.93 mg% and 131.53 ± 49.77 pg/ml to 244.30 ± 43.28 mg%, 272.10 ± 92.52 mg% and 115.33 ± 47.49 pg/ml respectively ($P > 0.05$, < 0.05 , > 0.05). This medicine had no side-effect. The results showed that the herbal formula might be useful to reduce serum TC, TG and TXB₂.

Key Words hyperlipemia, cholesterol, triglyceride, thromboxane B₂, 6-keto-prostaglandin F_{1α}, Jiang-Zhi Zhong-Yao-Pian

(Original article on page 20)

In Vitro Effect of Shen-Xue-Ling (生血灵) on Megakaryocytopoiesis in Patients with Idiopathic Thrombocytopenic Purpura

Zhou Yong-ming (周永明), Xue Zhi-zhong (薛志忠), Huang Zhen-qiao (黄振翘), et al

Yueyang Hospital Affiliated to Shanghai College of TCM, Shanghai (200031)

This paper used an improved methylulose-agar double layer method in vitro to culture megakaryocyte progenitors (CFU-meg) of 15 normal bone marrow donors and 20 idiopathic thrombocytopenic purpura (ITP) patients bone marrow cells in order to observe the action of Shen-Xue-Ling (SXL). The results showed that the colony efficiency of CFU-meg of ITP group ($13.75 \pm 6.93/10^5$ MNC) was obviously lower than that of the control group ($21.22 \pm 9.06/10^5$ MNC, $P < 0.01$). The serum of the patients whose PAIgG was markedly higher than normal value inhibited growth of CFU-meg derived from normal bone marrow cells ($P < 0.01$). SXL could increase the colony efficiency of