活血化瘀药对骨髓造血的影响

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内容提要　本文用粒系祖细胞(CFU-GM)及经液体培养一周骨髓细胞基质层，同种骨髓粒系祖细胞(称为SL-CFU-GM)，分别作为检测骨髓造血细胞及造血基质功能的方法。观察活血化瘀药对造血的影响。将小鼠给予川芎嗪、复方丹参液，并以环磷酰胺及生理盐水作对照，取骨髓测定CFU-GM、SL-CFU-GM。结果活血药均不能直接促进造血细胞增殖，但SL-CFU-GM明显高于对照组，加消炎痛后SL-CFU-GM更形增多。说明活血药促进基质功能，且与PGE有关。提示活血化瘀药在体内有改善骨髓造血微环境，从而促进造血。为临床应用活血药于造血系统疾病，提供了实验基础。

活血化瘀药是中医常用药物。我们曾用活血化瘀治疗骨髓增生性疾患等[1]，还有报告丹参治疗再生障碍性贫血有效者[2]。因此活血化瘀药对骨髓造血的作用，值得探讨。

骨髓是主要造血器官，其组成可大致区分为造血细胞及造血基质，二者关系密切。造血基质构成微环境，供造血细胞更新、增殖和分化。已有体外培养多能祖细胞、粒系祖细胞等方法。对于造血基质，则多用Dexter[3]建立的骨髓细胞液体培养，形成贴壁细胞层，能长期支持造血细胞在体外生长。是迄今认为研究基质的最好方法。我们仿此法[4]，在骨髓贴壁细胞或基质细胞层上，直接培养粒系祖细胞，可以显示基质功能。故用此法观察了川芎嗪等对造血的作用。

材料与方法

试剂：培养液用PRMI 1640（美国产），鼠肌条件培养液作为集落刺激因子(CSF)，健康马血清(HS)及日本产琼脂。

药物：川芎嗪：每支40mg/2ml（北京第四制药厂，批号860 616)；复方丹参液(每毫升含丹参及降香各1g，江苏盐城东台制药厂，批号850 128-1)；环磷酰胺(CTX) 200mg/支（上海市第十二制药厂，批号551 104)；消炎痛(美国Sigma产)。

动物：军事医学科学院提供昆明种小鼠，体重20～25g，每批实验动物性别相同。取股骨骨髓制成单个核细胞悬液。

粒系祖细胞(CFU-GM)：用单层琼脂平皿法[5]。含30%HS、20%CSF、有核细胞1×10^5的1640 1ml/皿，于5%CO_2 37°C培养5天。计数集落（>50个细胞)。基质层(SL)：含20%HS、有核细胞2×10^5的1640 1ml/皿，5%CO_2 37°C培养1周，去上清液，洗涤3次后，平皿底上贴壁细胞层即基质层。基质层上粒系祖细胞(SL-CFU-GM)：取上述1周SL，在其上直接种入CFU-GM培养体系，5天计数集落。

实验分组：共四组，每批每组小鼠5只，各组分别皮下注射川芎嗪、复方丹参液、生理盐水(NS)各0.2ml，每天2次，连续3天。另一组于实验前一天皮下注射环磷酰胺(CTX)0.15mg/g 1次。将小鼠脱颈白处死，取各组动物骨髓细胞分别培养CFU-GM及SL，SL培养一周后，种入同种骨髓CFU-GM。

统计学处理：每次实验多组结果用F检验，各组互比则用Student-Newman-Kenlt's检验。若仅是两组均数，则用t检验。

结　果

一、川芎嗪等对CFU-GM作用：见表1。

表1　川芎嗪等对CFU-GM(1×10^5)作用 (M±SD)

	第一批	第二批	第三批
川芎嗪	45.0±7.5	39.6±5.9	51.6±6.7
复方丹参	43.8±7.7	34.2±5.5	46.2±7.7
CTX	7.6±2.6*	7.4±2.1*	3.6±2.6*
NS	38.5±8.1	34.3±4.3	45.0±7.3
F值	60.02	48.50	48.92
P值	<0.01	<0.01	<0.01

注：与NS比 *P<0.01

川芎嗪、复方丹参液均不能促进CFU-GM，而CTX组却明显减少。

二、川芎嗪等对SL-CFU-GM影响：除分别测完各组小鼠骨髓SL-CFU-GM外，为观察基质层对CFU-GM的影响，特别用活血药后，是否与前列腺素

E(PGE)有关。在各组SL上的CFU-GM培养体系中加入消炎痛，最终浓度为1×10^{-7}M，然后观察SL-CFU-GM。结果见表2。

表2 活血药及加消炎痛(1×10^{-7}M)对SL-CFU-GM(1×10^{5})作用 (M±SD)

	第一批	%	第二批	%	第三批	%
川芎嗪	52.8 ±5.3**	75.9	37.8 ±3.7**	68.6	39.4 ±3.4*	6.91
加消炎痛	81.6 ±5.2	117.2	62.7 ±4.2	114.0	58.3 ±5.6	102.2
复方丹参	50.2 ±4.3**	72.1	30.7 ±3.1	55.8	41.5 ±4.9**	72.8
加消炎痛	82.0 ±5.0	117.8	51.7 ±7.6	94.0	63.2 ±5.2	110.9
CTX	4.4 ±5.0*	44.0	28.0 ±2.0*	50.9	30.6 ±2.7*	53.7
加消炎痛	65.0 ±5.6	93.4	42.8 ±5.3	77.8	46.4 ±4.2	81.4
NS	40.0 ±6.6	57.5	27.3 ±2.2	49.5	30.2 ±4.3	53.0
加消炎痛	78.8 ±7.9	113.0	58.3 ±6.8	106.0	48.8 ±4.0	85.6
无SL-CFU-GM	69.6 ±3.4	100	55.0 ±7.0	100	57.0 ±5.1	100

注：三批实验，未加消炎痛者，$P<0.01$；各组互比，P均<0.01，与NS组比*$P<0.05$，**$P<0.01$

川芎嗪能显著促进SL上CFU-GM增殖，复方丹参液似亦有一定作用，但不如川芎嗪肯定。

各组SL-CFU-GM，加入消炎痛后，产率明显增加，示一周基质层对CFU-GM的抑制，有PGEs的介入。

讨 论

川芎嗪[6]从川芎中提取，化学结构为四甲吡嗪，有扩张血管、改善微循环、抑制血小板聚集等作用，已用于临床多种与血瘀有关疾病，如肺心病[7]等。实验研究示丹参[8]对用肾上腺素阻断的肝窦血流之恢复，较阿托品、654-2为快。还有报告[9]川芎嗪及丹参在体外均抑制成纤维细胞株增殖，认为这与活血药治疗结缔组织疾患有效有关。至于活血药对骨髓造血的作用，我们[10]曾用川芎、当归混合剂在体外观察，未见能促进CFU-GM生长。本文也未见川芎嗪、复方丹参液有刺激造血细胞增殖的作用。

骨髓细胞经体外液体培养一周，形成基质细胞层，对在其上种入的CFU-GM有明显抑制，已为我们工作证实[4]，本次生理盐水组SL-CFU-GM，仅约为无基质层CFU-GM的50%，与上次报告一致。但当小鼠经用川芎嗪注射3天后，骨髓基质层上CFU-GM较生理盐水组者显著增多。复方丹参液也有相似效应。说明川芎嗪能影响骨髓基质层，有利于CFU-GM生长。从加入消炎痛后结果看，这一影响可能与对抗前列腺素有关。我们认为川芎嗪等活血药，不能直接作用于造血细胞本身，而可能在体内影响造血微环境，从而有利于造血细胞增殖。为临床应用活血化瘀药，治疗造血系统疾病，改善造血，提供了一定实验依据。

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·新药介绍·

沛心达 (Perhexiline maleate，简称 Pexid)，学名为1-(哌啶-2-基)-2，2二环已基乙烷马来酸盐，故又名环基哌啶。药理试验表明其具有奎尼丁样作用，钙拮抗作用。能扩张冠状动脉，增加心肌供氧，减轻左心室负荷，减少心肌耗氧作用，并降低心肌自律性，延缓心室传导时间，减低运动引起的心动过速而不影响休息时心律；尚有利尿，扩张支气管的作用。据中国医学科学院阜外医院，朝阳医院等13家医院229例临床观察，每日用量150～300mg，对冠心病心绞痛和心律失常的总有效率为70%；其中室性早搏42例，有效率达90.5%；副作用小。本品已于1987年由北京双桥制药厂正式批量生产。

(李良助)

Experimental Study on Treatment and Prevention of Asymmetrical Intrauterine Growth Retardation with Huoxue Huayu(活血化瘀) Prescription

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The therapeutical and preventive effect of the compound prescription of Huoxue Huayu (promoting blood circulation to remove stasis, PBCRS) and Yiqi Huoxue (益气活血, replenishing Qi to remove stasis, RQRS) on experimental intrauterine growth retardation (IUGR) of rat by passive smoking during gestation was observed. After the mother-rats were given PBCRS prescription during gestation the fetal average birth weight, crown-heel length, liver weight and someother growth parameters were markedly improved, the hemoglobin level and hematocrit level of pregnant rat were decreased and the pathological changes of placentae were alleviated. The results of present study indicated that blood stasis is one of pathogenetic mechanism of asymmetrical IUGR and that the PBCRS recipe could improve intrauterine growth environment of fetus by way of preventing the insufficiency of hemodilution, increasing in uteroplacental blood flow and alleviating of pathological lesions in placentae. The results were likely to provide some evidences for using PBCRS and RQRS in treating and preventing IUGR in clinical practice.

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Effect of Sodium Ferulate on ^{14}C-Arachidonic Acid Metabolism in Rabbit Platelets

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Sodium ferulate(SF) is one of the antiplatelet ingredients in *Angelica sinensis*. The effect of SF on ^{14}C-arachidonic acid metabolism in washed intact rabbit platelets was studied with radiochromatography and radioautography. SF(0.1~3.2mmol/L) inhibited the generation of platelet thromboxane B_2 in a dose-dependent manner (reduced by 16.7~93.8%), and the IC_{50} was shown to be 0.762mmol/L. The formation of PGE_2 and $PGF_{2\alpha}$ was also reduced significantly when SF was used at high concentration (0.8~3.2mmol/L). These results indicated that SF might be a platelet cyclo-oxygenase inhibitor. Since the utilization of ^{14}C-AA was obviously suppressed ($P<0.001$) at the concentrations higher than 0.8mmol/L, the activity of platelet 12-lipo-oxygenase might also be inhibited by SF.

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Effect of Promoting Blood Circulation to Remove Blood Stasis Herbs on Bone Marrow Hemopoiesis

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Our experiment have shown that human or mouse marrow stroma layer(SL) has markedly inhibited the granulocyte-macrophage progenitor cell (CFU-GM) grown over them, called SL-CFU-GM, was taken as a method to investigate the bone marrow hemopoietic cell and the function of hemopoietic stroma. The mechanism of inhibition is, at least partly through prostaglandin (PG) E produced in SL. Mice were treated with promoting blood circulation to remove blood stasis herbs as ligustrazine, a compound injection of *Salvia* (composed of *Salvia miltiorrhiza* and *Dalbergia odorifera* equally), and normal saline (control) for three days. Their bone marrow cells were taken on day 4 for CFU-GM and SL-CFU-GM determination. Both of them did not enhance CFU-GM. But the SL-CFU-GM of mice injected with ligustrazine increased significantly compared to the control ($P<0.05$). The promoting effect might be by overcoming, the suppression of PGE of SL, because when indomethacin was added, the SL-CFU-GM were further increased. The results suggested that ligustrazine may ameliorate the marrow microenvironment in vivo or the stroma function improved as to enhance hemopoiesis. It may be the reason that some blood disorders with hypoplasia marrow responded well to the PBCRS herbs.

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