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河车大造丸对小鼠粒系祖细胞的影响

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内容提要　本文为探索河车大造丸的益肾补血作用，采用造血干细胞的培养技术，观察该药对供体小鼠粒系祖细胞(CFU-C CFU-D)的影响，同时测定该药对受体小鼠 CFU-D 的作用，并以生理盐水作对照，结合中医理论略加讨论。

河车大造丸补阴阳气血、益肾，用于阴阳具虚、气血衰少、精血不足的证候[1]。我室应用复方大菟丝子饮、十四味建中汤，并合用河车大造丸治疗虚劳型再生障碍性贫血，获得满意的疗效[2~4]，并对以上二个复方中药的作用机理进行探讨[5]。本实验为探索河车大造丸对小鼠粒系祖细胞的作用，采用造血干细胞培养技术，测定体外琼脂培养 CFU-C (Colony Forming Unit Culture)与体内扩散盒琼脂培养CFU-D(Colony Forming Unit in Diffusion Chamber)，观察粒系祖细胞的细胞集落产率增殖情况，报告于下。

材料与方法

一、材料

1. 动物为Laca远交健康小鼠，体重25g左右，中医研究院动物室提供。

2. 健马血清，卫生部生物制品厂产品。

3. 集落刺激因子(小鼠胚胎)本室自制。

4. 培养液，按Fisher[6]配方本室自制。

5. 培养玻璃皿、有机玻璃扩散盒，军事医学科学院产品。

6. 微孔薄膜，孔径 0.45μm，上海医药工业研究所产品。

7. 琼脂粉，上海化学试剂采购供应站试剂厂（日本进口分装)。

8. 5%二氧化碳气体，北京分析仪器厂。

9. 河车大造丸：紫河车、熟地黄、龟版、杜仲、黄柏。北京同仁堂制药厂产品，批号，京Q/YS-3-006-255。

10. 环磷酰胺，上海第十二制药厂，批号840307。

二、方法

药液制备：取河车大造丸水煎后过滤，加乙醇使最后浓度为45%、75%先后两次，置4℃冰箱过夜，过滤后去乙醇，配制为100%药液(即每ml含丸药1g)，调整pH至7.2。

细胞悬液：取费氏培养液 10ml，冲出小鼠三根股骨的全部骨髓细胞，并通过 $4\frac{1}{2}$ 针头使成单细胞骨髓悬液，计数有核细胞。

小鼠随机分组：供体或受体小鼠分给药组与生理盐水对照组，每日二次皮内注射，每次 0.2ml 共注射三天。受体小鼠在埋盒前 12 小时按每克体重给0.3mg 环磷酰胺腹腔注射一次。

体外琼脂培养CFU-C：培养体系为健马血清25%、集落刺激因子20%、费氏液和适量细胞悬液，最后加5%琼脂使最终浓度为 0.3%，充分混匀，每个培养皿分装1ml，待凝固后置干燥器，加入5%二氧化碳，湿度 100%，置 37°C培养 7 天，计数细胞集落，以>50个细胞为一个集落。

体内扩散盒琼脂培养CFU-D：培养体系为健马血清 20%，费氏液和适量细胞悬液，5%琼脂使最终浓度为0.4%，充分混匀，每个扩散盒注入0.2ml，蜡封加样口，待凝固后埋入受体小鼠腹腔内，5 天后取出扩散盒，计数细胞集落，以>50个细胞为一个集落。

实验结果

一、河车大造丸对供体小鼠CFU-C的影响：结果表明河车大造丸给药组与生理盐水对照组比较，供体小鼠CFU-C细胞集落数，每根股骨细胞数有非常显著的差异($P<0.01$)，而且各实验批数结果相近。见表1。

二、河车大造丸对供体小鼠CFU-D的影响：河车大造丸给药组对供体小鼠CFU-D细胞集落、每根股骨细胞数明显高于对照组，两组比较有显著性差异($P<0.01$)，各批实验结果相似。见表2。

三、河车大造丸对受体小鼠CFU-D的影响：结果表明，供体小鼠正常骨髓细胞种入扩散盒后，分别埋入受体小鼠的给药组与对照组，细胞集落数同样有非常显著差异($P<0.001$)，各批结果均相近。见表 3 。

表1 河车大造丸对供体小鼠CFU-C的影响

(M±SD)

批号	给药组 CFU-C/10^5	对照组 CFU-C/10^5	给药组 CFU-C/F	对照组 CFU-C/F
1	88.70±12.35 (10)	71.50±10.13** (12)	10200±1417 (10)	9533.3±1350.8 (12)
2	179.20±53.47 (10)	60.00±30.04*** (10)	25386±7574 (10)	7512.5±3755.3*** (10)
3	124.8 ±20.76 (7)	64.50±14.73*** (10)	11445±1903 (7)	5912.5±1350.2*** (10)

( )代表样品数，**P<0.01，***P<0.001

表2 河车大造丸对供体小鼠CFU-D的影响

(M±SD)

批号	给药组 CFU-D/10^4	对照组 CFU-D/10^4	给药组 CFU-D/F	对照组 CFU-D/F
1	103.9±17.14 (10)	32.0±20.57*** (9)	135070±22277 (10)	52266±33592*** (9)
2	166.6±35.92 (9)	68.8±27.70*** (9)	119364±25740 (9)	56168±22598*** (9)
3	111.2±16.44 (10)	76.7±29.22*** (13)	101932±15067 (10)	68121±31181** (13)

( )代表样品数，**P<0.01，***P<0.001

表3 河车大造丸对受体小鼠CFU-D的影响

(M±SD)

批号	有核细胞 ×10^6	给药组 CFU-D/10^4	对照组 CFU-D/10^4	t	P
1	2.75	77.35±20.37 (20)	55.47±16.89 (17)	3.57	<0.001
2	6.90	75.38±25.01 (16)	45.50±15.70 (18)	4.11	<0.001
3	3.25	91.57±18.78 (14)	54.23±15.06 (13)	5.72	<0.001

( )代表样品数。

讨论与小结

一、实验结果说明河车大造丸对供体小鼠或受体小鼠均能促进粒系祖细胞（CFU-C CFU-D）产率增殖，与盐水对照组比较有非常显著差异，在给药于受体小鼠仍能使细胞产率提高，推想药物是通过体液因子而起作用的，当然造血干细胞的调节、增殖很复杂，有待进一步深入探索。

二、实验结果有助于阐明中医“补肾生血”的理论。河车大造丸滋阴益肾、填精补血、大养元气，可增强机体的自稳状态、调节内脏功能，补肾中药对造血机能有一定增殖分化的促进作用。我室于1980年应用体外琼脂培养、体内扩散盒琼脂培养、外源性脾结节三种方法，观察复方大菟丝子饮、十四味建中汤对小鼠骨髓造血干细胞均有促进增殖的作用[5]。

三、河车大造丸来源于明代张介宾《景岳全书》卷五十七，该药的加减，各地配方略有增减，但差异不大，我们实验采用北京同仁堂制药厂产品有批号。为方便给药途径，保证剂量，以水煎醇提法，皮内给药与对照组比较有明显效果。初步认为实验结果可为临床应用该成药丸提供依据，但由于中成药复方的研究，其有效成份及其药理作用较为复杂，有待今后进一步努力探索。

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Preliminary Study of Effects of Extract of *Codonopsis Pilosula* on Immunological Functions of Normal Mice and Immunosuppressed Mice Introduced by Cyclophosphamidum

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In this study the in vitro ^{3}H-TdR incorporation lymphocyte transformation test, the test of spleen cell mediated sheep red blood hemolysis quantitated spectrophotometrically, and hemagglutination titers test are practised. The paper reports the action of *Codonopsis pilosula* on immunological functions of normal mice and immunosuppressed mice introduced by cyclophosphamidum. The result shows that this extract has immunoenhancing action of humoral immunity and cell-mediated immunity on immunosuppressed mice introduced by cyclophosphamidum, but there is no apparent immunoenhancement action in normal mice.

The result coincides with the TCM theory "reinforcement is applied in case of deficiency", therefore it is considered that the extract of *Codonopsis pilosula* can enhance immunity of "deficiency symptom-complex" organism. (Original article on page 742)

An Observation of the Granulocytic Pregenitor Cells Affected by He Che Da Zao Wan (河车大造丸)

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Aplastic anemia patients, were generally treated with prescriptions or herbs which have effects to replenish the vital energy and essence of Kidney, i,e, "Bu Shen" (补肾, tonify the kidney). He Che Da Zao Wan (HCDZW) is one of the prescription commonly used. In order to see how aplastic anemia patients have a good response to Bu Shen herbs, HCDZW was injected hypodermically to a group of mice. Normal saline was given to another group of mice as control, The bone marrow granulocytic pregenitor cells or CFU-D of the experimental group assayed by the diffusion chamber technique, were significantly greater than those of the control ($P<0.01$). HCDZW does affect CFU-D through some humoral factors. This observation throws light on TCM theory: "Bu Shen" promotes hemopoiesis. (Original article on page 739)

Pharmacological Studies of the Extract of *Equisetum Pratense* on Tolerance Towards Myocardiac Hypoxia in Animals

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The extract isolated from the herb of *Equisetum pratense* was found to be effective on cardiovascular system. The ip. with 10-15 g/kg of this extract prolonged the survival time of mice under normobaric and hypobaric hypoxia significantly, increased notably the tolerance towards myocardiac hypoxia induced by isoproterenol in mice (under hypobaric and normobaric condition) and slowed down the oxygen consumption rate by 32.8% compared with the control group. When this extract was given intraperitoneally at dosage of 10 g/kg and 15 g/kg, the percentage of myocardial uptake of ^{86}Rb, as well as propranolol, decreased by 8.2% and 26.4% as compared with control group respectively. This indicates that myocardial blood flow was not augmented. After the ip. with 15 g/kg of this extract in mice, the increase of the cAMP content of myocardium and plasma by isoproterenol was checked significantly.

Experimental myocardial ischemia was reduced by iv. 1.5-3.0 g/kg of this extract as indicated by improvement of ECG. A widening of the QRS complex and a prolongation of the RR, PQ, and QT intervals on ECG was observed. The toxic dose and minimal lethal doses of this extract given intravenously to guinea pigs were found to be 24.4 ±1.95 g/kg and 31.3±1.7 g/kg respectively. (Original article on page 744)

A Study on the Active Anti-Inflammatory Constituents in a Chinese Decoction for the Dissipation of Urinary Stones

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The therapeutic effects of a Chinese traditional decoction Hua Yu Niao Shi Tang (化瘀尿石汤) for the dissipation of the calculus in urological system have been recognized and identified. This decoction is composed of 16 herbs. On the basis of qualitative analysis, main chemical compounds——volatile oils, phenolic acids and flavonoids compounds, were isolated from this decoction, and subsequently the pharmacological experiments were carried out to observe the acute and chronic inflammatory reactions in mice and rats. These laboratory experiments have been repeated with similiar results.

Our laboratory results indicate that the anti-inflammatory action of the mixture of flavonoids and phenolic acids as well as the ethanol soluble components of this decoction is quite satisfactory with statistical significance, while that of the volatile oils is not remarkable. (Original article on page 747)