

doi: 10.13241/j.cnki.pmb.2014.33.005

龙丹生血颗粒对骨髓抑制小鼠血象与巨核细胞影响的研究*

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摘要 目的: 用环磷酰胺(CTX)复制小鼠骨髓抑制动物模型,观察龙丹生血颗粒对模型动物血液学检查和骨髓巨核细胞的影响。**方法:** 70 只昆明种小鼠标记后,除空白组外,其余动物首次尾静脉注射给予环磷酰胺 200 mg/kg,第二天后改用每天腹腔注射 30 mg/kg,连续 6 天之后,断尾采血行血液学检查。挑选外周血血小板较正常组减少至 30 %以上的小鼠,随机分为模型、白介素 -11、升血小板胶囊、龙丹生血颗粒大、中、小剂量 6 组,每组 10 只。龙丹生血颗粒大、中、小剂量组分别灌胃龙丹生血颗粒浸膏粉混悬液 13.75 g 生药/kg、6.88 g 生药/kg、3.44 g 生药/kg; 升血小板胶囊组灌胃 1.125 g 内容物/kg; 白介素 -11 组皮下注射白介素 -11 250 μ g/kg; 正常组、模型组灌胃饮用水。各组每天给药 1 次,灌胃容积 0.2 mL/10g,连续 14 天。分别于给药第 7 天、14 天采血行外周血象检查,末次采血后处死动物,取股骨骨髓做骨髓涂片,光镜检查分类计数巨核细胞数量。**结果:** 以给药后与给药前差值统计,龙丹生血颗粒大、中剂量组小鼠外周血 PLT(给药 14 天)、WBC(给药 7 天)比模型组差值明显增大($P<0.01$ 或 $P<0.05$); 龙丹生血颗粒各剂量组给药 7 天、14 天的 RBC、Hgb 比模型组同期差值明显增大($P<0.01$ 或 $P<0.05$); 龙丹生血颗粒中剂量组小鼠骨髓巨核细胞总数、颗粒巨核细胞比模型组显著增加($P<0.05$)。**结论:** 龙丹生血颗粒对环磷酰胺导致小鼠外周血 PLT、WBC、RBC、Hgb 减少具有治疗作用,但对骨髓巨核细胞的恢复作用有待进一步证实。

关键词: 龙丹生血颗粒; 环磷酰胺; 骨髓抑制

中图分类号: Q95-3; R730.5 **文献标识码:** A **文章编号:** 1673-6273(2014)33-6415-05

Effect of Long Dan Sheng Xue Granule on the Peripheral Hemogram and Megakaryocyte of Myelosuppression Model Mice induced by Cyclophosphamide*

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ABSTRACT Objective: To observe the influence of Long Dan Sheng Xue granule on hematological examination and megakaryocyte of myelosuppression model mice induced by cyclophosphamide (CTX). **Methods:** Except blank group, the mice was administered CTX (200mg/kg) from the first tail vein, and after the 2nd day change to give intraperitoneal injection 30mg/kg a day, after six consecutive days, blood sample was collected to conduct hematological examination. The mice that the peripheral blood platelet was decreased to more than 30% of normal group was selected and divided randomly into model group, interleukin-11 group, Sheng Xuexiaoban capsule group, large, middle, and small doses of Long Dan Sheng Xue Granule groups, 6 groups total and 10 mice each group. To the large, middle, and small dose of Long Dan Sheng Xue Granule groups, the mice were administered by gavage Long Dan Sheng Xue Granule extract powder mixed suspension 13.75g crude drug/kg, 6.88g crude drug/kg, 3.44g crude drug, respectively; to Sheng Xuexiaoban capsule group, 1.125g content/kg was given by gavage; to interleukin-11 group, subcutaneous injection of interleukin-11 250 μ g/kg was performed; to normal and model groups, gavage potable water was performed. The dose was once a day and the volume of gavage was 0.2mL/10g, to continue 14 days. At the 7th 14th day, blood sample was conducted to peripheral hemogram examination respectively, after the last blood sampling, the animals were executed, and extracted femoral bone marrow for bone marrow smear. Light microscope examination was performed to get the quantities of megakaryocyte of differential counting. **Results:** According to statistics of difference value after and before dosing, the difference values of PLT (dosing 14 days) and WBC (dosing 7 days) of large and middle dose of Long Dan Sheng Xue Granule groups mice in peripheral blood was increased obviously ($P<0.01$ or $P<0.05$) compared with model group; the difference value of RBC and Hgb of each dose groups of Long Dan Sheng Xue Granule with 7 days and 14 days dosing was increased obviously ($P<0.01$ or $P<0.05$) compared with the same period of model group; the difference value of total marrow? megakaryocyte count and granular megakaryocyte count of middle dose group of Long Dan Sheng Xue Granule was increased obviously ($P<0.05$) compared with model group. **Conclusion:** Long Dan Sheng Xue Granule has therapeutical effect on the inhibition of peripheral blood PLT, WBC, RBC and Hgb induced by CTX in mice. But whether it can recover bone marrow megakaryocytes remains to be further confirmed.

Key words: Long Dan Sheng Xue Granule; Cyclophosphamide; Myelosuppression

Chinese Library Classification (CLC): Q95-3; R730.5 **Document code:** A

Article ID: 1673-6273(2014)33-6415-05

* 基金项目: 2013 北京中医药大学自主选题青年教师资助项目(2013-JYBZZ-JS-162)

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(收稿日期: 2014-06-13 接受日期: 2014-07-10)

前言

环磷酰胺(CTX)属烷化剂类细胞周期非特异性抗肿瘤药,临床应用广泛,对恶性淋巴瘤、急性或慢性淋巴细胞白血病、多发性骨髓瘤疗效较好^[1],对乳腺癌、睾丸肿瘤、卵巢癌、肺癌、头颈部鳞癌、鼻咽癌、神经母细胞瘤、横纹肌肉瘤及骨肉瘤也有一定疗效^[2-3]。其不良反应有骨髓抑制,肝、肾功能损害,消化道和膀胱刺激等。其中,骨髓抑制最为常见^[4,5]。本实验采用CTX复制小鼠骨髓抑制动物模型^[6-8],观察龙丹生血颗粒对模型动物血液学检查和骨髓巨核细胞的影响,探索龙丹生血颗粒治疗肿瘤化疗后血小板减少症的可行性。

1 材料与方法

1.1 实验动物

昆明种小白鼠,二级,雌雄各半,体重 18 g ~ 22 g,由第四军医大学实验动物中心提供,许可证号 SYXK(军)2007-007。饲料为小鼠全价营养饲料,饲养条件:陕西中医学院中药药理实验室(国家中医药管理局中医药科研三级实验室),实验动物使用许可证号 SYXK(陕)2007-006,室温 20℃ ~ 25℃,相对湿度 45% ~ 70%。

1.2 药品与试剂

龙丹生血颗粒药物浸膏粉(炙黄芪、穿山龙、丹参、风轮菜、生甘草),棕褐色细粉,每 g 相当于 4.34 g 生药,由西安华星医药研究所提供,批号 20071220、20071221、20071222。实验时,将三个批号的浸膏粉充分混合,用饮用水配成适当浓度的龙丹生血颗粒浸膏粉混悬液,备用;升血小板胶囊(0.45 g/粒,青黛、连翘、仙鹤草、牡丹皮、甘草),陕西郝其军制药股份有限公司生产,国药准字 Z20025029,批号 20090808;注射用重组人白介素-11,齐鲁制药有限公司生产,国药准字 S20030017,批号 20100207;环磷酰胺,规格 0.2 g/瓶,山西普德药业有限公司生

产,批号 20091103。

1.3 分组给药

取昆明种小鼠,标记,除空白组外,其余动物首次尾静脉注射给予环磷酰胺 200 mg/kg,第二天后改用每天腹腔注射 30 mg/kg,连续 6 天后,断尾采血进行血液学检查。挑选外周血小板较正常组减少至 30%以上的小鼠随机分为模型组、白介素-11 组、升血小板胶囊组、龙丹生血颗粒大、中、小剂量组共 6 组,每组 10 只动物,苦味酸标记。龙丹生血颗粒大、中、小剂量组分别灌胃龙丹生血颗粒浸膏粉混悬液 13.75 g 生药/kg、6.88 g 生药/kg、3.44 g 生药/kg;升血小板胶囊组灌胃 1.125 g 内容物/kg;白介素-11 组皮下注射白介素-11 250 μg/kg。各组每天给药 1 次,灌胃容积 0.2 mL/10g。正常组、模型组灌胃饮用水,每天 1 次,灌胃容积 0.2 mL/10g。实验各组连续给药、给水 14 天。

1.4 检测指标

主要观察指标与方法:①分别于给药第 7 天、14 天尾静脉采血,血液细胞分析仪检测外周血小板(PLT)、血小板平均体积(MPV)、血小板压积(PCT)、血小板平均宽度(PDW)、白细胞(WBC)、血红蛋白(Hgb)变化。②处死动物,取一侧股骨骨髓,用 25 μL 小牛血清混匀,迅速涂片,风干后做瑞氏染色,用光镜顺序计数 100 个巨核细胞,并观察巨核细胞形态及分类(原始巨、幼稚巨、颗粒巨、产板巨、裸巨)。再以每片以直径 6 mm 的圆内细胞数目为基准,计数光镜下的巨核细胞数。

1.5 统计方法

采用 SPSS 18.0 进行统计分析,计量资料以均数 ± 标准差描述,两样本比较正态分布采用 t 检验,非正态分布采用秩和检验。P < 0.05 为差异有统计学意义。

2 结果

2.1 外周血象

实验各组模型小鼠外周血象检测结果见表 1 与 2。

表 1 各组 PLT 及其参数检测结果($\bar{x} \pm s$)
Table1 Results of PLT and its parameters ($\bar{x} \pm s$)

Group	PLT($10^9/L$)			MPV(fL)		
	Before drug	Dosing 7 days	Dosing 14 days	Before drug	Dosing 7 days	Dosing 14 days
Normal	846 ± 97**	1194 ± 301 (348 ± 304)	1097 ± 292 (251 ± 267)	8.70 ± 0.51*	9.41 ± 0.63**	8.85 ± 0.69
Model	552 ± 179 ^{△△}	965 ± 394 ^{△△} (387 ± 437)	895 ± 100 ^{△△} (341 ± 164)	9.76 ± 1.33 [△]	8.58 ± 0.56 ^{△△}	9.16 ± 0.33
Long Dan large dose	492 ± 137 ^{△△}	1253 ± 456 ^{△△} (760 ± 433)	1162 ± 384 ^{△△◇◇} (684 ± 357)	10.10 ± 1.59 ^{△△△}	8.94 ± 0.78	8.98 ± 0.62 [◇]
Long Dan middle dose	484 ± 58 ^{△△}	1159 ± 287 ^{△△◇◇} (676 ± 327)	1148 ± 155 ^{△△◇◇} (666 ± 201**)	9.09 ± 0.62 ^{◇◇}	8.50 ± 0.56 ^{△△}	8.93 ± 0.42 [◇]
Long Dan small dose	504 ± 96 ^{△△}	1163 ± 378 ^{△△◇◇} (660 ± 390)	994 ± 148 ^{△△◇◇} (486 ± 163)	8.52 ± 0.50 [△]	8.92 ± 0.45	9.81 ± 1.39 ^{△△}
Sheng Xuexiaoban capsule	487 ± 86 ^{△△}	861 ± 221 ^{△△◇◇} (390 ± 214)	1016 ± 311 ^{◇◇} (545 ± 311)	9.36 ± 1.46 [◇]	8.77 ± 0.55 [△]	9.64 ± 1.57 ^{△△}
Interleukin-11	494 ± 193 ^{△△}	1258 ± 318 ^{△△} (764 ± 297 [△])	1332 ± 145 ^{△△△△} (838 ± 255 ^{△△})	10.18 ± 1.21 ^{△△△}	8.57 ± 0.70 ^{△△}	9.76 ± 1.17 ^{△△}

注:①与模型组比较,*P<0.05,**P<0.01;与正常组比较,△P<0.05,△△P<0.01;与升血小板胶囊组比较,△P<0.05,△△P<0.01;

与皮下注射白介素组比较, $^{\circ}P<0.05$, $^{\diamond}P<0.01$; ②括弧内是给药后与给药前差值的; ③给药前, 各组动物均为 10 只: 给药 7 天后, 除模型组 8 只、龙丹生血颗粒中、小剂量组及升血小板胶囊组 9 只外, 其余各组均 10 只; 给药 14 天后, 除模型组 7 只、龙丹生血颗粒中、小剂量组及升血小板胶囊组 9 只外, 其余各组均 10 只。④续表同。

Note: ① Compared with model group, $^{\circ}P<0.05$, $^{\diamond}P<0.01$. Compared with normal group, $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$. Compared with Sheng Xuexiaoban capsule group, $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$. Compared with interleukin-11 group, $^{\circ}P<0.05$, $^{\diamond}P<0.01$. ② Numbers in parentheses is of the difference with before and after drug. ③ Before drug, there are 10 mice in each group. At the 7th day, there are 8 mice in model group, 9 in Long Dan middle, small dose and Sheng Xuexiaoban capsule group, 10 in other groups. At the 14th day, there are 7 mice in model group, 9 in Long Dan middle, small dose and Sheng Xuexiaoban capsule group, 10 in other groups. ④ The same with continued 1.

续表 1 各组 PLT 及其参数检测结果($\bar{x} \pm s$)
Continued 1 Results of PLT and its parameters ($\bar{x} \pm s$)

Group	PDW(%)			PCT(%)		
	Before drug	Dosing 7 days	Dosing 14 days	Before drug	Dosing 7 days	Dosing 14 days
Normal	14.01 $\pm$ 1.46	14.66 $\pm$ 1.18	14.12 $\pm$ 1.90	0.74 $\pm$ 0.10 $^{\circ}\Delta\Delta^{\diamond}$	1.14 $\pm$ 0.33 $^{\Delta\Delta}$	0.97 $\pm$ 0.27 $^{\diamond}$
Model	15.55 $\pm$ 2.02	12.93 $\pm$ 1.36	15.04 $\pm$ 1.08	0.52 $\pm$ 0.14 $^{\Delta}$	0.83 $\pm$ 0.37 $^{\Delta}$	0.82 $\pm$ 0.11
Long Dan large dose	15.72 $\pm$ 2.36	13.64 $\pm$ 1.67	14.28 $\pm$ 1.55	0.49 $\pm$ 0.12 $^{\Delta}$	1.11 $\pm$ 0.37 $^{\Delta}$	1.06 $\pm$ 0.40
Long Dan middle dose	14.37 $\pm$ 1.65	12.74 $\pm$ 1.37	13.98 $\pm$ 1.13	0.44 $\pm$ 0.05 $^{\Delta\Delta}$	0.99 $\pm$ 0.27	1.03 $\pm$ 0.17 $^{\diamond}$
Long Dan small dose	12.82 $\pm$ 1.29 $^{\diamond\diamond}$	13.73 $\pm$ 1.28	15.60 $\pm$ 2.13	0.42 $\pm$ 0.07 $^{\Delta\Delta}$	1.04 $\pm$ 0.34 $^{\Delta}$	0.98 $\pm$ 0.21 $^{\diamond}$
Sheng Xuexiaoban capsule	14.62 $\pm$ 2.21	13.39 $\pm$ 1.68	14.88 $\pm$ 2.08	0.45 $\pm$ 0.10 $^{\Delta\Delta}$	0.75 $\pm$ 0.16 $^{\Delta\Delta\diamond}$	1.01 $\pm$ 0.50
Interleukin-11	16.47 $\pm$ 1.8	13.25 $\pm$ 1.93	16.02 $\pm$ 2.33	0.48 $\pm$ 0.15 $^{\Delta}$	1.09 $\pm$ 0.33 $^{\Delta}$	1.3 $\pm$ 0.22 $^{\circ}\Delta\Delta^{\Delta}$

表 2 各组 RBC、WBC、Hgb 检测结果($\bar{x} \pm s$)
Table 2 Results of RBC, WBC and Hgb ($\bar{x} \pm s$)

Group	RBC(10 ⁹ /L)			WBC(10 ⁹ /L)		
	Before drug	Dosing 7 days	Dosing 14 days	Before drug	Dosing 7 days	Dosing 14 days
Normal	10.47 $\pm$ 0.66 $^{\circ}\Delta\Delta^{\diamond\diamond}$	9.97 $\pm$ 2.52 $^{\Delta\Delta\diamond}$ (-0.50 $\pm$ 2.64)	9.99 $\pm$ 0.91 (-0.48 $\pm$ 0.85 $^{\circ}$)	9.93 $\pm$ 3.02 $^{\circ}\Delta\Delta^{\diamond\diamond}$	15.28 $\pm$ 3.36 $^{\Delta\Delta\diamond}$ (5.4 $\pm$ 3.4)	12.19 $\pm$ 3.00 $^{\Delta\Delta\diamond\diamond}$ (2.3 $\pm$ 2.9)
Model	8.60 $\pm$ 1.39 $^{\Delta\Delta}$	7.19 $\pm$ 2.29 $^{\Delta\Delta}$ (-1.82 $\pm$ 2.38)	10.09 $\pm$ 0.49 (1.28 $\pm$ 1.35)	5.96 $\pm$ 3.34 $^{\Delta\Delta}$	11.59 $\pm$ 4.08 $^{\Delta\Delta}$ (5.3 $\pm$ 6.5)	14.27 $\pm$ 2.54 $^{\Delta\Delta}$ (8.5 $\pm$ 5.1)
Long Dan large dose	7.85 $\pm$ 1.0 $^{\Delta\Delta}$	8.51 $\pm$ 1.91 $^{\Delta\Delta\Delta}$ (0.67 $\pm$ 2.40 $^{\circ}$)	9.61 $\pm$ 0.72 (1.83 $\pm$ 1.37)	2.82 $\pm$ 0.90 $^{\Delta\Delta\diamond\diamond}$	16.93 $\pm$ 9.08 $^{\Delta\Delta\diamond\diamond}$ (14.1 $\pm$ 9.2 $^{\circ}$)	10.41 $\pm$ 1.33 $^{\Delta\Delta*}$ (7.7 $\pm$ 1.8)
Long Dan middle dose	6.08 $\pm$ 0.58 $^{\Delta\Delta\Delta\Delta\diamond\diamond}$	8.44 $\pm$ 1.74 $^{\Delta\Delta\Delta}$ (2.31 $\pm$ 1.74 $^{\circ}$)	10.22 $\pm$ 0.69 (4.09 $\pm$ 0.86 $^{\circ}$)	2.09 $\pm$ 0.89 $^{\Delta\Delta\diamond\diamond}$	12.83 $\pm$ 3.04 $^{\Delta\Delta}$ (10.9 $\pm$ 2.6 $^{\circ}$)	10.75 $\pm$ 1.87 $^{\circ}\Delta\Delta^{\Delta}$ (8.8 $\pm$ 2.1)
Long Dan small dose	6.02 $\pm$ 0.87 $^{\Delta\Delta\Delta\Delta\Delta\diamond\diamond}$	8.43 $\pm$ 1.84 $^{\Delta\Delta\Delta\Delta}$ (2.55 $\pm$ 1.89 $^{\circ}$)	9.43 $\pm$ 1.15 (3.55 $\pm$ 1.77 $^{\circ}$)	3.86 $\pm$ 2.34 $^{\Delta\Delta\diamond\diamond}$	11.80 $\pm$ 3.65 $^{\Delta\Delta\Delta\diamond}$ (8.1 $\pm$ 3.6)	11.50 $\pm$ 1.51 $^{\circ}\Delta\Delta^{\Delta\diamond}$ (7.6 $\pm$ 1.8)
Sheng Xuexiaoban capsule	8.13 $\pm$ 0.82 $^{\Delta\Delta}$	6.97 $\pm$ 1.56 $^{\Delta\Delta\diamond\diamond}$ (-1.25 $\pm$ 2.22)	9.76 $\pm$ 0.85 (1.55 $\pm$ 0.98)	3.55 $\pm$ 1.59 $^{\Delta\Delta\diamond\diamond}$	13.46 $\pm$ 8.84 $^{\Delta}$ (9.8 $\pm$ 9.0)	9.36 $\pm$ 2.14 $^{\circ}\Delta\Delta^{\Delta}$ (5.7 $\pm$ 3.2)
Interleukin-11	8.88 $\pm$ 1.11 $^{\Delta}$	8.62 $\pm$ 1.80 $^{\Delta\Delta\Delta}$ (-0.26 $\pm$ 2.62)	10.18 $\pm$ 0.83 (1.30 $\pm$ 1.43)	5.13 $\pm$ 2.49 $^{\Delta\Delta\Delta}$	12.93 $\pm$ 5.02 $^{\Delta\Delta}$ (7.8 $\pm$ 7.0)	10.14 $\pm$ 2.24 $^{\circ}\Delta\Delta^{\Delta\Delta}$ (5.0 $\pm$ 3.3)

注: ①与模型组比较, $^{\circ}P<0.05$, $^{\circ\circ}P<0.01$; 与正常组比较, $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$; 与升血小板胶囊组比较, $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$; 与皮下注射白介素组比较, $^{\circ}P<0.05$, $^{\diamond}P<0.01$; ②括弧内是给药后与给药前的差值; ③给药前, 各组动物均为 10 只: 给药 7 天后, 除模型组 8 只、龙丹生血颗粒中、小剂量组及升血小板胶囊组 9 只外, 其余各组均 10 只; 给药 14 天后, 除模型组 7 只、龙丹生血颗粒中、小剂量组及升血小板胶囊组 9 只外, 其余各组均 10 只。④续表同。

Note: ① Compared with model group, $^{\circ}P<0.05$, $^{\circ\circ}P<0.01$. Compared with normal group, $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$. Compared with Sheng Xuexiaoban capsule group, $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$. Compared with interleukin-11 group, $^{\circ}P<0.05$, $^{\diamond}P<0.01$. ② Numbers in parentheses is of the difference with before and after drug. ③ Before drug, there are 10 mice in each group. At the 7th day, there are 8 mice in model group, 9 in Long Dan middle, small dose and Sheng Xuexiaoban capsule group, 10 in other groups. At the 14th day, there are 7 mice in model group, 9 in Long Dan middle, small dose and Sheng Xuexiaoban capsule group, 10 in other groups. ④ The same with continued 2.

续表 2 各组 RBC、WBC、Hgb 检测结果($\bar{x} \pm s$)Continued Table 2 Results of RBC, WBC and Hgb ($\bar{x} \pm s$)

Group	HGB(g/L)		
	Before drug	Dosing 7 days	Dosing 14 days
Normal	169.5 $\pm$ 10.4*	171.8 $\pm$ 12.8**▲▲◇ (2.3 $\pm$ 17.4 [◇])	156.4 $\pm$ 18.1 (-13 $\pm$ 15 [◇])
Model	138.5 $\pm$ 23.6	115.1 $\pm$ 36.8 ^{△△} (-30 $\pm$ 38)	155.9 $\pm$ 11.9 (14 $\pm$ 28)
Long Dan large dose	122.9 $\pm$ 17.2	136.8 $\pm$ 29.8 (14 $\pm$ 37 [◇])	156.8 $\pm$ 12.5 (35 $\pm$ 22)
Long Dan middle dose	94.6 $\pm$ 11.3 ^{△△◇◇}	137.6 $\pm$ 29.5 (43 $\pm$ 28 [◇])	165.1 $\pm$ 11.1 (70 $\pm$ 15 [◇])
Long Dan small dose	96.1 $\pm$ 13.3 ^{△△◇◇}	134.1 $\pm$ 31.2 (40 $\pm$ 30 [◇])	147.0 $\pm$ 17.3 (54 $\pm$ 23 [◇])
Sheng Xuexiaoban capsule	128.2 $\pm$ 13.4	111.6 $\pm$ 28.3 ^{△△} (-18 $\pm$ 38)	151.8 $\pm$ 12.4 (22 $\pm$ 23)
Interleukin-11	143.3 $\pm$ 18.3	135.4 $\pm$ 27.2 (-8 $\pm$ 39)	153.3 $\pm$ 12.6 (10 $\pm$ 22)

表 1、表 2 结果表明:①模型组动物给药前 PLT、RBC、WBC 和 Hgb 比正常组明显减少($P<0.01$ 或 $P<0.05$)。②给药 7 天、14 天时间段,龙丹生血颗粒各剂量组 PLT(7 天)、WBC、RBC、Hgb 与模型组比较无统计学意义($P>0.05$)。③龙丹生血颗粒中剂量组给药 14 天 PLT、PCT 较模型组显著升高($P<0.01$)。④按给药后减给药前差值统计表明,龙丹生血颗粒大、中剂量组 PLT(给药 14 天)、WBC(给药 7 天)与模型组比较差值增大($P<0.01$ 或 $P<0.05$);龙丹生血颗粒大、中、小剂量组给药 7 天、14 天的 RBC、Hgb 与模型组同期差值增大($P<0.01$ 或 $P<0.05$)。⑤与升

血小板胶囊组比较,龙丹生血颗粒给药 7 天、14 天时间段大、中剂量组 PLT 显著升高($P<0.05$),各剂量组 WBC、RBC 均显著升高($P<0.05$),HGB 无明显差异($P>0.05$)。⑥与白介素-11 组比较,龙丹生血颗粒大、中剂量组 PLT、RBC(14 天)显著升高($P<0.05$),大剂量组 WBC(7 天、14 天)显著升高($P<0.05$),HGB 无明显差异($P>0.05$)。

2.2 骨髓巨核细胞

各组慢性小鼠骨髓巨核细胞总数及其分类检测结果见表

3。

表 3 各组骨髓巨核细胞总数及其分类检测结果(个, $\bar{x} \pm s$)Table 3 Bone marrow megakaryocyte count and classification in each group (number, $\bar{x} \pm s$)

Group	Original	Immature	Granular	Platellate-forming	Naked nucleus	Micromegakaryocyte	Total
Normal	3 $\pm$ 1	19 $\pm$ 9	25 $\pm$ 7	1 $\pm$ 1	3 $\pm$ 3	0 $\pm$ 0	331 $\pm$ 73
Model	7 $\pm$ 5 [△]	15 $\pm$ 3	19 $\pm$ 8	2 $\pm$ 2	5 $\pm$ 5	0 $\pm$ 1	192 $\pm$ 111 ^{△△}
Long Dan large dose	4 $\pm$ 3	15 $\pm$ 5	22 $\pm$ 5 [▲]	5 $\pm$ 3	3 $\pm$ 5	1 $\pm$ 2	219 $\pm$ 145 ^{△△▲▲}
Long Dan middle dose	3 $\pm$ 1 [▲]	14 $\pm$ 4	27 $\pm$ 3 ^{▲▲}	2 $\pm$ 2	3 $\pm$ 1	2 $\pm$ 2	304 $\pm$ 80 ^{▲▲}
Long Dan small dose	4 $\pm$ 2 [▲]	15 $\pm$ 7	21 $\pm$ 6	3 $\pm$ 2	2 $\pm$ 2	1 $\pm$ 1	228 $\pm$ 233 ^{△△▲▲}
Sheng Xuexiaoban capsule	7 $\pm$ 7 ^{△◇}	11 $\pm$ 8 ^{△△}	13 $\pm$ 6 ^{△△◇◇}	3 $\pm$ 1	8 $\pm$ 9	0 $\pm$ 0	84 $\pm$ 55 ^{△△◇◇}
interleukin-11	3 $\pm$ 4	16 $\pm$ 8	24 $\pm$ 9 ^{▲▲}	3 $\pm$ 2	3 $\pm$ 3	0 $\pm$ 1	284 $\pm$ 204 ^{▲▲}

注:①与模型组比较,* $P<0.05$,** $P<0.01$;与正常组比较,△ $P<0.05$,△△ $P<0.01$;与升血小板胶囊组比较,▲ $P<0.05$,▲▲ $P<0.01$;与皮下注射白介素组比较,◇ $P<0.05$,◇◇ $P<0.01$;②各组动物数,除空白组 10 只、模型组和龙丹生血颗粒小剂量组 7 只、白介素-11 组 9 只以外,其余各组均 8 只。

Note: ① Compared with model group, * $P<0.05$, ** $P<0.01$. Compared with normal group, △ $P<0.05$, △△ $P<0.01$. Compared with Sheng Xuexiaoban capsule group, ▲ $P<0.05$, ▲▲ $P<0.01$. Compared with interleukin-11 group, ◇ $P<0.05$, ◇◇ $P<0.01$. ② There are 10 mice in normal group, 7 in model and Long Dan small dose group, 9 in interleukin-11 group, 8 in other groups.

表 3 结果表明:①与正常组比较,模型组小鼠骨髓巨核细胞总数显著减少,原始巨核细胞增加,差异有统计学意义($P<0.05$, 0.01)。其余各分类巨核细胞无明显差异($P>0.05$)。②与模型组比较,龙丹生血颗粒中剂量组巨核细胞总数、颗粒巨核细胞显著增加,差异有统计学意义($P<0.05$)。各剂量组其余各分类巨核细胞均无明显差异($P>0.05$)。③龙丹生血颗粒各剂

量组巨核细胞总数与白介素-11 组比较均无明显差异($P>0.05$)。④龙丹生血颗粒各剂量组、白介素-11 组巨核细胞总数均显著高于升血小板胶囊组,差异有统计学意义($P<0.01$)。

3 讨论

化疗后骨髓抑制指的是应用化疗药物后出现外周血单项

或全血细胞减少,骨髓增生减低^[9]。主要临床表现有贫血、不同程度的出血和感染^[10]。CTX 为常用的烷化剂类抗肿瘤药物,骨髓抑制为其限制性不良反应,能导致外周血象下降,尤其以 WBC、PLT 减少最为明显^[11]。因此,在实验研究中通常选用 CTX 复制骨髓抑制动物模型来研究药物抗骨髓抑制效能。

中医可骨髓抑制归属于“虚劳”、“血证”、“外感发热”或“内伤发热”等病证范畴^[12]。其病机主要为毒邪入侵机体,与气血相搏,毒邪过盛而导致气血两伤;药毒中伤脾胃,运化失常,水谷之精微物质缺乏,气血生化无源而导致气血两虚;药毒侵入骨髓,骨髓功能失司,血液生成减低,以致阴血亏虚;肾精亏损,精不养髓,髓不化血以致血液虚少;气血亏虚,进一步发展而致阴阳受损,使气血阴阳俱虚;气虚无以推运血行,血必有瘀;阴血虚少,脉道艰涩,血流不畅,血必有滞;阳虚鼓脉无力,或阳虚生内寒,血遇寒则凝,亦可形成血瘀^[13-15]。通过对中医药防治化疗后骨髓抑制的文献分析,目前骨髓抑制的治疗以益气养血为主,兼顾祛瘀,此类中药在化疗后外周血象恢复、化疗完成率、生活质量及中医症状改善方面都有明显优势^[16,17]。针对化疗后骨髓抑制的病机特点,以及文献分析,拟定“益气养血、活血化瘀”为治则。龙丹生血颗粒由炙黄芪、穿山龙、丹参、风轮菜、生甘草组成,具有益气养血、活血化瘀之功效。方中黄芪为补气之圣药,善疗诸气虚,为君药,本草论述黄芪“功用甚多,而其独效者,又在补血”“盖气无形,血则有形。有形不能速生,必得无形之气以生之”。臣以穿山龙、丹参活血祛瘀,凉血养血,古有“一味丹参功同四物”之说;丹参配黄芪益气养血、祛瘀生新。风轮菜清热解毒,凉血止血,炙甘草调和诸药,为方中佐使。诸药合用,共奏益气养血、活血化瘀之功。

本实验成功复制了环磷酰胺致小鼠外周血象下降的动物模型,并经龙丹生血颗粒干预治疗。结果证实,以临床实践为出发点的龙丹生血颗粒对化疗后骨髓抑制模型小鼠有一定的治疗作用,具体体现在:①龙丹生血颗粒大、中剂量可显著提升模型小鼠外周血 PLT 与 WBC。②龙丹生血颗粒大、中、小剂量可显著提升模型小鼠外周血 RBC 与 Hgb。③龙丹生血颗粒中剂量组对模型小鼠骨髓巨核细胞总数有一定的恢复作用。④与对照药物升血小板胶囊及白介素-11 比较,龙丹生血颗粒升高 PLT、WBC 疗效较显著,但对 HGB 及骨髓巨核细胞恢复无明显优势。以上研究结果说明,龙丹生血颗粒对环磷酰胺导致小鼠外周血 PLT、WBC、RBC、Hgb 减少^[18]有一定的治疗作用,但对骨髓巨核细胞的恢复作用有待进一步证实。

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