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补骨脂素加紫外光照对人白血病细胞株的抑制作用

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Inhibitory effects of psoralen plus ultraviolet irradiation on human leukemic cell lines

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ABSTRACT　A semi-solid cell culture technique was used to study the sensitivity of K562, HL-60, and Raji leukemic cell lines to the inhibitory effect of psoralen plus ultraviolet irradiation. Results indicated that: 1) the inhibition rate of K562, HL-60, and Raji cell lines were 86%, 35%, and 36%, respectively; and 2) K562, HL-60, and Raji cell lines were treated with psoralen (20 μg·ml^{-1}) for 1 h, then irradiated with ultraviolet (1 J/cm^2) for 10 min, none of the leukemic cell lines showed colony or cluster formation. These suggested that the cytocidal effect of psoralen plus ultraviolet might be useful to eradicate the residual leukemic cells in the bone marrow transplantation.

KEY WORDS　psoralens; leukemia; cell line; bone marrow; autologous transplantation; colony-forming units assay; ultraviolet therapy

摘要　用半固体细胞定量培养观察补骨脂素加长波紫外光照射对K562，HL-60和Raji白血病细胞株的抑制作用．发现补骨脂素对K562细胞克隆形成抑制率达86%；补骨脂素20 μg·ml^{-1}预孵1 h，加紫外光照1 J/cm^{2}后，上述细胞株均无集落和丛生长．提示补骨脂素加紫外光照对白血病细胞株呈非常显著光灭活作用（$P<0.01$），可用于自体骨髓移植前净化骨髓．

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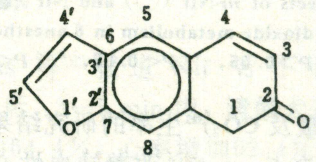
关键词　补骨脂素类；白血病；细胞株；骨髓；自体移植；集落形成单位测定；紫外治疗法

补骨脂素（psoralen）系中药补骨脂的提取物[1]，其在体内外均有一定抗肿瘤活性，当被365 nm波长光激发后能与细胞内DNA双链形成交联并释放单态氧等活性物质，使其抗肿瘤活性得到显著增强[2-4]，迄今未见其光动力学作用在骨髓净化实验研究的报道．本研究通过半固体细胞定量培养测定三种白血病细胞株对补骨脂素加紫外光照的敏感性来判断其在自体骨髓移植前体外净化残存白血病细胞的可能性．



M$_r$ 186.16

MATERIALS AND METHODS

细胞克隆形成分析　人红白血病细胞株K562和人B淋巴细胞白血病细胞株Raji本实验室传代保存．人早幼粒细胞白血病细胞株HL-60购于军事医学科学院．

收集液体培养的上述细胞（1000×g，10 min），在35 mm玻璃平皿中加含2×10^{4}细胞及0.3%琼脂的生长培养基1 ml．培养基由20%新鲜小牛血清，20%胎肌培养液的RPMI 1640液组成．将接种细胞后的平皿置入灭菌的150 mm玻璃平皿中，放入37℃、5% CO_2全湿度温育7 d，在倒置显微镜下计数40个细胞以上的集落，每测定点由3—4个平皿组成，取其平均数．

克隆生长抑制率＝100%×(1－实验组克隆生长率/对照组克隆生长率).

人胎肌培养液制备 取水囊引产胎儿(3－6个月)，无菌条件下取其股骨肌剪成0.3 mm 大小的碎块，用RPMI1640液洗涤3－4次，按1 g (湿重)肌肉加入含20%小牛血清的 RPMI1640液9 ml，放入37℃温育10 d，离心收集上清液，分装后－20℃保存.

光动力学作用敏感性测定 受试细胞悬液用补骨脂素（第三军医大学新桥医院生产，批号860506）20 $\mu g \cdot ml^{-1}$，在37℃，5% CO_2处理1 h 后装入石英玻璃瓶内，置主波长365 nm 的 XZY-B 量子血液治疗仪上固定(第四军医大学西京医院临床输血研究所)，紫外光源位于石英瓶正下方40 cm 处，时间10 min，辐射量1 J/cm^2(陕西省计量局光学测试所测定)，边照射边震荡，照射完毕即行半固体培养. 采用药物与细胞持续接触法.

RESULTS

长波紫外光照(1 J/cm^2) 10 min 对 K562，HL-60和 Raji 细胞株的克隆形成无明显影响. 补骨脂素20 $\mu g \cdot ml^{-1}$对 K562及 Raji 细胞株克隆的形成与对照组相比有抑制作用($P < 0.05$)，而对 HL-60细胞株无明显影响($P > 0.05$). 补骨脂素20 $\mu g \cdot ml^{-1}$在培养基温育1 h 后加长波紫外光照1 J/cm^2，10 min 则100%抑制这三种细胞株的形成(Tab 1).

Tab 1. Sensitivity of K562, HL-60, and Raji cell lines to psoralen (20 $\mu g \cdot ml^{-1}$) plus ultraviolet irradiation (1 J/cm^2, 10 min). $n = 3-5$ experiments, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

	Colony counts/2×10^4 cells (Inhibition%)		
	K562	HL-60	Raji
Control	61±51	189±54	154±37
Ultraviolet	52±18^a (15±7)	142±11^a (25±9)	128±30^a (17±17)
Psoralen	9±3^b (86±10)	122±24^a (35±21)	93±20^b (36±13)
Psoralen +ultraviolet	0±0^c (100)	0±0^c (100)	0±0^c (100)

DISCUSSION

在清除骨髓中残存白血病细胞方面，已报道的方法有将42℃高温，单克隆抗体偶联毒素或环磷酰胺衍生物等净化处理[5－6]. 光敏剂血卟啉衍生物虽被证实对白血病细胞有选择性杀伤作用[7]，但因其选择性不是很高，本身无抗肿瘤活性且机体正常组织含有相同成分，因此使之过渡到临床应用受到限制.

我们的研究表明：补骨脂素对 K562，HL-60和 Raji 等白血病细胞株均有一定程度的杀伤作用，初步显示其广谱抗瘤活性，但K562对补骨脂素更为敏感. 补骨脂素与上述细胞共孵1 h 后光照10 min，1 J/cm^2，均未见集落和丛形成，与补骨脂素组相比具有显著的光灭活作用($P < 0.01$).

我们曾观察补骨脂素20 $\mu g \cdot ml^{-1}$加长波紫外光照10 min 对小鼠脾结节形成单位(CFU-S)的影响较小，其中 d 13脾结节形成单位($CFU\text{-}S_1$)比 d 8脾结节形成单位($CFU\text{-}S_2$)要更为耐受(前者抑制率1.17%，后者为10.8%)[8]，而 $CFU\text{-}S_1$有较强自我更新潜能，在造血功能重建上有更重要意义. 因此采用补骨脂素加长波紫外光净化白血病患者完全缓解期骨髓中残存白血病细胞，有可能较少损伤正常造血干细胞而较彻底清除白血病细胞. 如能结合高温，单克隆抗体和脂质体等技术，则其更可能应用于临床.

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商陆多糖 I 对小鼠脾细胞体外产生集落刺激因子的影响[1]

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Effect of *Phytolacca acinosa* polysaccharides I on production of colony-stimulating factors of mouse splenocytes *in vitro*

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ABSTRACT *Phytolacca acinose* polysaccharides I (PAP-I), M_r=10 000, activated mouse splenocytes to produce colony-stimulating factors (CSF) *in vitro*. The level of CSF was tested by [^{3}H]TdR uptaken by bone marrow cells and rmGM-CSF was used as standard. PAP-I (10—500 μg·ml^{-1}) increased CSF production of the splenocytes treated with or without concanavalin A. When the concentration of PAP-I was 100 μg·ml^{-1}, the level of CSF was about equivalent to that of rmGM-CSF 11.8±1.8 ng·ml^{-1}. After a 3-d incubation of PAP-I with the splenocytes, CSF was assayed. The longer the incubation, the higher were the CSF concentrations. The CSF type in supernants of splenocytes induced by PAP-I was determined by IL-3 McAb, GM-CSF McAb, and M-CSF PcAb. The type of CSF was found to be interleukin-3.

KEY WORDS *Phytolacca acinosa*; polysaccharides; concanavalin A; colony-stimulating factors; interleukin-3; spleen

摘要 以 rmGM-CSF 作标准品, [^{3}H]TdR 骨髓细胞参入法定量检测 PAP-I 刺激小鼠脾细胞产生 CSF, 表明 PAP-I 10—500 μg·ml^{-1}显著促进脾细胞和 Con A 诱导的脾细胞产生 CSF. PAP-I 100 μg·ml^{-1}可刺激产生相当于 rmGM-CSF 11.8±1.8 ng·ml^{-1}的 CSF. PAP-I 与脾细胞培养前48 h 无 CSF 检出, 随着培养时间延长, CSF 含量越来越高. 经 CSF 的特异性抗体吸收实验证明 PAP-I 刺激产生的 CSF 为 IL-3.

关键词 商陆; 多糖; 刀豆球蛋白 A; 集落刺激因子; 白细胞介素3; 脾

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多糖的辐射防护作用可能与其促进造血过程、激活网状内皮系统、增强吞噬细胞活性、刺激淋巴组织、增强机体免疫功能有关[1]. 集落刺激因子(colony-stimulating factors, CSF)是一类促进造血祖细胞增殖、分化、成熟及存活的糖蛋白, 在造血功能调控中占有重要地位[2]. 商陆多糖-I (PAP-I) 是从中国