69-71, 图版2

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Jan; 17 (1): 69—71

索布佐生和阿霉素对小鼠白血病P388的协同作用

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Synergism of sobuzoxane in combination with doxorubicin against leukemia P388 in mice

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KEY WORDS sobuzoxane; doxorubicin; leukemia P388; myocardium; drug synergism; electron microscopy

AIM: To study the antitumor activity of sobuzoxane (Sob) in combination with doxorubicin (Dox) and the effect of Sob on Dox-induced cardiotoxicity. **METHODS**: DBA/2 mice bearing transplanted leukemia P388 were given iv Dox 2 $mg \cdot kg^{-1} \cdot d^{-1}$ for 3 d, 4 $mg \cdot kg^{-1} \cdot d^{-1}$ for 1 d combined with ig Sob 20, 40 $mg \cdot kg^{-1} \cdot d^{-1}$ for 7 d. The increase in life span (ILS) of each group was recorded in 30 d. The myocardium of moribund mice was examined by transmission electron microscopy. **RESULTS**: The ILS of combination therapeutic groups of Sob with Dox was 48.7 %, 57.3 %, 59.0 %, and 62.4 % respectively, which were 30 %—90 % higher than the sum of ILS of two groups treated with Dox and Sob separately ($P<0.01$). The ultrastructural injury of cardiomyocytes of P388-bearing mice caused by combination chemotherapy with Dox plus Sob was markedly attenuated compared with Dox alone. **CONCLUSION**: Sob with Dox exhibited an antitumor synergistic effect on leukemia P388, and the cardiotoxicity of Dox was reduced by Sob.

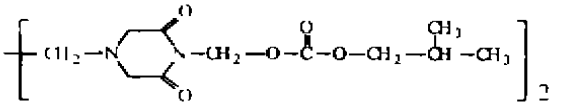
R979.1 R733.7

关键词 索布佐生；阿霉素；白血病P388；心肌；药物协同作用；电子显微镜检查 鼠

目的：研究索布佐生(Sob)与阿霉素(Dox)联合应用的抑瘤作用以及Sob对Dox诱导的心脏毒性的影响。**方法**：DBA/2小鼠接种P388细胞，次日起iv Dox 2 $mg \cdot kg^{-1} \cdot d^{-1} \times 3$ d及4 $mg \cdot kg^{-1} \cdot d^{-1} \times 1$ d，ig Sob 20 $mg \cdot kg^{-1} \cdot d^{-1} \times 7$ d及40 $mg \cdot kg^{-1} \cdot d^{-1} \times 7$ d，配对联用。统计各组的生命延长率(ILS)；电镜观察荷瘤鼠垂死前的心肌毒性表现。**结果**：联合用药组的ILS分别为48.7 %，57.3 %，59.0 %和62.4 %，是Sob和Dox单药应用ILS之和的130 %—190 %；荷瘤鼠心肌细胞的超微结构损伤较单用Dox组明显减轻。**结论**：Sob与Dox联用对小鼠白血病P388有显著的抑瘤协同作用；Sob能降低Dox所致的心肌毒性。

Received 1995-01-17 Accepted 1995-05-18

索布佐生(sobuzoxane，Sob，代号MST-16)系我所首先合成的乙亚胺(ICRF-154)的衍生物[1]。与同类化合物乙亚胺、丙亚胺(razoxane)相比，Sob脂溶性强，体内吸收容易，化疗指数提高一倍左右[2]。临床试用对恶性淋巴瘤、成人T-细胞白血病有明显的疗效[3,4]。日本厚生省业已批准上市[5]。ip razoxane与Dox联用对小鼠白血病L1210有抑瘤协同作用[6]，但临床口服给药，骨髓毒性增加，与Dox联用效果不理想[7]。为了寻找Sob临床扩大应用的可能性，我们以小鼠白血病P388为模型，观察ig Sob和Dox联合应用的抑瘤作用和Sob对Dox所诱导的心肌超微结构毒性的影响。



Chemical structure of sobuzoxane
4,4'-ethylenebis[1-(hydroxymethyl)-2,6-piperazinedione]bis(isobutyl carbonate)

MATERIALS AND METHODS

Sob由我所合成，用0.5 %羧甲基纤维素钠(粘度RH 300—600 cp 上海化学试剂站分装)配制成混悬液。盐酸阿霉素系浙江省海门制药厂生产，批号920606，以生理盐水溶解稀释。

清洁级DBA/2小鼠123只，♀性，体重18—22 g。由中国科学院上海实验动物中心提供(合格证号：中科005)。

给药前一日(d_0) ip 接种 P388细胞5×10^5个. 随机分成9组, 生理盐水(NS)对照组19只, 其余各组均为13只, 每日定时给药, iv 后1 h ig. 各组给药方案如下:

A) d_1-d_7 iv NS 0.2 mL; B) d_1 iv Dox 4 mg·kg^{-1}; C) d_1 iv Dox 4 mg·kg^{-1}, d_1-d_7 ig Sob 20 mg·kg^{-1}; D) d_1 iv Dox 4 mg·kg^{-1}, d_1-d_7 ig Sob 40 mg·kg^{-1}; E) d_1, d_3, d_5 iv Dox 2 mg·kg^{-1}; F) d_1, d_3, d_5 iv Dox 2 mg·kg^{-1}, d_1-d_7 ig Sob 20 mg·kg^{-1}; G) d_1, d_3, d_5 iv Dox 2 mg·kg^{-1}, d_1-d_7 ig Sob 40 mg·kg^{-1}; H) d_1-d_7 ig Sob 20 mg·kg^{-1}; I) d_1-d_7 ig Sob 40 mg·kg^{-1}.

各组从d_0起, 每天称重, 观察30 d 内死亡情况, 统计体重变化和生命延长率[8]. 根据预初试验的结果, 在预测各组荷瘤鼠出现死亡前1 d (个别前2 d)各组随机剖取3鼠心脏, 将心肌组织切割成1 mm^{3}左右小块, 经2.5 %戊二醛前固定, 1 %锇酸后固定, 丙酮系列浓度脱水, 醋酸铀块染, 环氧树脂618包埋, LKB-III 型超薄切片机切制50 nm 左右切片, 柠檬酸铅染色后, 在透射电镜下观察摄片[9].

RESULTS

Sob 和 Dox 联用对小鼠白血病 P388的作用

Dox 2和4 mg·kg^{-1}组以及 Sob 20和40 mg·kg^{-1}组单药应用对小鼠白血病 P388的生命延长率除了 Sob 40 mg·kg^{-1}组有抑瘤边缘效应(生命延长率 26.5 %)外, 其余各组均不显著. 而 Sob 和 Dox 联合用药组对小鼠 P388白血病的生命延长率非常显著($P<0.01$). 分别为48.7 %, 57.3 %, 59.0 %和62.4 %, 是 Sob 和 Dox 单药应用生命延长率之和的130 %—190 %. 两药联用有明显的抑瘤协同作用. 此外, 生理盐水对照组荷瘤鼠死亡前平均体重增加了2.27 g; 单用 Dox 组平均体重减轻了0.55 g 和0.37 g. 单用 Sob 组及 Sob 和 Dox 联用组平均体重增加1 g 左右, 显示因药物毒性所致的体重减少较 Dox 组为轻(Tab 1).

Tab 1. Effects of Sob ig in combination with Dox iv on mice bearing leukemia P388. $\bar{x}\pm s$.
$^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* NS.

Group	Dose/ mg·kg^{-1}	On day	Times	Mice	Weight change/ g	Lifespan/ d	ILS/ %
NS		1—7	7	16	2.27	11.7±2.2	
Dox	4	1	1	10	−0.55	13.2±1.4	12.8[a]
Dox + Sob	4 20	1 1—7	1 7	10	1.41	17.4±3.2	48.7[c]
Dox + Sob	4 40	1 1—7	1 7	10	0.47	18.4±2.5	57.3[c]
Dox	2	1,3,5	3	10	−0.37	13.1±0.7	12.0[a]
Dox + Sob	2 20	1,3,5 1—7	3 7	10	0.95	18.6±3.2	59.0[c]
Dox + Sob	2 40	1,3,5 1—7	3 7	10	0.98	19.0±3.0	62.4[c]
Sob	20	1—7	7	10	0.37	14.0±1.7	19.7[a]
Sob	40	1—7	7	10	0.56	14.8±1.3	26.5[b]

各组荷瘤鼠垂死前的心肌细胞的超微结构观察

1 P388对照组 荷瘤鼠心肌细胞超微结构基本正常. 电镜下可见排列规则的肌原纤维. 肌纤维附近线粒体密集, 内嵴丰富. 脂滴偶见. 细胞核大多单个; 核质分布均匀; 异染色质有边集现象. 相邻细胞间可查见闰盘结构, 其纵切面呈阶梯状, 水平部分主要由粘合带和桥粒组成, 相邻膜之间有20 nm 左右间距. 在心肌细胞纵切面上, 肌浆网为扁囊状, 与横管构成偶合体(Fig 1A, Plate 2).

2 单用 Sob 组 Sob 20和40 mg·kg^{-1}组荷瘤鼠心肌细胞超微结构改变轻微. 镜下个别视野可见线粒体稍有肿胀, 少数线粒体内嵴小量丢失或模糊. 其余细胞器未见明显异常(Fig 1B).

3 单用 Dox 组 Dox 4 mg·kg^{-1}组心肌细胞超微结构有明显改变, 主要表现为 1) 线粒体严重肿胀, 外膜破损, 内嵴减少或排列混乱, 甚至出现空泡, 个别可见髓样结构. 2) 局部视野肌原纤维部分溶解, 肌球蛋白丢失, 肌原纤维变细, 细丝间透明区扩大. 3) 个别视野肌浆网扩大; 闰盘间隔局部增宽(Fig 1C). Dox 2 mg·kg$^{-1}\times3$组的心肌细胞超微结构变化与 Dox 4 mg·kg^{-1}组相似, 但可查见的损伤范围稍大(Fig 1D).

4 Sob 和 Dox 联用组 Sob 20 mg·kg^{-1}, 40 mg·kg^{-1}与 Dox 4 mg·kg^{-1}联用组, 荷瘤鼠的心肌超微结构较单用 Dox 组损伤明显减轻. 线粒体肿胀、内嵴丢失现象减少, 肌原纤维、肌浆网大多正常. 闰盘扩张未查见(Fig 1E). Sob 20和40 mg·kg^{-1}与 Dox 2 mg·kg$^{-1}\times3$联用组的心肌细胞超微结构特点与上述联用组基本相似(Fig 1F).

DISCUSSION

Sob 口服毒性低, 小鼠急性 $LD_{50}>5$ g·kg^{-1}.

本实验采用 ig Sob 与 Dox 若干剂量配对联用，对于小鼠白血病 P388有显著的抑瘤协同作用，为临床合并应用提供了实验依据.

电镜观察显示，单用 Dox 组荷瘤鼠垂死前心肌细胞损伤范围较大，线粒体、肌原纤维、肌浆网和闰盘等均可查见不同程度的改变. 联合用药组荷瘤鼠心肌损害较单用 Dox 组明显减轻. 实验提示，Sob 能降低 Dox 所致的心肌毒性. 此结果对于扩大 Sob 的临床应用范围有积极意义. 此外，联合用药组较单用 Dox 组荷瘤鼠的平均体重增加1 g 左右. 鉴于因胃肠道损伤所致的体重减轻是蒽环类抗生素的常见毒性之一[10]，因此，Sob 对 Dox 所致胃肠道损伤是否有改善作用值得研究.

乙双吗啉(bimolane)降低柔红霉素的心脏毒性是代谢过程 ICRF-154起作用[11]，Sob 的主要代谢产物也是 ICRF-154[12]，由于 Sob 体内吸收容易，血中 ICRF-154浓度更高. ICRF-154的进一步代谢产物是依地酸二酰胺化合物[13]. 依地酸类似物能与 Fe^{2+} 螯合，减少 Fe-Dox 复合物的形成，从而使 Dox 所诱导的超氧自由基减少，降低对心脏的损害[14]. 因此，推测 Sob 降低 Dox 心脏毒性可能与其代谢产物减少 Dox 所诱导的自由基形成有关.

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