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8种抗癌药物对裸小鼠肾包膜下接种的人肺腺癌移植瘤(LAX-83)生长的影响

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Effects of 8 antitumor drugs against the growth of human lung adenocarcinoma (LAX-83) transplanted under the kidney capsule of nude mice

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ABSTRACT Nude mice, inoculated with LAX 83 in bilateral subrenal capsules, were used in experimental therapy with 8 antitumor drugs. Treatment was initiated 2 d after tumor inoculation. All the drugs were ip to the nude mice daily for 7 d. At the daily doses VCR 0.4, MMC 2, CCNU 16, cis-DDP 2, AdM 2.5, 5-Fu 30, CTX 40 and MTX 2-6 mg/kg, the inhibition of the tumor growth were 100, 95.8, 91.3, 79.2, 65.2, 60.7, 62.3 and 0%, respectively. The results indicated that the effects of the drugs on nude mice inoculated with LAX-83 in subrenal capsule not only exhibited a good correlation to those in sc, but also shortened the period of experiment from 22 to 11 d. Furthermore, when LAX-83 was inoculated into the subrenal capsule of Swiss +/+ mice, the tumor tissues degenerated and disintegrated 2 d after the inoculation and replaced by inflammatory granuloma tissues 6 d later.

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KEY WORDS nude mice; subrenal capsule assay; lung neoplasms; adenocarcinoma; drug therapy; antineoplastic agents

提要 在裸小鼠双侧肾包膜下接种LAX-83快速筛选抗肿瘤药物。在所试的8种抗癌药中，7种有明显的抗肿瘤作用，它们是：VCR，AdM，CCNU，cis-DDP，CTX，5-FU，MMC，仅MTX无效。将LAX-83接种在普通有毛小鼠的肾包膜内，种后d 2肿瘤组织出现退化变性，d 4有炎性细胞浸润，d 6为炎性肉芽组织取代。

关键词 裸小鼠；肾包膜下检定；肺肿瘤；腺癌；药物疗法；抗肿瘤药

Bogden 等[1]首先报道在裸小鼠肾包膜下(subrenal capsule, SRC)接种人癌移植瘤，用以快速筛选抗癌药物，并认为此法对评价临床前药物有实际应用价值，且节约时间和费用。其后也有用普通有毛小鼠进行类似试验的报道[2,3]。我们用人肺腺癌移植瘤LAX-83[4]接种在裸小鼠双侧SRC部位，同时用临床常用的8种治疗肺癌药物进行药敏试验。此外我们还

观察了在普通有毛小鼠的SRC部位接种LAX-83后肿瘤组织生长的情况。

MATERIALS AND METHODS

实验动物 试验用BALB/c-nu/nu和Swiss-nu/nu裸小鼠以及Swiss+/+小鼠均由我所实验动物研究室提供，鼠令6-8 wk，每批药敏试验用20只裸小鼠。每一药物剂量组用2只裸小鼠(4个肾)，对照组用3-4只裸小鼠。裸小鼠及+/+小鼠的饲养及实验条件同前文[1]报道。

接种方法及治疗 参照SRC接种法[1]，将小鼠用水合氯醛麻醉后，分别在二侧SRC植入瘤径为1.0-1.5 mm的瘤块，并用放有测微器的国产C-XTL-1型双目体视显微镜测量瘤块的长、宽。接种鼠于手术后d 2开始ip治疗，Qd连续7 d，于术后d 11解剖，并测量瘤块长、宽，按公式(长+宽)/2求出各组在d 0与d 11时瘤径平均值(mm)，然后计算出各组给药前后瘤径的净增值，以给药组与对照组净增值比较，按C-T/C%计算出给药组肿瘤生长抑制率，并经t测验。

为了观察人癌移植瘤在有免疫机能的普通有毛小鼠SRC部位的生长情况，用Swiss-nu/nu裸小鼠及Swiss+/+小鼠各14只，分别在裸小鼠左右二侧及+/+小鼠左侧SRC处接种LAX-83其后每隔2 d，各组解剖2只小鼠，测量瘤的长、宽，并绘成瘤的生长曲线，同时对瘤组织作病理切片观察。

药物 阿霉素(doxorubicin, adriamycin, AdM)意大利Farmitalia Carlo Erba SPA生产，治疗剂量为1.5-3 mg/kg；氯氨铂(cis-dichlorodiaminoplatinum, cis-DDP)齐鲁制药厂生产0.5-2 mg/kg；环磷酰胺(cytoxan CTX)10-40 mg/kg；洛莫司汀(lomustine, CCNU，原名环己亚硝脲)4-16 mg/kg；5-氟尿嘧啶(5-fluorouracil, 5-FU)20-50 mg/kg；甲氨蝶呤(methotrexate, MTX)2-6 mg/kg以及长春新碱(vincristine, VCR)0.05-0.4 mg/kg均为上海第十二制药厂生产；丝裂霉素C(mitomycin C, MMC) 0.5-2 mg/kg日本协和发酵工业株式会社生产。

RESULTS

接种在裸小鼠二侧SRC的LAX-83，瘤组织生长速度相似(Fig 1)。种后d 10所测得瘤块大小，与接种时相比，增加2倍，对种后d 8的肿瘤组织作病理观察时。发现接种肿瘤部位的肾皮质因瘤组织生长而受压内陷，瘤组织呈片块状，细胞核大，透明，核膜厚，核仁清晰，胞浆丰富(Fig 2 A, Plate 4)。种后d 10，可见肾包膜下大片癌组织浸润到肾皮质中，癌细胞活跃，核分裂相多见。

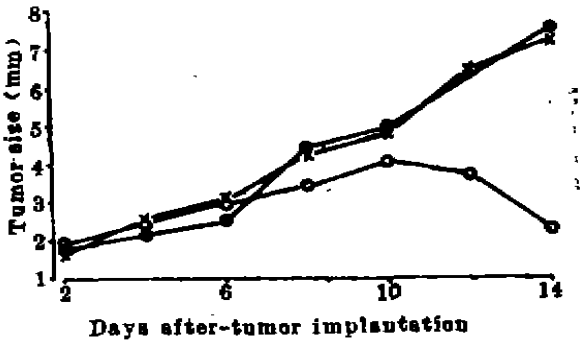

Fig 1. Growth of human lung adenocarcinoma LAX-83 implanted under renal capsule of nude mice [left (●), right (×) subrenal capsule] and Swiss mice +/+ (○) left subrenal capsule. $n=14$.

LAX-83接种在Swiss +/+小鼠的SRC后，瘤块虽然有所增大，但经病理切片观察表明，种后d 2，瘤组织与肾皮质间分界清楚，部分瘤组织出现退化变性，核染色质模糊不清，核膜崩解，种后d 4，肾包膜下的瘤块与肾皮质间被纤维组织分隔，瘤组织大多退化变性，胞浆中出现明显空泡，核结构模糊，瘤组织边缘查见少量炎细胞浸润，种后d 6，在显微镜下观察发现，肾包膜下增大癌灶为炎性肉芽组织所取代，其中可见新生毛细血管、组织细胞以及少量癌细胞残骸(Fig 2 B)。

用接种在裸小鼠二侧SRC的LAX-83作药敏试验时发现，在所试的8个抗肿瘤药物中，7个药物对LAX-83产生明显的抑制作用，其中VCR，MMC及CCNU对肿瘤的抑制率可达91.3-100%，特别是用VCR最大耐受剂量0.4 mg/kg治疗后，瘤径较接种时缩小。cis-DDP，CTX，5-FU及AdM对LAX-83的抑制率分别在60.7-79.2%之间，在所试的药物中MTX对LAX-83无效(Tab 1)。

Tab 1. Effects of anti-tumor drugs on growth of LAX-83 implanted subrenal capsule in nude mice. n = 4-8 capsules, $\bar{x}\pm SD$ *$P>0.05$, **$P<0.05$, ***$P<0.01$

Drug	Dose	Mean tumor size (mm)			Body wt	Inhibition rate (Tumor size)
	mg/kg	d_0	d_{11}	d_{11-0}	d_{11}/d_0	%
Control	—	1.44	3.7	2.3±0.4	1.03	—
AdM	1.5	1.18	2.79	1.6±0.7	1.05	30.4**
	2	1.5	2.66	1.2±0.4	0.89	47.8***
	2.5	1.21	2.1	0.8±0.4	0.87	65.2***
	3	1.66	1.3	—	0.45	Toxicity
CCNU	4	1.29	2.34	1.05±0.26	1.05	54.3***
	8	1.31	2.09	0.78±0.25	1.05	66.1***
	12	1.2	1.83	0.6±0.3	1.00	73.9***
	16	1.24	1.54	0.2±1	0.97	91.3***
Control	—	1.44	4.63	3.18±0.23	1.15	—
CTX	10	1.41	4.28	2.8±0.4	1.19	11.0*
	20	1.6	3.75	2.2±0.6	1.19	30.8**
	30	1.38	3.59	1.9±0.6	1.00	40.3**
	40	1.4	2.64	1.2±0.3	0.94	62.3***
VCR	0.05	1.45	2.94	1.5±0.6	1.17	52.8***
	0.1	1.44	2.55	1.1±0.4	1.19	65.4***
	0.2	1.26	1.83	0.5±0.6	1.11	84.3***
	0.4	1.49	1.39	−0.1±0.3	1.00	100
Control	—	1.38	4.34	2.8±0.4	1.06	—
5-Fu	20	1.45	3.53	2.08±0.27	1.10	25.7**
	30	1.54	2.7	1.1±0.4	1.01	60.7**
	40	1.26	2.48	1.23±0.27	0.91	56.1***
	50	1.56	—	—	—	Toxicity
MTX	2	1.43	4.23	2.8±0.1	1.00	—
	4	1.5	4.05	2.6±0.6	1.01	—
	6	1.33	3.78	2.45±0.21	1.13	12.0*
Conirol	—	1.42	3.82	2.4±0.13	1.10	—
MMC	0.5	1.39	3.69	2.3±0.5	0.97	—
	1	1.49	3.08	1.7±0.5	0.93	29.2***
	1.5	1.51	2.14	0.6±0.9	0.93	75.0***
	2	1.28	1.35	0.1±0.8	0.96	95.8***
Cis-DDP	0.5	1.38	3.71	2.3±0.8	1.06	—
	1	1.34	3.1	1.7±0.5	0.96	29.2**
	1.5	1.48	2.79	1.31±0.3	0.84	45.3***
	2	1.36	1.95	0.5±0.7	0.91	79.2***

DISCUSSION

Bogden首先报道在裸小鼠单侧SRC接种人癌组织进行药敏试验[1,2]；我们在裸小鼠双侧SRC接种LAX-83，亦获得较满意的结果。虽然双侧SRC接种方法在技术操作上较单侧的复杂些，但其优点是可在同一个体中获得2个样本数据，节省用鼠数。试验结果表明，接种在裸小鼠双侧SRC的LAX-83，其生长速度相似，癌灶均匀，误差小。在4批试验中，各批对照组d 0的平均瘤大小相差甚小，接种后d 11的瘤组织较d 0的大1-2倍，病理切片证实增长的癌灶为活跃的肿瘤组织，根据LAX-83在SRC生长曲线中，癌组织的倍增时间，确定药敏试验的全程为11 d。在所试8种药物中，除MTX无效外，其余药物对LAX-83均产生明显的抑制作用，这与LAX-83接种在裸小鼠皮下时的药敏试验结果一致，而试验时间及用鼠数则缩短和减少一半。

AdM剂量为3 mg/kg时虽然产生明显的抑制作用，但d 11/d 0为0.45明显地小于允许范围0.7，这是药物引起宿主毒性反应的结果[5]。此外我们还观察到在cis-DDP及MMC大剂量中，受治的宿主肾脏变小，且色淡，此时缩小的瘤块可能与药物引起宿主肾循环障碍，因而造成局部缺血有一定关系。

LAX-83接种在具有免疫功能的Swiss+/+小鼠的SRC后，d 2即出现排异反应，表现为部分肿瘤组织退化变性，d 4可见炎细胞浸润，而d 6虽然测出瘤块大小较接种时有所增长，但此增长部分实际上是炎性肉芽组织，并非肿瘤组织。Levi等亦报道在$B_6D_2F_1$普通小鼠SRC部位接种人体肿瘤后d 4出现排异反应，显然这种排异现象使得有免疫功能的小鼠不适于在SRC部位移值人癌组织进行药敏试验。

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Effects of *Tremella* polysaccharides on immune function in mice[1]

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ABSTRACT It was found *in vitro* that *Tremella* polysaccharides (TP) (50, 100, 150 and 200 μg/ml) augmented lymphocyte proliferation induced by Con A and did not antagonize the suppressive effect of hydrocortisone on lymphocyte proliferation. *In vivo* TP promoted the plaque-forming cell (PFC) response to SRBC in mice. TP 50 and 100 mg/kg ip for 5 d produced 77.6% and 81.8% increases in PFC response respectively. At the doses of 150 and 200 μg/ml, TP decreased the interleukin 2 (IL-2) activities in the supernatant of culture media of mouse spleen cells. TP (50 μg/ml) enhanced the lymphocyte proliferation induced by Con A and increased the PFC response to SRBC by 47.1 % in 14-month-old mice.

KEY WORDS *Tremella fuciformis*; polysaccharides; lymphocytes; hydrocortisone; concanavalin A; plaque assay; interleukin 2

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Tremella polysaccharides (TP) are important components isolated from *Tremella fuciformis* Berk. TP showed antitumor activity in mice[1,2]. Our previous researches demonstrated that TP increased phagocytosis of intraperitoneal macrophages and production of hemolysin in normal and immunosuppressive mice[3,4].

In order to further observe the effects of TP on immune function in mice, we examined the effect of TP on the lymphocyte proliferation induced by Con A in normal mouse spleen cells and immunosuppressive mouse spleen cells caused by hydrocortisone and the effect of TP on humoral immunity with plaque forming cell response. We also examined the effect of TP on the interleukin 2 (IL-2) production