原药[11, 12]，而主要是测得其主要代谢物AlbSO和阿苯达唑砜，前者证明有抗原头节[13]和损害细粒棘球蚴囊生发层的作用。因此，在用Alb治疗时，实际上是AlbSO起作用，故是否因个体对药物的吸收及代谢有差异，从而影响药效，尚待阐明。

REFERENCES

1 Davis A, Pawlowski ZS, Dixon H. Multicentre clinical trials of benzimidazole carbamates in human echinococcosis. *Bull WHO* 1986; **64** : 383

2 You JQ, Xiao SH, Jiao PY, Guo HF, Chai JJ, Zhang WL. Effects of mebendazole, albendazole and praziquantel on mice infected with secondary cysts of *Echinococcus granulosus*. *Endemic Dis Bull* 1989; **4** (3) : 16

3 Horton RJ. Chemotherapy of *Echinococcus* infection in man with albendazole. *Trans Roy Soc Trop Med Hyg* 1989; **83** : 97

4 Qiu JM, Luo CX, Chen HC, *et al*. Primary observation in the therapy of experimental mice and human hydatidosis with albendazole. *Endemic Dis Bull* 1988; **3** (3) : 19

5 Schantz PM, Van den Bossche H, Eckert J. Chemotherapy for larval echinococcosis in animals and humans : Report of a workshop. *Z Parasitenkd* 1982; **67** : 5

6 Verheyen A. *Echinococcus granulosus*: The influence of mebendazole therapy on the ultrastructural morphology of the germinal layer of hydatid cysts in humans and mice. *Z Parasitenkd* 1982; **67** : 55

7 Eckert J. Prospects for treatment of the metacestode stage of *Echinococcus*. In : Thompson RCA, ed. *The biology of Echinococcus and hydatid disease*. London : George Allen & Unwin, 1986 : 250–77

8 Van en Bossche H. Commentary, Peculiar targets in anthelmintic chemotherapy. *Biochem Pharmacol* 1980; **29** : 1981

9 Xiao SH, You JQ, Jiao PY, *et al*. Studies on the effects of mebendazole, albendazole and its metabolites in experimental therapy of mice infected with secondary cysts of *Echinococcus granulosus*. *Endemic Dis Bull* 1990; **5** (in press)

10 Tao IH, Ma LJ, Lin KH, Wu K. Chemical determination of the glycogen content of *Schistosoma japonica*. *Acta Biochim Sin* 1958; **1** : 218

11 Marriner SE, Bogan JA. Pharmacokinetics of albendazole in sheep. *Am J Vet Res* 1980; **41** : 1126

12 Marriner SE, Morris DL, Dickson B, Bogan JA. Pharmacokinetics of albendazole in man. *Eur J Clin Pharmacol* 1986; **30** : 705

13 Chinnery JB, Morris DL. Effects of albendazole sulphoxide on viability of hydatid protoscoleces *in vitro*. *Trans Roy Soc Trop Med Hyg* 1986; **80** : 815

中国药理学报 *Acta Pharmacologica Sinica* 1990 Nov; **11** (6) : 549–553

4–[4″–(2″,2″,6″,6″–四甲基哌啶氮氧自由基)氨基]–4′–去甲表鬼臼毒素体外抗肿瘤作用

贾正平[1]、张培林、梁重栋(兰州医学院药理教研室，兰州，730000 中国)
王彦广[2]、陈耀祖、田 瑄、李景新(兰州大学化学系，兰州，730000 中国)

Antitumor activity of 4–(4″–(2″,2″,6″,6″–tetramethyl–1″–piperidinyoxy)amino)–4′–demethyl epipodophyllotoxin *in vitro*

JIA Zheng–Ping[1], ZHANG Pei–Yan, LIANG Zhong–Dong (*Department of Pharmacology, Lanzhou Medical College, Lanzhou 730000, China*)
WANG Yan–Guang[2], CHEN Yao–Zu, LI Jin–Xin, TIAN Xuan (*Department of Chemistry, Lanzhou University, Lanzhou 730000, China*)

Received 1989 Aug 25 Accepted 1990 Jul 12

[1] Now in *Department of Clinical Pharmacology, General Hospital of Lanzhou Command of PLA, Lanzhou 730050, China*
[2] Now in *Department of Chemistry Tianjin University, Tianjin 300072, China*

ABSTRACT The antitumor activity of a new podophyllotoxin spin–labeled derivative, 4–(4″–(2″,2″,6″,6″–tetramethyl–1″–piperidinyoxy)amino)–4′–demethylepipodophyllotoxin (GP–7) first syn–

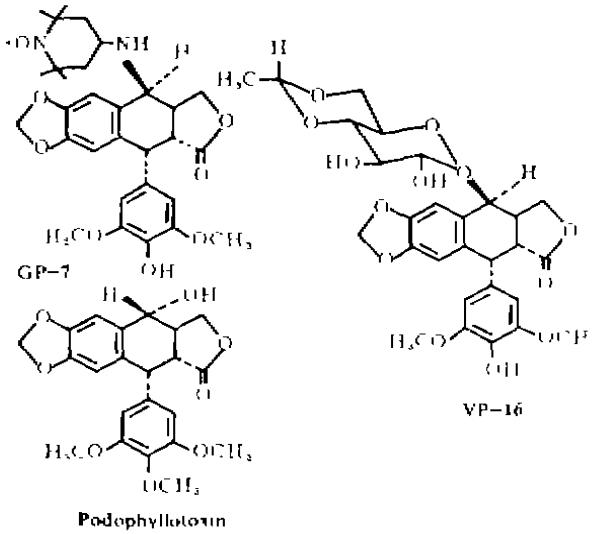
thesized by us, was studied *in vitro*. It was found that the proliferation of SGC-7901 cells was markedly inhibited by GP-7 depending the concentration and exposure time. At concentrations of 0.04–100 mg/L, the inhibition rates were 15.5–92.6 %, ID_{50} was 0.42 mg/L. After exposure to GP-7 > 0.5 mg/L for 24, 48, 72 and 96 h, the inhibition rates of cells were 25.1, 49.0, 71.4 and 84.9 % respectively. The dose-response curve of GP-7 on SGC-7901 cell was similar to that of etoposide (VP-16). The colony formation of SGC-7901 cell was also inhibited by GP-7 in a concentration dependent fashion with ID_{50} 1.63 mg/L. At concentrations of 0.1–0.5 mg/L, the inhibitory effects were stronger than that of VP-16. GP-7 decreased the mitotic index (MI) of SGC-7901 cell and had no effect on microtubule assembly and disassembly *in vitro*, which suggested that GP-7 did not act on M phase.

KEY WORDS podophyllotoxin; free radicals; cell line; clone cells; mitotic index; tubulin; etoposide

提要 4-[4″-(2″,2″,6″,6″-四甲基哌啶氮氧自由基)氨基]-4′-去甲表鬼臼毒素(GP-7)对 SGC-7901 细胞生长抑制作用和浓度及处理时间正相关；72 h ID_{50} 为 0.42 mg/L，剂量反应曲线同鬼臼乙叉甙(VP-16). GP-7 0.1–0.5 mg/L 对 SGC-7901 细胞克隆形成抑制作用较 VP-16 强. GP-7 降低 SGC-7901 细胞有丝分裂指数，对微管蛋白体外聚合和解聚无影响；提示其抗肿瘤机理主要不在 M 期

关键词 鬼臼毒素；自由基；细胞株；克隆细胞；有丝分裂指数；微管蛋白；去甲鬼臼甙

4-[4″-(2″,2″,6″,6″-四甲基哌啶氮氧自由基)氨基]-4′-去甲表鬼臼毒素[4-(4″-(2″,2″,6″,6″-tetramethyl-1″-piperidinyoxy)amino)-4′-demethylepipodophyllotoxin, GP-7]是我们根据将稳定自由基引入抗癌剂母体可降低毒性[1,2]，提高或不影响疗效的理论半合成的鬼臼毒素氮氧自由基衍生物[3]，体内实验 GP-7 显著抑制小鼠移植性肿瘤白血病 P388 和实体瘤 S180、HePS. 抑瘤率和鬼臼乙叉甙[4′-demethyl-epipodophyllo-toxin-9-(4,6-*O*-ethylidene-β-D-glucopyranoside), VP-16]相同. 连续给药 10 d，对荷瘤小鼠胸腺和脾指数的影响明显小于 VP-16[3]. 本实验从体外角度进一步观察了 GP-7 和 VP-16 对人胃腺癌 SGC-7901 细胞生长、克隆形成和有丝分裂指数(MI)及对微管蛋白体外聚合和解聚的影响.



MATERIALS

GP-7 为杏黄色结晶性粉末，结构稳定，水溶性差，易溶于二甲亚砜(DMSO). VP-16 为北京医药工业研究所产品. 2-(*N*-morpholine)-ethane sulfonic acid (MES), adenosine 5′-triphosphate (ATP)为 Sigma 产品. 药品均用 DMSO 或 10%DMSO 溶解或稀释. 部分纯化的猪脑微管蛋白由中国医学科学院药物研究所提供.

人胃腺癌 SGC-7901 细胞系中国科学院上海药物研究所引来；用 RPMI 1640 培养液[Difco 产品，内含 20%小牛血清，青霉素(penicillin)、链霉素(streptomycin)各 100 IU (μg)/ml, pH 7.2]常规培养[4].

METHODS AND RESULTS

GP-7 和 VP-16 对 SGC-7901 细胞生长的影响 将 SGC-7901 细胞用 RPMI 1640 培

养液配成 5×10^4/ml 悬液，每瓶分装 4 ml，37℃ 培养 48 h 后随机分组，每组 4 瓶，换培养液后实验组加入不同浓度 GP-7 和 VP-16，对照组加等量溶媒，培养一定时间后取出，消化、染色，计拒染活细胞数；取均值计算抑制百分率．结果 GP-7 和 VP-16 对 SGC-7901 细胞生长抑制作用与浓度和处理时间正相关．72 h ID_{50} 分别为 0.42 和 0.26 mg/L，二者剂量反应曲线相似(Fig 1)．GP-7 和 VP-16 5 mg/L 处理 24，48，72，96 h 对 SGC-7901 细胞生长抑制率分别为 41.5，60.1，81.4，91.4 % 和 51.5，64.9，84.1，96.2%．

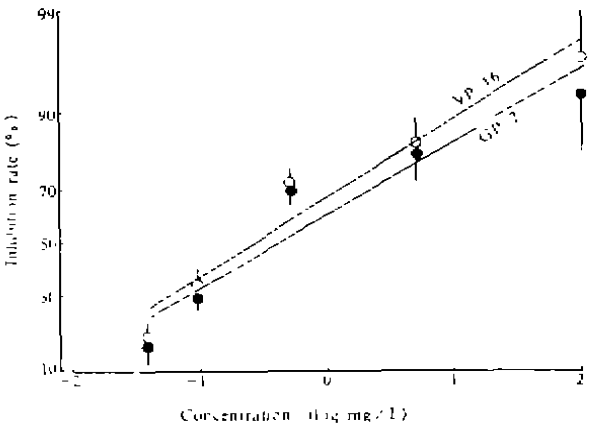


Fig 1. Effects of GP-7 (●) and VP-16 (O) on proliferation of SGC-7901 cells which were exposed to the drugs for 72 h *in vitro*. $n=4$, $\bar{x}\pm SD$.

对细胞相对增殖存活率的影响 取 GP-7 和 VP-16 处理 6 h 的 SGC-7901 细胞用 RPMI 1640 培养液制成 100/ml 的悬液，每皿 6 ml，接种到直径 60 mm 培养皿内，置 CO_2 孵箱培养 12 d 取出，甲醇固定，Giemsa 染色，解剖显微镜下计克隆数(50 个细胞以上者为 1 克隆)，每组 4 皿，按均值据公式：

克隆形成率 = 克隆数/接种细胞数 × 100%

细胞相对增殖存活率 = 给药组克隆形成率/对照组克隆形成率 × 100%

Tab 1. Effects of GP-7 and VP-16 on cloning efficiency of SGC-7901 cells *in vitro*. 600 cells were planted on each plate. $n=4$, $\bar{x}\pm SD$. $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* 10% DMSO control.

Drugs	Concn (mg/L)	Clone number	Cloning efficiency (%)	Relative survival rate(%)
Control		285±21	47.5	
GP-7	0.1	248±14	41.3	87.0**
	0.5	207±12	34.5	72.8***
	1.0	142±20	23.7	49.8***
	2.0	74±9	12.3	26.2***
	5.0	12±2	2.0	4.1***
	20.0	0	0	0***
VP-16	0.1	254±29	42.3	89.2*
	0.5	231±12	38.5	81.1***
	1.0	138±9	23.0	48.4***
	2.0	65±18	10.8	22.7***
	5.0	9±2	1.5	3.3***
	20.0	0	0	0***

计算克隆形成率和细胞相对增殖存活率(Tab 1)．

结果 GP-7 和 VP-16 对 SGC-7901 细胞克隆形成抑制作用随浓度增高而增强；ID_{50} 分别为 1.63 和 2.23 mg/L，20.00 mg/L 组均无克隆形成．在 0.1-0.5 mg/L 范围内 GP-7 对 SGC-7901 细胞克隆形成抑制作用较 VP-16 强．

对 MI 的影响 取不同浓度 GP-7 和 VP-16 处理 48 h 的 SGC-7901 细胞，消化、低渗，1∶3 冰醋酸甲醇固定，Giemsa 染色，光镜下计 1000 个细胞中分裂细胞数，每组 4 瓶细胞，取均值计算抑制百分率(Tab 2)．结果 GP-7 和 VP-16 均降低 SGC-7901 细胞 MI，0.5-20 mg/L，MI 分别下降 12.5-95.8% 和 62.5-95.8%．浓度小于 2 mg/L 时，VP-16 降低 MI 作用较 GP-7 强．

对微管蛋白体外聚合和解聚的影响 参照文献[5,6]．微管蛋白 1.5 mg/mL，ATP 1 mmol/L．聚合和解聚过程采用岛津 2000 型多功能记录分光光度计时间扫描法记录；波长 350 nm(Fig 2)．结果鬼臼毒素 5 mg/L 对微管蛋白体外聚合有显著抑制作用．GP-7 对微管

蛋白聚合和解聚过程无影响.

Tab 2. Effects of GP-7 and VP-16 on mitotic index of SGC-7901 cells which were exposed to the drugs for 48 h *in vitro*. $n=4$, $\bar{x} \pm SD$. $^{*}P>0.05$, $^{***}P<0.01$ *vs* DMSO control.

Drugs	Concn (mg / L)	Mitotic index(‰)	Inhibition rate(%)
Control		24 ± 2	
GP-7	0.5	21 ± 3	12.5*
	1.0	17 ± 3	29.2***
	2.0	5 ± 3	79.2***
	5.0	1 ± 1	95.8***
	20.0	1 ± 1	95.8***
VP-16	0.5	9 ± 2	62.5***
	1.0	5 ± 1	79.3***
	2.0	3 ± 1	87.5***
	5.0	2 ± 1	91.7***
	20.0	1 ± 1	95.8***

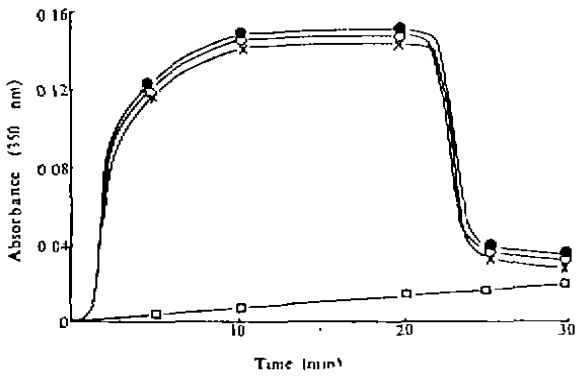


Fig 2. Effects of GP-7 and podophyllotoxin on microtubule assembly and disassembly *in vitro*. Control (O), GP-7 20 mg / L (●), GP-7 100 mg / L (×), podophyllotoxin 5 mg / L (□). (tubulin 1.5 mg / ml, ATP 1 mmol / L and 0.1 mol / L MES buffer were used).

DISCUSSION

4′-去甲基表鬼臼毒素衍生物具有通过作用于 DNA 拓扑异构酶Ⅱ(topoisomerase Ⅱ)使 DNA 断链而有较高抗肿瘤活性[7]. 氮氧自由基可作为抗肿瘤药载体使其优先积累于肿瘤组织中[2]; 从而降低毒性, 提高疗效. 为此, 我们以鬼臼毒素为母体半合成了 4′-去甲基表鬼臼毒素氮氧自由基衍生物 GP-7, 结构和 VP-16 相似. 结果表明 GP-7 显著抑制 SGC-7901 细胞生长和克隆形成; 抑制作用同剂量及处理时间正相关; 剂量效应曲线同 VP-16; 表现出 4′-去甲基表鬼臼毒素衍生物的特点[8]. GP-7 降低 MI, 对微管蛋白体外聚合和解聚无影响; 完全改变了母体鬼臼毒素的作用机理[9]; 说明其抗肿瘤作用不是通过抑制微管蛋白聚合所产生, 作用机理主要不在 M 期. 有文献报道, 鬼臼毒素对微管蛋白聚合的影响与其 C-4 取代基团及构型有密切关系, C-4 可能是鬼臼毒素与微管蛋白键合的重要部位. GP-7 在 C-4 接有四甲基哌啶氮氧自由基, 阻止了它与微管蛋白的键合; 这可能是其对微管蛋白体外聚合无影响的重要原因. GP-7 对细胞生长、克隆形成、MI 和微管蛋白体外聚合和解聚的影响均与 VP-16 相似, 提示其结构中四甲基哌啶氮氧自由基主要影响其作用强度和毒性大小, 对作用特点影响不大.

REFERENCES

1 Emanuel NM, Konovalova NP, Djachkovskaya RF. Toxicity, antitumor activity, and pharmacokinetics of spin-labeled thioTEPA analogs. *Cancer Treat Rep* 1976; **60** : 1605
2 Sosnovsky G, Rao NUM, Li SW. In the search for new anticancer drugs. 19. A predictive design of *N,N*: *N′,N′* : *N″*, *N‴*-tri-1,2-ethane-diylphosphoric triamide (TEPA) analogues. *J Med Chem* 1986 : **29** : 2225
3 Chen YZ, Wang YG, Li JX, Tian X, Jia ZP, Zhang PY. Anticancer drugs Ⅱ. Synthesis and biological evaluation of spin labeled derivatives of podophyllotoxin. *Life Sci* 1989; **45** : 2569
4 Yang JL, Shen ZM, Han JX. A study on the biological characteristics of an established human gastric adenocarcinoma cell line (SGC-7901). *Kexue Tongbao* 1982; **27** : 1355
5 Loike JD, Horwitz SB. Effects of podophyllotoxin and VP-16-213 on microtubule assembly *in vitro* and nucleoside transport in HeLa cells. *Biochemistry* 1976; **15** : 5435

6 Li ZR, Liu ZL, Sun RH, Han R, Yin MB, Ye QR. Isolation and identification of microtubule protein and its application in anticancer drug screening. *Acta Pharm Sin* 1986; **21** : 651
7 Ross W, Rowe T, Glisson B, Yalowich J, Liu L. Role of topoisomerase II in mediating epipodophyllotoxin-induced DNA cleavage *Cancer Res*. 1984; **44** : 5857
8 Hunag CC, Hou Y, Wang JJ. Effects of a new antitumor agents, epipodophyllotoxin, on growth and chromosomes in human hematopoietic cell lines. *Ibid*. 1973; **33** : 3123
9 Loike JD. VP 16-213 and podophyllotoxin, a study on the relationship between chemical structure and biological activity. *Cancer Chemther Pharmacol*. 1982; **7** : 103
10 Loike JD, Brewer CF, Sternlicht H, Gensler WJ, Horwitz SB. Structure-activity study of the inhibition of microtubule assembly *in vitro* by podophyllotoxin and its congeners *Cancer Res*. 1978; **38** : 2688

中国药理学报 *Acta Pharmacologica Sinica* 1990 Nov; **11** (6) : 553-555

DNA 链间交联的检测用于估计肿瘤细胞对烷化剂的敏感性

贺 涛、夏寿萱、章扬培 (军事医学科学院放射医学研究所, 北京 100850, 中国)

Application of DNA interstrand cross-link assay to estimate the sensitivity of tumor cells to alkylating agents.

HE Tao, XIA Shou-Xuan, ZHANG Yang-Pei.(*Institute of Radiation Medicine, Academy of Military Medical Sciences, Beijing 100850, China*)

ABSTRACT Fluorometric method was modified and used to detect DNA interstrand crosslinks (ISC) in tumor cells after treatment with cisplatin. Linear dose-response curve was obtained. The difference of DNA ISC formation between HeLa S3 (containing O^6-methylguanine methyltransferase, Mer+) and HeLa MR (devoid of O^6-methylguanine methyltransferase, Mer-) cells was studied after treatment with alkylating agent 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride (ACNU). The survival fraction was also observed in Mer+ and Mer- cells treated with ACNU. It seems that DNA ISC formation may be used as one of the possible criteria in estimating the sensitivity of tumor cells to bifunctional alkylating agent and forecasting the efficacy of tumor chemotherapy.

KEY WORDS experimental neoplasms; alkylating agents; DNA damage, DNA repair, drug therapy

提要 改进 Jong 等检测 DNA 链间交联(ISC)的荧光方法,获得了顺铂所致 HeLa S3 细胞 DNA 链间交联的剂量效应曲线. 不含 O^6-甲基鸟嘌呤 DNA 甲基转移酶(O^6-MT)的细胞(Mer^-)较含 O^6MT 的细胞(Mer^+)经双功能烷化剂 ACNU 处理后产生的 ISC 明显为多. 同时测定了 Mer^+与 Mer^-细胞经 ACNU 处理后的活存率. 认为 ISC 形成的多少可作为估计肿瘤细胞对烷化剂敏感性并预测药物化疗效果的指标之一.

关键词 实验性肿瘤; 烷化剂; DNA 损伤; DNA 修复; 化学治疗

Received 1989 Nov 9 Accepted 1989 Jul 11

某些双功能烷化剂能使细胞 DNA 分子中的碱基受到损伤, 如果这种损伤得不到修复, DNA 双链形成共价结合的 ISC, 细胞正常生命活动会受到干扰[1]. 目前, 有不少双功能烷化剂作为临床使用的抗癌药物, 因此检测 DNA ISC 对于评价此类药物的效价和毒性并阐明其作用机制具有重要意义. Jong 等[2]建立了检测 ISC 的荧光方法, 所得结果与 Kohn 等建立的碱洗脱方法[3]一致. 本文在改进检测 DNA ISC 荧光方法的基础上, 比较了 Mer^+与 Mer^-细胞受烷化剂嘧啶亚硝脲(ACNU)处