ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Sep; 22 (9): 827-830
http://www.chinaphar.com

Relationship between lactone ring forms of HCPT and their antitumor activities

ZHOU Jian-Jun, LIU Jian, XU Bin[1] (*Department of Pharmacology, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China*)

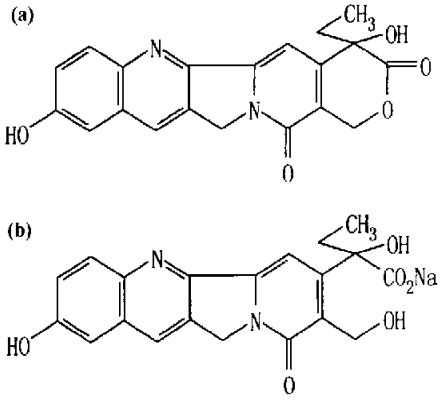
KEY WORDS 10-hydroxycamptothecin; lactones; antineoplastic agents; cell culture; high pressure liquid chromatography

ABSTRACT

AIM: To study the relationship between the lactone forms of 10-hydroxycamptothecin (HCPT) and their antitumor activities. **METHODS**: Antitumor activity of the two forms of HCPT was studied *in vitro* using seven cultured human and mouse tumor cell lines. Mice bearing sarcoma 180 and solid hepatoma were treated with HCPT (0.5, 1, and 2 mg/kg, ip) and tumor growth inhibition was assayed. HPLC method was employed to investigate the conversion of two forms of HCPT in different pH conditions and cultured tumor cells. **RESULTS**: It was found that both forms of HCPT (O-HCPT and C-HCPT) showed similar activities *in vitro* against a number of tumor cell lines at the same concentration; but C-HCPT was more effective (about two times) than O-HCPT *in vivo*. The difference between *in vitro* and *in vivo* results could be explained by the conversion of O-HCPT into C-HCPT in a certain condition, which was shown by the HPLC analysis of HCPT at different pH values and in cultured tumor cells. **CONCLUSION**: Both forms of HCPT were effective against tumor growth, but C-HCPT was more effective than O-HCPT, the latter could be converted into the former under certain conditions.

INTRODUCTION

10-Hydroxycamptothecin (HCPT) is an effective natural anticancer agent developed by Shanghai Institute of Materia Medica[1]. There is a lactone in the E ring in the chemical structure of camptothecin and its analogues (CPT). It has been reported that the lactone of CPT in ring-closed form (C-CPT) is active while in ring-opened form (O-CPT) is inactive[2,3], but this opinion is lack of enough experimental support. In this study, the antitumor activity of two forms of HCPT (C-HCPT and O-HCPT) was compared both in *in vitro* and *in vivo*.



Chemical structure of hydroxycamptothecin. (a) Lactone ring-closed form of HCPT (C-HCPT); (b) Lactone ring-opened form of HCPT (O-HCPT).

MATERIALS AND METHODS

Drugs HCPT powder (>98.5 %) was obtained from Shanghai Institute of Materia Medica. O-HCPT was prepared by adding several drops of NaOH 0.5 mmol/L to the powder of HCPT and then dissolved with normal saline; C-HCPT was dissolved with drops of Me_2SO and then diluted with double distilled water.

Animals and drug treatment[4] Kuming strain female mice (n = 160, Grade Ⅱ, Certificate № 005, weighing 20 g ± 2 g) were obtained from Shanghai Experimental Animal Center of Chinese Academy of Sciences. The mice bearing sarcoma 180 (S_{180}) and solid hepatoma (HepS) were treated ip with O-HCPT or C-HCPT for 7 d at 0.5, 1.0, and 2.0 $mg \cdot kg^{-1} \cdot d^{-1}$ and

[1] Correspondence to Prof XU Bin.
Phn 86-21-6431-1833, ext 311. Fax 86-21-6437-0269.
E-mail bxu@mail.shcnc.ac.cn
Received 2000-11-28 Accepted 2001-06-14

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Sep; 22 (9)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

the tumor growth inhibition was recorded. Cyclophosphamide (CTX, obtained from Shanghai Hualian Pharmaceuticals) was used as positive control.

Cell culture and cytotoxicity assay Six human tumor cell lines (SGC-7901, BEL-7404, BEL-7402, Bcap-37, HL-60, and K562) and one mouse tumor cell line P388 were suspended in RPMI-1640 (Gibco), supplemented with 10 % calf serum, at 37 ℃ in 5 % CO_2 + 95 % air atmosphere. Each tumor cell line at the cell concentration of $(1-5) \times 10^8$/L was treated with O- or C-HCPT at different concentrations for 3 – 5 d (HL-60 cells for 12 h and P388 cells for 48 h) and the antitumor activity was measured by MTT[5] or trypan blue dye exclusion method[6].

Observation of lactone ring form conversion of O-HCPT in the tumor cells Human hepatoma cell line BEL-7404 or 7402 was suspended with RPMI-1640 medium (1×10^{11} cells/L, pH 7.5) without calf serum and exposed to O-HCPT at 100 μmol/L. After incubation at 37 ℃ for different hours, the suspension was centrifuged, washed with PBS solution for several times, and then extracted with 1 mL of cold methanol, shaken for 15 min, centrifuged again, and the sediment was determined by HPLC method.

HPLC assay[7] HPLC system was consisted of Waters Model 510 pump, Lambda-Max Model 481 LC spectrophotometer. μBONDPAK C_{18} column (3.9 mm × 300 mm); UV detection: 370 nm; flow rate: 0.5 mL/min; chart speed: 5 cm/min. Mobile phase was a mixture of methanol and phosphate buffer 0.02 mmol/L (40 : 60, v/v, pH 6.0); Under such condition, the retention time (RT) of O-HCPT was 7 min and C-HCPT was 12 min, respectively.

Statistical analysis Data were expressed as $x \pm s$ and compared with t-test.

RESULTS

***In vitro* cytotoxicity comparison** Either O-HCPT or C-HCPT had similar activity against the same tumor cell line at the same concentration, ie, the IC_{50} values of both forms of HCPT showed no significant difference ($P > 0.05$, Tab 1).

Tab 1. Cytotoxicity comparison of two forms of HCPT in tumor cell lines *in vitro*. $n = 3$.

Tumor cells	IC_{50}/mmol·L^{-1} (Logit method)		
	O-HCPT	C-HCPT	P value
BEL-7404	0.20	0.26	> 0.05
BEL-7402	0.05	0.05	> 0.05
SGC-7901	5.80	5.20	> 0.05
Bcap-37	0.45	0.40	> 0.05
HL-60	0.78	0.72	> 0.05
K562	0.08	0.08	> 0.05
P388	0.24	0.28	> 0.05

BEL-7402 and BEL-7404: human heptoma cell lines; SGC-7901: human gastric cancer cell line; Bcap-37: human breast cancer cell line; HL-60 and K562: human leukemia cell lines; P388: murine leukemia cell line.

***In vivo* experiment** Growth inhibitory effect of C-HCPT on either S_{180} or HepS was more effective (about two times) than that of O-HCPT (Tab 2, 3).

Conversion of O-HCPT caused by pH change Either C-HCPT or O-HCPT was dissolved in the buffered phosphate solution 0.02 mmol/L with different pH value at 37 ℃. The results showed that the lactone ring forms

Tab 2. Effect of two forms of HCPT on the growth of HepS (ip) in mice. $n = 10$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* normal saline group.

Treatment	Dosage/ mg·kg^{-1}·d^{-1}	Survival number (Initial/End)	Body weight/g (Initial/End)	Tumor weight/g	Inhibition rate/ %
Normal saline		10/10	20.1/27.6	1.62 ± 0.23	
CTX	30	10/10	20.1/22.0	0.50 ± 0.21^c	69.1
O-HCPT					
	0.5	10/9	20.0/26.8	1.4 ± 0.5^a	10.4
	1.0	10/10	20.0/25.2	1.09 ± 0.29^b	32.7
	2.0	10/10	20.0/23.7	1.1 ± 0.3^b	33.3
C-HCPT					
	0.5	10/10	20.0/23.8	0.83 ± 0.19^c	48.8
	1.0	10/10	20.0/21.9	0.70 ± 0.22^c	56.8
	2.0	10/10	20.1/22.0	0.32 ± 0.10^c	80.2

Tab 3. Effect of two forms of HCPT on the growth of S_{180} (ip) in mice. $n=10$. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* normal saline group.

Treatment	Dosage/ mg·kg^{-1}·d^{-1}	Survival number (Initial/End)	Body weight/g (Initial/End)	Tumor weight/g	Inhibition rate/%
Normal saline		10/10	18.3/24.9	1.5±0.3	
CTX	30	10/10	18.3/21.4	0.43±0.19^c	71.1
O-HCPT					
	0.5	10/9	18.2/24.3	1.2±0.3^a	18.8
	1.0	10/10	18.1/23.1	1.2±0.5^a	19.5
	2.0	10/10	20.0/21.1	0.8±0.6^b	45.6
C-HCPT					
	0.5	10/10	18.3/23.5	0.8±0.3^c	45.0
	1.0	10/10	18.3/21.7	0.7±0.3^c	53.0
	2.0	10/10	18.2/18.4	0.43±0.19^c	68.5

of HCPT could be changed along with pH alteration, C-HCPT predominated in acidic condition (pH<6.5) while O-HCPT did in basic condition (Fig 1).

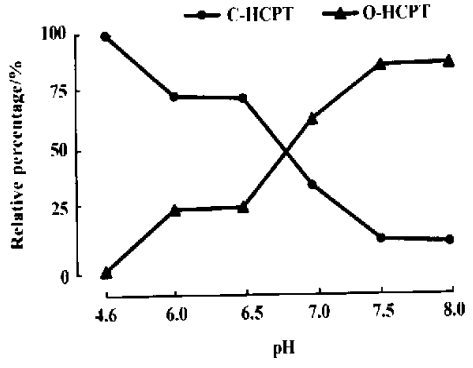


Fig 1. The kinetic change of O-HCPT and C-HCPT at different pH values of phosphate buffer 0.02 mmol/L (37 ℃, measured by HPLC method). $n=3$ samples.

Conversion of O-HCPT into C-HCPT in heptoma cells The lactone form change of O-HCPT was observed in the human heptoma cell lines BEL-7404 and BEL-7402 by HPLC method. The percentage of O-HCPT decreased (from more than 90 % to about 50 %) along with the increase of incubation time (from 0 to 4 h) while the percentage of C-HCPT increased from less than 10 % to about 50 % (Fig 2). Such phenomenon may explain partly the difference between the *in vitro* and *in vivo* antitumor activity of O-HCPT in comparison with C-HCPT experiments, ie, under certain condition, O-HCPT could be converted into C-HCPT which can exert stronger antitumor activity.

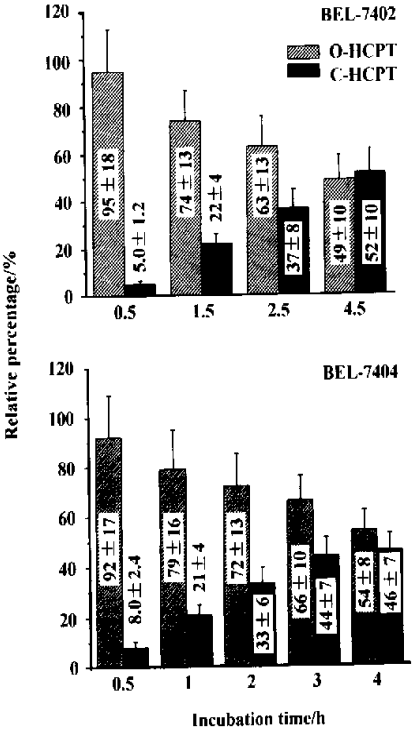


Fig 2. Conversion of O-HCPT into C-HCPT in cultured BEL-7402 and BEL-7404 hepatoma cells (measured by HPLC method). $n=3$ samples. $\bar{x}\pm s$.

DISCUSSION

In the present study, we found that the antitumor activities of the ring-closed and ring-opened forms of HCPT were nearly the same. Such results were contradictory to the report of Wani *et al*[2] and Wall *et al*[3]. We think that their experiments were mainly

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Sep; 22 (9)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

performed in topoisomerase Ⅰ activity determination. In contrast, we used various tumor cell lines and the cytotoxicity of the two forms of HCPT was compared in detail. In fact, the results obtained by Hertzberg *et al*[9] also supported our findings, indicating that both forms of camptothecin showed the similar activities on L1210 leukemia cells. Besides, our results demonstrated that the conversion of two forms of HCPT could occur in certain conditions, ie, O-HCPT could be converted into C-HCPT under acidic condition, such as in cultured tumor cells which were more acidic.

Our work suggests that O-HCPT is also an effective form of HCPT in experiment *in vivo* (ip) but less active than C-HCPT. The cause of difference between *in vitro* and *in vivo* results may be inferred as: the molecular polarity of O-HCPT is stronger (and also difficult to be converted into C-HCPT in basic body fluid, pH≈7.40) and easier to be metabolized and eliminated in animal body; in contrast, the polarity of C-HCPT is weaker and can pass through cell membrane more freely. Thus, more C-HCPT molecules can enter tumor cells and exert stronger antitumor activities.

ACKNOWLEDGEMENTS The authors wish to thank Mrs ZHANG Zhen-De, XU Yong-Ping, TANG Wei-Dong, and Ms ZHOU Pei-Qing for their technical helps.

REFERENCES

1 Xu B, Yang JL. Hydroxycamptothecin as an antitumor agent. In: Chang HM, Yeung HW, editors. Advances in Chinese medicinal materials research. Singapore: World Scientific Publishing Co; 1985. p 377-89.
2 Wani MC, Ronman PE, Lindley JT, Wall ME. Plant antitumor agents. 18' Synthesis and biological activity of camptothecin. J Med Chem 1980; 23: 554-60.
3 Wall ME. Alkaloids with antitumor activiting. In: International symposium on biochemistry and physiology of the alkaloids. Methes K, Schreiber K, Schutte HR, editors. Berlin: Akademic-Verlag; 1969. p 77-87.
4 Hsu B, Chang SY, Cheng JT, Le XF, Yang JL, Wu FG, *et al*. Studies of the sensitivity of several tumour models in screen of anticancer agents. Chin Sci Bull 1975; 20: 242-4.
5 Zhou JJ, Yue XF, Han JX, Yang WY. Improved MTT assay for activity of antitumor agents. Chin J Pharm 1993; 24: 455-7.
6 Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res 1987; 47: 936-42.
7 Yao PH. Determination of camptothecin by HPLC method. Chin Pharm J 1994; 29: 165-6.
8 Jaxel C, Kohn KW, Wani MC, Wall ME, Pommier Y. Structure-activity study of the actions of camptothecin derivatives on mammalian topoisomerase Ⅰ: evidence for a specific receptor site and a relation to antitumor activity. Cancer Res 1989; 49: 1465-9.
9 Hertzberg RP, Caranfa MJ, Holden KG, Jakes DR, Gallagher G, Mattern MK, *et al*. Modification of the hydroxy lactone ring of camptothecin: Inhibition of mammalian topoisomerase Ⅰ and biological activity. J Med Chem 1989; 32: 715-20.

羟基喜树碱内酯环形式与其抗癌活性的关系

周建军，刘 键，胥 彬[1]
(中国科学院上海生命科学研究院上海药物研究所药理室，上海 200031，中国)

关键词 10-羟基喜树碱；内酯类；抗肿瘤药；细胞培养；高压液相色谱法

目的：研究10-羟基喜树碱(HCPT)的内酯环形式与抗癌活性之间的关系. **方法**：采用MTT和台盼蓝排染法测定两种内酯环形式的HCPT在体外对多种肿瘤细胞的作用，并在体内(0.5、1.0和2.0 mg/kg, ip)评价它们对S_{180}肉瘤和HepS肝癌的抑瘤效果；应用HPLC方法观察了HCPT内酯环在肿瘤细胞内的转化情况. **结果**：两种形式的HCPT对同一种肿瘤细胞均显示类似的细胞毒性($P>0.05$)；但在体内实验中，闭环HCPT(C-HCPT)的抑瘤率明显高于(约两倍)开环HCPT(O-HCPT). 采用HPLC方法分析证明，两种类型的HCPT能在一定条件(如pH改变等)下相互转化. 因此，O-HCPT体内、外作用的明显差异可归因于其在偏酸性环境(包括肿瘤细胞体外培养时)可较多地转化成C-HCPT，而在偏碱性环境(如正常体液pH约为7.40)中多呈开环形式. **结论**：闭环和开环HCPT羟基喜树碱均具有抗癌活性，但前者的活性较强；开环HCPT可在一定条件下转化成闭环形式.

(责任编辑 吴民淑)