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Antitumor effect of CPT-11, a new derivative of camptothecin, against pleiotropic drug-resistant tumors in vitro and in vivo

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Summary. CPT-11, a new derivative of camptothecin, was effective against tumor cells, especially vincristine (VCR)- and adriamycin (ADM)-resistant P388 leukemia, compared to either VCR or ADM. The drug showed superior chemotherapeutic effects over VCR and ADM in sensitive P388 leukemia-bearing mice, and was also effective in VCR- and ADM-resistant P388 leukemia-bearing mice. These latter survival advantages with CPT-11 were almost equal to those obtained by CPT-11 against sensitive P388 leukemia. CPT-11 was found to be effective against human tumor cells, especially various pleiotropically drug-resistant human tumor lines, compared to VCR and ADM. CPT-11 should be considered for further development as a new chemotherapeutic agent potentially effective against pleiotropically drug-resistant tumors.

Introduction

Camptothecin, an antitumor alkaloid isolated from *Camptotheca acuminata* [20], is a potent inhibitor of DNA synthesis and has shown significant antitumor activity against mouse L1210 leukemia [20], rat Walker carcinosarcoma [20], and several experimental tumors [6]. In spite of its activity against murine tumors, this compound has been disappointing because of both its low response rate in clinical trials and its significant and unpredictable toxicity [4, 7, 10, 11].

To avoid toxicity and to improve therapeutic efficacy, several derivatives of camptothecin have been synthesized [9]. One of them, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (CPT-11), has shown strong antitumor activity against various kinds of murine tumors [12]. This compound is now undergoing phase I clinical trials in Japan. In this paper, we examined the activity of CPT-11 against vincristine (VCR)- and adriamycin (ADM)-resistant tumor sublines of rodent and human origins. We found that this compound is equally effective against drug-sensitive tumor-cell lines and their drug-resistant derivatives in vivo and in vitro. Although further clinical and toxicological studies are needed, CPT-11 could be an interesting compound against both drug-sensitive and drug-resistant tumors.

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Materials and methods

Tumor cells and culture. P388 leukemia cells were supplied by Simonsen Laboratories, Inc. (Gilroy, Calif), under the auspices of the National Cancer Institute (NIH, Bethesda, Md). P388 cells resistant to VCR (P388/VCR) and to ADM (P388/ADM) were kindly supplied by the Mammalian Genetics and Animal Products Section, NCI, NIH. The human myelogenous leukemia K562 cell line was provided by Dr. Ezaki, and its sublines resistant to VCR (K562/VCR) and to ADM (K562/ADM) were established in the laboratory of one of the authors [16, 17]. The human ovarian cancer line A2780 and its ADM-resistant subline (2780AD) were provided by Dr. R. Ozols, Medicine Branch NCI, NIH [18]. The acute lymphoblastic leukemia cell line (CCRF-CEM) and its vinblastine-resistant subline (CEM-VLB100) were provided by Dr. W. Beck, St. Jude Children's Hospital [3]. A cloned human epidermoid carcinoma cell line (KB3-1) and its colchicine-resistant subline (KBC-4) were provided by Dr. I. Pastan, NCI, NIH [1].

Drugs. CPT-11 (molecular weight, 677) was the product of Yakult Co. Ltd, Tokyo, Japan. The drug was dissolved in phosphate-buffered saline (PBS). All other antitumor agents were formulated for clinical use. The drugs were obtained from the following sources; ADM, from Kyowa Hakko Co. Ltd., Tokyo, Japan; VCR and VLB, from Shionogi and Co., Ltd., Osaka, Japan; colchicine was the product of Sigma, St. Louis, Missouri.

Drug treatment of the cells. For the drug treatment experiments, tumor cells (2×10^4 for P388, P388/VCR, and P388/ADM cells, and 4×10^4 for K562, K562/ADM, K562/VCR, A2780, 2780AD, KB3-1, KBC-4, CCRF-CEM, and CEM-VLB100 cells) were cultured at 37° C for 24 h in Costar 6-well tissue-culture clusters (for A2780, 2780AD, KB3-1, and KBC-4, which grow on the surface of the dish), or for 5 h in Falcon No. 2054 culture tubes (for other cell lines, which grow in suspension) containing 2 ml growth medium (RPMI 1640 medium containing 5% fetal bovine serum and 100 μg/ml kanamycin) in a humidified atmosphere of 5% CO_2. Then the cells were treated with graded drug concentrations, reincubated for 72 h in the presence of drugs, and counted with a Model ZBI Coulter counter as described previously [13, 14]. Three samples were used for each drug concentration. In the control cultures, tumor cells grew exponentially during the incubation period.

IC_{50} was determined by plotting the logarithm of the drug concentration vs the growth rate (percentage of control) of the treated cells [13, 14].

Evaluation of antitumor activity. For evaluation of antitumor activity, 1/10 ml of diluted ascites fluid containing 10^6 P388, P388/VCR, or P388/ADM cells was transplanted i.p. into $CD2F_1$ mice [14, 15]. Drugs were dissolved in 0.9% NaCl solution and administered i.v. on days 1, 5, and 9 after tumor inoculation. Six mice were used for each experimental group. Antitumor activity was evaluated by the mean survival time of a group of mice, and also expressed by the ILS (increase in life span: percentage value) [19].

Results

Growth-inhibitory effect of CPT-11 on sensitive and vincristine- and adriamycin-resistant P388 leukemia

Cytotoxicity of CPT-11 against drug-resistant as well as drug-sensitive tumor cells would increase interest in the development of this compound as a new antitumor agent. P388/VCR and P388/ADM cells showed 25- and 47-fold resistance to VCR, and 3.4- and 27-fold resistance to ADM, respectively, when IC_{50} values of these tumor lines and the parent cells were compared (Table 1). P388/VCR cells, however, showed almost equal sensitivity to CPT-11 as did the parent cells, and P388/ADM showed only 2.6-fold resistance to CPT-11. These results clearly indicate that CPT-11 is effective against tumor cells, especially the VCR- and ADM-resistant tumor cell lines, compared to either VCR or ADM. This information suggests that CPT-11 should be effective in vivo in animals bearing VCR- or ADM-resistant tumors.

Chemotherapeutic effects against P388 leukemia

The chemotherapeutic effect of CPT-11 was compared with VCR and ADM against P388 leukemia inoculated i.p. into $CD2F_1$ mice. Drugs were given i.v. on days 1, 5, and 9 after tumor inoculation. CPT-11 was more effective against P388 leukemia than either VCR or ADM in this experiment (Fig. 1). A maximum ILS of 145% was observed at 200 mg/kg CPT-11 (total dose, given on days 1, 5, and 9), whereas a slightly lower maximum ILS (90%–95%) occurred with VCR at 4 mg/kg (total dose, given on days 1, 5, and 9), and with ADM at 15–30 mg/kg (total dose, given on days 1, 5, and 9).

Table 1. Cytotoxity of CPT-11, vincristine, and adriamycin in mouse P388 leukemia sensitive and resistant to vincristine and adriamycin

Cell line	IC_{50}(nM)		
	VCR	ADM	CPT-11
P388	1.6±0.5[a]	31± 0.5	3,400± 40
P388/VCR	41 ±3 (25)[b]	105± 12 (3.4)	3,000±140 (0.9)
P388/ADM	75 ±2 (47)	850±100 (27)	9,000±310 (2.6)

[a] Mean ± SD of three determinations
[b] Numbers in parentheses, degree (*X*-fold) of resistance as compared to parent cells

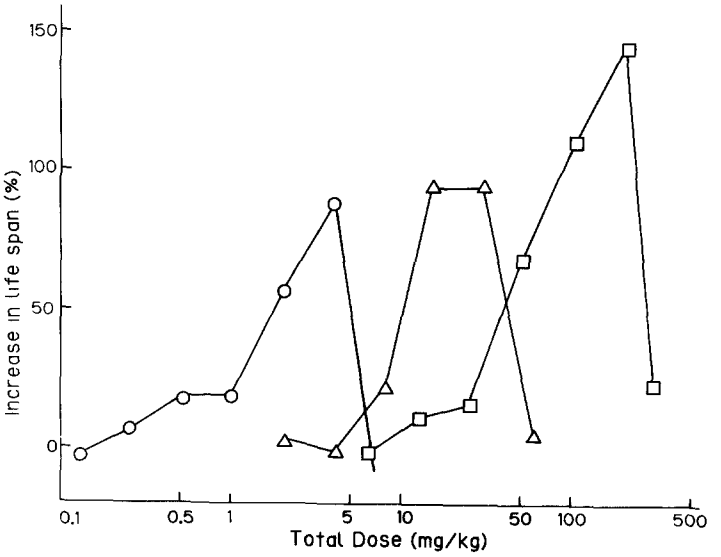

Fig. 1. Antitumor activity of VCR, ADM, and CPT-11 against P388 leukemia. P388 leukemia cells (10^6 cells/mouse) were implanted i.p. into female $CD2F_1$ mice (six mice per group) on day 0, and VCR (○), ADM (△), or CPT-11 (□) was administered i.v. on days 1, 5, and 9. The dose is expressed as total dose of 3 injections. Mean survival time of the control group in days was 10.1 ± 1.6 (SD)

Chemotherapeutic effect of CPT-11 in VCR- and ADM-resistant tumor-bearing mice

VCR at doses ranging from 1 to 4 mg/kg (total dose) given i.v. on days 1, 5, and 9 after tumor inoculation showed marginal chemotherapeutic effect (ILS, <20%) in mice bearing P388 leukemia resistant to VCR (Fig. 2). CPT-11 given on the same schedule resulted in a 118% ILS at 100 mg/kg (total dose) and a maximum ILS of about 130% at a dose of 200 mg/kg (total dose). These survival advan-

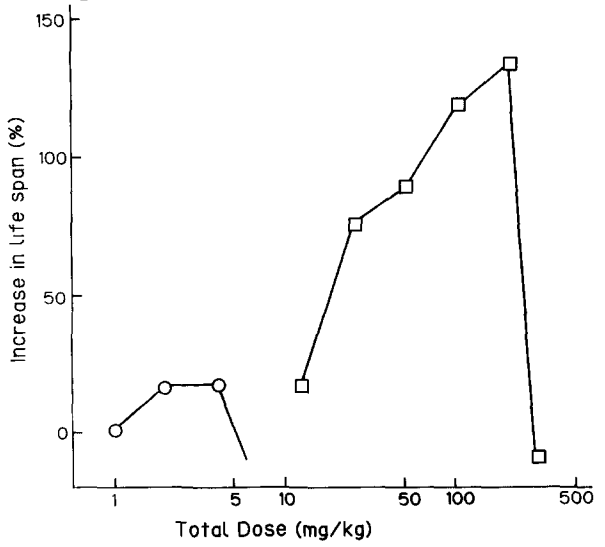

Fig. 2. Antitumor activity of VCR and CPT-11 against P388 leukemia resistant to VCR. P388 leukemia cells resistant to VCR (10^6 cells/mouse) were implanted i.p. into female $CD2F_1$ mice (six mice per group) on day 0, and VCR (○) or CPT-11 (□) was administered i.v. on days 1, 5, and 9. The dose is expressed as total dose of 3 injections. Mean survival time of the control group in days was 9.0 ± 0 (SD)

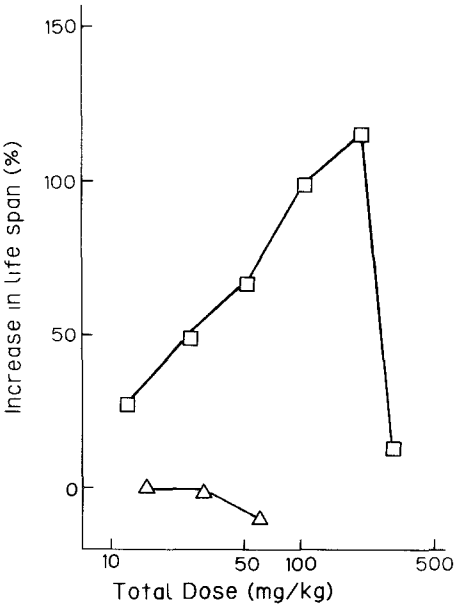

Fig. 3. Antitumor activity of ADM and CPT-11 against P388 leukemia resistant to ADM. P388 leukemia cells resistant to ADM (10^6 cells/mouse) were implanted i.p. into female $CD2F_1$ mice (six mice per group) on day 0, and ADM (△) or CPT-11 (□) was administered i.v. on days 1, 5, and 9. The dose was expressed as total dose of 3 injections. Mean survival time of the control group in days was 8.0±0 (SD)

tages were almost equal to those (ILS 110%–140%) conferred by CPT-11 at similar doses in the sensitive P388 leukemia-bearing mice (Fig. 1). It is evident that CPT-11 is effective against VCR-resistant tumor cells in vivo.

Similar results were also obtained with ADM in ADM-resistant P388 leukemia-bearing mice (Fig. 3). ADM at doses ranging from 15 to 60 mg/kg (total dose) given i.v. on days 1, 5, and 9 after tumor inoculation showed no chemotherapeutic effect in mice bearing P388 leukemia resistant to ADM. CPT-11 given on the same schedule resulted in a 100% ILS at 100 mg/kg (total dose) and a maximum ILS of about 115% at a dose of 200 mg/kg (total dose). Although these survival advantages were slightly smaller than those conferred by CPT-11 at similar doses in the sensitive P388 leukemia-bearing mice, it is evident that CPT-11 is also effective against ADM-resistant tumor cells in vivo.

The survival advantages of CPT-11 in VCR- and ADM-resistant P388 leukemia-bearing mice were equal or superior to those obtained with VCR and ADM in drug-sensitive P388 leukemia-bearing mice.

Growth-inhibitory effect of CPT-11 on sensitive and pleiotropic drug-resistant human tumor lines

The cytotoxicity of CPT-11 against drug-resistant human tumor lines is of interest for clinical use. K562/VCR, K562/ADM, 2780AD, KBC-4, and CEM-VLB100 cells showed 88-, 644-, 970-, 1020-, and 500-fold resistance to VCR, and 10-, 128-, 1000-, 37-, and 10-fold resistance to ADM, respectively, when the IC_{50} values of these drug-resistant derivatives and their parent cell lines were compared (Table 2). These tumor-cell lines, however, showed only 5.2-, 12-, 14-, 6.4-, and 8.7-fold resistance to CPT-11. These results clearly indicate that CPT-11 is more effective against tumor cells than either VCR or ADM, especially in pleiotropic drug-resistant human tumor lines.

Drug-sensitive human tumor lines K562, A2780, and CCRF-CEM were about 4-fold more sensitive to CPT-11 than mouse P388 leukemia. This suggests that CPT-11 may be effective against human tumors, as well as against human tumors clinically resistant to VCR and ADM. Animal experiments in the nude mouse system would be rewarding.

Discussion

In this study, we found that CPT-11 is equally effective against P388 leukemia, sensitive and resistant to VCR or ADM in vitro and in vivo. The drug also showed potent effects against human tumor-cell lines resistant to VCR, ADM, colchicine, and vinblastine, compared to the drugs used for the induction of primary resistance. These resistant tumor cells are called pleiotropically drug-resistant because of their wide resistance patterns. The common mechanisms of pleiotropic drug resistance are mainly attributed to defects in drug transport (outward transport) [5, 8, 14, 15]. While antitumor agents are clinically very useful for cancer therapy, the use of these agents has often been hampered by the emergence of resistance.

Table 2. Cytotoxicity of CPT-11, vincristine, and adriamycin in human tumor lines sensitive and pleiotropically resistant to vincristine and adriamycin

Cell line	IC_{50}(nM)		
	VCR	ADM	CPT-11
K562	1.6 ± 0.3[a]	13 ± 1	860± 60
K562/VCR	140 ± 36 (88)[b]	130 ± 12 (10)	4,500± 70 (5.2)
K562/ADM	1,030 ±200 (644)	1,660 ±100 (128)	10,000± 350 (12)
A2780	0.3 ± 0.02	1.3± 0.1	870± 10
2780AD	290 ± 12 (970)	1,300 ±190 (1,000)	12,400± 180 (14)
KB3-1	0.47± 0.01	3.6± 0.1	2,800± 60
KBC-4	480 ± 16 (1020)	133 ± 2.9 (37)	17,700±1,600 (6.4)
CCRF-CEM	0.21± 0.01	10 ± 0.29	810± 30
CEM-VLB1100	104 ± 1.1 (500)	97 ± 5.2 (10)	7,100± 240 (8.7)

[a] Mean ± SD of three determinations

[b] Numbers in parentheses, degree (*X*-fold) of resistance as compared to parent cells

Camptothecin inhibits both DNA and RNA synthesis in mammalian cells. Inhibition of DNA synthesis is more prominent than the inhibition of RNA synthesis [20]. A rapid and reversible fragmentation of cellular DNA in cultured mammalian cells was observed with the drug. This fragmentation did not occur with purified DNA, although extensive single-strand DNA breaks have occurred in reactions containing purified mammalian DNA topoisomerase I [21]. Camptothecin has been shown to block the rejoining step of the breakage-reunion reaction of mammalian DNA topoisomerase I by stabilizing the enzyme-DNA complex [21]. Thus, the mode of action of camptothecin differs from that of VCR and ADM, which induce pleiotropic drug resistance in tumor cells. The mechanism of resistance to camptothecin has recently been attributed to the resistance of topoisomerase I to the drug [2]. This mechanism also differs from the resistance mechanisms observed for VCR and ADM. These observations may provide the rationale behind the significant effects of camptothecin (CPT-11) against pleiotropic drug-resistant tumor cells. Inhibitors of topoisomerase I should be evaluated as a new class of antitumor agents because of their effects against pleiotropic drug-resistant tumor cells.

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