[CANCER RESEARCH 51, 3052-3055, June 1, 1991]

Advances in Brief

Complete Growth Inhibition of Human Cancer Xenografts in Nude Mice by Treatment with 20-*(S)*-Camptothecin[1]

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Abstract

20-*(S)*-Camptothecin (CAM), a plant alkaloid, was tested against 13 human cancer xenograft lines carried by immunodeficient (nude) mice. The drug, formulated in 20% intralipid and given i.m., was more effective than any other clinically available drug tested. It was found that: (*a*) CAM, at nontoxic doses, suppressed growth and induced regression of cancer of the colon (3 lines), lung (4 lines), breast (2 lines), stomach (1 line), ovary (1 line), and malignant melanoma (2 lines); (*b*) the drug was equally effective administered i.m. or p.o. Both routes are significantly better than i.v. administration; (*c*) CAM is substantially more effective and less toxic than its sodium salt, which was unsuccessfully tested in cancer patients. CAM should be further tested against responsive cancers as a drug which is easy to isolate and formulate for large-scale studies.

Introduction

CAM,[3] a plant alkaloid, was isolated in 1966 from *Camptotheca acuminata* of the *Nyssaceae* family and tested for anticancer activity (1). Following preclinical research, its water-soluble sodium salt (CAM-Na^+) was used in phase I and phase II clinical trials (2–5). Although early tumor-specific effects were reported in some studies (2, 3), the general consensus, based on detected toxicities (discussed in Ref. 6), led to discontinuation of the clinical application of the sodium salt.

More recent developments, namely preparation of semi- and fully synthetic analogues of CAM (7, 8), and studies on the mechanism of their action have paved the way for further exploration. It was established that a cellular enzyme, DNA topoisomerase I, was the target for the cytotoxic action of the parent drug as well as its analogue (9–12). Among CAM analogues screened for their antitumor potential, 9-AC and 10,11-MDC were selected for further tests against human colon cancer xenografts (6, 13). Unlike other chemotherapeutic drugs, the two analogues induced long-term disease-free remissions. Drug toxicity was low and allowed for repeated courses of therapy. Recently, both drugs were tested using xenograft lines of six main human cancer types (14). The results are overviewed in Table 1.

The purpose of this study was to evaluate the efficacy of CAM in the treatment of various human cancer xenografts, to compare the effects of CAM to those of CAM-Na^+, and to find the best way to administer camptothecins.

Materials and Methods

Nude Mice. Swiss immunodeficient (nude) mice of the NIH-1 high fertility strain, bred and maintained in a pathogen-free environment, were used for the experiments (17).

Human Cancer Xenografts. All lines, established as heterotransplants into nude mice using surgical specimens of cancerous tissue, were maintained by serial passages in nudes. The type and histology of xenograft lines are listed in Table 1, as well as a review of the line sensitivity to chemotherapy by nine clinically available chemotherapeutic agents and three analogues of camptothecin, (9-NC, 9-AC, and 10,11-MDC). For an implant, approximately 50 mg, wet weight, of finely minced tumor tissue in 0.5 of Eagle's minimum essential medium (Gibco, Long Island, NY) were injected under the skin over the right dorsal chest region on Day 0. Tumor-implanted animals were randomly assigned to drug-treated or control groups. The treatment started on Day 7 and continued once or twice a week for the indicated time periods. The tumors were measured in three dimensions using a caliper, and their volumes were calculated. Responses to chemotherapy were evaluated in terms of partial growth inhibition, growth inhibition, PR, and CR, as defined in the legend to Table 1. Control tumor-bearing mice were given injections of the drug-formulating vehicle at the same intervals as the treated ones.

Camptothecin Formulation and Administration. 20-*(S)*-Camptothecin was purchased from the Institute of Materia Medica, Academia Sinica, Shanghai, China, and purified to homogeneity as determined by analytical methods (12). Camptothecin sodium salt was prepared as described (7).

Finely ground drug was dispersed in intralipid 20% (Kabil Vitrum, Inc., Alameda, CA) by sonication, 3 pulses for 30 s each (Ultrasonic Processor, Model GE600, Sonics and Materials, Inc., Danbury, CT) at 1 mg/ml. The volume injected was adjusted to 0.1 ml/25 g of body weight. For i.m. injections, a 27-gauge needle was inserted into the deep muscles of the posterior leg. Injections were also given i.v. through a 27-gauge needle into a tail vein. In none of the experiments was an adverse reaction registered during the injections or thereafter. For p.o. administration, the required amount of the drug, formulated in intralipid 20%, was mixed with a dietary supplement (whole wheat bread saturated with milk and mixed into a paste). Once a day the mice were fed a 1-g supplement containing the required dose of the drug, followed by 5 g of autoclavable mouse food. By itself, this feeding regimen had no adverse effects on the health of experimental animals; this feeding regimen can be continued indefinitely for at least 6 months.

Results and Discussion

Currently, numerous studies are available which demonstrate that human tumor xenografts retain the histological, biochemical, and antigenic characteristics (reviewed in Refs. 18 and 19), as well as drug sensitivity pattern of the original tumor tissue. At the maximum tolerated dose, treatments of various human cancer lines in nude mice by a chemotherapeutic agent correlate well with its clinical effectiveness (15). Based on these and related studies (19), the predictive value of xenografts in chemotherapy testing is well established.

Received 3/19/91; accepted 4/5/91.

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[1] The research was supported in part by USPHS-NIH Grant CA 11655, Grants RD-300 and CH-348C from the American Cancer Society, and the Stehlin Foundation for Cancer Research.

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[3] The abbreviations used are: CAM, 20-*(S)*-camptothecin; CAM-Na^+, 20-*(S)*-camptothecin sodium salt; 9-AC, 9-aminocamptothecin; 10,11-MDC, 10,11-methylenedioxycamptothecin; 9-NC, 9-nitrocamptothecin; CR, complete remission; PR, partial remission.

Table 1 *Chemotherapy of human cancer xenografts*

Xenograft line	Type	Histology[a]	ADR[b]	5-FUra	MTX	CYT	ALK	VCR	VBL	MCCNU	BCNU	9-NC	9-AC	10,11-MDC	Ref.
CASE	Colon	P	0[c]	0	0	0	0	0	0	0	0	PR	CR	CR	6, 13, 15, 16
SW 48	Colon	P	0	0	0	0	0	0	0	0	0		CR		
SQU	Colon	M	0	0	0	0	0	0	0	0	0		CR	PR	
BRE	Stomach	M													
DOY	Lung	UN	0	0	0	0	0	0	0	0	0			CR	
HAR	Lung	SC	0	0	gi	0	pi	0	0	0	0	CR		PR	
SPA	Lung	AD										CR		CR	
DIL	Lung	EP										PR		CR	
CLO	Breast	ID	0	pi	0	gi	gi	gi	gi	0	0	PR	PR		
MUR	Breast	ID	0	0	0	0	0	0	0	0	0	CR		CR	
LAN	Ovary	UN										CR			
BRO	Malignant melanoma		0	0	0	pi	0	0	0	0		CR	PR		
SCH	Malignant melanoma		0	0	pi	pi	pi	pi	0	0		gi			

[a] Adenocarcinoma of the colon or stomach: P, poorly differentiated; M, moderately differentiated. Lung: non-small cell carcinoma; UN, undifferentiated carcinoma; EP, epidermoid; AD, adenocarcinoma; SC, small-cell carcinoma; ID, infiltrating duct carcinoma of the breast; U, undifferentiated ovarian carcinoma.

[b] Chemotherapy by maximum tolerated doses (mg/kg body weight/dose x 8-12): ADR, doxorubicin (1 mg); 5-FUra 5 fluorouracil (20 mg); MTX, methotrexate (8 mg/kg), CYT, cytoxan (20 mg), ALK, alkeran (1 mg); VCR, vincristine (0.2 mg); VBL, vinblastine (0.3 mg); MCCNU, methyl-1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (0.4 mg); BCNU (0.4 mg); 9-NC (9-nitrocamptothecin (4 mg)1; 9-AC, 9-aminocamptothecin (10 mg); 10,11-MDC, 10,11-methylenedioxycamptothecin (10 mg). Doxorubicin was injected i.v. 9-Aminocamptothecin was injected i.m.; all other drugs were injected s.c.

[c] 0, no response to treatment or tumor growth inhibition <80%; pi, partial growth inhibition of 80–99%; gi, 100% growth inhibition; PR, partial remission defined as 50–100% tumor regression followed by regrowth within 1 month posttreatment; CR, complete remission, *i.e.*, tumor disappearance in all treated mice sustained for >1 month.

CAM Treatment of Human Cancer Xenografts. Thirteen human cancer xenografts lines were treated with CAM administered i.m., i.v., or p.o. (Table 2). These lines were resistant to most of the chemotherapeutic agents available to the oncologist (Table 1). The treatment of a colon carcinoma line CASE with CAM, 4 mg/kg of body weight (twice a week schedule) injected i.m., had optimal effectiveness and minimal toxicity (Table 2). Doses of 3 mg/kg and lower were less effective, while 6 and 8 mg/kg were toxic. CAM i.v. had no inhibitory effects on the growth of CASE cancer line, and this route of application resulted in toxic deaths. In contrast, all i.m. CAM treatments were well tolerated, and the overall toxicity was acceptable. Consequently, an i.m. dose of 4 mg/kg, delivered twice a week, was selected for most of the experiments. The weight loss of mice treated with 53–232 mg/kg did not exceed 15% of the initial body weight. The treated animals regained the weight during the late phase of the treatment or shortly thereafter.

CAM induced complete remission in the majority of the treated animals bearing 10 of 13 tumors studied. The lines included cancer of the colon (CASE), lung (DOY, HAR, SPA, and DIL), breast (CLO, MUR), ovary (LAN), and stomach (BRE) and a malignant melanoma (BRO). Breast carcinoma CLO was highly sensitive to the treatment, and a once a week schedule, 116-mg/kg total dose, sufficed in the induction of CR. The CR lasted at least 3–4 weeks following the termination of the treatments. Mice implanted with the rest of the tested cancer lines responded either with CR in some and PR in others or with PR in all animals. The poorest responders were mice implanted with colon cancers SW 48 and SQU or with melanoma SCH. Treatment p.o. was tested in mice carrying SPA lung adenocarcinomas. Four to 8 mg/kg on a daily schedule resulted in CR. The remissions were maintained with minimal toxicity during the whole course of treatment. An important difference between CAM and derivatives, however, was that after 6 months of continuous treatment, we observed regrowth of 5 of 7 lines treated with CAM, but no regrowth of any of the 4 lines treated with 9-NC. This suggests that resistance to CAM can become a problem under prolonged treatment.

Table 3 compared CAM with its sodium salt (CAM-Na^+). Consistent with results shown in Table 2, CAM i.m., 4 mg/kg body weight, was highly effective against CASE colon cancer xenografts. CAM-Na^+ was ineffective in this tumor line, which otherwise was sensitive to CAM and analogues 9-AC, 9-NC, or 10,11-MDC. Dose escalation of CAM-Na^+ was accompanied by severe toxicity and did not improve the antitumor efficacy. These results were consistent with the early clinical trials which were conducted with the less effective and more toxic form of CAM. Low effectiveness was also observed in treatments with 9-AC sodium salt, while another water-soluble drug, 10,11-MDC sodium salt, was highly toxic.[4] This is in sharp contrast with the high efficacy of lipophilic congeners 9-AC or 10,11-MDC.

Several conclusions resulting from the presented studies can be suggested: (*a*) CAM appears by far more effective against 13 human cancer xenograft lines than any other clinically available drug tested; (*b*) CAM formulated in intralipid 20% i.m. is almost equally effective p.o. as it is i.m., and both forms of application are significantly more effective than the i.v. route; (*c*) in several treated lines (SW 48, BRE, DOY, DIL, and BRO), a pattern of emerging drug resistance is encountered; and (*d*) in view of the very low anticancer activity and toxicity of CAM-Na^+, it is not surprising that it failed in clinical tests.

When compared to CAM, 9-AC or 10,11-MDC achieve the onset of CR with lower total dose and within a shorter time period (14). Both analogues can be used for a second course of treatment, without signs of an apparent secondary drug resistance. Unlike CAM, 9-NC, 9-AC, and 10,11-MDC induce CR in several tumor lines such as SW 48, DOY, or DIL. Finally, the treatment with the three analogues often has long-term curative effects. Under these conditions, CAM should be further tested against selected human cancer types as a drug which can be isolated and formulated with relative ease and cost effective-

[4] B. C. Giovanella, M. E. Wall, and M. Potmesil, unpublished observations.

Table 2 *20-(S)-Camptothecin treatment of human cancer xenografts, twice a week schedule*

Tumor[a]	Route	Single/total dose (mg/kg)	Efficacy T/A[d]	Efficacy PR/CR	Maximum decrease in body wt[b] (%)	Comments[c]
CASE	i.m.	0/0	4/2	0/0		Day 100
		1/22	4/1	0/0		
		2/44	4/1	1/0		
		3/66	4/2		−5	
		4/88	4/3	0/3	−6	
	i.m.	0/0	4/1	0/0		Day 48
		4/36	5/5	0/5	−8	
		6/54	5/5	0/5	−22	
		8/72	5/5	0/5	−34	
	i.v.	0/0	5/0	0/0		
		4/36	4/0	0/0		
		6/54	5/2	0/0		
		8/72	5/3	0/0		
SW 48	i.m.	0/0	6/7	0/0		Day 100
		4/53[b]	7/7	7/0	<−5	
		4/116	7/7	7/0	−5	
SQU	i.m.	0/0	7/3	0/0		Day 90
		4/84	7/7	0/0	−6	
BRE	i.m.	0/0	5/2	0/0		Day 130
		6/180	5/5	0/5[a]	−14	
	i.v.	6/180	5/5	5/0	−15	
DOY	i.m.	0/0	6/0	0/0		Day 146
		4/168	6/6	0/6[e]	−12	
HAR	i.m.	0/0d	4/3	0/0		Day 167
		4/164	5/4	0/4	−8	
SPA	i.m.	0/0	6/0	0/0		Day 153
		4/188	6/5	0/5	−5	
	p.o.	0/0	12/11	0/0		Day 56
		4/204	4/4	0/4	−8	
		6/306	4/4	0/3	−14	
		8/408	4/4	0/4	−10	
DIL	i.m.	0/0	6/6	0/0		Day 145
		4/128	6/6	0/6[e]	−8	
CLO	i.m.	0/0	6/0	0/0		Day 187
		4/116[f]	6/5	0/5	<−5	
		4/232	6/5	0/5	<−5	
		0/0	5/5	0/0		Day 55
		4/56	5/5	0/5	−10	
MUR	i.m.	0/0	6/1	0/0		Day 180
		4/220	6/5	0/5	−12	
LAN	i.m.	0/0	8/1	0/0		Day 83
		4/108	8/8	0/8	−8	
BRO	i.m.	0/0	7/0	0/0		Day 130
		4/148	7/5	0/5[e]	−15	
		0/0	6/3	0/0		Day 54
		4/72	6/6	0/6	−12	
SCH	i.m.	0/0	6/2	0/0		Day 60
		4/100	8/8	0/0	−13	

[a] See Table 1 for tumor type, histology, and senstivity to various chemotherapeutic agents.
[b] Decrease in body weight evaluated in mice with total tumor reduction or with a stable tumor size.
[c] Day when efficacy data obtained; Day 0 = tumor implantation.
[d] T/A, total number of experimental animals/alive; PR/CR, partial remission/complete remission.
[e] Regrowth of some tumors during treatment.
[f] Once a week schedule.

Table 3 *Treatment of CASE xenografts with a 20-(S)-camptothecin or 20-(S)-camptothecin sodium salt, twice a week schedule*

Drug	Route and vehicle	Single/total dose (mg/kg)	Efficacy T/A[a]	Efficacy PR/CR
	i.m., intralipid 20%	0/0	6/2	0/0
CAM Na^+	i.v., saline	4/52	6/4	0/0
CAM Na^+	i.v., intralipid 20%	4/52	6/2	0/0
CAM Na^+	i.m., intralipid 20%	4/52	6/4	0/0
CAM	i.v., intralipid 20%	4/52	6/5	0/0
CAM	i.m., intralipid 20%	4/52	6/5	0/5

[a] T/A, total number of experimental animals/alive; PR/CR, partial remission/complete remission, Day 60. Evaluated in animals with total tumor regression.

ness. However, the data available thus far (summarized in Table 1) showed an unprecedented effectiveness of three analogues of camptothecin, 9-NC, 9-AC, and 10,11-MDC, against all human cancer xenograft lines tested. Further development of these highly potent CAM analogues is warranted. These drugs may provide a potential tool combating major therapy-resistant cancers.

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