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Short report

Camptothecin cytotoxic effects *in vitro*: dependency on exposure duration and dose

Lawrence Helson, Christiane Helson and Sterling Ainsworth
NaPro BioTherapeutics, Inc., 4725 Walnut Street, Suite 100, Boulder, CO 80301, USA

A survey of *in vitro* cytotoxic effects of camptothecin in human epithelioid sarcoma, colon, breast and ovarian carcinomas, glioblastoma, and neuroblastoma (PNET) cell lines, was done. We chose the MTT assay to measure survival and observed that 24 h exposures to camptothecin caused consistently greater toxicity than 1 h exposures. The LD_{50} for camptothecin was in the 12.5–25 ng/ml range. There was a 10-fold range of growth rates measured by OD after 5 days exposure and varied expression of MDR1 in these cell lines—none of which could be correlated with tumor sensitivity to drug. The most sensitive cell lines were colon and glioblastoma, and the most resistance were ovarian, breast and epithelioid sarcoma.

Key words: Camptothecin, HT-29, OVCAR-3, MDR1, SK-N-AS, SK-N-DZ, SK-N-FI, U373-MG, VA-ES-BJ, ZR-75-1.

Introduction

Among the factors contributing to camptothecin cytotoxicity, intrinsic sensitivity, MDR1, dose and exposure duration all appear to play a role. Intrinsic sensitivity and MDR1 are variables which are constitutive and difficult to control or manipulate. For this reason, we undertook this study of a panel of eight human cell lines to clarify the impact of dose and exposure duration.

Materials and methods

All experiments were *in vitro*. Camptothecin was purchased from Sigma (St Louis, MO). It was solubilized in alcohol and further diluted serially in Eagle's minimal essential medium with 10% fetal bovine serum from 100 to 1.6 ng/ml final concentrations. The tumor cell lines SK-N-AS, SK-N-DZ, SK-N-FI and VA-ES-BJ were established by CH. The tumor cell lines U373-MG, HT-29, OVCAR-3 and ZR-75-1 were obtained from the ATCC (Rockville, MD). The origin and detailed conditions for *in vitro* growth and analysis of these cell lines have been published.[1–3]

The method for determining *in vitro* cytotoxicity was trypsin–EDTA harvesting of stock tumor cells, electronic enumeration and inoculation of 4000–15 000 cells into three to six replicate wells in a 96-well microtiter plate. Tumor cells were exposed for 1 or 24 h to camptothecin. An MTT assay was used to gauge viability.[4]

Results

There was a significant decrease in survival with 24 h exposures at concentrations of 100–6.25 ng/ml when compared with 1 h exposures (Table 1). A dose–response relationship was observed for 1 h exposure at 25–100 ng/ml and for 24 h at 1.6–100 ng/ml. The toxicity engendered by camptothecin in each cell line was less consistent at different dosages at the 1 h than at the 24 h exposure level. At 6.5 ng/ml for 24 h, the least sensitive cell lines were VA-ES-BJ, OVCAR-3 and ZR-75-1, while the epithelioid sarcoma, neuroblastoma, colon carcinoma and glial tumors were more sensitive (Table 2). The relative growth, based on mean OD/microwell containing 4000–15 000 inoculated untreated cells, revealed the less sensitive cells VA-ES-BJ and ZR-75-1 to have high growth rates.

Discussion

The data are consistent with camptothecin functioning as a cycle active agent. Contrary to expectation,

Correspondence to L Helson, NaPro BioTherapeutics Laboratory, St Agnes Hospital, 305 North Street, White Plains, NY 10605, USA. Tel: (+1) 914 681-4604.

Table 1.

Cell line	Camptothecin													
	100 ng/ml		50 ng/ml		25 ng/ml		12.5 ng/ml		6.25 ng/ml		3.1 ng/ml		1.6 ng/ml	
	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h
U373 MG	48	10	63	11	68	19	87	20	91	34	88	66	77	90
(Glioblastoma)	62	9	67	8	73	12	87	17	91	31	95	80	95	96
VA-ES-BJ	67	2	79	1	92	2	100	8	100	62	100	100	100	96
(Epithelioid sarcoma)	57	1	67	2	94	4	100	29	99	90	93	91	88	87
	80	3	85	2	93	5	95	13	97	79	96	94	100	100
SK-N-AS	51	9	64	13	100	15	100	18	100	43	100	100	100	100
(PNET Neuroblastoma)	65	14	72	11	96	13	100	21	100	59	100	95	84	93
	60	13	72	12	100	13	100	18	100	57	100	100	100	100
HT-29	43	4	46	6	75	7	92	13	94	40	92	77	80	84
(Colon CA)	70	6	78	6	100	11	100	14	100	30	100	82	100	97
	51	3	62	5	89	10	100	12	100	21	100	68	100	90
SK-N-DZ	71	6	63	4	74	4	87	5	100	12	100	68	100	73
(Neuroblastoma)	46	7	67	7	100	8	100	16	100	54	100	100	100	100
	34	10	39	7	50	7	74	7	100	32	100	83	100	90
	57	5	77	4	98	3	81	4	100	7	100	57	100	83
SK-N-FI	63	68	75	65	88	78	93	75	87	81	82	95	69	84
(PNET Neuroblastoma)	60	13	77	10	86	8	100	8	100	18	100	70	100	84
	94	66	100	71	100	78	100	73	100	68	100	85	100	90
OVCAR-3	54	42	65	52	82	55	95	58	100	59	100	65	100	96
(Ovary CA)	45	55	72	76	88	89	99	89	100	89	100	100	100	100
ZR-75-1	70	58	85	62	90	66	91	69	100	80	100	96	100	100
(Breast CA)	59	55	75	54	76	56	88	58	85	69	95	77	90	79

Table 2.

Cell line	Growth[a]	Toxicity[b] (%)
VA-ES-BJ (Epithelioid sarcoma)	1.309	23
HT-29 (Colon CA)	1.254	70
U-373 MG (Glioblastoma)	0.812	68
ZR-75-1 (Breast CA)	0.740	26
SK-N-AS (PNET Neuroblastoma)	0.269	44
SK-N-DZ (Neuroblastoma)	0.230	65
OVCAR-3 (Ovary CA)	0.135	26
SK-N-FI (PNET Neuroblastoma)	0.065	44

[a] Average MTT assay optical density of six microwells from each of three experiments containing 4000–6000 tumor cells plated 5 days previously.
[b] Average cytotoxicity caused by 6.25 ng/ml camptothecin for 24 h.

there was no evidence that rapidly proliferating cells are more sensitive to camptothecin than the slower cycling cells. The presence of high or low MDR1 expression did not correlate with response as SK-N-FI, SK-N-DZ and SK-N-AS are known high intermediary and low MDR1 expressors, respectively, yet SK-N-FI and SK-N-AS remain equally sensitive to camptothecin.[5]

In conclusion, camptothecin appears to be most cytotoxic in the colon cancer cell line, and least in breast, ovarian and epitheliod sarcoma cell lines. Extension of these studies to a cohort of tumors of different histiotypic origin may exhibit other sensitivities. The unusual sensitivity observed in colon cancer cells suggests it be the subject of further investigation.

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