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Therapeutic efficacy of vinorelbine against pediatric and adult central nervous system tumors

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Abstract *Purpose*: The activity of vinorellbine, a new semisynthetic vinca alkaloid, was evaluated against a battery of human tumor xenografts derived from adult and pediatric CNS malignancies. *Methods*: Tumors included adult high-grade gliomas (D-54 MG, D-245 MG), childhood high-grade gliomas (D-212 MG, D-456 MG), medulloblastomas (D-341 MED, D-487 MED), ependymomas (D-612 EP, D-528 EP), and a mismatch repair-deficient procarbazine-resistant glioma [D-245 MG (PR)]. Tumors were grown subcutaneously in athymic nude mice and vinorelbine was administered at a dose of 11 mg/kg on days 1, 5, and 9. Additionally, vinorelbine was also administered in combination with BCNU against D-54 MG. *Results*: Vinorelbine produced statistically significant growth delays in D-456 MG, D-245 MG, and D-245 MG (PR). No statistically significant growth delays were observed in D-54 MG, D-487 MED, D-212 MG, D-528 EP, D-341 MED or D-612 EP. The antitumor effects of the vinorelbine/BCNU combination were additive. Growth delays observed in the procarbazine-resistant line [D-245 MG (PR)] were greater than twofold the delays seen in the parent line (D-245 MG). Vincristine was equally potent against D-245 MG and D-245 MG (PR). Taxol demonstrated little activity against D-245 MG but produced 32- and 18-day growth delays in D245 MG (PR). *Conclusions*: These studies indicate that vinorelbine possesses antitumor activity against several glioma tumor xenografts with marked activity in a mismatch repair deficient-tumor.

Key words Vinorelbine · Vinca alkaloid · CNS tumors Xenografts · Mismatch repair

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Introduction

Tumors of the central nervous system (CNS) including medulloblastoma, astrocytoma, and glioblastoma multiforme account for the majority of common solid neoplasms in children under the age of 15 years [3]. Although conventional treatment options include surgery, radiotherapy, and chemotherapy, success is often limited, particularly with high-risk medulloblastoma and malignant glioma [8]. Similarly, adult malignant glioma is very challenging, with the majority of patients dying within 5 years of diagnosis. Although chemotherapy plays an important role in the battle against brain tumors, there are several obstacles specific to pharmacotherapy which limit its success, including delivery of drug to privileged intracranial sites, activity of drug against a particular tumor type, *de novo* presence or emergence of drug resistance, and/or failure to achieve therapeutic dosing levels due to dose-limiting toxicity.

Vincristine, a natural vinca alkaloid, is widely utilized in the therapy of central nervous system and other tumors [14]. Vinca alkaloids exert their cytotoxic effect by depolymerizing microtubules, although at clinically achievable concentrations the mechanism of action is probably inhibition of microtubule polymerization with associated disruption of mitotic spindle dynamics. The dose-limiting toxicity of vincristine is neurotoxicity. Vinorelbine is a new semisynthetic vinca alkaloid with significant neoplastic activity against a variety of tumor types, including lung, breast, head and neck, melanoma,

and lymphoma [12]. Vinorelbine, unlike vincristine, produces dose-limiting myelosuppression [5]. These differences in toxicity between vinorelbine and vincristine can be explained by a preferential depolymerization of the mitotic microtubules over axonal microtubules [2]. The sparing of axonal microtubules by vinorelbine accounts for the reduction in neurotoxicity.

We evaluated vinorelbine against a panel of human tumor xenografts derived from adult and pediatric CNS malignancies growing subcutaneously in athymic mice. Tumors included adult high-grade gliomas (D-54 MG, D-245 MG), childhood high-grade gliomas (D-212 MG, D-456 MG), medulloblastomas (D-341 MED, D-487 MED), ependymoma (D-612 EP, D-528 EP), as well as a glioma with a deficiency of mismatch repair activity, D-245 MG (PR).

Materials and methods

Animals

Female athymic BALB/c (*nu*/*nu* genotype, 6 weeks or older) mice were bred, handled and used for all experiments as previously described [4].

Xenografts

A panel of eight human CNS tumor-derived xenografts were used for all in vivo studies and were maintained as previously described [9].

Drugs

Vinorelbine was provided by GlaxoWellcome (Research Triangle Park, N.C.). BCNU and Taxol were provided by the Drug Synthesis and Pharmacology Laboratory, NCI (Bethesda, Md.). Vincristine was provided by Bristol Myer Squibb (Wallingford, Ct.).

Subcutaneous xenograft transplantation

Subcutaneous tumor transplantation into the right flank of the animals was performed as described previously, with inoculation volumes of 50 μl [7].

Tumor measurements

Tumors were measured twice weekly with hand-held vernier calipers (Scientific Products, McGraw, Ill.). Tumor volume was calculated according to the formula: $[(\text{width})^2 \times (\text{length})]/2$.

Xenograft therapy

In replicate experiments, vinorelbine was given to mice via intraperitoneal (i.p.) injection in doses ranging from 8.75 to 11 mg/kg per dose in 0.9% normal saline on days 1, 5, and 9 with 11 mg/kg representing the dosage lethal to 10% of the treated animals (LD_{10}). BCNU was administered to mice via i.p. injection at a dose of 50 mg/m^2 (LD_{10}) in 10% ethanol in 0.9% normal saline on day 1. Taxol was administered via i.p. injection at a dose of 20 mg/kg (LD_{10}) in 10% Cremophor in 0.9% normal saline on days 1–5. Vincristine was administered at a dose of 3.26 mg/kg (LD_{10}) in 0.9% normal saline via i.p. injection on day 1. Groups of ten randomly selected mice began receiving treatment when the median

Table 1 Effect of vinorelbine treatment on growth of s.c. human CNS xenografts in mice. Vinorelbine was administered via i.p. injection at a dose of 11 mg/kg in 0.9% normal saline on days 1, 5, and 9. The experiments are replicate studies on groups of eight to ten animals each

Xenograft	Histology	T − C (days)[a]	Regressions[b]	*P*-value[c]	Deaths
D54 MG	Adult high grade glioma	3.9	0/10	0.000	0/10
D54 MG	(anaplastic astrocytoma)	4.9	2/9	0.047	1/10
D487 MED	Medulloblastoma	2.3	0/10	0.264	0/10
D487 MED		3.5	2/8	0.000	2/10
D456 MG	Childhood high-grade glioma	14.1	7/8	0.000	2/10
D456 MG		10.2	3/9	0.000	1/10
D212 MG	Childhood high grade glioma	1.9	0/10	0.109	0/10
D212 MG		1.5	1/8	0.061	2/10
D245 MG	Adult high-grade glioma	11.6	0/10	0.000	0/10
D245 MG		9.8	2/10	0.000	0/10
D245 MG (PR)	Adult high-grade glioma	28.5	8/8	0.000	1/9
D245 MG (PR)	(procarbazine resistant)	20.6	8/8	0.000	2/10
D612 EP	Ependymoma	3.3	2/10	0.012	0/10
D612 EP		1.4	1/10	0.176	0/10
D341 MED	Medulloblastoma	2.8	0/10	0.018	2/10
D341 MED		1.3	0/9	0.027	0/10
D528 EP	Ependymoma	5.3	7/10	0.200	0/10
D528 EP					

[a] T − C, growth delay in days, is defined as the difference between the median time required for tumors in treated (T) and control (C) animals to reach five times the volume measured at the initiation of treatment
[b] Regression is defined as a decrease in tumor volume over two successive measurements
[c] Values are statistically significant if $P < 0.01$

Table 2 Effect of vinorelbine in combination with BCNU on the growth of subcutaneous glioma xenograft (D-54 MG) in mice. Vinorelbine was administered at a dose of 8.75 mg/kg in 0.9% normal saline via i.p. injection on days 1, 5, and 9. BCNU was given at a dose of 50 mg/m^2 in 10% ethanol via i.p. injection on day 1

Drug	T − C (days)	Regressions	*P*-value	Deaths
BCNU alone	2.0	0/10	0.006	0/10
Vinorelbine alone	4.3	1/9	0.000	1/10
Vinorelbine/BCNU	8.4	3/10	0.000	0/10

tumor volume exceeded 100 mm^3 and were compared with control animals receiving no drug.

Assessment of response

The response of s.c. xenografts was assessed by delay in tumor growth and by tumor regression. Growth delay, expressed as T − C, is defined as the difference in days between the median time required for tumors in treated (T) and control (C) animals to reach a volume five times greater than that measured at the start of treatment. Tumor regression was defined as a decrease in tumor volume over two successive measurements. Statistical analyses were performed using a personalized SAS statistical analysis program, the Wilcoxon rank order test for growth delay, and the Fisher's exact test for tumor regression as described previously [7].

Results

Vinorelbine was active against D-456 MG, D-245 MG, and D-245 MG (PR) with statistically significant ($P < 0.01$) growth delays ranging from 9.8 days to 28.5 days. Tumor regressions were noted against D-456 MG and D-245 MG (PR). No tumor regressions were noted in control animals. No activity was seen against the D-612 EP, D-528 EP, D-54 MG, D-212 MG, D-341 MED, or D-487 MED (Table 1). Vinorelbine was twofold more active against D-245 MG (PR) compared with D-245 MG (Table 1), with growth delays of 28.5 and 20.6 days versus 11.6 and 9.8 days, respectively.

The combination of BCNU (50 mg/m^2) plus vinorelbine (8.75 mg/kg per dose) yielded a growth delay that was additive against D-54 MG (Table 2), although a formal set of studies to define the presence of a synergistic relationship between vinorellbine and BCNU using isobologram analysis was not conducted.

The activity of vincristine against D-245 MG and D-245 MG (PR) was similar, with growth delays of 25.3 and 21.6 days compared with 18.8 and 13.9 days, respectively. Taxol demonstrated more activity against D-245 MG (PR) than against the parent line, with growth delays of 32.1 and 14.3 days compared with 2.8 and 4.5 days, respectively (Table 3).

Among 189 animals tested, 13 deaths were attributable to vinorelbine toxicity (Table 1). The median nadir weight loss was 10.3%. No neurologic toxicity, including seizure activity, was noted. Taxol produced one death in 40 animals, while vincristine produced four toxic deaths in 40 animals tested (Table 3).

Table 3 Effect of Taxol and vincristine on growth of a procarbazine-resistant subcutaneous human glioma xenograft, D-245 MG (PR), as compared with the parent line, D-245 MG. Taxol was administered at a dose of 20 mg/kg in nine parts 0.9% normal saline and one part cremophor via i.p. injection on days 1–5. Vincristine was administered at a dose of 3.26 mg/kg in 0.9% normal saline via i.p. injection on day 1

Line	Drug	T − C (days)	Regressions	*P*-value	Deaths
D245 MG	Taxol	4.5	0/9	0.005	1/10
	Vincristine	21.6	6/9	0.039	1/10
	Taxol	2.8	0/10	0.001	0/10
	Vincristine	25.3	8/10	0.000	0/10
D245 MG (PR)	Taxol	32.1	9/10	0.000	0/10
	Vincristine	18.8	8/8	0.000	2/10
	Taxol	14.3	5/9	0.000	0/10
	Vincristine	13.9	7/9	0.000	1/10

Discussion

The vinca alkaloids represent a class of antineoplastic agents which play a role in the therapy of CNS tumors. Vinorelbine is a unique, new semisynthetic vinca alkaloid which differs from other members of the class in its chemical structure, selectivity for microtubules, and toxicity profile. Vinorelbine is characterized by a modification in the catharanthine nucleus which is distinct from other vincas. Vinorelbine is the only vinca to be configured around an eight-member catharanthine moiety, while the other vincas are defined by a nine-member catharanthine ring. Vinorelbine, in a similar manner to other vinca alkaloids, interferes with tubulin depolymerization and arrests the cell in metaphase by inhibiting mitotic spindle formation [12]. Thus, vinorelbine acts as a cell cycle-dependent antimitotic agent blocking progression in the G-2 and M cell phases. However, it appears that vinorelbine's distinct structural differences may be responsible for the drug's selective binding to microtubule-associated proteins over axonal microtubules [7]. This observation has clinical implication since neurotoxicity with vinorelbine appears to be less of a problem than with other vincas.

Early phase I and II trials have demonstrated the activity of vinorelbine against a wide array of cancers, but the drug has not been studied against an extensive spectrum of CNS tumors. The results presented here demonstrate that vinorelbine possesses antitumor activity against several human glioma xenografts. The heterogeneity of growth delays in the childhood glioma and adult glioma groups demonstrate that the antitumor effects of vinorelbine, as with other agents, cannot be predicted by tumor histology alone. Mechanisms of drug resistance to vinorelbine are not clearly established, although, in a similar manner to the other vinca alkaloids,

vinorelbine appears to be susceptible to p-glycoprotein-mediated multidrug resistance [1, 11].

Laboratory and clinical studies have demonstrated that vincas exhibit enhanced activity in combination with a number of other cancer agents, such as cyclophosphamide, procarbazine, BCNU/CCNU, and cisplatin. Previous in vivo studies have shown that vinorelbine demonstrates increased activity without enhanced toxicity when combined with cisplatin [6]. No increased activity or toxicity has been observed in combinations using cyclophosphamide [6]. Our study exploring the combination of BCNU/vinorelbine produced effects which were only additive.

The greater activity of vinorelbine against the methylator resistant DNA mismatch repair-deficient xenograft, D-245 MG (PR), compared with the parent line is interesting and unexplained. The only known mechanisms of resistance to methylators such as procarbazine include the activity of O^6- alkylguanine-DNA alkyl transferase (AGT), an enzyme which removes the methyl adduct at the O^6 position of guanine [13], and a deficiency of mismatch repair activity [10]. D-245 MG (PR) has no measurable AGT activity, indicating that the only identified mechanism of resistance operating in this methylator-resistant glioma appears to be related to the deficiency of the mismatch repair protein hMSH2 [10]. The enhanced activity of vinorelbine in the presence of DNA mismatch repair deficiency led us to explore two other microtubule inhibitors, vincristine and Taxol. Vincristine, a vinca alkaloid with microtubule depolarizing actions similar to those of vinorelbine, produced significant and similar growth delays in both the parent and resistant lines. Taxol, a microtubule drug which prevents depolymerization by acting in a manner different from both vinorelbine and vincristine, specifically hyperstabilization of cellular microtubules, had minimal activity in the parent line while producing marked growth delays in the resistant line. Although the reason for this difference is not yet apparent, these results are potentially clinically relevant since there is no known way to reverse methylator resistance secondary to a deficiency of DNA mismatch repair activity. Agents which are active against mismatch repair-deficient tumors are clinically needed.

In summary, vinorelbine demonstrated antitumor activity against several human glioma xenografts and evaluation may be warranted against these tumors in phase II clinical trials, particularly but not exclusively in the presence of DNA mismatch repair deficiency.

References

1. Adams DJ, Knick VC (1995) P-glycoprotein mediated resistance to 5′-nor-anhydro-vinblastine (Navelbine). Invest New Drugs 13: 13
2. Binet S, Fellous A, Lataste H, Krikorian A, Couziner JP, Meininger V (1989) In situ analysis of the action of Navelbine on various types of microtubules using immunofluorescence. Semin in Oncol 16: 5
3. Boring CC, Squires TS, Tong T (1993) Cancer statistics. CA Cancer J Clin 43: 7
4. Bullard DE, Schold SC Jr, Bigner SH, Bigner DD (1981) Growth and chemotherapeutic response in athymic mice of tumors arising from human glioma-derived cell line. J Neuropathol Exp Neurol 40: 410
5. Burris HA III, Fields S (1994) Summary of data from in vitro and phase I vinorelbine (Navelbine) studies. Semin in Oncol 21: 14
6. Burroughs Wellcome Co., Navelbine investigators brochure, 12. Sept 1993
7. Friedman HS, Colvin OM, Skapek SX, Ludeman SM, Elion GB, Schold SC Jr, Jacobsen PF, Muhlbaier LH, Bigner DD (1988) Experimental chemotherapy of human medulloblastoma cell lines and transplantable xenografts with bifunctional alkylating agents. Cancer Res 48: 4189
8. Friedman HS, Horowitz ME, Oakes WJ (1991) Tumors of the central nervous system. Improvement in outcome through a multi-modality approach. Pediatr Clin North Am 38: 381
9. Friedman HS, Houghton PJ, Schold SC, Keir S, Bigner DD (1994) Activity of 9-dimethylaminomethyl-10-hydroxycamptothecin against pediatric and adult central nervous system tumors xenografts. Cancer Chemother Pharmacol 34: 171
10. Friedman HS, Johnson SP, Dong Q, Schold SC, Rasheed BK, Bigner SH, Ali-Osman F, Dolan E, Colvin OM, Houghton P, Germaine G, Drummond JT, Keir S, Marcelli S, Bigner DD, Modrich P (1997) Methylator resistance mediated by mismatch repair deficiency in a glioblastoma multiforme xenograft. Cancer Res 57: 2933
11. Knick VC, Miler CG (1992) Invitro derivation of a murine P388 leukemia subline resistant to 5′ nor-anhydrovinblastine (navelbine) and its cross resistance profile (abstract). Proc Am Assoc Cancer Res 33: 462
12. Potier P (1989) The systhesis of Navelbine prototype of a new series of vinblastine derivatives. Semin Oncol 16: 2
13. Schold SC, Brent TP, Hofe E von, Friedman HS, Mitra S, Bigner DD, Swenberg JA, Kleihues P (1989) O^6-alkylguanine-DNA alkyltransferase and sensitivity to procarbazine in human brain tumor xenografts. J Neurosurg 70: 573
14. Schold CS, Burger PC, Mendelsohn DB, Glatstein EJ, Mickey BC, Minna JD (1997) Primary tumors of the brain and spinal cord. Butterworth-Heinemann, Newton, Mass.