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Original Paper

Superior *in vivo* Experimental Antitumour Activity of Vinflunine, Relative to Vinorelbine, in a Panel of Human Tumour Xenografts

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The antitumour activity of vinflunine, 20′,20′-dichloro-3′,4′-dihydrovinorelbine, a fluorinated *Vinca* alkaloid obtained by reaction in superacid media, was evaluated in comparison with vinorelbine against a series of subcutaneously-implanted human tumour xenografts. The tumours studied were established from bladder (BXF1299), pancreas (PAXF546), kidney (RXF944LX), colon (DLD-1, HT-29, TC37), central nervous system (SF-295), small cell lung (NCI-H69) and prostate (PC-3). Vinflunine or vinorelbine was administered as four weekly intraperitoneal treatments, within dose ranges of 5–80 or 0.63–10 mg/kg/injection, respectively. The overall antitumour activity of vinflunine was superior to that of vinorelbine. Vinflunine showed high activity against RXF944LX and NCI-H69 xenografts and moderate activity against PAXF546, PC-3 and TC37 tumours, achieving an overall response of 64%. This contrasts with a 27% response with vinorelbine, which proved only moderately active against RXF944LX and TC37 xenografts. These results confirm and extend our previous report of the broad spectrum of *in vivo* antitumour activity of vinflunine and reinforce its potential as a valuable addition to current chemotherapeutic agents.

Key words: vinflunine, vinorelbine, human tumours, xenografts, cancer chemotherapy
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INTRODUCTION

VINFLUNINE IS a new *Vinca* alkaloid, uniquely fluorinated by the use of superacid chemistry in a little exploited region of the catharanthine moiety [1]. Vinflunine resulted in significant prolongation of survival in two murine tumour models considered relatively refractory to anticancer agents [2]. In the intravenous (i.v.) grafted P388 leukaemia, administered either as a single dose by the intraperitoneal (i.p.) or i.v. routes or as multiple i.p. doses according to three different schedules, vinflunine extended survival from between 1 and 3.57-fold. In comparison, the effects of other tubulin-interacting agents tested concurrently were limited to 0.29–1.14-fold [3]. Significant survival prolongation (log rank $P < 0.001$) and tumour growth inhibition (optimal reduction in volume of treated (T) versus control (C) tumours expressed as the ratio of T/C equal to 24%) were also shown by treating the subcutaneously (s.c.) grafted B16 melanoma with multiple doses of vinflunine [2]. The extent of this activity was again superior to that noted for vinorelbine under the same experimental conditions [2]. Growth inhibition of human tumour xenografts LX-1 (lung) and MX-1 (breast) was also observed following four weekly i.p. injections of vinflunine as reflected by optimal T/C values of 23 and 26%, respectively [2]. It was also noticeable that vinflunine induced

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considerably more pronounced inhibitory effects on tumour growth than vinorelbine [2, 3]. Overall, these results demonstrate that vinflunine is well tolerated and is active against a range of experimental animal tumour models. *In vitro* studies have confirmed the mitotic-arresting and tubulin-interacting properties of vinflunine and have identified certain quantitative differences relative to other *Vinca* alkaloids, although it participates in P-glycoprotein-mediated multidrug resistance (MDR) [4–6]. Indeed, vinflunine-resistant murine P388 tumour cells recently established *in vivo* proved cross-resistant to drugs implicated in classic MDR and showed overexpression of P-glycoprotein [5].

To examine in more depth the overall spectrum of *in vivo* antitumour activity of vinflunine, a panel of nine human solid tumours xenografted onto nude mice, including various histological types of bladder, pancreas, kidney, colon, central nervous system (CNS) and prostate cancers, were studied. These evaluations were carried out concurrently in four independent research centres using standardised procedures and evaluation criteria in accordance with National Cancer Institute (U.S.A.) and EORTC guidelines. The activity of vinflunine, administered by the i.p. route as single weekly injections for 4 consecutive weeks, a protocol with proven efficacy in various murine and human experimental tumours [2], was compared with that of vinorelbine given according to a similar protocol.

MATERIALS AND METHODS

Drugs

Vinflunine ditartrate, or 20′,20′-difluoro-3′,4′-dihydrovinorlebine (Figure 1) was synthesised at the Centre de Recherche Pierre Fabre (Castres, France) as described elsewhere [1]. Vinorelbine ditartrate was obtained from Pierre Fabre Medicament (Gaillac, France). The drugs were dissolved in sterile 0.9% sodium chloride solution before their administration to animals at 10 ml/kg body weight. Doses refer to the free base weights.

Mice

Female athymic nude mice (5–6 weeks old) of the Swiss nu/nu strain (Iffa Credo, Les Arbresles, France) were used for the DLD-1 human tumour xenografts. Athymic nude mice (NMRI nu/nu strain) at 5–7 weeks, grown in the breeding facilities of the Drug Development Laboratory (Oncotest GmbH, Freiburg, Germany), were used for the BXF1299, PAXF546 and RXF944LX xenografts. NCr-nu female nude mice at 5–6 weeks old (Taconic Farm, Germantown, New York, U.S.A. or Charles River Laboratories, Raleigh, North Carolina, U.S.A.) were used for SF-295 and NCI-H69 xenografts. Young, 5–6 weeks old, adult NCr-nu male mice (Taconic Farm or Frederick Cancer and Research Development Centre, Frederick, Maryland, U.S.A.) were used for the PC-3 xenografts. TC37 and HT-29 colon tumour were xenografted on to Swiss (nu/nu) female mice (6–8 weeks old) purchased from Iffa Credo.

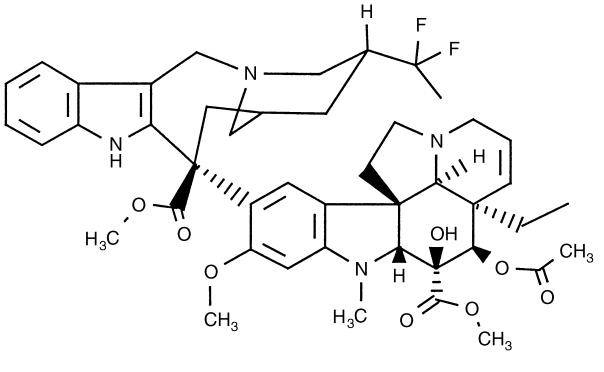

Figure 1. Chemical structure of vinflunine ditartrate, or 20′,20′-difluoro-3′,4′-dihydrovinorelbine.

All mice were housed in either sterile isolators or confined rooms (Institut Curie) and fed with irradiated nutrients and filtered water *ad libitum*.

Xenografts

The colon DLD-1 and HT-29 xenografts were obtained by injection of cultured cells (American Type Culture Collection, Rockville, Maryland, U.S.A.) into nude mice and then transplanted as a solid tumour from mouse to mouse. The BXF1299 (bladder), RXF944LX (renal) and PAXF546 (pancreas) tumours were obtained by biopsy and transplantation of patient tissue (Department of Internal Medicine, University of Freiburg, Freiburg, Germany) into nude mice using procedures detailed previously [7, 8]. A similar procedure was originally reported [9] for the TC37 (colon) xenografts (designated 'Leo', Institut Curie), which were used at the 10th passage. The three human tumours (SF-295 CNS, PC-3 prostate and NCI-H69 small cell lung) were obtained from the NCI/Frederick Cancer Research and Development Center and maintained in routine passage *in vivo* at the Southern Research Institute (Birmingham, Alabama, U.S.A.). DLD-1, TC37 and HT-29 xenografts were established as one tumour per mouse by s.c. implantation into the flank region. Drug treatments were started using tumours within the size range of 4–8 mm in diameter for DLD-1 and within the volume range of 32–256 mm^3 for TC37 and HT-29. With BXF1299, two tumours were implanted s.c. in the flanks of athymic nude mice, whilst only one tumour per mouse was used with PAXF546 and RXF944LX, since these tumours induced a tumour volume-related body weight loss which interfered with the monitoring of drug-induced body weight loss. Characterisation of these models have been previously published [7, 8]. Drug treatments with these xenografts were started as soon as the tumours reached diameters of 6–8 mm, depending on the doubling times (see Table 1). All these mice were randomly assigned to control groups (5 to 10 animals) or one of four treatment groups (5 to 10 animals per group) immediately before treatment began.

Mice xenografted with SF-295, PC-3 and NCI-H69 tumours were randomised to one of four treatment groups (5 animals per group) or to control groups (10 animals) following tumour implantation. Treatment began when the median tumour size ranged from 75 to 126 or 141 mg or mm^3 (NCI-H69), within three individual experiments from 113 to 162, 125 to 180 and 144 to 196 mg or mm^3 (SF-295) or, from 94 to 144, 63 to 100 and 113 to 196 mg or mm^3 (PC-3). The characteristics of each tumour studied are listed in Table 1.

Experimental chemotherapy

The protocol required that tumour-bearing mice were treated using weekly i.p. administrations on days 1, 8, 15 and 22. Generally, four different dose levels of either vinflunine (40, 20, 10 and 5 mg/kg) or vinorelbine (5, 2.5, 1.25 and

Table 1. *Characteristics of the human tumour xenografts studied*

Xenograft	Tumour type	Histology	Tumour DT (days)	Reference
BXF1299	Bladder (primary-recurrence)	Transitional cell carcinoma	8.0	[7, 8]
DLD-1	Colon (primary)	Poorly differentiated adenocarcinoma	3.6	[10]
HT-29	Colon (primary)	Well differentiated adenocarcinoma	4.0	[11]
TC37	Colon (primary)	Well differentiated adenocarcinoma	4.0	[9]
SF-295	CNS (primary)	Glioblastoma	1.9	[12]
NCI-H69	Lung (bone marrow metastasis)	Small cell carcinoma	6.1	[13]
PAXF546	Pancreas (primary)	Adenosquamous carcinoma	8.8	[7, 8]
PC-3	Prostate (bone metastasis)	Adenocarcinoma	4.0	[14]
RXF944LX	Kidney (lung metastasis)	Hypernephroma	2.9	[8]

CNS, central nervous system; DT, doubling time.

0.63 mg/kg) were evaluated in three separate experiments. The mice were checked daily for survival and any adverse clinical signs. The mice were weighed one to four times weekly (more often during treatments than afterwards). A dose producing a weight loss nadir of $\geq 15\%$ of the initial body weight was considered toxic. Tumour measurements were recorded two to three times weekly and the tumour volume (or mass, assuming unit density) was estimated from two-dimensional tumour measurements as: tumour volume (mm^3) = 0.5 (length$\times$width^2). Relative tumour volumes were calculated for each individual tumour by dividing the tumour volume on day x by the tumour volume on day 1, the first day of treatment. Each test compound was evaluated in three individual experiments.

Evaluation of antitumour activity

Antitumour activity was assessed by three main criteria: (i) relative tumour volumes of the treated (T) group of mice expressed as a percentage of the tumour volumes of the control (C) groups, namely ratios of T/C [15]; (ii) on the basis of delay in tumour growth, calculated as specific growth delay (SGD) and (iii) tumour regressions. Relative tumour volumes were calculated as the ratio of the median tumour volumes (masses) of the treated versus control groups: T/C (%) = (median relative tumour volume (mass) of the treated group on day x/median relative tumour volume (mass) of the control group on day x)$\times$100, the optimal value being the minimal T/C ratio that reflects the maximal tumour growth inhibition achieved. According to NCI standards, the criterion for efficacy for the T/C ratio is $\leq 42\%$ [16], with a value of $< 10\%$ being judged as a high level of activity. Optimal T/C values were derived from data obtained from tumour-bearing mice that had received at least two weekly injections. The SGD was calculated as follows: $SGD = Td_{treated}\text{-}Td_{control}/Td_{control}$, where Td is the time required for the tumour volume to double (SGD_{200}) or to quadruple (SGD_{400}). Tumour regressions were defined as partial (PR) if the tumour volume decreased to 50% or less of the tumour volume at the start of treatment, without dropping below measurable size and regressions were defined as complete (CR) if the tumour burden became unpalpable [17, 18]. The antitumour activity was also scored according to Fodstad [19] on the basis of optimal %T/C and SGD values (Table 2).

RESULTS

Dose-finding studies (data not shown) with vinflunine and vinorelbine identified respective doses of 40 or 5 mg/kg/injection administered i.p. as single weekly injections for 4 consecutive weeks as approximating to the maximum tolerated dose (MTD), either on the basis of induced mortality or major body weight loss or by the demonstration that either a 1.5- or 2-fold higher dose resulted in pronounced toxicity and death of treated animals before controls. The sole exception being those mice xenografted with DLD-1 tumours which tolerated a single weekly dose of 10 mg/kg$\times$4 of vinorelbine, with on average only 6% weight loss recorded.

Vinflunine demonstrated definite *in vivo* activity against five of the nine xenograft models studied (Tables 3–5), contrasting with minimal activity recorded for vinorelbine against only two of the tumours. A high level of activity was noted for vinflunine at 40 mg/kg/injection against the NCI-H69 (small cell lung) tumours (Figure 2 and Table 3). Optimal T/C values were below 10% in two of the three experiments and generally T/C values remained below 42% for periods of 43, 29 and 54 days in the three tests, indicating the sustained nature of this activity with vinflunine. In all these experiments, SGDs exceeded 3.0. These effects were achieved with some body weight loss, which was not judged significant and was not associated with any marked toxicities or any drug-related mortality. Notably, in each of the three experiments one or two CRs were obtained amongst the groups of five tumour-bearing animals treated with the weekly dose of 40 mg/kg$\times$4 vinflunine. In contrast, vinorelbine (Table 3) showed only moderate activity against these NCI-H69 xenografts at the weekly dose of 5 mg/kg$\times$4 in one of the three experiments, yielding an optimal T/C of 30%, with one CR noted, but without reaching a significant SGD value.

Vinflunine was also judged as exhibiting a high level of activity against RXF944LX xenografts (Table 3 and Figure 3), in terms of the optimal T/C values recorded being 17, 6 and 8% in the three individual experiments, with corresponding SGD values being 1.7, 6.3 and 2.7. One CR and one PR were identified in the groups of animals tested in the second and third experiments. Moderate activity, with T/C values of $\leq 42\%$, was also noted in two of the three experi-

Table 2. *Antitumour activity scores*

Optimal % T/C		SGD	Score
≥ 50	and	≤ 1.0	–
≤ 50	or	≥ 1.0	+/–
≤ 50	and	≥ 1.0	+
≤ 40	and	≥ 1.5	++
≤ 25	and	≥ 2.0	+++
≤ 10	and	≥ 3.0	++++

– inactive; +/–, marginally active; +, ++, +++, ++++, minimally to highly active. T, treated; C, control; SGD, specific growth delay.

ments in which the lower dose level of 20 mg/kg/injection was employed. With vinorelbine activity was noted in one of the three experiments at 5 mg/kg/injection with an optimal T/C value of 28% and a SGD value of 1.7, whilst in one of the other two experiments an optimal T/C of 24% was identified, but without a significant SGD value. These effects with both vinflunine and vinorelbine were again obtained without any major toxicities (Table 3), the one exception being the second experiment with 40 mg/kg/injection of vinflunine, where a significant maximum body weight loss of 20% was recorded, but this was on the last day of the experiment, day 21 (cf. Figure 3).

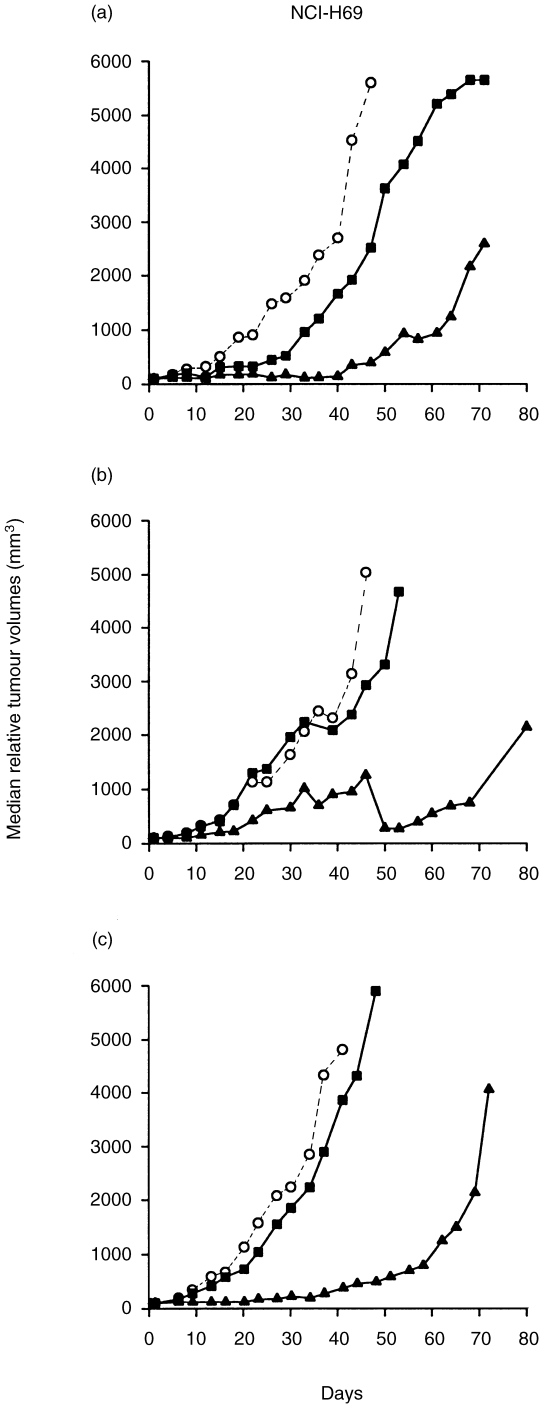

Figure 2. Responses of NCI-H69 (small cell lung) xenografted tumours to treatments with maximum tolerated doses (MTD) of either vinflunine (40 mg/kg/injection; ▲) or vinorelbine (5 mg/kg/injection; ■) administered intraperitoneally (i.p.) weekly×4, relative to control vehicle-treated mice (O). Median values for each treatment group are plotted for each of three individual series of experiments (a–c).

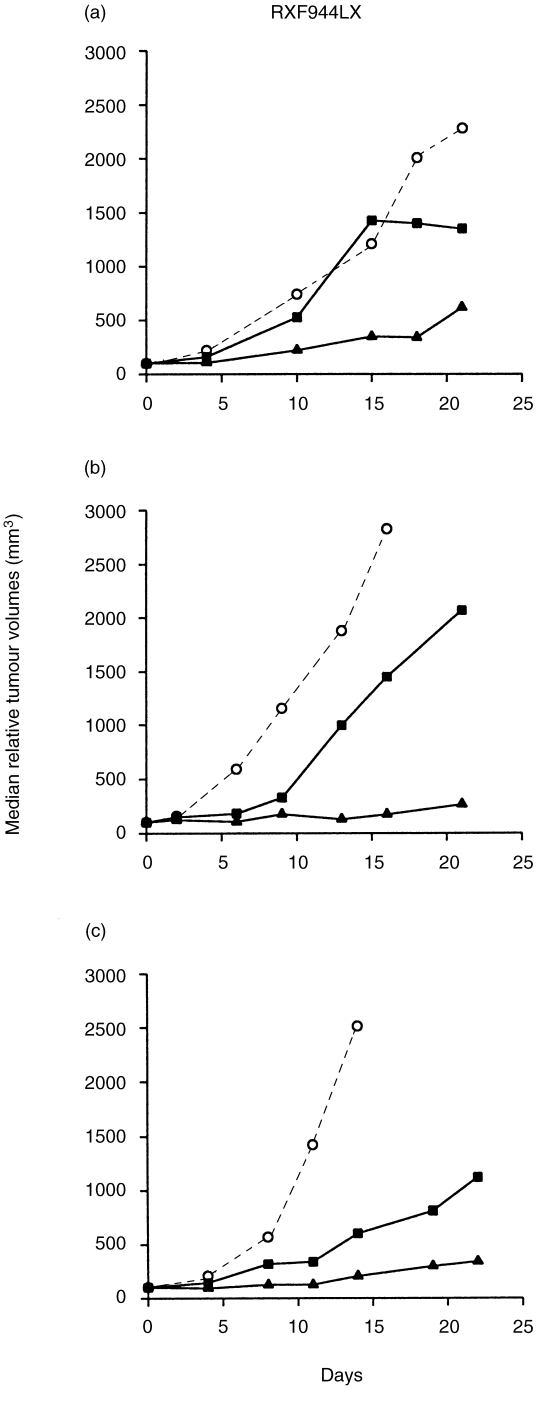

Figure 3. Responses of RXF944LX (renal) xenografted tumours to treatments with maximum tolerated doses (MTD) of either vinflunine (40 mg/kg/injection; ▲) or vinorelbine (5 mg/kg/injection; ■) administered intraperitoneally (i.p.) weekly ×4, relative to control vehicle-treated mice (O). Median values for each treatment group are plotted for each of three individual series of experiments (a–c).

Table 3. Antitumour activity of vinflunine or vinorelbine against NCI-H69 and RXF944LX xenografts

Tumour (origin)	Test compound	Dose (mg/kg/injection)	No. of weekly injections given (*n* of mice)	Maximum body weight change (%)	(day)	Optimal T/C (%)	(day)	Tumour growth inhibition SGD (200%)	Efficiacy	No. of CRs or PRs
NCI-H69 (small cell lung)	Vinflunine	40	4 (5)	Gain		5	(36)	3.2	++++	2CR
		20	4 (5)	Gain		36*	(47)	0.2	+/–	–
		40	4 (5)	– 9.1	(25)	28	(36)	3.1	++	2CR
		20	4 (5)	Gain		54	(46)	0.3	–	–
		40	4 (5)	– 8.7	(9)	6	(37)	3.7	++++	1CR
		20	4 (4); 3 (1)	– 4.1	(16)	52	(41)	0.4	–	–
	Vinorelbine	5.0	4 (4); 2 (1)	– 9.1	(19)	30	(26)	0.9	+	1CR
		2.5	4 (5)	Gain		59	(26)	0.1	–	–
		5.0	4 (5)	Gain		76	(43)	–	–	–
		2.5	4 (5)	Gain		91	(15)	–	–	–
		5.0	4 (5)	– 4.3	(6)	64	(20)	0.3	–	–
		2.5	4 (5)	Gain		79	(9)	0.2	–	–
RXF944LX (renal)	Vinflunine	40	4 (5)	– 4.7	(21)	17	(18)	1.7	++	–
		20	4 (4); 2 (1)	Gain		98	(18)	–	–	–
		40	3 (6)	– 19.9	(21)	6	(16)	6.3	++++	1CR
		20	3 (6)	– 3.2	(7)	38*	(9)	0.7	+/–	–
		40	4 (5)	– 4.9	(14)	8	(14)	2.7	+++	1PR
		20	4 (5)	Gain		36	(11)	0.4	+/–	–
	Vinorelbine	5.0	4 (3); 3 (1); 2 (1)	– 7.1	(21)	59	(21)	0.4	–	–
		2.5	4 (4); 3 (1)	Gain		> 100		–	–	–
		5.0	3 (6)	– 7.1	(21)	28	(9)	1.7	++	–
		2.5	3 (5); 2 (1)	– 2.0	(7)	51	(9)	1.0	+/–	–
		5.0	4 (3); 1 (1)	Gain		24	(11)	0.4	+/–	1PR
		2.5	4 (4); 3 (1)	Gain		21	(11)	0.6	+/–	–

*Value noted only on a single day. CR, complete responder; PR, partial responder; T, treated; C, control; SGD, specific growth delay.

Against the pancreatic PAXF546 xenografted tumours there was evidence of activity with vinflunine at the highest dose tested of 40 mg/kg/injection in all three experiments, with optimal T/C values of < 42% (Table 4). In the second and third experiments these respective values of 36% and 29% were only recorded on a single day, although the activity was sustained in the first experiment with an optimal T/C of $\leq$ 42% recorded throughout the last week of the experiment. The lower doses of vinflunine proved inactive. In terms of SGD measurements, a value of > 1 was only recorded at the highest dose of vinflunine tested in the first experiment. Overall, therefore, only moderate activity was ascribed to vinflunine against these xenografted tumours, but this was superior to that noted with vinorelbine, under comparable experimental conditions. Vinorelbine induced a significant T/C ratio value only on a single day in one of the three experiments at the highest dose evaluated (Table 4), reflected in a SGD value of only 0.4. Vinorelbine was, therefore, judged as inactive against this tumour model. In mice bearing PAXF546 xenografts drug-associated toxicity appeared more marked, with definite body weight loss being recorded, not only in all experiments using the highest dose levels of each test compound, but also in the first series of experiments at all doses evaluated. In subsequent experiments though, lower dose levels of both vinflunine and vinorelbine were non-toxic. These data, suggestive of drug- and dose-related toxicities, need to be interpreted in the light of the significant weight losses experienced by the control animals in the first and third experiments.

Mice bearing PC-3 prostate tumours, both drug-treated and control groups, all had marked body weight losses during the course of these experiments (Table 4), exceeding 15% in all three control experiments and in drug-treated groups in the first and third experiments. Such weight loss, however, appeared to be related to the tumour itself rather than to any of the administered treatments. Moderate activity, measured in terms of SGD values over four doublings (but not over two doublings), was recorded in one of the three experiments at the weekly dosage of 40 mg/kg vinflunine, namely 1.4, and this was coupled with an optimal T/C ratio of 44%. In the first experiment, moderate activity was also noted with a T/C value of 40%, which reflected a sustained response over an 8 day period. Comparable activity was noted when testing vinorelbine at the highest weekly dose of 5 mg/kg, but in only one of the three experiments. Vinorelbine was, therefore, judged as inactive against this xenograft model by NCI criteria.

Neither vinflunine nor vinorelbine resulted in any major weight loss in mice xenografted with either the TC37 or the HT29 tumours. Some antitumour efficacy was observed in studies using the TC37 xenografts (Table 4), with both vinflunine and vinorelbine. These effects were clearly dose related, and at best judged as moderate, but appeared comparable for both drugs tested. With vinflunine, at the weekly dose of 40 mg/kg, optimal T/C values ranged from 12 to 46% in the three experiments, considered as evidence of minimal activity according to NCI criteria and this activity was sustained in the first two experiments over 30 days. Sig-

Table 4. *Antitumour activity of vinflunine or vinorelbine against PAXF*946, *PC*-3 *and TC*37 *xenografts*

Tumour (origin)	Test compound	Dose (mg/kg/injection)	No. of weekly injections given (no. of mice)	Maximum body weight change (%)	(day)	Tumour growth inhibition: Optimal T/C (%)	(day)	SGD (200% or 400%†)	Efficacy
PAXF 546	Vinflunine	40	4 (3); 2 (3)	− 37.0	(28)	32	(29)	1.51	++
(pancreas)		20	4 (3); 3 (2); 2 (1)	− 15.3	(14)	61	(24)	0.29	−
				Controls: − 21.2	(28)				
		40	3 (5); 2 (1)	− 33.8	(28)	36★	(28)	0.58	+/−
		20	4 (6)	− 12.1	(28)	50	(17)	0.70	−
				Controls: − 0.5	(7)				
		40	4 (5)	− 38.3	(28)	29★	(25)	0.51	+/−
		20	4 (5)	− 2.9	(28)	73	(18)	< 0	−
				Controls: − 18.3	(28)				
	Vinorelbine	5.0	4 (3); 3 (1); 2 (2)	− 25.2	(21)	32★	(24)	0.40	+/−
		2.5	4 (3); 3 (2); 1 (1)	− 34.9	(28)	52	(29)	< 0	−
				Controls: − 21.2	(28)				
		5.0	3 (5); 2 (1)	− 17.3	(21)	> 100		< 0	−
		2.5	4 (6)	− 13.8	(28)	80	(10)	0.26	−
				Controls: − 0.5	(7)				
		5.0	4 (5)	− 21.6	(28)	> 100		< 0	−
				Controls: − 18.3	(21)				
PC-3	Vinflunine	40	4 (5)	− 20.0	(28)	40	(24)	0.8†	+/−
(prostate)		20	4 (5)	− 19.0	(28)	51	(24)	0.4†	
				Controls: − 15.4	(24)				
		40	4 (5)	− 4.3	(15)	54	(19)	0.4†	−
		20	4 (5)	− 13.0	(15)	74	(22)	0.1†	−
				Controls: − 15.4	(15)				
		40	4 (5)	− 20.0	(24)	44	(17)	1.4†	+
		20	4 (5)	− 20.0	(24)	68	(17)	0.5†	−
				Controls: − 20.0	(14)				
	Vinorelbine	5.0	4 (5)	− 18.5	(28)	40	(17)	0.7†	+/−
		2.5	4 (5)	− 3.8	(28)	> 100		< 0†	−
				Controls: − 15.4	(24)				
		5.0	4 (5)	− 16.6	(15)	80	(12)	0.3†	−
		2.5	4 (5)	− 12.5	(19)	> 100		0†	−
				Controls: − 15.4	(15)				
		5.0	4 (5)	− 19.2	(17)	75	(10)	0.3†	−
		2.5	4 (5)	− 19.2	(17)	30★	(17)	> 0.4†	−
				Controls: − 20.0	(14)				
TC37	Vinflunine	40	4 (8)	− 6.4	(4)	12	(39)	1.2	+
(colon)		20	4 (8)	− 5.5	(4)	40★	(33)	< 0	−
		40	4 (7)	Gain		13	(41)	2.3	+++
		20	4 (7)	Gain		16	(23)	2.8	+++
		40	4 (10)	Gain		46	(24)	< 0	+/−
		20	4 (10)	Gain		> 100		0.6	−
	Vinorelbine	5.0	4 (8)	Gain		23	(34)	0.5	+/−
		2.5	4 (8)	− 2.8	(4)	40★	(39)	0	−
		5.0	4 (8)	− 5.2	(5)	11	(32)	1.4	+
		2.5	4 (9)	Gain		34	(22)	0.7	+/−
		5.0	4 (10)	Gain		48	(22)	1.4	+
		2.5	4 (10)	Gain		59	(22)	0.3	−

★Value ≤ 42 noted only on a single day. †Value ≤ 50 noted only on a single day. T, treated; C, control; SGD, specific growth delay.

nificant SGD values of 1.2 and 2.3 were also recorded in two of the three experiments. Similar results were obtained at the lower dose of 20 mg/kg/injection of vinflunine in the first two experiments, although a sustained response was only noted in the second experiment. Treatments with vinorelbine resulted in comparable effects, with optimal T/C ratios of 11–48 being recorded in the three experiments and significant SGD values of 1.4 being noted in the second and third experiments, using the MTD dose of vinorelbine of 5 mg/kg weekly×4. Again this activity with vinorelbine was sustained over periods of 3–4 weeks before the experiment was terminated. Moderate activity was also recorded in two of the three experiments at the lower vinorelbine dose of 2.5 mg/kg/injection.

Neither vinflunine nor vinorelbine showed any definite activity against the two refractory colon tumours (DLD-1 and HT-29), nor against the BXF1299 (bladder) xenografts or the rapidly growing SF295 (glioma) tumours. In the studies using the HT-29 xenografts all the recorded optimal T/C

Table 5. Antitumour activity of vinflunine or vinorelbine against DLD-1, BXF1299 and SF-295 xenografts

Tumour (origin)	Test compound	Dose (mg/kg/injection)	No. of weekly injections given (no. of mice)	Maximum body weight change (%)	(day)	Tumour growth inhibition: Optimal T/C (%)	(day)	SGD	Efficiacy
DLD-1 (colon)	Vinflunine	40	4 (5)	−4.5	(8)	74	(26)	<0	–
		20	4 (5)	Gain		65	(26)	0.3	–
		40	4 (5)	Gain		44	(23)	0.4	+/–
		20	4 (5)	Gain		39*	(30)	0.4	–
		40	4 (5)	−2.0	(8)	82	(33)	<0	–
		20	4 (5)	−1.0	(8)	48	(19)	<0	–
	Vinorelbine	10	4 (5)	−5.3	(8)	53	(26)	0.6	–
		5	4 (5)	−1.3	(8)	32*	(33)	0.3	–
		10	4 (5)	−6.5	(19)	20	(12)	4.6	+++
		5	4 (5)	−3.6	(18)	51	(37)	0.1	–
		10	4 (5)	−5.8	(19)	45	(33)	<0	+/–
		5	4 (5)	−3.7	(15)	88	(9)	0.2	–
BXF1299 (Bladder)	Vinflunine	40	4 (5); 3 (2)	−23.4	(21)	47†	(21)	0.96	–
		40	3 (6)	−17.5	(28)	48†	(21)	0.43	–
		40	4 (5)	−9.4	(28)	51	(18)	0.60	–
				Controls: −17.6	(21)				
	Vinorelbine	5	4 (2); 3 (4); 1 (1)	−18.8	(7)	89	(13)	0.16	–
		5	3 (5); 2 (1)	−21.0	(7)	53	(18)	0.54	–
		5	3 (4); 1 (1)	−7.8	(21)	46†	(11)	0.69	–
				Controls: gain					
SF-295 (glioma)	Vinflunine	40	4 (5)	−4.5	(11)	23	(11)	0.97	+
		40	4 (5)	Gain		46	(11)	0.49	+/–
		40	4 (5)	Gain		56	(11)	0.46	–
	Vinorelbine	5	4 (5)	−13.6	(11)	12	(11)	1.94	++
		5	4 (5)	−4.8	(11)	39*	(11)	0.87	+/–
		5	4 (5)	−5.0	(8)	59	(15)	0.23	–

*Value ≤ 42 noted only on a single day. †Value ≤ 50 noted only on a single day. T, treated; C, control; SGD, specific growth delay.

ratios exceeded 42% (data not shown) and the groups of treated and control animals all died or were sacrificed on the basis of tumour size at similar times. Similarly, in studies using the BXF1299 xenografts, neither vinflunine nor vinorelbine exhibited any significant inhibitory activity in terms of optimal T/C ratios (Table 5), according to NCI criteria. According to the Fodstad [19] criteria of efficacy, vinflunine proved marginally active in two of the three experiments at the MTD of 40 mg/kg/injection, whilst overall vinorelbine was judged inactive at its MTD dose. In the first studies with the SF295 tumours, both vinflunine and vinorelbine exhibited minimal activity with optimal T/C values $\leq 42\%$, but in the second and third series, neither compound showed any appreciable activity nor resulted in any major body weight loss.

No activity was recorded for either *Vinca* alkaloid in animals bearing DLD-1 xenografts at respective doses of vinflunine or vinorelbine of 40 or 5 mg/kg administered weekly×4. However, since this dose of vinorelbine resulted in no major body weight loss in tumour-bearing animals, the higher dose level of 10 mg/kg/injection was also evaluated (Table 5), but overall this dose also failed to produce an optimal T/C of $\leq 42\%$. Increasing the vinflunine dose by a factor of two to 80 mg/kg weekly×4, resulted in early deaths and toxicity expressed in terms of major body weight loss, rendering the data uninterpretable. Overall, therefore, both vinflunine and vinorelbine were judged inactive against these DLD-1 human colon xenografts.

DISCUSSION

Human tumour xenografts are now well-established tools for the preclinical screening of anticancer drugs and an integral part of the current NCI and EORTC disease-orientated strategies for drug screening [20]. Results are presented here of a comparative evaluation of a novel fluorinated *Vinca* alkaloid, vinflunine, which entered phase I clinical trials in late 1998 and vinorelbine, the more recent *Vinca* with a widely acclaimed spectrum of antitumour activity already identified during its early clinical development [21]. These evaluations were set up essentially as a joint study involving four different research centres working concurrently, using a standard schedule of drug administration, namely four weekly i.p. doses, and eventually a common procedure for data analyses. A range of human tumour xenografts were selected for study, as summarised in Table 6: three colon tumours were included, two (DLD-1 and HT-29) known to be refractory to most antitumour agents generally [18, 22], and specifically to vinblastine for HT-29 tumours [18], whilst the third example (TC37) was considered a more responsive model (M.F. Poupon, Institut Curie, Pans, France); one small cell lung cancer tumour (NCI-H69) and one prostate tumour (PC-3) were selected on the basis of their known insensitivities to vinblastine [18], whilst the CNS tumour (SF-295) provided an example of a xenograft with known sensitivity to vinblastine [18]; finally, three tumours were identified on the basis of their characterised responses to vindesine (H.H. Fiebig, University of Freiburg), with the choice of a bladder tumour

Table 6. *Human tumour xenografts studied and their* in vivo *responses to* Vinca *alkaloids*

Responses to	Insensitive	Moderate sensitivity (T/C > 25 and < 50%)	High level of sensitivity (T/C < 10%)
Data based on literature reports and personal communications			
Vinblastine specifically	HT-29 [18] NCI-H69 [18] PC-3 [18]	–	SF-295 [18]
Vindesine specifically	PAXF546*	BXF1299* RXF944LX*	–
Vincristine specifically	BXF1299*	–	–
Cytotoxic agents generally	DLD-1 [18, 22] SF-295 [18]	TC37†	–
Data based on experimental studies presented			
Vinflunine specifically	BXF1299 DLD-1 HT-29 SF-295	PAXF546 PC-3 TC37	NCI-H69 RXF944LX
Vinorelbine specifically	BXF1299 DLD-1 HT-29 NCI-H69 PAXF546 PC-3 SF-295	RXF944LX TC37	

*H.H. Fiebig. †M.F. Poupon.

(BXF1299) which had proved unresponsive to vincristine, but showed moderate sensitivity to vindesine, like the renal cancer tumour (RXF944LX) selected and a pancreatic tumour (PXF546) which had proved insensitive.

The objective of these studies was to establish whether vinflunine and vinorelbine had any differing spectra of activities and whether the previously published data describing the efficacy of the earlier *Vincas*, exemplified by vinblastine, vincristine or vindesine, could be significantly improved upon by either of these two molecules. Kruczynski and colleagues [2, 3] had already documented activity for vinflunine in terms of survival prolongation in experimental murine tumours (i.v. grafted P388 leukaemia and s.c. implanted B16 melanoma) and of tumour growth inhibition in both murine (B16 melanoma), as well as human solid tumour xenograft models (LX-1, lung and MX-1, breast). The schedule selected for this collaborative study was that of four weekly i.p. injections which had been used in these latter studies.

An overall summary of the data obtained is provided in Table 6. Essentially, as far as the novel fluorinated *Vinca* alkaloid is concerned, a higher level of activity, relative to that of vinorelbine, was noted, with two xenografts proving highly responsive (RXF944LX and NCI-H69) and three proving moderately sensitive to vinflunine. It is also evident from the data summarised in Table 6 that following treatment with vinflunine, responses were noted in three xenografted tumours (PAXF546, PC-3, NCI-H69), not previously observed with any of the other classic *Vincas*, where data are available, given that a number of differing schedules of administration were employed. Increased sensitivity to vinflunine *vis-à-vis* vindesine and vinorelbine was also noted in the renal RXF944LX tumours, whilst comparable, moderate activity was recorded in the TC37 xenografts. The other two colon tumours studied proved unresponsive to both drugs. In addition, neither vinflunine nor vinorelbine affected the growth of the SF-295 gliomas, despite this model being described as sensitive to vinblastine [18]. One reason for this apparent discrepancy may relate to the schedule of administration used in our study involving 'long-term' intermittent therapy, since this tumour has a very rapid doubling time of approximately 2 days (see Table 1). Indeed, in two of the series of three experiments tumour-bearing mice being treated with the MTD of vinflunine received only three of their prescribed four weekly doses before death intervened.

These data might be considered as suggestive of a wider spectrum of activity for vinflunine and they certainly serve not only to confirm our earlier *in vivo* data [2, 3], but to greatly extend the observed overall superiority of vinflunine *vis-à-vis* vinorelbine in this range of human solid tumour xenografts. Including the earlier published results [2] with the MX-1 and LX-1 tumours, it is evident that antitumour activity has been recorded for vinflunine against seven of the 11 (64%) tumours studied, versus only three of 11 (27%) for vinorelbine. Of particular note, this activity was generally sustained and was obtained using vinflunine doses which were not associated with any excessive toxicity, as judged by body weight monitoring of tumour-bearing mice. In general, these data provide a favourable profile for further development and initiation of clinical trials with vinflunine.

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