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# Preclinical in vivo antitumor activity of vinflunine, a novel fluorinated *Vinca* alkaloid

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**Abstract** Vinflunine, or 20′,20′-difluoro-3′,4′-dihydrovinorelbine, is a novel *Vinca* alkaloid obtained by hemisynthesis using superacidic chemistry. The most impressive structural modification of this vinorelbine derivative was the selective introduction of two fluorine atoms at the 20′ position, a part of the molecule previously inaccessible by classic chemistry. The antitumor activity of vinflunine was evaluated against a range of transplantable murine and human tumors. Vinflunine exhibited marked activity against murine P388 leukemia grafted i.v. when given i.p. in single or multiple doses according to various schedules or in single i.v. or p.o. doses. Increases in life span achieved with vinflunine, as assessed by T/C ratios, ranged from 200% to 457% and proved markedly superior to those of 129–186% obtained with the other *Vinca* alkaloids tested. Against s.c.-implanted B16 melanoma, multiple i.p. administration of vinflunine proved active in terms of both survival prolongation and tumor growth inhibition, with optimal T/C values and relative areas under the tumor growth curves (rAUC) being 24% and 36%, respectively. The extent of this activity was superior to that noted for vinorelbine under the same experimental conditions. Growth inhibition of human tumor 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, and significant differences in the rAUCs noted for treated versus control animals. It was also noticeable that vinflunine induced considerably more prolonged inhibitory effects on tumor growth than did vinorelbine. These results demonstrate that vinflunine is well tolerated and is definitively active against a range of experimental animal tumor models. Vinflunine activity has been documented in terms of both survival prolongation and tumor growth inhibition, with definite superiority over vinorelbine being shown in each tumor model evaluated.

**Key words** Vinflunine · Murine tumors · Human tumor xenografts · Cancer chemotherapy

**Abbreviations** *CRPF* Centre de Recherche Pierre Fabre · *NCI* National Cancer Institute · *CNRS* Centre National de la Recherche Scientifique · *UKCCCR* United Kingdom Coordinating Committee on Cancer Research · *ICLAS* International Council for Laboratory Animal Science · *rAUC* relative area under the tumor growth curve · *Opt.* optimal

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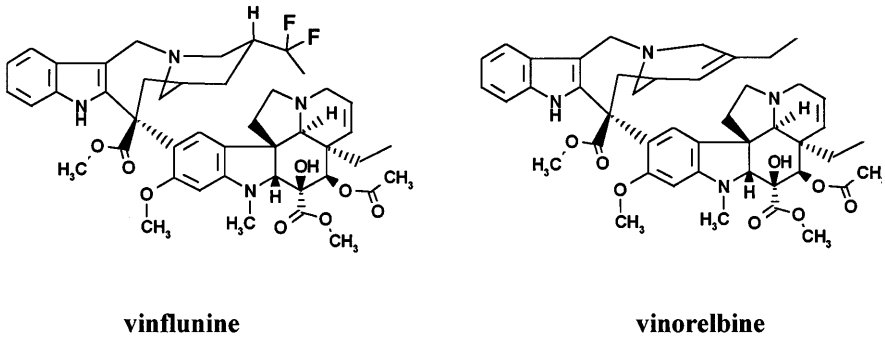
## Introduction

Many currently used antineoplastic agents are derived from natural products originally isolated from plants. One of the best-known classes of these agents are the *Vinca* alkaloids vinblastine and vincristine, found in the periwinkle plant *Catharanthus roseus* [5, 17, 27], and currently widely used for the clinical treatment of leukemias and certain solid tumors [10]. The binding of *Vinca* alkaloids to tubulin and the subsequent arrest of cells in mitosis are generally accepted as key events in



**vinflunine** **vinorelbine**

**Fig. 1** Molecular structures of vinflunine (20′,20′-difluoro-3′,4′-dihydrovinorelbine) and vinorelbine

their mechanisms of action [34]. These alkaloids posses a dimeric structure composed of vindoline and velbanamine (coming from catharanthine) units linked together by a carbon-carbon bridge [29]. That vinblastine and vincristine differ from each other structurally by only a single modification in the vindoline unit, whereby this minor distinction appears responsible for substantial differences in their clinical efficacies and toxicities [2, 18], has provided the impetus for synthesis of new analogues. These endeavors, many of which were centered on the use of vinblastine as the starting molecule since it was the only product readily available in sufficient quantity, resulted in the identification of a third clinically active *Vinca* alkaloid, namely, vindesine, a desacetyl carboxyamide derivative of vinblastine [2, 7]. In the meantime, new methods of coupling the two precursor alkaloids catharanthine and vindoline had enabled chemists to obtain large amounts of the intermediate 3′,4′-anhydrovinblastine [22], allowing the preparation of new derivatives, which differed from the natural compounds by having an eight-membered rather than a nine-membered ring in the velbanamine moiety [23]. Among these derivatives, vinorelbine was selected for development and has shown marked clinical efficacy [14, 19]. Therefore, four *Vinca* alkaloids are presently widely used worldwide in the clinic. It is noteworthy that these four compounds were obtained by modification of reactive parts of the bisindole alkaloid molecules using classic chemistry [29].

Within this context we were interested in identifying an original chemical approach that could conceivably induce dramatic changes in the skeleton of these complex molecules. We decided to investigate the reactivity of these highly functionalized compounds in superacidic media. Superacids can induce modifications at non-activated bonds [28]. Furthermore, under these unusual conditions, indoles and indolines remain sufficiently stable to react with various electrophiles [3]. Via this approach a new family of fluorinated *Vinca* alkaloids has been synthesized [12], from which vinflunine, or 20′,20′-difluoro-3′, 4′-dihydrovinorelbine, has been selected for further studies on the basis of its initial activity in primary pharmacological screening. The most impressive structural modification of this vinorelbine derivative was the selective introduction of two fluorine atoms at the 20′ position, a part of the molecule previously inaccessible by classic chemistry (Fig. 1). The 3′,4′ double bond was also reduced during the course of the reaction.

The purpose of the present study was to determine the in vivo antitumor activity of vinflunine against a panel of transplantable tumor models with different biological properties and chemosensitivities, namely, i.v.-implanted murine P388 leukemia, s.c.-implanted B16 melanoma, and human tumor xenografts MX-1 (breast) and LX-1 (lung) [9].

## Materials and methods

### Drugs

Vinflunine ditartrate, or 20′, 20′-difluoro-3′, 4′-dihydrovinorelbine (Fig. 1), was synthesized at CRPF as described elsewhere [12]. Reference compounds obtained from various supplies included vinorelbine ditrartrate, vincristine sulfate (Pierre Fabre Médicaments, Gaillac, France), vinblastine sulfate (Centre de Recherche Pierre Fabre, Castres, France), and vindesine sulfate (Lilly, Indianapolis, USA). 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 DBA/2 (Iffa Credo, L'Arbresle, France), hybrid $CDF_1$ (Balb/c × DBA/2), and C57B1/6 (Charles River, St. Aubin les Elbeufs, France) mice were used for implantation of murine P388 leukemia (i.v.) and B16 melanoma (s.c.), respectively. Female athymic nude mice of the Swiss strain (Iffa Credo) homozygous for the nude gene (*nu*/*nu*) were used for the LX-1 and MX-1 human tumor xenografts. Food and drinking water were provided ad libitum. A quarantine period of 5–14 days was imposed on each animal before the initiation of experiments. Manipulations of all mice were conducted in laminar-flow biosafety hoods located in specific pathogen-free barrier facilities. The temperature in the barrier facilities was maintained at 25 ± 1 °C for the Swiss mouse strain or at 21 ± 1 °C for the other strains, and the relative humidity was maintained at 55 ± 5%. Lighting was controlled automatically on a 12-h light/dark cycle (lights on at 7:00 a.m). Screening for rodent viruses of blood samples from sentinel mice housed in each enclosure containing experimental animals was performed once a month (ICLAS, International Council for Laboratory Animal Science, Laboratory of the University Hospital of Nijmegen, The Netherlands).

### Tumor models

The tumor models used for in vivo evaluations were the murine P388 leukemia and B16 melanoma models (generous gifts from

Dr. S. Cros, CNRS, Toulouse, France, obtained earlier from the Division of Cancer Treatment, Tumor Repository, NCI, Frederick, Md., USA) and the human LX-1 lung and MX-1 breast carcinomas (Division of Cancer Treatment, Tumor Repository, NCI). Solid tumors were transplanted as s.c. fragments. P388 leukemia was passaged as i.p. implants. For chemotherapy trials, tumors were transplanted into the same strain used for passages or in the appropriate $F_1$ hybrid. All mice weighed over 18 g at the start of the chemotherapy.

### Experimental chemotherapy

All experiments were conducted in accordance with established CRPF guidelines and were based on the UKCCCR guidelines for the welfare of animals in experimental neoplasia [37]. A total of $10^6$ P388 cells/mouse were implanted i.v. in $CDF_1$ mice on day 0. After randomization of the animals into treatment cages, test compounds were given i.p., i.v., or p.o. on day 1 in a single dose. Multiple treatments were also tested using the i.p. route, i.e., daily injections (days 1–4), intermittent treatments over 2 weeks (days 3, 5, 7, 10, 12, and 14), or four once-weekly injections (days 1, 8, 15, and 22). For the B16 melanoma tumor model, 0.5 ml of a tumor brei at 1 g/ml, made by disruption and homogenization of tumor fragments in sterile 0.9% sodium chloride, was inoculated s.c. into C57B1/6 mice. After randomization, test compounds were given i.p. 3 days later as intermittent treatments over 2 weeks (days 1, 3, 5, 8, 10, and 12) or as four weekly injections (days 1, 8, 15, and 22). Experiments using human tumor xenografts were started by implantation of tumor fragments into Swiss mice by tocar; the xenografts were then allowed to increase in size to a median volume of 100–200 $mm^3$. The animals were then randomly assigned to treatment and control groups, and treatment was initiated using weekly i.p. administrations on days 1, 8, 15, and 22. In each chemotherapy trial, mice were checked daily throughout each experiment, with all adverse clinical reactions being noted and deaths being recorded. Mice were weighed two to four times weekly during the treatments and once weekly thereafter. A dose producing a weight loss nadir of ≥15% of the initial body weight was considered toxic [21]. For solid tumor models, tumors were measured by calipers twice weekly and tumor volumes (in cubic millimeters) were estimated from two-dimensional tumor measurements (in millimeters) as: *tumour volume* $= 0.5$ ($length \times width^2$).

Results are presented for experiments involving (a) a minimum of 7 and a maximum of 15 mice per treated group for animals implanted with P388 leukemia and (b) a minimum of 15 mice per experimental group for mice carrying the s.c.-implanted solid tumors.

### Evaluation of antitumor activity

#### *Life span*

An increase in life span was defined as the *median survival of treated mice/median survival of control mice*) × 100 (T/C, in percent). According to NCI standard criteria for the P388 tumor model, 120% ≤ T/C < 175% is the minimal level for activity (L) and T/C ≥ 175% corresponds to a high level of antileukemic activity (H); 0 represents a T/C value of <120% [36]. Survival curves generated for the treated and control groups of B16 tumor-bearing mice were compared using the log-rank test [24, 25]. If the result of the test gave $P > 0.05$, then the conclusion was that no significant difference was detected between the treated and control groups, whereas $P < 0.05$ was taken to be indicative of a significant difference between the two groups.

#### *Tumor growth*

Treatment efficacy was assessed in terms of the effects of each compound on the tumor volumes of tumor-bearing mice relative to solvent-treated control animals. Two evaluation criteria were used in parallel: tumor growth inhibition as reflected by ratios of T/C [16] and relative areas under the tumor growth curve (rAUC) [20]. Growth inhibition was calculated as the ratio of the median tumor volumes of the treated versus control groups: $T/C(\%) = (median\ tumor\ volume\ of\ the\ treated\ group\ on\ day\ X / median\ tumor\ volume\ of\ the\ control\ group\ on\ day\ X) \times 100$, the optimal value (Opt.) being the minimal T/C ratio that reflects the maximal tumor growth inhibition achieved. According to NCI standards, the criteria for efficacy for the T/C ratio is ≤42% [4].

rAUC (%), representative of the tumor growth curve as a whole, therefore reflects the overall effect of a test compound on tumor growth over time. rAUC was expressed as a percentage of the median rAUC value recorded for the control group: $rAUC = median$ [(*area under the tumor tumor growth curve of an individual experimental mouse/median area under the tumor growth curve of the control group*) × 100]. The more active the compound, the lower the rAUC value. Comparisons of the rAUC population values noted for the treated and the control groups were performed using the nonparametric Mann-Whitney rank-sum test [26]. Similarly as for the log-rank test (survival), if the result of the test gave $P > 0.05$, then the conclusion was that no significant difference was detected between the treated and control groups, whereas $P < 0.05$ was taken to be indicative of a significant difference between the two groups.

## Results

### P388 leukemia

Against i.v.-implanted P388 leukemia, vinflunine was found to be active when given i.p. in a single dose on the day following tumor grafting. The optimal dose, i.e., the dose inducing the greatest increase in life span as reflected by a maximal T/C ratio with minimal side effects, was 40 mg/kg, producing T/C values of 200%, which would be considered indicative of a high level (H) of antileukemic activity according to NCI criteria (Table 1). Furthermore, this optimal dose resulted in minimal body weight loss of only 2.5% of the initial body weight (Table 1). The clinically active *Vinca* alkaloids, namely, vinblastine, vincristine, vindesine, and vinorelbine, were also active when given by the i.p. route against this i.v.-implanted P388 leukemia but achieved T/C values of 143–157% at the most effective doses, which are indicative of only a minimal level (L) of activity (Table 1).

For evaluation of the influence of the schedule of administration of vinflunine on its antitumor activity and on the dose that could be injected without resulting in undue toxicity, in addition to the single-dose schedule, three multiple-dose schedules were tested (Table 2). The effects of single-dose administration, multiple daily injections (days 1–4), or intermittent treatments over 2 weeks (days 3, 5, 7, 10, 12, and 14) at optimal doses, differing by only a factor of 2 or 3, produced similar maximal increases in life span as assessed by a T/C ratio of 200%. However, vinflunine induced a marked prolongation of survival as reflected by a T/C ratio of 457% at a 4-fold greater optimal total dose (160 mg/kg) when the intertreatment interval was increased to 7 days, i.e., with the once-weekly schedule over 4 weeks (days 1, 8, 15, and 22). This intertreatment interval of 7 days was

**Table 1** Antitumor activity of vinflunine given i.p. in a single dose against i.v.-implanted P388 murine leukemia: comparison with vinblastine, vincristine, vindesine, and vinorelbine. The most effective dose ranges are represented for vinblastine, vincristine, vindesine, and vinorelbine

| Compound | Dose (mg/kg) | Body weight change[a] (%) [day] | Presumed drug-related deaths[b] (%) | Maximal T/C[c](%) | Activity rating[d] |
|---|---|---|---|---|---|
| Vinflunine | 20 | −1.7 [4] | 0 | 143 | L |
| | 40 | −2.5 [4] | 0 | 200 | H |
| | 80 | −14.4 [4] | 28 | – | Toxic |
| Control | | +0.7 [4] | | | |
| Vinblastine | 1.25 | −1.1 [4] | 0 | 114 | 0 |
| | 2.5 | −6.7 [4] | 20 | 143 | L |
| Control | | +0.2 [4] | | | |
| Vincristine | 1.25 | −13.3 [4] | 0 | 143 | L |
| | 2.5 | −15.1 [4] | 0 | 143 | L |
| Control | | 0 [4] | | | |
| Vindesine | 2.5 | −11.7 [4] | 0 | 143 | L |
| | 5 | −10.4 [4] | 20 | 157 | L |
| Control | | +1.2 [4] | | | |
| Vinorelbine | 5 | −9.4 [4] | 0 | 114 | 0 |
| | 10 | −11.7 [4] | 0 | 157 | L |
| Control | | +1.2 [4] | | | |

[a] Body weight changes reported are maximal weight losses or minimal weight gains expressed as a percentage of the initial body weight. According to NCI criteria, a dose is considered toxic if the induced body weight loss is greater than 15% of the initial body weight
[b] A death was presumed to be drug-related if it preceeded the first death in the solvent-treated group
[c] $T/C$ = (*median survival of the drug-treated group*/*median survival of the solvent-treated group*) × 100
[d] According to NCI standard criteria for the P388 tumor model, 120% ≤ T/C < 175% is the minimal level for activity (*L*) and T/C ≥ 175% corresponds to a high level of antileukemic activity (*H*); *0* represents a T/C value of < 120%

**Table 2** Effects of the schedule of administration on the antitumor activity of vinflunine against i.v.-implanted P388 leukemia[a]

| Compound | Schedule (days) | Dose (mg/kg/inj) | Total dose[b] (mg/kg) | Body weight change (%) [day] | Presumed drug-related deaths (%) | Maximal T/C (%) | Activity rating |
|---|---|---|---|---|---|---|---|
| Vinflunine | 1,2,3,4 | 2.5 | 10 | +1.5 [2] | 0 | 100 | 0 |
| | "1 week" | 5 | 20 | +0.8 [8] | 0 | 143 | L |
| | | 10 | 40 | +1.0 [2] | 0 | 200 | H |
| | | 20 | 80 | −16.7 [8] | 0 | 143 | L |
| | | 40 | 80–160 | −8.1 [4] | 28 | – | Toxic |
| Control | | | | 0 [2] | | | |
| Vinflunine | 3,5,7,10,12,14 | 5 | 10–15 | −0.5 [7] | 0 | 100 | 0 |
| | "2 weeks" | 10 | 30–60 | −12.9 [14] | 0 | 143 | L |
| | | 20 | 80–120 | −8.3 [14] | 0 | 200 | H |
| | | 40 | 120–240 | −13.5 [10] | 0 | 143 | L |
| | | 80 | 160–140 | −16.6 [7] | 0 | 100 | 0 |
| Control | | | | +1.5 [5] | | | |
| Vinflunine | 1,8,15,22 | 10 | 10–20 | +4.5 [8] | 0 | 128 | L |
| | "4 weeks" | 20 | 40–60 | +2.1 [8] | 0 | 214 | H |
| | | 40 | 160 | +3.0 [8] | 0 | 457 | H |
| | | 80 | 80–160 | −9.1 [8] | 0 | 186 | H |
| | | 160 | 160 | – | 86 | – | Toxic |
| Control | | | | 0 [1] | | | |

[a] See footnotes to Table 1
[b] Compounds were given i.p. by the indicated schedule of administration. The total dose varied according to the day of death of each animal and, hence, to the number of injections (*inj*) received

indeed shown to be optimal in terms of achieving the maximal T/C value with vinflunine, as is shown in Fig. 2. These data depict the effects on survival of vinflunine given i.p. for a total of four consecutive treatments at a single dose of 40 mg/kg (identified as the maximal nontoxic single dose in preliminary studies; cf. Table 1) at intervals of 1, 3, 7, 10, and 14 days. Results of the comparative study conducted with vinorelbine showed that the maximal T/C ratio achieved with this compound was only 186% for the same four schedules of administration (Table 3). However, although a marked prolongation of survival was not achieved with

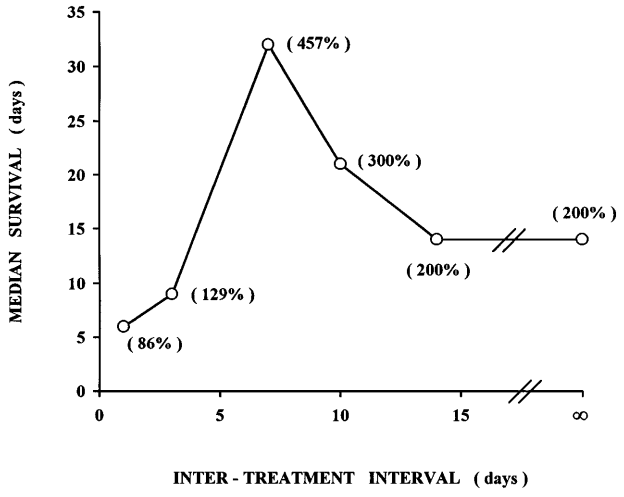



**Fig. 2** Antitumor effects of vinflunine given i.p. at 40 mg/kg per injection for 4 consecutive weeks according to varied intertreatment intervals against i.v.-implanted P388 leukemia. On day 0, $10^6$ P388 cells were inoculated i.v. into $CDF_1$ mice and treatments were initiated beginning on day 1. *Values in* parentheses correspond to the T/C ratio obtained with each schedule of treatment

vinorelbine by increasing the intertreatment interval to 7 days, i.e., by using the once weekly (x4) schedule, this was nonetheless the schedule with which the maximal T/C ratio of 186% was obtained (Table 3). The total tolerated dose of vinorelbine given in a single dose or according to the daily schedule was 10 mg/kg, which was only 2- to 4-fold lower than the total dose that could be given as intermittent treatments over 2 weeks (days 3, 5, 7, 10, 12, and 14) or as four weekly injections (days 1, 8, 15, and 22; Table 3). Multiple treatments with optimal doses of either vinflunine or vinorelbine never resulted in body weight losses in excess of 15% (Tables 2, 3).

When given by other routes, namely, i.v. or p.o., in a single dose, vinflunine was also active against i.v.-implanted P388 leukemia, producing maximal T/C ratios of 200% and 229% for the i.v. and p.o. routes of administration, respectively. These values were similar (i.v. route) or slightly superior (p.o. route) to those obtained with vinorelbine (Table 4).

Vinflunine given i.p. in a single dose also had a positive effect in terms of survival prolongation against i.p.-implanted P388 leukemia, achieving a maximal T/C value of 178% at a dose of 40 mg/kg (data not shown).

## B16 melanoma

The antitumor activity of vinflunine against s.c.-implanted B16 melanoma was evaluated using intermittent treatments either over 2 weeks, i.e., on days 1, 3, 5, 8, 10, and 12, or over 4 weeks, i.e., on days 1, 8, 15, and 22 (Table 5). Vinflunine given i.p. intermittently over 2 weeks induced a significant increase in the survival of B16 tumor-bearing mice as assessed by the log-rank test ($P < 0.001$–$0.05$) at doses of 5, 10, and 20 mg/kg per injection, the best effect being achieved at a dose of 10 mg/kg per injection as illustrated in Fig. 3. This activity was associated with a significant inhibitory effect on tumor growth at 10 and 20 mg/kg per injection as reflected by optimal T/C values of 38% and 24% and rAUC values (relative area under the tumor growth curve, expressed as a percentage of the area under the tumor growth curve of the control group) of 48% and 36%, respectively (Table 5). It is also noteworthy that T/C values representative of a minimal level of activity (T/C ≤ 42%) according to the NCI criteria were recorded from day 10 to day 21 for vinflunine at the highest nontoxic dose tested, i.e., 20 mg/kg per injection, indicative of long-lasting tumor growth inhibition (data not shown). The vinflunine dose of 40 mg/kg per injection was toxic since 80% of the animals treated with this dose had died by day 9, whereas only 2% of the control animals had died by this day. Using the weekly (× 4)

**Table 3** Effects of the schedule of administration on the antitumor activity of vinorelbine, the parent molecule of vinflunine, against i.v.-implanted P388 leukemia[a]

| Compound | Schedule (days) | Dose (mg/kg/inj) | Total dose (mg/kg) | Body weight change (%) [day] | Presumed drug-related deaths (%) | Maximal T/C (%) | Activity rating |
|---|---|---|---|---|---|---|---|
| Vinorelbine | 1,2,3,4 | 2.5 | 10 | −8.9 [4] | 0 | 128 | L |
| | "1 week" | 5 | 20 | −11.3 [4] | 0 | 100 | 0 |
| | | 10 | 30–40 | −10.7 [4] | 71 | – | Toxic |
| Control | | | | +0.2 [2] | | | |
| Vinorelbine | 3,5,7,10,12,14 | 2.5 | 7.5 | −5.9 [7] | 0 | 143 | L |
| | "2 weeks" | 5 | 10–25 | −11.3 [10] | 0 | 143 | L |
| | | 10 | 20–30 | −14.1 [7] | 0 | 143 | L |
| | | 20 | 20–40 | −9.5 [5] | 28 | – | Toxic |
| Control | | | | −1.6 [5] | | | |
| Vinorelbine | 1,8,15,22 | 5 | 5–10 | −0.5 [8] | 0 | 128 | L |
| | "4 weeks" | 10 | 20–30 | −12.9 [15] | 0 | 143 | L |
| | | 20 | 20–40 | −12.0 [8] | 0 | 186 | H |
| | | 40 | 40 | – | 100 | – | Toxic |
| Control | | | | 0 [1] | | | |

[a] See footnotes to Table 2

**Table 4** Antitumor activity of vinflunine given i.v. or p.o. in a single dose against i.v.-implanted P388 murine leukemia: comparison with vinorelbine[a]

| Compound | Dose (mg/kg) | Route | Body weight change (%) [day] | Presumed drug-related deaths (%) | Maximal T/C (%) | Activity rating |
|---|---|---|---|---|---|---|
| Vinflunine | 20 | i.v. | +1.4 [8] | 0 | 157 | L |
| | 40 | i.v. | −1.5 [8] | 0 | 200 | H |
| | 80 | i.v. | – | 71 | – | Toxic |
| Control | Saline | i.v. | +1.5 [4] | | | |
| Vinorelbine | 10 | i.v. | +3.4 [4] | 0 | 143 | L |
| | 20 | i.v. | −6.8 [4] | 0 | 200 | H |
| | 40 | i.v. | – | 71 | – | Toxic |
| Control | Saline | i.v. | +1.1 [4] | | | |
| Vinflunine | 40 | p.o. | +2.7 [4] | 0 | 157 | L |
| | 80 | p.o. | +0.5 [8] | 0 | 229 | H |
| | 160 | p.o. | −10.3 [4] | 0 | 200 | H |
| | 320 | p.o. | −10.1 [4] | 43 | – | Toxic |
| Control | Saline | p.o. | +2.6 [4] | | | |
| Vinorelbine | 10 | p.o. | +2.7 [4] | 0 | 114 | 0 |
| | 20 | p.o. | +1.2 [8] | 0 | 129 | L |
| | 40 | p.o. | −5.2 [4] | 0 | 186 | H |
| | 160 | p.o. | −13.5 [4] | 57 | – | Toxic |
| Control | Saline | p.o. | +1.9 [4] | | | |

[a] See footnotes to Table 1

schedule (days 1, 8, 15, and 22), a significant increase in survival ($P < 0.001$) was also observed for vinflunine, but only at 20 mg/kg per injection, and this was associated with a transient inhibitory effect on tumor growth as reflected by the T/C ratio of 32% recorded on day 23. The effect on tumor growth was more marked at the dose of 40 mg/kg per injection, resulting in a significant ($P < 0.05$) rAUC value of 59%, corresponding to an overall tumor growth inhibition of 41% (Table 5). Therefore, administration over 2 weeks, i.e., on days 1, 3, 5, 8, 10, and 12, appeared to be superior for the use of vinflunine against the s.c.-implanted B16 model as compared with the once-weekly schedule for 4 consecutive weeks.

**Table 5** Effects of vinflunine given i.p. against s.c.-implanted murine B16 melanoma: (comparison with vinorelbine. *NS* Not significant $P > 0.05$)

| Compound | Dose (mg/kg/inj) | Schedule (days) | Body weight change[a] (%) [day] | Survival Log-rank test | Tumor growth inhibition | |
|---|---|---|---|---|---|---|
| | | | | | Opt. T/C[b] (%) [day] | rAUC[c] (%, Mann-Whitney test) |
| Vinflunine | 2.5 | 1,3,5,8,10,12 | +2.5 [3] | NS | 35 [23] | 71 NS |
| | 5 | "2 weeks" | +3.4 [3] | ** | 57 [23] | 75 NS |
| | 10 | | +0.5 [3] | *** | 38 [10] | 48 * |
| | 20 | | −0.9 [8] | * | 24 [8] | 36 * |
| | 40 | | – | Toxic | – | – |
| Control | | | +1.4 [3] | | | |
| Vinflunine | 10 | 1,8,15,22 | +3.2 [5] | NS | 66 [23] | 99 NS |
| | 20 | "4 weeks" | +1.9 [3] | *** | 32 [23] | 72 NS |
| | 40 | | −12.2 [17] | NS | 40 [8] | 59 * |
| Control | | | +2.1 [3] | | | |
| Vinorelbine | 0.63 | 1,3,5,8,10,12 | +1.9 [3] | NS | 71 [19] | 110 NS |
| | 1.25 | "2 weeks" | +2.6 [5] | NS | 82 [10] | 94 NS |
| | 2.5 | | +2.6 [3] | NS | 55 [19] | 79 * |
| | 5 | | – | Toxic | – | – |
| Control | | | +1.3 [3] | | | |
| Vinorelbine | 1.25 | 1,8,15,22 | +3.2 [3] | NS | 70 [15] | 92 NS |
| | 2.5 | "4 weeks" | +3.0 [3] | * | 60 [15] | 74 * |
| | 5 | | −7.6 [3] | ** | 38 [8] | 73 NS |
| Control | | | +2.1 [3] | | | |

*$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

[a] Body weight changes are maximal losses or minimal gains expressed as a percentage of the initial body weight

[b] T/C = (median tumor volume of the drug-treated group/median tumor volume of the solvent-treated group) × 100

[c] Relative area under the tumor growth curve

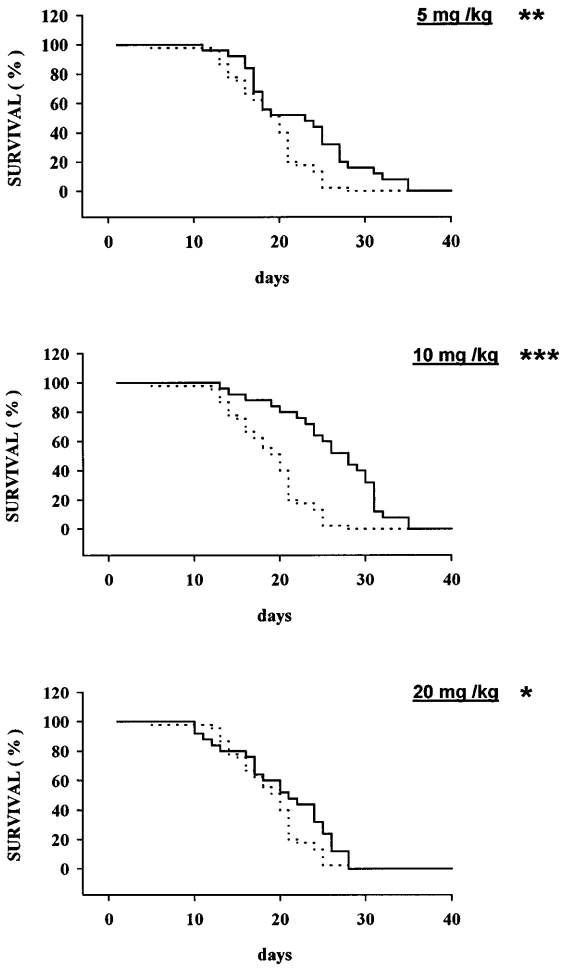



**Fig. 3** Effects of vinflunine given i.p. intermittently over 2 weeks (days 1,3, 5, 8, 10, and 12) at 5, 10, and 20 mg/kg per injection on the survival of B16-bearing mice. Initially, 0.5 ml of B16 tumor brei at 1 mg/ml was inoculated s.c. into C57B16 mice, and drug treatments were initiated 3 days later. The *solid* and *dotted lines* correspond to the vinflunine-treated and solvent-treated groups, respectively. The survival of vinflunine-treated and solvent-treated B16-bearing mice was compared using the log-rank test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

A comparative study conducted in parallel with vinorelbine showed that its administration via the 2-week schedule (days 1, 3, 5, 8, 10, and 12) induced only marginal inhibitory activity (rAUC = 79%) against s.c.-implanted B16 melanoma. However, a significant ($P < 0.001 - 0.05$) increase in the survival of B16 tumor-bearing mice was recorded when vinorelbine was given once weekly for 4 consecutive weeks (days 1, 8, 15, and 22) at doses of 2.5 and 5 mg/kg per injection, although this was associated with only a transient inhibitory effect on tumor growth (T/C = 38% on day 8 after two injections) at the highest dose tested, i.e., 5 mg/kg per injection (Table 5). These intermittent injections of vinflunine or vinorelbine over 2 or 4 weeks at the most effective doses resulted in body weight losses of less than 15% of the initial body weight, which is again indicative of a high level of tolerance of these compounds (Table 5).

## Human tumor xenografts

The antitumor activity of vinflunine was evaluated in two human tumor xenografts: LX-1, originally established from a small-cell lung carcinoma, and MX-1, established from a breast carcinoma.

Vinflunine demonstrated definite in vivo activity against s.c.-implanted LX-1 human lung xenografts as judged by its effects on tumor growth in LX-1 tumor-bearing mice (Table 6). This activity was obtained following four weekly treatments (days 1, 8, 15, and 22) by the i.p. route at the two highest doses tested, namely, 20 and 40 mg/kg per injection, and was reflected in significant ($P < 0.05$) rAUC values of 53% and 36%, respectively, corresponding to overall tumor growth inhibition of 47% and 64%, respectively, (Table 6). Optimal tumor growth inhibition of 60% and 77% (T/C 40% and 23%) was reached on days 16 and 26, respectively, at these same doses of vinflunine (Table 6). It is also noteworthy that T/C values representative of a minimal level of activity (T/C ≤ 42%) according to the NCI criteria were recorded from day 9 to day 33 at the optimal dose of 40 mg/kg per injection, indicative of sustained tumor inhibition (Fig. 4A). In comparison, T/C values of ≤42% were achieved with vinorelbine at the equitoxic dose of 5 mg/kg per injection only from day 12 to day 23 (Fig. 4B). In addition, the rAUC value recorded for vinorelbine at this optimal dose was 56% versus the value of 36% obtained with vinflunine at the optimal dose of 40 mg/kg per injection (Table 6).

The response of s.c.-implanted MX-1 human breast xenografts to vinflunine given i.p. weekly for 4 consecutive weeks (days 1, 8, 15, and 22) is defined in Table 6. This weekly schedule of vinflunine administration resulted in definite in vivo activity against MX-1 xenografts at 20 and 40 mg/kg per injection, producing optimal T/C ratios of 42% and 30%, respectively, and a significant ($P < 0.05$) rAUC value of 39% at 40 mg/kg per injection. The vinflunine dose of 80 mg/kg per injection appeared toxic since 60% of the animals treated by this dose had died by day 9, whereas none of the control animals had died by this day (Table 6). T/C values representative of a minimal level of activity (T/C ≤ 42%) were recorded from day 16 to day 19 and from day 26 to day 37 at 40 mg/kg per injection (Fig. 4C), which again reflects a definite persistence of the antitumor activity of vinflunine. In contrast, vinorelbine, studied in parallel, did not result in any T/C value lower than 42% at the highest nontoxic dose of 5 mg/kg on the same schedule of administration (Fig. 4D, Table 6). The only dose of vinorelbine inducing some significant tumor growth inhibition, reflected by an optimal T/C value of 7% and an rAUC value of 31%, was 10 mg/kg per injection. However, this dose was

**Table 6** Effects of vinflunine given i.p. against s.c.-implanted human lung LX-1 and breast MX-1 xenografts: comparison with vinorelbine[a]

| Model | Compound | Dose (mg/kg/inj) | Schedule (days) | Body weight change (%) [day] | Tumor growth inhibition | |
|---|---|---|---|---|---|---|
| | | | | | Opt. T/C (%) [day] | rAUC (%, Mann-Whitney test) |
| Lung, LX-1 | Vinflunine | 5 | 1,8,15,22 | −4.4 [22] | 37 [40] | 92 NS |
| | | 10 | "4 weeks" | −2.5 [18] | 39 [47] | 73 NS |
| | | 20 | | −2.6 [18] | 40 [16] | 53 * |
| | | 40 | | −11.0 [18] | 23 [26] | 36 * |
| | Control | | | −1.6 [22] | | |
| | Vinorelbine | 0.63 | 1,8,15,22 | −4.7 [18] | 53 [44] | 106 NS |
| | | 1.25 | "4 weeks" | −3.2 [15] | 48 [30] | 75 NS |
| | | 2.5 | | −4.0 [15] | 20 [44] | 52 * |
| | | 5 | | −9.3 [18] | 30 [12] | 56 * |
| | Control | | | −1.6 [22] | | |
| Breast, MX-1 | Vinflunine | 10 | 1,8,15,22 | +4.9 [8] | 70 [9] | 110 NS |
| | | 20 | "4 weeks" | +6.4 [8] | 42 [44] | 71 NS |
| | | 40 | | +4.0 [8] | 30 [30] | 39 * |
| | | 80 | | – | Toxic | – |
| | Control | | | +3.1 [4] | | |
| | Vinorelbine | 1.25 | 1,8,15,22 | +5.2 [8] | 71 [19] | 115 NS |
| | | 2.5 | "4 weeks" | +5.7 [8] | 35 [47] | 79 NS |
| | | 5 | | +6.7 [18] | 49 [30] | 72 NS |
| | | 10 | | −16.8 [4] | 7 [26] | 31 * |
| | Control | | | +2.3 [4] | | |

[a] See footnotes to Table 5

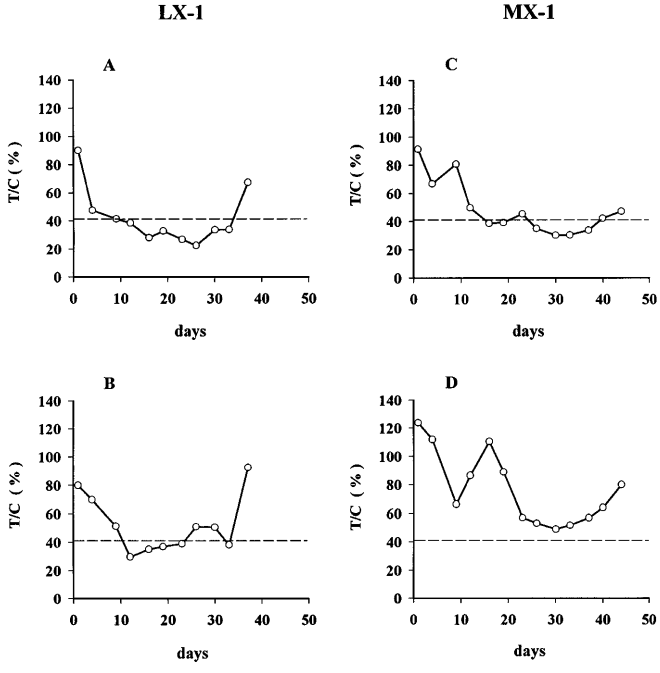



**Fig. 4A–D** Antitumor effects of **A, C** vinflunine given at 40 mg/kg per injection or **B, D** vinorelbine given at 5 mg/kg per injection against **A, B** human LX-1 lung and **C, D** MX-1 breast xenografts. Tumor fragments were implanted s.c. by trocar into athymic mice and, when tumors had reached a volume of 100–200 $mm^3$, the treatments were given i.p. on days 1, 8, 15, and 22. T/C = (*median tumor volume of the drug-treated group*/*mediantumor volume of the solvent-treated group*) × 100. The *dotted line* represents 42%, which is the minimal T/C value judged to be active according to NCI criteria

associated with some toxicity since the maximal body weight loss was 16.8% (Table 6).

## Discussion

Vinflunine, or 20′, 20′-difluoro-3′, 4′-dihydrovinorelbine, is a novel fluorinated *Vinca* alkaloid obtained by reaction of vinorelbine in superacid media [12]. In this study its pharmacological activity in vivo was evaluated against a panel of murine and human tumors, namely, P388 leukemia, B16 melanoma, and the human tumor xenografts MX-1 (breast) and LX-1(lung).

Against i.v.-implanted P388 leukemia, vinflunine demonstrated marked activity following multiple i.p. injections resulting in a maximal T/C ratio of 457%. This result is superior to the maximal T/C value of 186% recorded for vinorelbine under the same experimental conditions. The activity of vinflunine given in a single dose against this tumor model was also greater than that recorded for the clinically active *Vinca* alkaloids, namely, vinblastine, vincristine, vindesine, and vinorelbine. Cros et al. [8] had previously reported antitumor activity for these same clinically active *Vinca* alkaloids given i.v. against i.p.-implanted P388 leukemia. When given in a single dose by the i.v. route to mice bearing the i.v.-implanted leukemia, vinflunine induced the same level of activity as that reached using the i.p. route. Although the optimal dose for the oral route was 2-fold that used via the i.p or i.v. route, the results clearly showed that vinflunine retained the level of efficacy obtained using the other routes, even producing a slightly higher T/C ratio. These results indicate that vinflunine

crosses the physiological barriers and is well absorbed by the mouse. However, although vinflunine proved to be superior to vinorelbine when given i.p. or p.o. in a single dose, both compounds exhibited a similar degree of activity when given i.v., which may be explained by differing half-lives and/or distribution profiles. Detailed pharmacokinetics studies are currently under way.

Comparison of the various schedules of administration of vinflunine used in the i.v.-implanted P388 model reveals that although the single-dose regimen, the multiple daily injections (days 1–4), and the intermittent treatments over 2 weeks (days 3, 5, 7, 10, 12, and 14) did not markedly influence either the resultant antitumor activity or the total dose that could be given, the schedule of longest duration, i.e., the weekly schedule over 4 weeks (days 1, 8, 15, and 22) achieved the greatest antitumor effect (T/C = 457%) and permitted administration of the highest total tolerated dose (160 mg/kg). Therefore, weekly treatments for 4 consecutive weeks appeared to be the best schedule of administration of vinflunine in this i.v.-implanted P388 model.

The s.c.-implanted B16 melanoma was selected because it is considered to constitute a relatively drug-refractory tumor model [35]. Indeed, the natural *Vinca* alkaloids vinblastine and vincristine have been described as having activity mostly against experimental leukemia models, showing only a limited spectrum of solid-tumor activity and, in particular, no activity against s.c.-grafted B16 melanoma [35]. The results presented in this study, however, show that vinflunine exhibited significant activity against this s.c.-implanted B16 melanoma as judged in terms of both increased survival and inhibition of tumor growth. This activity was noted using two different schedules of administration involving multiple injections given over either 2 or 4 weeks. Furthermore, the extent of this activity was superior to that noted for vinorelbine under the same experimental conditions. Earlier, Cros et al. [8] reported activity for vinorelbine in this s.c.-implanted B16 model that was superior to that obtained with vinblastine or vincristine.

In spite of experimental limitations and considerable expense, the nude mouse-human tumor model has become widely integrated into the evaluation of and screening for new compounds and therapies. This model also appears to offer the potential of improved predictivity for clinical activity as exemplified by several research groups [6, 13, 21]. The present study shows that vinflunine induced significant activity in terms of growth inhibition of human LX-1 (lung) and MX-1 (breast) xenografts. A direct comparison with vinorelbine showed clear superiority for vinflunine against both tumor xenografts at equitoxic doses. Vinflunine induced overall growth inhibition of the LX-1 tumors that was 1.5-fold that obtained with vinorelbine as reflected by respective rAUC values of 36% and 56%. Activity for vinblastine against the LX-1 model has previously been reported [31], with an optimal T/C value of 27% resulting from a single i.v. drug administration at the maximum tolerated dose, whereas vincristine was inactive under these experimental conditions. Antitumor activity against LX-1 xenografts for both vinorelbine and vincristine given i.v. has also been claimed by Cros et al. [8], with optimal T/C values being 32% and 20%, respectively. It is also noteworthy that two reports concluded that vinorelbine was the most effective *Vinca* alkaloid tested against non-small-cell lung carcinoma xenografts [7, 8].

Against human MX-1 breast tumor xenografts, vinflunine produced an overall growth inhibition of 61% (rAUC = 39%), whereas vinorelbine did not result in any significant inhibition under these experimental conditions. Interestingly, vinblastine has also been described as inactive according to standard NCI criteria in this human MX-1 breast model [11]. However, Cros et al. [8] claimed definite activity for vinorelbine against MX-1 s.c. xenografts when the compound was given i.v. in a single dose. More recently the effects of vinorelbine given i.v. intermittently (on days 0, 4, and 8) against four different types of human breast carcinoma xenografts have been reported by Tsuruo et al. [33]. The T/C ratios recorded in that study for these models on a specified day were only 64%, 35%, 75%, and 81%, which could be interpreted overall as being indicative of marginal activity.

It would perhaps be instructive to try to place the results of these initial evaluations of the antitumor activity of vinflunine against these LX-1 and MX-1 tumor xenografts in the context of activity reported for other *Vinca* alkaloids as a group against human tumor xenografts in general. Such a comparison is complicated by the lack of a universal method of defining or reporting activity and by the diversity of schedules and routes of administration used. However, data have been published by two groups working with over 40 different human tumor xenograft models. Dykes et al. [11] reported that when vinblastine was tested as an example of a mitotic inhibitor, tumor inhibition (T/C < 40%) was noted in 12 of 41 tumors, giving a response rate of 29%. In the study of Fiebig and co-workers [13], who reported activity as tumor regressions (tumor reduction of 11–75% of the initial size), significant responses were recorded for vincristine in 5 of 30 (17%) xenografts and for vinblastine in 2 of 19 (10%) human xenografts tested. Clearly, the extent of activity of *Vinca* alkaloids such as vincristine or vinblastine against human tumor xenografts appears moderate as defined by these two studies. Complete regressions were rarely achieved with *Vinca* alkaloids (2/30 were recorded for vincristine by Fiebig et al. [13]). Therefore, as compared with the response rates obtained with other *Vinca* alkaloids against human xenografts, the extent of activity of vinflunine against the two human LX-1 and MX-1 xenografts described herein appears promising.

The latest new semisynthetic *Vinca* alkaloid that has shown improved efficacy and reduced toxicity both experimentally and in the clinic is vinorelbine. Results of preclinical studies generally show that vinorelbine exhibits a broader spectrum of activity with reduced

toxicity than do the natural *Vinca* alkaloids and that in several cases vinorelbine has proved to be more active than other *Vinca* alkaloids, especially against some human transplantable non-small-cell carcinoma xenografts [1, 8, 15, 30]. In the clinic as well, vinorelbine has proved to be an active and useful agent against non-small-cell lung cancer, advanced breast cancer, and ovarian carcinoma [14, 32]. In addition, a broader range of indications for the use of vinorelbine seems likely to emerge, since it has shown promise in the management of lymphomas, esophageal cancer, and prostatic carcinomas [19]. Since the results of the present preclinical study provide evidence for the superiority of vinflunine over vinorelbine in each experimental model system used, results are eagerly awaited to see if this enhanced efficacy translates into an improved spectrum of clinical activity for vinflunine.

In summary, the results of this preclinical in vivo study show that vinflunine has a good spectrum of activity against a panel of experimental tumors with different biological properties and chemosensitivities. This level of activity was consistently superior to that of vinorelbine, and although it was achieved at higher doses, their administration was not associated with any increased toxicity as judged by monitoring of body weights of tumor-bearing animals. Preliminary studies of acute toxicity in non-tumor-bearing mice also suggested a high level of tolerance of vinflunine, but these observations need to be validated by complete toxicology studies, which are currently under way. If confirmed, this high level of tolerance of vinflunine in these experimental in vivo models will provide a favorable profile for this new compound in its further development.

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