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Vinflunine

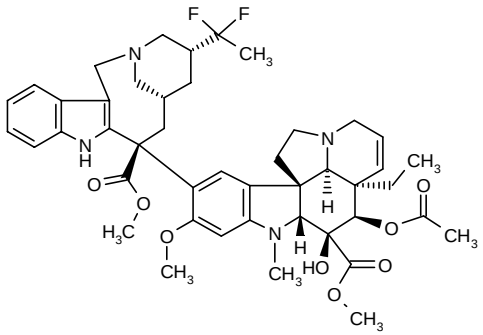
Prop INN

Antimitotic
Vinca Alkaloid

F-12158
L-0070
Javlor®

4(*R*)-Acetoxy-9-[4(*R*)-(1,1-difluoroethyl)-8(*S*)-(methoxycarbonyl)-2,3,4,5,6(*R*),7,8,9-octahydro-1*H*-2,6-methanoazecino-[4,3-*b*]indol-8-yl]-3a(*R*)-ethyl-5(*S*)-hydroxy-8-methoxy-6-methyl-3a,4,5,5a(*R*),6,11,12,12a(*R*)-octahydro-1*H*-indolizino-[8,1-*cd*]carbazole-5-carboxylic acid methyl ester

4'-Deoxy-20,20'-difluoro-8'-norvincaleukoblastine



$C_{45}H_{54}F_2N_4O_8$
Mol wt: 816.9510
CAS: 162652-95-1
EN: 219585

Abstract

Vinflunine is a second-generation *Vinca* alkaloid distinguished from other related compounds by differences in tubulin binding affinities and its effects upon microtubule dynamics. Although *in vitro* studies have demonstrated that vinflunine is consistently the least potent of the *Vinca* alkaloids, *in vivo* studies in a range of transplantable murine and human tumor models in mice have shown that vinflunine was markedly superior to vinorelbine and the other *Vinca* alkaloids in terms of increase in life span and inhibition of tumor growth. The potential of vinflunine as a component of combination chemotherapy has also been demonstrated. Preliminary evidence from phase I and phase II studies indicated that vinflunine was a promising anticancer agent with manageable toxicity, at least comparable in efficacy to the most active currently available agents. Antitumor activity has been observed in patients with advanced transitional cell cancer of the bladder, metastatic breast cancer and non-small cell lung cancer. Phase III trials are ongoing in non-small cell lung and bladder cancer, with programs planned in advanced breast cancer and ovarian cancer.

Synthesis

Fluorination of vinorelbine bitartrate (I) with SbF_5 and NBS, NCS, Br_2 or $Ca(OCl)_2$ (1), with SbF_5 and CCl_4 (2, 3) or with SbF_5 and $CHCl_3$ (3), all in anhydrous HF, gives directly vinflunine (1). Scheme 1.

Alternatively, fluorination of vinblastine (II) or 3',4'-anhydrovinblastine (III) with SbF_5 and $CHCl_3$, 2,2-dichloropropane, CBr_4, BBr_3 or CH_2Br_2 in anhydrous HF yields 4'-deoxy-20',20'-difluorovinblastine (IV), which is submitted to a *C*'-ring contraction by treatment with trifluoroacetic acid and NBS in CH_2Cl_2, then neutralization with $NaHCO_3$ and finally hydrolysis in $THF/H_2O/CH_2Cl_2$, optionally in the presence of $AgBF_4$ (4). Scheme 1.

Introduction

The *Vinca* alkaloids are widely and successfully used in the treatment of cancer. Their antineoplastic properties arise from their interaction with tubulin and the resultant inhibition of microtubule assembly, which thereby halts cell division at metaphase. Vinflunine (F-12158, Javlor®) is a second-generation *Vinca* alkaloid, synthetically derived from vinorelbine by the introduction of two fluorine atoms using superacidic chemistry. Although this compound also acts as a specific inhibitor of tubulin, it has demonstrated qualitative differences in its tubulin-binding properties compared to other *Vinca* alkaloids, which may account for its superior antitumor efficacy (2, 5-9).

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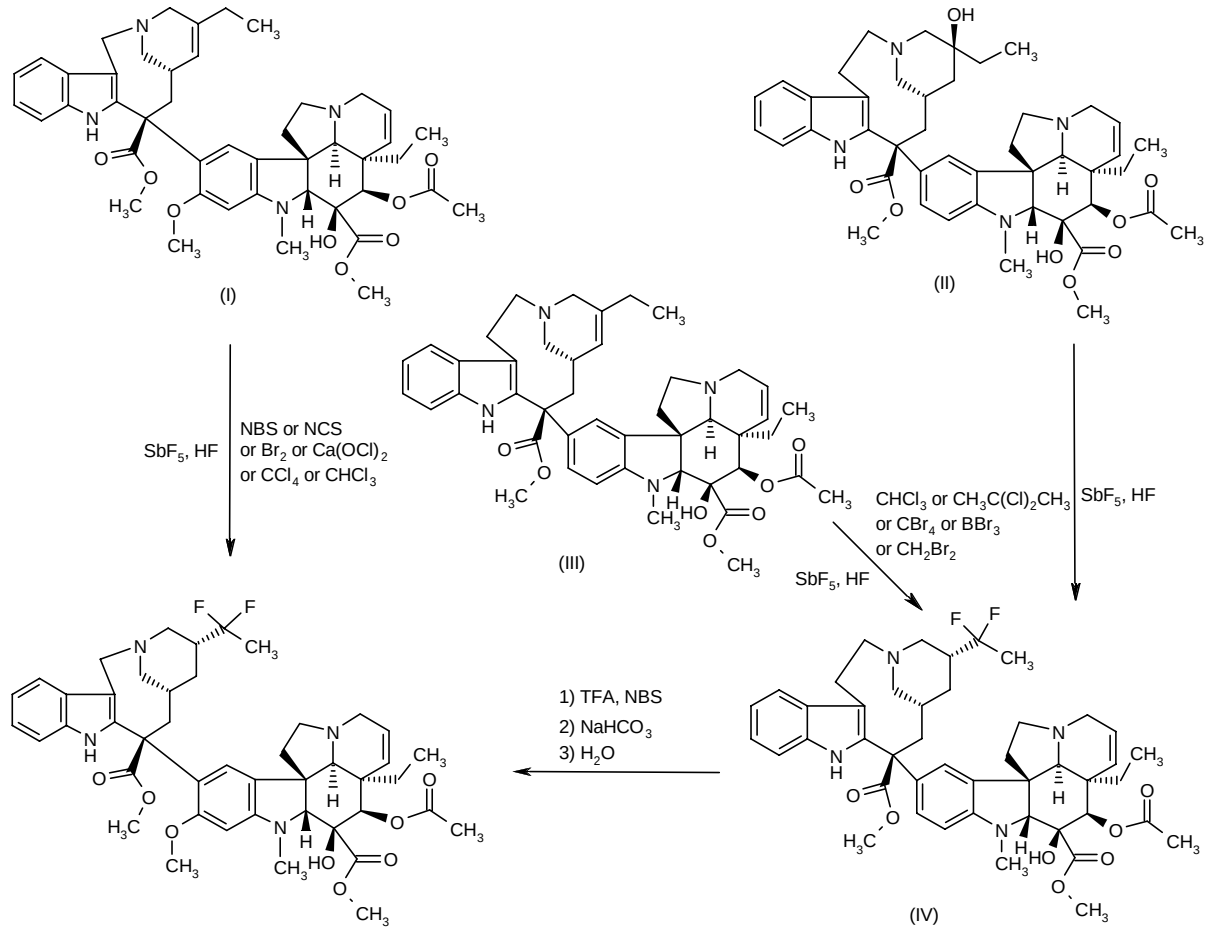
Scheme 1: Synthesis of Vinflunine



Pharmacological Actions

The mechanism of action of vinflunine was investigated in a panel of murine and human tumor cell lines. The cytotoxicity of vinflunine against cultured murine L1210 leukemia cells was strongly dependent on both concentration and duration of exposure. However, consistent with the classical *Vinca* alkaloids, a plateau-shaped dose-response curve indicated that there was a maximum concentration at which additional activity was dependent upon duration of exposure. The concentrations of vinflunine at which cell number or absorbance was reduced to 50% of that of control cells (IC_{50}) were 3-17-fold higher against murine leukemias compared with those for vinorelbine, vinblastine or vincristine. Vinflunine was also consistently the least potent against a panel of 9 human tumor cell lines. Vinflunine induced arrest at the G_2^+M phase of the cell cycle, with mitotic accumulation and reduction in the microtubular network of interphase cells. The capacity of vinflunine to bind to tubulin was undetectable by centrifugal gel filtration, and of the 4 *Vinca* alkaloids studied, it had the lowest affinity for binding to tubulin (5).

Further studies on the self-association of porcine brain tubulin used sedimentation velocity to investigate the tubulin-binding properties of vinflunine. Consistent with the findings of the previous study, vinflunine interacted with tubulin with 3-16-fold lower overall affinity compared to vinorelbine, depending on the model used. Stopped-flow light-scattering studies showed that vinflunine induced the shortest relaxation times, consistent with formation of the shortest spirals, in relation to the other *Vinca* alkaloids. These results indicated that vinflunine may be associated with reduced neurotoxicity, as smaller spirals have the potential for faster clearance from cells and shorter intracellular drug retention. The sedimentation velocity studies provided direct evidence that vinflunine is a tubulin-binding drug (6). The findings in this study correlated with studies in rat kangaroo (Pt K2) cells, in which intracellular accumulation of vinflunine was approximately 4-9-fold higher than that of other *Vinca*

alkaloids, while its interaction with tubulin was the most readily reversible (7, 8).

Quantitative video microscopy was used to assess the effect of vinflunine on microtubule dynamics. Microtubules exhibit two types of behavior which are important for progression through mitosis: dynamic instability and treadmilling. Microtubule protein preparations were isolated from bovine brain. Vinflunine slowed microtubule growth rate, increased growth duration and reduced shortening duration. These effects are different from those of the classical *Vinca* alkaloid vinblastine, which reduces the rate of shortening and increases the time microtubules spend in an attenuated state. Vinflunine suppressed treadmilling 7-fold less strongly than vinblastine. The diverse actions of the classical and second-generation *Vinca* alkaloids on microtubules may result in different effects upon cell cycle progression and cell killing (9).

The mechanism of mitotic block by vinflunine was studied in cultures of human cervical epithelioid carcinoma HeLa S3 cells. Mitotic block occurred at the metaphase/anaphase transition, and although the intracellular concentrations of vinflunine required to inhibit HeLa cell proliferation and block mitosis were 32-fold greater than those of vinblastine, their effects on spindle organization were very similar. These results indicated that overall suppression of microtubule dynamics rather than inhibition of specific parameters of microtubule instability was of greater significance in the antiproliferative actions of the *Vinca* alkaloids (10). Further studies used high-resolution time-lapse confocal video microscopy in living human osteosarcoma U-2 OS cells. A fluorescently labeled, centromere-binding protein was used to quantitate the effects of vinflunine on the dynamic behavior of spindle microtubules. The suppression of microtubule dynamics was demonstrated by a concentration-dependent decrease in centromere relaxation rates, stretching durations and transition frequencies. This suppression reduces tension across centromeres and prevents the cell cycle signal for progression into anaphase (11).

The mechanism of cell death induced by vinflunine was investigated in sensitive and vinflunine-resistant murine P388 leukemia cells. Apoptosis was characterized by DNA fragmentation and proteolytic cleavage of poly(ADP-ribose) polymerase (PARP). The mechanisms involved caspase 3 and 7 and c-Jun *N*-terminal kinase 1 (JNK1) activation, but did not require Bcl-2 phosphorylation (12). A further study on the mechanism of apoptosis was performed in neuroblastoma SK-N-SH cells. Bcl-2 phosphorylation only occurred at the high, clinically relevant concentration of 500 nM vinflunine, which induced microtubule depolymerization and mitotic block, whereas these effects were not observed at IC_{50} (50 nM) and IC_{70} (80 nM) concentrations. The results of this study indicated that vinflunine induces apoptosis independently of mitotic block and that mitochondria are involved in apoptotic pathways. Additional signal transduction pathways are also probably involved (13).

The preclinical *in vivo* antitumor activity of vinflunine was evaluated against a range of transplantable murine and human tumors. Increases in life span (median survival of treated mice/median survival of control mice [T/C%]) in mice bearing murine P388 leukemia and treated with vinflunine, up to a maximum of 160 mg/kg weekly for 4 weeks, ranged from 200% to 475%. These values were markedly superior to those of other *Vinca* alkaloids tested. Vinflunine was also active in terms of both prolongation of survival and tumor growth inhibition against B16 melanoma, considered to be a relatively drug-refractory tumor model, as well as against the human tumor xenografts LX-1 (lung) and MX-1 (breast). The study demonstrated that vinflunine was well tolerated and active in these animal tumor models when given intraperitoneally as single or multiple doses, showing improved efficacy over vinorelbine in each experimental model system used (14).

The experimental antitumor activity of vinflunine relative to vinorelbine was evaluated in further studies using subcutaneously implanted human tumor xenografts from the bladder, pancreas, kidney, colon, central nervous system, lung and prostate. In these studies in mice, antitumor activity was assessed by tumor volumes (T/C%), specific tumor growth delay and tumor regressions. Vinflunine was administered as 4 weekly i.p. treatments of 5-80 mg/kg each. Vinflunine demonstrated activity against 5 of the tumors, including 3 tumors unresponsive to other classical *Vinca* alkaloids. The overall response rate was 64% compared with 27% for vinorelbine, and the results confirmed the findings from the above study. Vinflunine was shown to have a broad spectrum of *in vivo* antitumor activity, although it was not active against bladder BXF1299, colon DLD-1 or HT-29, or glioma SF-295 tumor xenografts (15).

A number of other studies have confirmed the antitumor activity of vinflunine in various xenograft models. In nude mice implanted subcutaneously with human gastric cancer and melanoma xenografts, the maximum tolerated dose (MTD) was determined to be 40-50 mg/kg/week i.v. Antitumor activity was observed in both these models (16). In another study, antitumor activity was documented against pancreatic, non-small cell lung and renal tumor xenografts following weekly i.v. injections (MTD = 30-40 mg/kg/week) (17). Vinflunine resulted in 92% growth inhibition of intracecally grafted human orthotopic colorectal tumors, and also markedly reduced the number of experimental hepatic metastases induced by intrasplenic injection of these cancer cells (18). In a human small cell lung cancer model (NCI-H69), the mean doubling time of subcutaneous tumors was significantly longer after vinflunine injections of 4.0 and 6.0 mg/kg (15.1 and 14.2 days, respectively) than after vehicle injection (5.8 days); significant antitumor activity was also evidenced by T/C% values of 36-42% compared to controls (19).

In an orthotopic murine model of transitional cell carcinoma of the bladder, there was an 8-fold increase in the MTD of vinflunine compared to vinorelbine (40 mg/kg *versus* 4.8 mg/kg) following i.p. administration twice weekly

for 4 weeks. Intravesical tumor incidence on day 21 was significantly reduced in mice treated with 10 and 20 mg/kg vinflunine compared with all vinorelbine-treated and control groups (17% and 0%, respectively, *versus* 75-83%). There was also a significant difference between the groups in terms of survival. All mice treated with 20 mg/kg vinflunine remained alive 60 days after tumor implantation, whereas most of the control and vinorelbine-treated mice died before day 32 (20).

These *in vivo* studies clearly demonstrated the markedly superior antitumor efficacy of vinflunine compared to other *Vinca* alkaloids, and comparative studies have shown that vinflunine has a wider therapeutic window in experimental models of metastasis and exerts more potent antiangiogenic effects (21).

Vinflunine, like other *Vinca* alkaloids, participates in P-glycoprotein (Pgp)-mediated multidrug resistance (MDR). Studies of drug resistance mechanisms associated with vinflunine have been performed using vinflunine-resistant murine P388 leukemia cells (P388/VFL) and various resistant human tumor cell lines *in vitro*. The MDR tumor cell lines were generally less cross-resistant to vinflunine than to the other *Vinca* alkaloids (22). In further studies, vinflunine showed markedly reduced induction of drug resistance compared with vinorelbine both *in vitro* and *in vivo* using P388 cells and human lung carcinoma A549 cells (23).

The antitumor activity of vinflunine has also been investigated in combination with a number of other anticancer drugs. Using the A549 cancer cell line, it was shown to have synergistic cytotoxicity with cisplatin, mitomycin C, doxorubicin, 5-fluorouracil (5-FU) and camptothecin; no antagonism was observed in any case (24). In another study using A549 cells, vinflunine showed synergistic activity with the farnesyltransferase inhibitor Sch-66336 (25). However, in a transplantable murine colon adenocarcinoma model, the antitumor effects of cisplatin were potentiated, but vinflunine had little effect in combination with 5-FU. In the latter study, the functional vasculature was assessed and showed clear evidence of vascular shutdown in treatment groups which included vinflunine. These effects have been seen in other studies, with histological analysis of tumor tissue showing substantial hemorrhagic necrosis. The antitumor effects of vinflunine may therefore be attributable in part to these antivascular effects, due to the presence of dividing endothelial cells in newly forming tumor blood vessels. Vascular shutdown occurs at doses below the MTD for vinflunine, suggesting that combination therapies using drugs with different mechanisms of action at doses below their MTD may result in synergistic activity with minimal toxicity (26, 27). Further evidence for the potential of vinflunine as a component of combination chemotherapy has also been obtained *in vivo* using the P388 murine leukemia model (28, 29).

Pharmacokinetics and Metabolism

The *in vitro* distribution of [^{3}H]-vinflunine in human blood was examined at concentrations corresponding to those achieved at doses equivalent to 400 mg/m^2 administered every 3 weeks. The binding of vinflunine to blood cells was slight, while binding to platelets was negligible. The main binder was high-density lipoproteins (HDL). The binding of vinflunine to blood cells and serum proteins was nonsaturable (30).

The first clinical trial conducted in humans assessed the pharmacokinetic characteristics of vinflunine in 31 patients with advanced solid malignancies. Vinflunine was administered once every 3 weeks as a 10-min i.v. infusion, according to an accelerated dose-escalation schedule at doses between 30 and 400 mg/m^2. Pharmacokinetic assessments were made over a 96-h period during the first cycle of treatment only. The volume of distribution was large, indicating substantial tissue distribution. The elimination half-life was 25.5 ± 3.9 h. The area under the curve (AUC) and C_{max} increased in a dose-proportional manner, but no saturation of elimination was observed. The pharmacokinetic/pharmacodynamic (PK/PD) relationship demonstrated a high correlation between vinflunine AUC (body exposure) and the decrease in neutrophil count at nadir. Three metabolite peaks were observed in blood at lower levels than vinflunine; 2 metabolites were rapidly cleared, but 4-*O*-deacetylvinflunine persisted and reached the same level as the parent compound at 96 h. Urinary excretion of vinflunine was identified as a minor route of elimination (31, 32).

A phase I and pharmacokinetic study was also conducted in 16 patients with solid tumors administered vinflunine given on days 1 and 8 of a 21-day cycle at dose levels of 210, 190 and 170 mg/m^2. With predose sampling on day 8, the elimination half-life of vinflunine was more accurately defined as 39 ± 6 h. The metabolite 4-*O*-deacetylvinflunine had an estimated half-life of between 3 and 8 days. At steady state, a linear increase in the AUC of this metabolite was observed, suggesting that this dose schedule might result in a moderate metabolite accumulation, possibly having an impact on the tolerability profile of vinflunine (33, 34).

Vinflunine was also administered on a weekly schedule to patients with solid tumors at doses ranging from 120 to 190 mg/m^2/week in a parallel-group design. Blood samples were collected up to week 4. A dose-proportional increase in blood concentrations was observed for vinflunine and its metabolites. An increase in blood concentrations of the 4-*O*-deacetyl metabolite was also observed in this study at steady state; there was a 50% linear accumulation of this metabolite at week 4 compared to the first administration. The hematological toxicity was also additive based on the PK/PD relationship with AUC (35).

The pharmacokinetics, metabolism and excretion of vinflunine were studied in 5 patients using [^{3}H]-vinflunine. In blood, the main circulating compound was vinflunine and the principal metabolite was 4-*O*-deacetylvinflunine, which was the only active metabolite. Consistent with other phase I studies, this metabolite was slowly formed and eliminated, while the other metabolites were more

rapidly cleared from blood. Excretion was via the bile and kidneys (36).

Clinical Studies

In the phase I studies described above (31-35), MTDs and recommended doses for further phase II development were established, and preliminary antitumor activity was also assessed. In the first trial in humans, a total of 96 cycles of vinflunine were delivered, with a median number of 2 per patient. The MTD was 400 mg/m^2 every 3 weeks, at which 3 of 5 patients experienced dose-limiting toxicity (DLT). Toxicity mainly consisted of mucositis, constipation and neutropenia. According to the protocol rules, the recommended dose was established at 350 mg/m^2. At this dose level, 2 of 6 patients experienced DLT. No patients experienced DLT by hematological criteria alone and none of the patients treated at the lower dose levels of vinflunine (30-250 mg/m^2) experienced any DLT. In terms of efficacy, 3 partial responses were achieved, 2 in patients pretreated for metastatic breast carcinoma, and 1 in a chemotherapy-naïve patient with metastatic renal cell carcinoma (31).

In phase I studies with alternative dosing schedules, recommended doses were 170 mg/m^2 for vinflunine given on days 1 and 8 every 3 weeks (33), and 120 mg/m^2/week when given on a weekly schedule (35, 37). In a further phase I study, 26 previously untreated patients with advanced solid tumors were treated with vinflunine at 5 consecutive dose levels ranging from 150 to 250 mg/m^2/week. Five patients received 150 mg/m^2/week for at least 6 consecutive weeks without either DLT or dose delay; the recommended dose for chemotherapy-naïve patients was thus established at this dose (38).

The efficacy and safety of vinflunine were assessed in pretreated patients with advanced transitional cell cancer of the bladder. Patients had progressed after first-line platinum-containing chemotherapy for metastatic disease. Vinflunine was administered initially at a dose of 350 mg/m^2, but this was reduced to 320 mg/m^2 after the first 6 patients. Preliminary results were reported for 53 patients who were given a total of 175 cycles of vinflunine (median of 2). Nine patients achieved a partial response, confirmed by an independent panel. There were 3 episodes of fatal neutropenic sepsis (1 at 350 mg/m^2) and grade 3 and 4 neutropenia occurred in 30% and 37% of patients, respectively. The authors concluded that vinflunine had substantial activity and manageable toxicity, which was comparable to the most active currently available agents (39).

Preliminary results were also presented from a phase II study of vinflunine given as second-line therapy in patients with metastatic breast cancer progressing after first-line therapy with an anthracycline/taxane-based regimen. Forty-three patients had been enrolled, of whom 28 had evaluable data. Vinflunine was given as a single dose of 320 mg/m^2 administered every 3 weeks for a total of 114 cycles (median of 6). Grade 3 and 4 neutropenia occurred in 18% and 36% of patients, respectively. The main grade 3 toxicities were fatigue, abdominal pain, nausea and constipation. A partial response was achieved in 8 patients, which was confirmed by an independent panel. Vinflunine was considered a promising therapy for patients with metastatic breast cancer (40).

A trial to determine the recommended dose of vinflunine in combination with cisplatin, as well as the response rate and safety of the combination, was conducted in chemotherapy-naïve patients with advanced non-small cell lung cancer. Doses of vinflunine of 250, 280 and 320 mg/m^2 were administered in combination with cisplatin 80 mg/m^2 once every 3 weeks. As there were no DLTs at the first 2 doses (3 patients each), the recommended dose was established at 320 mg/m^2 vinflunine. Of 40 intended patients, preliminary results are available for 15 subjects. Five of these achieved a partial response, assessed by independent radiological review, and 7 had stable disease. The median number of cycles administered was 5, with grade 3 or 4 neutropenia observed in 52% of cycles (41).

Two phase III trials of vinflunine are ongoing. A study in non-small cell lung cancer will randomize approximately 550 patients with locally advanced disease previously treated with a platinum-containing regimen. Patients receive either vinflunine 320 mg/m^2 or docetaxel. A study in bladder cancer will enroll 330 patients who have either not responded to platinum derivative associations, or who have relapsed. In this study, vinflunine is compared with best supportive care. Enrollment in both studies is expected to be complete by the end of 2004. Other planned phase III programs include studies in advanced breast cancer and ovarian cancer (42, 43).

Sources

Pierre Fabre Médicament S.A. (FR); licensed to Bristol-Myers Squibb Co. (US).

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