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Pharmacokinetics, Tissue Distribution and Excretion of Vinflunine

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SUMMARY

The plasma pharmacokinetics, tissue distribution, excretion and binding to plasma proteins of vinflunine, were investigated after intravenous (iv) administration. We obtained plasma profiles after iv administration of vinflunine at the doses of 3.5, 7 and 14 mg/kg in rats. The $t_{1/2}$ values for vinflunine were estimated to be 18.38±1.20, 17.05±0.77, 18.35±1.57 h, and the mean AUC_{0-t} values were 3.48±0.38, 6.54±0.68, 12.79±2.93 μg·h/ml, respectively. Of the various tissues tested, vinflunine was widely distributed into tissues, with the highest concentrations of vinflunine being found in well perfused organs. Maximal concentration of vinflunine was reached at 0.5 h postdose in the majority of tissues. In tumor-bearing mice, the similar pattern of tissue distribution was observable, except that vinflunine can be distributed into tumor. The binding of vinflunine in human and rat plasma proteins were 39.6% and 58.4% respectively. Within 96 h after administration, 9.58%, 15.36% and 0.71% of the given dose was excreted in urine, feces and bile, respectively. In conclusion, Vinflunine had a longer terminal half-life, a wide tissue distribution and less than 25% of the given dose was excreted as unchanged drug, suggesting metabolism as a major style of elimination.

INTRODUCTION

Vinca alkaloids, including the natural products vinblastine (VBL) and vincristine (VCR) and the semisynthetic derivatives vindesine and vinorelbine, are antimitotic drugs that are widely and successfully used in the treatment of cancer (1). Their interactions with tubulin, the major component of microtubules in the mitotic spindle, and the subsequent arrest of cell in mitosis are generally accepted as key events in their mechanisms of action (2,3). The indole alkaloids VBL and VCR, initially extracted from the common *Madagascar periwinkle* have been used in the clinic for over 40 years. However, clinical applications of these agents are sometimes limited because of neuro- and hemato-toxicities (1). Semi-synthetic development of the vinca alkaloids has produced a successful second-generation compound, vinorebine, which has shown improved efficacy and reduced toxicity (4). It is effective in the treatment of Non-Small-Cell lung cancer, metastatic breast cancer, and ovarian cancer, and it showed promise in the management of lymphomas, esophageal cancer, and prostatic carcinomas (5-7). Now, it has been further modified through super-acidic chemistry to generate new and more active derivatives. The process of production involves the insertion of two fluorine atoms at the 20' position and reduction of the 3'4' double bond to produce 20', 20'-difluoro-3'4'-dihydrovinorelbine, known as vinflunine (Fig. 1) (4).

In vitro investigations have confirmed the mitotic-arresting and tubulin-interacting properties of vinflunine shared by other Vinca alkaloids (8, 9). However, vinflunine exerted markedly superior on antitumor activities against a panel of 13 murine and human tumour models compared

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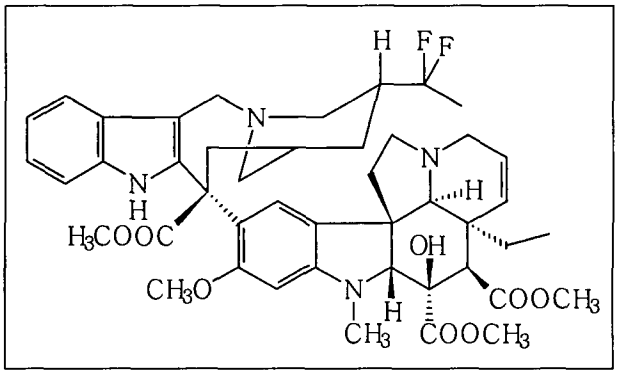

Fig. a: Structure of vinflunine

to the parent compound, vinorelbine (10-12). Differences in terms of the inhibitory effects of vinflunine on microtubules dynamics and its tubulin binding affinities have been identified, which appeared to distinguish it from the other Vinca alkaloids (13). Furthermore, an in vivo study has suggested that vinflunine mediates its antitumour activity at least in part via an antivascular mechanism, even at sub-cytotoxic doses (14). Vinflunine induced smaller spirals with a shorter relaxation time, which might be associated with reduced neurotoxicity(15). Although vinflunine appeared to participate in P-glycoprotein-mediated drug resistance mechanisms, it had proved only a weak substrate for this protein and a far less potent inducer of resistance than vinorelbine (16).

Compared to the extensive literatures on pharmacodynamic investigations, very little information is available on the pharmacokinetics and disposition of vinflunine. Resently, Bennouna studied the pharmacokinetics of vinflunine after infusion administration in patients with advanced solid tumours(15). In the present investigation, the pharmacokinetic behavior of vinflunine in rats was characterized as: 1) the pharmacokinetic disposition of vinflunine in rats; 2) excretion of parent drug in the urine, feces and bile of rats; 3) tissue distribution of vinflunine after iv administration to rats and tumor-bearing mice; 4) Plasma protein binding to rat and human plasma proteins.

MATERIAL AND METHODS

Chemicals

Vinflunine (purity 99.87%) was supplied by Qilu Pharmaceutical Co., Ltd (China). The internal standard (IS), finasteride (purity 99.10%), was a gift from Organic Chemistry Laboratories of China Pharmaceutical University. Sodium hydroxide and ethyl acetate were of analytical grade, and methanol (Merck, Germany) was of HPLC grade. All the other chemicals were purchased from Nanjing Chemical Reagent Co. (Nanjing, China). Purified water from a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout the experiment.

Animal

Sprague–Dawley rats were obtained from the experimental animal center of China Pharmaceutical University, and the studies were approved by the Animal Ethics Committee of China Pharmaceutical University.

Pharmacokinetics

18 Sprague-Dawley rats (both sexes) weighing 180-220 g were assigned randomly into three groups for iv administration of 3.5, 7 and 14 mg/kg vinflunine. A cannula was inserted into the carotid artery of a rat under ether anesthesia. Blood samples were collected immediately before and at 0.033, 0.25, 0.75, 1, 2, 4, 8, 12, 24, 36, 48, 72 h after drug administration. The blood samples were withdrawn into heparinized Eppendoff tubes, and were centrifuged at 2000 rpm for 10 min at 4 °C. A 0.2 ml volume of plasma was obtained and stored at -20 °C until analysis.

Excretion

Excretion - The urinary and fecal excretion of vinflunine was evaluated by treating a group of six rats with vinflunine 7 mg/kg. The animals were housed in stainless steel metabolic cages and given food and water *ad libitum* during the experiment. Urine and feces were collected at 0-6, 6-12, 12-24, 24-36, 36-48, 48-72 and 72-96 h following drug administration, and the feces were dried at room temperature for 24 h.

Biliary excretion - Experiments on biliary excretion of vinflunine were performed in another group of six rats. The bile duct was cannulated with PE10 tubing 1 h before dosing under anesthesia by ethyl ether. The drug was administered in the same fashion as in the experiments on urinary and fecal excretion. Bile samples were collected at 2 h intervals for the first 12 h and then from12 to 24 h after drug administration.

The specimens were stored at -20 °C until analysis after measuring the urine (or bile) volume and feces dry weight for each collection period. Blank excreta from untreated rats were similarly obtained during a 12 h period.

Tissue distribution

Rats - Eighteen rats were dosed with vinflunine intravenously at 7 mg/kg. Each six animals were sacrificed

at 0.5, 4 and 24 h after dosing. The following tissues and organs were collected at the time of sacrifice for determination of vinflunine concentration: brain, fat, heart, kidneys, small intestine, liver, lungs, muscle, plasma, skin, spleen, stomach, testes and ovaries. Rat tissues (0.1g) were homogenized with water (1:5, w/v) (500μl).

Tumor-bearing mice - Thirty mice were administrated with vinflunine intravenously at 20 mg/kg. Blood drawn from the eye socket, was collected 0.5, 2, 4, 12 and 24 h (six animals at each sampling point) after dosing, and the mice were then killed by cervical dislocation. The following tissues and organs were collected and homogenized at the time of sacrifice: brain, fat, heart, kidneys, liver, lungs, muscle, spleen and tumor.

Plasma protein binding determinations

Samples of rat and human plasma (1 ml) containing vinflunine at concentrations of 0.05, 0.4, 3.12 μg/ml were dialyzed (spectrapor 1 dialysis membrane 6000-8000 molecular weight cut-off; Spectrum Medical Industries, Ramcho Dominquez, CA) against isotonic Krebs-Ringer-bicarbonate buffer, pH 7.4 (20ml), for 72 h at 4 °C. After dialysis, the concentrations of drug in plasma and buffer were determined using liquid-liquid extraction and LC-MS.

Extraction of vinflunine

From rat plasma - Plasma samples were thawed in a water-bath at 37 °C. 0.1 ml volume of the plasma sample was transferred to a 15ml plastic test tube together with 20 μl of IS solution and 20 μl of 0.25 M sodium hydroxide solution. After vortex shaking for 1 min, 2 ml of ethyl acetate was added and the mixture was vortexed for 3 min. After centrifugation at 3500 rpm for 10 min, the upper organic layer was removed and evaporated to dryness under nitrogen gas at 50°C. The residue was reconstituted in 150 μl of the mobile phase, and then vortex-mixed. After centrifugation at 14000 rpm for 10 min, a 40-μl aliquot of the supernatant solution was injected into the LC/MS system for analysis.

From bile, urine and tissue homogenate - For extraction of vinflunine from bile, urine and tissue homogenate, bile (200 μl), urine (200 μl) and tissue homogenate (500 μl) were treated in a similar manner to plasma as described above.

From feces - The feces samples were freeze-dried overnight and pulverized with a mortar and pestle. Water (1:5, w/v) (200 μl) was added and homogenized with the pulverized feces (40 mg). The supernatant was treated as described above prior to analysis.

LC-MS Analysis

HPLC analyses were performed using a Hewlett-Packard HP1100 LC system (Hewlett-Packard, USA) with a Shim-pack C_{18} column(150 x 4.6 mm I.D., 5 μm, Shimadzu, Japan). The mobile phase was methanol-10 mM ammonium acetate (80:20, v/v), and the column temperature was maintained at 25 °C. A constant mobile phase flow-rate of 1.0 ml/min was employed throughout the analyses. LC-ESI-MS was carried out using nitrogen to assist nebulization. A quadrupole mass spectrometer equipped with an electrospray ionization source was used in positive ion selected ion monitoring (SIM) mode, set with a drying gas (N_2) flow of 10 l/min, nebulizer pressure of 40 psig, drying gas temperature of 350 °C and capillary voltage of 4 kV. The fragmentor voltage was 120 V. Target ions were monitored at *m/z* 817.3 for vinflunine and *m/z* 373.2 for Finasteride (IS) in the SIM mode.

Pharmacokinetic analysis of data

Pharmacokinetic parameters were estimated from the plasma concentration-time data with noncompartment analysis. The elimination half-life ($t_{1/2}$) was determined by linear regression of the terminal portion of the plasma concentration-time data. The area under the plasma concentration-time curve from zero to the last measurable plasma concentration point ($AUC_{0-\tau}$) was calculated by the linear trapezoidal method. Extrapolation to time infinity ($AUC_{0-\infty}$) was calculated as follows: $AUC_{0-\infty} = AUC_{0-\tau} + C_t/k_e$, where C_t is the last measurable plasma concentration and k_e is the terminal elimination rate constant. The total body clearance (CL_t) was calculated as dose/AUC_∞. The apparent volume of distribution (V_d) was calculated as CL_t/k_e. To evaluate dose proportionality, the *AUC*-relationships were analyzed by linear regression, and $t_{1/2}$ and CL_t were compared by an analysis of variance (ANOVA) model.

RESULTS

Pharmacokinetics after iv administration

The mean plasma concentration versus time profiles for viflunine after iv administration are presented in Fig. 2, and the pharmacokinetic parameters are summarized in Table I. After iv administration of vinflunine at doses of 3.5, 7 and 14mg/kg, The $t_{1/2}$ values for vinflunine were estimated to be 18.3, 19.4, and 18.7 h, and the mean $AUC_{0-\tau}$ values were 3.48, 6.54 and 12.79 μg·h/ml, respectively. CL_{tot} was relatively constant from 179.3 to 217.7 mL·h^{-1}.

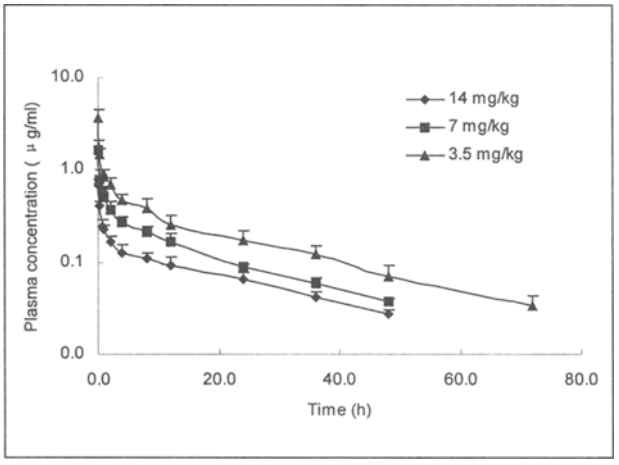

Fig. 2: Profiles of mean plasma concentration of vinflunine versus time after iv administration of vinflunine to rats (◆ 3.5 mg/kg; ■ 7 mg/kg; ▲ 14 mg/kg).

The analysis of variance of the $t_{1/2}$ and CL_{tot} showed no differences among the three doses of treatments after iv administration ($P>0.05$). These data indicated that the pharmacokinetic parameters did not vary with doses. The AUC increased with increasing doses for iv administration, and the regression analysis of the AUC-dose plot indicated good linearity($r>0.85$, $P<0.01$).

Excretion of vinflunine

The cumulative excretions of unchanged drug in urine, feces and bile after iv administration of 7 mg/kg were illustrated in Fig. 3. After iv administration of vinflunine for 96 h, 9.58% of the dose was found in the urine. During this time, 15.36% of the dose was detected in the feces, primarily in the first 48 h (14.1%). The billary excretion of vinflunine was 0.71% by the iv injection.

Tissue distribution

Table II summarized the distribution of vinflunine in different tissues such as brain, fat, heart, kidneys, small intestine, liver, lungs, muscle, plasma, skin, spleen, stomach, testes and ovaries after 0.5, 4, and 24 h post-administration. Vinflunine in each organ was expressed in μg of unchanged drug per g of wet organ. After iv administration, vinflunine was widely distributed into all tissues except brain and skin. The most significant levels of vinflunine were observed in the blood well perfused organs, that is heart, spleen, lung, kidneys and livers. At 0.5 h, concentrations of vinflunine in these tissues can be ranked, liver > lungs > heart > kidneys > spleen. The

Table I: Pharmacokinetic parameters of vinflunine after iv administration to rats. n=6. Mean±SD.

Parameter	Dose (mg/kg)		
	3.5	7	14
$t_{1/2}$(h)	18.38±1.20	17.05±0.77	18.35±1.57
CLtot(ml/h)	179.28±14.44	210.60±35.07	217.70±24.9
V(l/kg)	24.74±3.01	26.49±3.67	28.51±2.82
MRT(h)	26.52±1.73	24.61±1.11	26.47±2.27
$AUC_{0\to t}$(μg·h·ml^{-1})	3.48±0.38	6.54±0.68	12.79±2.93
$AUC_{0\to\infty}$(μg·h·ml^{-1})	4.27±0.40	7.35±0.63	13.72±3.09

Table II: Tissue distribution (μg/g) of vinflunine after iv administration at a dose of 7 mg/kg in rats.

Tissue	Concentration		
	0.5 h	4 h	24 h
plasma	0.61±0.07	0.15±0.03	0.08±0.01
heart	6.66±2.80	1.81±0.21	0.94±0.22
liver	16.00±3.39	9.56±5.80	1.01±0.32
lung	13.36±5.38	16.71±4.17	1.56±0.78
spleen	4.57±2.41	6.26±3.36	1.70±0.93
stomach	2.59±0.97	0.86±0.48	0.32±0.05
intestine	1.26±0.68	0.47±0.28	0.27±0.09
kindey	5.67±1.72	14.33±7.24	1.12±0.37
brain	0.15±0.05	0.11±0.04	0.11±0.02
uterus	1.05±0.72	1.36±0.92	0.09±0.04
testicle	0.09±0.02	0.17±0.10	0.07±0.08
skin	0.14±0.08	0.09±0.05	0.08±0.04
muscle	0.26±0.14	0.16±0.10	--
fat	0.89±0.40	0.13±0.04	0.12±0.06

-- not detected

amount of the parent drug found in the rest tissues was relatively low. The time course analysis indicates that most tissues had the highest vinflunine concentration at 0.5 h after iv administration, followed by a slow decrease.

The mean tissue concentrations of vinflunine versus time profiles after single intravenous doses of 20 mg/kg to tumor-bearing mice are presented in Fig. 4. Vinflunine was distributed mainly in blood well perfused organs like liver, kidney, lung, spleen and heart at all time points, which was in good agreement with that in rats. In addition, Vinflunine can be distributed into tumor, the target tissue, in which the concentration of vinflunine was higher than that in other peripheral tissue. The time course analysis indicates that kidney had the highest vinflunine concentration at 0.5 h postdose, followed by a rapid decrease. A similar pattern is observable in the other investigated tissues except spleen and tumor, which had the highest point at 2 h.

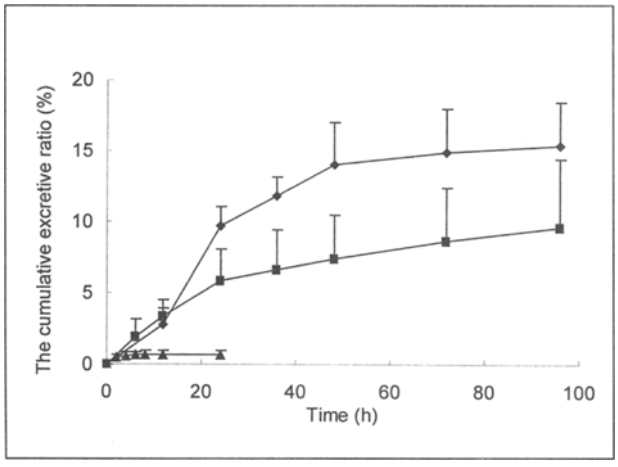

Fig. 3: Mean plots of cumulative excretion of vinflunine into urine, feces, and bile after iv administration of vinflunine to rats(◆ feces; ■ urine; ▲ bile).

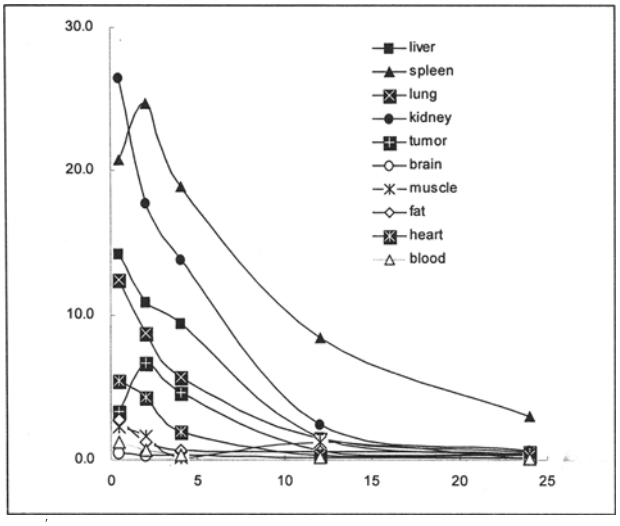

Fig. 4: The mean tissue concentrations of vinflunine versus time profiles after single intravenous doses of 20 mg/kg to tumor-bearing mice.

Determination of binding to plasma proteins

The results of binding to plasma proteins were shown in Table III. The free fractions (means) in human and rat plasma were 60.4% and 41.6% respectively, which indicated that the binding of vinflunine to plasma proteins of rats and humans is low. No concentration dependence was observed.

DISCUSSION

In the present study, we determined the pharmacokinetics of vinflunine in rats. In addition, drug excretion, tissue distribution and binding to plasma proteins results were also included to better understand the disposition of vinflunine.

Table III: The binding of vinflunine to plasma proteins of rats and humans.

Concentration μg/ml	The binding of vinflunine plasma proteins (%)	
	rat	human
0.05	60.8 ± 6.5	38.3 ± 9.3
0.4	59.0 ± 5.8	38.4 ± 5.4
3.2	55.4 ± 4.7	42.0 ± 5.0

After iv injection, drug levels fell rapidly during the distribution phase and then showed a slower decline phase. In rats, plasma concentration-time kinetics were apparently biphasic, differing from the thriphasic kinetics observed in man (15). The resulting $t_{1/2}$ was relatively longer, suggesting that the elimination of vinflunine from rat plasma was rather slow. The apparent volume of distribution (Vd) is relatively large, indicating a wide tissue distribution which was certified by the tissue distribution findings. For the dose range studied, AUC values were clearly dose proportional, while $t_{1/2}$ and CL were not dose-dependent. These results showed the linear pharmacokinetics of vinflunine in rats after iv administration.

After iv treatment with vinflunine, the feces and urine excretion of the parent drug was higher than that excreted in the bile. Less than 25% of the administered dose was recovered in the urine and feces after 96 h, suggesting metabolism as a primary mechanism of elimination.

After an iv dose of vinflunine to rats, the most significant levels of vinflunine were observed in the liver, lungs, heart, kidneys and spleen, with the highest concentration in liver. However, less than 1% of unchanged drug is observed in the bile, suggesting extensively metabolism of vinflunine by liver and relatively significant in vivo instability, which may represent a disadvantage for its potential oral administration. The results of pharmacodynamic investigation showed that vinflunine is effective in the treatment of bladder cancer and Non-Small-Cell lung cancer [17], which may be attributed to high level of vinflunine in the kidney and lungs. Lower levels in the brain tissue indicate that penetration of vinflunine across the blood-brain barrier is limited. The distribution to tissues is rapid with the maximal concentration occurring in most tissues at about 0.5 h, which explained the rapid drug disappearance in the distribution phase of concentration-time kinetics. Total drug concentrations were below background in many tissues at 24 h post-dose, indicating that there was no retention of drug in the animals.

In tumor-bearing mice, vinflunine was distributed into normal tissues with a similar pattern as in rats and reached

higher concentration in tumor than in other peripheral tissues. The superior antitumor activities of vinflunine may be attributed to this.

In summary, present studies have shown the linear pharmacokinetics of vinflunine after iv administration to rats. The disposition of vinflunine is characterized by long terminal half-life and extensive tissue distribution with no tissue accumulation. Feces excretion was the primary excretion route of the parent drug. Metabolism may be the most important elimination style.

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REFERENCES

1. Donehover R.C., Rowinsky E.K., Devita V.T., Hellman S., Rosenberg S.A. (1993). In Cancer: Principles and Practice of Oncology, JB Lippincott Co., Philadelphia, PA, 409-417.
2. Jordan M.A., Thrower D., Wilson L (1991): Mechanism of inhibition by Vinca alkaloids. Cancer Res., 51, 2212-2222.
3. Wilson L., Panda D., Jordan M.A. (1999): Modulation of microtubule dynamics by drugs: a paradigm for the actions of cellular regulators. Cell Struct. Funct., 24, 329-335.
4. Fahy J., Duflos A., Ribet J.P. (1997): Vinca alkaloids in superacidic media: a method for creating a new family of antitumor derivatives. J. Am. Chem. Soc., 119, 8576-8577.
5. Bunn. P.A. Jr., Kelly K. (1998): New chemotherapeutic agents prolong survival and improve quality of life in non-small cell lung cancer: a review of the literature and future directions. Clin. Cancer Res., 4, 1087-1100.
6. Crown J. (1997): Optimising treatment outcomes: a review of current management strategies in first-line chemotherapy of metastatic breast cancer. Eur. J. Cancer, 33 suppl. 7, S15-S19.
7. Johnson S.A., Harper p., Hortobagyi G.N., Pouillart P. (1996): Vinorelbine: an overview. Cancer Treat. Rev., 22, 127-142.
8. Jordan M.A., Wilson L. (1990): Kinetic analysis of tubulin exchange at microtubule ends at low vinblastine concentrations. Biochemistry, 29, 2730-2739.
9. Kruczynski A., Barret J.M., Etievant C., Colpaert F., Fahy J., Hill B.T. (1998): Antimitotic and tubulin-interacting properties of vinflunine, a novel fluorinated Vinca alkaloid. Biochem. Pharmacol., 55, 635-648.
10. Kruczynski A., Colpaert F., Tarayre J.P., Mouillard P., Fahy J., Hill B.T. (1998): Preclinical in vivo antitumor activity of vinflunine, a novel fluorinated Vinca alkaloid. Cancer Chemother. Pharmacol., 41, 437-447.
11. Hill B.T., Fiebig H.H., Waud W.R., Poupon M.F., Colpaert F., Kruczynski A. (1999): Superior in vivo experimental antitumour activity of vinflunine, relative to vinorelbine, in a panel of human tumour xenografts. Eur. J. Cancer, 35, 512-520.
12. Kruczynski A., Hill B.T. (2001): Vinflunine, the latest Vinca alkaloid in clinical development. A review of its preclinical anticancer properties. Crit. Rev. Oncol. Hematol., 40, 159-173.
13. Singer W.D., Himes R.H. (1992): Cellular uptake and tubulin binding properties of four Vinca alkaloids. Biochem. Pharmacol., 43, 545-551.
14. Holwell S.E., Hill B.T., Bibby M.C. (2001): Anti-vascular effects of vinflunine in the MAC 15A transplantable adenocarcinoma model. Br. J. Cancer, 84, 290-295.
15. Bennouna J., Fumoleau P., Armand J.P., Raymond E., Campone M., Delgado F.M., Puozzo C., Marty M. (2003): Phase I and pharmacokinetic study of the new vinca alkaloid vinflunine administered as a 10-min infusion every 3 weeks in patients with advanced solid tumours. Ann. of Oncol., 14, 630-637.
16. Etiévant C., Barret J.M., Kruczynski A. Perrin D. Hill B.T. (1998): Vinflunine (20',20'-difluoro-3',4'-dihydrovinorelbine), a novel Vinca alkaloid, which participates in P-glycoprotein (Pgp)-mediated multidrug resistance in vivo and in vitro. Inveat. New Drugs, 16, 3-17.
17. Jacquesy J.C., Jouannetaud M.P. (2005): Vinflunine: a new anti-cancer fluorinated agent derived from Vinca-alkaloids. Ann. Pharm. Fr., 63, 28-34.