Cancer Chemother Pharmacol (1993) 32: 487–490

Cancer Chemotherapy and Pharmacology

Short communication

Biliary elimination and pharmacokinetics of vinorelbine in micropigs

Dominique Levêque[1], Myriam Merle-Melet[3], Laurent Bresler[4], Jean Paul Didelot[4], Jean Pierre Aymard[5], Jeannette Wihlm[2], François Jehl[1]

[1] Laboratory of Pharmacokinetics, Institute of Bacteriology, 67 000 Strasbourg, France
[2] Laboratory of Biology and Pharmacokinetics, Paul Strauss Anticancer Center, 67 000 Strasbourg, France
[3] Department of Infectious Diseases, Hospital University Center, 54 000 Nancy, France
[4] Laboratory of Experimental Surgery, Hospital University Center, 54 000 Nancy, France
[5] Laboratory of Hematology, Hospital University Center, 54 000 Nancy, France

Received 1 March 1993/Accepted 4 May 1993

Abstract. The biliary elimination and pharmacokinetics of vinorelbine (NVB) were investigated in five conscious micropigs provided with a double-terminal choledocal fistula allowing the collection and reinstillation of bile. After the i.v. administration of NVB (0.5 mg/kg), serum and bile samples were collected over a 48-h period. The concentrations of NVB were measured by high-performance liquid chromatography. The serum concentrations decreased rapidly from a maximal value of 208.6 ng/ml (SD, 111.7 ng/ml). The mean half-life was 10.9 h (SD, 8.6 h) and the mean $AUC_{0-48\,h}$ was 292.8 ng ml^{-1} h (SD, 79.4 ng ml^{-1} h). The bile concentrations were high, amounting to 16.0 µg/ml (range, 5.4–27.7 µg/ml). The 0- to 48-h biliary excretion of unchanged NVB accounted for 25.8% (SD, 5.7%) of the injected dose, with 21.5% (SD, 4.0%) being eliminated during the 0- to 8-h period. Desacetyl-NVB was found in an inconstant manner and in very low amounts in bile samples. In addition, no glucuronide of NVB could be detected. Thus, in the micropig, biliary excretion represents an important route of elimination for NVB.

Introduction

Vinorelbine (5′-noranhydrovinblastine, Navelbine; NVB) is a new semisynthetic vinca alkaloid that chemically differs from its analogues by the alteration of the catharanthine moiety [15, 16]. NVB is currently used in the treatment of advanced non-small-cell lung cancer [4, 12] and advanced breast cancer [3] but is also active in ovarian cancer [5]. The pharmacokinetic profile of NVB in humans has recently been obtained using a specific high-performance liquid chromatographic (HPLC) method [8, 13]. It is best described by a three-compartment model characterized by a terminal half-life of 42 h. However, little is known about its elimination pathways since urinary excretion represents only 11% of the injected dose [8]. As for other vinca alkaloids, the major pathway for NVB should be hepatic clearance [1] via biliary elimination and/or hepatic biotransformation. Thus, we investigated the 48-h biliary elimination and serum pharmacokinetics of NVB in conscious micropigs provided with a double-terminal choledocal fistula allowing the collection and reinstillation of bile. The pig's physiology closely resembles that of the human in a number of respects; therefore, this animal represents an appropriate model for predicting the extent of biliary elimination of drugs in humans. Desacetyl-NVB, a minor urinary metabolite in humans, and the glucuronide of NVB were also sought in bile samples.

Materials and methods

Experimental animal model. Five female Yucatan micropigs (Charles River, Cléon, France) with a mean weight of 19.4 kg (SD, 1.8 kg) were used in this study. Following a 12-h fast, the micropigs were anesthetized. Ketamine was injected to sedate the animals. General anesthesia was induced with propofol, fentanyl, and vecuronium injected i. v. The anesthesia was maintained by i. v. perfusion of propofol and by injections of fentanyl given every 15 min and of vecuronium given every 30 min. An endotracheal tube was inserted and a median laparotomy was performed. The main biliary duct was dissected, the choledocal duct was cut below the junction with the cystic duct, and each end was catheterized with a Pedinelli drain. A catheter was also inserted in the jugular vein to allow the further collection of blood samples. Water was provided immediately after the procedure had been completed and food, the day thereafter. The pharmacokinetics study was performed 3 weeks after the operation. In our experiment, bile was reintroduced into the duodenum via the Pedinelli drain after each sampling. This restitution of bile flow was required to prevent changes in bile secretion and restore a semblance of normality.

Abbreviations: NVB, vinorelbine; HPLC, high-performance liquid chromatography; $t_{1/2}$, terminal half-life; AUC, area under the serum concentrations versus time curve

Correspondence to: Dominique Levêque, Institute of Bacteriology, Laboratory of Pharmacokinetics, 3 rue Koeberlé, F-67 000 Strasbourg, France

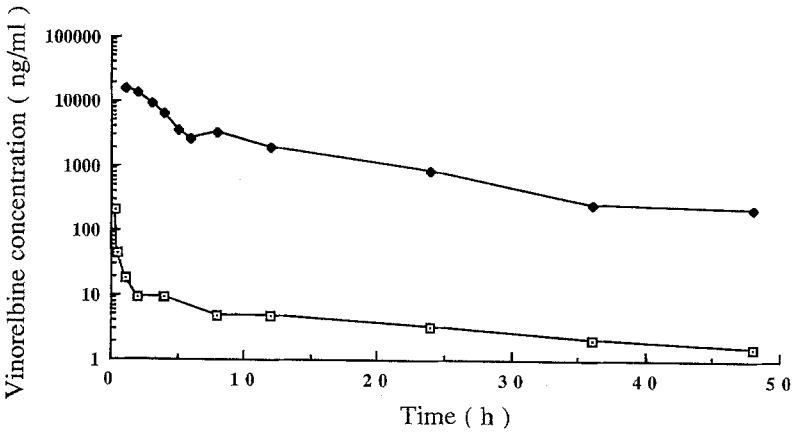

Fig. 1. Mean serum (□) and bile (◆) concentrations (versus time) of NVB measured in five micropigs after the i. v. (15-min) administration of 0.5 mg/kg

Protocol. Micropigs received NVB (0.5 mg/kg in normal saline) by a short i. v. infusion lasting 15 min. Blood was sampled at the catheterized site at time zero (before NVB injection) and at 15 and 30 min as well as 1, 2, 4, 8, 12, 24, 36, and 48 h after the injection. Samples were then centrifuged and the serum was frozen at –80° C until analysis. Bile was collected in a plastic infusion bag before treatment and at 0–1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–8, 8–12, 12–24, 24–36, and 36–48 h after drug administration. The volume of each bile fraction was measured and an aliquot was sampled and frozen at –80° C until analysis. The remaining volume was reintroduced into the duodenum through the Pedinelli drain.

Chemicals. NVB (as the ditartrate salt) and desacetyl-NVB were provided by Pierre Fabre Médicament as pure powders. Vinblastine (the internal standard) was obtained commercially (Velbé, Eli Lilly). All of the solvents used were of HPLC-grade. β-Glucuronidase type IXa (from *Escherichia coli*) was purchased from Sigma Chemical Co. (St. Louis, Mo.).

Drug analysis. NVB and desacetyl-NVB were measured in biological fluids by a specific HPLC method as previously described [7]. Briefly, an aliquot (1 ml) of serum or bile diluted in methanol : hydrochloric acid (20 : 80, v/v; 1 : 20) containing 100 ng vinblastine and 1 ml 66 m*M* phosphate buffer (pH 7) was added to 3 ml diethyl ether in a 60-ml screw-capped glass tube. After mixing, the tubes were gently shaken for 30 min by rotation and then centrifuged for 10 min at 1000 *g*. The upper organic phase was transferred to another glass tube and evaporated to dryness under a stream of nitrogen at 37° C. The dry residue was then dissolved in 120 µl methanol : hydrochloric acid (pH 2; 20 : 80, v/v).

The chromatograph consisted of a 126 programmable solvent-delivery module (Beckman, Fullerton, Calif.), a model 210 sample injection valve with a 50-µl loop (Beckman), and a model 166 programmable wavelength detector (Beckman). Chromatograms were processed by a GOLD chromatographic data system (Beckman). The assay was carried out using a 250- × 4-mm (inner diameter) cyanoanalytical column (5-µm particle size; SGE, Paris, France). The mobile phase consisted of 55% acetonitrile in 40 m*M* ammonium acetate (final concentration) adjusted to pH 2.9 and delivered at a flow rate of 1 ml/min. Quantitation was based on UV detection at 268 nm. The limit of detection of NVB and desacetyl-NVB in serum and bile was 1 ng/ml. Within- and between-day reproducibilities for NVB in bile were less than 10% in terms of coefficients of variation.

NVB from potential glucuronide was assayed following enzymatic hydrolysis of diluted bile samples with β-glucuronidase. This hydrolysis required 18 h incubation at 37° C with 2000 U/ml (final volume) of the enzyme. The hydrolyzed samples (1 ml) were then extracted as described above.

Pharmacokinetic analysis. The serum half-life was estimated using computerized software (Sifar; Simed, Créteil, France). The area under the concentration versus time curve (AUC) was calculated by the trapezoidal rule and included all experiment data points.

Results

As shown in Fig. 1, the serum concentrations decreased rapidly from a maximal value of 208.6 ng/ml (SD, 111.7 ng/ml). The mean terminal half-life was 10.9 h (SD, 8.6 h), and the mean AUC value was 292.8 ng ml^{-1} h (SD, 79.4 ng ml^{-1} h; Table 1). The mean biliary excretion profile is shown in Fig. 2. Most of the excretion occurred during the first 8 h. The bile concentrations of NVB were high, amounting to 16.1 µg/ml (range, 5.4–27.6 µg/ml) and remained around 220 ng/ml at 48 h after the injection (Fig. 1). The biliary excretion of unchanged NVB mea-

Table 1. Individual pharmacokinetic parameter of the five pigs

Pig number	C_{max} (ng/ml)	$t_{1/2}$ (h)	AUC (ng ml^{-1} h)	C_{max} bile (µg/ml)	Dose excreted during the 0- to 8-h period (%)	Dose excreted during the 0- to 48-h period (%)
1	108.8	8.2	272.9	15.6	22.0	27.0
2	144.9	25.5	307.1	5.4	14.0	16.2
3	264.8	5.3	406.4	15.5	24.9	31.0
4	402.7	ND	ND	16.5	21.5	ND
5	121.6	4.5	184.7	27.6	25.0	29.0
Mean	208.6	10.9	292.8	16.1	21.5	25.8
SD	111.7	8.6	79.4	7.1	4.0	5.7

ND; Not determined

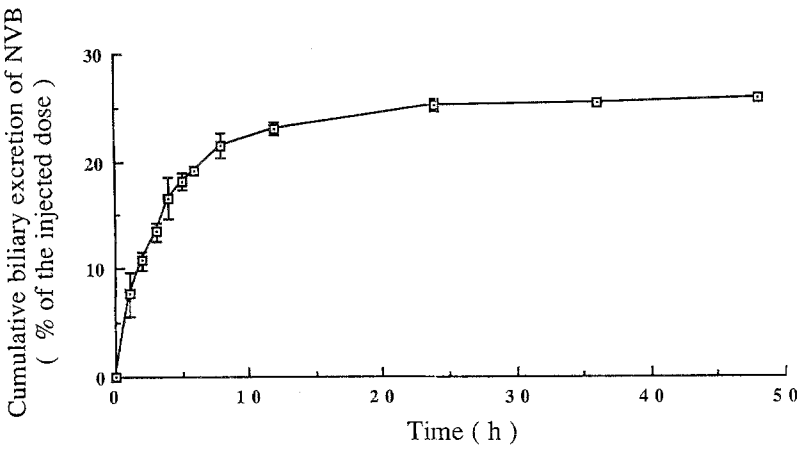

Fig. 2. Mean cumulative biliary excretion of NVB determined in five micropigs after the i. v. (15-min) administration of 0.5 mg/kg

sured up to 48 h accounted for 25.8% (SD, 5.7%) of the injected dose, with about 21.5% (SD, 4.0%) being eliminated during the 0- to 8-h interval. Low amounts of desacetyl-NVB were found in bile samples from two pigs and accounted for less than 5% of the injected dose. Furthermore, neither a glucuronide of NVB nor an additional peak could be detected.

Discussion

Little information is available about NVB elimination pathways. As is the case for other vinca alkaloids, NVB is poorly excreted in the urine. The mean urinary recovery obtained in our previous study in humans was 11% [8]. Thus, hepatic metabolism and/or excretion in bile should play an important role in the elimination of NVB. Unfortunately, difficulties are encountered in bile sampling in humans, and the use of an experimental model should therefore prove helpful. The pig is one of the most appropriate experimental models for the study of the biliary excretion of drugs [10, 11]. Like the human, the pig has only one stomach and is omnivorous. In addition, it easily tolerates ingrafting of instruments concerned with the pharmacokinetic investigation, especially the micropig. We recently used this model for the study of the biliary elimination of temafloxacin, an antibacterial agent [9]. Furthermore, no study on biliary elimination of unlabeled vinca alkaloids has been performed using an HPLC method.

In our present work, the serum concentrations were lower than those previously measured in humans, although the doses were similar [8]. The C_{max} values measured at the end of the 15-min infusion were 5 times lower than those observed in humans: 208.6 ng/ml (SD, 111.7 ng/ml) vs 1130 ng/ml (SD, 621 ng/ml). The biliary excretion of NVB was important and accounted for 25% of the injected dose during the 0- to 48-h period. Biliary concentrations exceeded plasma levels by 100 times at peak levels and remained high, around 200 ng/ml, at 48 h after drug administration. We found low amounts of desacetyl-NVB, and this compound, which is a minor urinary metabolite in humans, does not seem to be of importance in the hepatic clearance of NVB in the pig. Furthermore, the search for conjugated NVB by β-glucuronidase hydrolysis did not result in significant levels of glucuronide conjugates being found. As is the case for other vinca alkaloids [6, 17], the recovery of NVB remains incomplete, indicating the presence of other metabolites [2] and/or a sustained retention of the drug in tissue. Indeed, NVB is likely to be intensively distributed in tissues, as has been shown in human lung tissue, in which concentrations exceeded serum levels by up to 300 times at peak levels after i. v. administration [14].

In conclusion, the biliary excretion of unchanged NVB as determined by HPLC represents an important route of elimination for NVB in this micropig model. Furthermore, this model proved to be useful in allowing the investigation of bilary excretion of drugs.

Acknowledgements. The authors thank Laurence Linger and Mireille Pélégrin for their excellent technical assistance and Pierre Fabre Laboratories for the kind gift of vinorelbine.

References

1. Bender RA, Hamel E, Hande KR (1990) Plant alkaloids. In: Chabner BA, Collins JM (eds) Cancer chemotherapy, principles and practice. J. B. Lippincott, Philadelphia, pp 253–275
2. Boré P, Rahmani R, Van Cantfort J, Focan C, Cano JP (1989) Pharmacokinetics of a new anticancer drug, navelbine, in patients. Cancer Chemother Pharmacol 23: 247–251
3. Canobbio L, Boccardo F, Pastorino G, Brema F, Martini C, Resasco M, Santi L (1989) Phase-II study of Navelbine in advanced breast cancer. Semin Oncol 16: [Suppl 4]: 33–36
4. Depierre A, Lemarie E, Dabouis G, Garnier G, Jacoulet P, Dalphin JC (1991) A phase II study of Navelbine (vinorelbine) in the treatment of non-small-cell lung cancer. Am J Clin Oncol 14: 115–119
5. George MJ, Heron JF, Kerbrat P, Chauvergne J, Goupil A, Lebrun D, Guastalla JP, Namer M, Bugat R, Ayme Y, Toussaint C, Lhomme C (1989) Navelbine in advanced ovarian epithelial cancer: a study of the French oncology centers. Semin Oncol 16 [Suppl 4]: 30–32
6. Jackson DV Jr, Castle MC, Bender RA (1978) Biliary excretion of vincristine. Clin Pharmacol Ther 24: 101–107
7. Jehl F, Debs J, Herlin C, Quoix E, Gallion C, Monteil H (1990) Determination of navelbine and desacetylnavelbine in biological fluids by high performance liquid chromatography. J Chromatogr 525: 225–233
8. Jehl F, Quoix E, Levêque D, Pauli G, Breillout F, Krikorian A, Monteil H (1991) Pharmacokinetic and preliminary metabolic fate of navelbine in humans as determined by high performance liquid chromatography. Cancer Res 51: 2073–2076

9. Jehl F, Bresler L, Koechlin C, Merle-Melet M, Didelot JP, Hazebroucq J (1992) Pharmacokinetics and biliary elimination of temafloxacin in pigs. J Antimicrob Chemother 30: 189–196
10. Juste C, Corring T, Le Coz Y (1983) Bile restitution procedures for studying bile secretion in fistulated pigs. Lab Anim Sci 33: 199–202
11. Juste C, Corring T, Le Coz Y (1983) Bile secretion in the fistulated pig: effect of the method used for bile reinfusion. Reprod Nutr Dev 23: 765–773
12. Le Chevalier T, Pujol JL, Douillard JY, Alberola V, Monnier A, Rivière A, Lianes P, Chomy P, Cigolari S, Berthaud P, Gottfried M, Panizo AG, Besenval M, Brisgand D (1992) A European multicentre randomized study comparing navelbine alone vs navelbine-cisplatin vs vindesine-cisplatin in 612 patients with advanced non small cell lung cancer (NSCLC). Proc Am Soc Clin Oncol 11: 289
13. Levêque D, Jehl F, Quoix E, Breillout F (1992) Clinical pharmacokinetics of vinorelbine alone and combined with cisplatin. J Clin Pharmacol 32: 1096–1098
14. Levêque D, Quoix E, Dumont P, Massard G, Hentz JG, Charloux A, Jehl F (1992) Tumoral and healthy lung tissue concentrations of navelbine in patients undergoing surgery. Proc Am Assoc Cancer Res 33: 527
15. Mangeney P, Andriamialisoa RZ, Lallemand JY, Langlois N, Langlois Y, Potier P (1979) 5′-Noranhydrovinblastine. Prototype of a new class of vinblastine derivatives, Tetrahedron 35: 2175–2179
16. Mangeney P, Andriamialisoa RZ, Langlois N, Langlois Y, Potier P (1979) A new class of antitumor compounds: 5′-nor and 5′, 6′-seco derivatives of vinblastine type alkaloids. J Org Chem 44: 3765–3768
17. Owellen RJ, Hartke CA, Hains FO (1977) Pharmacokinetics and metabolism of vinblastine in humans. Cancer Res 37: 2597–2602