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Pharmacokinetic and Preliminary Metabolic Fate of Navelbine in Humans as Determined by High Performance Liquid Chromatography

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ABSTRACT

The pharmacokinetics and metabolism of Navelbine (NVB) were investigated in 20 patients by a specific high performance liquid chromatographic methodology allowing the monitoring of NVB, deacetyl-NVB, and *N*-oxide NVB. After the i.v. (15 min) administration of 30 mg/m² of drug, blood and urine samples were collected for, respectively, 144 and 48 h. NVB is characterized by a three compartmental kinetics, with a C_{max} of 1130 ± 139 (SEM) ng/ml. The total body clearance and apparent volume of distribution, as defined by high performance liquid chromatography, are 1.26 ± 0.09 liter/h/kg (48.6 ± 4.1 liters/h/m²) and 75.6 ± 9.2 liters/kg (2918.4 ± 307.2 liters/m²). No metabolite could be detected in serum; the urinary excretion of NVB represented 11% of the administered dose. Deacetyl-NVB could be identified as a minor urinary metabolite when no *N*-oxide NVB appeared in the urine samples. Two additional peaks appeared in most of urinary chromatograms as trace amounts. Thus, the major pathway of NVB, as for other *Vinca* alkaloids, should be hepatic clearance, as biliary elimination and/or hepatic biotransformation.

INTRODUCTION

Navelbine (5′-noranhydrovinblastine) is a new semisynthetic *Vinca* alkaloid developed by Mangeney *et al.* (1, 2) who demonstrated its pharmacological property, *i.e.*, a potent inhibition of tubulin polymerization and a weak induction of tubulin spiralization. Experimental models, such as L1210, P388 leukemia, and B16 melanoma as numerous human tumors grafted on nude mice, were used to demonstrate its important antitumor activity (3). Moreover, this new drug is also characterized by a low cross-resistance with other *Vinca* alkaloids (4). Navelbine has proved to be very effective in at least three cancer types: non-small cell lung cancer; breast cancer; and Hodgkin's disease. Some data also indicate that Navelbine has significant activity as a single agent in second or third line treatment of patients with advanced ovarian epithelial cancer (5).

The up to now published data on the pharmacokinetic behavior of Navelbine result either from RIA[2] measurement or from direct radioactive determinations after injection of [³H]Navelbine into cancer patients (6–9). Because there is evidence for the existence of an important metabolism of this drug, there was a need for a specific, reliable methodology for the measurement of Navelbine in biological fluids. This is why we recently developed a high performance liquid chromatographic (HPLC) method for the determination of Navelbine and two of its potential metabolites, deacetylnavelbine and *N*-oxide Navelbine, in biological fluids (10). We present here the first pharmacokinetic data of the new anticancer drug Navelbine, determined in 20 patients, by a parent drug as well as metabolite specific HPLC methodology.

MATERIALS AND METHODS

Patients. Twenty patients (19 men and 1 woman) with non-small cell lung cancer were included in this pharmacokinetic study after written informed consent. Their ages ranged from 41 to 74 years [58.5 ± 8.4 (SEM)] and their weights from 49 to 97 kg [69.1 ± 13.3). All had normal liver and renal functions.

Protocol. All of the patients were undergoing a single agent Navelbine therapy. The drug, at a dose of 30 mg/m², was infused over a 15-min period. Blood samples were drawn before infusion and then 0.25, 0.5, 1, 2, 4, 12, 24, 48, 72, 96, 120, and 144 h after the beginning of infusion. They were centrifuged at 4000 rpm for 10 min and then the serum were frozen at −80°C until analysis.

Urine samples were collected during the following intervals: 0–4 h; 4–8 h; 8–12 h; 12–24 h; and 24–48 h. Each volume was measured and an aliquot was frozen for analysis.

Blood and urine samples were obtained during the first cycle of treatment.

Analytical Method. Navelbine (as the ditartrate salt), deacetylnavelbine and *N*-oxide Navelbine were kindly provided by Pierre Fabre Medicament Company as pure powders. The concentrations of these compounds in biological fluids were measured by high performance liquid chromatography as described previously (10). Briefly, the chromatographic separations were performed on a cyano analytical column, 5 μm particle size. Navelbine, its metabolites, and internal standard (vinblastine) were eluted using a mobile phase consisting of 40% acetonitrile and 10% methanol in 5 mM ammonium acetate (final concentration). The pH was adjusted to 2.5 with hydrochloric acid, and the mixture was delivered at 1 ml/min. The extraction procedure involved the addition of the internal standard (100 μl of stock solution of 1 μg/ml of vinblastine) to 1 ml of biological sample in a screw capped glass tube. After a mixing on a vortex mixer, 1 ml of 66 mM phosphate buffer (pH 7) and 3 ml (serum) or 5 ml (urines) of diethyl ether were added. The tubes were then gently shaken by rotation for 30 min (20 rpm). After 10 min centrifugation at 1000 × *g*, the supernatant 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 of methanol:hydrochloric acid (20:80, v/v) (pH 2). A 50-μl aliquot was injected into the chromatograph. In this described methodology, *N*-oxide Navelbine eluted between deacetyl-NVB and NVB itself (Fig. 1).

The extraction recoveries of the drugs ranged from 66.8 ± 0.9 to 68.1 ± 1.7%. The limits of detection, respectively, were 0.5 and 1.0 ng/ml in serum and urine samples.

Pharmacokinetic Parameters. AUCs were evaluated using the trapezoidal rules, including all experimental data points. The pharmacokinetic parameters were estimated using a three compartment linear model suggested by the triphasic serum concentrations decay. All the parameters were calculated according to the method of Gibaldi and Perrier (11):

$$Cl_t = D/\text{AUC}$$

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[2] The abbreviations used are: RIA, radioimmunoassay; HPLC, high performance liquid chromatography; NVB, Navelbine (5′-noranhydrovinblastine; AVD, apparent volume of distribution.

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where D is the administered amount and Cl_t is total clearance,

$$t_{1/2\gamma} = \frac{0.693}{\gamma}$$

where γ is the slope of the γ phase (last elimination phase) of the serum concentration-time curve and $t_{1/2}$ is the half-life of elimination

$$AVD = Cl_t/\gamma$$

$$Cl_r = Cl_t \times f$$

where f represents the percentage of total dose eliminated in the urines as unchanged form and Cl_r is renal clearance.

RESULTS

As shown in Fig. 2, a curve stripping on the semilog plot of the serum concentrations *versus* time data revealed that the kinetics of NVB best fits a three compartment model. The equation of the triphasic decay is

$$C(\text{ng/ml}) = 3073\ e^{-5.17t} + 56.2\ e^{-0.247t} + 5.09\ e^{-0.0165t}$$

The mean serum concentrations obtained in 20 patients are summarized in Table 1. The 2 first hours are characterized by a rapid decrease from a C_{max} value of 1130 ± 139 ng/ml. After the 24th h, concentrations declined very slowly. The calculated elimination half-life was 42.1 ± 4.7 h. The half-lives of the α and β phases, respectively, were 0.14 ± 0.02 and 2.80 ± 0.35 h. All the pharmacokinetic parameters are reported in Table 2.

The urinary excretion of NVB, measured up to 48 h, amounted to 10.9 ± 0.7% of the administered dose, with about 8% being eliminated in the 0–8-h interval. When no deacetyl-NVB could be detected in serum, 0.24 ± 0.07% of the admin-

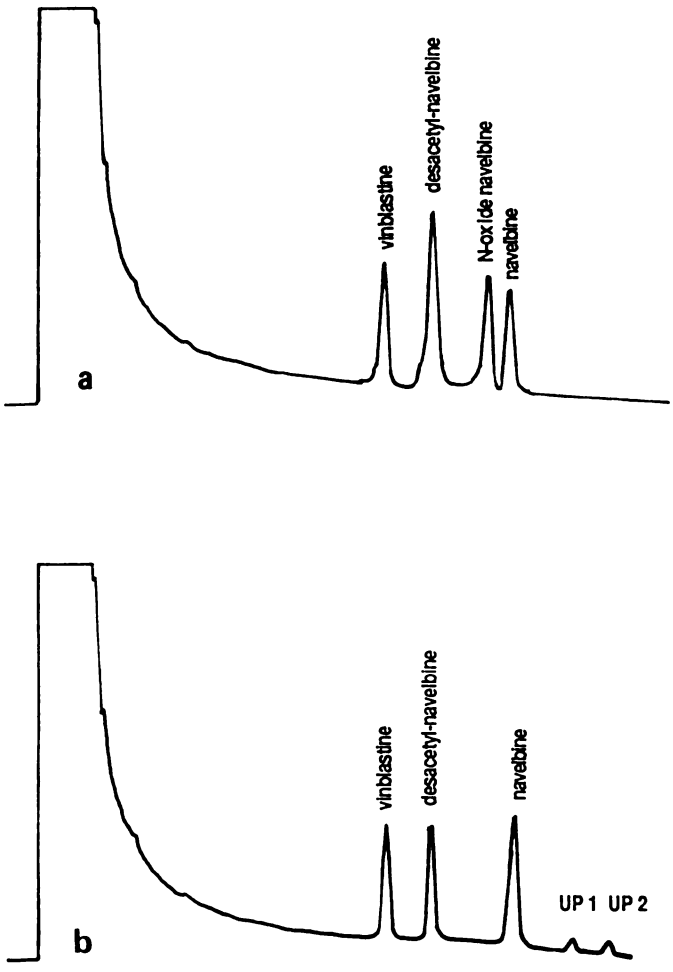

Fig. 1. Chromatograms of (*a*) human blank urine supplemented with vinblastine (internal standard), deacetylnavelbine, *N*-oxide navelbine, and Navelbine and (*b*) human urine sampled from a treated patient 4 h after the i.v. administration of 30 mg/m² of Navelbine.

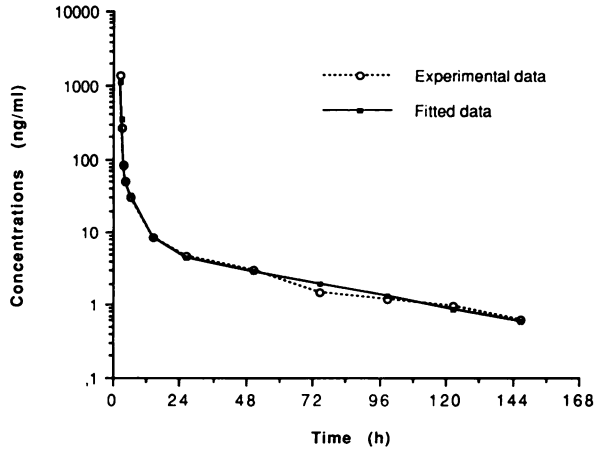

Fig. 2. Mean serum concentrations versus time of Navelbine in 20 patients after the i.v. (15 min) administration of 30 mg/m².

Table 1 *Mean serum concentrations of Navelbine obtained in 20 patients after a single i.v. dose of 30 mg/m²*

Time (h)	Concentrations (ng/ml)	SEM
0.25	1130	139
0.5	212	84
1	68	4.5
2	40.7	2.9
4	24.5	2.0
12	7.1	0.5
24	3.8	0.4
48	2.5	0.4
72	1.2	0.25
96	0.95	0.22
120	0.77	0.31
144	0.50	0.30

Table 2 *Individual pharmacokinetic parameters of the 20 patients included in this study*

Patient	AUC (ng/h/ml)	Cl_t (liters/h/kg)	$t_{1/2}\gamma$ (h)	AVD (liters/kg)
1	1357	0.62	24.0	21.5
2	422	1.62	24.0	56.1
3	825	1.18	33.0	56.2
4	586	1.22	26.7	47.0
5	709	1.14	24.2	39.4
6	662	1.38	60.0	119.5
7	580	1.39	48.1	96.2
8	588	1.25	24.2	43.2
9	565	1.47	24.0	50.9
10	577	1.42	43.2	98.3
11	474	1.92	24.2	66.4
12	883	0.93	69.3	93.0
13	682	1.15	99.0	157.6
14	816	0.98	22.3	31.5
15	816	0.95	33.0	45.2
16	440	2.00	58.1	167.0
17	1112	0.68	72.1	70.6
18	902	0.68	44.0	43.2
19	571	1.28	48.3	88.6
20	406	2.00	41.9	121.2
Mean ± SEM	701.2 ± 54.4	1.26 ± 0.09	42.1 ± 4.7	75.6 ± 9.2

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istered dose of NVB was eliminated in the urine as deacetyl-NVB. No *N*-oxide NVB was found in serum or in urine samples.

Furthermore, 2 minor peaks (UP_1 and UP_2) appeared on most urinary chromatograms of patients as trace amounts.

DISCUSSION

Some experimental data obtained either *in vitro* or *in vivo* in animals (12) revealed the existence of 1 to 3 metabolites of Navelbine.

Furthermore, investigations carried out in two patients by Bore *et al.* (9) revealed that the AUC determined by RIA were 23 and 31% of that measured by radioactivity.

Taking into account these evidences for Navelbine metabolism, we developed a specific HPLC method assay that can discriminate between the parental drug and two potential metabolites namely deacetyl-NVB and *N*-oxide NVB (10). This specific methodology was then used to redefine the pharmacokinetic parameters of this new anticancer drug in a large population of 20 patients.

The chromatographically measured serum concentrations are specific from parent drug and thus are lower than those resulting from RIA especially after the 12th h after administration. The most direct consequences of this fact concern the area under the serum concentrations *versus* time curve that are lower in our study (Table 3): 701.2 ± 54.4 ng/h/ml (0–144 h) *versus* 779 ± 162.5 ng/h/ml (0–72 h) or 1782.5 ± 3.5 ng/h/ml (0–240 h) for the same dosage. Consequently, AUC-derived parameters (in term of data reduction), such as Cl_t or AVD, are characterized by equivalent discrepancies: our total body clearance is the highest ever published for Navelbine (1.26 liters/h/kg) and the AVD is from 1.5 to 3 times higher than those resulting from RIA.

The biological half-life is not significantly affected by the analytical method used (Table 3).

As for other *Vinca* alkaloids (13–16), Navelbine is poorly excreted in the urine, both as unchanged form and as metabolites. The mean urinary recovery obtained in this study was 11% (range, 6.4–15.8%). This value is to be compared with the 4% recovery (RIA; 11 patients) published by Rahmani *et al.* (8) and with the 18.5–24.5% recovery (RA-RIA; 2 patients) published by Bore *et al.* (9). A radiochromatogram performed on one urine sample by these authors revealed the existence of metabolite, and thus interferences with RIA of Navelbine were likely to occur, leading to somewhat too high concentrations.

From the possible metabolites of NVB, the deacetyl derivative shows the same activity and toxicity as the parent compound, while the *N*-oxide derivative is inactive and nontoxic.

Using our HPLC methodology, we were able to measure the deacetylnavelbine concentrations in serum and urine samples.

Table 3 *Comparison of the pharmacokinetic parameters of NVB as determined by RIA or HPLC after i.v. administration of 30 mg/m²*

Authors	Ref.	No. of patients	Assay	$t_{½}$ (h)	Cl_t (liters/h/kg)	AVD (liters/kg)	AUC (ng/h/ml)
Rahmani	7	5	RIA	31.2	0.92	51.4	780[a]
Bore	9	2	RIA	62–97	23.5[b]		1782[c]
Rahmani	8	7	RIA	38	0.66	25.0	
Rahmani	8	6	RIA	40.2	0.78	27.2	
Our study		20	HPLC	42.1	1.26	75.6	701[d]

[a] $AUC_{0\rightarrow72}$.
[b] Liters/h.
[c] $AUC_{0\rightarrow240}$.
[d] $AUC_{0\rightarrow144}$.

Although we never found deacetyl-NVB in the serum of our 20 patients, this compound was present in almost all urine samples, but in very low amounts, representing only 0.25% of the administered navelbine (range, 0–0.70%). Thus, deacetylnavelbine must be considered as a metabolite of Navelbine, even if it is a minor metabolite. Nevertheless, these data should lead to search deacetyl-NVB in human bile, since the biliary elimination and hepatic biotransformation seem to be the major pathways of this new anticancer drug (12). On the other hand, we never detected *N*-oxide NVB in any biological fluids, urine or serum. Nevertheless, before concluding that *N*-oxide NVB is not a metabolite of NVB, investigation should be performed on human bile.

Two other compounds, UP_1 and UP_2 (for unknown peaks) eluted after NVB on about one-half of the patients' urinary chromatograms (Fig. 1). They could be considered as NVB metabolites as they all were absent from the chromatograms resulting from the same patients' urine sampled just before NVB administration. Of particular interest is that the amounts of deacetyl-NVB and UP_1-UP_2 varied in the opposite site as a function of time. This indicates that UP_1 and UP_2 could be degradation products of deacetyl-NVB, but this remains to be proved. Nevertheless, UP_1 and UP_2 should not be of any significant importance in the NVB metabolism schedule as they were present as trace amounts.

The formation of conjugates of Navelbine, essentially glucuro- and sulfo- conjugates, remains to be investigated, and work in this area is currently underway using urine from patients. The high hepatic clearance of this drug also calls for the quantitation of elimination of NVB and the research of deacetyl-NVB and *N*-oxide NVB in human bile.

In conclusion pharmacokinetic properties of NVB are similar to those of other *Vinca* alkaloids (13–16); they all are best described by a three compartment model and characterized by a low urinary excretion, indicating a predominant hepatic clearance. Among these drugs, NVB is characterized by a much more increased body clearance and a wider volume of distribution than what was previously shown by Rahmani *et al.* (7). These findings may, in part, explain its lower toxicity and raise the potential interest in this new antitumor drug for the treatment of solid tumors.

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