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Pharmacokinetics of a new anticancer drug, navelbine, in patients

Comparative study of radioimmunologic and radioactive determination methods

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Summary. A study was designed to investigate the fate of navelbine (NVB) and its excretion routes in two cancer patients treated with tritiated NVB (30 mg/m^2) by i.v. bolus injection. Plasma and red blood cell concentrations and urine and stool elimination were monitored over long periods of time. NVB plasma and urine concentrations were measured by both radioimmunoassay (RIA) and direct radioactive (RA) determination. Samples were also analyzed by high-performance liquid chromatography to evaluate the importance of NVB metabolism. Whereas the major excretion route for NVB was the stool (from 34% to 58.4% of the total dose given over 21 days), urinary excretion was low (about 21% within the same time period), corresponding mainly to that of unchanged drug. Thus, a good correlation was found between RIA and RA determinations in urine. In contrast, plasma area under the curve (AUC) values obtained after RA and RIA analysis differed markedly (AUC RIA/AUC RA = 0.23–0.31), demonstrating that a significant proportion of the plasma-circulating drug was biotransformed, mainly during the last elimination phase. This could have important pharmacologic and toxicologic implications in clinical practice.

Introduction

Navelbine (5'-noranhydrovinblastine; NVB) is a novel synthetic antitumor vinca alkaloid currently under clinical investigation. It is structurally related to vinblastine (VLB) and its congeners, and its properties are similar to those of other vinca alkaloids [10]. Tolerance to NVB was evaluated in two phase I studies [4, 16] involving a total of 42 advanced cancer patients (mainly solid tumors) who were given 2 weekly bolus injection at doses ranging from 3.6 to 43 mg/m^2. Leukopenia was the principal dose-limiting toxicity; other side effects were extremely mild. No neurotoxicity was observed at doses $\leq$30 mg/m^2.

Recent phase II studies in nonoperable patients with non-small-cell carcinoma have indicated a very encouraging rate of response to NVB as a single agent (30 mg/m^2 weekly injection), confirming the extremely high activity observed in vitro with this new agent in a subline of epidermoid carcinoma [17]. The pharmacokinetic properties of this compound were assessed following i.v. bolus administration to 11 patients as part of a phase I clinical trial (15–30 mg/m^2) using a radioimmunological method [12], which demonstrated that NVB's plasma concentration decay was triphasic and thus characteristic of vinca alkaloids given by i.v. bolus [2, 7, 14].

To investigate better the fate of NVB and its excretion after a single i.v. dose, a new study was designed in cancer patients treated with [^{3}H]-NVB. In this study, plasma and red blood cell (RBC) concentrations and urine and stool excretion were monitored over a long period. The concentration of NVB in plasma and urine samples from treated patients were measured by both radioimmunoassay (RIA) and direct radioactive determination (RA). When possible, samples were also analyzed by HPLC to evaluate the importance of NVB metabolism in man.

Materials and methods

Chemicals. [^{3}H]-NVB bitartrate (5 Ci/mmol) was prepared by the C.E.A. (Commissariat à l'énergie Atomique, Saclay, France). NVB bitartrate was synthesized by Pierre Fabre Médicament (Castres, France). The chemical and radiochemical purities of NVB were assessed by HPLC; all other chemicals were analytical grade.

NVB (240 mg) was dissolved in 20 ml NaCl (0.75%) aqueous solution. [^{3}H]-NVB bitartrate (200 μCi) in 200 μl ethanol was added to 13 ml NVB saline solution. This preparation was passed through 0.45- and 0.2-μm Millipore filters and stored in sterile vials. The final solution, 10 mg NVB base/ml, was tested for sterility; it was isotonic and had a specific activity of 3,338,700 dpm/mg NVB base.

Clinical data. Two patients (aged 64 and 67 years) entered the study after giving informed consent. Their eligibility was based on the following criteria: serum creatinine $<$130 μM, WBC count $>$3000/mm^3, and platelet count $>$100,000/mm^3. No clinical or biochemical abnormalities of hepatic or renal function were observed. Both patients were hospitalized for 5 days. After a fasting period of 15 h, they were given 30 mg/m^2 injectable preparation by i.v. bolus; patient 1 received 46.5 mg (69.9 μCi) and patient 2, 49.5 mg (14.4 μCi). Food was reintroduced 4 h after treatment.

Sampling. Blood was drawn into heparinized tubes from the arm opposite the injection site at the following times:

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0 (pretreatment), 5, 10, 20, 30, and 45 min and 1, 1.5, 2, 3, 4, 8, 12, 24, 48, 72, 96, 168, and 240 h posttreatment. Urine was collected over a period of 3 weeks at the following intervals: 0–4, 4–8, 8–12, 12–24, 24–36, and 36–48 h, and then by 24-h fractions. Stools were collected for 3 weeks at daily intervals. Samples were stored at −20° C until analysis.

Determination of radioactivity. Radioactivity in plasma and urine was directly determined in triplicate in a scintillation spectrometer (Beckman LS 7800). The protein binding of radioactivity was evaluated in the plasma samples obtained 5 and 20 min and 2, 24, and 96 h after injection. Plasma (1 ml) was centrifuged at 2500 *g* for 3 h at 25° C on an Amicon centrifree filter for the accurate separation of free and protein-bound material. Radioactivity in the ultrafiltrate was then determined. Samples of feces, collected in weighed containers, were lyophilized. The dry residue was precisely weighed and homogenized in a Waring blender. Five 200- to 300-mg aliquot samples were compressed as pellets and precisely weighed, and radioactivity was determined in a Packard model 360 sample oxidizer. The yield of combustion (95% ± 2%) was reevaluated for every 10 samples, providing a variation coefficient of <6%. RBC radioactivity was determined in triplicate by liquid scintillation after direct combustion (sample oxidizer) in exactly weighed 500-µl samples.

Determination of NVB levels by RIA. NVB concentrations in plasma and urine were measured by RIA according to the technique of Rahmani et al. [12]. Briefly, plasma and urine samples were diluted when necessary in phosphate-buffered (50 m*M*, pH 7.4) saline (0.15 *M*) containing 1 g/l bovine serum albumin (Fraction V, Sigma) and incubated with rabbit anti-NVB antiserum and ^{125}I-NVB-glycyl-tyrosine conjugate at 4° C for 22 h. Human plasma was added when necessary to maintain a constant amount of human plasma components in the incubation medium and to ensure reproducible precipitation of immune complexes (healthy donors, Centre de Transfusion Sanguine, Marseille). At the end of the incubation, polyethylene glycol 6000 (Merck) was added, for a final concentration of 12.5% (w/v). Precipitated immune complexes were separated by centrifugation (2000 *g*, 10 min) after 5 min incubation at −20° C and counted for 1 min on a Kontron MR 252 γ counter. Nonspecific inhibition in pre-treatment plasma was taken into account to calculate NVB concentrations, which were determined by interpolation of the logit-log linearized standard curve (useful range, 0.25–25 ng/ml; variation coefficient, 15%).

High-performance liquid chromatography. Samples were analyzed on a Hewlett Packard 1084B chromatograph equipped with a UV detector (Waters model 440). The column used was a µBondapack phenyl (10 µm) prepacked reverse-phase column (Waters Associates). A mobile phase of methanol and sodium perchlorate (45 m*M*)-perchloric acid (6 m*M*) was used, and the solvent system consisted of a linear gradient going from 50% to 75% methanol in 35 min at a flow rate of 1 ml/min. Detection was carried out at 269 nm. The eluent was directly collected into scintillation vials for 35 min (timed fractions, 30 s). Radioactivity was measured after the addition of 5 ml scintillation liquid (PCS, Amersham) using a Beckman LS 2800 liquid scintillation spectrophotometer. HPLC separation was checked with several vinca alkaloid analogs presenting minor changes in the vindoline or catharantine moieties.

Data analysis. NVB plasma clearance (Cl_p) was determined according to the relationship

Cl_p (l/h) = IV dose/AUC.

The area under the NVB plasma concentration-time curve (AUC) was calculated according to the trapezoidal rule [5] using all experimental data points. Apparent elimination half-lives ($t_{½}$) were estimated by least-squares regression on terminal data points. The renal clearance (Cl_r) of NVB was expressed as follows:

Cl_r (l/h) = Cl_p × Qu%,

where Qu% = the percentage of the total injected dose in urine. Cumulative urinary excretion was calculated by summing up the amount of NVB excreted in the different samples, expressed as a percentage of the dose.

Results

Plasma kinetics

Each of two patients was given 30 mg/m^2 NVB by rapid i.v. injection. Pharmacokinetic parameters were evaluated after RA and RIA determinations. For patient 2, plasma concentration curves after RA and RIA determinations are shown in Fig. 1. In both cases, plasma concentrations declined sharply in the first 2 h after drug injection, followed by a slow decline in levels determined by RA. The levels obtained at 96 h were about half those obtained at 2 h . At 168 h (7 days), significant levels determined by RA were still detectable in both patients. The extremely slow plasma elimination and some rebounds observed in the curve (probably due to drug recirculation) made it difficult to determine $t_{½}$ values, especially for the last phase.

In contrast, after RIA analysis the kinetics presented multiexponential decays, with $t_{½}$s of 62.4 h and 97.3 h, respectively. As shown in Table 1, the AUCs calculated after RA and RIA quantifications differed significantly. The AUC ratios (RIA/RA) observed after the injection were 0.23 and 0.30 for patients 1 and 2, respectively. Systemic clearances were 23.33 l/h (0.436 l/h per kg) and 23.65 l/h

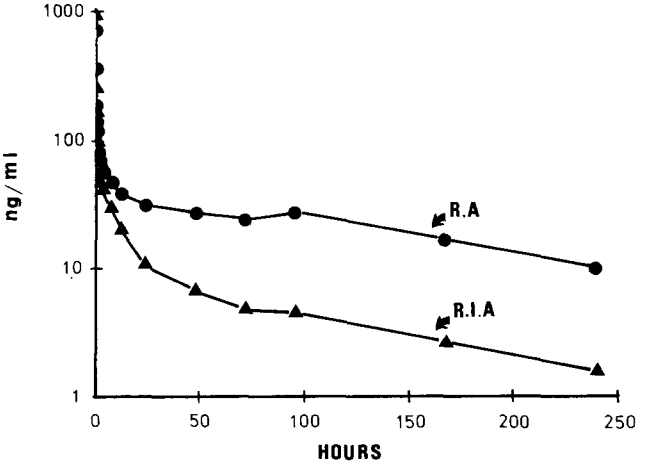

Fig. 1. Plasma concentrations of NVB in patient 2 after the administration of 30 mg/m^2 of [^{3}H]-NVB. *RA*, radioactive determination (●); *RIA*, radioimmunoassay (▲)

Table 1. Pharmacokinetic parameters of NVB

Patients	Doses (mg)	AUC (experimental)	(ng/ml × h) 5′–2 h/0–∞	$t_{1/2}$	C_p (l/h)	C_r (l/h)	AUC ratio (RIA/RA)
1 RA	46.5	7529	324/–	–	6.18	0.98	0.23
RIA		1785	389/1992.4	62.4	23.33	4.84	
2 RA	49.5	5758	297/–	–	8.60	1.81	0.31
RIA		1780	380/2092.8	97.3	23.65	6.74	

C_p, plasma clearance; C_r, renal clearance; RA, radioactive determination; RIA, radioimmunoassay

(0.400 l/h per kg) for patients 1 and 2, respectively. These values are in complete agreement with the pharmacokinetic data obtained during a previous phase I study [15].

The correlation between RIA and RA measurements was rationalized by linear least-squares regression of the data plotted as RIA vs RA. For experimental points ranging between 5 min and 3 h, the slope of the linear regression averaged 0.6, with a correlation coefficient of 0.983 (18 paired points). For the data obtained by these two methods between 5 min and 20 min, the coefficient of variation (CV) between the analytical methods was 35.2 ± 12.2% (mean ± SD); from 30 min to 3 h, the CV was 12.0% ± 4.7% (mean ± SD). However, the AUC portions corresponding to the first points (5 min–2 h) were in the same range (Table 1). In contrast, kinetic data points between 24 h and 168 h exhibited large discrepancies when estimated by either RA or RIA. This was confirmed by the slope of linear regression (1.6) and the lack of correlation (0.547) between the two methods. Moreover, the CV estimated point by point was 81.3% ± 10.2% (mean ± SD).

Blood cell concentration and protein binding

In both patients, radioactivity linked to RBC was equal to or higher than that found during the first 2 h in plasma. Thereafter, the general tendency was a faster concentration decay for erythrocytes, with a plasma/RBC ratio of approximately 2. Moreover, whereas 79%–85% and 72%–75% of the [^{3}H]-NVB radioactivity was bound by plasma proteins at 5–20 min and 2 h, respectively, this proportion reached only 43%–58% at 24 h and 26%–59% at 96 h.

Urinary excretion

An example of the urinary excretion rate over time is shown in Fig. 2. Within the 21 days of urine collection, the total recovered excretion represented 24.6% ± 5.4% (RIA) and 18.5% ± 3.5% (RA) of the injected dose. Regardless of the determination method, an average of 61%–65% of the total amount was excreted within the first 24 h, and 68%–76%, within the first 48 h (Table 2). RIA and RA values were well correlated. The slopes of the regression line were 0.74 and 1.01, with correlation coefficients of 0.99 and 0.97, respectively (22 and 24 paired points for patients 1 and 2, respectively). When possible, urine samples were analyzed by HPLC. Figure 2 (*inset*) illustrates the radiochromatographic profile of samples collected from 0–7 h (patient 1); at these times, unchanged NVB represented 95.2% ± 4.5% (n = 2) of the total injected radioactivity.

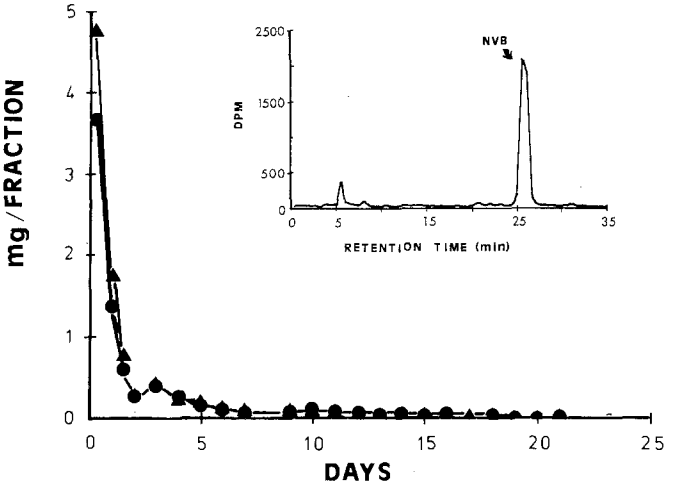

Fig. 2. Urinary excretion of NVB measured by RA (●) and RIA (▲) for patient 1. *Inset:* Radiochromatogram obtained after HPLC analysis of urinary sample taken at 0–7 h

Table 2. Total tritium excretion for each patient (20–21 days), percentage of total dose

Patients	Doses (mg)	Urine (%)	Stool (%)	Total (urine + stool)
1	46.5	15.8	33.9	49.7
2	49.5	21.0	58.4	79.4

Fecal excretion

Within the 21-day collection period, 34% and 58.4% of the injected radioactivity was recovered in feces of patients 1 and 2, respectively. In contrast to the urinary excretion kinetics, where the maximal value was obtained during the 1st day, the highest fecal excretion value was observed at day 3 for both patients (17%–25% of the total dose). Whereas up to 85% of the total fecal excretion occurred within the 1st week for patient 1, only 47% took place in that period for patient 2. At the last fecal collection (days 20–21), from 0.16% to 0.9% of the total dose was excreted. Radioactivity excreted in feces as tritiated water was negligible (0.02%–0.04%) as measured after lyophilization.

Discussion

NVB was first introduced in a phase I study on its high affinity for tubuline and potential anticancer activity in humans as demonstrated in vivo and in vitro in animal models. In mice, NVB was equiactive to vincristine (VCR) on L1210 leukemia cells, whereas vinblastine (VLB) showed no significant effect. On P388 leukemia cells, NVB exhibited a marked effect, with a therapeutic index of 19 compared with that of 8 for VLB and VCR [18]. Moreover, a low cross-resistance to VCR and vindesine (VDS) has been observed in a P388-resistant leukemia subline [6].

As has been demonstrated in rat, mouse and monkey (Pierre Fabre Médicament, personal communication), the urinary excretion of NVB in man is low (<20% within 21 days), being in the same range as that previously described for other vinca alkaloids [9, 13]. The results obtained in the present study by RA and RIA urine analysis showed a good correlation. Moreover, HPLC analysis demonstrated that the unchanged NVB corresponded to 92% ± 2% of the total tritium excreted by this route. These results are in agreement with those of Owellen et al. [8] for VLB. In contrast, a non-negligible amount of VCR, which is also poorly excreted in urine, has been reported to be eliminated in a metabolized form (46% of the total amount) [1].

Regardless of the determination method used, the first phase of NVB plasma disappearance was rapid. This is in agreement with other studies on vinca alkaloids in animals and humans [2, 7, 14]. All vinca alkaloids are characterized by a triphasic plasma concentration decay. In the present study, systemic clearances and half-lives determined by RIA, were in accordance with the pharmacokinetic data obtained during a previous study [15]. However, AUC values obtained after RA and RIA analyses differed markedly (AUC RIA/AUC RA = 0.23–0.31). Over the first 2 h, these values were similar, but the RIA value was slightly overestimated because of the high concentration. In contrast, the last kinetic data points show large discrepancies between RA and RIA data, which could be explained by the fact that the RIA method does not quantify all of the NVB-related compounds in plasma and thus may be more specific for the parent compound. Actually, due to the immunogenic form used, the antisera specificity is primarily directed towards the catharantine moiety of the molecule. Thus, the modifications reported to occur on the vindoline moiety (near C16–C17 and N_a) did not affect antibody-antigen binding [11, 12]. The large quantity of these biotransformation products suggests the presence of a hepatobiliary clearance of circulating metabolites.

As previously described by Owellen and Hartke [8] and Bender et al. [1], the initial phase probably represents tissue distribution and binding to the RBC. As reported for VLB [8] and VCR [1], 20 min after NVB injection, 50% of the drug was detected in red and withe blood cells. Moreover, tritium binding to the plasma protein decreased over time (20 min, 82%; 96 h, 42%), which suggests that metabolites are less strongly bound to the plasma proteins.

As has been shown for other vinca alkaloids [1, 8], the major route of excretion for NVB in humans is the stool. This can probably be explained by the high liver uptake and biliary excretion previously observed in animals [3]. The same excretion pattern has been described for VLB [8] and VCR [1] in patients. The latter evaluations, however, were done over a short period. In our study, urine and feces samples were monitored up to 21 days after a single i.v. treatment, after which time the recovery was not complete, reaching approximately 50% (patient 1) and 80% (patient 2) of the total dose. In animals, the same incomplete recovery has also been observed 7 days (rats) [3] or even 1 month (monkeys) after drug administration (PF Med, personal communication), possibly due to the large distribution volume previously described for NVB [15], suggesting a high binding to tissues.

The metabolic fate of NVB has not yet been determined, but previous studies carried out on animal models [3] have demonstrated a large biliary excretion of this drug as both unchanged compound and metabolites. Furthermore, preliminary studies undertaken on fresh human hepatocytes in suspension or culture have shown a significant hepatic metabolism of navelbine. In vitro studies are now under way to isolate NVB metabolites from liver cells or their subcellular fractions so as to characterize the molecular structure and test the potential activity and/or toxicity of these metabolites.

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