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Oral administration of [^{3}H]navelbine in patients: comparative pharmacokinetics using radioactive and radioimmunologic determination methods

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[^{3}H]Navelbine (NVB) was administered orally to two patients. Drug levels in biological fluids were monitored by radioimmunoassay (RIA) and direct radioactivity (RA) determinations. NVB absorption was rapid: maximum plasma concentrations appeared in the first 2 h after oral administrations. Pharmacokinetic parameters estimated from RIA data were in complete accordance with those obtained from iv injections. Bioavailability (iv/po) estimated from RIA and RA data averaged 40.6 and 93.0%, respectively. NVB urine excretion was low. Fecal excretion remained its main elimination route. Moreover, large differences were observed in area under NVB plasma concentration–time curve (AUC) values obtained by the two methods, implying intense drug biotransformations.

Key words: Bioavailability, navelbine, oral administration, pharmacokinetics.

Introduction

Vinca alkaloids are molecules which are closely related in their chemical structures. In this family, only vinblastine (VLB) and vincristine (VCR) are naturally occurring. These compounds have a high level of antimitotic activity and are widely used in cancer chemotherapy. In order to improve their antitumor index and reduce neurological and hematological toxicities, which are common side effects of vinca alkaloids in the clinic,[1] efforts have been directed at developing new compounds through structural modifications. In this context, vindesine (VDS) was synthesized from vinblastine, and shown to exhibit oncolytic activity in several human neoplasms.[2] Navelbine (NVB) is the latest analog in the antitumor vinca alkaloid family; it was semisynthesized by modification of the catharantine moiety of VLB,[3,4] and it is more liposoluble than vinblastine, vincristine or vindesine. Although this new compound is structurally similar to other vinca alkaloids, NVB differs markedly from its congeners in tolerance and toxicity. This was demonstrated by some phase I and phase II studies[5–7] in which NVB was given by iv injection to cancer patients (with mainly solid tumors), in weekly doses of up to 43 mg/m^2, i.e., more than 20-fold higher than those prescribed for VCR. Leukopenia was found to be the principal dose-limiting factor. No neurotoxicity was observed at doses under 30 mg/m^2. NVB is as active as VCR on L1210 leukemia cells,[8] and it exhibits antitumor activity against the VCR resistant cell line P388/VCR.[9]

No significant gastrointestinal irritation has been observed in animal toxicological studies. This prompted us, for the first time, to carry out a pharmacokinetic study of radiolabeled NVB given orally to cancer patients. Plasma concentrations, and urine and fecal excretion of navelbine were monitored by radioimmunoassay and direct count of radioactivity. The pharmacokinetic parameters describing the absorption, distribution and elimination processes estimated from the two determination methods were evaluated.

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Materials and methods

Protocol

Two patients (aged 64 and 67 years) were entered in this study after giving informed consent. Their eligibility was based on the following criteria: WBC count $>3000/mm^3$; platelet count $>100\,000/mm^3$; serum creatinine <130 μM. No clinical or biochemical abnormalities of hepatic or renal function were observed during treatment. NVB bitartrate (P.F. Médicament, Paris, France) and [^{3}H]NVB bitartrate (Commissariat à l'énergie Atomique, Saclay, France) were mixed to give a final specific activity of 1.58 μCi/mg NVB base. After a fasting time of 15 h, two capsules containing 46.5 mg (73.5 μCi) and 49.5 mg (78.2 μCi) of the above preparation were given orally to the patients (30 mg/m^2). Food was reintroduced 4 h after treatment. Venous blood samples were drawn into heparinized tubes 5 min before, then at 0.5, 1, 2, 4, 8, 12, 24, 48, 72 and 168 h after administration. The tubes were immediately centrifuged for 10 min at $1000 \times g$, and plasma was then separated. Urine samples were collected for 3 weeks at the following intervals: 0–4, 4–8, 8–12, 12–24, 24–36, 36–48 h, then by daily fractions. Stools were collected every day for the same period. All samples were stored at -20°C until analysis.

Radioimmunoassay (RIA)

Plasma and urine NVB concentrations were determined by the radioimmunoassay method developed by Rahmani *et al.*[10] Briefly, plasma and urine samples were diluted when necessary in phosphate-buffered (50 mM, pH 7.4) saline (0.15 M) containing 1 g/l bovine serum albumin (fraction V, Sigma, USA) 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, France). At the end of the incubation, polyethylene glycol 6000 (Merck, Germany) was added to reach a final concentration of 12.5% (w/v). Precipitated immune complexes were separated by centrifugation ($2000 \times \boldsymbol{g}$, 10 min) after a 5-min incubation at -20°C and counted for 1 min on a Kontron MR 252 gamma counter. Nonspecific inhibition in pretreatment 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%).

Radioactivity determination (RA)

Radioactivity in plasma and urine was directly determined in triplicate in a scintillation spectrometer (Beckman LS 7800). Fecal samples were collected in weighed containers and then lyophilized. The dry residue was weighed and homogenized in a Waring blender. Five 200–300 mg aliquot samples were compressed as pellets and precisely weighed, and radioactivity was measured in a Packard model 360 sample oxidizer. The radioactivity was converted into NVB concentrations as a function of the specific activity.

High performance liquid chromatography (HPLC)

Urine samples were analyzed on a Hewlett Packard 1084B liquid chromatograph equipped with a radioactive flow detector (Flo-one Beta, Radiomatic Instruments and Chemical Co., Inc.). The column used was a μBondapak phenyl (10 μ) prepacked reversed-phase column (Waters Associates). The mobile phase consisted of sodium perchlorate (45 mM), perchloric acid (6 mM) and methanol. Elution was performed using a linear gradient from 50 to 75% methanol in 35 min at a flow rate of 0.9 ml/min.

Data analysis

The bioavailability ($F_{po/iv}$) was estimated using iv data already obtained from the same patients.[11] The area under the NVB plasma concentration–time curve (AUC) was calculated according to the trapezoidal rule with all experimental data. Apparent elimination half-lives ($t_{1/2}$) were obtained by least-squares regression on terminal data points. The other pharmacokinetic parameters were either obtained directly from the plasma concentration–time curves (T_{max}, C_{max}) or estimated from standard pharmacokinetic equations (plasma clearance, Cl_p; urine clearance, Cl_r; apparent total distribution volume, V_t). Cumulative urine and fecal excretion were calculated by summing the amount of NVB

recovered in all collected fractions, and expressed as percentages of the total absorbed dose.

Results

Plasma kinetics

[^{3}H]NVB was administered orally to two patients (30 mg/m^2). For patient 2, plasma concentration–time curves obtained by RA and RIA determinations are shown in Figure 1. For both patients, plasma concentrations determined by RA were always higher than those measured by RIA. For data obtained by RIA determination, plasma drug concentration decay patterns were biphasic after a rapid absorption phase. By contrast, the kinetics resulting from RA measurements were apparently monophasic, and drug disappearance was very slow: 168 h after administration, 29.0 and 16.1% of peak values remained for patients 1 and 2, respectively. The half-lives ($t_{1/2}$) of the apparent terminal elimination phase estimated from RA data (88.0 ± 15.6 h) were much longer than those obtained from RIA data (55.1 ± 1.1 h). However, irrespective of the analytical method used, maximum concentrations (C_{max}) were rapidly reached: 30 min after drug administration NVB plasma levels represented about 80% of the peak values. T_{max} and C_{max} averaged 1.5 h and 58.5 mg/ml from RIA data and 1.5 h and 93.5 ng/ml from RA data, respectively. The AUC values determined by RA were much higher than those obtained by RIA. The AUC ratios (AUC_{RIA}/AUC_{RA}) were 0.09 and 0.19 for patient 1 and patient 2, respectively. The mean

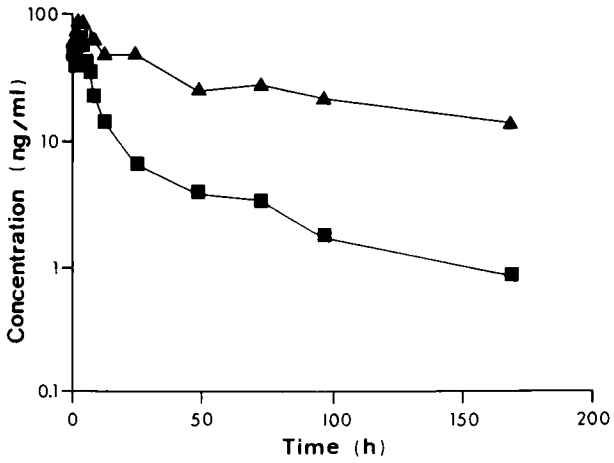

Figure 1. Plasma concentration–time curves of NVB in patient 2 after oral administration of 49.5 mg NVB. RIA (■) and R (▲).

plasma clearances (Cl_p) calculated from RIA and RA data were respectively 0.439 ± 0.005 and 0.137 ± 0.064 l/h/kg. The apparent total distribution volumes (V_t) were 34.6 ± 1.1 and 18.2 ± 11.1 l/kg for RIA and RA data, respectively. The bioavailabilities (F) were estimated taking into account the data already reported after iv injections of the same NVB doses to the same patients.[11] They were 40.6 ± 8.5% for RIA and 93.0 ± 14.6% for RA. Individual pharmacokinetic parameters are reported in Table 1. The parameters estimated from RIA data are in complete agreement with those obtained during previous phase I studies.[11–13]

The correlation analyses between RIA and RA measurements were performed using linear least-

Table 1. Pharmacokinetic parameters obtained with plasma, urine and fecal experimental data determined by RIA and RA

		Patient 1		Patient 2	
		RIA	RA	RIA	RA
Dose	(mg)	46.5		49.5	
T_{max}	(h)	1.0	1.0	2.0	2.0
C_{max}	(ng/ml)	58.9	100.0	58.2	87.0
AUC	(ng/ml × h)	685.2	7780.5	904.5	4757.0
$F_{po/iv}$	(%)	34.6	82.6	46.6	103.3
$t_{1/2}$	(h)	55.9	76.9	54.3	99.0
Cl_p	(l/h/kg)	0.439	0.092	0.431	0.182
Cl_r	(l/h/kg)	0.047	0.003	0.107	0.015
V_t	(l/kg)	35.4	10.3	33.8	26.0
Urinary excretion[a]		10.8	3.8	24.9	8.6
Fecal excretion[a]		—	70.0	—	70.4

[a] % of the total absorbed dose.
(—) not determined.

squares regression with all experimental data points plotted as RIA versus RA (12 pairs compared for patient 1 and 13 pairs for patient 2). For both patients, a significant correlation was demonstrated between the two methods, as shown by the slope of the regression lines (1.00) and the correlation coefficient ($r = 0.87$, $p < 0.01$). However, high y-axis intercepts were observed in both cases (49.4 ng/ml for patient 1 and 26.9 ng/ml for patient 2).

Urinary excretion

After oral administration of NVB, urinary drug excretion was low. Figure 2 shows the urinary excretion rate over time for patient 2. Regardless of the determination method used, similar results were obtained; within 3 weeks of collection, 17.9 ± 10.0% nd 14.1 ± 7.1% of the total absorbed dose was recovered in urine as determined by RIA data and RA data respectively. Quantitatively, 47.7% (mean value of the two methods) of the total amount of NVB eliminated in urine was excreted in the first 24 h and 62.0% within the first 48 h. Renal clearances (Cl_r) estimated from RIA and RA data were respectively 0.077 ± 0.042 and 0.009 ± 0.008 l/h/kg. Moreover, when analyzed by HPLC, excreted drug was found mainly as unchanged NVB (Figure 2, insert). A better correlation between RIA and RA determinations was observed from urine data than from plasma data. These results were rationalized in the same way (19 and 24 paired points for patients 1 and 2 respectively): the mean

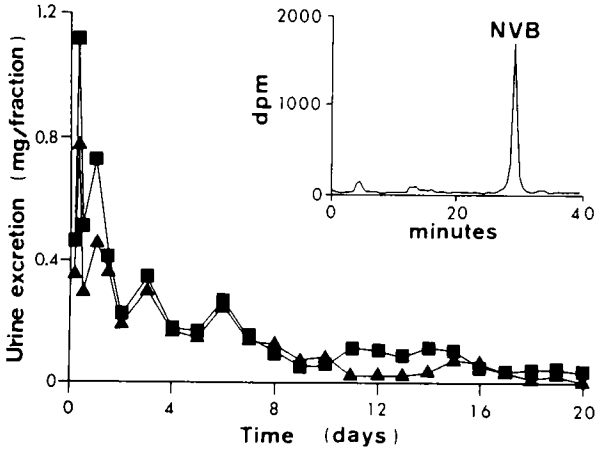

Figure 2. Urinary excretion of NVB in patient 2 after oral administration of 49.5 mg NVB. RIA (■) and RA (▲). Insert: HPLC analysis of urine samples (4–8 h fraction).

slope of regression lines was 1.1, with average correlation coefficients of 0.95 ($p < 0.01$). In contrast with plasma data, y-axis intercepts were nearly null (0.012 ± 0.005 ng/ml).

Fecal excretion

Fecal samples were collected every day for 3 weeks and monitored for NVB content by RA determination. During this period, recovered radioactivity represented 70.2 ± 0.3% of the total absorbed dose. This was much higher than the urinary excretion. The highest fecal excretions of NVB and/or its metabolites occurred at the third day for patient 1 (24% of the total absorbed dose) and at the seventh day for patient 2 (34% of the total absorbed dose). The level of tritiated water in stools was negligible, representing only 0.08% of the recovered radioactivity as analyzed after lyophilization.

Discussion

To date, the pharmacokinetic data published on vinca alkaloids have been based on iv bolus injection or iv perfusion of these drugs. No published study has dealt with the pharmacokinetics of these anticancer agents after oral administration. Although oral administration is practical, and useful in ambulatory treatment, this route of administration has remained rare for vinca alkaloids until the appearance of NVB, the most liposoluble derivative of these agents. NVB shows some original and overlapping antitumor activities as compared with its congeners. Its toxicity is relatively low, and the maximum tolerated dose is very high. These properties prompted us to undertake a pilot study with tritiated NVB given orally to two patients. Two determination methods (RIA and RA) were compared.

NVB was shown to be rapidly absorbed in these patients. Whatever the determination method used, maximum plasma concentrations were reached within the first 2 h after drug administration; rapid NVB absorption may be explained by its highly liposoluble character. The AUC obtained by RA and RIA determinations were significantly different: AUC_{RIA}/AUC_{RA} ratios ranged from 0.09 to 0.19, implying a marked formation of metabolites. Indeed, the RA method measured total radioactivity and therefore could not distinguish the unchanged drug from metabolites and/or degraded com-

pounds; this method is therefore less specific. By contrast, RIA determination is more specific; the anti-NVB antiserum used in this study was primarily directed towards the catharantine moiety.[10] The NVB-related metabolites whose existence is seen by the discrepancy between RA and RIA values may therefore not cross-react with the antibody used and they might exhibit certain modifications on the catharantine heterocycle of the molecule. However, when analyzed by linear regression, plasma concentrations from RA and RIA measurements were significantly correlated, with a mean slope of regression lines near to 1 and high y-axis intercepts. The latter represents the mean differences between the two determination methods.

The two patients were also treated with the same doses by iv bolus injections, thus allowing estimation of the bioavailability. Large discrepancies were shown between the bioavailability calculated with RA data (93.0%) and RIA data (40.6%). The relatively low F value of the latter might be due to an intense hepatic or intestinal first-pass effect.

As has been demonstrated in rat, mouse and monkey (P.F. Médicament, personal communication), urine excretion of NVB in man was low (<20% within 21 days), being in the same range as that previously reported for other vinca alkaloids. HPLC analysis of urine samples demonstrated that NVB was eliminated mainly in an unchanged form. These results are in agreement with those described for VLB.[14] In contrast, a significant amount of VCR, which is also poorly excreted in urine, has been reported to be eliminated as metabolites (46% of total excreted VCR).[15] The absence of NVB metabolites in urine could explain the good correlation between urine RIA and RA data. As has been shown for VCR and VLB, the major elimination route for NVB is the stool (about 70% within 3 weeks). This can probably be explained by the high hepatic uptake and biliary excretion of these drugs, previously observed in animals.[16,17]

The metabolic pathways of NVB remain unknown, but some *in vitro* studies in animal models have demonstrated that high levels of NVB are excreted in bile as both unchanged drug and metabolites.[18] Significant biotransformation was also observed when NVB was incubated with freshly isolated or cultured human hepatocytes.[19] Efforts are being made in our laboratory to prepare these metabolites in quantities permitting their structural identification and pharmacologic and/or toxicologic evaluation.

Conclusion

These results demonstrate that oral administered NVB exhibits a pharmacokinetic behavior similar to that found for iv injections. The latter has been characterized by: high Cl_p (0.27–1.49 l/h/kg); large V_t (8.2–48.2 l/kg); and long $t_{1/2}$ (22.1–67.8 h).[11–13] Further investigations should be carried out to define the spectrum of antitumor activity, and the side effects, and to ensure a reproducible bioavailability of oral NVB. This will probably lead to a novel administration route alternative to iv injections in chemotherapy with the antitumor vinca alkaloids.

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