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Pharmacokinetics of navelbine after oral administration in the dog and the monkey

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Navelbine (NVB) pharmacokinetics has been investigated in the dog after p.o. administration of increasing doses (0.5–8 mg/kg) and in the monkey after a single dose of 40 mg/ml. In the dog, NVB pharmacokinetic parameters, C_{max}, t_{max}, AUC, $t_{1/2}$ and Cl, were 49.0–1021.0 ng/ml, 1.14–3.69 h, 370.0–11754.5 ng/ml h, 19.3–64.3 h and 0.46–1.47 l/h/kg, respectively. As the dose increased, the plasma concentration peak appeared more slowly and $t_{1/2}$ increased significantly with a marked reduction in Cl. Moreover, NVB pharmacokinetics in the dog exhibited significant dose-dependency as demonstrated by analysis of variance (ANOVA) of dose-normalized AUC and C_{max}. Pharmacokinetic parameters estimated from monkey data were essentially the same as those of the dog (C_{max}, 877.6 mg/ml; t_{max}, 1.14 h; AUC, 7004,8 ng/ml h; $t_{1/2}$, 18.2 h) except for Cl (10.40 l/h/kg), which was about 10-fold that of the dog.

Key words: Dose-dependency, navelbine, oral administration, pharmacokinetics.

Introduction

Navelbine (NVB, 5'-noranhydrovinblastine) is a new semisynthesized antitumor vinca alkaloid currently under clinical trials.[1,2] This compound is structurally related to vinblastine (VLB) and its congeners (vincristine, VCR; vindesine, VDS), and has similar antitumor mechanism to other vinca alkaloids, i.e. a high affinity for tubulin and a potent inhibition of tubulin polymerization.[3–5] NVB has shown interesting antitumor activity in various experimental tumor models. In mice, it was as active as VCR against L1210 leukemia cells, whereas VLB had no marked effect.[6] On P388 leukemia cells, NVB exhibited a significant effect with a therapeutic index of 19 compared with 8 for VCR and VLB. Moreover, NVB showed a high activity on VCR resistant P388/VCR leukemia subline. Low cross-resistance was found between NVB and other vinca alkaloids.[7,8] For clinical antitumor activity, NVB was highly active as single agent against at least three cancer types: non-small cell lung cancer, breast cancer and Hodgkin's disease.[9,10] Phase I clinical trials demonstrated that leukepenia was the main dose-limiting toxicity and its neurotoxicity was mild even at high doses of up to 30 mg/m^2. NVB was much less toxic than other vinca alkaloids. Its maximum tolerated dose is about 40 mg/m^2/week.[11,12] NVB pharmacokinetics have been studied in cancer patients after i.v. bolus injection and p.o. administration of tritiated and non-labeled drug, and is characterized by a large apparent volume of distribution (25.0–75.6 l/kg), a long terminal phase half-life (31.2–79.8 h), and a high systemic clearance (0.42–1.26 l/h/kg).[13–18] Moreover, NVB exhibited time- and dose-dependent pharmacokinetics in humans.[15]

The aim of the present paper was to characterize NVB pharmacokinetics after p.o. administration in two animal species, the dog and the monkey. Parameters describing NVB kinetic behavior were estimated and analyzed by using various statistical tests to address problems such as the dose-dependency of NVB pharmacokinetics.

Materials and methods

Protocol

In all, 24 beagle dogs (12 male and 12 female) and four rhesus monkeys (two male and two female)

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were used in this study. NVB (bitartrate salt; PF Médicament, Paris, France) was given in capsules to overnight fasted animals. Food was reintroduced 4 h after administration. Each dog received a single oral NVB dose. Five doses [0.5 ($n = 4$), 1 ($n = 4$), 2 ($n = 6$), 4 ($n = 6$) and 8 ($n = 4$) mg/kg] were tested. Each monkey was orally treated with a single dose. Only one dose (40 mg/kg) was studied in the monkey.

Venous blood samples were drawn into heparinized glass tubes 5 min before, and then at 1, 2, 4, 8, 12, 24, 36 and 48 h after administration and immediately cooled at 4°C. The tubes were centrifuged for 5 min at 1000 *g* and the resulting plasma was stored frozen at −20°C until analysis.

Drug analysis

Plasma NVB concentrations were determined by the radioimmunoassay method described by Rahmani *et al.*[13] Briefly, plasma samples were diluted if 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 from healthy donors (Centre de Transfusion Sanguine, Marseille, France) was added when necessary in order to maintain a constant amount of human plasma components in the incubation medium and to ensure reproducible precipitation of immune complexes. At the end of incubation, polyetylene glycol 6000 (Merck, Germany) was added to reach a final concentration of 12.5% (v/w). Precipitated immune complexes were separated by centrifugation (2000 *g*, 10 min) after a 5 min incubation at −20°C and counted for 1 min on a Kontron MR 252 gamma counter. Non-specific inhibition in pretreated 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%).

Data analysis

The area under the plasma concentration–time curves (AUC) after p.o. administration of NVB in dogs and monkeys were calculated with all experimental data according to the trapezoidal rule. Apparent elimination half-lives ($t_{1/2}$) were obtained by least-squares regression on terminal data points. Maximum plasma concentrations (C_{max}) and times to C_{max} (t_{max}) were obtained directly from the plasma concentration-time curves. The systemic clearances (Cl) were estimated according to the relationship: $Cl = dose_{p.o.}/AUC$, on the assumption that the drug was totally absorbed. The dose-dependency (linearity) of NVB pharmacokinetics in the dog after p.o. administration of increasing doses was evaluated by using statistical tests: AUC and C_{max}, which were dose-dependent parameters, were dose-normalized to 2 mg/kg and compared by ANOVA and Student's *t*-test. Cl and $t_{1/2}$, which were dose-independent parameters in the linear model, were analyzed by the same tests. Since t_{max} was not normally distributed, it was examined by Kruskal–Wallis distribution free test. The statistical significance level was set at $p = 0.05$.

Results

In the dog, whatever the administered dose was, the NVB plasma level showed a biphasic decay pattern after a rapid absorption phase (Figure 1). t_{max} ranged from 1.14 to 3.69 h (harmonic mean). Kruskal–Wallis non-parametric test demonstrated significant differences between the values of t_{max} obtained after different doses. Indeed, t_{max} increased significantly with oral dose as demonstrated by correlation analysis ($r = 0.9888$, $p < 0.001$). C_{max} and AUC ranged between 49.0–1021.0 ng/ml and 370.0–11754.5 ng/ml h, respectively. These parameters were dose-normalized to 2 mg/ml and analyzed by ANOVA. Results showed significant differences between the parameters obtained with

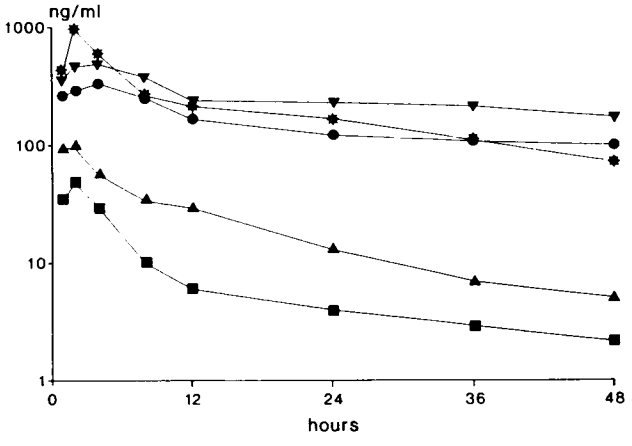

Figure 1. Mean plasma concentration–time curves obtained after oral administration of NVB in the dog at doses of 0.5 (■), 1 (▲), 2 (★), 4 (●) and 8 (▼) mg/kg.

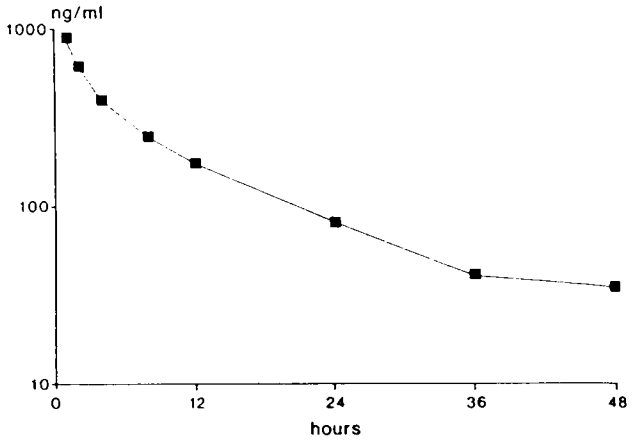

Figure 2. Mean plasma concentration–time curve obtained after oral administration of NVB in the monkey at a single dose of 40 mg/kg (■).

different doses ($p < 0.05$), i.e. there was no direct proportionality between the parameters and administered doses. Cl and $t_{1/2}$ were 0.46–1.47 l/h/kg and 19.3–64.3 h, respectively. Results of ANOVA indicated significant differences between the values of $t_{1/2}$ ($p < 0.05$). For Cl, similar results were obtained ($p < 0.05$). Further analysis performed on Cl and $t_{1/2}$ by using Student's *t*-test demonstrated that the values of the parameters obtained with doses of 2, 4 and 8 mg/kg (averaged 0.63 l/h/kg and 48.9 h) were significantly lower and higher, respectively, than those estimated with doses of 0.5 and 1 mg/kg (averaged 1.45 l/h/kg and 22.5 h) ($p < 0.01$). These results indicate non-linear NVB elimination at higher doses. In the monkey, the mean NVB plasma concentration decay curve was apparently triphasic (Figure 2). A plasma concentration peak was not observed on the curve; hence, the first point of the curve represented C_{max} (877.6 ng/ml) and T_{max} (1.14 h). Other pharmacokinetic parameters including AUC, $t_{1/2}$ and Cl averaged 7004.8 ng/ml h, 18.2 h and 10.40 l/h/kg, respectively. Table 1 summarizes the mean ± SD pharmacokinetic parameters of NVB after p.o. administration in the dog and the monkey.

Discussion

Modifications on the structure of the catharantine moiety of antitumor vinca alkaloids have led to the hemisynthesis of NVB, the most liposoluble analog among the vinca series. The increased lipophilicity produced some characteristic pharmacokinetic properties that could be at the origin of its low hemato- and neurotoxicity. Moreover, unlike other vinca alkaloids which are administered i.v., NVB was expected to be orally active because of its high lipophilicity, large tubulin binding affinity and high tolerated dose. Thus, in a first step, tritiated NVB was administered p.o. and i.v. to two patients; the results appeared encouraging, as the bioavailability was about 40% and as pharmacokinetic parameters following p.o. administration were similar to those with i.v. injection.[16,17]

In the present study, we evaluated NVB pharmacokinetics after p.o. administration in the dog and the monkey. We found that in the dog, the NVB plasma level exhibited biophasic decay pattern, as was the case in man.[17] However, as the dose increased, NVB absorption was delayed, as demonstrated by a significant increase in t_{max}. NVB pharmacokinetic parameters from the dog were in the same range as those from man. Table 2 summarizes the so far published data on NVB pharmacokinetics. Moreover, as has been shown in patients after i.v. injection,[15] NVB pharmacokinetics after p.o. administration was characterized by a significant dose-dependency as revealed by the comparison of dose-normalized AUC and C_{max}. The non-linearity of NVB pharmacokinetics was further confirmed by a significant increase in $t_{1/2}$ and a significant reduction in Cl as the dose increased. Such a decrease in Cl has also been reported for VDS[19,22] VLB,[23–25] VCR[26,27] and NVB[14] after i.v. bolus injection and long-term infusion, indicating non-linear drug elimination. There has been no complete elucidation of these phenomena. However, since the antitumor vinca alkaloids are largely metabolized and eliminated via the hepatobiliary

Table 1. Mean ± SD pharmacokinetic parameters of NVB after oral administration in the dog and the monkey

Species	Dose (mg/kg)	No.	AUC (ng/ml h)	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}$ (h)	Cl (l/h/kg)
Dog	0.5	4	370.0 ± 119.8	49.0 ± 18.1	1.14	26.5 ± 9.5	1.47 ± 0.51
	1	4	1023.5 ± 655.0	121.4 ± 72.0	1.33	19.3 ± 3.1	1.44 ± 1.10
	2	6	9537.5 ± 7804.0	1021.0 ± 1003.9	2.00	31.8 ± 16.1	0.46 ± 0.44
	4	6	7272.5 ± 3123.4	356.5 ± 156.0	2.56	64.3 ± 40.6	0.69 ± 0.44
	8	4	11754.5 ± 5210.7	525.8 ± 250.5	3.69	50.3 ± 18.9	0.81 ± 0.39
Monkey	40	4	7004.8 ± 5880.6	877.6 ± 833.9	1.14	18.2 ± 12.1	10.40 ± 7.92

Table 2. Comparison of the data published up to date on NVB pharmacokinetics after i.v. and p.o. administration in patients and animal species

References	Species	Dose (mg/m^2)	AUC (ng/ml × h)	$t_{1/2}$ (h)	Cl (1/h/kg)	V_t (1/kg)
Rahmani *et al.*[13]	rat	1.2[a] (i.v.)	—	8.9	1.9	—
Rahmani *et al.*[14]	human	15–30 (i.v.)	780[b]	31.2	0.92	51.4
Rahmani *et al.*[15]	human	15–30 (i.v.)	—	37.3–40.2	0.66–0.83	25.0–28.6
Boré *et al.*[16]	human	30 (i.v.)	1783[c]	79.8	0.42	—
Jehl *et al.*[18]	human	30 (i.v.)	701[d]	42.1	1.26	75.6
Rahmani *et al.*[17]	human	30 (p.o.)	794[e]	55.1	0.44	34.6

[a] mg/kg.
[b] AUC_{0-72h}.
[c] AUC_{0-240h}.
[d] AUC_{0-144h}.
[e] AUC_{0-168h}.

system,[28,29] the apparent decrease in their elimination may be explained by an inhibition, due to prolonged treatment and/or high dose, of the hepatic enzyme system responsible for their biotransformation and elimination. The enzyme system may include cytochromes P450 that have recently been demonstrated to be involved in the hepatic biotransformation of VDS and VLB in man.[30] We also investigated the pharmacokinetic behavior of NVB in the monkey treated orally with a very high dose of 40 mg/kg. The latter is about 20-fold the dose prescribed for patients. The high dose may inhibit the gastrointestinal absorption of the drug and result in a low bioavailability, leading to the overestimation of Cl. Moreover, plasma level decay followed triphasic kinetics, and the concentration peak was not observed because of the lack of blood sample during absorption phase. Other pharmacokinetic parameters were comparable with those of the dog and human (Tables 1 and 2).

Conclusion

The results demonstrate that NVB orally administered in the dog exhibits plasma kinetic characteristics similar to those found in patients. Therefore, the dog may be considered an appropriate model for toxicological and pharmacological study of the drug. However, so as to ensure a reliable extrapolation of animal data to man, NVB metabolic pathway and its interspecies variability should be established. These investigations will allow a better understanding of the relationship between the characteristic pharmacokinetics (time- and/or dose-dependency) of the drug and its biotransformation.

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