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# Comparative pharmacokinetics of antitumor Vinca alkaloids: intravenous bolus injections of navelbine and related alkaloids to cancer patients and rats

**Roger Rahmani[1], Francoise Guéritte[2], Marie Martin[1], Sylvaine Just[1], Jean-Paul Cano[1], and Jacques Barbet[3]**

[1] Groupe de Recherche en Immunologie et Pharmacologie des Anti-Cancéreux, INSERM U278, Institut Paoli Calmettes, 232 Bd de Sainte Marguerite, F-13273 Marseille Cedex 9, France
[2] Institut de Chimie des Substances Naturelles du CNRS, F-91190 Gif-sur-Yvette, France.
[3] Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, F-13288 Marseille Cedex 9, France

**Summary.** The kinetics of distribution and elimination in rats of the antitumor drug navelbine and of two of its analogues, Na-formyl navelbine and deacetyl navelbine amide, have been studied by radioimmunoassay and compared with the kinetics obtained with vinblastine and vincristine. Fitting to two-exponential curves was used to derive pharmacokinetic parameters. Clearance was found to parallel toxicity for all drugs: it increases from 0.19 l $h^{-1}$ $kg^{-1}$ for vincristine to 0.41 for Na-formyl navelbine, 1.4 for vinblastine, 2.3 for navelbine, and 2.6 for deacetyl navelbine amide. Terminal half-lives were longer for the Na-formyl-substituted alkaloids (around 13 h) than for the others (8–10 h). We have also studied navelbine kinetics in cancer patients entered in recent navelbine clinical trials and found that navelbine pharmacokinetics are characterized by fast and extensive distribution, high clearance ($0.92 \pm 0.27$ l $h^{-1}$ $kg^{-1}$), and a relatively long terminal half-life ($31.2 \pm 4.4$ h). Relationships between chemical structure, pharmacokinetic properties, and toxicity or therapeutic efficiency within the Vinca alkaloid series are discussed, together with the relevance of animal models such as the rat in the screening of new antitumor drugs.

## Introduction

The design of pharmacologically active molecules, especially of new antitumor drugs, often relies upon hypotheses about their interaction with putative or established intracellular target molecules. Antitumor Vinca alkaloids are tested in the first place for their ability to prevent tubulin polymerization [14]. However, the best candidates selected by this preliminary screening sometimes display very disappointing in vivo activity. In such cases stability and metabolism of the molecule and its rate of elimination should be investigated. For instance, although vincristine, vindesine, and vinblastine are very similar in their binding affinity to tubulin [14], they differ considerably in toxicity and therapeutic dose. Nelson et al [7] observed a correlation between clinical weekly doses and systemic clearances for these drugs, which might explain the differences.

Navelbine is a new antitumor drug [3, 4] closely related to vinblastine, and is currently under clinical investigation [11, 12]. The maximal tolerated doses are unexpectedly high, and it seemed interesting to find out whether there was a correlation with pharmacokinetic properties and whether navelbine and its derivatives (Na-formyl navelbine, deacetyl navelbine amide [2]) could be considered members of the Vinca alkaloid series.

In this paper we report comparative pharmacokinetic studies of navelbine and congeners, vinblastine, and vincristine in rats. We also present a preliminary pharmacokinetic study of navelbine in five cancer patients entered in clinical trials of navelbine. The drugs were injected as single IV doses, and plasma concentrations were monitored by radioimmunoassay as reported earlier [9, 10, 13]. Results are discussed in terms of possible relationships between structures and in vivo behavior (kinetics, toxicity, antitumor activity) and of the predictive value of pharmacokinetic experiments in rats.

## Materials and methods

*Radioimmunoassays.* The radioimmunoassays used to measure plasma concentrations of the various Vinca alkaloids have been described previously. Vinblastine, vincristine and vindesine were measured as described by Rahmani et al. [9] using either a rabbit antiserum kindly provided by the Eli Lilly Research Laboratories [13] or a rat monoclonal antibody against vinblastine recently developed by ourselves [8]. The monoclonal antibody (Vinca 4) gave higher sensitivity but otherwise essentially the same results as the antiserum. A detailed comparison of these assays will be published elsewhere. Navelbine, deacetyl-navelbine amide, and Na-formyl navelbine were measured using a specific rabbit anti-navelbine antiserum and $^{125}$I-navelbine-glycyl-L-iodo-tyrosine as previously described [10].

*Pharmacokinetic studies in rats.* Rats (male Sprague-Dawley received injections in the lateral tail vein of nontoxic doses of the various alkaloids (Table 1). The injection duration was always less than 15 s. Blood samples (around 300 µl) were then collected from the tail in heparinized tubes by cutting the tail tip. After centrifugation, plasma samples were stored frozen until analyzed.

*Navelbine kinetics in cancer patients.* Blood samples from five patients given navelbine as single IV doses were collected at selected time intervals and centrifuged. The injection duration was less than 2 min. Plasma samples were

*Offprint requests to:* J. Barbet

**Table 1.** Two-exponential fits of Vinca alkaloid kinetics in rats

| Drug | Dose (mg/kg) | First exponential | | Second exponential | |
|---|---|---|---|---|---|
| | | Amplitude (μg/l) | Half-life (h) | Amplitude (μg/l) | Half-life (h) |
| Vinblastine | 0.6 | 87 ± 52[a] | 0.51 ± 0.15 | 47 ± 13 | 7.5 ± 2.8 |
| ($n$ = 7)[b] | | 66 ± 4 | 0.51 ± 0.06 | 46 ± 2 | 5.7 ± 0.1 |
| Vincristine | 0.1 | 714 ± 383 | 0.78 ± 0.21 | 12 ± 10 | 14.3 ± 6.3 |
| ($n$ = 3) | | 454 ± 23 | 0.68 ± 0.02 | 4.2 ± 0.5 | 12.3 ± 0.6 |
| Navelbine | 1.2 | 137 ± 31 | 0.57 ± 0.21 | 36 ± 9 | 9.3 ± 1.7 |
| ($n$ = 7) | | 116 ± 7 | 0.45 ± 0.04 | 36 ± 1 | 8.5 ± 0.1 |
| Na-formyl navelbin | 0.3 | 838 ± 64 | 0.58 ± 0.09 | 12 ± 5 | 11.8 ± 5.3 |
| ($n$ = 2) | | 588 ± 40 | 0.67 ± 0.03 | 8.2 ± 0.9 | 14.1 ± 1.0 |
| Deacetyl navelbine amide | 0.85 | 142 ± 28 | 0.55 ± 0.19 | 20 ± 7 | 10.0 ± 2.2 |
| ($n$ = 3) | | 124 ± 8 | 0.55 ± 0.04 | 17 ± 1 | 9.0 ± 0.4 |

[a] For pairs of pre-exponential terms (amplitude) and half-lives, the first line gives the mean and standard deviation over all individual estimations and the second the estimation of each parameter and of its standard deviation when all experimental data points are fitted simultaneously to a two-exponential curve

[b] $n$ is the number of animals

stored at −20 °C until analyzed. These patients were part either of the phase I clinical trial conducted at the Hôpital Paul Brousse, Villejuif, France (plasma samples obtained by courtesy of Dr P. Ribaud and Prof. G. Mathé [11, 12]) or of the phase II clinical trial recently initiated at the Institut Paoli Calmettes, Marseille, France (plasma samples obtained by courtesy of Dr R. Favre and Prof. Y. Carcassonne).

*Mathematical analysis of plasma kinetics.* For rats, the plasma concentration-time curves from individual kinetics were fitted to two-exponential curves using the modeling program CONSAM [1] run on a VAX 11/780 (Digital Equipment Corporation) at the Laboratory of Mathematical Biology (NCI-NIH, Bethesda, Md). The macroconstants were then expressed as pre-exponential terms (μg/l) and half-lives (h). Systemic clearances were calculated from area under the simulated plasma concentration-time curves from zero to infinite time. Two exponential curves were also fitted to all experimental data available for each drug and pharmacokinetic parameters were derived as above using the same program. For humans, because of the non-negligible injection duration, simple multi-exponential fit would give wrong estimations of volumes and clearances. Calculations were thus performed using CONSAM assuming a three-compartment mamorillary model taking account of injection durations. Clearances were then evaluated as the product of the central compartment volume times the elimination rate constant. Terminal half-lives were estimated through direct semilogarithmic regression on data collected at times longer than 12 h.

## Results

In rats, the plasma concentration-time kinetics were best fitted by two exponential curves (Fig. 1). The difference with the kinetics generally observed in human, where three exponentials are necessary for reasonable fits [7], is most probably explained by the faster kinetics, which means that the first distributive phase is not represented in our sampling schedule. Apart from obvious practical problems, the total amount of blood withdrawn from the animals is limiting: with eight to ten time points the blood volume collected over 2 days represents about 10% of the total blood volume. The maximum error on the clearance introduced by this sampling problem could be estimated by assuming the initial distribution volume equal to plasma volume and single exponential decay to the first recorded data point: this error was found to be around 10%.

The rats were inbred animals of the same age and the same weight (213 ± 34 g). They were given a dose calculated from body weight (Table 1). Inter-individual variability was thus kept to a minimum and observed differences reflected essentially experimental errors. All kinetics for a given drug could be represented reasonably well by a single curve, which was fitted simultaneously to all experimental data points (Fig. 1). The curves simulated using the means of the individual parameters also represented a reasonable fit for the data points taken together. In Fig. 1, F shows the curves simulated for a theoretical dose of 1 mg/kg of each drug. Vincristine would then give the highest concentrations, followed by Na-formyl navelbine, vinblastine, and then navelbine and deacetyl navelbine amide, reflecting the increase in systemic clearance. A simple non-parametric analysis using Wilcoxon's test showed that vincristine and Na-formyl navelbine have smaller clearances than vinblastine ($P < 0.01$ and 0.05) and that vinblastine has a smaller clearance than either navelbine ($P < 0.01$) or deacetyl navelbine amide ($P < 0.05$). Navelbine and deacetyl navelbine amide have almost identical clearances. The difference between vincristine and Na-formyl navelbine was not significant. The sequence navelbine, deacetyl navelbine amide, vinblastine, Na-formyl navelbine, vincristine for clearance presents a striking parallel with the sequence for toxicity (Table 2).

Navelbine plasma concentration kinetics in one of the patients are shown in Fig. 2. A total of ten kinetics were analyzed (Table 3). Graphically, all resembled those of vinblastine, with a dramatic decline of the concentration in the first hours followed by a much slower phase. Concentrations measured at equal times, however, were far from being proportionate to the doses, which were almost

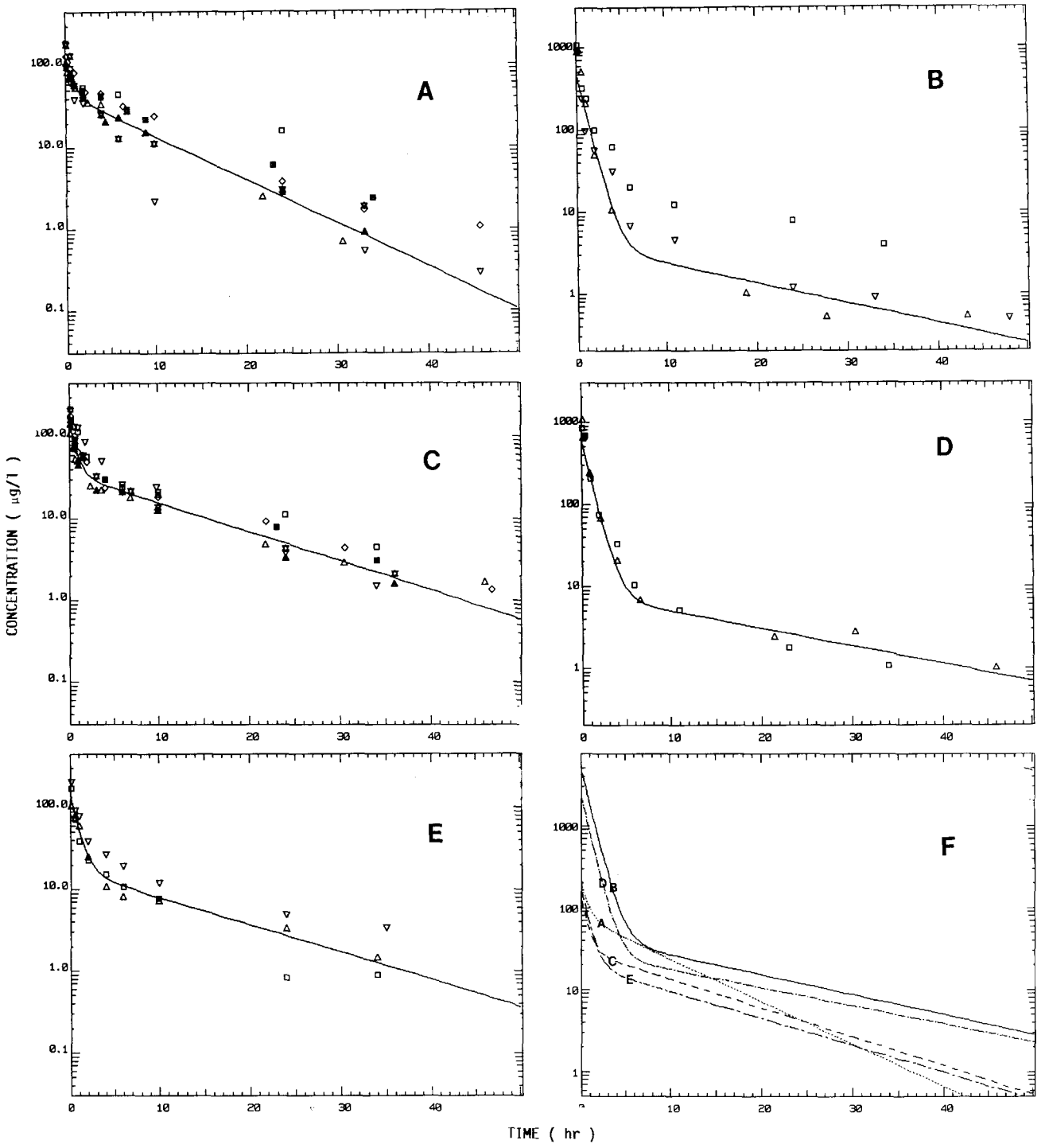


**Fig. 1 A–F.** Kinetics of distribution and elimination of the various alkaloids. Blood was collected at the time intervals shown in the figure, rapidly centrifuged and plasma was stored frozen until analyzed. Data obtained in individual animals are reported in the figure using different symbols. Two-exponential curves were fitted simultaneously to all data points. **A** Vinblastine, 0.6 mg/kg; **B** vincristine, 0.1 mg/kg; **C** navelbine, 1.2 mg/kg; **D** Na-formyl navelbine, 0.3 mg/kg; **E** deacetyl navelbine amide, 0.85 mg/kg; **F** simulations of concentration-time curves for a dose of 1 mg/kg: *curve A*, vinblastine; *curve B*, vincristine; *curve C*, navelbine; *curve D*, Na-formyl navelbine; *curve E*, deacetyl navelbine amide

ten times higher than for a bolus injection of vinblastine. The mathematical analysis of human data was complicated by the fact that the injection duration was not always negligible compared with the distribution rates. A three-compartment mamillary model was thus used to fit the experimental curves, which definitely showed three exponential phases, as already reported for vinblastine, vincristine, and vindesine [7]. This allowed us to take account of the injection duration and to obtain good estimations of systemic clearances. A more detailed analysis of navelbine pharmacokinetics in the human, addressing the questions of the linearity and time dependence of the kinetics, will be reported elsewhere. Systemic clearances were found to be very high, as were the distribution volumes (Table 3).

**Table 2.** Comparison between clearance and toxicity

| Drug | Clearance ($l\ h^{-1}\ kg^{-1}$) Rat | | $LD_{50}$ (mg/kg) Rat | $LD_{50}$ (mg/kg) Mouse | $LD_{10}$ (mg/kg) Mouse |
|---|---|---|---|---|---|
| Vinblastine | 1.2 ± 0.4[a] | 1.4[b] | 2.9 ± 1.5[c] | 10.8 ± 0.8[c] | 10.0[d] |
| Vincristine | 0.13 ± 0.07 | 0.19 | 1.0 ± 0.1[c] | 2.1 ± 0.1[c] | 1.4[d] |
| Navelbine | 2.1 ± 0.5 | 2.3 | N.D.[e] | 24.0[d] | 20.0[d] |
| Na-formyl navelbine | 0.27 ± 0.14 | 0.41 | N.D. | 6.0[d] | N.D. |
| Deacetyl navelbine amide | 2.4 ± 0.7 | 2.6 | N.D. | 17.0[d] | N.D. |

[a] Mean and standard deviation over all individual estimations
[b] Evaluation from simultaneous fit
[c] Todd et al. [15]
[d] Maral et al. [6]
[e] Not determined

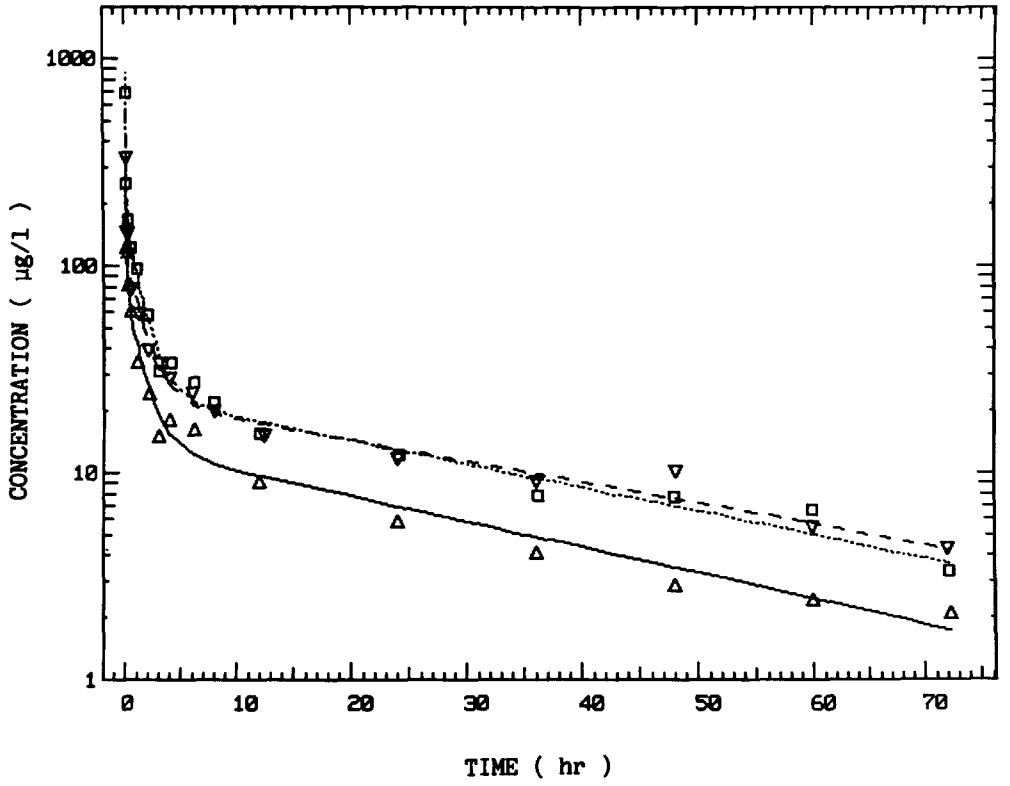


**Fig. 2.** Distribution and elimination kinetics of navelbine in patient 1, who received three injections, one of 26.3 mg (15 $mg/m^2$) (△) and two of 52.6 mg (30 $mg/m^2$) (□ and ▽). The interval between two curves was 2 weeks or longer by which time navelbine concentrations in plasma had fallen below the detection limits of the assay (0.1 µg/l). *Solid lines* represents the respective best fits used to calculate clearances.

**Table 3.** Pharmacokinetic parameters for navelbine in human

| Patient | Weight (kg) | Cure | Dose (mg) | Clearance ($l\ h^{-1}\ kg^{-1}$) | Terminal half-life (h) | Distribution volume (l/kg) |
|---|---|---|---|---|---|---|
| 1 | 66 | 1 | 26.3 | 0.66 ± 0.14 | 27.9 ± 1.7 | 37.2 ± 2.8 |
| 1 | 66 | 2 | 52.6 | 0.64 ± 0.04 | 33.5 ± 2.4 | 39.9 ± 2.8 |
| 1 | 66 | 3 | 52.6 | 0.65 ± 0.02 | 29.2 ± 2.2 | 38.1 ± 2.9 |
| 2 | 41 | 1 | 21.1 | 0.84 ± 0.03 | 34.4 ± 3.3 | 59.8 ± 5.7 |
| 2 | 41 | 2 | 42.1 | 0.93 ± 0.05 | 30.0 ± 2.2 | 60.4 ± 3.6 |
| 3 | 50 | 1 | 26.3 | 1.25 ± 0.05 | 37.8 ± 3.1 | 101.9 ± 7.1 |
| 3 | 50 | 2 | 52.6 | 1.47 ± 0.04 | 25.1 ± 2.2 | 77.5 ± 8.4 |
| 4 | 66 | 1 | 55.0 | 0.91 ± 0.10 | 39.5 ± 2.8 | 71.8 ± 3.7 |
| 4 | 66 | 2 | 55.0 | 0.92 ± 0.08 | 30.1 ± 4.0 | 52.5 ± 6.3 |
| 5 | 56 | | 70.0 | 0.93 ± 0.05 | 34.8 ± 2.4 | 59.2 ± 2.8 |

**Table 4.** Comparison of pharmacokinetic parameters and weekly clinical doses in human

| Drug | Clearance ($l\ h^{-1}\ kg^{-1}$) | Half-life (h) | Distribution volume (l/kg) | Weekly clinical dose ($mg/m^2$) |
|---|---|---|---|---|
| Vinblastine[a] | 0.74 ± 0.32 | 24.8 ± 7.5 | 27.3 ± 14.9 | 8.0 |
| Vincristine[a] | 0.11 ± 0.06 | 85.0 ± 68.9 | 8.4 ± 3.2 | 1.4 |
| Vindesine[a] | 0.25 ± 0.10 | 24.2 ± 10.4 | 8.8 ± 4.3 | 3.5 |
| Navelbine | 0.92 ± 0.24 | 31.2 ± 4.4 | 51.4 ± 16.0 | 30.0–43.0[b] |

[a] Nelson et al. [7]
[b] Ribaud et al. [11, 12]

This reflected the very significant plasma concentration decay during the first two phases. By contrast, the terminal half-lives were not shorter than those of vinblastine (Table 3).

## Discussion

Nelson et al [7] compared vinblastine, vindesine, and vincristine with respect to weekly clinical doses and clearances in human and found them in direct proportion. This implies that differences in binding affinity for the target protein tubulin are largely overriden by differences in pharmacokinetics. Thus the analysis of the pharmacokinetics of the navelbine series [2–4] provides an opportunity for testing such relationships and comparing these new alkaloids with vinblastine and congeners. The data we have obtained from patients entered in clinical trials of navelbine are numerically limited, but several conclusions are possible. Qualitatively, the triphasic kinetics resembles that of vinblastine, and the low percentage of immunoreactive navelbine found in urine (less than 10%, data not shown) indicates that navelbine, like other Vinca alkaloids, is most probably eliminated primarily via the liver. Quantitatively, concentrations of navelbine measured a few hours after injection were in the same order as those observed with the other alkaloids, although the doses were 4–20 times higher. These low concentrations are accounted for by increased clearances ($0.92 \pm 0.27\ l\ h^{-1}\ kg^{-1}$) and by larger distribution volumes ($51.4 \pm 16.0$ l/kg) than those seen with vinblastine (Table 4). Terminal half-lives are comparable to those of vinblastine or vindesine (Table 4). Thus, there is indeed a relationship between the pharmacokinetics of Vinca alkaloids, including navelbine, and toxicity in human, but it is more complex than a direct proportionality between clearance and tolerated doses [7].

Similar results were obtained in rats. Navelbine has a significantly faster clearance than vinblastine. Analogues of navelbine that have not yet been released for human clinical trials were studied. The derivatization of navelbine to the deacetyl-amide, which produces the navelbine analogue of vindesine, did not significantly reduce the clearance. This is in contrast to the result obtained with vindesine [7]. The Na-formyl analogue of navelbine, which is similar to vincristine in this way, has a definitely lower clearance than navelbine, as vincristine has a lower clearance than vinblastine. In addition the half-lives of the two phases were longer for both Na-formyl navelbine and vincristine. Thus, chemical modifications change the pharmacokinetic properties of Vinca alkaloids in a consistent way: the 5'-nor-anhydro modification of the original catharanthine ring increases clearance, while the the change to a formyl substituant on the vindoline ring reduces it and increases the half-life. Toxicities, and possibly antitumor activities (5, 6, 15), follow the reduction of clearances.

No explanation of this structure-pharmacokinetics relationship is yet available. Clues might be found by studying the binding to proteins and tissues and the mechanisms of elimination and metabolism. These differences could eventually be rationalized, which would generate new ideas for the design of more potent antitumor drugs: this work already calls for further evaluation of compounds such as Na-formyl navelbine and shows that preliminary evaluation of pharmacokinetics in animals may yield information that is of interest for therapeutic innovation.

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