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Clinical pharmacokinetics of intravenously injected tritiated vinzolidine*

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Summary. Vinzolidine (VZL), a novel, semi-synthetic vinca alkaloid showing evidence of oncolytic activity in phase I/II clinical trials, was studied in six patients for its pharmacokinetic and metabolic behavior. Following i.v. administration of [^{3}H]-VZL at doses of 5, 6.7, and 9 mg/m^2, blood and urine samples were collected and analyzed by sample oxidation and HPLC. Following a single i.v. dose, decay of total tritium in plasma was tetraphasic, with a rapid initial $t_{1/2\alpha}$ of 0.044 ± 0.013 h, followed by a $t_{1/2\beta}$ of 0.54 ± 0.22 h and a $t_{1/2\gamma}$ of 9.48 ± 4.89 h; the terminal $t_{1/2\gamma}$ was 219 ± 57 h. The mean plasma clearance of total tritium was 0.054 ± 0.044 l·kg/h, and the mean volume of distribution was 14.3 ± 5.4 l/kg; mean urinary excretion was $13.6\% \pm 4.3\%$ of the delivered radioactivity. Qualitative analysis of plasma and urine revealed the predominance of unchanged VZL plus two unidentified metabolites with different elution times. In comparison with oral VZL, as previously reported [7], i.v. injected VZL showed comparable values with respect to the volume of the central compartment (V_C), plasma clearance (Cl_p), and terminal $t_{1/2}$ for total tritium. Qualitatively, the metabolites observed in plasma and urine were comparable in number and quantity with values obtained in analyses after oral administration.

Introduction

Vinzolidine (VZL) is a semi-synthetic vinca derivative with a substituted heterocyclic oxazolidine-dione ring at the 4" position of the vinblastine molecule. These modifications contribute more rigid and lipophilic areas to the vinca alkaloid, possibly modifying the binding of the molecule to receptor sites. The β-chloroethyl side chain is nonfunctional with respect to alkylation. The presumptive mechanism of action of VZL involves binding to tubulin, as has been reported for other vinca alkaloids [1].

In tumor screens, VZL was active against B16 melanoma, P1534J leukemia, Gardner's lymphosarcoma, 6C3HED lymphosarcoma, 755 adenocarcinoma, C3H mammary carcinoma, and CA115 carcinoma. No activity, however, was found against Ridgeway osteosarcoma, L1210 leukemia, or Lewis lung carcinoma [2].

Originally tested by oral administration in phase I and II studies, VZL showed significant antitumor activity [3, 6, 9, 10, 13]. However, there was a wide variation in the oral dose of VZL that could be tolerated by patients, resulting in unpredictable and severe toxicity in some cases. The mechanism of this unpredictable toxicity was never clearly defined but may have been due to differences in the extent of systemic absorption or differences in metabolism among patients.

Because of its unpredictable clinical toxicity when given orally, this drug was studied in a phase I trial of i.v. bolus administration once every 2 weeks [4, 5]. This paper presents the results of a.pharmacokinetic study with radiolabeled VZL that was carried out in six patients with cancer during evaluation of the drug's clinical pharmacology after i.v. bolus administration.

Patients and methods

All patients treated with tritiated VZL met the same entry criteria outlined for the phase I study by Budman et al. [4]. Special informed consent was obtained for the use of radioactive VZL as per institutional guidelines.

The doses used for the present studies were 5, 6.7, and 9 mg/m^2, comparable with those used in the then ongoing phase I study [5]. Tritiated VZL was synthesized by Lilly Research Laboratories. The radiolabeled preparation was supplied in vials containing 5 mg lyophilized powder (sp. act. 99.2 μCi/mg). Chemical purity tests revealed no other UV-absorbing material, and the radioactive purity was 94.8% as evaluated by HPLC in the laboratories of Eli Lilly and Company.

The tritiated material was injected only once per patient and was given either alone (to the two patients receiving 5 mg/m^2) or mixed with unlabeled material in the syringe used for bolus i.v. injection. The drug

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Table 1. Pharmacokinetics after a single i. v. dose of [^{3}H]-vinzolidine: 4-exponential model[a]

Patient	Age (years)	Sex	Weight (kg)	Dose (mg)	Dose (mg/kg)	Dose (mg/m^2)	A′ (kg/l)	α (h^{-1})	$t_{1/2\alpha}$ α(h)	B′ (kg/l)	β (h^{-1})	$t_{1/2\beta}$ β(h)	C′ (kg/l)	γ (h^{-1})	$t_{1/2\gamma}$ δ(h)	D′ kg/l	δ (h^{-1})	$t_{1/2\delta}$ δ(h)	$V_{d\delta}$ l/kg	Cl_p l/kg/h	AUC_0^∞ (μg h ml^{-1})
LC	66	F	60	7.7	0.128	5	11.09	14.2	0.049	0.771	1.56	0.44	0.269	0.0679	10.19	0.094	0.002	233	9.1	0.027	4.73
SJ	68	M	57	8.5	0.149	5	6.17	15.7	0.044	0.493	1.58	0.44	0.158	0.1158	5.98	0.043	0.003	223	20.1	0.063	2.38
NN	70	M	83	13.3	0.161	6.7	5.25	12.8	0.054	0.623	0.99	0.70	0.169	0.0802	8.69	0.069	0.003	236	12.7	0.037	4.30
SC	57	M	56	11.2	0.198	6.7	2.99	11.9	0.058	0.470	0.81	0.86	0.131	0.0700	9.90	0.028	0.006	109	22.1	0.141	1.41
FF	47	M	86	18.4	0.213	9	7.16	16.4	0.042	0.802	1.17	0.58	0.171	0.0381	18.17	0.077	0.003	240	10.7	0.031	6.91
DH	65	M	71	16.6	0.234	9	14.30	32.6	0.021	1.370	3.05	0.23	0.366	0.1742	3.97	0.081	0.002	274	11.2	0.028	8.23
Mean	62		69	12.6	0.181		7.83	17.3	0.044	0.755	1.52	0.54	0.211	0.0910	9.48	0.065	0.003	219	14.3	0.054	
SD	8.7		13	4.3	0.041		4.14	7.7	0.013	0.331	0.81	0.22	0.089	0.0478	4.89	0.025	0.001	56.8	5.4	0.044	

[a] All values were calculated from quantitative evaluations of [^{3}H]-VZL-derived tritium. A′, B′, C′, and D′ represent the dose-in variant y-intercept coefficients corrected for the 1-min infusion interval; α, β, γ and δ represent the exponents in the polyexponential equation; $t_{1/2}$ represents plasma half-lives α, β, γ, and δ; AUC is expressed over time O→∞ or O→t; $V_d\delta$ represents the volume of distribution (area); and Cl_p represents plasma clearance

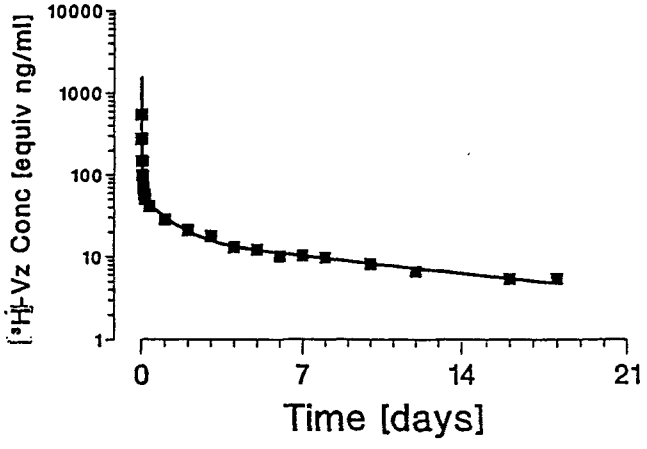

Fig. 1. Plasma vinzolidine (VZL) equivalents after i. v. administration of 9 mg/m^2 [^{3}H]-VZL to patient FF

was always injected i. v. over 1 min to patients who had fasted overnight. Each patient received only one radiolabeled dose of VZL, given with the first course of therapy. Two patients were treated for each of the three doses evaluated, namely, 5, 6.7, and 9 mg/m^2. At time 0 (before drug administration) and at 5, 10, and 30 min as well as at 1, 2, 3, 4, 8, 16, and 24 h and days 2–8, 10, 12, 14, 16, and 18, blood was collected in siliconized tubes containing 150 units heparin; urine was collected quantitatively at 0–4, 4–8, and 8–24 h and, subsequently, every 24 h until day 18. Plasma was prepared from the specimens by centrifugation at 2,000 rpm for 10 min in a Damon IEC HN-S centrifuge (Damon, Needham Heights, Mass.). Samples were stored at –70° C until analysis.

Extraction and analytical procedures, such as combustion analysis and HPLC methodology, were identical to those reported in our previous study using oral VZL [7]. All values presented are expressed as VZL equivalents.

Both a 3-exponential and a 4-exponential model were used to evaluate the data. Initial estimates for these polyexponential models were obtained using CSTRIP [12] and were further refined by nonlinear least-squares regression analysis using the program NONLIN 84 [15]. The weighting scheme used in the nonlinear least-squares regression was 1/Y. The resulting fits indicated that the 4-exponential model best describes the data. The equation for the four-compartment (postinfusion) model was:

$$Cp = \text{Dose}\,(Ae^{-\alpha t}+Be^{-\beta t}+Ce^{-\gamma}+De^{-\delta t}), \quad \text{Eq. (1)}$$

where Cp represents the concentration in plasma or whole blood; t, the time from the start of infusion; and Dose, the dose in milligrams per kilogram body weight.

The coefficients and exponents of this 4-exponential equation were fit to the data observed for each patient. Subsequently, the corresponding i. v. equivalent bolus coefficients were calculated using Eq. 2 to "correct" the coefficients for the infusion period:

$$Z' = (Z\lambda\tau)/(1-e^{-\lambda\tau}), \quad \text{Eq. (2)}$$

where Z′ represents the i. v. equivalent coefficient (A′, B′, C′, or D′); Z, the postinfusion coefficient (A, B, C, or D); τ, the duration of the infusion; and λ, the exponent (α, β, γ, or δ).

In the analysis of these kinetic models, a 1-min infusion period was assumed for all doses evaluated. For i. v. bolus equivalent pharmacokinetic variables, such as the area under the curve, clearance, and the volume of distribution, the linear pharmacokinetic equations published by Wagner [14] were used. The trapezoidal rule area (AUC_0^t) is the area calculated by a triangulation of the experimental data points. It differes from the AUC_0^∞ because it does not include the "tail end" of the curve AUC_t^∞). The AUC_0^∞ was calculated by integration of the pharmacokinetic model (Eq. 1).

Results

After a single i. v. bolus injection of [^{3}H]-VZL, plasma decay of total tritium was best fitted to a 4-exponential curve, with a rapid mean initial-distribution α-phase half-life of 0.044±0.013 h for all six patients evaluated and two middle distribution-phase half-lives of (β) 0.54±0.22 and (γ) 9.48±4.89 h. The terminal half-life was characterized by a mean of 219±57 h (range, 109–274 h) (Table 1). A representative decay curve for total tritium is demonstrated in Fig. 1 for a dose of 9 mg/m^2.

Whereas $t_{1/2\alpha}$, $t_{1/2\beta}$, and $t_{1/2\gamma}$ values showed little interpatient variation, the $t_{1/2\delta}$ value for patient SC was substantially lower than that obtained for cohort NN, who received the same dose of 6.7 mg/m^2 (108.9 h vs 235.7 h). There was no obvious correlation of any of the $t_{1/2}$ values with the delivered dose. The volume of distribution, V_d (14.3±5.4 l/kg), deviated substantially from the mean only in patients LC and SC.

By dividing the trapezoidal area AUC_0^t by the modeled area AUC_0^∞, we found that 87% of the theoretical tritium curve area was included during 18 days of sampling. Mean plasma clearance was 0.054±0.044 l/kg/h. When the AUC (in ng/h/ml) was plotted vs dose (in mg/kg), the

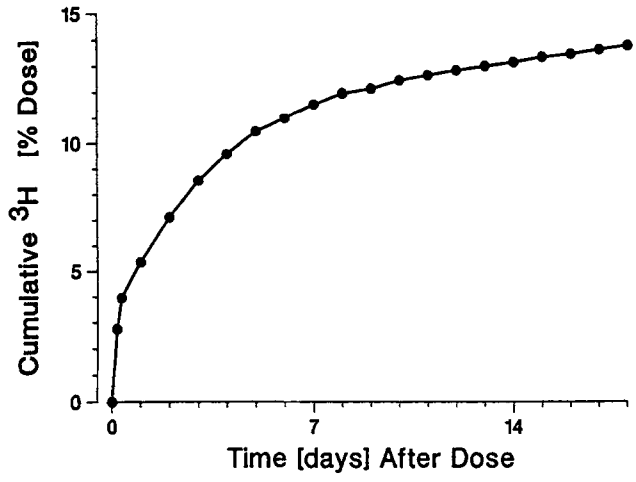

Fig. 2. Cumulative urinary excretion of tritium after administration of a 9 mg/m² i. v. bolus of [³H]-VZL to patient FF

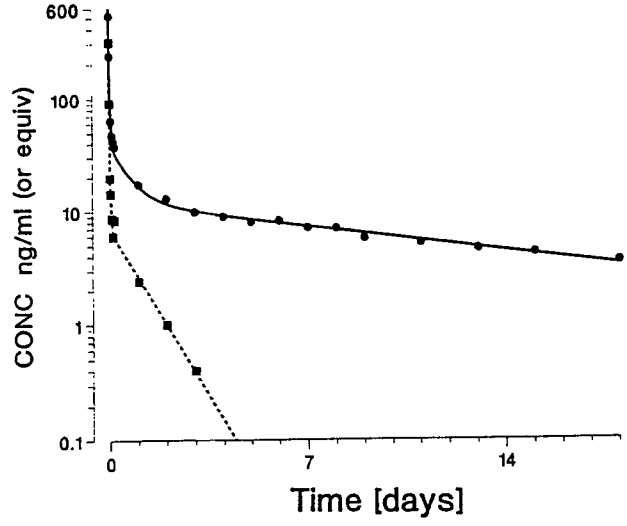

Fig. 4. Comparison of plasma levels of total tritium (●) vs VZL (■) in patient LC following administration of 7.7 mg (5.0 mg/m², 0.128 mg/kg) [³H]-VZL. ———, NONLIN84 fit for total tritium; – – – –, NONLIN84 fit for VZL

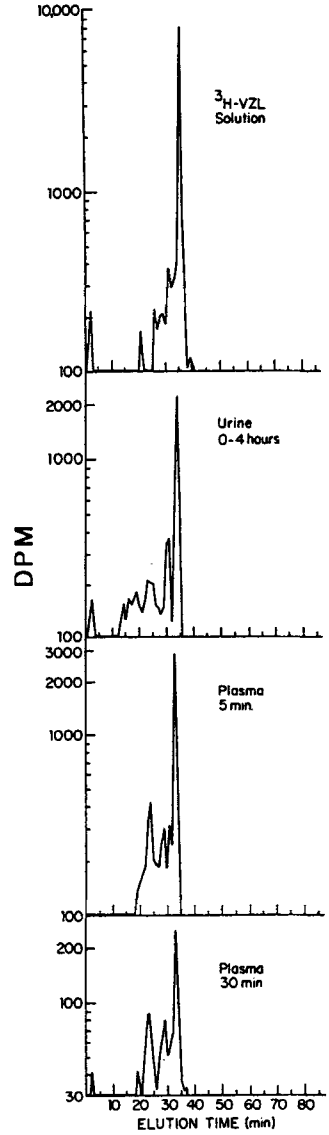

Fig. 3. Elution pattern of VZL and its derivatives in the plasma and urine of patient FF in comparison with a [³H]-VZL solution

Table 2. Pharmacokinetic variables for total tritium vs [³H]-VZL based on an i. v. bolus of 5 mg/m² [³H]-VZL given to patient LC

Pharmacokinetic variable	total tritium	[³H]-VZL
AUC^{∞}_0 (μg·h/ml)	4.73	0.27
$t_{1/2\alpha}$ (min)	2.94	2.73
$t_{1/2}$ terminal (h)	(δ)233	(γ)15.2
Clearance (l/kg/h)	0.027	0.461
Volume of distribution ($V_{d\beta}$)(l/kg)	9.10	10.1

presence of a relationship between the AUC and the dose was suggested (not shown), in that a larger AUC was achieved for a greater dose of VZL ($r^2 = 0.280$). However, these data are insufficient to establish whether the kinetics of tritiated VZL were linear or nonlinear with respect to the dose.

Urinary excretion amounted to 13.6% ± 4.3% of the delivered radioactivity. A representative cumulative urinary excretion of tritium is shown in Fig. 2 for patient FF.

Qualitatively, analysis of plasma extracts by HPLC revealed that the predominant product was unchanged VZL; moreover, significant amounts of unidentified metabolites or breakdown products were detected (Fig. 3). It is obvious that the major radioactivity in plasma was represented by material other than VZL, including metabolites, breakdown products, and tritiated water (Fig. 4). When the pharmacokinetic variables for total tritium vs parent compound were compared (Table 2), large differences were especially noted in the AUC^{∞}_0, terminal $t_{1/2}$, and clearance values, whereas the initial $t_{1/2\alpha}$ and $V_{d\delta}$ values and the terminal half-lives were comparable. The same prevalence of VZL was also noted in urine; the observed metabolites eluted at times comparable with those seen in plasma. No attempts were made to identify these substances. When the data for radioactivity in plasma were compared with those

in whole blood (not shown), it was found that the VZL-derived radioactivity was located predominantly in the plasma.

Discussion

In contrast to the data obtained after oral administration of VZL, the i.v. injected drug displayed a 4-exponential decay curve (Fig. 1) for total radioactivity. Whereas the mean terminal half-life following i.v. administration of the drug evaluated as total radioactivity, is comparable with that obtained after its oral administration (219 vs 172 h) (Table 1), both values are considerably greater than the corresponding values for i.v. vincristine, vinblastine, and vindesine (85, 25, 24 and 50 h, respectively) [8, 11].

As expected, the dose-normalized area under the curve (AUC_0^∞) showed a substantial increase over the value reported for oral administration at doses higher (29.2 – 36.5 mg/m^2) than that used in the present study (5 – 9 mg/m^2) (mean, 25.75 vs 11.76 kg/h/l). Unlike the situation with oral VZL, a relationship between AUC_0^∞ and injected dose was less obvious; this may be attributable to the small dose increments given in the present study, to the limited number of patients evaluated, or to interpatient variability in this respect.

Mean plasma clearance (Cl_p) for total tritium was 0.054 ± 0.044 l/kg/h, with little interpatient variation being observed except in patient SC. This clearance was comparable with the mean value observed after oral VZL treatment (0.093 ± 0.030 l/kg/h) but lower than those reported for vindesine (0.252 ± 0.1 l/kg/h) and vinblastine (0.74 ± 0.32 l/kg/h) [8]. Comparison of the volumes of the central compartment (V_C) revealed that VZL had a value of 0.138 l/kg in the present study; for oral VZL, the value was 6.7 ± 2.9 l/kg. Corresponding values for i.v. vincristine, vinblastine, and vindesine were 0.328, 0.696, and 0.045 l/kg, respectively [8]. The higher value obtained in the present study, along with those reported for vincristine and, especially, vinblastine may reflect rapid binding to formed blood elements. The high values for the volume of distribution (V_d) probably reflect extensive, reversible tissue binding. In this respect, VZL is comparable with vincristine and vindesine but not vinblastine, with values obtained for these four compounds being 11.4 ± 4.0, 8.42 ± 3.17, 8.84 ± 4.35, and 27.3 ± 14.9 l/kg, respectively.

Analysis by HPLC of a representative series of plasma samples obtained from patient LC after a bolus injection of 5 mg/m^2 [^{3}H]-VZL (Fig. 3, Table 2) revealed significant amounts of radioactivity no longer associated with [^{3}H]-VZL. This suggests metabolism, chemical breakdown, or exchange of tritium for hydrogen. Whereas minor metabolites (or related substances) of VZL as evaluated by HPLC were obvious in plasma as early as 5 min after the injection (Fig. 3), the initial ($t_{1/2\alpha}$) half-lives and volume of distribution were comparable for total tritium and VZL-derived tritium (Table 2), but the AUC, terminal $t_{1/2}$, and clearance showed large differences. This implies that metabolism, chemical degradation, and tritium exchange were taking place predominantly after the distribution phase, i.e., intracellularly.

Urinary excretion amounted to a mean of 13.6% of the delivered radioactivity. In comparison, following oral administration, the mean radioactivity excreted in urine amounted to only 3.6% over a comparable duration of urine collection. By calculation, the oral absorption of VZL amounted to about 25% of the delivered dose.

From these studies we concluded that i.v. injection of tritiated VZL results in a half life for total radioactivity that is comparable with that observed after oral administration. VZL has a considerably longer terminal half-life for total tritium than that reported for other commonly used vinca alkaloids. Qualitative HPLC showed breakdown products and true metabolites that were similar to those previously reported by us in a study using oral VZL [7].

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