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Clinical pharmacology of vinzolidine*

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Summary. Vinzolidine (VZL), a new semisynthetic vinca alkaloid, was studied by using ^{3}H-labeled VZL administered PO in four patients. At single doses from 1.5 to 36.5 mg/m^2 (0.034–0.919 mg/kg) radioactivity was rapidly absorbed with a half-life of absorption of 1 h and a peak at 4 h. Plasma decay of radiolabel followed a biphasic pattern with an alpha half-life of 10.48 h and a beta half-life of 172 h. The apparent plasma clearance was dose-dependent.

The total radiolabel recovered was 52.9% ± 11.4% of the administered label, with 90% in the feces.

HPLC analysis revealed that in all extracted plasma, urine, and feces the predominant material was unchanged VZL. Several metabolites were observed but not identified.

Introduction

The two naturally occurring vinca alkaloids, vincristine and vinblastine, are characterized by small differences in their molecular structure, but significantly different therapeutic indications and toxic side effects. Vinzolidine (VZL) (Fig. 1) is a semisynthetic vinca modification produced by the introduction of a substituted heterocyclic oxazolidine dione ring at the 4″ position of the vinblastine molecule. The β-chloroethyl side chain is a new group in this class of alkaloids. It is nonfunctional with respect to alkylation, but increases the lipophilicity of the molecule. The presumptive mode of action of VZL is binding to tubulin, as reported for other vinca alkaloids [1].

In phase I and phase II studies, orally administered VZL had significant antitumor activity [2, 3, 5, 7, 10].

For further definition of the clinical pharmacology of this new vinca alkaloid a radiolabeled oral pharmacokinetic study in humans was carried out.

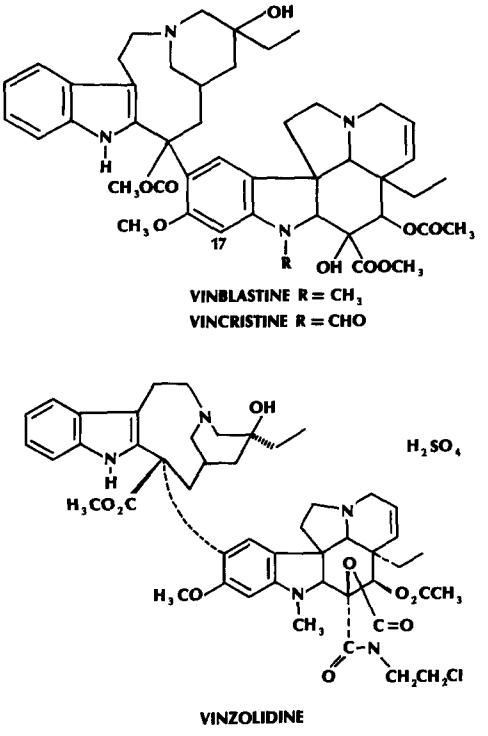

Fig. 1. Structures of vinblastine, vincristine, and vinzolidine

Materials and methods

All patients treated with (^{3}H)-vinzolidine had to meet the same criteria as were outlined for the phase I study by Budman et al. [3]. A special informed consent was obtained for the use of radioactive VZL as per institutional guidelines.

The doses used in these studies, with the exception of the one for the first patient (AM), were chosen according to the then ongoing phase I study [3]. The doses chosen for the last three patients conform with the dose established by Budman et al. [3]. Tritiated (^{3}H)-vinzolidine was synthesized by the Lilly Research Laboratories. The radiolabeled preparation was supplied in capsules containing 3.36 mg (^{3}H)-VZL, with specific activity of 104.3 μCi/mg. Chemical purity tests revealed no other UV-absorbing material, and the radioactive purity of the drug was 94.8%, as evaluated by HPLC in the laboratories of the Eli Lilly Company. The capsules were administered to patients after

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Chemical name: 3″ = (2-chloroethyl)-3-de(methoxycarbonyl)-3-deoxy-2″, 4″-dioxospiro [oxazolidine-5″, 3-vincaleukoblastine]

Table 1. Pharmacokinetics after a single PO administration of ^{3}H-vinzolidine

	(Dosage and serum Parameters)																
Pt	Wt kg	Dose mg	Dose mg/kg	mg/m^2	A kg/l	α h^{-1}	$t^1/_2$ α h	B kg/l	β h^{-1}	$t^1/_2$ α h	C kg/l	γ h^{-1}	$t^1/_2$ γ h	V_C l/kg	Cl_P l · kg^{-1} h^{-1}	AUC_{∞} kg·h·l^{-1}	TRAP·AUC kg·h/l
AM	97.5	3.4	0.034	1.5	0.072	0.074	9.37	0.025	0.00356	194	0.155	1.051	0.66	10.3	0.128	7.83	5.72
HE	63.5	58.4	0.919	36.5	0.203	0.073	9.53	0.079	0.00511	136	0.346	0.675	1.03	3.5	0.057	17.71	16.32
MG	108.8	68.4	0.628	29.2	0.140	0.111	6.23	0.035	0.00388	179	0.197	0.407	1.70	5.7	0.102	9.86	7.44
MZ	83.9	60.1	0.716	30.0	0.104	0.041	16.79	0.036	0.00388	178	0.186	0.902	0.77	7.2	0.086	11.62	9.31
Mean	88.4	–	0.574	–	0.130	0.075	10.48	0.044	0.00411	172	0.221	0.759	1.04	6.7	0.093	11.76	9.70
SD ±	19.5	–	0.380	–	0.056	0.029	4.47	0.024	0.00069	25	0.085	0.281	0.47	2.9	0.030	4.26	4.65

* All values calculated from quantitative evaluations of (^{3}H–) vinzolidine-derived tritium. A, B, C, dose-invariant y-intercept coefficients (kg/l); α, β, γ; exponents in (1/h or h^{-1}; also slope of plasma decay and absorption; $t^1/_2$, plasma half-life (α, β) and half time of absorption (γ)

fasting overnight, either alone (patient AM) or together with the unlabeled material in the same form. The first three patients (Table 1) received one each, and the fourth patient three radioactive capsules. The administered doses used ranged from 1.5 to 36.5 mg/m^2. Blood was collected at time 0 (before drug administration) and 30 min and 1, 2, 3, 4, 6, and 24 h and subsequently every 24 h until day 13, in siliconized tubes containing 150 units of heparin; urine was collected quantitatively at 0–1 h; 1–4 h; 4–24 h and subsequently every 24 h up to 15 days; and daily feces collected over 14 days were diluted with and homogenized in water. Plasma was prepared from the specimens by centrifugation at 2000 rpm for 10 min in a Damon IEC HN-S centrifuge (Damon, Needham Hts., Mass). Samples were stored at −20° until analysis.

Quantitative analyses were done by combustion analysis in a Packard Sample Oxidizer, Model 306 (United Technologies-Packard, Downers Grove, Ill).

For qualitative analysis an extraction procedure was developed. Following adjustment of the pH of 1 ml plasma or urine to pH 8.0 with 1.48 *N* NH_4OH, 1.5 ml 95% ethanol was added. Following centrifugation at 3000 *g* for 10 min, the supernatant was removed and the precipitate re-extracted twice with 0.25 ml ethanol. To the combined ethanol fractions, 2 ml dichloromethane was added. After mixing and centrifugation at 3000 *g* for 10 min the top layer was discarded and the bottom layer evaporated under N_2 at room temperature. Reconstitution of the dried material was in 1 ml of a mixture of solution A (0.001 *M* K_2HPO_4 buffer, pH 7.5) and acetonitrile 80:20, to which 20 µg desipramine (Pertofrane) was added. The yield of radioactivity recovered from plasma amounted to 93.2%±5.7%. Sensitivity for the evaluation of the radioactive material was 0.5 ng for combustion and 1.0 ng VZL for the HPLC assay.

High-pressure liquid chromatography was used to separate unchanged VZL from metabolites, using a µ Bondapak C18 column (Waters Assoc., Milford, Mass). A stepwise gradient of acetonitrile (containing 25 mg desipramine/100 ml) 20%–100% in 0.001 *M* K_2HPO_4 buffer pH 7.5 was used for elution over 90 min. A combination of Waters pump (Waters, Milford, Mass). and Perkin-Elmer LC-85 detector was used with a Sigma 15 Integrator (Perkin-Elmer, Norwalk, Conn). Desipramine was necessary to increase the recovery of radioactivity from the column to 85.1%±14.6% of the input.

A Packard Tri-Carb Scintillation Spectrophotometer, Model 300, (United Technology) with external standardization was used for the measurement of radioactivity.

Table 2. Pharmacokinetics after a single PO administration of

	(Absorption and excretion)				
Patient	Absorption		Excretion		
	Half-time (h)	Peak at (h)	Urine (% of dose)	Feces (% of dose)	Total (% of dose)
AM	0.66	2	3.0	61.2	64.2
HE	1.03	4	5.2	47.8	53.0
MG	1.70	4	3.2	38.2[a]	41.4[a]
MZ	0.77	4	3.0	N.D.	N.D.
Mean	1.04	3.5	3.6	49.1[b]	52.9[b]
SD ±	0.47	1	1.1	11.6[b]	11.4[b]

All values calculated from quantitative evaluations of (^{3}H–)-vinzolidine-derived tritium

[a] Incomplete collection

[b] Approximate

All values in Tables 1 and 2 and Fig. 2 and 3 are expressed as VZL equivalents.

Initial estimates of the pharmacokinetic parameters A, B, C, α, β, γ [y intercepts and slopes for distribution (A, α), elimination (B, β) and absorption (C, γ) phases] of the plasma concentration-time curves were obtained from semilogarithmic plots [11]. The six parameters were next fitted to the plasma concentrations vs time data (Eq. 1) for each patient separately, using the nonlinear regression program MLAB [4] on a DEC-10 computer:

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

The regression was weighted with the reciprocal of the plasma concentration values during computer fitting. In all cases, a line of best fit was obtained that was in reasonable agreement with the actual data values according to visual inspection of the model-predicted line of best fit superimposed on the data values. The volume of the central compartment (V_c) and the area under the plasma concentration-time curve (AUC_0^∞) (Table 1) are apparent volumes. The trapezoidal rule area (TRAP AUC) is the actual trapezoidal area calculated from the experimental data points. It is normally less than the AUC_0^∞, since the collection of plasma was not carried out for four to five half-lives in this study.

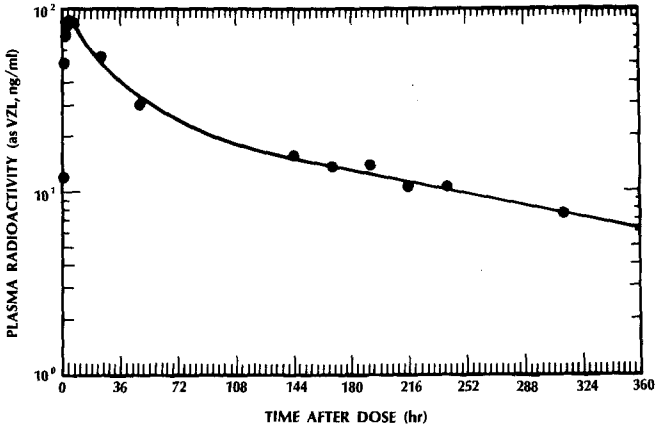

Fig. 2. Plasma vinzolidine equivalents after PO administration of (H)-vinzolidine (30 mg/m^2) to patient MZ

Results

Following a single oral dose of (^{3}H)-VZL, plasma was obtained for analytical purposes at times demonstrated in Fig. 2 (for patient MZ). When plotted semilogarithmically, total plasma tritium was observed to rise rapidly with a half-time of absorption of about 1 h and a time to peak of about 4 h. Plasma decay revealed a biphasic pattern (Fig. 2), the terminal points being very well fit in all cases by a straight line. This same pattern was observed in all four patients studied, and corresponds to Eq. 1.

With the exception of patient MZ, the $T_{1/2}\alpha$ shows little variation (6.23 to 9.53 h) (Table 1) and appears to be independent of the dosage administered. The terminal half-life ($t_{1/2}\beta$) is comparable in all four patients, with a mean of 172 h and a range of 136–194 h, with no obvious correlation with the dosage administered. The apparent central compartment volumes (V_C) deviate significantly from the mean (6.7 l/kg), only for patient AM (Table 1), who had had the smallest dosage of VZL of all four patients. Since the bioavailability factor could not be calculated from the data provided in this study, the absolute volumes of distribution could not be evaluated.

By division of $\frac{\text{TRAP} - \text{AUC}}{\text{AUC}_0^\infty}$, 82% of the theoretical curve area was covered during the 2 weeks of sampling.

When the trapezoidal areas under the curve of the four patients are plotted against time (Fig. 3) a striking "bend" occurs at about 0.7 mg/kg, corresponding to about 28 mg/m^2. This indicates a dose-dependent nonlinearity in this small series of four patients who received different doses of VZL.

When the contribution of the radioactivity of the plasma was compared with the radioactivity obtained by combustion of the corresponding whole blood (not shown) it was found that the predominant amount of (VZL-derived radioactivity was located in the plasma.

Urinary excretion of radioactivity (Table 2) in the four patients was consistently small, amounting on average to only 3.6% of the dose administered. Figure 4 (Pt HE) de-

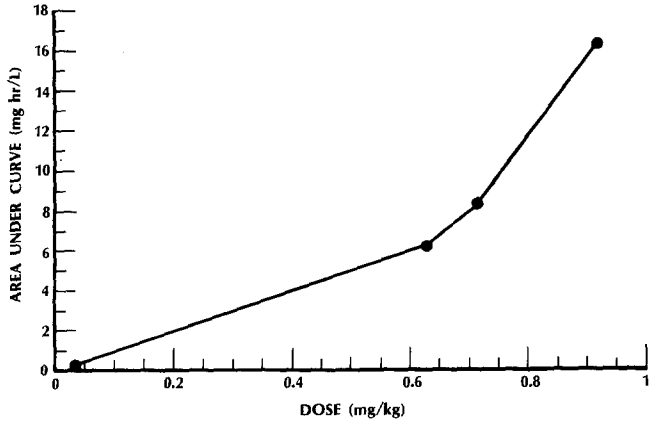

Fig. 3. Correlation of area under curve (AUC) with dose (mg/kg) of vinzolidine administered PO. The value for AUC was calculated by multiplying AUC_0^∞ from Table 1 by the dose administered to each patient in mg/kg

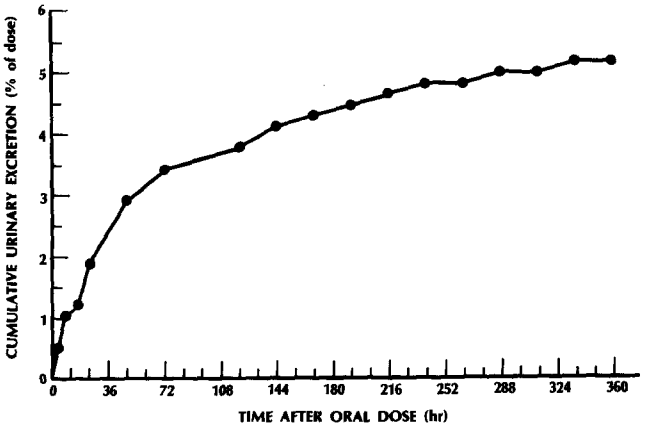

Fig. 4. Cumulative urinary excretion of tritium after administration of 36.5 mg/m^2 PO to patient HE

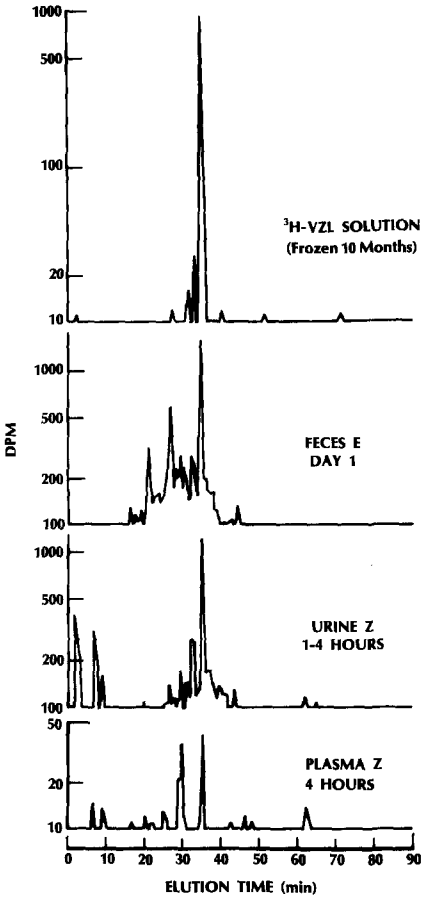

Fig. 5. Elution pattern of vinzolidine and derivatives in feces (patient HE), urine (patient MZ), and plasma (patient MZ) in comparison with a (^{3}H)-vinzolidine stock solution prepared from a capsule such as was used for the pharmacokinetic studies. For separation of peaks by HPLC, see text

monstrates the cumulative urinary excretion, indicating that the elimination by the kidneys of (^{3}H)-VZL-derived radioactivity extended beyond the 2 weeks of sampling. This was confirmed in two patients from whom urinary samples collected 5 and 12 weeks after administration of the drug still contained measurable (^{3}H)-radioactivity. Similarly, the excretion in bile of VZL-derived radioactivity must have extended beyond the 14 days of feces analysis, since on the last day of collection, feces still contained 0.03%–0.69% of the amount of radioactivity administered.

Qualitatively, analysis by HPLC revelaed that the predominant product was unchanged VZL, besides which significant amounts of unidentified metabolites or breakdown products were present (Fig. 5). When freshly prepared solutions of (^{3}H)-VZL were analyzed by HPLC and compared with such solutions that had been stored for 10 months at −70°, stored samples exhibited significant peaks other than the VZL peak; some of these peaks aligned with the metabolite peaks observed in feces, urine, and plasma obtained from all four patients.

A possible metabolite, prepared by acid hydrolysis of VZL and analyzed as being an oxazolidinedione ring-opened VZL derivative, prepared by Dr G. Thompson, did not coelute with any of the metabolic products observed in plasma, urine, and feces.

Discussion

The tritiated radioactive material used for this study provided adequate sensitivity and, in combination with the high-resolution HPLC system used, high specificity.

When (^{3}H)-VZL was given PO to four patients in doses varying from 1.5 mg/m^2 to 36.5 mg/m^2, rapid uptake from the GI tract was observed, with a half-life of 0.76 h and a peak plasma level at about 4 h. A biphasic plasma decay pattern was observed, which fit a two-compartment open model. The terminal (elimination) half-life for VZL is considerably higher (172 h) than the corresponding half-lives reported for vincristine, vinblastine, and vindesine (85 h, 25 h, 24 h and 50 h respectively) after IV administration to patients [6, 8]. In comparison with patient studies with VZL analyzed by radioimmunoassay [7] the absorption half-life and peak of absorption correlated well, whereas the mean α and, especially, the β half-lives were higher in our study. The relatively long half-lives support schedules spaced 2–3 weeks apart to avoid cumulative toxicity. If Eq. 1 and the mean values for A, B, C and α, β, γ of all 4 patients (Table 1) are used, plasma levels for any patient within a reasonable weight range can be calculated for a given dose.

The rate of drug elimination did not change significantly with dose. Thus, while β remains relatively constant the areas under the curve, when plotted against dose, show a striking nonlinear increase as the dose increases, with a bend at 0.7 mg/kg (about 28 mg/m^2, Fig. 3). Possible explanations for this finding are: (a) nonlinear capacity-limited hepatic first-pass effect; *or* (b) nonlinear intestinal absorption kinetics; *or* (c) complex nonlinear tissue binding.

In the clinical studies with VZL, significant marrow toxicity was observed at doses above 28 mg/m^2 [3].

The total recoverable amount excreted in urine and feces amounted (Table 2) to 52.9% (mean; it did not exceed 64.2%). Tritiated VZL adsorbs extensively to a large number of substances, including glass and plastic containers. Our extraction procedure is effective for plasma and urine only, and not for feces. Particulate matter in feces was most likely responsible for the incomplete recovery of radioactivity.

Qualitatively, unchanged VZL was the predominant peak consistently observed in HPLC analysis. "Metabolites" derived in plasma, urine, and feces are due in part to chemical decomposition of the original VZL, as can be seen on prolonged storage but not in freshly prepared material.

In all instances, unextracted urine and extracted feces and plasma always exhibited peaks in the chromatograms that were not observed in freshly prepared or stored (^{3}H)-VZL, implying cellular metabolism. From these studies with tritiated VZL it was concluded that VZL administered PO is absorbed rapidly from the GI tract, has a considerably longer half-life than reported for other commonly used vinca alkaloids, and is probably excreted mostly through the bile. Qualitative HPLC analysis shows both unidentified breakdown products and true metabolites. When the areas under the curve are plotted against dose a substantial deviation from linearity is observed at about 0.7 mg/kg (= 28 mg/m^2). At this dosage and above significant marrow toxicity has been noted in clinical trials with this new agent.

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