Cancer Chemother Pharmacol (1998) 41: 299–306

ORIGINAL ARTICLE

D.A. Garteiz · T. Madden · D.E. Beck
W.R. Huie · K.T. McManus · J.L. Abbruzzese
W. Chen · R.A. Newman

Quantitation of dolastatin-10 using HPLC/electrospray ionization mass spectrometry: application in a phase I clinical trial

Received: 17 March 1997 / Accepted: 13 June 1997

Abstract A highly sensitive and specific assay for the quantitation of the anticancer agent dolastatin-10 (DOL-10) in human plasma is described. The method was based on the use of electrospray ionization-high-performance liquid chromatography/mass spectrometry (ESP-LC/MS). The analytical procedure involved extraction of plasma samples containing DOL-10 and the internal standard (DOL-15) with *n*-butyl chloride, which was then evaporated under nitrogen. The residue was dissolved in 50 µl mobile phase and 10 µl was subjected to ESP-LC/MS analysis using a C_{18} microbore column. A linear gradient using water/acetonitrile was used to keep the retention times of the analytes of interest under 5 min. The method exhibited a linear range from 0.005 to 50 ng/ml with a lower limit of quantitation (LLQ) at 0.005 ng/ml. Absolute recoveries of extracted samples in the 85–90% range were obtained. The method's accuracy (≤5% relative error) and precision (≤10% CV) were well within industry standards. The analytical procedure was applied to extract DOL-10 metabolites from samples obtained following incubation of the drug with an activated S9 rat liver preparation. Two metabolic products were detected and were tentatively identified as a *N*-demethyl-DOL-10 and hydroxy-DOL-10. Structural assignments were made based on the fragmentation patterns obtained using the electrospray source to produce collision-induced dissociation (CID). The method was also applied to the measurement of DOL-10 in the plasma of patients treated with this drug. Preliminary investigation of the pharmacokinetics suggested that drug distribution and elimination may be best described by a three-compartment model with $t_{1/2}\alpha = 0.087$ h, $t_{1/2}\beta = 0.69$ h and $t_{1/2}\gamma = 8.0$ h. Plasma clearance was 3.7 l/h per m^2.

Key words Dolastatin-10 · Dolastatin-15 · Liquid chromatography atmospheric pressure · Mass spectrometry · Phase I trial

This work was supported in part by the National Institutes of Health (U01CA62461) and was presented as an abstract at the American Association for Cancer Research Meeting, Washington, DC, 20–24 April, 1996 (Proc Am Assoc Cancer Res 37: 393)

D.E. Beck · J.L. Abbruzzese · W. Chen · R.A. Newman
Department of Clinical Investigation, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA

T. Madden · W.R. Huie
Department of Experimental Pediatrics, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA

D.A. Garteiz · K.T. McManus
TEXms Inc., 15701 West Hardy Road, Houston, Tx, 77060

R.A. Newman (✉)
Clinical Pharmacology Resource Facility, Box 52, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA
Tel. +713-792-3608; Fax +713-792-6759

Introduction

Dolastatin-10 (DOL-10, Fig. 1), one of a series of related compounds isolated from the sea hare *Dolabella auricularia*, [1, 2], is a unique linear, lipophilic pentapeptide which has been shown to be a very potent antineoplastic and antimitotic agent. The compound has demonstrated cytotoxicity against several human tumor cell lines as well as antitumor efficacy against murine leukemia tumor models [3–7]. DOL-10 is composed of several unusual amino acids unique to this animal species. These include an α-amino acid (dolavaline) and two γ-amino acids (dolaisoleucine and dolaproline) linked to an unusual primary amine (dolaphenine) at its carboxyl terminus [8–10].

DOL-10 was chosen by the National Cancer Institute for further evaluation as a potential antitumor agent for patients with advanced refractory solid tumors. In anticipation of clinical testing of this compound, a highly sensitive and specific assay for detection and quantitation of DOL-10 in human plasma was developed using LC/MS analyses. Starting doses of DOL-10 in phase I clinical trials were very low (e.g. low mg/m^2) which

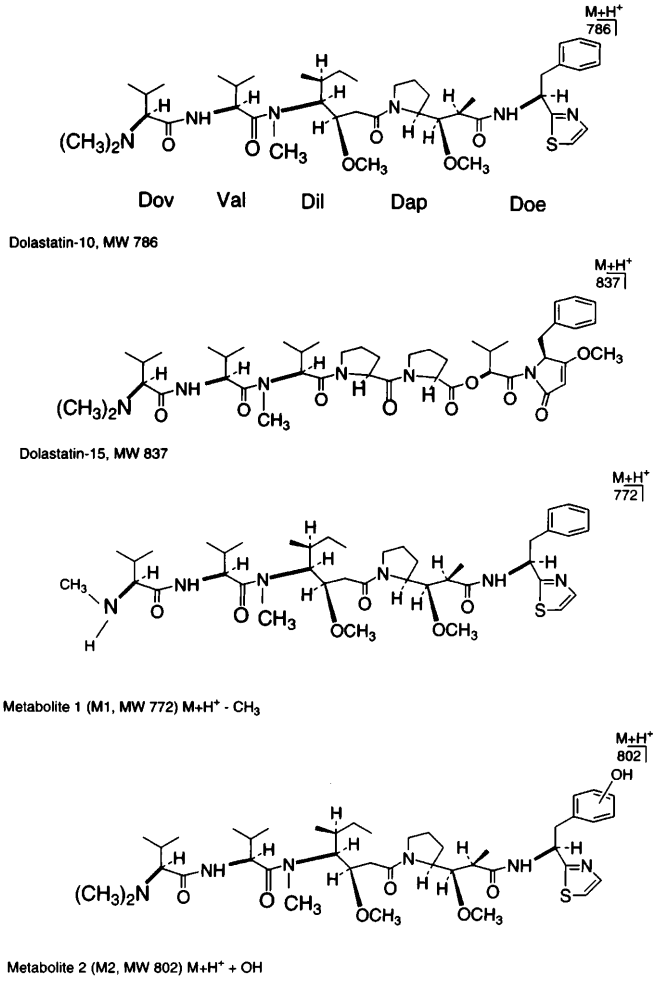

Fig. 1 Structures of dolastatin 10, dolastatin 15, demethyl-DOL-10 (metabolite 1) and hydroxy-DOL-10 (metabolite 2)

suggested that routine analytical procedures such as HPLC would not be appropriate for determination of the clinical pharmacology of this new drug. The present LC/MS assay for DOL-10 provided excellent sensitivity (5 pg/ml) and readily meets other validation criteria typically required for routine analysis of patient-derived samples. We applied this method to an evaluation of DOL-10 metabolites. Structural assignments for two DOL-10 metabolites, an *N*-demethylated species and a hydroxylated species, were based on fragmentation patterns obtained using the electrospray (ESP) source to produce collision-induced dissociation (CID). In addition, the method was used to demonstrate that the *N*-demethylated metabolite can be found circulating in the plasma of patients treated with DOL-10.

Materials and methods

Chemicals

DOL-10 and DOL-15 were obtained as dry powders from the National Cancer Institute (Bethesda, Md.). Clinical grade drug was supplied by the NCI as a sterile solution containing 200 μg/ml DOL-10 in 100 m*M* potassium phosphate buffer (pH 7). Butyl chloride and acetonitrile were HPLC grade and were purchased from Burdick and Jackson (Muskegon, Mich.). Drug-free human plasma was obtained from Biological Specialty Corp. (Houston, Tx.) and was centrifuged to remove clotted material. The S9 (9000 *g* supernatant) fraction of rat liver homogenate (Aroclor 1254-activated) was obtained from Microbiological Associates (Rockville, Md.). In experiments involving incubations of drug with S9, magnesium chloride (hexahydrate), D-glucose-6-phosphate (98%, disodium salt hydrate), glucose-6-phosphate dehydrogenase (1000 units as suspension in 3.2 m*M* ammonium sulfate) and β-nicotinamide adenine dinucleotide phosphate (NADP, 98%, sodium salt) were purchased from Sigma Chemical Co. (St. Louis, Mo.). The potassium monohydrogen phosphate (anhydrous, ACS-certified) was obtained from Fisher Scientific Co. (Houston, Tx.).

Preparation of standard drug solutions

Stock solutions of DOL-10 and DOL-15 (1 mg/ml in 1.00 ml acetonitrile) were stored at −20 °C. Serial dilutions from the original stock solution of DOL-10 provided solutions of 100, 10 and 0.5 μg/ml DOL-10 in acetonitrile. The 0.5 μg/ml solution was used to serially prepare the DOL-10 plasma-spiking solutions which ranged from 0.25 to 125 ng/ml. For the internal standard (IS), serial dilution of the stock DOL-15 solution was used to obtain a final concentration (0.25 mg/ml) which was used as the plasma-spiking solution.

Preparation of QC, calibration standards and subject samples

A 1-ml aliquot of plasma from treated subjects was transferred into an 8-ml nonsilanized culture tube and an aliquot of the IS spiking solution (20 μl) was added. *n*-Butyl chloride (5 ml) was added and the sample was shaken for 1 h at 250 strokes/min (Eberbach, Ann Arbor, Mich.). Tubes were then centrifuged at 2500 rpm for 10 min, and frozen for a minimum of 30 min, then the organic layer was transferred into a 10-ml nonsilanized conical centrifuge tube. The extract was evaporated to dryness under nitrogen at about 40 °C and reconstituted by adding 20 μl of a 60:40 (v:v) mixture of acetonitrile/water to the dried extract. Sonication for 5 min followed by vortexing at a high setting for 5 min was sufficient to insure complete dissolution of the extract. Prior to injection, the samples were centrifuged for 10 min. When samples were not analyzed within 8 h, they were stored as dry residues at −80 °C. Dry residues were kept at −80 °C for 24 h before reconstitution. Preparation of calibration standards or QC samples consisted of addition of IS and 20 μl of the appropriate DOL-10 spiking solution.

Electrospray ionization conditions

ESP conditions were optimized for maximum sensitivity of the protonated molecular ionic species (MH^+) of DOL-10 at m/z 786. The needle was held at ground potential relative to the capillary entrance which was held at −5000 V. Other key voltages were: the capillary exit, the first skimmer and the second skimmer which were set at +110 V, +34 V and +34.4 V, respectively. Nitrogen was used as the nebulizing gas (80 psi), as well as the drying gas (170 psi). Both gases assisted in the evaporation of the droplets formed by the ESP process. The nebulizing gas also had the effect of driving the droplets toward the capillary entrance.

HPLC-MS conditions

Quantitation of DOL-10 and detection of its *N*-demethylated metabolite in human plasma was accomplished by injecting 5 μl of the extracted drug onto a Zorbax microbore C18 reverse-phase column (50 × 1 mm, 5 μm particle size, Micro-Tech Scientific, Sunnyvale,

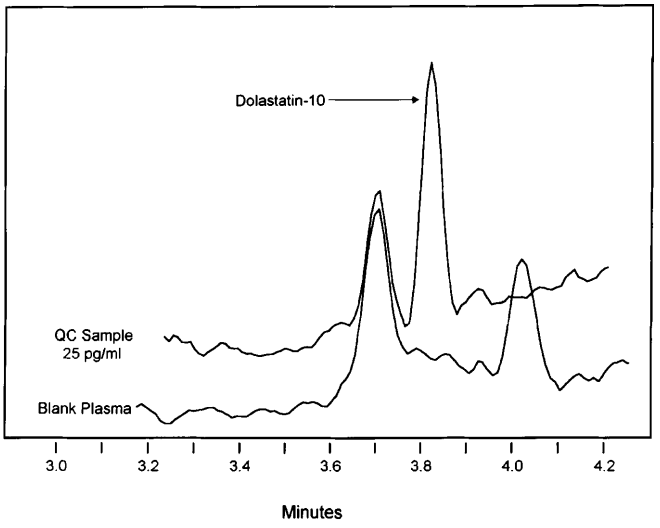

Fig. 2 LC/MS ion chromatographs of extracted human plasma blank and a trace showing the presence of a low concentration of DOL-10 (25 pg/ml)

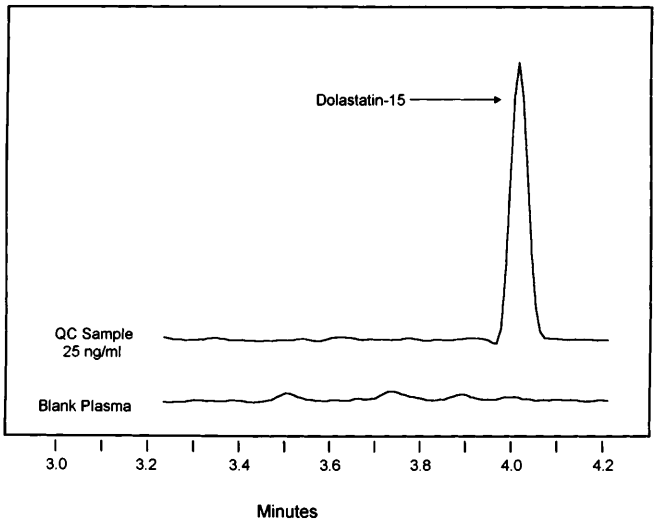

Fig. 3 LC/MS ion chromatographs of extracted human plasma blank and a trace showing the presence of the internal standard DOL-15 (25 ng/ml)

Calif.) at ambient temperature. The mobile phase consisted of water and acetonitrile. A linear gradient using water (A) and acetonitrile (B) was used to keep the retention times of the analytes of interest under 5 min. The following linear gradient was used: 98% A to 30% A in 3.0 min; at 3 min, conditions were held for 1 min then continued to 97% B in 0.6 min. At 5.2 min, the HPLC was returned to the initial conditions. Flow was set at 75 µl per min. Under these conditions the retention times for DOL-10 and the IS were about 4 min.

Representative chromatograms obtained following the analysis of drug-free plasma and a QC sample are shown in Fig. 2 (DOL-10) and Fig. 3 (DOL-15) revealed the absence of interfering mass peaks in the retention time window for both compounds.

Assay validation

Three calibration curves were run with a linear range of 0.005 to 2.5 ng/ml of plasma. For each curve, duplicate analyses of each of three QC samples (0.025, 0.5, and 1 ng/ml) were carried out. Calibration curves were generated following the analyses of duplicate 1-ml aliquots of drug-free human plasma fortified with 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1 or 2.5 ng DOL-10. In addition, 0.4 ng DOL-15 was spiked into each sample as the IS. Results obtained for a typical DOL-10 standard curve (Fig. 4) revealed excellent linearity. The mean slope and intercept for all curves were 3.9 and 0.034, respectively, with an average correlation coefficient of 0.9985.

Within-day and between-day precisions and accuracies for DOL-10 extracted from human plasma are presented in Table 1. One concentration of drug (0.50 ng/ml) was tested for intraassay precision and accuracy; three different concentrations of drug (0.03, 0.50 and 1.0 ng/ml) were tested for interassay precision and accuracy. For precision, the coefficient of variation (CV) ranged from 3.5% to 9.6%. For accuracy, the relative errors ranged from 0.3% to 4.5% from the nominal values. Elution times for DOL-10 ranged from 3.7 to 3.9 min while those for DOL-15 averaged about 0.2 min longer (3.9 to 4.1 min). The absolute extraction recovery of DOL-10 (at 0.5 ng/ml) and DOL-15 (at 0.4 ng/ml) was determined to be 89.8% and 100%, respectively. The relative extraction recovery of DOL-10 from the plasma samples was excellent when tested in triplicate at three different levels of drug: 0.75, 5.0 and 15.0 ng/ml. Mean recoveries were 106%, 104%, and 98.4%, respectively, with an overall mean recovery of 103%. The CVs were 4.9%, 5.7%, and 4.1%, respectively, indicating good reproducibility of the assay. The limit of quantitation (LOQ), defined as the lowest drug concentration for which acceptable accuracy and acceptable precision were obtained, was 0.005 ng DOL-10/ml plasma. The mean recovery (accuracy) for the LOQ was 95.69% with a CV of 11.56%.

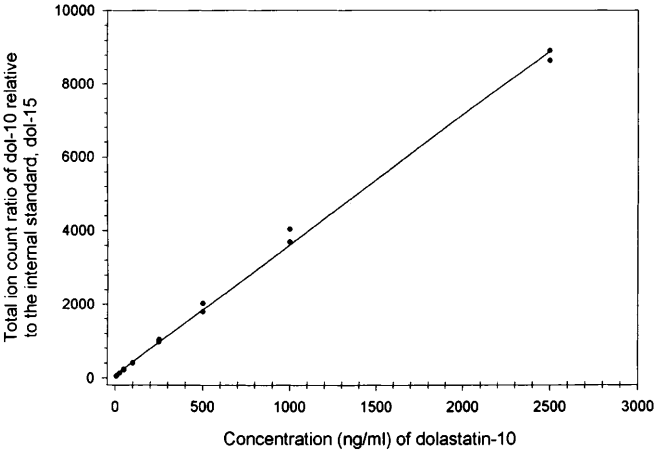

Fig. 4 Standard curve for determination of DOL-10. Data are plotted as individual determinations of nine concentrations of drug extracted from human plasma. Samples were run in duplicate

Drug metabolism in vitro

To 6 µg DOL-10 were added 1 ml (20 mg protein) S9 fraction and 1 ml NADPH-generating system (final concentrations: 1 m*M* NADP, 10 m*M* glucose-6-phosphate, 4 m*M* magnesium chloride, and 100 m*M* potassium phosphate buffer, pH 7.4). Samples were incubated for 10 min. A "0" time control was prepared by immersing the sample in ice after the addition of the reagents. At the end of the reaction, samples were centrifuged to remove debris. Methanol was added to the supernatants to precipitate proteins and the samples were extracted twice with methylene chloride. Extracts were combined and dried under nitrogen; the residue was dissolved in methanol in preparation for analysis by LC/MS.

Table 1 LC/MS assay of DOL-10: precision and accuracy

Nominal concentration (ng/ml)	Mean ± SD calculated concentration (ng/ml)	% CV[a]	% Relative error[b]
Intraassay (n = 12)			
0.5	0.52 ± 0.04 (high 0.58, low 0.44)	7.6	3.7
Interassay (n = 6 at each concentration)			
0.03	0.03 ± 0.002 (high 0.036, low 0.02)	9.6	1.2
0.5	0.52 ± 0.02 (high 0.54, low 0.49)	3.5	4.5
1.00	0.99 ± 0.04 (high 1.06, low 0.96)	4.0	0.3

[a] % CV = (SD/mean concentration) × 100
[b] % Relative error = [(mean calculated concentration – nominal concentration)/nominal concentration] × 100

Clinical pharmacology

Preliminary clinical pharmacology data were obtained from the initial patient entered into a phase I trial of DOL-10 at M.D. Anderson Cancer Center (Houston, Tx.). The DOL-10 starting dose of 65 μg/m^2 was administered as a short (30-s) bolus given intravenously. Blood samples were collected in tubes containing heparin as anticoagulant immediately prior to dosing and immediately after the bolus injection ("0" time). Additional samples were taken at 5, 10, 15, 30, 45, 60 and 90 min and at 2, 3, 4, 6, 8 and 12 h following drug administration. Plasma was obtained from the blood samples and stored frozen until analysis. The protocol was approved by the M.D. Anderson Cancer Center Institutional Review Board for Human Research and the study was sponsored by the National Cancer Institute.

Pharmacokinetics

A preliminary examination of DOL-10 pharmacokinetics was made by fitting the plasma drug concentration-time data using the ADAPT II program [11]. The pharmacokinetic parameters were calculated from the coefficients and exponents of the triexponential regression equation using standard equations [12].

Results

In vitro metabolism of DOL-10

Following assay validation, an in vitro study of the metabolism of DOL-10 was undertaken in order to investigate the formation of metabolites which might be expected in humans enrolled in the phase I clinical study. The metabolism study used Aroclor-activated rat liver S9 fraction. There was rapid conversion of parent drug to more polar, drug-derived metabolites. Two metabolites were identified by LC/MS using selected ion monitoring (SIM; Fig. 1). These consisted of a major *N*-demethylated species (M1, m/z 772) and a minor hydroxylated species (M2, m/z 802). Average retention times of both metabolites M2 and M1 at 12.6 and 13.1 min, respectively, were less than that of the parent drug, DOL-10, at 13.3 min. Following a 1-min incubation of DOL-10 with S9 preparation and subsequent extraction of the metabolite mixture, there was conversion of 39% of parent drug into M1 and M2 with relative concentrations of 81% and 19%, respectively (data not shown). Following a 30-min incubation, over 90% of DOL-10 had been converted to metabolites (90% M1 and 10% M2 in relative amounts). The control "0 min" incubation time sample, where the sample was immediately extracted after addition of drug to plasma, did not generate any detectable metabolites and resulted in apparent full recovery of DOL-10.

HPLC-ESP/CID/MS analysis of DOL-10 and its metabolites

A capillary exit setting of 150 V was sufficient to induce CID fragment ions for DOL-10. Its ESP/CID/MS spectrum is shown in Fig. 5. The pattern obtained was characterized by an intense protonated molecular ion (MH^+) at m/z 786. In addition, key "diagnostic" fragment ions were observed at m/z 100, m/z 188, m/z 560 and m/z 754 (see Table 1). The ion at m/z 100 resulted from α-cleavage to the nitrogen on the dolavaline portion of the molecule. Shifts in the mass of this fragment for any metabolite would indicate whether a metabolic change had taken place on the dolavaline end of the molecule. Furthermore, the mass shift observed would help to elucidate what metabolic change had taken place.

The ion fragment at m/z 188 resulted from cleavage between the NH functionality and the adjoining carbon on the dolaphenine portion of the molecule. It is also a key "diagnostic" ion. Shifts in the mass of m/z 188 for any metabolite would indicate metabolic changes on the dolaphenine end of the molecule, and the nature of the structural change. Other important fragments were m/z 560 which arose from cleavage between the carbonyl and the NH functionality of the dolaisoleucine portion of the molecule and m/z 754. The fragment at m/z 754 was derived from the parent ion by the loss of 32 D which corresponds to a neutral loss of CH_3OH.

The ESP/CID/MS spectrum of the major metabolite of DOL-10 is shown in Fig. 6. The spectrum is characterized by an intense protonated molecular ion (MH^+) at m/z 772. In addition, fragment ions were observed at

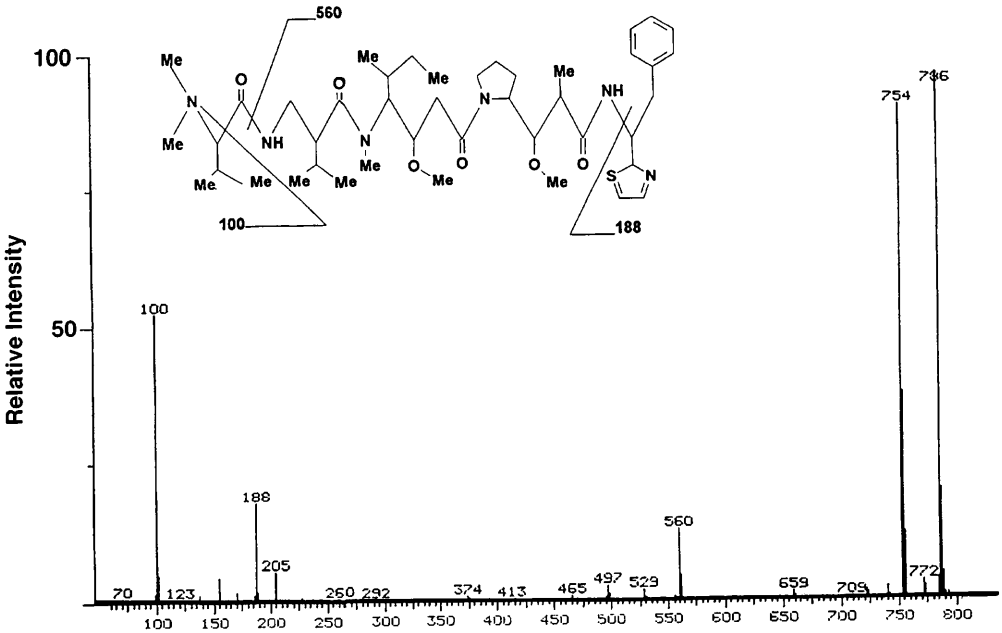

Fig. 5 Mass spectrum of DOL-10 showing the base peak abundance at m/z 786 and the fragmentation pattern (*inset*)

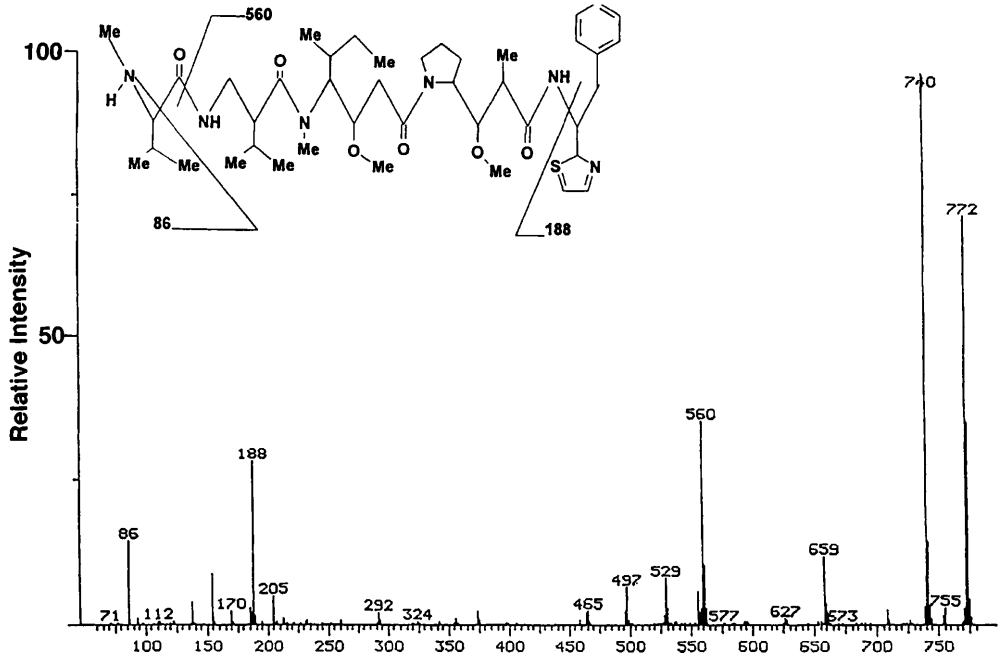

Fig. 6 Mass spectrum of M1, the *N*-demethyl metabolite of DOL-10 (m/z 740), and the corresponding fragmentation pattern (*inset*)

m/z 86, m/z 188, m/z 560 and m/z 740. As in the case of DOL-10, m/z 560 arose from cleavage between the carbonyl and the NH functionality of the dolaisoleucine portion of the molecule and m/z 754 arose from the parent ion by a neutral loss of CH_3OH (Table 1). The ion at m/z 86 resulted from α-cleavage at the nitrogen on the dolavaline portion of the molecule. For this metabolite, the protonated molecular ion, m/z 772, was 14 Da smaller than the corresponding protonated molecular ion for DOL-10 at m/z 786. The loss of 14 Da relative to the parent drug is consistent with the loss of a methyl group as occurs in the well-known metabolic pathway of oxidative demethylation. The position of demethylation was pinpointed by comparing the fragment ions arising from α-cleavage to the nitrogen on the dolavaline portion of the molecule for both parent drug (m/z 100) and major metabolite (m/z 86). It is clear that for the major metabolite, a loss of 14 Da in the structure of this fragment ion had occurred. This is strong evidence that the major metabolite of DOL-10 is an *N*-demethylated species.

The ESP/CID/MS spectrum of the minor metabolite of DOL-10 is shown in Fig. 7. The spectrum is characterized by an intense protonated molecular ion at

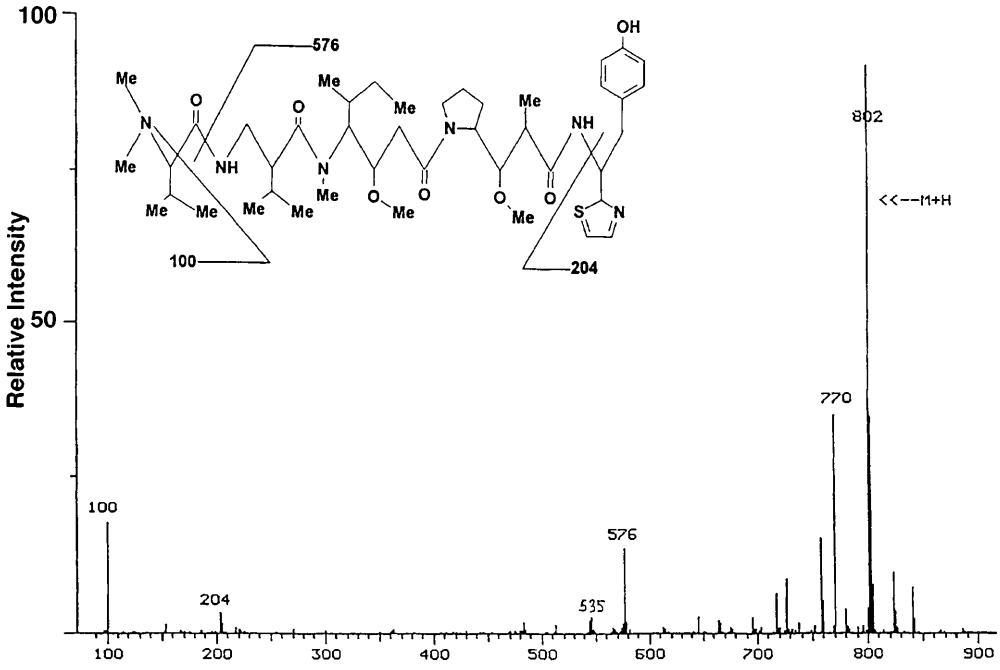

Fig. 7 Mass spectrum of M2, a hydroxy-DOL-10 metabolite, m/z 802. The fragmentation pattern is shown in the inset

m/z 802. In addition, fragment ions were observed at m/z 100, m/z 204, m/z 576 and m/z 770 (see Table 1). As in the case of DOL-10, m/z 100 resulted from α-cleavage at the nitrogen on the dolavaline portion of the molecule and m/z 770 arose from the parent ion by a neutral loss of CH_3OH. The ion fragment at m/z 204 resulted from cleavage between the NH functionality and the adjoining carbon on the dolaphenine portion of the molecule. Finally, the ion at m/z 576 arose from cleavage between the carbonyl and the NH functionality of the dolaisoleucine portion of the molecule.

For this metabolite, the protonated molecular ion, m/z 802, was 16 Da higher in mass than the corresponding protonated molecular ion for DOL-10 at m/z 786. The 16 Da increase for the protonated molecular ion of this metabolite relative to the parent drug is consistent with hydroxylation, also a very well-known metabolic pathway. Other fragment ions that were observed to shift by 16 Da were present at m/z 576 and m/z 204. The fragment ion at m/z 576 rules out the dolavaline portion of the molecule as the site for hydroxylation. However, the position of hydroxylation could be narrowed down by comparing the fragment ions arising from cleavage between the NH functionality and the adjoining carbon on the dolaphenine portion of the molecule for the parent drug (m/z 188) and minor metabolite (m/z 204). It is clear that for the metabolite, an increase of 16 Da in the structure of the dolaphenine portion of the molecule had occurred. This is strong evidence that hydroxylation had taken place in the dolaphenine portion of the molecule. The precise site of hydroxylation could not be determined with certainty. However, since aromatic hydroxylation is one of the most commonly encountered metabolic pathways, it is believed that hydroxylation takes place in the phenyl ring of DOL-10.

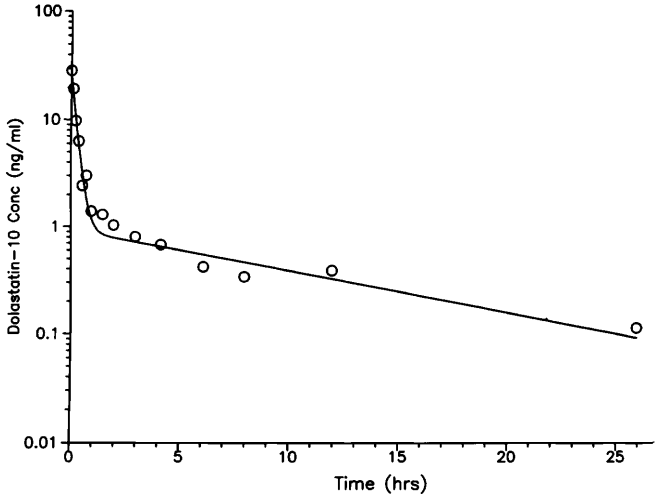

Fig. 8 Concentration-time data of DOL-10 obtained from a patient with cancer who was given the drug by rapid intravenous bolus injection at a dose of 65 μg/m^2 in a phase I trial

Clinical utility of the LC/MS assay

The principal application of this analytical technique was the serial determination of DOL-10 plasma concentrations in human subjects treated with this agent in a phase I toxicity study. While a discussion of DOL-10 human pharmacokinetics is beyond the scope of this report, the data presented in Fig. 8 demonstrate our initial experience in applying this analytical methodology to understanding the clinical pharmacology of this drug. These data, from a patient receiving DOL-10 at a dose of 65 μg/m^2 by rapid intravenous injection, were best described by a three-compartment open model. The

Table 2 Structural-indicating CID fragments

Compound	MH^+	Isovaline	Isoleucine	Isophenine
Dolastatin-10	*m/z* 786	100	560	188
Demethyl DOL-10	*m/z* 772	86	560	188
Hydroxy DOL-10	*m/z* 802	100	576	204

peak plasma concentration for DOL-10 was 29.3 ng/ml, with plasma clearance estimated to be 3.7 l/h per m^2, and a volume of distribution of 46.7 l/m^2. The only detectable metabolite in these human plasma samples was M1, the *N*-demethyl species, which peaked approximately 0.75 to 1.5 h after DOL-10 administration, and never exceeded 1/50th of the simultaneous DOL-10 plasma concentration (data not shown). The concentration of DOL-10 exceeded the limits of quantitation throughout the 24-h sampling period, demonstrating the utility of this procedure for the study of DOL-10 pharmacokinetics.

Discussion

The aim of this study was to develop a highly specific and sensitive assay for DOL-10 and its metabolites in plasma samples. Although a radioimmunoassay-based assay has recently been reported for determination of DOL-10 in mouse plasma samples [13], the present report is the first to describe an analytical assay suitable for separation, detection, quantitation and structural characterization of DOL-10 and its metabolites in human plasma samples, including plasma from patients receiving this drug in phase I trials. Our recent work has shown that DOL-10 can be analyzed by high-performance capillary electrophoresis using low wavelength UV detection; the sensitivity of the assay using 1 ml of plasma, however, is only 25 ng/ml [14]. Pettit et al. [15] have reported a reverse-phase HPLC assay for both DOL-10 and DOL-15 in a synthetic mixture matrix; this method, however, was developed to determine the purity of both drugs without consideration to biological samples and the necessity for sufficient sensitivity. A radioimmunoassay for DOL-10 has been reported by Aherne et al. [13] with a limit of detection (LOD) in mouse plasma of 2.5 ng/ml. DOL-15 cannot be used as an IS in this procedure as it cross-reacts with the rabbit antiserum. A nonspecific bioassay for determination of DOL-10 which is based on growth inhibitory activity against the L1210 murine leukemia cell has been recently reported by Reid et al. [16]. Besides DOL-10, all cytotoxic species produced in this assay are quantified. Although the assay is sensitive with a lower LOD of 0.5 ng/ml, cytotoxicity produced by metabolites cannot be distinguished from that produced by DOL-10 in this procedure. In contrast to these published methods for determination of DOL-10, the LC/MS assay presented here was shown to provide excellent sensitivity with acceptable quantitation at 5 pg/ml extracted from human plasma. The relative simplicity and ruggedness of this assay make it potentially useful in the routine analysis of clinical plasma samples.

Previous analytical studies of DOL-10 have been performed by Pettit et al. [1] in an attempt to ascertain the complete structure of this novel oligopeptide. Some of these experiments involved the use of high resolution electron impact MS to determine the correct amino acid sequence. Their CID data on the protonated DOL-10 (m/z 786) showed fragmentation of the precursor ion to give a demethoxylated species (m/z 783), a fragment ion from the tripeptide C-terminus (m/z 560), a fragment ion from dolaphenine (m/z 188), a fragment ion from dolavaline (m/z 100) and other product ions which are in agreement with our MS results for DOL-10.

Preliminary results obtained from analyses of a patient treated with DOL-10 showed parent compound as well as a *N*-demethylated species present; no hydroxylated species generated in vivo were detected. There was rapid distribution and elimination of the parent drug after intravenous bolus administration of DOL-10 associated with a rapid appearance of the *N*-demethylated metabolite. Overall, DOL-10 appears to be rapidly metabolized to more polar products: in vitro by rat liver to the major *N*-demethylated species and the minor hydroxylated species and, presumably, in vivo in humans to the *N*-demethylated species. Determination of the relative pharmacodynamic and cytotoxic properties of the *N*-demethylated DOL-10 compound may be important in future studies. Miyazaki et al. [17], for example, have reported that *N*-demethylated DOL-10 (synthesized in their laboratory) retained activity comparable to DOL-10 when evaluated against P388 leukemia in mice. Finally, it is also worth noting that DOL-15, the IS used in the present study, may also soon be developed as an anticancer compound [7, 18]. Replacing DOL-15 with DOL-10 as an IS using the present LC/MS method would provide a suitable method for the quantitation of this novel dolastatin compound.

References

1. Pettit GR, Kamano K, Herald CL, Tuinman AA, Boettner FE, Kizu H, Schmidt JM, Bacztnskyi L, Tomer KB, Bontems RJ (1987) The isolation and structure of a remarkable marine animal antineoplastic constituent: dolastatin-10. J Am Chem Soc 109: 6883
2. Pettit GR, Singh SB, Hogan F, Lloyd-Williams P, Herald DL, Burkett DD, Clewlow PJ (1989) The absolute configuration and synthesis of natural (−)dolastatin-10. J Am Chem Soc 111: 5463
3. Bai R, Pettit GR, Hamel E (1990) Dolastatin-10, a powerful cytostatic peptide derived from a marine animal. Inhibition of tubulin polymerization mediated through the vinca alkaloid binding domain. Biochem Pharmacol 39: 1941
4. Jacobsen SEW, Ruscetti FW, Longo DL, Keller JR (1991) Antineoplastic dolastatins: potent inhibitors of hematopoietic progenitor cells. J Natl Cancer Inst 83: 1672
5. Steube KG, Grunicke D, Pietsch T, Gignac SM, Pettit GR, Drexler HG (1992) Dolastatin-10 and dolastatin-15: effects of two natural peptides on growth and differentiation of leukemia cells. Leukemia 6: 1048

6. Hu ZB, Gignac SM, Quentmeier H, Pettit GR, Drexler HG (1993) Effects of dolastatins on human B-lymphocytic leukemia cell lines. Leukemia Res 17: 333
7. Beckwith B, Urba WJ, Longo DL (1993) Growth inhibition of human lymphoma cell lines by the marine products dolastatins 10 and 15. J Natl Cancer Inst 85: 483
8. Pettit GR, Kamano Y, Herald L, Fujii Y, Kizu H, Boyd MR, Boettner FE, Doubek DL, Schmidt JM, Hapuis JC, Michel C (1993) Isolation of dolastatins 10–15 from the marine mollusc *Dolabella auricularia*. Tetrahedron 49: 9151
9. Bai R, Pettit GR, Hamel E (1990) Structure-activity studies with chiral isomers and with segments of the antimitotic marine peptide dolastatin 10. Biochem Pharmacol 40: 1859
10. Bai R, Roach MC, Jayaram SK, Barkoczy J, Pettit GR, Luduena RF, Hamel E (1993) Differential effects of active isomers, segments and analogs of dolastatin-10 on ligand interactions with tubulin. Correlation with cytotoxicity. Biochem Pharmacol 45: 1503
11. D'Argenio DZ, Schmitzky A (1979) A program package for simulation and parameter estimation in pharmacokinetic systems. Comput Programs Biomed 9: 115
12. Gibaldi M, Perrier D (1982) Pharmacokinetics. Marcel Dekker, New York, pp 92–109
13. Aherne W, Hardcastle A, Valenti M, Bryant A, Rogers P, Srirangam JK, Pettit GR, Kelland LR (1995) In vitro and in vivo antitumor evaluation of dolastatin (Dol) 10 and 15 and their measurement in plasma by radioimmunoassay (RIA). Proc Am Assoc Cancer Res 36: 385
14. Huie WR, Madden T, Newman RA (1997) High-performance capillary electrophoresis measurement of dolastatin-10. J Chromatogr B (in press)
15. Pettit GR, Kantoci D, Herald DL, Barkoczy J, Slack JA (1994) Procedures for the analyses of dolastatins 10 and 15 by high performance liquid chromatography. J Liq Chromatogr 17: 191
16. Reid JM, Walker DL, Ames MM (1995) Evaluation of an L1210 bioassay and HPLC for determination of dolastatin 10 in human and murine plasma. Proc Am Assoc Cancer Res 36: 364
17. Miyazaki S, Kobayashi M, Natsume T, Gondo M, Mikami T, Sakakibara K, Tsukagoshi S (1995) Synthesis and antitumor activity of novel dolastatin 10 analogues. Chem Pharm Bull 43: 1076
18. Bai R, Fredman SJ, Pettit GR, Hamel E (1992) Dolastatin 15, a potent antimitotic depsipeptide derived from *Dolabella uricularia*. Interaction with tubulin and effects on cellular microtubules. Biochem Pharmacol 43: 2637