Chemical Characterization of the Degradation Products of Vinblastine Dihydrogen Sulfate

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ABSTRACT

Vinblastine was incubated in 0.2 M glycine buffer (pH 7.4 or 8.8) containing bovine serum albumin (1%) at 37°C for 72 h. The reaction mixture was extracted with CH2Cl2, and the extract gave 6 major peaks (A, B, C, D, E, and F) with retention times of 5.0, 7.5, 11.0, 13.0, 23.0, and 30.0 min, respectively, in a high-performance liquid chromatography system (μBondapak C18 column; solvent, 50% MeOH in 10 mM KH2PO4, pH 4.5; flow rate, 1.2 ml/min; detector, 254 nm). Vinblastine in this system corresponded with peak C, and its spectral data were identical to those of the parent compound. The UV, infrared, and mass spectral properties of these peaks were as follows [UV (λmax): infrared (cm⁻¹); mass spectrum (m/z)]: peak A: 214, 266, and 315 nm; 3464, 2850, and 1730; and 769 (MH⁺); peak B: 213, 258, 285, and 295 nm; 3457, 2951, 2580, and 1734; and 809 (MH⁺); peak C: 214, 266, 292, and 312 nm; 3457, 2951, and 1734; and 811 (MH⁺); peak D: 212, 265, 283, and 312 nm; 3467, 2915, and 1734; and 825 (MH⁺); peak E: 212, 260, 285, 294, 313 nm; 3479, 2850, and 1734; and 852 (MH⁺); and peak F: 212, 265, 283, and 312 nm; 3407, 2857, and 1734; and 807 (MH⁺). These data suggest the following tentative structures for the degradation products: peak A, 4-deacetylvinblastine; peak B, 19'-hydroxy-3',4'-dehydrovinblastine; peak D, an isomer of vinblastine; peak E, 19'-oxovinblastine; and peak F, 3',4'-dehydro-19'-oxovinblastine. The structure of peak A as 4-deacetylvinblastine was further confirmed by chemical synthesis.

INTRODUCTION

Catharanthus alkaloids VBL¹ and vincristine (Chart 1A) are clinically important drugs in the treatment of various cancers in humans. They act as powerful inhibitors of cell division by binding with tubulin and thus interfere with the polymerization of microtubules (1, 2). These alkaloids differ only slightly in structure; namely, the —CH3 group on the vindoline N-atom in VBL is substituted by a —CHO group in vincristine. VBL is widely used in the treatment of Hodgkin's disease and testicular neoplasm. The clinical use of VBL is limited due to its myelosuppression. Their action is potentiated in persons with myeloma and other types of tumors. They act as powerful inhibitors of cell division by binding with tubulin and thus interfere with the polymerization of microtubules (1, 2). These alkaloids differ only slightly in structure; namely, the —CH3 group on the vindoline N-atom in VBL is substituted by a —CHO group in vincristine. VBL is widely used in the treatment of Hodgkin's disease and testicular neoplasm. The clinical use of VBL is limited due to its myelosuppression. Their action is potentiated in persons with myeloma and other types of tumors.

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² The abbreviations used are: VBL, vinblastine; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; IR, infrared; MS, mass spectrum; DCI MS, desorption chemical ionization mass spectra; BSA, bovine serum albumin. Received 3/14/85; revised 8/8/85; accepted 8/9/85.

MATERIALS AND METHODS

Chemicals. Vinblastine dihydrogen sulfate (NSC 49842) was obtained from the Drug Development Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. The purity of this alkaloid was checked by thin-layer chromatography on a 0.25-mm silica gel glass plate (Anatech, Newark, DE) in a solvent system of diethylether:n-propanol:triethylamine (v/v), 24:16:2, and a single spot with a Rf value of 0.76 was obtained. The purity was also checked by HPLC, and a single sharp peak with retention time of 11.0 min was obtained under the conditions described below. In mass spectroscopy (Ribermag, R10-10C, GC-MS), a protonated mass of 811 (M⁺H) was obtained. Glycine was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI), and BSA (96-99%) was obtained from Sigma Chemical Company (St. Louis, MO). Tween 20 (polyisorbate 20) was obtained from ICI American, Inc., Wilmington, DE. The alkaloid and BSA were stored at 4°C in a refrigerator. All reagents and solvents used in this work were of analytical grade quality.

Preparation of Glycine Buffer. A 0.2 M solution of glycine buffer was prepared by dissolving 1.502 g of glycine and 1 g of BSA in deionized water. The pH of this solution was adjusted to 4.0, 7.4, or 8.8 with aqueous NaOH or HCl solution, and the volume was made up to 100 ml.

High-Performance Liquid Chromatography. HPLC was carried out on a Waters Associates (Milford, MA) model ALC-201 and 202 with a solvent delivery system model 6000A, universal liquid chromatographic injector model U6K, a differential UV detector, and a Linear Instruments Corporation recorder. The experimental conditions were as follows: Waters Associates μBondapak C18 P/N 27324, 10-μm reverse-phase steel column (4.6 mm x 30 cm); isotropic solvent system, 50% MeOH in 10 mM KH2PO4 (pH 4.5); flow rate, 1.2 ml/min; detector, 254 nm.

Instrumentation. UV spectra of vinblastine dihydrogen sulfate and its degradation products were scanned in the range of 200–400 nm on a Varian 5000 binary liquid chromatograph provided with HP 1040A multichannel LC detector after injection of the methanolic solution of the sample. IR spectra of each HPLC peak fraction were obtained in the form of a thin film on a KBr window with a Perkin-Elmer model 580-B spectrophotometer attached to a data station. The absorption maxima reported in wave numbers (cm⁻¹) were calibrated with respect to the absorption band of polystyrene at 1601 cm⁻¹. The weak spectrum of each sample was accumulated 5 to 8 times on the data station, and the accumulated spectrum was recorded on chart paper after smoothing of the spectrum and was followed by base line correction. The ammonia DCI MS of the samples was obtained at 300–950 amu and 300–450 mA on a Ribermag R10-10C GC-mass spectrometer with an upper mass limit of 1500 amu having desorption chemical ionization capabilities.

Synthesis of 4-Deacetylvinblastine. 4-Deacetylvinblastine was synthesized according to the procedure of Hargrove (3, 4). Three mg of vinblastine dihydrogen sulfate dissolved in 2 ml anhydrous methanol were saturated at 0°C with dry hydrogen chloride gas prepared by adding concentrated HCl to concentrated H2SO4. The reaction mixture was kept at room temperature for 12 h. It was quantitatively transferred into a separating funnel, diluted with water, adjusted in its pH to 9–10, and extracted three times with 1500 amu having desorption chemical ionization capabilities.

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several times after multiple injections. The sample was extracted with methylene chloride at pH 9-10 and evaporated in a current of N2. The spectral data on the sample were as follows: UV (λmax): 214, 266, and 315 nm; IR (cm⁻¹): 3457, 3045, 2915, 2850, 1727, 1611, 1496, 1466, 1376, 1232, 1108, 1036, 816, 732, 684, and 586; and MS (m/z): 769 (MH⁺), 755 (MH⁺ - OCH₃+NH₃), and 571 (MKT - CsH₂O). These data were in agreement with those of the published reports on 4-deacetylvinblastine (3, 4).

RESULTS

Incubation of VBL at 37°C in glycine buffer of pH 8.8 for 72 h gave, by HPLC analysis, six major peaks, A, B, C, D, E, and F, with retention times of 5.0, 7.5, 11.0, 13.0, 23.0, and 30.0 min, respectively (Chart 2). Similar results were obtained at pH 4.0 and 8.8 for 72 h at 37°C in a degradation of 8, 20, and 26%, respectively (data not shown). By 24 h at 37°C, about 8.5% of VBL was degraded into its products at pH 8.8 (data not shown). No apparent growth of microorganisms was noted during the 72-h incubation period, and degradation products formed in the presence of sodium azide (1 mw) or Tween 20 (0.05%) gave a HPLC profile similar to that of the control (data not shown).

Characterization of the Degradation Products. Peak A co-chromatographed in the HPLC system with the synthetically prepared sample of 4-deacetylvinblastine. The UV spectrum of this peak fraction showed the absorbance maxima (λmax) at 214, 266, and 315 nm. The IR (cm⁻¹) spectrum exhibited the bands at 3457, 2915, 2850, 1734, 1607, 1456, 1372, 1235, 1036, 732, 664, and 588; and MS (m/z): 769 (MH⁺), 755 (MH⁺ - H₂O), and 779 (MH⁺ - CH₃OH) (Chart 3A). These data are consistent with the published report on the structure of 19'-hydroxy-3',4'-dehydronvinblastine (5), and therefore this structure is tentatively assigned for peak A.

Peak C coeluted with the standard VBL with a retention time of 11.0 min in the HPLC profile. The UV λmax values of 214, 266, 292, and 312 nm were found for peak C. The IR spectrum showed the bands at 3457, 3038, 2951, 2856, 2850, 1734, 1611, 1500, 1452, 1366, 1293, 1235, 1138, 1036, 932, 823, 736, and 584 cm⁻¹. The mass spectral study revealed the formation of the protonated molecular ion (MH⁺) at m/z 809. Other mass peaks were observed at m/z 795 (MH⁺ - OCH₃+NH₃), 779 (MH⁺ - OCH₃), 770 (MH⁺ - C₂H₅), and 752 (MH⁺ - OC₆H₅) (Chart 3C). These data are consistent with the published report on the structure of 19'-hydroxy-3',4'-dehydronvinblastine (5), and therefore this structure is tentatively assigned for peak B.

Peak E coeluted with the standard VBL with a retention time of 11.0 min in the HPLC profile. The UV λmax values of 214, 266, 292, and 312 nm were found for peak E. The IR spectrum showed the bands at 3467, 2915, 2850, 1734, 1683, 1640, 1611, 1542, 1452, 1366, 1293, 1235, 1138, 1036, 932, 823, 736, and 584 cm⁻¹. The mass spectral study revealed the formation of the protonated molecular ion (MH⁺) at m/z 811. The loss of H₂O from the parent ion gave the peak at m/z 793 for the ion (MH⁺ - H₂O). Other peaks were observed at m/z 779 (MH⁺ - CH₃OH) and 753 (MH⁺ - OOC₆H₅) (Chart 3D). These spectral data were in agreement with the data on a standard HPLC purified sample of vinblastine.

Partial chemical characterization of peak F was based on its UV, IR, and MS data. The UV spectrum showed λmax at 212, 265, 283, and 312 nm. The IR (cm⁻¹) spectrum showed the bands at 3479, 2922, 2850, 1734, 1658, 1500, 1459, 1380, 1238, 1163, 1043, and 660. Ammonia DCI MS revealed the formation of the protonated molecular ion (MH⁺) at m/z 825. The daughter ion was observed at m/z 809 due to the loss of oxygen (Chart 3E). These data are in agreement with those of the literature report on 19’-oxovinblastine (6).

DISCUSSION

There is limited information in the literature on the metabolism of VBL. 4-Deacetylvinblastine has been reported to be a urinary metabolite of VBL in dog (7) and in human (8) based on thin-layer chromatographic analysis. Following i.p. injection of [³H]-VBL into tumor-bearing mice, five peaks of radioactivity have
DEGRADATION OF VINBLASTINE SULFATE

Chart 2. Separation and isolation of the degradation products of vinblastine. Two mg of vinblastine dihydrogen sulfate were dissolved in 1 ml methanol. To this solution, 10 ml of glycine buffer (pH 8.8) were added. The test tube was wrapped with aluminum foil and incubated at 37°C for 72 h in a shaking water bath. At the end of incubation, the reaction mixture was transferred into a separating funnel, and the pH of this mixture was adjusted to between 9 and 10 with sodium carbonate solution. It was extracted three times with methylene chloride (% vol of the reaction mixture each time). The organic extracts were combined and reextracted with 0.01 N H₂SO₄ thrice. The combined sulphuric acid fractions were adjusted between pH 9-10 as above and extracted with methylene chloride three times. The combined organic phase was dried with anhydrous Na₂SO₄ and evaporated under a stream of N₂. The residue was dissolved in 90% MeOH-0.01 N H₂SO₄ and analyzed by HPLC according to the conditions described in “Methods and Materials.” Six peaks, A, B, C, D, E, and F, with retention times of 5.0, 7.5, 11.0, 13.0, 23.0, and 30.0 min, respectively, were obtained. Peaks A, B, C, and D were collected at Aₑₓₜ = 2.0, and peaks E and F were collected at Aₑₓₜ = 0.15. Each peak fraction was collected separately many times after multiple injections, and these fractions were extracted with methylene chloride and dried under N₂. These samples were used for UV, IR, and MS analysis.

been obtained by HPLC (9). Among these, one peak coelutes with a standard preparation of 4-deacetylvinblastine. The chemical nature of other peaks is not known. 4-Deacetylvinblastine has been also shown to be 4 to 5 times more potent as an antitumor agent relative to vinblastine (8, 10).

The spectral data of peak A are in agreement with the spectral properties of the synthetic 4-deacetylvinblastine. Hence, peak A is assigned the structure of 4-deacetylvinblastine. Tentative structures for other degradation products B, D, E, and F have been suggested as 19'-hydroxy-3',4'-dehydrovinblastine, an isomer of vinblastine, 19'-oxovinblastine, and 3',4'-dehydro-19'-oxovinblastine, respectively, on the basis of their UV, IR, and MS data. The UV spectral data indicate that both the indole and dihydroindole chromophores are intact in the degradation products. MS data also reveal the dimeric nature of these compounds. IR spectral data show the presence of characteristic...
functional groups of the VBL type molecules in these compounds. Confirmation of these structures through synthesis and nuclear magnetic resonance spectroscopy is under investigation.

In conclusion, the present paper describes the preliminary spectral characterization of five degradation products of VBL formed following in vitro incubation in glycine buffer. These studies should serve as a precursor to the investigations on the metabolism of VBL in experimental animals.

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