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Note

High-performance liquid chromatographic determination of deoxyspergualin in dog plasma with ultraviolet detection

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Deoxyspergualin (NKT-01; 1-amino-19-guanidino-11-hydroxy-4,9,12-triazanonadecane-10,13-dione trihydrochloride, I) (Fig. 1) is a synthetic spermidine derivative with strong immunosuppressive and antitumoral activity [1–4]. In preclinical study, I has demonstrated several potential advantages over inhibitory effect for rejection after organ transplantation [5–10].

Recently, we developed a gas chromatographic–mass spectrometric (GC–MS) method that exhibits high sensitivity and selectivity for determination of I in plasma [11]. However, this method requires a tedious derivatization process, and it is inevitable to convert I into a volatile derivative without fission of α-hydroxyglycine moiety. Thus we have attempted to develop a simpler assay for quantification of I as an intact molecule in dog plasma. We report

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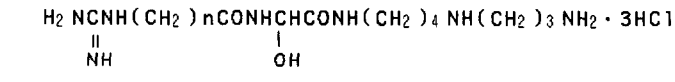

1: n=6

2: n=7

Fig. 1. Structures of I (1) and the internal standard (2).

here a simple, sensitive and specific ion-pair reversed-phase high-performance liquid chromatographic (HPLC) method for the determination of I. Rapid assay by this method appears to be of particular interest for therapeutic monitoring and/or pharmacokinetic study of I in dogs.

EXPERIMENTAL

Samples and reagents

Compound I (NKT-01) and glyoxyloylspermidine were supplied by Takara Shuzo (Shiga, Japan). Sodium pentanesulphonate was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). 8-Guanidinooctanamide was purchased from Aldrich (Milwaukee, WI, U.S.A.). CM-Sephadex C-25 and Sep-Pak C_{18} cartridges were purchased from Pharmacia (Uppsala, Sweden) and Millipore-Waters Assoc. (Milford, MA, U.S.A.), respectively. Other solvents and reagents were of analytical or HPLC grade. The internal standard was synthesized in our laboratories from 8-guanidinooctanamide and glyoxyloylspermidine according to the method of Umeda et al. [12].

Chromatography

The chromatographic system consisted of a Hitachi Model 635A pump, a Hitachi Model 056 recorder, a Shimadzu Chromatopac E1A integrator and a Rheodyne Model 7125 sample injector. A Shimadzu Model SPD-2AS variable-wavelength UV detector set to a wavelength of 205 nm was employed. Chromatographic separation was obtained on a reversed-phase Cosmosil $5C_{18}$-P (150 mm × 4.6 mm I.D., Nacalai Tesque, Kyoto, Japan), protected by a Guard Pak™ pre-column module with Guard Pak C_{18} cartridge (Millipore-Waters). The mobile phase consisted of 9% acetonitrile in 0.01 *M* phosphate buffer (pH 3) containing 5 m*M* sodium pentanesulphonate. The mixture was filtered and degassed, and the flow-rate was 1.0 ml/min.

Sample preparation

To 1 ml of dog plasma spiked or infused with I, was added 10 μg of internal standard. The resulting plasma was mixed well, then diluted with distilled water

(10 ml) and transferred to a small column of CM-Sephadex C-25 (5 cm×0.6 cm I.D.). The column was washed with 0.3 *M* sodium chloride (10 ml). Then I and the internal standard were eluted with 0.4 *M* sodium chloride (10 ml) and extracted from the eluate by passing through a Sep-Pak C_{18} cartridge. The cartridge was washed with distilled water (5 ml), and then the compounds were eluted with methanol (10 ml). The eluate was evaporated to dryness under reduced pressure. The residue was dissolved in the mobile phase (0.2 ml) and vortex-mixed, and a 50-μl aliquot was injected into the column.

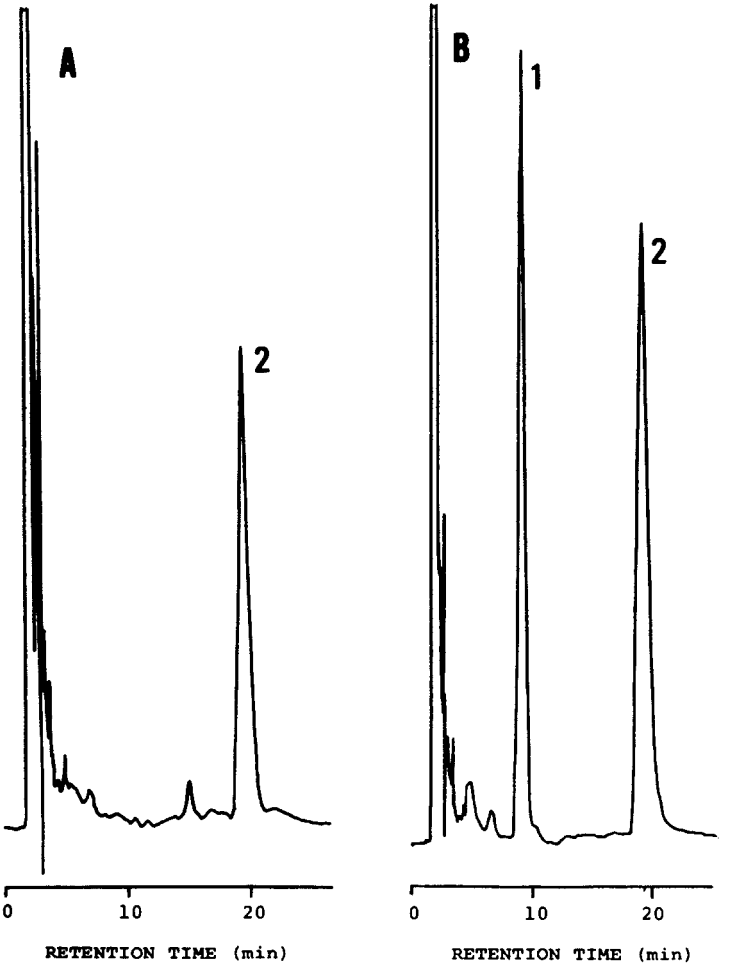

Fig. 2. Chromatograms of the extract obtained from dog plasma spiked with internal standard (A) and with I and internal standard (B). Peaks: 1 = I; 2 = internal standard.

RESULTS AND DISCUSSION

On the basis of our previous GC–MS study [11], we decided to extract and purify I using a CM-Sephadex C-25 column and subsequent elimination of sodium chloride by a Sep-Pak C_{18} cartridge. I and the internal standard are coeluted from CM-Sephadex C-25 with 0.4 *M* sodium chloride. The reversed-phase HPLC with sodium pentanesulphonate as a modifier is a simple and sensitive way of monitoring the therapeutic concentration of I in dog plasma.

TABLE I

PRECISION OF THE HPLC METHOD

	Concentration (μg/ml)	Coefficient of variation (%)
Inter-assay ($n=5$)	1	7.8
	5	1.8
Intra-assay ($n=5$)	1	3.9
	5	6.5

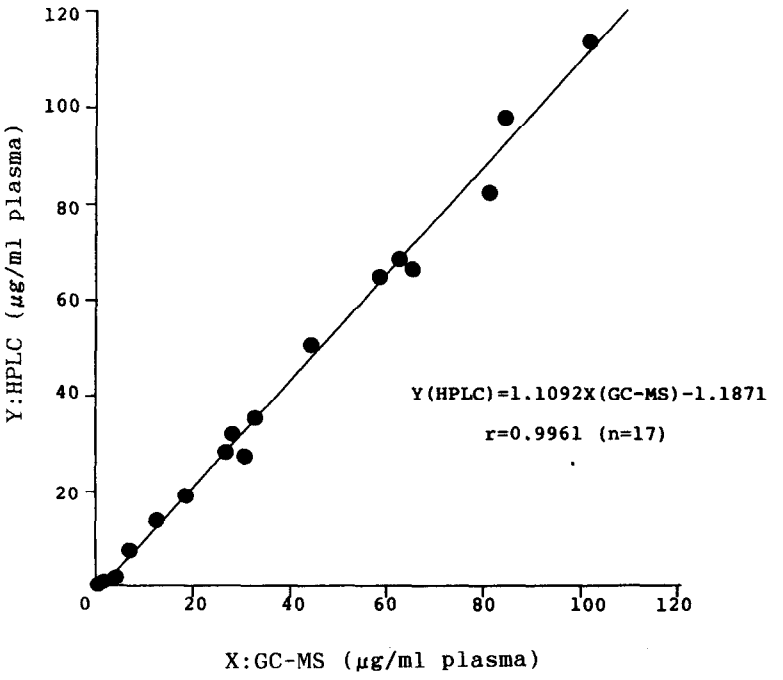

Fig. 3. Correlation between I levels obtained from the same samples of dog plasma ($n=17$) by the present HPLC method (Y) and a GC-MS method (X).

Chromatograms obtained from drug-free and spiked plasma are shown in Fig. 2. Peaks of I and internal standard were identified by their retention times: under the chromatographic conditions described, these were 8.6 min and 18.6 min, respectively. The sharp resolution of the I peak and the absence of interfering peaks indicates the high degree of selectivity achieved.

The reproducibility of the procedure was found to be satisfactory (Table I). The inter-assay coefficients of variation (C.V.) for replicate analysis of 1 and 5 μg/ml plasma samples were 7.8 and 1.8%, respectively. The intra-assay C.V. values for the same concentrations were 3.9 and 6.5%, respectively. The calibration graph was constructed by adding known amounts of I to drug-free dog plasma in the range 1–10 μg/ml. Least-squares analysis of peak-height ratio versus I concentration showed a linear relationship with a correlation coefficient of 0.9975.

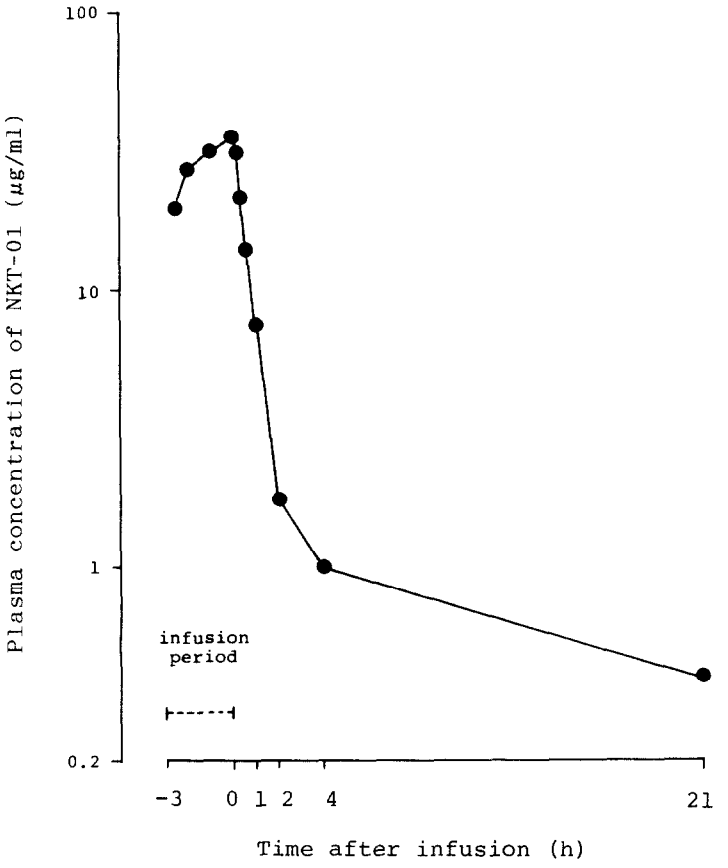

Fig. 4. Plasma concentration of I in a dog during and after intravenous infusion of I at a dose of 15 mg/kg per h.

In order to confirm the validity of the method, each plasma sample was analysed by this method and also by a conventional GC–MS method. The results (Fig. 3) indicate a good correlation between the two methods ($r = 0.9961$). The limit of quantification was ca. 50 ng/ml for dog plasma at a signal-to-noise ratio of 5.

In conclusion, the present study demonstrated that I in dog plasma can be accurately and precisely determined by CM-Sephadex C-25 column and Sep-Pak C_{18} cartridge purification, followed by isocratic reversed-phase HPLC and detection at 205 nm. This method is simple, selective, sensitive and rapid. It has now been successfully applied to the pharmacokinetics study of I in dogs, and a typical result is shown in Fig. 4.

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