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Note

High-performance liquid chromatographic determination of tranilast in plasma

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Tranilast [N-(3,4-dimethoxycinnamoyl)anthranilic acid] is a new remedy for allergic diseases, which has been developed in Japan as a result of extensive drug design based on research into the antiallergic properties of some ingredients of nandin (*Nandina domestica*). Unlike the symptomatic drugs used so far, the mechanism of action of tranilast lies in the inhibition of release of chemical mediators and it approaches closer to the basic allergic reaction [1, 2]. Tranilast has been used for the treatment of bronchial asthma and is usually combined with theophylline and other antiasthmatic drugs.

We found that free levels of theophylline in plasma increased when given in combination with tranilast [3]. It is important to investigate the relationship between tranilast plasma levels and the variation in theophylline plasma levels. However, there are very few reports about the determination of tranilast in plasma [1]. In this paper we describe a simple and reproducible high-performance liquid chromatographic (HPLC) method using a reversed-phase column and UV detector.

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EXPERIMENTAL

Materials

Tranilast and N-cinnamoyl anthranilic acid were kindly supplied by Kissei, Matsumoto, Japan. All chemicals were of reagent grade and used without further purification.

Procedures

A 100-μl volume of methanol containing N-cinnamoyl anthranilic acid as an internal standard (25 μg/ml) were added to 100 μl of plasma in a test tube, and agitated with a vortex mixer. After centrifugation at 12 000 *g* for 5 min, 100 μl of the supernatant were centrifuged at 12 000 *g* for 5 min once again; 10 μl of the supernatant were injected into the HPLC system.

Chromatographic conditions

A liquid chromatograph (Hitachi 638-50) equipped with a multi-wavelength UV detector (Hitachi 635 M) was used. A reversed-phase column (Radial Pak C_{18}, 10 μm, 10 cm × 5 mm I.D.; Waters Assoc.) was used at room temperature. The mobile phase consisted of 0.01 *M* dipotassium hydrogen phosphate buffer—acetonitrile (4:3). Before mixing, the buffer was brought to pH 3.5 with phosphoric acid. The flow-rate was 1.5 ml/min. The wavelength was 280 nm at 0.04 a.u.f.s.

Calibration graph

Two plasma standard curves were generated over the range 0.5—10 μg/ml and 5—50 μg/ml tranilast. The ratios of the peak height of tranilast to that of N-cinnamoyl anthranilic acid (internal standard) were used to construct a calibration graph.

Monitoring of plasma concentrations

The experiment was performed on two healthy subjects each aged 32 years, weighing 63 and 80 kg. Tranilast (200 mg in capsule form, Rizaben; Kissei) were administered orally with 100 ml of tap water. Plasma samples were obtained just before the administration and at 0.5, 1, 2, 3, 4, 6, 8, 10 and 24 h after administration. Food and beverages were not restricted after administration.

RESULTS AND DISCUSSION

Selectivity

Fig. 1 shows the chromatogram of blank plasma, plasma sample spiked with 30 μg/ml tranilast, and a plasma sample at 3.0 h after the administration of tranilast to a healthy subject. Tranilast and internal standard were well separated from endogenous substances.

Two calibration curves of peak height ratio were linear with a correlation coefficient of 0.996 (0.5—10 μg/ml) and 0.997 (5—50 μg/ml). The coefficients of variation at 0.5, 5 and 23 μg/ml of plasma were 4.28% (n = 8), 2.43% (n = 10) and 1.31% (n = 15), respectively. The relative recovery of tranilast from

plasma containing 30 μg/ml was estimated by comparing it with the recovery from an aqueous sample (distilled water) and was found to be 99.3 ± 1.8% (mean ± S.D., n = 10). Plasma was spiked with tranilast by the same procedure as described for the calibration graph. The limit of sensitivity for quantitation was 0.5 μg/ml plasma.

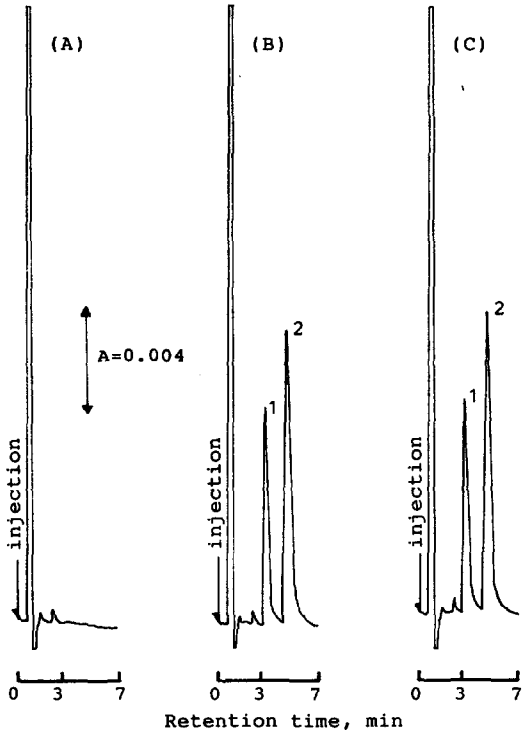

Fig. 1. High-performance liquid chromatograms of (A) blank plasma, (B) plasma sample spiked with 30 μg/ml tranilast and (C) a healthy subject. Peaks: 1 = tranilast, 2 = internal standard.

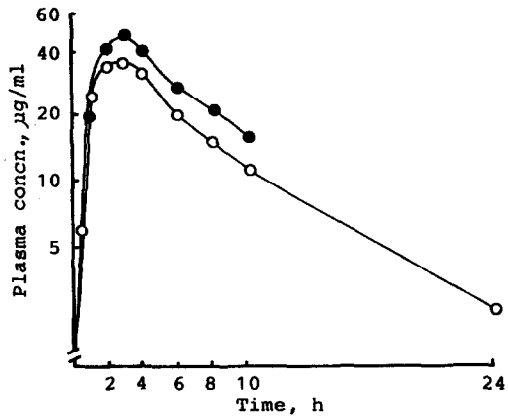

Fig. 2. Plasma concentration profiles of tranilast after oral administration of 200 mg of tranilast to two volunteers.

Plasma concentration profile

Plasma concentrations of tranilast were monitored using the newly developed assay method. The plasma concentration profiles are shown in Fig. 2. Peak plasma concentrations of tranilast were 48.0 and 34.4 μg/ml, and the elimination half-lives were 3.8 and 4.1 h, respectively.

It is possible to determine low plasma concentrations of tranilast rapidly, reproducibly and sensitively by the method described in this report. Our results suggest that the method is useful for both therapeutic drug monitoring and pharmacokinetic studies.

The effect of the concentration of tranilast in plasma on the variation of theophylline plasma levels is currently being examined.

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