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Note

Simultaneous determination of carbidopa, levodopa and 3,4-dihydroxyphenylacetic acid using high-performance liquid chromatography with electrochemical detection

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The combination of levodopa (3,4-dihydroxyphenylalanine) and carbidopa (L-α-hydrazino-3,4-dihydroxy-L-α-methylcinnamic acid) in the treatment of Parkinson's disease has proved to be very successful [1]. The effect of levodopa on Parkinson's disease is considered to be due to its metabolic product dopamine (3-hydroxytyramine), which is produced by decarboxylation of levodopa [2]. Carbidopa instead is a decarboxylase inhibitor and is used to block the extracerebral metabolism of levodopa allowing more levodopa to reach the brain for conversion to dopamine [2].

Carbidopa, levodopa and its metabolites dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) have so far been determined separately or simultaneously by rather tedious methods [3—5]. Of the available methods high-performance liquid chromatography (HPLC) with electrochemical detection offers the best specificity and sensitivity for determination of catecholamines in any tissue [6]. However, the recent reports for the simultaneous determination of carbidopa and levodopa [7—9] are not sensitive or selective enough for quantification of these compounds with their metabolites in human plasma.

We report here a method for the simultaneous determination of these compounds from plasma using adsorption on alumina and HPLC with electrochemical detection. This method has been applied to the quantification of carbidopa, levodopa and DOPAC sampled continuously in human plasma after an oral dose of levodopa with carbidopa.

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EXPERIMENTAL

Reagents

Dihydroxybenzylamine (DHBA), dopamine and DOPAC were purchased from Sigma (St. Louis, MO, U.S.A.). Levodopa and carbidopa were obtained from Orion Pharmaceutical (Helsinki, Finland). Sodium octanesulfonic acid was from Eastman Kodak (Rochester, NY, U.S.A.). Alumina, activity grade I for chromatography, was from Merck (Darmstadt, G.F.R.) and it was prepared by the method of Anton and Sayre [10]. All other chemicals were of reagent grade and purchased from commercial sources.

Sample preparation

Blood samples (5 ml) were drawn by venipuncture and transferred immediately to tubes containing 0.1 ml of 10% metabisulfite and 0.1 ml of 1% EDTA in physiological saline solution. Samples were centrifuged at 600 *g* at 4°C; the plasma was removed and stored at −20°C until analysis.

Plasma samples (1 ml) were spiked with 50 ng of DHBA (the internal standard) in tubes containing 0.1 ml of 2.5 m*M* metabisulfite. Acid-washed alumina (100 mg) was added followed by 1.0 ml of 0.5 *M* Tris—EDTA, pH 8.6. The tubes were stoppered and shaken mechanically 5 min on a multitube vortexer. The samples were centrifuged, the supernatants discarded and the alumina was washed twice with 10 ml of water. The catecholamines were eluted with 0.5 ml of 0.2 *M* perchloric acid, and 20 μl of the eluate were injected into the liquid chromatograph.

Chromatography

The liquid chromatography—electrochemical detection system consisted of a Waters Model 6000A pump (Waters Assoc., Milford, MA, U.S.A.), a Rheodyne Model 7125 injector valve with a 20-μl sample loop (Rheodyne, Cotati, CA, U.S.A.), and a 3.2 mm × 250 mm 5 μm ODS Spherisorb column fitted with a 3.2 mm × 30 mm precolumn (Altex Scientific, Berkeley, CA, U.S.A.).

The electrochemical detector consisted of a TL-5 glassy carbon electrode and LC-4A potentiostat (Bioanalytical Systems, West Lafayette, IN, U.S.A.). The working electrode was set at +0.70 V relative to an Ag/AgCl reference electrode. The sensitivity was set 5 nA full-scale.

The elution of catecholamines from the column was carried out in isocratic mode at ambient temperature. The mobile phase was composed of 100 m*M* NaH_2PO_4, 20 m*M* citric acid, 1.25 m*M* sodium octanesulfonic acid, 0.15 m*M* sodium EDTA in 8% methanol. The pH was adjusted to 3.2 with sodium hydroxide. The flow-rate was 1 ml/min.

RESULTS AND DISCUSSION

As seen in Fig. 1A, all the compounds studied — levodopa, carbidopa, DOPAC, dopamine and DHBA (the internal standard) — are completely separated in 10 min, thus allowing the handling of about 40 samples per day. Fig. 1B shows a chromatogram of blank plasma after adsorption on alumina

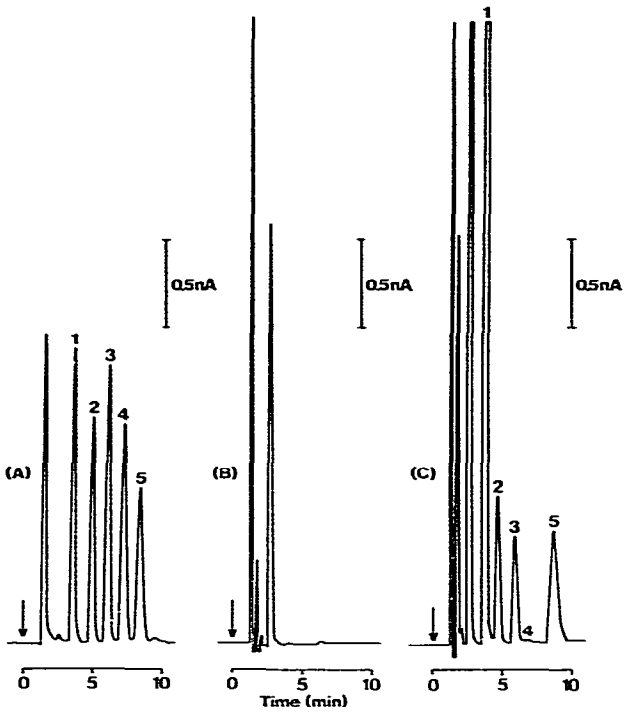

Fig. 1. Chromatograms of (A) catecholamine standards, (B) predose control plasma, and (C) plasma obtained from a volunteer 60 min after oral administration of 100 mg of levodopa with 25 mg of carbidopa and containing the internal standard (50 ng/ml). Peaks: 1 = levodopa; 2 = DHBA; 3 = DOPAC; 4 = dopamine; 5 = carbidopa. Chromatographic conditions as described under Experimental.

and perchloric acid extraction. Using the chromatographic conditions described no interfering peaks were detected. Fig. 1C demonstrates a typical chromatogram of a plasma sample from a volunteer after 1 h of concomitant oral administration of levodopa and carbidopa.

The recoveries of added catecholamines (50 ng/ml, n = 10) from plasma were: levodopa 60.7 ± 7.6%, carbidopa 51.3 ± 7.3%, DOPAC 54.1 ± 8.5% and DHBA 72.2 ± 7.8%. Repeated determinations (n = 10) of four parallel standard preparations at the concentration 50 ng/ml gave the following coefficients of variation: levodopa 1.7%, carbidopa 3.6%. DOPAC 3.8% and DHBA 3.2%. Standard curves were linear over the range 1—500 ng/ml, except for carbidopa, for which the lower limit of detection was 15 ng/ml.

The described HPLC method with electrochemical detection provides a fast, reliable, uncomplicated method to determine serum levels of levodopa—carbidopa combination dosage up to 12 h after administration. It has been successfully applied to the analysis of over 700 plasma samples from human volunteers with various levodopa—carbidopa dosage combinations.

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