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Concentrations of L-dopa in plasma and plasma ultrafiltrates[1]

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Abstract

A sensitive and specific procedure is described for the determination of therapeutically relevant concentrations of L-dopa in plasma and plasma ultrafiltrates (free fraction) by high performance liquid chromatography with electrochemical detection. In plasma samples from healthy adult subjects ($n = 15$) spiked with L-dopa (500 $\mu g\,l^{-1}$) the free fraction averaged $76 \pm 8\%$ (range 61–84%). Free fraction values increased by 38% with increasing plasma concentrations of L-dopa from 100–5000 $\mu g\ l^{-1}$. L-dopa free fraction was not affected by the presence of 3-*o*-methyl-dopa at concentrations up to 10 000 $\mu g\ l^{-1}$.

Keywords: Endogenous L-dopa; 3-*o*-Methyl-dopa; Non-protein-bound; Parkinson's disease; Ultrafiltration; Total L-dopa

1. Introduction

L-dopa still represents the mainstay for the management of Parkinson's disease. Although the pharmacokinetic profile of total (free + plasma protein-bound) L-dopa has been extensively characterized [1], virtually no information appears to be available on its degree of binding to plasma proteins. The issue may be clinically relevant, because only the unbound drug fraction is considered to create the driving gradient for diffusion across biological membranes [2]. Although in the case of L-dopa diffusion is a less important mechanism because the drug is actively transported across the blood–brain barrier, differences in plasma protein binding can not be excluded as a contributory factor to the interindividual variability in L-dopa response. A rapid, sensitive and specific procedure for the separation of free L-dopa from protein-bound L-dopa in human plasma and subsequent high performance liquid chromatography (HPLC) determination has been

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recently developed by this group [3]. In the present study, this method was applied to the evaluation of factors affecting L-dopa binding to plasma proteins in vitro.

2. Materials and methods

Blood samples were collected using vacutainers containing sodium heparinate. After centrifugation the plasma was stored at −80°C until analysis within 3 weeks. The assays were carried out exactly as described by Melzi d'Eril and Rizzo [3].

For the determination of total L-dopa 300 μl of 60.0 mM trichloroacetic acid was added to 1.0 ml of plasma. After 10 min in an ice bath, the mixture was centrifuged (5000*g*, 10 min) and 20 μl of the supernatant was injected directly into the chromatographic system. For the determination of unbound L-dopa concentration, 250 μl of plasma was centrifuged (8000*g*, 30 min) at 25°C through a membrane Minicent 10 (Bio-Rad, Richmond, CA). Before ultrafiltration, 5 μl of 4 M orthophosphoric acid was added to the bottom of the cone. 20 μl of the ultrafiltrate was injected into the chromatographic system. The HPLC assay utilized a 5 μm C18 (45 mm × 4.6 mm i.d.) reverse phase column (Bio-Rad) eluted isocratically at 1.0 ml min^{-1} with potassium dihydrogen ortophosphate (50 mM)–sodium dodecylsulphate (1.4 mM)–acetonitrile (42:42:16, v/v/v) (pH*2.8). The electrochemical system utilized was a three-electrode coulometric detector (Coulochem 5100, ESA, Bedford, MA) consisting of one conditioning cell (+300 mV) and one analytical cell equipped with two electrodes (+50 mV; −300 mV). The signal generated by the third electrode was converted to a chromatographic trace by a Bio-Rad Model 1322 detector.

For experiments with spiked samples, plasma from untreated subjects was mixed with the appropriate amount of L-dopa and, if required, 3-*o*-methyl-dopa (3-OMD), for 30 min at room temperature, to allow equilibration of the drug with plasma protein binding sites.

Results are expressed as means ± SD.

3. Results

Using the above described assay method, separation and elution of L-dopa could be achieved rapidly (< 5 min) in both deproteinized plasma and plasma ultrafiltrates (Fig. 1). The assay had a detection limit of 0.2 μg l^{-1} and within-day and day-to-day precisions better than 4%. The recovery of L-dopa from plasma samples spiked with a 500 μg l^{-1} concentration of the drug was 101 ± 2.7% (range 96–105%, $n = 10$). Response was linear over the examined concentration range (up to 5000 μg l^{-1}).

In a first set of experiments, endogenous total and unbound L-dopa concentrations were assayed

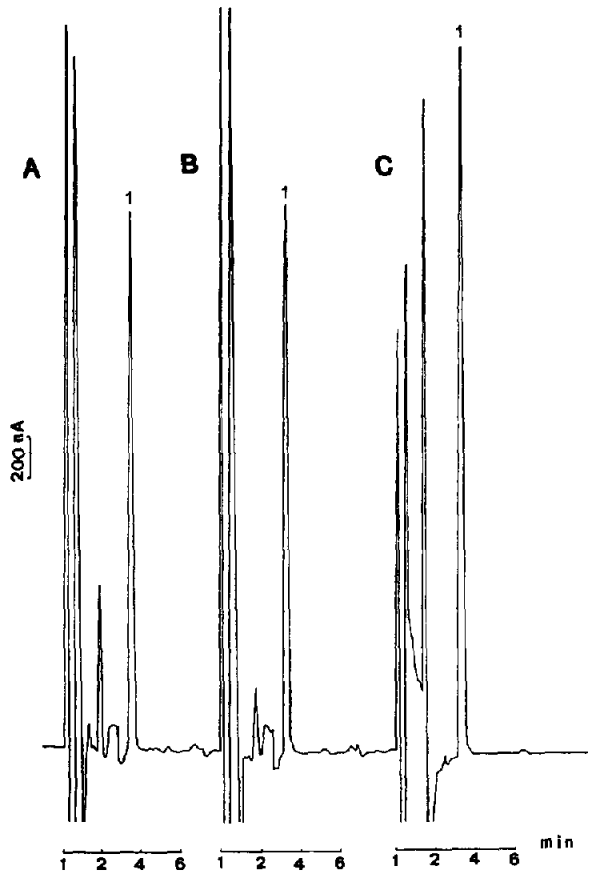

Fig. 1. Chromatograms obtained by injecting 20 μl of (A) deproteinized plasma containing 497.7 μg l^{-1} of total L-dopa, (B) the same plasma ultrafiltered containing 385.7 μg l^{-1} of unbound L-dopa and (C) standard solution of 500 μg l^{-1} of L-dopa. Peak identification: 1, L-dopa.

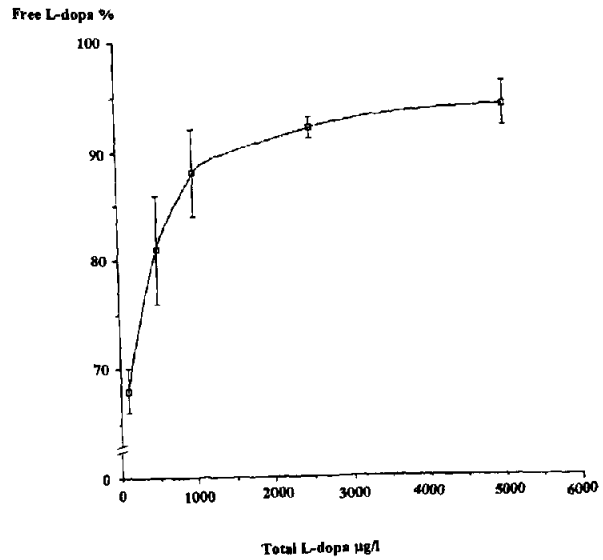

Fig. 2. Free fraction of L-dopa in plasma samples ($n = 4$) spiked with increasing amounts of the drug. The results are expressed as means ± SD.

in plasma samples collected from five healthy subjects. Total and unbound concentrations in these samples were 3.8 ± 1.2 $\mu g\ l^{-1}$ respectively, which correspond to a free fraction of $55 \pm 15\%$.

In an additional set of experiments, 15 samples collected from healthy subjects were spiked with L-dopa at a 500 $\mu g\ l^{-1}$ concentration, which is within the range encountered in patients receiving therapeutic dosages. The free fraction in these samples was $76 \pm 8\%$.

To assess the relationship between plasma protein binding and total L-dopa concentration, unbound fraction was assessed in four samples spiked with increasing concentrations (100–5000 $\mu g\ l^{-1}$). Free fraction values in these samples increased with increasing concentration from $68 \pm 2\%$ at 100 $\mu g\ l^{-1}$ to $94 \pm 4\%$ at 5000 $\mu g\ l^{-1}$ (Fig. 2).

To explore a possible influence of the L-dopa metabolite 3-OMD on L-dopa binding, binding studies were performed in samples ($n = 5$) spiked with 500 $\mu g\ l^{-1}$ L-dopa and 3-OMD concentrations increasing from 500 to 10 000 $\mu g\ l^{-1}$.

L-dopa unbound fraction was not affected by changes in 3-OMD concentration. On average, L-dopa unbound fraction values were $81 \pm 5\%$ at 500 $\mu g\ l^{-1}$ 3-OMD compared with $86 \pm 3\%$ at 10 000 $\mu g\ l^{-1}$ 3-OMD.

4. Discussion and conclusions

The method used in the present study exhibited performance characteristics adequate for a precise evaluation of L-dopa binding in human plasma. The assay has a high sensitivity to allow determinations to be performed in samples containing both endogenous and spiked concentrations of the test compound.

In all sets of samples examined, L-dopa concentrations were consistently lower in plasma ultrafiltrates than in plasma, indicating a significant degree of protein binding. The unbound fraction appeared to be influenced by the total concentration originally present in the sample and increased from an average of about 68% in plasma containing 100 $\mu g\ l^{-1}$ L-dopa to 94% at 5000 $\mu g\ l^{-1}$. These concentrations are within the range observed in patients with Parkinson's disease receiving chronic treatment with the drug [4]. It should be noted that experiments were carried out in vitro, and that the therapeutic situation in vivo may differ due to potential binding interference by concurrently present metabolites. In this study, however, increasing concentrations of the metabolite 3-OMD were not associated with changes in the degree of L-dopa protein binding in spiked samples.

Overall, the observation that in virtually all spiked samples the unbound fraction was greater than 60–70% indicates that L-dopa binds to plasma proteins only to a minor extent and suggests that protein binding should not be a major limiting factor in the distribution of L-dopa across tissues. In a similar way, interindividual variation in plasma protein binding would not be expected to play an important role in explaining differences in magnitude of clinical response at any given plasma L-dopa concentration. For the purpose of therapeutic drug monitoring and pharmacokinetic studies, measurement of total (free + protein-bound) concentrations is likely to provide clinically meaningful information.

References

[1] G.F. Wooten, in C.D. Marsden and S. Fahn (Eds.), Movement Disorders 2, Butterworths, London, 1987, pp. 231–248.
[2] E. Perucca and A. Richens, in C.D. Marsden and S. Fahn (Eds.), Drug Concentrations in Neuropsychiatry, Excepta Medica, Amsterdam, 1980, pp. 52–68.
[3] G.V. Melzi d'Eril and V. Rizzo, J. Chromatogr., 553 (1991) 265–269.
[4] J.C. Nutt and J.H. Fellman, Clin. Neuropharmacol., 7 (1994) 35–49.