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Note

Rapid liquid chromatographic determination of probenecid in human cerebrospinal fluid

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Probenecid [*p*-(di-*n*-propylsulfamyl)-benzoic acid] has been used to study the metabolic turnover of dopamine and serotonin in the central nervous system. Previous reports [1–6] have shown that the degree of the probenecid-induced accumulation of the acid metabolites [homovanillic acid (HVA) and 5-hydroxy-indoleacetic acid (5-HIAA)] and the standard dose concentration of probenecid vary considerably in the cerebrospinal fluid (CSF) of individual patients. Thus, the probenecid levels in CSF must be determined before any correlation between the increase of the acid metabolites and the CSF levels of probenecid can be established.

Probenecid concentrations in biological fluids have been measured by both fluorometric and radioenzymatic methods [5, 7]. The general problems associated with these methods were tedious sample preparation and relative nonspecificity. Gas chromatography coupled with electron-capture detection [8, 9] or with mass spectrometry [6] have also been employed to measure probenecid in CSF samples. Although these two techniques have the adequate sensitivity and specificity for the probenecid determination, they required the derivatization procedure prior to analysis. Recently, a technique utilizing high-performance liquid chromatography (HPLC) with UV detection has been described for the determination of probenecid in plasma and urine [10].

In the present paper we have modified this method for the measurement of probenecid in human CSF. Probenecid is isolated from acidified CSF with toluene extraction and then quantitated by liquid chromatography with UV detection at 245 nm. An internal standard is used for correction of the losses during the extraction procedure.

EXPERIMENTAL

Chemicals

Probenecid standard was obtained from Merck Sharp and Dohme (West Point, PA, U.S.A.). Internal standard [*m*-(diisobutylsulfamyl)benzoic acid (DSB)] was synthesized according to the procedure of Mieler [11]. Sodium octylsulfate was purchased from Pfaltz and Bauer (Stamford, CT, U.S.A.). All other reagents were of analytical grade. Water was deionized and then double-distilled in glass.

Apparatus

The liquid chromatograph was constructed from five components: Consta-Metric IIG solvent delivering system (Laboratory Data Control, Riviera Beach, FL, U.S.A.); Model 450 variable-wavelength detector (Waters Assoc., Milford, MA, U.S.A.); Model 7125 injection valve (Rheodyne, Cotati, CA, U.S.A.); a 25 cm × 4.6 mm reversed-phase column of 5- μ m octadecyl silica (Bioanalytical Systems, West Lafayette, IN, U.S.A.); and a 3 cm × 4.6 mm guard column of 5- μ m RP-18 (Brownlee Labs., Santa Clara, CA, U.S.A.). The mobile phase was prepared by mixing 540 ml of methanol, 260 ml of water, 9 ml of glacial acetic acid, and 750 mg of sodium octylsulfate. The flow-rate was fixed at 1.5 ml/min at ambient temperature. At the end of each day, the system was flushed with methanol-water (1:1, v/v) to prolong the lifetime of the column.

Procedure

Lumbar CSF samples were collected from patients administered probenecid orally (a total dose of 100 mg/kg body weight) at the Bronx Veterans Administration Medical Center, New York. The CSF was immediately frozen and stored at -80°C until analyzed.

To an aliquot of CSF (0.5 ml), 100 μ l of 0.15 mM DSB and 0.3 ml of 6 M hydrochloric acid solution were added. The mixture was extracted twice with 3 ml of toluene and the combined extracts evaporated to dryness under a stream of dry nitrogen at 37°C. The drying procedure was carefully repeated after 1 ml methanol was used to wash the dried residue. The dried residue was dissolved in 200 μ l of the mobile phase. Standard curves were prepared by plotting the relative height ratios ($H_{\text{probenecid}}/H_{\text{DSB}}$) in a series of samples containing a known quantity of DSB to which proportionate amounts of probenecid had been added. The free probenecid concentrations were calculated by inverse linear regression analysis. The total probenecid concentrations of five CSF samples were determined as described in the above section except CSF samples were first incubated with an aryl sulphatase + β -glucuronidase enzyme preparation (Type H-1, Sigma, St. Louis, MO, U.S.A.) in 1 M sodium acetate buffer (pH 6.2, 200 μ l) at 37–38°C for 6 h prior to extraction in order to disassociate probenecid from its conjugate. A small volume (20 μ l) of each CSF sample was also injected directly onto the column and the free probenecid level presented in the original sample was calculated from the peak height using a standard curve.

RESULTS AND DISCUSSION

Fig. 1 shows representative chromatograms of human CSF extracts. Identification of the probenecid and DSB peaks observed in CSF samples was based on retention times and cochromatography with the authentic compounds. Under the chromatographic conditions used, the variable-wavelength detector (245 nm) gave better detector responses for probenecid and DSB than the fixed-wavelength detector (254 nm). Therefore, an UV-absorbing wavelength at 245 nm and a flow-rate of 1.5 ml/min were chosen because of the desirable sensitivity and adequate separation time for probenecid and DSB in standard and CSF solution.

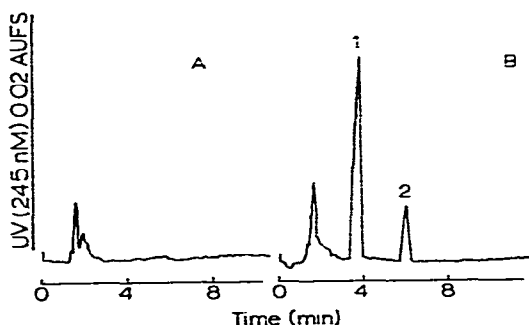


Fig. 1. Chromatograms of human CSF extracts: (A) probenecid-free (blank) CSF without DSB internal standard; (B) CSF containing probenecid (1) and 4.6 μ g DSB (2). Conditions were as given in the Experimental section.

No endogenous compounds of CSF interfered with the detection of probenecid and DSB. Linearity between the detector response and the concentration of probenecid injected was observed over the range 2.4–24 μ g. The percentage recoveries, estimated by peak height ratios of extracted standard solutions to non-extracted ones, for probenecid and DSB, were 85 ± 5 and $92 \pm 3\%$ ($n = 4$), respectively. The detection limit of probenecid was 250 ng/ml of CSF.

Table I shows free probenecid CSF concentrations determined by both direct injection and toluene extract of CSF samples from fourteen patients administered the same total dose of probenecid (100 mg/kg body weight). The wide range of those CSF probenecid values is in general agreement with previous data using different techniques [5, 6, 8, 9]. Small percentages (2–10%) of probenecid existing in conjugated form [9] were found in the five human CSF samples analyzed for total probenecid.

Although there were small differences among individual CSF probenecid concentrations obtained by direct injection and toluene extraction methods, no statistical discrepancy between the mean value of both methods was noticed (by paired *t*-test, not significant, Table I). However, we preferred the toluene extraction method since it yielded cleaner chromatograms and gave a longer lifetime of the column. Also, toluene can selectively extract probenecid and DSB from CSF samples and leave the acid metabolites (e.g. DOPAC, HVA, and

TABLE I

COMPARISON OF FREE CSF PROBENECID CONCENTRATIONS BY DIRECT INJECTION AND AFTER TOLUENE EXTRACTION

All patients were administered 100 mg/kg body weight of probenecid in divided oral doses over an 18-h period.

Patient No.	Free CSF probenecid ($\mu\text{g/ml}$)	
	Direction injection	Toluene extraction
1	17.8	17.0
2	19.0	18.8
3	15.0	15.2
4	14.0	13.0
5	20.9	21.2
6	30.4	29.3
7	22.0	22.3
8	25.4	25.6
9	20.8	21.4
10	18.0	16.2
11	17.8	17.3
12	16.2	15.7
13	13.4	14.8
14	20.2	19.8
\bar{X}	19.4	19.1
S.D.	4.3	4.5

5-HIAA) in aqueous solution [12]. Those acids could be reextracted back into an organic phase (e.g. ethyl acetate) and then determined by an appropriate technique.

In conclusion, the procedure described offers a simple and accurate HPLC determination of CSF probenecid concentration and can be done within an hour.

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