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Note

Determination of chloramphenicol-glucuronide in urine by high-performance liquid chromatography

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The recently renewed interest in chloramphenicol (CAP) for treating serious infections has created a need for fast, reliable, and accurate measurement of the drug in body fluids [1]. Recently Aravind et al. [2] described a high performance liquid chromatographic (HPLC) method for the simultaneous measurement of CAP and its succinate ester (CAPS) in serum, cerebrospinal fluid, and urine. The purpose of this communication is to extend the original methodology to include analysis of chloramphenicol glucuronide (CAPG) in urine.

MATERIALS AND METHODS

Chromatography

All assays were performed on a Perkin-Elmer Series II high-performance liquid chromatograph and interfaced with a Sigma 10-B data system (Perkin-Elmer, Norwalk, CT, U.S.A.). The chromatographic conditions and data handling were as previously reported [2].

Reagents

Bovine liver β-glucuronidase type B (β-Glu) containing no sulphatase activity was obtained from Sigma (St. Louis MO, U.S.A.). A working β-Glu solution containing 5000 Fishman units of enzyme per ml was prepared in 0.1 *M* sodium acetate buffer (pH 5.0). Saccharo-1,4-lactone monohydrate, a specific inhibitor for β-glucuronidase was obtained from Calbiochem-Behring (La Jolla, CA, U.S.A.). A working standard containing 200 μg/ml of CAP was prepared in

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drug-free urine. All other chemicals and reagents were as previously described [2].

Procedure

All urine samples were placed in 10 × 75 mm disposable culture tubes and were analyzed for CAP and CAPG as detailed in Table I.

The tubes were gently vortexed and then covered with Parafilm® and incubated in a water bath at 37°C for 3 h. At the end of the incubation, the tubes were centrifuged for 5 min at 2000 *g*. A 10-μl aliquot of the supernatant was injected onto the column.

TABLE I

INCUBATION CONDITIONS

Tube A	Tube B	Tube C
0.1 ml of standard or patient urine + 0.9 ml sodium acetate buffer (pH 5.0)	0.1 ml of standard or patient urine + 0.9 ml of working β-Glu solution	0.1 ml of standard or patient urine + 0.9 ml of working β-Glu solution + 10 mg saccharo-1,4-lactone

RESULTS AND DISCUSSION

Fig. 1 shows three chromatograms which were obtained after analysis of a patient's urine sample. This patient had been maintained on chloramphenicol palmitate. Fig. 1A shows the chromatogram obtained when the sample was not treated with β-Glu. The retention times for CAPG and CAP were 1.6 and 2.9 min, respectively. Fig. 1B shows the chromatogram obtained after hydrolysis of the same sample with β-Glu. In this chromatogram the CAPG peak is absent and there is a corresponding increase in the CAP peak. Fig. 1C shows the chromatogram after incubation of the sample with both β-Glu and saccharo-1,4-lactone (inhibitor of β-glucuronidase). This chromatogram is virtually identical to that of Fig. 1A, confirming that the peak at 1.6 min was CAPG. Analysis of the area count under the peak provided additional support that the peak at 1.6 min was indeed CAPG. In Fig. 1A, the CAPG and CAP had area counts of 8.6 and 3.5, respectively. In Fig. 1B, the area count for CAP increased to 11.9, an amount (8.4) virtually equal to that of the CAPG peak. In Fig. 1C, the area counts for CAPG and CAP were 8.5 and 3.5, the values obtained in Fig. 1A.

Table II shows data obtained from several patients. Since standard CAPG was not readily available, the CAPG concentrations were reported as CAP equivalents. The data in Table II substantiate our earlier reports [1, 2] of unpredictable and variable excretion of CAPS and CAP in urine. The data indicate that CAP is also glucuronidated to a variable extent as well. The mean percentages determined from the data in Table II for CAPS, CAP and CAPG were 51.4, 22.5 and 26.2, respectively.

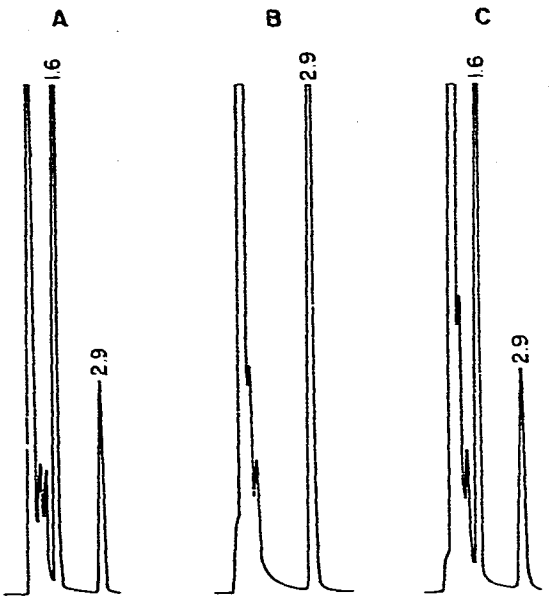

Fig. 1. (A) Chromatogram obtained from urine not treated with β-glu; (B) chromatogram of the same sample after hydrolysis with β-glu; and (C) chromatogram of the same sample treated with saccharo-1,4-lactone with β-glu. The retention times for CAPG and CAP were 1.6 and 2.9 min, respectively.

TABLE II

AMOUNTS OF CAP, CAPS AND CAPG RECOVERED IN PATIENT URINE

The percentage values of CAPS, CAP and CAPG are determined from the total recovered.

Patient no.	Dose of CAP (mg)*	CAPS (mg)	CAP (mg)	CAPG (mg)	Total (mg)	CAPS (%)	CAP (%)	CAPG (%)
1	355	187.5	81.7	77.1	346.3	54	24	22
2	250	108.4	23.1	42.8	174.3	62	13	25
3	200	160.6	23.6	6.2	190.4	84	13	3
4	400	108.5	114.6	241.0	464.1	23	25	52
5	200	56.3	35.4	40.6	132.3	42	27	31
6	200	60.7	24.1	30.6	115.4	53	21	31
7	450	217.0	37.0	108.5	362.5	60	10	30
8	750	258.8	63.6	134.4	556.8	64	11	24
9	50	13.5	19.2	11.2	43.9	31	44	25
10	66	24.6	23.6	15.1	63.3	39	37	24

*Dose administered as CAPS.

Patient 3 (Table II) was a therapeutic failure. Unpon analysis of his urine, it was evident that this failure was not due to excessive glucuronidation of the drug, but rather to almost complete excretion of the unhydrolyzed succinate ester in his urine. Patient 4, on the other hand, initially failed to achieve a therapeutic level of CAP. Analysis of his urine indicated a larger-than-average capacity to glucuronidate the drug. Thus, his dose was subsequently increased

with the knowledge that he was capable of tolerating the increased dose because of his high glucuronidation ability.

The method presented here extends our previously reported method [2] to include the determination of CAPG. It is important to evaluate this metabolite to assess liver function in regards to CAP as well as provide additional information when evaluating toxicity or therapeutic failures.

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