Journal of Chromatography, 121 (1976) 401–404

CHROM. 9015

Note

Fractionation of tritiated digoxin and dihydrodigoxin with DEAE-Sephadex LH-20

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(Received January 7th, 1976)

Recently Clark and Kalman[1] stated that it has not been possible to separate digoxin from dihydrodigoxin using solvent partitioning, column chromatography, paper chromatography or thin-layer chromatography. Quantitation of dihydrodigoxin has, however, been reported using gas–liquid chromatography[1] and the combination of radioimmunoassay and mass spectroscopy[2]. These studies have suggested that an average of 13%[1] and 16.4%[2] and up to 47%[1] of the ingested digoxin may be excreted as dihydrodigoxin.

We have successfully used diethylaminoethoxypropylated Sephadex LH-20 (DEAE-Sephadex LH-20) column chromatography to separate tritiated dihydrodigoxin from tritiated digoxin following their extraction from urine.

EXPERIMENTAL

Materials

2-Chlorotriethylamine hydrochloride was purchased from Eastman-Kodak (Rochester, N.Y., U.S.A.), and Sephadex LH-20 from Pharmacia (Uppsala, Sweden). Reagent grade sodium chloride, sodium hydroxide, hydrochloric acid and ethyl alcohol were purchased from Fisher Scientific (Montreal, Canada).

Dihydrodigoxin was a gift from Burroughs (Research Triangle Park, N.C., U.S.A.). Dihydrodigoxin was tritiated by catalytic exchange by New England Nuclear (Boston, Mass., U.S.A.). [^{3}H]Digoxin-12α, specific activity 23 mCi/mg, was obtained from New England Nuclear. [^{3}H]Dihydrodigoxin, specific activity 4.4 mCi/mg, was purified by LH-20 column chromatography[3] and [^{3}H]digoxin-12α by both paper and LH-20 chromatography[3]. The purity was checked using DEAE-Sephadex LH-20 column chromatography.

Procedures

The use of DEAE-Sephadex LH-20 was described by Dittmer[4] for the separation of membrane phospholipids. Preparation was by a method similar to that described by Peterson and Sober[5] for the synthesis of DEAE-cellulose, substituting LH-20 for cellulose. During the course of this synthesis a limited number of diethylamino-

ethoxy groups were linked to the Sephadex LH-20 by heating alkaline LH-20 with 2-chlorotriethylamine hydrochloride according to the general reaction scheme

$$Cl\text{–}C_2H_4\text{–}N(C_2H_5)_2 + NaOH + LH\text{-}20 \rightarrow (LH\text{-}20)\text{–}O\text{–}C_2H_4\text{–}N(C_2H_5)_2 + Na\,Cl$$

For chromatography, a 40 × 1.0 cm glass column was packed by gravity with a chloroform–methanol (85:15) slurry of the gel. Digoxin and its metabolites were extracted from 20 ml of urine with 3 × 40-ml volumes of chloroform–ethanol (50:50), reconstituted in chloroform–methanol (85:15), and after solvent evaporation eluted from the column with chloroform–methanol (85:15). Samples were applied in 0.2 to 0.5-ml volumes of the eluting solvent. The flow-rate was 0.25 ml/min at 25°. Extraction of tritiated digoxin and dihydrodigoxin from urine using chloroform–ethanol (50:50) was complete[3].

RESULTS AND DISCUSSION

DEAE-Sephadex LH-20 column chromatography has achieved complete separation of [^{3}H]dihydrodigoxin ($V_e/V_t = 0.25$) from [^{3}H]digoxin ($V_e/V_t = 0.34$) in extracts of urine to which these compounds had been added. Complete separation was also achieved in extracts of urine from five volunteers and two patients on maintenance digoxin dosage following the ingestion of 150 μCi [^{3}H]digoxin-12α.

The elution profile shown in Fig. 1 demonstrates the separation achieved when [^{3}H]dihydrodigoxin and [^{3}H]digoxin-12α were added to a sample of urine in proportions of approximately 1:20 and extracts from the urine chromatographed. When [^{3}H]dihydrodigoxin was added to urine alone or with [^{3}H]digoxin-12α, 95–96% of the radioactivity added as dihydrodigoxin was present under the dihydrodigoxin peak.

From 0.2 to 1.9% of the radioactivity excreted in urine specimens collected during the first 10 h after ingestion of [^{3}H]digoxin-12α by seven volunteers was found to be dihydrodigoxin (Table I). Only in one patient was a dihydrodigoxin peak observed after the first 10 h and no comparable peak was found after 24 h. There was no appreciable difference between volunteers with normal renal function and those who were dialysis dependent and had creatinine clearances of 2 and 5 ml/min.

Tritiated dihydrodigoxin and digoxin have also been separated from digoxigenin and its mono- and bisdigitoxosides using DEAE-Sephadex LH-20 column chromatography, after extraction from urine[6]. Background levels were achieved between peaks and the V_e/V_t values were the same as those obtained when only tritiated dihydrodigoxin and digoxin were added to urine (Fig. 1).

DEAE-Sephadex LH-20 gave better separation of digoxin and dihydrodigoxin than Sephadex LH-20 alone. The latter achieved only a partial separation[3] (Fig. 2).

The clinical importance of dihydrodigoxin relates to its relative inactivity on the heart compared with digoxin, and to its lesser affinity for antibodies to digoxin in radioimmunoassay systems[7].

In conclusion, we have presented a method for the separation of tritiated dihydrodigoxin from digoxin by column chromatography using DEAE-Sephadex LH-20, after extraction from urine. This method detects less than 1 ng of dihydrodigoxin, gives complete separation of eluted fractions and clearly identifies dihydrodigoxin. As little as 300 dpm have been resolved into a peak.

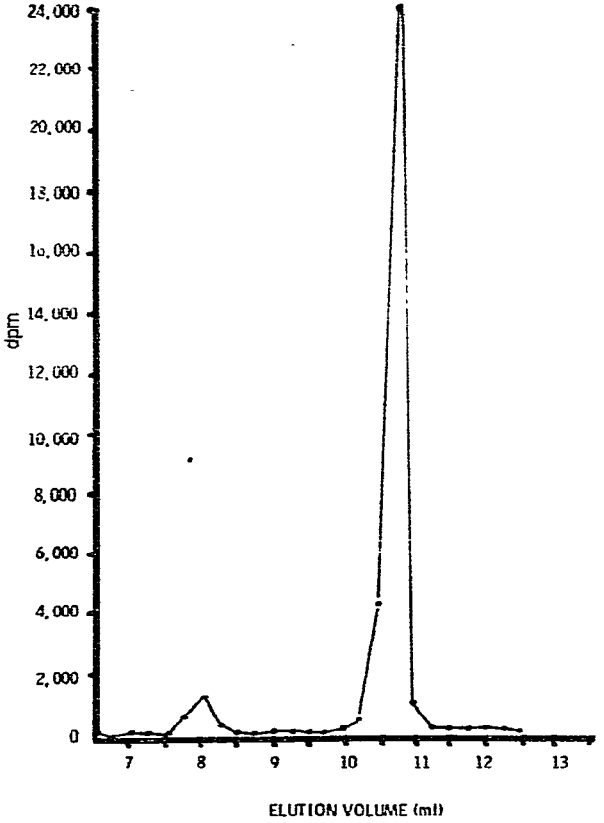

Fig. 1. Fractionation of [^{3}H]dihydrodigoxin ($V_e/V_t = 0.25$) and [^{3}H]digoxin-12α ($V_e/V_t = 0.34$) employing DEAE-Sephadex LH-20. A 40 × 1.0 cm column was used at a flow-rate of 0.25 ml/min at 25°.

TABLE I

PER CENT RADIOACTIVITY EXCRETED IN URINE AS DIHYDRODIGOXIN AFTER INGESTION OF 150 μCi[^{3}H]DIGOXIN-12α

	A. Volunteers with normal renal function						
	Hours after ingestion of digoxin						
	0–2	*2–4*	*4–7*	*7–10*	*10–14*	*14–24*	*24–48*
J.C.	0.5	0.9	1.0	0.2	0	0	0
P.D.	1.0	1.9	1.9	0.2	0	0	0
A.G.	0.9	0.9	1.4	0.4	0	0	0
H.G.	0.9	1.0	1.1	0.5	0	0	0
C.M.	1.3	1.5	0.5	0.5	0	0	0
$\bar{x}$	0.9	1.2	1.2	0.4			
S.D.	0.3	0.4	0.5	0.2			

	B. Dialysis-dependent patients		
	Hours after ingestion of digoxin		
	0–10	*10–24*	*24–48*
E.H.	1.8	0.3	0
E.P.	1.1	0	0

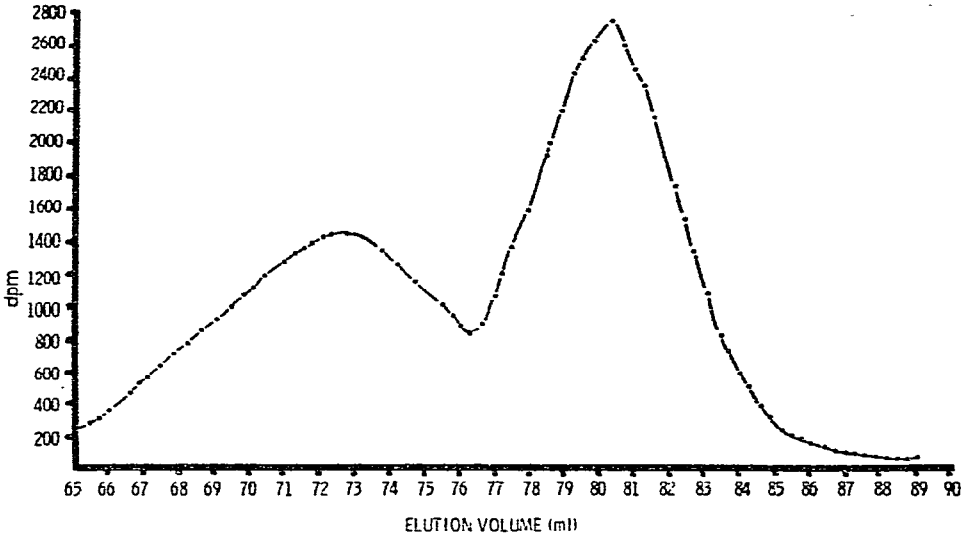

Fig. 2. Fractionation of [^{3}H]dihydrodigoxin ($V_e/V_t = 0.43$) and [^{3}H]digoxin-12α ($V_e/V_t = 0.48$), employing Sephadex LH-20. A 36 × 2.5 cm column was used at a flow-rate of 0.20 ml/min at 4°.

ACKNOWLEDGEMENTS

This study was supported by grants from the Department of Veterans' Affairs, Canada (N-15-74) and Burroughs Wellcome Co., Research Triangle Park, N.C. 27709, U.S.A.

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