Journal of Chromatography, 181 (1980) 497—503
Biomedical Applications

CHROMBIO. 501

Note

Rapid and sensitive determination of chlorthalidone in blood, plasma and urine of man using high-performance liquid chromatography

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(Received August 20th, 1979)

Chlorthalidone is a diuretic drug widely used in anti-hypertensive therapy [1]. This sulphonamide-like diuretic differs chemically from the thiazides by the nature of the heterocyclic ring, although its pharmacological action is indistinguishable from that of the thiazides. The drug is administered orally, in a dose of 25—100 mg daily and is claimed to have a prolonged action [2—5], mainly due to the long biological half-life of the compound (30—80 h) [6—9].

The volume of distribution of chlorthalidone appears to be relatively large, 200—400 l [9], resulting in maximum plasma concentrations of 100—400 ng/ml. However, this compound reaches 10—30 times greater concentrations in red blood cells [7—10]. For the determination of the low concentrations in plasma only a limited number of methods are available. A spectrophotometric method for the determination of chlorthalidone in whole blood and urine has been published by Tweeddale and Ogilvie [6], permitting the determination of a minimum concentration of 1 mg/ml. Gas—liquid chromatographic (GLC) methods have also been published [11—13]; most of these methods, based on the conversion of chlorthalidone to its tetramethyl derivative by extractive alkylation [11, 12], are still quite laborious and may pose considerable methodological problems for a large series of samples. A sensitive and selective

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direct GLC method has been developed by Fleuren and Van Rossum [13]; although plasma concentrations as little as 10 ng/ml can be measured, this method still requires 1 ml of plasma, which can only be obtained by venipuncture. For kinetic studies in volunteers, large series are required, which can easily be obtained and analysed by the proposed method.

In an earlier high-performance liquid chromatography (HPLC) paper from our group, dealing with the determination of diazoxide [14], the separation characteristics of chlorthalidone were mentioned. However, the method was still not sensitive enough for the routine determination of chlorthalidone in kinetic studies. Therefore, a new HPLC method for the analysis of chlorthalidone in plasma, urine and whole blood was developed. Analytical details and some results of the pharmacokinetics of chlorthalidone in healthy volunteers are presented in this paper.

MATERIALS AND METHODS

Apparatus

A Spectra Physics 3500B high-performance liquid chromatograph was used, equipped with a variable wavelength spectrophotometric detector (Model SP 770). A stainless-steel column (15 cm × 4.6 mm I.D.) was packed with LiChrosorb RP-18, particle size 5 μm, obtained from Chrompack (Middelburg, The Netherlands). An injection loop of 100 μl was used. Detection of chlorthalidone was effected at 226 nm. The detection limit is 30 ng/ml.

Solvents

The solvent used was a mixture of 0.01 *M* sodium acetate in water and acetonitrile (400:100, v/v) and the flow-rate was 1.6 ml/min, at a pressure of 165 atm.

Drugs

Chlorthalidone was obtained from Ciba-Geigy (Arnhem, The Netherlands) and probenecid from Sigma (Brunschwig Chemicals, Amsterdam, The Netherlands).

Subjects

Two healthy, caucasian subjects, both employees of the Department of Clinical Pharmacy, Nijmegen, participated in this study. Chlorthalidone was administered orally either as a dose of 100 mg as Hygroton® tablets (Ciba-Geigy) or as an experimental generic preparation (Pharmachemie, Haarlem, The Netherlands).

Blood samples of 0.7 ml were collected at scheduled time intervals by finger-tip puncture (Microlance No. 433, Becton-Dickinson). An amount of 0.1 ml of blood was used for the determination of the whole blood chlorthalidone concentration. The remaining 0.6 ml was centrifuged at 2600 *g* for 5 min and the plasma was immediately separated from the red blood cells. Spontaneously voided urine was collected over a period of 70 h. The pH of the urine was not affected.

Sample preparation

Whole blood. Whole blood (0.1 ml) is mixed with 0.4 ml of 0.33 *N* perchloric acid at a temperature of 4° in a vortex mixer. Deproteinization and haemolysis are complete after standing for 5 min. The mixture is centrifuged for 5 min at 2600 *g* in a Heraus Christ centrifuge. A 100-μl aliquot of the clear supernatant is injected onto the column.

Plasma. A 0.2-ml aliquot of a potassium dihydrogen phosphate solution in water (0.067 *M*) containing probenecid (1 mg/l) as internal standard, was added to 0.2 ml of plasma and 1 ml of diethyl ether, and was mixed for 1 min in a vortex mixer. The mixture is then centrifuged in another tube and evaporated to dryness. The residue is dissolved in 0.15 ml of eluent and 0.1 ml injected onto the column.

Urine. Urine (10 μl) is added to 0.2 ml of the eluent and 0.1 ml of this mixture is injected directly onto the column.

Recovery

The recovery of chlorthalidone added to human whole blood in the concentration range of 1—10 μg/ml was found to be 47.6 ± 2.7% (S.D.) and in urine 100 ± 2% (S.D.). The recovery of the chlorthalidone extracted from plasma in the concentration range of 50—500 ng/ml was found to be 70 ± 3% (S.D.). The calibration curves were linear over the concentration ranges measured, and the sensitivity limit for chlorthalidone was 30 ng/ml.

RESULTS

Fig. 1 shows high-performance liquid chromatograms of whole blood, plasma and urine samples obtained from a volunteer after the intake of 100 mg chlorthalidone. As shown in the blank samples no interfering peaks are found either in direct injections (urine) or after deproteinization (whole blood), or extraction (plasma).

Fig. 2 shows the concentration—time profiles of whole blood and plasma and the renal excretion rate—time profile of chlorthalidone in a volunteer after an oral dose of 100 mg. The calculated pharmacokinetic parameters of the volunteers are summarized in Table I. The plasma half-life time of elimination in this study varies from 26.5—42 h, whereas the half-life time measured in whole blood appeared to be somewhat longer (33—57 h).

About 40% of chlorthalidone is excreted in urine unchanged, although big variations are found. The renal excretion rate hardly appeared to be affected by urinary flow or urinary pH, but was strongly influenced by the plasma concentration. The relationship between the renal excretion rate and the plasma concentration (the proportionality factor being the renal clearance) clearly shows a biphasic character. The renal clearance of chlorthalidone was much higher (161 ml/min) during the absorption and distribution phase of the drug in the body than during the elimination phase (59 ml/min) (Fig. 3).

The concentration—time courses of chlorthalidone in plasma and whole blood are not synchronous. A linear relation between blood and plasma concentration was found (r = 0.929) after absorption and distribution processes were complete. The uptake of chlorthalidone in the red blood cells was slower than the drug supply after absorption, resulting in altering whole blood:plasma

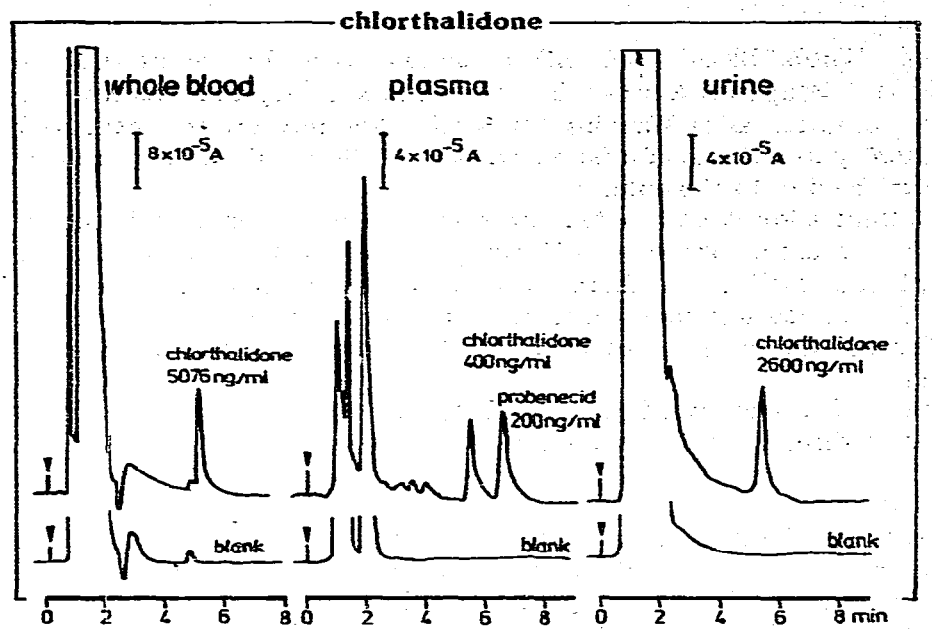

Fig. 1. HPLC chromatograms of chlorthalidone in whole blood, plasma and urine samples obtained from volunteers after the intake of 100 mg chlorthalidone and their respective blanks, sampled prior to drug intake.

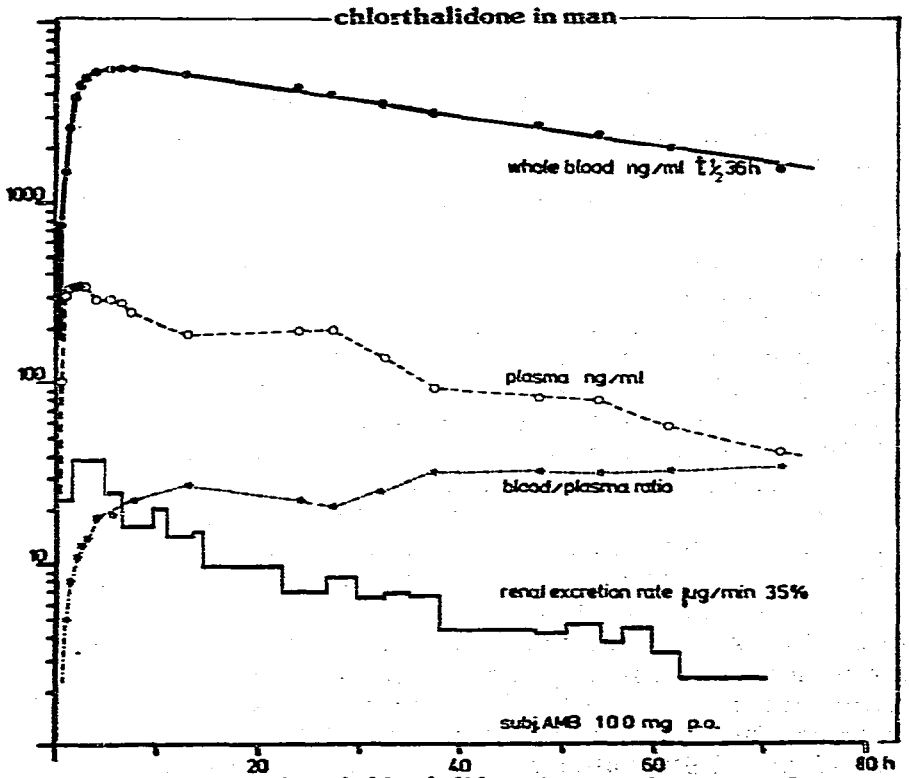

Fig. 2. Pharmacokinetics of chlorthalidone in man after an oral dose of 100 mg. The plasma elimination curve is much lower than the whole blood elimination curve, due to the selective uptake of chlorthalidone in red blood cells.

TABLE I

SOME PHARMACOKINETIC PARAMETERS OF CHLORTHALIDONE AFTER ORAL ADMINISTRATION OF 100 mg TO VOLUNTEERS

Subject	Body weight (kg)	Plasma $t_{½}$ (h)	Whole blood $t_{½}$ (h)	Concentration ratio whole blood:plasma	Percentage excreted in urine* (%)	Renal clearance** (ml/min)
JED	60	26.5	—***	—***	50.9	67.5 ± 16.8
		26.5	36	28.3 ± 5.4	42.7	60.6 ± 35.4
		30.5	34	26.1 ± 3.0	44.9	54.0 ± 15.3
		28.5	33	39.8 ± 8.4	38.3	62.4 ± 15.3
AMB	57	40.0	—***	—***	23.4	67.5 ± 11.8
		29.0	36	20.5 ± 5.2	34.8	65.1 ± 22.3
		42.0	57	25.9 ± 5.5	27.2	51.9 ± 13.7
		40.0	33	26.6 ± 4.6	19.2	39.0 ± 14.3

*Percentage of the dose excreted in urine as the unchanged drug during 70 h (ca. 2 × $t_{½}$).
**Calculated from plasma concentrations and renal excretion rates during the elimination phase.
***Not measured.

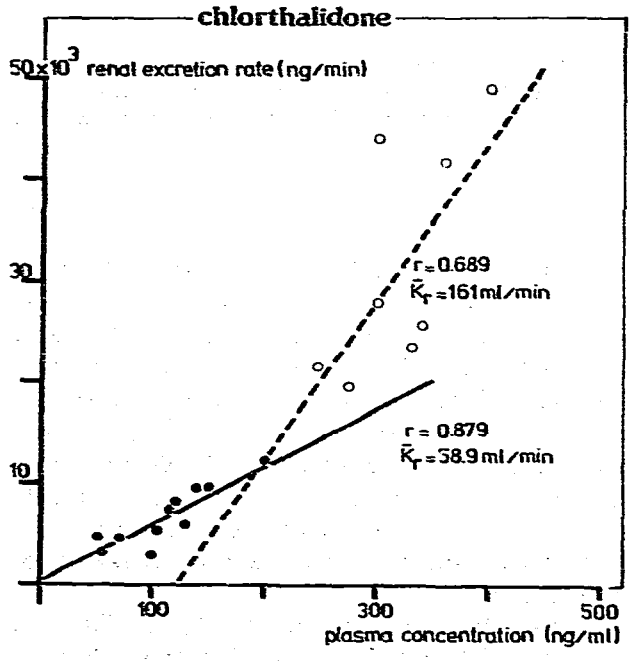

Fig. 3. The relationship between the renal excretion rate and the plasma concentration, the proportionality factor being the renal clearance. The renal clearance of chlorthalidone is much higher during the absorption and distribution phase of the drug than during the elimination phase.

concentration ratios during the first 8 h after drug intake (Fig. 4). Chlorthalidone could not be measured in saliva in this experiment, because the concentrations appeared to be below the detection limit (30 ng/ml).

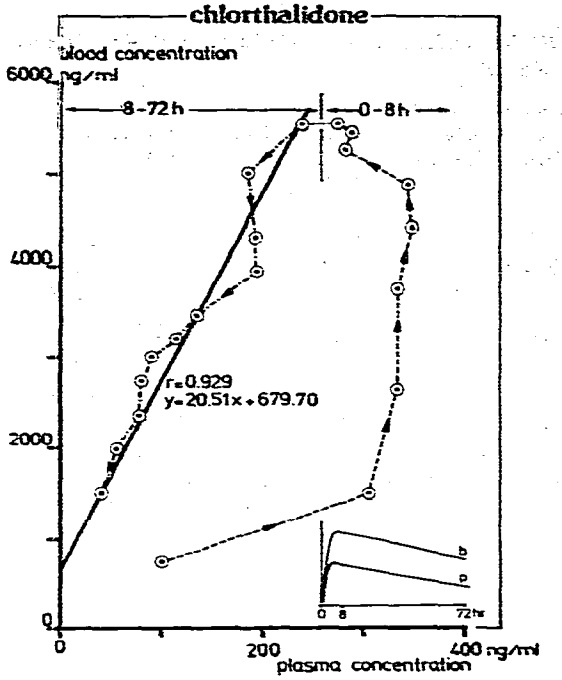

Fig. 4. The relationship between whole blood and plasma concentrations of chlorthalidone. The uptake of chlorthalidone in the blood cells is slower than the drug supply after absorption, resulting in changing blood:plasma concentration ratios during the first 8 h after drug intake.

DISCUSSION

The HPLC method described in this paper permits the determination of chlorthalidone in biological fluids at concentrations as low as 30 ng/ml. The method has advantages over the spectrophotometric method of Tweeddale and Ogilvie [6] with respect to sensitivity. The published GLC methods [11—13] are sensitive, but are mainly based on derivatization, with the exception of the method of Fleuren and Van Rossum [13], which still needs 1.0 ml of plasma to attain a detection limit of 10 ng/ml. These volumes can only be obtained from venipuncture, which may pose practical problems when large sample series are required for kinetic studies. With our method large numbers of samples can easily be obtained by fingertip puncture and the concentration of chlorthalidone can be measured in plasma, whole blood and urine.

Preliminary pharmacokinetic results as shown in this paper (Fig. 2, Table I) are in good agreement with earlier reports [6—10, 15, 16]. Chlorthalidone is excreted mainly unchanged in urine. In this study about 40% of the administered dose could be traced in 72 h ($2 \times t_{\frac{1}{2}}$) with wide inter- and intra-individual variations (Table I). These differences are reported earlier in literature [8—10, 15, 16] and are attributed to variations in bioavailability, which is relatively poor for chlorthalidone [8, 9]; non-linear binding of this drug to red blood cells [10, 15] and dose-dependent urinary excretion [16].

Some of these phenomena are shown in Figs. 3 and 4.

Detailed results of the bioavailability of some chlorthalidone preparations and the clinical implications will be the subject of further publication.

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