Journal of Chromatography, 277 (1983) 261—272
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1768

THIN-LAYER CHROMATOGRAPHIC DETERMINATION OF PROCAINAMIDE AND N-ACETYLPROCAINAMIDE IN HUMAN SERUM AND URINE AT SINGLE-DOSE LEVELS

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(First received August 4th, 1982; revised manuscript received May 6th, 1983)

SUMMARY

Thin-layer chromatographic methods were applied for bioavailability studies of procainamide in serum and urine. Detection of the parent compound and the major metabolite was performed in the ultraviolet range at 275 nm. Using 100-μl samples, detection limits were 60 ng of procainamide—HCl per ml serum and 7 μg/ml urine, and 60 ng of N-acetylprocainamide—HCl per ml serum and 5 μg/ml urine. Advantages over previous methods are discussed. From serum and urine data of five volunteers, the bioavailability of procainamide from a 250-mg dragee preparation compared with an intravenous dose was verified. Pharmacokinetic data were computed using one-compartment open models. Results corresponded well with values previously published.

INTRODUCTION

Procainamide (Fig. 1) is an effective and widely used anti-arrhythmic drug [1]. Pharmacokinetic and metabolism data reported in the literature are primarily concerned with long-term therapy [1—9]. N-Acetylprocainamide, the potent [2] major metabolite, has to be regarded separately, since it has a comparatively lower clearance and a longer half-life than the parent drug. Two groups of human patients, related to their metabolite serum levels, should be discriminated, i.e. slow and fast acetylators. The latter are expected to have

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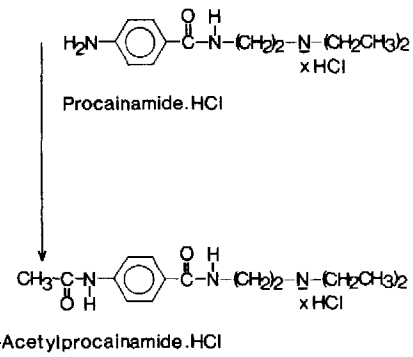


Fig. 1. Structure of procainamide · HCl and its metabolite.

a comparatively lower risk [4] of developing a syndrome resembling lupus erythematosus which may be induced by the parent drug.

For therapeutic monitoring in serum, detection limits of 1 μg/ml procainamide · HCl and 1 μg/ml N-acetylprocainamide · HCl, respectively, suffice. Assays published in the last decade have utilized gas chromatography (GC) [10–12], high-performance liquid chromatography (HPLC) [13—19], and thin-layer chromatography (TLC) [20—22]. In most cases, serum aliquots of 0.5—1 ml were used.

For single-dose pharmacokinetics, however, especially for bioavailability studies of various preparations, analytical methods for serum and urine are required which are practicable, selective and accurate. They should allow the simultaneous assay of both compounds at the 100 ng/ml serum and 10 μg/ml urine levels. Since large numbers of individual blood samples are usually needed, preferably small serum aliquots should be used for each analysis.

The present report describes sensitive TLC assays for procainamide and N-acetylprocainamide in serum and urine. In one simple extraction step, 100-μl samples are cleaned-up for chromatographic analysis.

EXPERIMENTAL

Reagents

The reagents used were 1 mmol/l carbonate buffer pH 10.9 AR, ethyl acetate AR, 1,4-dioxane AR, methanol AR, concentrated ammonia solution (25%) AR.

Equipment

A Zeiss KM3 chromatogram spectrometer with microoptics and a Servogor® 210 (Metrawatt) recorder were used. Separation was performed on silica gel HPTLC plates F 254 (no. 5642, E. Merck, Darmstadt, F.R.G.) in a Camag twin-trough HPTLC chamber 20 cm × 10 cm (no. 25254). For sample clean-up and spotting, a Vortex® mixer, a centrifuge, glass-stoppered tubes (ca. 8 ml), conical glass-stoppered tubes (ca. 8 ml) and a Desaga Autospotter® were used.

Serum procedure

In a glass-stoppered tube, 100 μl of serum were treated with 1 ml of buffer

pH 10.9. The serum was extracted with 5 ml of ethyl acetate for 30 sec on a Vortex mixer. The phases were separated by centrifugation (5 min), and 4 ml of the organic phase were transferred into a conical tube and evaporated to dryness at 40°C under a stream of nitrogen. The residue was dissolved in 100 µl of ethyl acetate.

Using the Desaga Autospotter*, 75 µl were transferred onto the HPTLC plate as a series of consecutive droplets of approx. 100 nl each. Since each drop evaporated before the next was applied, narrow spots were obtained which were suitable for HPTLC.

Urine procedure

In a glass-stoppered tube, 100 µl of urine were treated with 1 ml of buffer pH 10.9. The urine was extracted with 5 ml of ethyl acetate for 30 sec on the Vortex mixer. The phases were separated by centrifugation (5 min) and 75 µl were transferred onto the HPTLC plate.

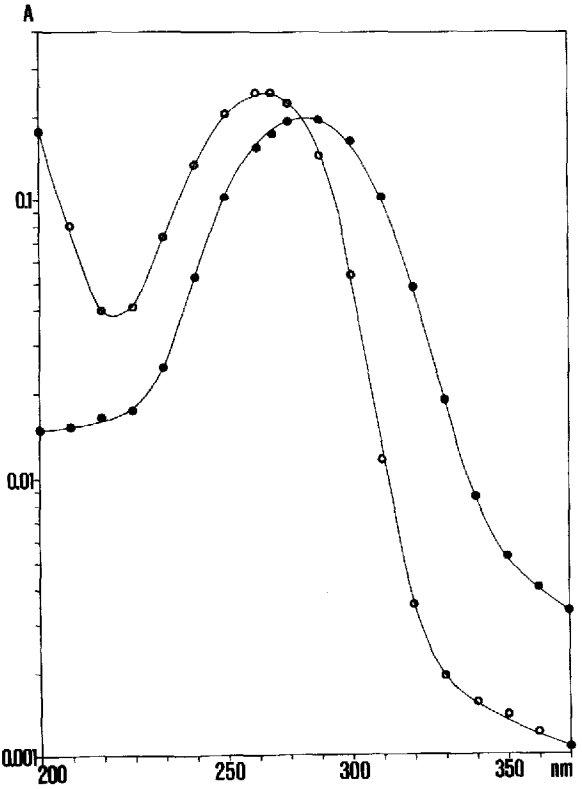


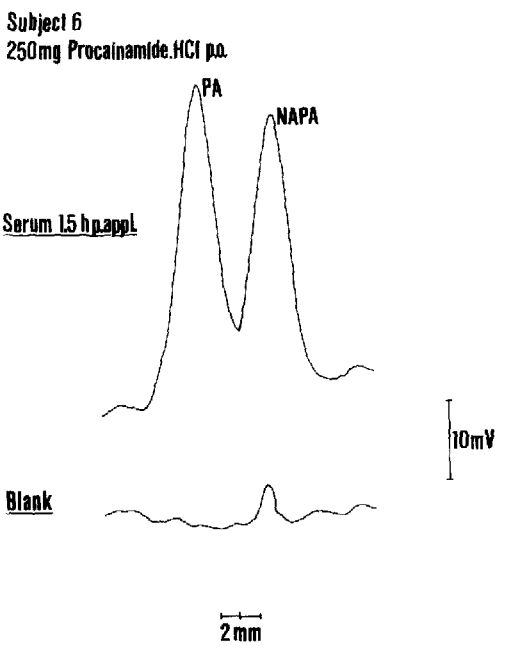
Fig. 2. In situ ultraviolet spectra of procainamide · HCl (•) and N-acetylprocainamide · HCl (○) on an HPTLC plate; 0.5 µg per spot.

*Modified version, Tygon® tubes of larger diameter [Technicon flow-rated, code 116-0549 09 (white)] and 60-cm long Hostaflon® tubes were used.

Chromatography

The twin-trough HPTLC developing chamber contained 10 ml of solvent in one compartment. The plate was developed with dioxane–concentrated ammonia (9:1) in the dark, without previous saturation, over a distance of 4 cm. R_F values were procainamide 0.55, N-acetylprocainamide 0.45.

Measurements were carried out in the direction of the solvent flow with an effective slit (microoptics) of 4.5 mm × 0.15 mm at a wavelength of 275 nm (Fig. 2), scanning speed 50 mm/min and paper speed 240 mm/min (Figs. 3 and 4).



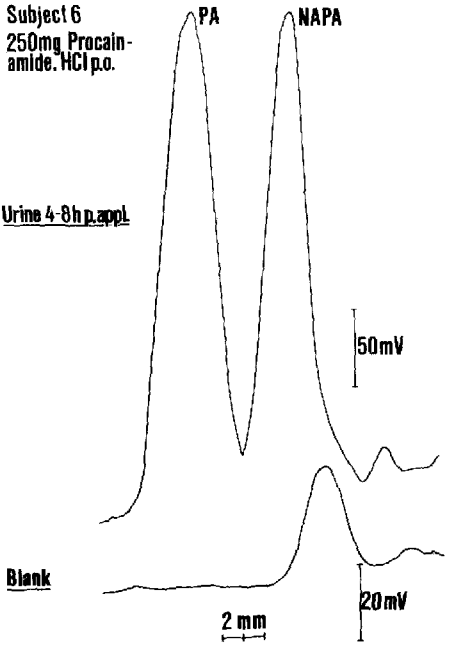


Fig. 3. Determination of procainamide · HCl (PA, 0.90 μg/ml) and N-acetylprocainamide · HCl (NAPA, 0.55 μg/ml) in serum, compared to a blank.

Fig. 4. Determination of procainamide · HCl (PA, 103 μg/ml) and N-acetylprocainamide · HCl (NAPA, 53 μg/ml) in urine, compared to a blank.

Quantitation

Peak height evaluation leads to a better precision and accuracy compared to peak area determinations, since these results depend much less on the quality of peak separation and on the baseline noise. Therefore, calibration functions were determined for each compound from the peak heights of the standards (Fig. 5).

These functions were non-linear. Deviation from linearity, however, could be expressed in terms of a parameter k_m according to Kufner and Schlegel [23]:

$$C = \frac{C_{max} \times E_{rel} \times k_m}{1 - E_{rel} + k_m}$$

where C = unknown concentration, C_{max} = maximum calibration standard,

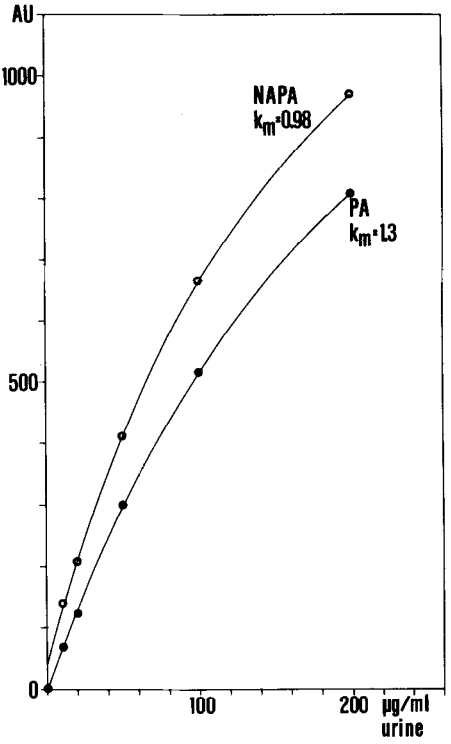


Fig. 5. Non-linear calibration graphs for procainamide · HCl (PA, •) and N-acetylprocainamide · HCl (NAPA, ○).

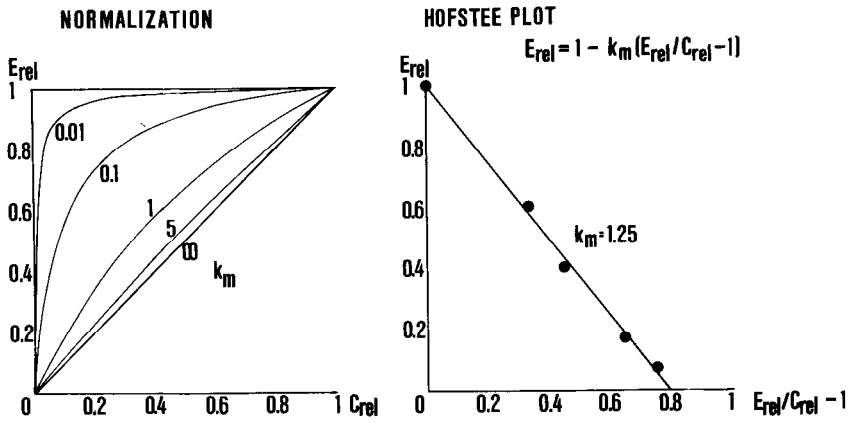


Fig. 6. Determination of k_m, parameter of non-linearity, from a Hofstee plot.

E_{rel} = peak height/maximum peak height, and k_m = parameter of non-linearity. The latter was obtained from a Hofstee plot following normalization of peak heights and concentrations (Fig. 6).

RESULTS

Serum

The compounds were added to blank serum in five concentrations over the range 0.1—2.0 μg/ml serum. Each sample was split into six portions of 0.1 ml, so that six equal series were formed. Each series was then analyzed in turn so that a total of six independent analytical results were available for each concentration.

Quality criteria of an analytical method are selectivity, accuracy, reproducibility and sensitivity [24, 25]. The corresponding parameters were derived from the analytical results given in Table I (except inter-assay reproducibility).

TABLE I

RECOVERY, PRECISION AND ACCURACY OF PROCAINAMIDE AND N-ACETYLPROCAINAMIDE IN SERUM

n = 6 determinations, concentration in μg/ml.

Procainamide · HCl			N-Acetylprocainamide · HCl		
Added	Found (mean ± S.D.)	Accuracy	Added	Found (mean ± S.D.)	Accuracy
2.00	2.01 ± 0.02	—0.01	2.00	2.00 ± 0.00	0.00
1.00	1.02 ± 0.08	—0.02	1.00	1.00 ± 0.07	0.00
0.50	0.45 ± 0.03	+0.05	0.50	0.49 ± 0.04	+0.01
0.20	0.21 ± 0.02	—0.01	0.20	0.21 ± 0.02	—0.01
0.10	0.10 ± 0.01	±0.00	0.10	0.09 ± 0.02	+0.01
Blank	0		Blank	0	

Selectivity. The serum blank does not contain any peak which could interfere with the assay (Fig. 3).

Accuracy. This was expressed by the deviation (bias) of the mean value of the results from the theoretical value: accuracy = $C_{added} - \bar{C}_{found}$. In each case the average accuracy was <0.01 μg/ml serum. Regression coefficients were greater than 0.998.

Precision (intra-assay reproducibility). This was defined in terms of standard deviations of the results obtained from each sample. It was constant in the concentration range considered: procainamide · HCl, S.D. = 0.03 ± 0.03 μg/ml serum; N-acetylprocainamide · HCl, S.D. = 0.03 ± 0.03 μg/ml serum.

The limit of detection was 60 ng/ml serum for both compounds. It was calculated as precision × 2 [26].

Inter-assay reproducibility. This was tested in two spiked control sera. From values obtained between September and November 1979, the following degrees of inter-assay reproducibility were determined (μg/ml, mean ± S.D.): For control 1, procainamide · HCl = 2.07 ± 0.22, and N-acetylprocainamide · HCl = 1.95 ± 0.12. For control 2, procainamide · HCl = 0.54 ± 0.13, and N-acetylprocainamide · HCl = 0.56 ± 0.08.

Urine

The compounds were added to blank urine in five concentrations over the range 10—200 μg/ml urine. Each sample was split into six portions of 0.1 ml, so that six equal series were formed. Each series was than analyzed in turn so that a total of six independent analytical results were available for each concentration.

Quality criteria of the urine method were defined by the corresponding parameters calculated from the analytical results given in Table II (except inter-assay reproducibility).

TABLE II

RECOVERY, PRECISION AND ACCURACY OF PROCAINAMIDE AND N-ACETYLPROCAINAMIDE IN URINE

n = 6 determinations, concentration in μg/ml.

Procainamide · HCl			N-Acetylprocainamide · HCl		
Added	Found (mean ± S.D.)	Accuracy	Added	Found (mean ± S.D.)	Accuracy
200	200 ± 0	0	200	201 ± 2	−1
100	101 ± 9	−1	100	99 ± 5	+1
50	49 ± 6	+1	50	48 ± 2	+2
20	21 ± 3	−1	20	23 ± 2	−3
10	10 ± 2	0	10	11 ± 1	−1
Blank	0		Blank	0	

Selectivity. The assay is practically free from blanks for both substances; a small blank as observed near the metabolite R_F was in the range of the detection limit (Fig. 4).

Accuracy. For each substance, the average accuracy was <0.3 μg/ml urine. Regression coefficients were greater than 0.998.

Precision (intra-assay reproducibility). This was constant in the concentration range considered. For procainamide · HCl, S.D. = 3.3 ± 3.6 μg/ml urine; for N-acetylprocainamide · HCl, S.D. = 2.4 ± 1.5 μg/ml urine.

The limit of detection (DL), taken as precision × 2, was 7 μg/ml urine for procainamide · HCl and 5 μg/ml urine for N-acetylprocainamide · HCl.

Inter-assay reproducibility. This was tested in two spiked control urines. From values obtained between September and November 1979, the following degrees of inter-assay reproducibility were determined (μg/ml, mean ± S.D.): For control 1, procainamide · HCl = 196 ± 9, N-acetylprocainamide · HCl = 194 ± 12. For control 2, procainamide · HCl = 50 ± 9, N-acetylprocainamide · HCl = 50 ± 7.

Pharmacokinetics

In a cross-over study, procainamide · HCl was administered intravenously and orally in doses of 250 mg to six healthy male volunteers (age 25—31 years, height 165—189 cm, weight 56—74 kg). Blood was sampled and urine collected in fractions up to 24 h post administration.

Using TLC, serum levels were found in the ranges 0.05—2 μg/ml for procainamide · HCl and 0.05—0.7 μg/ml for N-acetylprocainamide · HCl. Urine levels were found in the ranges 5—600* μg/ml for procainamide · HCl and 5—90 μg/ml for N-acetylprocainamide · HCl.

Pharmacokinetic profiles were calculated based on one-compartment open models. This is demonstrated by the serum kinetic results of subject 2 following intravenous and oral doses (Figs. 7 and 8) and by mean cumulative excretion values of five** volunteers (Fig. 9).

Following intravenous dosage, the initial serum concentration of 1.7 ± 0.3 μg/ml procainamide · HCl corresponded to a volume of distribution of 144 ± 20 l and a distribution coefficient of 2.1 ± 0.2 l/kg. The drug was eliminated with a half-life of 2.4 ± 0.4 h. The area under the curve was 6.0 ± 0.9 μg h/ml procainamide · HCl. The total clearance was 711 ± 106 ml/min.

N-Acetylprocainamide serum levels rose after a lag time of 10 ± 14 min with a half-life of 4 ± 0 min. Maximum levels of 0.4 ± 0.1 μg/ml N-acetylprocainamide · HCl were reached 0.7 ± 0.2 h post administration. The metabolite was eliminated with a half-life of 8 ± 2 h. The area under the curve was 4.3 ± 1.0 μg h/ml N-acetylprocainamide · HCl.

Following oral treatment, the drug was absorbed after a lag time of 13 ± 10 min with a half-life of 9 ± 6 min. Maximum serum levels of 1.2 ± 0.2 μg/ml were reached 0.8 ± 0.3 h post administration. The elimination half-life of 2.5 ± 0.3 h and the area under the curve of 4.8 ± 1.0 μg h/ml corresponded well with the data after intravenous dosage (see Tables III and IV). Metabolite serum levels rose after a lag time of 17 ± 10 min with a half-life of 16 ± 10 min.

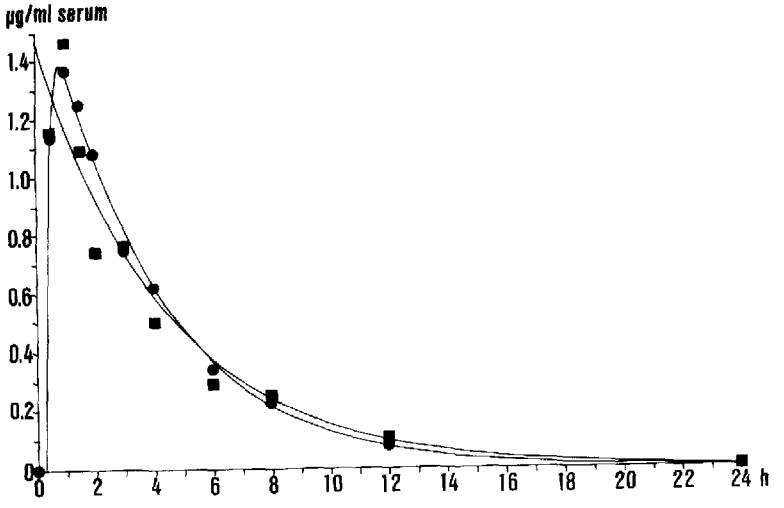


Fig. 7. Procainamide · HCl serum levels of subject 2 after a single dose of 250 mg intravenously (▪) and orally (•).

*For levels greater than 200 μg/ml urine, smaller aliquots were used.

**Subject 3 was excluded since, following the oral dose, higher serum levels and a higher degree of renal excretion compared to the intravenous dose were observed.

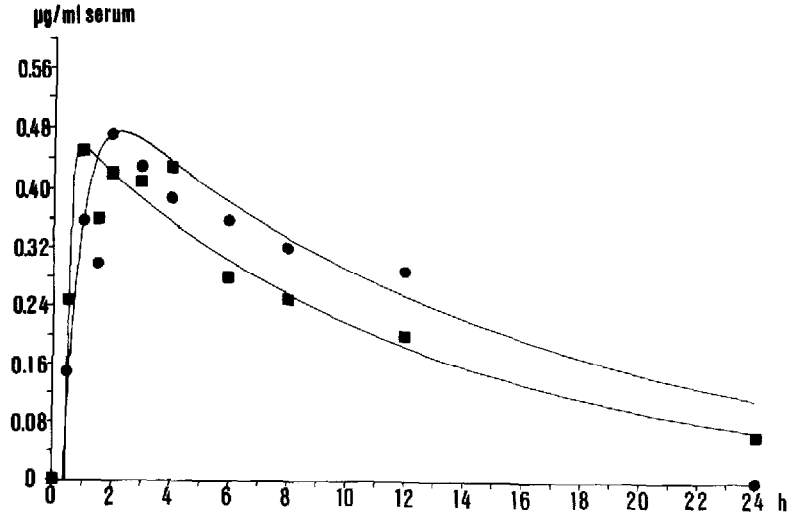


Fig. 8. N-Acetylprocainamide · HCl serum levels of subject 2 after a single dose of 250 mg procainamide · HCl intravenously (▪) and orally (•).

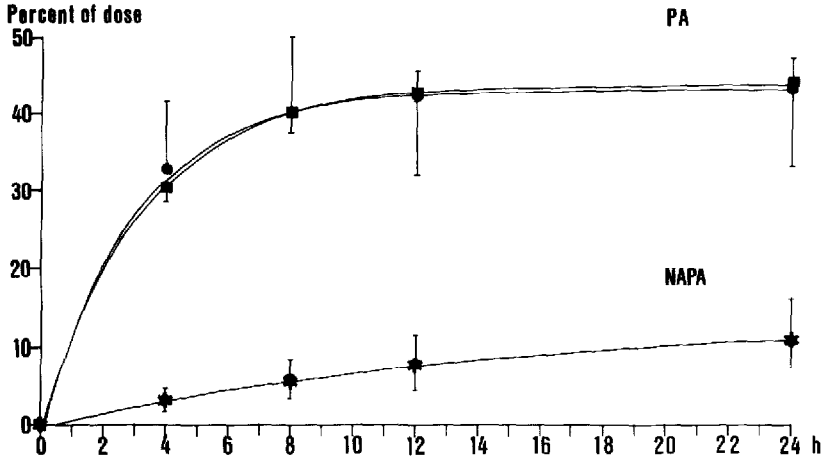


Fig. 9. Mean cumulative renal excretions after a single dose of 250 mg procainamide · HCl to five volunteers intravenously (PA, ▪; NAPA, *) and orally (PA, •; NAPA, ○).

Maximum serum levels of 0.5 ± 0.1 µg/ml were reached 1.6 ± 0.7 h post administration.

The elimination half-life of 8 ± 2 h and the area under curve of 5.8 ± 1.4 µg h/ml corresponded well with the data after intravenous dosage (see Tables III and IV).

From the cumulative renal excretions (Fig. 9), urinary half-lives were identical to serum half-lives (Table IV).

Cumulative renal excretions up to 24 h post procainamide administration are presented in Table V in comparison to the extrapolated amounts.

Renal clearances were calculated from data up to 24 h post administration. Values after oral dose were 385 ± 85 ml/min in the case of procainamide and

TABLE III

AREAS UNDER CURVES

Values are given in μg h/ml.

	Procainamide · HCl	N-Acetylprocainamide · HCl	Sum as procainamide · HCl
Intravenous	6.0 ± 0.9	4.8 ± 1.0	9.7 ± 1.4
Oral	4.3 ± 1.0	5.8 ± 1.4	9.9 ± 1.9

TABLE IV

HALF-LIVES

Values are given in hours.

	Procainamide · HCl		N-Acetylprocainamide · HCl	
	Serum	Urine	Serum	Urine
Intravenous	2.4 ± 0.4	2.4 ± 0.5	8 ± 2	8 ± 5
Oral	2.5 ± 0.3	2.3 ± 0.6	8 ± 2	10 ± 4

TABLE V

CUMULATIVE RENAL EXCRETION

Results* are expressed as percentage of dose administered.

	Procainamide · HCl	N-Acetylprocainamide · HCl	Sum
Intravenous dose			
$U^{24\ h}$	44 ± 4	11 ± 5	55 ± 8
U^{∞} (calc.)	44 ± 4	14 ± 9	58 ± 12
Oral dose			
$U^{24\ h}$	44 ± 10	11 ± 3	55 ± 11
U^{∞} (calc.)	44 ± 10	13 ± 4	57 ± 11

*U = urinary excreted drug.

TABLE VI

BIOAVAILABILITY OF PROCAINAMIDE FROM A 250-mg DRAGEE COMPARED WITH AN INTRAVENOUS DOSE

Ratio*	Drug (%)	Drug and metabolite (%)
$AUC^{\infty}_{p.o.}/AUC^{\infty}_{i.v.}$	81 ± 13	102 ± 11
$U^{\infty}_{p.o.}/U^{\infty}_{i.v.}$	100 ± 26	101 ± 26

*AUC = area under the curve, U = urinary excreted drug, i.v. = intravenous dose, p.o. = oral dose.

113 ± 36 ml/min in the case of N-acetylprocainamide corresponding to those after intravenous dose which were 316 ± 63 ml/min (drug renal clearance) and 161 ± 117 ml/min (metabolite renal clearance), respectively. Following oral and intravenous administration of 250 mg procainamide · HCl to five subjects, drug bioavailability was determined from individual ratios of serum areas under the curves and from cumulated renal excretions [27]. The average drug bioavailability was greater than 81%. Complete bioavailability can be calculated from both serum and urine when the data for the major equally potent metabolite are also considered (Table VI).

DISCUSSION

Analytical methods were developed for procainamide and N-acetylprocainamide in serum and also in urine. They proved to be practicable, selective, and sensitive enough to follow pharmacokinetics in serum and urine after a single intravenous or oral dose of 250 mg of procainamide · HCl to volunteers.

The HPLC method for serum of Su and Au [17] may be taken for comparison. They used 200-μl samples and, following back-extraction steps, could measure minimum serum levels of 300 ng/ml. For the TLC assay described here, even smaller samples of 100 μl suffice and, following one simple extraction step, improved detection limits of 60 ng/ml are obtained for both compounds. In general, from our experience, TLC may be used in most cases when low amounts of drug have to be determined. Since automatization of TLC [28] has become possible, even large series of samples can be processed with reasonable work and cost factors.

Pharmacokinetic data presented here corresponded well with values published previously. The drug volume of distribution (144 ± 20 l) and the distribution coefficient (2.1 ± 0.2 l/kg) agreed with the results of Galeazzi et al. (184 ± 24 l) [7] and of Koch-Weser and Klein (1.74—2.12 l/kg) [1], respectively. The drug half-life of 2.4 ± 0.4 h was in the range 2.5—4.7 h as reported by Koch-Weser and Klein [1]. Total clearance (711 ± 106 ml/min) was identical with data from Galeazzi et al. (851 ± 220 ml/min) [7] and Lima et al. (637 ± 215 ml/min) [8]. Drug renal clearance of 316 ± 63 ml/min was identical to the 341 ± 68 ml/min reported by Galeazzi et al. [7].

The metabolite distribution coefficient of 1.4 ± 0.1 l/kg was determined by Stec et al. [29] following single intravenous doses. The metabolite half-life of 8 ± 2 h from the present study agreed with that reported by Lima et al. (6 h) [8], Ludden et al. (7.5 h) [30] and Roden et al. (7.5 ± 2.2 h) [31]. The total clearance of 254 ± 15 ml/min from Stec et al. [29] was the same as that reported by Ludden et al. (283 ± 52 ml/min [30]. The renal clearance of 161 ± 117 ml/min from the present study agreed with that determined by Strong et al. (179 ± 40 ml/min) [6], Stec et al. (216 ± 25 ml/min) [29] and Ludden et al. (183 ± 50 ml/min) [30]. Deacetylation of N-acetylprocainamide was reported by Stec et al. [29] to be a minor route of elimination with a clearance of 6.5 ml/min (≈ 2.8% of total clearance).

In the present study, within 24 h post administration, 44 ± 4% of the intravenous dose was accounted for as unchanged drug in urine compared with 11 ± 5% as metabolite. The same values, 43 ± 10% and 10 ± 5%, respectively, were observed by Galeazzi et al. [10] following a single intravenous dose of 500 mg of procainamide · HCl to four volunteers.

All subjects of the present study were slow acetylators [32].

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