ACETYLATION OF PROCAINE AMIDE IN MAN STUDIED WITH A NEW GAS CHROMATOGRAPHIC METHOD

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1 A specific gas-chromatographic method was developed for determination of *N*-acetylprocaine amide in plasma and urine, using 4-amino-*N*-(2-piperidinoethyl)benzamide as an internal standard. The investigation was performed in 50 cardiac patients who had reached steady-state plasma concentrations of procaine amide.

2 The plasma concentrations of the acetylated metabolite varied between 1.0 and 15.0 µg/ml and were thus of the same order of magnitude as those of the parent drug. Especially high plasma levels of the metabolite were seen in patients with poor kidney function.

3 The urinary excretion of the metabolite varied markedly between individuals and ranged between 6 and 52% of the administered daily dose. There was a clear tendency towards higher rates of acetylation of procaine amide in patients with short plasma half-lives of isoniazid, i.e. the phenotype rapid acetylators. Because of coexisting therapy with other drugs and impaired renal function in some patients studies are required in healthy human volunteers to ascertain whether the acetylation of procaine amide and isoniazid are mediated by the same enzyme system.

Introduction

Procaine amide has been widely used as an antiarrhythmic drug for many years but its metabolism is not yet completely understood. It has been shown that 40-60% of a given dose is excreted unchanged in the urine and 2-10% as *p*-aminobenzoic acid or its conjugate (Mark, Kayden, Steele, Cooper, Berlin, Rowenstine & Brodie, 1951; Koch-Weser & Klein, 1971). Two other metabolites have been reported by Dreyfuss, Bigger, Cohen & Schreiber (1972), one of these was identified as *N*-acetylprocaine amide, which appears to be a main metabolite in man (Karlsson, 1973; Karlsson, Åberg, Collste, Molin, Norlander & Sjöqvist, 1974).

In several investigations it has now been shown that isoniazid (INH), hydralazine and some sulphonamides are acetylated in the liver by a common enzyme system. The population consists of slow and fast acetylators, the former representing an autosomal recessive trait in a polymorphic system (Evans, 1968; Gelber, Peters, Gordon, Glazko & Levy, 1971; Schröder & Evans, 1972). It has been demonstrated that slow

acetylators are most prone to develop side-effects on these drugs, e.g. a systemic lupus erythematosus-like (SLE) syndrome after hydralazine therapy (Perry, 1973). As a similar SLE-like syndrome can be induced by procaine amide (Dubois, 1969) we found it of great interest to investigate the quantitative aspects of its acetylation in a patient material and to relate these data to the acetylator phenotype determined from the isoniazid elimination rate. Further incentive for the study stems from the recent demonstration by independent workers that *N*-acetylprocaine amide has antiarrhythmic effects in animals (Karlsson *et al.*, 1974; Elson, Strong & Atkinson, 1974; Drayer, Reidenberg & Sevy, 1974).

Methods

Patients

Fifty patients with suspected or proven acute myocardial infarction treated initially with

lidocaine and then with procaine amide because of ventricular arrhythmias were studied. It has been shown previously that no kinetic interaction occurs between these two drugs (Karlsson, Collste & Rawlins, 1974). Some characteristics of the patients are given in Table 1.

All patients received an oral or intravenous loading dose of procaine amide followed by ordinary tablets (Pronestyl^R, Squibb) every 3rd-4th hour or sustained release tablets t.d.s. (Karlsson, 1973) with a total dose of about 50 mg kg⁻¹ day⁻¹. In order to determine the plasma concentrations of procaine amide and *N*-acetylprocaine amide at least three blood samples were drawn from all patients on the day of investigation. A 24 h urinary specimen was also collected. The blood samples were drawn just before the morning and early afternoon doses and at expected maximum plasma levels during a dosage interval. The time for the peak plasma levels had been determined previously for the two dosage forms (Collste & Karlsson, 1973; Karlsson, 1973).

The investigation was performed after 3-6 days of procaine amide therapy, i.e. during steady-state. As expected there was no difference in the renal excretion of *N*-acetylprocaine amide between days 3 and 6 (unpublished observations). The urinary recovery of unchanged procaine amide and *N*-acetylprocaine amide was the same after the two dosage forms when given in a cross-over fashion

(Karlsson, 1973). It should be added that most patients had to be given other drugs during the course of the study such as digitalis, diuretics, analgesics and sedatives. At least 24 h after procaine amide therapy was discontinued the acetylation phenotype was determined in 19 patients as follows. The fasting subject received isoniazid (INH) as the commercial preparation Tibinide^R (Ferrosan) in a dose of approximately 10 mg/kg orally. Heparinized blood samples were drawn at 3, 5, 7, 9 and 12 h thereafter and analysed according to Maher, Whitney, Chambers & Stanonis (1957). The plasma half-lives of INH and the overall elimination rate constant (*k*) were calculated from the regression lines representing the logarithmic decay of the concentration with time. The correlation coefficients for all the regression lines used for the calculations were at least 0.98.

Analytical procedures

Reagents and chemicals. Procaine amide hydrochloride was of pharmacopoeial grade. The internal standard, 4-amino-*N*-(2-piperidinoethyl) benzamide (PANBA) was synthesized according to Thyrum & Day (1965). *N*-acetylprocaine amide base was synthesized from procaine amide hydrochloride according to the principles described by Dreyfuss *et al.* (1972), but substituting acetic anhydride for acetyl chloride.

The melting points were: PANBA hydrochloride 208-210°C and *N*-acetylprocaine amide base 136.5-138°C. Other chemicals and solvents were of analytical grade.

Extraction procedures

(a) **Urine:** A solution of PANBA (100 µl, 5 mg/ml) (pH = 4.0) was added to urine (1 ml) in a conical 12 ml glass-stoppered centrifuge tube. The urine was alkalized with 2.5 *N* NaOH (100 µl) and then mixed with dichloromethane (5 ml, Merck p a, redistilled). After extraction for 15 min the tube was centrifugated at 2500 rev/min for 10 minutes. The aqueous phase was removed and the organic phase (3 ml) was transferred to a 12 ml silanized centrifuge tube. The solvent was evaporated by a gentle stream of nitrogen at 40°C. The residue was dissolved in dichloroethane (400 µl) and 0.5 µl was injected into the gas chromatograph.

(b) **Plasma:** 100 µl of a solution of procaine amide (1 mg/ml)—added to decrease the adsorption of *N*-acetylprocaine amide to the column—was mixed with plasma (1 ml), 100 µl of the internal standard PANBA (80 µg/ml, pH = 4.0), 100 µl of 2.5 *N* NaOH and 5 ml dichloromethane (BDH

Table 1 Clinical details of the patients

Characteristics	Number
Sex	
Male	43
Female	7
Age (years)	
Mean	62
Range	39-82
Diagnosis	
Acute myocardial infarction	36
Nonproven myocardial infarction	14
Renal function	
Creatinine clearance (ml/min)*	
>80	31
61-80	5
41-60	2
21-40	3
Liver function	
Bilirubin (mg %) (normal < 1.2)	
<1.2	48
>1.2	2

* Information lacking in 9 patients.

Quality, redistilled) in a 12 ml conical glass-stoppered pyrex centrifuge tube and extracted for 15 minutes. After centrifugation at 3000 rev/min for at least 10 min the plasma was removed and discarded. The remaining dichloromethane was transferred to a new conical centrifuge tube and 0.1 N H_2SO_4 (1 ml) was added. After extraction for 15 min and centrifugation the aqueous phase was transferred to a new tube containing 2.5 N NaOH (100 μl) and dichloromethane (5 ml). The samples were re-extracted for 15 min and centrifuged for 5 minutes. The aqueous phase was discarded and the dichloromethane phase was transferred to a silanized glass tube and evaporated by a gentle stream of nitrogen at 40°C. The residue was dissolved in dichloroethane (25 μl) and 2 μl injected into the gas chromatograph.

Spectrophotofluorimetry. The concentration of procaine amide in plasma was determined according to the spectrophotofluorimetric method described by Koch-Weser & Klein (1971), but substituting benzene for toluene. In this procedure *N*-acetylprocaine amide gives minor interference.

Gas chromatography. The instrument used was a Varian 1400 gas chromatograph equipped with a hydrogen flame ionization detector and a Varian A25 strip-chart recorder.

(a) Urine analysis: The column used was a 2 m $\times$ 1.5 mm (i.d.) silanized glass coil packed with 0.75% OV 17 (Varian Inc, Walnut Creek, California) on Chromosorb G AW DMCS conditioned at 290°C for 24 h and at 270°C for 90 hours. It was silanized by injection of Silyl 8 (Pierce Chemical Co, Rockford, Illinois). The temperatures used were: injector 275°C, column 270°C, and detector 275°C. Gas flow rates were: nitrogen 25 ml/min, hydrogen 25 ml/min and oxygen 250 ml/minute.

Standard samples containing 200, 400, 800, 1200 and 1600 μg procaine amide and 200, 400, 600, 800 and 1000 μg *N*-acetylprocaine amide/ml urine were prepared by adding 50 μl of water solutions (pH 5.0) of the compounds to drug-free human urine.

The collected urine had been stored at +4°C for up to 11 days before analysis. Both procaine amide and *N*-acetylprocaine amide were found to be stable in urine stored at +4°C for up to 2 months. *N*-acetylprocaine amide was stable in frozen urine for up to 3 months. However, the concentration of procaine amide in frozen urine decreased within a few days and after one week it reached a plateau of 75% of the original value, which was maintained after 2 months (Graffner & Skånberg, personal communication; and personal observations).

(b) Plasma analysis: The column used was a 1.7 m $\times$ 1.5 mm (i.d.) silanized glass coil packed with 5% OV 17 on Chromosorb W HP 80/100 mesh conditioned in the following way: the column was kept at 90°C with a nitrogen flow for 1 h, at 340°C for 6 h without nitrogen flow and 36 h at 305°C with a nitrogen flow. When the column was not in use the column temperature was maintained at 180°C. During chromatography the temperatures were: injector 285°C, column 280°C and detector 285°C. Gas flow rates were: nitrogen 25 ml/min, hydrogen 25 ml/min and oxygen 250 ml/minute.

Standard samples containing 1, 3, 5, 10 and 15 μg *N*-acetylprocaine amide/ml plasma were prepared by adding 50 μl of water solutions (pH 5.0) of *N*-acetylprocaine amide to drug-free human citrate plasma.

The collected plasma was stored at -20°C for 1-2 weeks before determination of procaine amide and for 3-12 months before analysis of *N*-acetylprocaine amide. Both procaine amide and *N*-acetylprocaine amide were stable at -20°C plasma for at least 6 months.

Statistical methods

Significance of differences between mean values was tested by Student's *t* test.

Results

Analysis of *N*-acetylprocaine amine by gas chromatography

A typical chromatogram from analysis of procaine amide and *N*-acetylprocaine amide in urine is shown in Fig. 1 and representative standard curves in Figure 2. Linear standard curves were obtained in the concentration range 200-3000 μg procaine amide/ml urine and 100-2000 μg *N*-acetylprocaine amide/ml urine. Since the standard curves did not go through the origin, indicating adsorption phenomena, a new standard curve was prepared for every run. The recovery of procaine amide and *N*-acetylprocaine amide from urine was $97 \pm 7\%$ (s.d.) and $94 \pm 7\%$ (s.d.) respectively.

A typical chromatogram from analysis of *N*-acetylprocaine amide in plasma is shown in Figure 3. A linear standard curve was obtained in the concentration range 1-15 μg /ml plasma (Figure 4). The recovery of *N*-acetylprocaine amide from plasma was found to be $85 \pm 7\%$ (s.d.).

The identity of the chromatographic peaks was ascertained by comparing the retention times and mass spectra with those of reference compounds. As a further control of specificity, urine and

Fig. 1 Chromatograms of extracts of patient urine. Left: Patient urine containing procaine amide (P, 1200 µg/ml) and *N*-acetylprocaine amide (N, 800 µg/ml). I, internal standard, 4-amino-*N*-(2-piperidinoethyl)benzamide. Right: Patient urine blank.

plasma were collected from the patients before procaine amide was given. The patients were then on other drugs (see above). No interfering gas chromatographic peaks were found.

Urinary excretion of *N*-acetylprocaine amide

The renal excretion of unchanged procaine amide, *N*-acetylprocaine amide and both during 24 h were $50.3 \pm 13.6\%$, $23.3 \pm 10.8\%$ and $73.6 \pm 15.9\%$ of administered dose respectively (mean $\pm$ s.d.; $n = 50$). There was a marked inter-individual variation in the excretion of *N*-acetylprocaine amide which ranged between 6.3 and 52.6%. Of the recovered procaine amide and *N*-acetylprocaine amide in the urine the former constituted $68 \pm 12\%$ and the latter $32 \pm 12\%$.

The percentage of administered dose of procaine amide excreted as *N*-acetylprocaine

Fig. 2 Standard curves (peak height ratios v. concentrations) for the estimation of (a) *N*-acetylprocaine amide and (b) procaine amide in urine.

amide in the individual patients is shown in Figure 5. The histogram in Fig. 6 depicts *N*-acetylprocaine amide in % of the total amount of unchanged drug and metabolite recovered in the urine in the entire patient material. Since the excretion of the two compounds is dependent on kidney function a similar plot has been made of the data obtained in 29 patients with normal renal function, i.e. creatinine clearance of 80 ml/min or more (Figure 7).

Plasma concentrations of procaine amide and *N*-acetylprocaine amide

The procaine amide levels in plasma were mostly within the apparent therapeutic range of 4–8 µg/ml (Koch-Weser & Klein, 1971).

Fig. 3 Gas chromatograms of extracts of patient plasma.

Left: Patient plasma blank. The prominent peak at retention time = 2.1 min is an unidentified impurity, which interferes with procaine amide in the plasma method.

Right: Patient plasma containing 5 µg *N*-acetylprocaine amide (N, 5 µg/ml). I, internal standard, 4-amino-*N*-(2-piperidinoethyl)benzamide.

There was a great inter-individual variation in the plasma concentrations of *N*-acetylprocaine amide with values ranging from 1.0 to 15.0 µg/ml. In some patients the procaine amide level was about 3-4 times higher than the metabolite level in plasma while in other cases the situation was the

Fig. 4 Standard curve (peak height ratio v. concentration) for the glc estimation of *N*-acetylprocaine amide in plasma.

reverse. The fluctuation of the metabolite concentration during each dosage interval appeared to be less than that of unchanged drug (Table 2).

Acetylation of procaine amide in relation to acetylator phenotype determined with isoniazid

Fifteen of the above patients were available for a study of the plasma half-life of a single oral dose (10 mg/kg) of isoniazid (INH). The results are given in Table 3. In accordance with previous results obtained in Swedish subjects (the antimode between fast and slow acetylators being 2 h, Hanngren, Borgå & Sjöqvist, 1970) two groups can be distinguished: one with half-lives ranging between 91 and 117 min and one with half-lives ranging between 128 and 360 minutes.

Fig. 5 *N*-acetylprocaine amide in urine (24 h) expressed as % of administered dose of procaine amide. Entire patient material ($n = 50$), steady-state conditions.

Fig. 6 *N*-acetylprocaine amide in urine (24 h) expressed as % of recovered procaine amide and *N*-acetylprocaine amide. Entire patient material ($n = 50$), steady-state conditions.

Fig. 7 The same plot as in Fig. 6—patients with normal kidney function ($n = 29$).

In fast acetylators (INH half-life < 2 h) the urinary excretion of *N*-acetylprocaine amide accounted for $43.1 \pm 10.3\%$ ($n = 5$) of the sum of procaine amide and its main metabolite in urine. The corresponding figure in slow acetylators (defined as INH half-life > 2 h) was $23.8 \pm 4.7\%$ ($n = 10$). This difference reaches statistical sig-

nificance ($P < 0.01$) but has to be interpreted with caution due to the predominance of patients with impaired kidney function in the fast acetylator group.

The correlation coefficient between recovered *N*-acetylprocaine amide in urine and the overall elimination rate constant of INH for all the

Table 2 Plasma concentrations of procaine amide and *N*-acetylprocaine amide during a dosage interval

	Plasma concentration ($\mu\text{g/ml}$), mean $\pm$ s.d.	
	Procaine amide ($n = 42$)	<i>N</i> -acetylprocaine amide ($n = 40$)
Before morning dose	3.4 ± 1.7	3.6 ± 2.7
1-2 h after morning dose	5.9 ± 2.0	4.0 ± 2.3
Before early afternoon dose	3.4 ± 1.5	3.6 ± 2.6

Table 3 Plasma isoniazid (INH) half-lives and urinary *N*-acetylprocaine amide in % of the sum of excreted procaine amide and *N*-acetylprocaine amide in 15 patients

Subject	Plasma half-life of INH (min)	Elimination constant (k) of INH	Acetylator phenotype according to INH test	N-acetylprocaine amide in urine %	Creatinine clearance (ml/min)
RJ	91	0.46	Rapid	49.5	67
UJ	101	0.41	Rapid	37.6	59
ER	102	0.41	Rapid	58.2	37
BO	114	0.37	Rapid	42.0	40
EK	117	0.36	Rapid	28.0	84
Group mean	105	0.40		43.1	
± s.d.	9.4	0.04		10.3	
LH	128	0.32	Slow	36.8	146
EJ	162	0.26	Slow	24.4	—
HJ	190	0.22	Slow	20.8	100
NK	215	0.19	Slow	24.5	88
AJ	215	0.19	Slow	21.9	117
NS	220	0.19	Slow	21.6	102
NE	246	0.17	Slow	23.5	—
KEK	256	0.16	Slow	20.5	116
AR	281	0.15	Slow	24.4	91
HT	360	0.12	Slow	19.6	99
Group mean	227	0.20		23.8	
± s.d.	61	0.05		4.65	
Significance of difference between group means (<i>P</i>)				<0.01	

Table 4 Plasma procaine amide and *N*-acetylprocaine amide, given in % of total amount of unchanged drug and metabolite, in relation to renal function and acetylator phenotype (if available)

Subject	Creatinine clearance (ml/min)	Acetylator phenotype (INH-test)	Plasma concentration before dose			
			Procaine amide (µg/ml, % of total amount)		N-acetylprocaine amide (µg/ml, % of total amount)	
Patients with impaired renal function (<i>n</i> = 8)						
ER	37	Rapid	5.1	25	15.0	75
EB	56	—	5.8	34	11.3	66
UJ	59	Rapid	3.6	47	4.1	53
OA	62	—	4.3	51	4.1	49
RJ	67	Rapid	3.2	29	7.7	71
JS	71	—	3.0	33	6.0	67
AK	72	—	4.9	44	6.3	56
JA	75	—	3.3	41	4.7	59
Group mean			4.2	38	7.4	62
± s.d.			1.0	8.6	3.6	8.6
Patients with normal renal function (<i>n</i> = 9)						
EK	84	Rapid	5.9	64	3.3	36
NK	88	Slow	3.8	68	1.8	32
AJ	93	Slow	2.6	57	2.0	43
HT	99	Slow	2.2	61	1.4	39
HJ	100	Slow	2.2	69	1.0	31
NS	102	Slow	2.2	61	1.4	39
KEK	116	Slow	1.3	57	1.0	43
NE	135	Slow	5.8	73	2.1	27
LH	146	Slow	1.4	30	3.2	70
Group mean			3.0	60	1.9	40
± s.d.			1.6	12	0.80	12
Significance of difference between group means (<i>P</i>)			<0.02		<0.02	

patients and for patients ($n = 10$) with creatinine clearance of 80 ml/min or more were 0.87 ($P < 0.01$) and 0.76 ($P < 0.02$) respectively.

In Table 4 are listed the concentrations in plasma of the parent drug and its acetylated metabolite, the creatinine clearance and the acetylator phenotype, if available. A comparison is made between eight patients with impaired renal function and nine patients with normal kidney function. In the first group the plasma concentration of the metabolite was 24% higher than that of the unchanged drug. In the latter group the situation was the reverse with a difference of 20%. The difference between the two groups reached statistical significance ($P < 0.02$).

There was a significant correlation between *N*-acetylprocaine amide in plasma and in urine ($r = 0.82$; $P < 0.001$) both measured as percentage of the sum of parent compound and its metabolite.

Discussion

Considerable emphasis has been put on the importance of kidney function for the elimination rate of procaine amide (Weily & Genton, 1972), but there are a few studies on the quantitative aspects of its metabolism in man and none in a large patient material. Using a new gas chromatographic procedure for the analysis of *N*-acetylprocaine amide great inter-individual variations were demonstrated in the present study in plasma concentrations and renal excretion of this metabolite. Thus, the extent of acetylation of procaine amide may explain some of the variability in plasma concentration of the drug seen in patients treated with similar doses.

The plasma concentration of *N*-acetylprocaine amide was of the same order of magnitude as that of the unchanged drug but in a few patients it was even three times as high. Therefore, it is important to evaluate the antiarrhythmic potency and possible side-effects of this metabolite in man. Koch-Weser & Klein (1971) have previously found

a good relationship between the plasma level of procaine amide and its clinical effects. Therefore the acetylated metabolite may be of minor pharmacodynamic importance in most patients. However, it would nevertheless play a role in patients with poor kidney function since they seem to achieve higher plasma levels than other patients.

One of the great disadvantages when using procaine amide for long-term prophylaxis against ventricular arrhythmias, is its inclination to induce a SLE-like syndrome. In a prospective investigation the antinuclear factor became positive in eight out of sixteen patients within 9 months and two of the patients also developed clinical symptoms of a SLE-like syndrome (Blomgren, Condemi, Bignall & Vaughan, 1969). The distribution of the *N*-acetylprocaine amide data (expressed in % of the sum of urinary procaine amide and *N*-acetylprocaine amide) in patients with normal renal function may very well be bimodal. Moreover, there is a significant correlation between the plasma INH half-lives and the acetylation rate of procaine amide, the shorter the INH half-life, the higher is the urinary excretion of *N*-acetylprocaine amide. Furthermore, four patients who previously had shown SLE-like syndromes during treatment with procaine amide, were phenotyped using the plasma INH half-life. All of them were slow acetylators. However, the results of this part of our investigation are not conclusive since it cannot be excluded that metabolism via the acetylation pathway is increased as the renal clearance of procaine amide decreases. The difficulty in studying the genetic aspects of the acetylation of procaine amide was further enhanced by the fact that most patients also received other drugs. In order to establish whether procaine amide is acetylated by the same enzyme system as INH studies are in progress in healthy drug-free human volunteers.

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