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Preparation of the glucosylamines of procainamide and evaluation of the pharmacokinetics of the β-glucosylamine of procainamide in the conscious rabbit

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Abstract

An aqueous solution of the glucosylamines formed when procainamide reacts with glucose has been prepared and the kinetics of the elimination of the β-anomer has been investigated in the conscious rabbit. Analytical methods were developed for the β-glucosylamine and for both procainamide and *N*-acetylprocainamide. The β-glucosylamine was not converted to procainamide in vivo and showed monoexponential elimination kinetics with a mean ($\pm$ 1 S.D.) $t_{1/2}$ of 0.92 $\pm$ 0.11 h and a V_z of 0.16 $\pm$ 0.03 l/kg as opposed to 0.93 $\pm$ 0.10 h and 6.09 $\pm$ 1.17 l/kg, respectively for procainamide. Simulations using this data indicated that administration of an infusion of procainamide hydrochloride in 5% glucose to human subjects could result in steady-state plasma concentrations of the β-glucosylamine which are fifteen times greater than for procainamide. The biological activity of the glucosylamines of procainamide remains to be studied. © 1997 Elsevier Science B.V.

Keywords: Procainamide hydrochloride; Glucose; Glucosylamines; Pharmacokinetic studies; *N*-acetylprocainamide; Plasma assay

1. Introduction

Procainamide hydrochloride is frequently administered following admixture of the injection to glucose intravenous infusion. A number of studies have demonstrated that the procainamide is lost from this admixture and this loss has been usually interpreted as involving the formation of a complex [1–6]. Recently it has been shown that the reaction involves the formation of an equilibrium mixture of the corresponding α- and β-glucosylamines and that these compounds are formed at a rate of 1–2% per h at room temperature following admixture of commercially available products [7].

Therefore, a significant proportion of the administered dose of the procainamide would be present as glucosylamines when the admixture is administered under the usual conditions. It is of interest to discover if the glucosylamines are converted back to procainamide in-vivo and gain an appreciation of the pharmacokinetics of these compounds. This paper reports the preparation of the glucosylamines free of contamination by procainamide and the pharmacokinetics of these compounds in rabbits.

2. Materials and methods

2.1. Materials

Procainamide hydrochloride, sulphadiazine and triethylamine (Sigma, USA) were used as supplied. All other chemicals were either analytical or high-performance liquid chromatography (HPLC) quality.

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2.2. Chromatographic equipment and conditions

The HPLC system consisted of a pump (501, Waters Associates, USA), autoinjector (K65B, Kortec, Australia), variable wavelength detector (481, Waters Associates) and integrating recorder (Hewlett–Packard, USA) with a column of octadecyl-bonded silica, 10 μm particle size, 30 cm × 3.9 mm ID (Phenomenex, USA). For the glucosylamines the mobile phase consisted of acetonitrile–water (6:94) containing 0.5% acetic acid and 0.35% triethylamine with the pH adjusted to 5.2 with sodium hydroxide and for procainamide and *N*-acetylprocainamide, acetonitrile–water (8:92) containing 0.1% phosphoric acid and 0.15% triethylamine. In both cases the monitoring wavelength was 279 nm and the flow-rate 1.5 ml/min.

2.3. Preparation of glucosylamines

A mixture of procainamide hydrochloride (3.0 g), anhydrous glucose (7.5 g) and water (2.5 ml) was agitated at 37°C for 10 days. The syrup, 1 ml, was diluted with water (3.5 ml), made alkaline with 1 M sodium hydroxide (0.5 ml) and extracted three times with methylene dichloride (5.0 ml). The aqueous solution was then purged with nitrogen at 50°C for 10 min to remove residual organic solvent and the resulting solution adjusted to pH 7.4 by the dropwise addition of 1 M phosphoric acid and the solution made to 10 ml with water. A sample was diluted with 0.05 M disodium phosphate solution and submitted to HPLC analysis.

2.4. Hydrolysis of glucosylamines

A mixture of procainamide hydrochloride (0.5 g) and anhydrous glucose (5.0 g) was dissolved in water to 10 ml and the solution allowed to stand at 37°C for 10 days. This syrup, 1 ml, was diluted with 9 ml of disodium phosphate solution (0.5 M) and submitted to chromatography under the conditions used for analysis. The two fractions corresponding to the two glucosylamines were collected into vials containing 0.1 ml of disodium phosphate solution (0.5 M). Hydrochloric acid, 40 μl, (5.0 M) was added to the collected fractions and the resulting solutions immediately submitted to analysis by HPLC at regular time intervals for 3 h.

2.5. Plasma assay of β-glucosylamine

To 50 μl of rabbit plasma was added 0.5 ml of a solution of sulphadiazine (5×10^{-4}% w/v) and the solution shaken for 2 min and submitted to centrifugation. The supernatant was transferred to a tube containing 10 μl of disodium phosphate solution (0.1 M) and the solution evaporated to dryness under a stream of nitrogen. The residue was dissolved into 100 μl of mobile phase and submitted to HPLC analysis. The assay was validated and standards were prepared by addition to blank rabbit plasma of standard solutions of glucosylamines made by diluting 1 ml of the injection to 250 ml with disodium phosphate solution (0.1 M).

2.6. Plasma assay of procainamide and N-acetyl procainamide

To 0.25 ml of rabbit plasma was added 50 μl of a solution of practolol hydrochloride (0.1 mg/ml), 0.25 ml of a solution of sodium hydroxide (1 M) and 3 ml of methylene dichloride and the solution was shaken for 2 min and the two layers separated by centrifugation. The upper layer was removed by aspiration and the lower layer transferred to a second tube and extracted with 0.25 ml of a solution of sulphuric acid (0.1 M). Following separation by centrifugation 50 μl of the upper layer was submitted to analysis by HPLC. The assay was validated and standard curves prepared by addition of a solution of the hydrochloride salts of procainamide and *N*-acetyl procainamide in water (2.5×10^{-2}% w/v) to blank rabbit plasma.

2.7. Rabbits

Male or female New Zealand half lop-cross rabbits (2.0–2.6 kg) were obtained from the Animal Resources Centre, Murdoch, W.A.

2.8. Pharmacokinetic studies

Procainamide (10 mg/kg) was administered as an i.v. bolus via a marginal ear vein over 1 min to three male and two female conscious rabbits. Blood samples (1 ml in heparinised tubes) were collected by venipuncture from a marginal ear just before drug administration and 5, 10, 20, 30, 40 min and 1, 2, 3, 4 5 and 6 h after drug administration. The solution of the mixed α- and β- *N*-glucosylamines prepared as described above (α:β = 10:90) was administered at a dose rate of 16.9 mg/kg (equivalent to 10 mg/kg free procainamide) via a marginal ear vein over 1 min to three male and three female conscious rabbits. Blood samples (1 ml in heparinised tubes) were collected by venipuncture from a marginal ear just before drug administration and 0.5, 1, 1.5, 2, 3, 4, and 5 h after drug administration. Plasma was separated by centrifugation and procainamide and *N*-acetylprocainamide or procainamide β-glucosylamine concentrations in the samples were analysed immediately by HPLC as described above. Plasma drug concentration-time data were subjected to noncompartmental pharmacokinetic analysis using the program Topfit 2.0 [8]. The terminal half-life ($t_{1/2}$) for procainamide, *N*-acetylprocainamide and procainamide β-

glucosylamine was determined by log-linear regression analysis of the last six, last five or all data pairs for procainamide, *N*-acetylprocainamide and procainamide β-glucosylamine respectively. Area under the plasma concentration-time curves for procainamide and procainamide β-glucosylamine was calculated by the trapezoidal rule and clearance (Cl), mean residence time (MRT), volume of distribution in pseudo distribution equilibrium (V_z) and steady-state volume of distribution (V_{ss}) were calculated using standard formulae [9].

3. Results and discussion

Previous studies using nuclear magnetic resonance spectroscopy and HPLC have demonstrated that procainamide reacts with glucose in aqueous solution to form a mixture of the α- and β-anomers of the corresponding glucosylamines with the β-anomer predominating in the ratio of 88–12% with no detectable amounts of the intermediate imine [7]. This is very similar to the values measured by Capon and Connett [10] using polarimetry of a range of analogous *N*-arylglucosylamines (10% α- and 90% β-anomer). Unlike the chromatographic conditions used in the study of Sianipar et al., the conditions developed for this study successfully resolved the two anomers, the ratio of the area of the two peaks being 90:10 for the glucosylamines formed in concentrated glucose solution. To confirm the identity of the two peaks as being due to the α- and β-anomers, chromatography was performed on a concentrated solution of the glucosylamines and the eluate fractions corresponding to the two peaks collected into disodium phosphate solution. The resulting alkaline solutions were stable, the major peak consisting of one component and the minor being contaminated with 16% of the major component. By addition of hydrochloric acid to these fractions it was possible to study the acid-catalysed anomerisation and hydrolysis of the two components and confirm their identity as the two possible glucosylamines. Both glucosylamines underwent rapid anomerisation to establish a mixture of anomers in the ratio of 88:12 and a slower hydrolysis to procainamide and glucose (Fig. 1). This behaviour is identical to that encountered by Capon and Connett [10] in their study of *N*-arylglucosylamines. Fig. 1 demonstrates the results for the β-anomer and identical results were obtained for the minor α-form.

As all attempts to produce a pure crystalline sample of the glucosylamines was unsuccessful [7] an alternate approach had to be made to quantitate the glucosylamines. In the hydrolytic experiment, if it is accepted that only glucosylamines and procainamide appear in the chromatogram and that no other side reactions are involved, it is possible to use the hydrolytic data (Fig. 1) to measure the relative absorbance of equimolar concentrations of procainamide and glucosylamines and from this derive a factor which permits glucosylamines to be quantitated using procainamide as an external standard. This factor was found to be 0.7451 ($\pm$ 2.2%, $n = 4$) utilising the peak areas of measurements between 2 and 24 min and assessment of the peak area for procainamide at 2 and 3 h. This approach has been used previously for the glucosylamines [7] where, using an alternate assay but the same monitoring wavelength and deriving values from the formation of the glucosylamines a factor of 0.7440 was determined. Therefore this value was considered satisfactory for the subsequent study.

A pure sample of a solution of the glucosylamines suitable for the pharmacokinetic study was prepared by reaction of a concentrated solution of procainamide hydrochloride in saturated glucose syrup. The high concentrations of reactants ensures that the reaction proceeds almost to completion and dilution of a sample of the syrup with disodium phosphate solution followed by HPLC analysis demonstrated that only 2.3% of the procainamide remained unreacted at equilibrium. The residual procainamide was removed by making the solution alkaline and selectively extracting the procainamide base with methylene dichloride. Under these conditions the highly water soluble glucosylamines were not extracted. Removal of residual organic solvent, adjustment of the pH to 7.4 and dilution with water afforded a solution of the mixed glucosylamines which was completely free of procainamide base and contained approximately 2% w/v of procainamide as the glucosylamines. The precise concentration of the compounds was determined by dilution and HPLC analysis. This solution was stable for 24 h when stored at 4°C and was used in the pharmacokinetic study and, upon

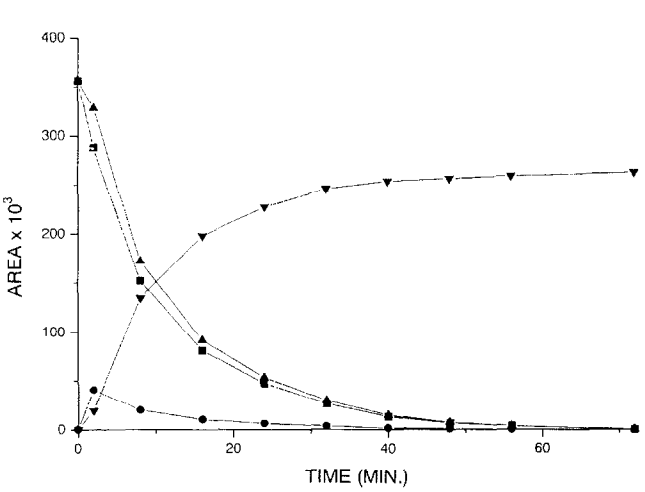

Fig. 1. Hydrolysis under acidic conditions of the chromatographic fraction containing the β-glucosylamine of procainamide. ■-β-glucosylamine, ●-α-glucosylamine, ▲-total area of the two glucosylamine peaks and ▼-procainamide.

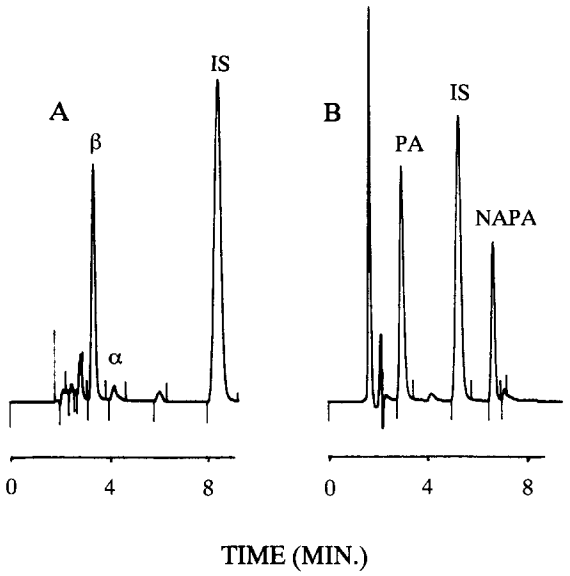

Fig. 2. Representative chromatograms derived from the assays employed in the study. A: for glucosylamines. α = α-glucosylamine, β = β-glucosylamine (23.4 mg/l) and IS = internal standard (sulphadiazine). B: for procainamide and *N*-acetylprocainamide. PA = procainamide (1.4 mg/l), NAPA = *N*-acetylprocainamide (1 mg/l) and IS = internal standard (practolol).

dilution, for the preparation of standards for development of the plasma assay.

The glucosylamines were determined in plasma by selective precipitation of proteins with acetonitrile. The supernatant was then evaporated to dryness following the addition of disodium phosphate to make the solution alkaline. Under these conditions no evidence of hydrolysis of the glucosylamines during work-up could be detected. Analysis by HPLC of the reconstituted solution in mobile phase afforded a chromatogram in which the β-anomer of the glucosylamine was well resolved from other peaks in the chromatogram (Fig. 2A). The minor α-anomer co-eluted with an endogenous component and could not be readily quantitated. The calibration line for the β-anomer was linear over the range 2.5–50 mg/l according to the equation: peak area ratio = 1.60×10^{-2} concentration (mg/l) + 4.40×10^{-3} ($r = 0.999$; $n = 10$) with coefficients of variation (CV) of $\pm 2.7\%$ at 25 mg/l and $\pm 11.7\%$ at 2.5 mg/l ($n = 6$).

The procainamide and *N*-acetylprocainamide were quantitated by extraction into methylene dichloride and back-extraction into 0.1 M sulphuric acid. The compounds eluted without interference from either endogenous materials or the glucosylamines which are not extracted into methylene dichloride (Fig. 2B). The calibration line for the procainamide was linear over the range 0.05 to 10 mg/l according to the equation: peak area ratio = 3.76×10^{-1} concentration (mg/l) – 4.51×10^{-2} ($r = 0.9996$, $n = 7$) with a CV of $\pm 3.3\%$ at a concentration of 0.5 mg/l and $\pm 8\%$ at 0.05 mg/l ($n = 6$). The line for the *N*-acetylprocainamide over the range 0.03–6.70 mg/l was: peak area ratio = 3.61×10^{-1} concentration (mg/l) – 6.55×10^{-2} ($r = 0.9980$, $n = 7$) with a CV of 2.8% at 0.26 mg/l.

Typical plasma concentration-time data from one rabbit following the administration of procainamide are shown in Fig. 3A. The disposition profile for procainamide was biexponential with a rapid distribution phase in the first 30 min followed by a slower elimination phase with a mean $t_{1/2}$ of 0.93 h. *N*-acetyl procainamide levels were either steady or fell slowly in the first hour following dosage and then showed an apparent slower elimination phase with a mean apparent $t_{1/2}$ of 2 h. The various pharmacokinetic descriptors for both procainamide and *N*-acetyl procainamide are summarised in Table 1.

Typical data for the disposition of procainamide β-glucosylamine following administration of 16.9 mg/kg of a 90:10 mixture of the α- and β-procainamide

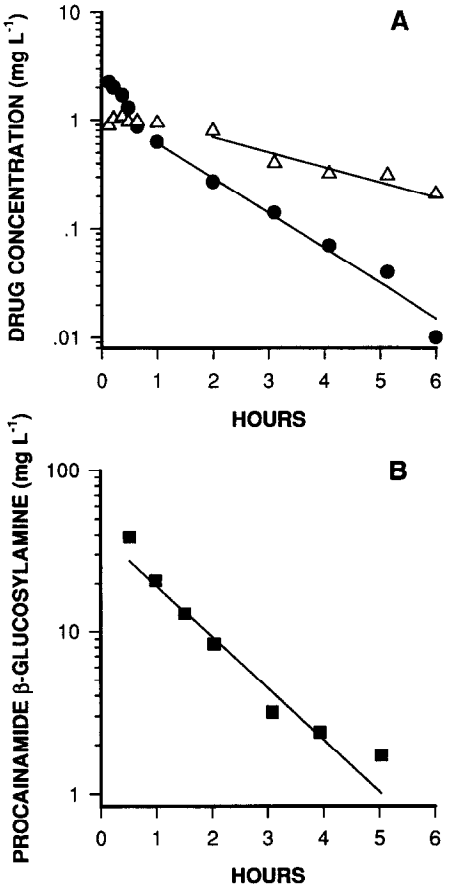

Fig. 3. Disposition of procainamide (●) and *N*-acetylprocainamide (△) following an i.v. dose of 10 mg procainamide per kg (panel A) and disposition of procainamide β-glucosylamine (■) following an i.v. dose of 16.9 mg mixed (10:90) α- and β-glucosylamines per kg (equivalent to 10 mg/kg of procainamide) (panel B). The solid lines are the predicted lines of best fit determined by log-linear least squares regression analysis. Data from rabbit # D.

Table 1
Pharmacokinetic descriptors[a] for procainamide[b], *N*-acetylprocainamide[b] and procainamide β-glucosylamine[c] in the conscious rabbit

Drug	$t_{1/2}$ (h)	MRT (h)	Cl (l/h per kg)	V_z (l/kg)	V_{ss} (l/kg)
Procainamide	0.93 ± 0.1	0.94 ± 0.11	4.55 ± 0.72	6.09 ± 1.17	4.23 ± 0.83
N-Acetylprocainamide	2.0 ± 0.4	ND	ND	ND	ND
Procainamide β-Glucosylamine	0.92 ± 0.11	1.07 ± 0.13	0.12 ± 0.02	0.16 ± 0.03	0.12 ± 0.02

[a]Data as mean ± S.D.
[b]Following an i.v. dose of 10 mg procainamide per kg; $n = 5$.
[c]Following an i.v. dose of 16.9 mg mixed (10:90) α- and β-glucosylamines per kg; $n = 6$.

glucosylamines are shown in Fig. 3B. The disposition profile was monoexponential with a mean $t_{1/2}$ of 0.92 h. Preliminary experiments in which sampling was more frequent in the first hour after drug administration failed to show a distribution phase. The various pharmacokinetic descriptors for procainamide β-glucosylamine are also summarised in Table 1. The α-anomer was detectable up to 3 h after dosage, but eluted very close to an endogenous component of the plasma extracts and could not be adequately quantified to enable the determination of pharmacokinetic descriptors. However, the α-anomer appeared to display a similar disposition profile to that of the β-anomer. Moreover, there was no chromatographic evidence for deconjugation of the glucosylamines to form the parent procainamide. While the mean MRT and $t_{1/2}$ values for procainamide and its β-glucosylamine were very similar, the mean V_z for the β-glucosylamine was some 24 times less than that for procainamide, resulting in a correspondingly lower Cl value for the β-glucosylamine. The small volumes of distribution for the β-glucosylamine are in keeping with its highly polar nature. Assuming an average clearance value of 36 l/h for procainamide in humans [11] and that the ratio of the clearance of procainamide β-glucosylamine to that of procainamide (0.026) in the rabbit is applicable to humans, simulations indicate that plasma levels of the β-glucosylamine during a steady-state infusion of 150 mg procainamide per h in 5% dextrose (containing approximately 30% of the dose as the β-glucosylamine) could be around 47 mg/l and approximately 15 times those of procainamide. In addition the steady state procainamide level would be 30% lower than that achievable in the absence of glucose. Lower concentrations of procainamide are unlikely to be a problem since procainamide dose is usually adjusted by plasma concentration monitoring. Whether the glucosylamines have any biological activity is unknown. Although the predicted high plasma concentrations for the β-glucosylamine would enhance any biological actions, its low volume of distribution may limit tissue penetration and any effects.

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