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Metabolism of Beclamide after a Single Oral Dose in Man: Quantitative Studies

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Abstract

A simple reverse phase HPLC assay is described for the determination of the anticonvulsant compound, beclamide and its 3- and 4-hydroxyphenyl metabolites in urine.

Following oral administration of 1 g beclamide to a panel of healthy volunteers, less than 0·4% of the dose was excreted unchanged in the 24-h urine and unconjugated 3- and 4-hydroxyphenyl metabolites were not detected. Based on examination of the urine after incubation with β-glucuronidase and aryl sulphatase, it was found that these hydroxyl metabolites were excreted as both glucuronide and sulphate conjugates. For each metabolite the glucuronide was the major excretory product (approximately 10 : 1). The 24-h excretion of the combined conjugated metabolites was 7% (for the 3-hydroxy metabolite) and 24% (for the 4-hydroxy metabolite) of the dose. Approximately 22% of the administered dose of beclamide was excreted as hippuric acid.

In view of the simplicity of assay, beclamide may be a useful tool substance with which to examine factors influencing the xenobiotic metabolic pathways of benzene ring hydroxylation and glucuronide and sulphate conjugation in man.

Beclamide (*N*-benzyl-β-chloropropionamide, Fig. 1) has been used in the management of both epilepsy and behavioural disorders associated with epilepsy (Sharpe et al 1958). There is continued interest in the drug because it and a novel analogue display a useful degree of anti-aggressive activity without sedation (Darmani et al 1990). Earlier studies in man (Nicholls et al 1979) found that the drug was rapidly metabolized, products appearing in the urine only. The major pathways (Fig. 1) are oxidation of the benzene ring (yielding the 3- and 4-hydroxyphenyl metabolites, excreted as glucuronide and sulphate conjugates) and oxidation of the benzyl methylene, yielding benzoic acid, excreted as the glycine conjugate, hippuric acid. The present study examines the quantitative aspects of these pathways.

Materials and Methods

Chemicals

Aryl sulphatase (Type H1), β-glucuronidase (Type H1), saccharo-1, 4-lactone, benzene sulphonyl chloride and hexobarbitone were purchased from Sigma (Poole, Dorset). Acetonitrile (far-UV HPLC grade) and methanol were from Fisons PLC (Loughborough, Leicestershire). Beclamide (Nydrane) was a gift from Lipha Laboratories (Hitchin, Hertfordshire). All of the other reagents and chemicals used were of the highest quality available and purchased from BDH (Poole, Dorset) except for the 3- and 4-hydroxyphenyl metabolites of beclamide which were synthesized by coupling the appropriate methoxybenzylamine with β-chloropropionyl chloride (Nicholls & Luscombe 1979) followed by *O*-demethylation.

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Volunteers

Eight healthy male subjects (age range 24–30 years, weight range 54–85 kg) participated in the study which was approved by the local ethics committee. Subjects were requested to adhere to an identical diet on both the day of drug administration and the day before (control day). The volunteers swallowed beclamide (2 × 500 mg Nydrane tablets) with 150 mL water after an overnight fast and the 0–24-h urine was collected from each subject. The urine was stored at −70°C until analysed.

Assay of beclamide and its hydroxymetabolites

Urine (0·5 mL) was mixed with 1 M phosphate buffer (0·1 mL, pH 7·4) and internal standard solution (hexobarbitone, 3 μg in 0·1 mL). The mixture was extracted by shaking with diethyl ether (10 mL) for 15 min. The ether layer was then washed by shaking for 10 min with 1 M sodium bicarbonate solution (1 mL). After separation, the ether layer was dried over anhydrous sodium sulphate. Following filtration, the ether extract was evaporated to dryness at 60°C. The residue was reconstituted in methanol (0·1 mL) and 20 μL aliquots were used for HPLC.

For the ether extraction of the glucuronide and sulphate conjugates of the hydroxyphenyl metabolites, aliquots of urine, adjusted to pH 5·0, was first incubated for 24 h at 37°C with either β-glucuronidase or aryl sulphatase according to previously described conditions (Nicholls et al 1979). In the case of the sulphatase, saccharo–1, 4-lactone (50 mM) was included in the incubation mixture to inhibit the glucuronidase present in the enzyme preparation.

HPLC was performed at ambient temperature on a 5-μm Apex ODS column (250 × 4·5 mm, Jones Chromatography, Tir-y-Berth, Mid Glamorgan). The mobile phase was acetonitrile/water (22·5 : 77·5, v/v) containing KH_2PO_4

($1{\cdot}4\,g\,L^{-1}$) and the flow rate was 2 mL min^{-1}. Detection of eluted compounds was at 215 nm. Retention times were 3·0, 3·5, 8·8 and 14·0 min for 4-hydroxybeclamide, 3-hydroxybeclamide, beclamide and hexobarbitone (internal standard), respectively (Fig. 2). The method was capable of detecting at least $0{\cdot}2\,\mu g\,mL^{-1}$ of beclamide and its hydroxyl metabolites and for each of these compounds the assay was linear over the range of $0{\cdot}5$–$500\,\mu g\,mL^{-1}$. Good accuracy (coefficient of variation 5·5–6·3%) and precision (mean coefficient of variation at $2\,\mu g\,mL^{-1}$ 2·7%, and at $500\,\mu g\,mL^{-1}$ 5·0%) were obtained. Mean ($n = 5$) recovery from solutions of the compounds in urine ($500\,\mu g\,mL^{-1}$) was 86·8, 92·8 and 96·9% for beclamide, 4-hydroxybeclamide and 3-hydroxybeclamide, respectively.

Determination of hippuric acid

The earlier study (Nicholls et al 1979) which identified hippuric acid as a metabolite of beclamide had been performed with [^{14}C]-beclamide and this allowed the ready differentiation in urine between endogenous hippuric acid and that derived from beclamide. In man, physiological concentrations of hippuric acid in urine may vary widely ($0{\cdot}3$–$1{\cdot}4\,g\,L^{-1}$) and daily variation may occur in a single subject (Wilczok & Bieniek 1978).

As the use of [^{14}C]-beclamide was not possible in the present study, the strategy adopted was to collect a control urine sample from the subjects when on an identical diet to that eaten on the day of dosing with beclamide. Hippuric acid was determined directly in the urine by a colorimetric method (410 nm) based on interaction with benzene sulphonyl chloride in pyridine (Tomokuni & Ogata 1972). Mean ($n = 5$) recovery from a solution of hippuric acid in urine ($1\,mg\,mL^{-1}$) was 98·8%.

Results and Discussion

The results for the 24-h excretion of beclamide and its two main hydroxylphenyl metabolites in urine are presented in Table 1. No chromatographic peaks corresponding to these compounds were detected in the control urine samples. It may be observed that less than 1% of the administered dose was excreted unchanged, only between 0·13 and 0·54% being detected in the urine of the volunteers and this is consistent with earlier findings (Nicholls et al 1979). As the urine samples had been collected in two 12-h fractions, it was possible to show, in all but one subject, that at least 80% of the fraction of unchanged beclamide had been excreted in the first 12 h after dosing. In the outlier, only 59% of the unchanged drug in urine had been eliminated in this time.

The 3- and 4-hydroxybeclamides could not be detected in extracts of the 24-h urines of the volunteers unless the urine had been incubated, prior to extraction, with either β-glucuronidase or aryl sulphatase. This indicates that both of these hydroxyphenyl metabolites are excreted extensively (or entirely) as their glucuronide and sulphate conjugates. From the data presented in Table 1, it may be observed that the glucuronide is the major excreted hydroxyphenyl beclamide conjugate in urine. It is also evident that 4-hydroxybeclamide is present in urine to a greater extent than 3-hydroxybeclamide, indicating the importance of the 4-hydroxylation pathway for beclamide metabolism. However, it may be seen that, in total, these hydroxy

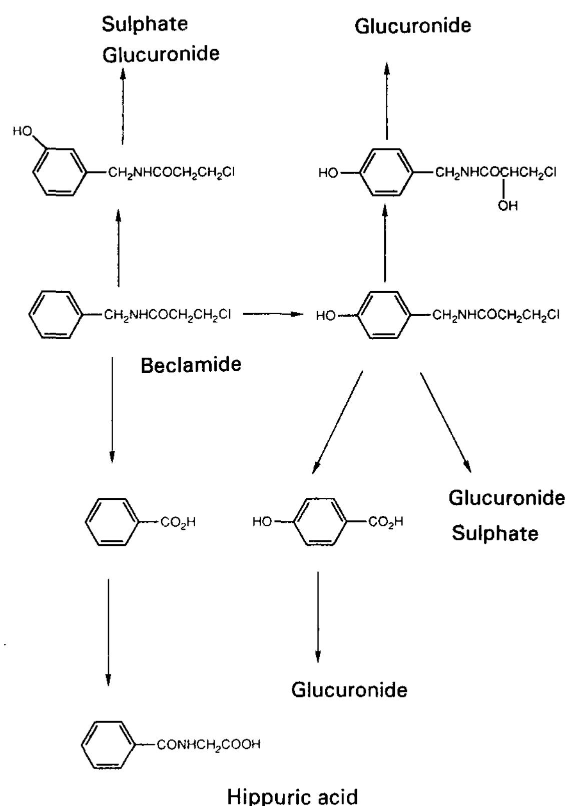

FIG. 1. Metabolic pathways of beclamide in man (Nicholls et al 1979).

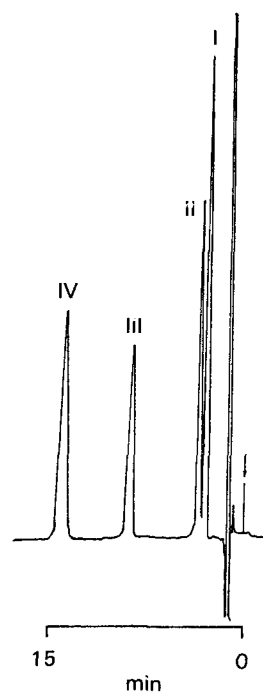

FIG. 2. Chromatogram of a mixture in methanol of 4-hydroxybeclamide (I, $0{\cdot}4\,\mu g$), 3-hydroxybeclamide (II, $0{\cdot}4\,\mu g$), beclamide (III, $0{\cdot}8\,\mu g$) and hexobarbitone (IV, $0{\cdot}8\,\mu g$).

Table 1. Excretion of beclamide, its 3- and 4-hydroxyphenyl metabolites and hippuric acid (% dose) in 24-h urine of volunteers receiving beclamide (1 g) orally.

Volunteer	Elimination in 24-h urine Beclamide	4-OH (as glucuronides)	3-OH (as glucuronides)	4-OH (as sulphates)	3-OH (as sulphates)	Hippuric acid*
A	0·49	28·4	8·2	2·9	0·7	18·6
B	0·13	21·4	6·2	1·4	0·5	18·3
C	0·29	23·5	7·0	2·2	0·6	32·2
D	0·17	21·9	6·0	2·4	1·1	19·7
E	0·54	21·2	6·1	2·0	0·4	11·3
F	0·36	20·6	5·1	1·7	0·5	30·9
G	0·38	26·3	7·1	2·9	0·6	22·4
H	0·24	14·4	4·3	1·2	0·4	21·7
Mean	0·33	22·2	6·3	2·1	0·6	21·9
s.d.	0·1	4·2	1·2	0·6	0·2	6·8

* Hippuric acid excreted during the control period has been deducted from these values

metabolites account for about only 31% of the administered dose.

Beclamide is a benzyl derivative and the formation of hippuric acid via benzoic acid is a common pathway for benzyl compounds (Nicholls et al 1979). Study of the hippuric acid pathway for beclamide was complicated because of the difficulty of distinguishing between endogenous and beclamide-derived hippurate and of the daily variation in the excretion of this normal urinary constituent. During the control period, a mean value of 648 ± 45 mg of hippuric acid was excreted in the 24-h urine of this panel of volunteers and this is within the range previously reported for the physiological excretion of this metabolite (Wilczok & Bieniek 1978). Following administration of beclamide (1 g), the 24-h urine contained a mean value of 847 ± 63 mg of hippuric acid which is significantly ($P < 0{\cdot}05$) greater than that present in the control urine. Results for hippuric acid derived from metabolism of beclamide and excreted in the 24-h urine of each subject are presented in Table 1. It may be observed that, when expressed in terms of equivalent amounts of beclamide, $21{\cdot}9 \pm 6{\cdot}8$% of the dose was excreted as hippuric acid. There were no apparent correlations between the excretion of hippuric acid and of any of the hydroxyphenyl metabolites in urine (Table 1). After oral administration of [^{14}C]beclamide to human subjects, approximately 20% of the radiolabel was eliminated as hippuric acid (Nicholls et al 1979).

In the present study, beclamide and its hydroxyphenyl metabolites were found not to interfere with the colorimetric assay for hippuric acid. However, because of the day to day variation in spontaneous hippurate excretion, the accuracy of this indirect estimate of increased urinary hippuric acid arising from the metabolism of beclamide cannot be high.

It is of interest to note that, in unpublished studies in the mouse, neither the above hydroxy metabolites nor hippuric acid share the CNS effects of beclamide.

Overall, not more than 54% of beclamide-derived compounds were accounted for in the form of the metabolites examined in the present work. As previous work with [^{14}C]beclamide has shown that after an oral dose to man, 90% of the radiolabel is eliminated in the 24-h urine (Nicholls et al 1979), the present results indicate that the other metabolites (Fig. 1), which were not examined, must account for the bulk of the remaining 46% of the dose. However, the ease and reliability of the HPLC assay for the hydroxyphenyl metabolites of beclamide and their glucuronide and sulphate conjugates suggest that the drug may be a useful tool for examining factors which influence xenobiotic hydroxylation and conjugation by these pathways in man.

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