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The pharmacokinetics of lamotrigine (BW430C) in healthy subjects with unconjugated hyperbilirubinaemia (Gilbert's Syndrome)

J. POSNER, A. F. COHEN*, G. LAND, C. WINTON & A. W. PECK
Wellcome Research Laboratories, Langley Court, Beckenham BR3 3BS

A single oral dose of lamotrigine was administered to seven volunteers with Gilbert's Syndrome (unconjugated hyperbilirubinaemia). Plasma samples were assayed by high performance liquid chromatography (h.p.l.c.) and pharmacokinetic parameters were compared with those of a group of nine normal volunteers. In the subjects with Gilbert's Syndrome mean oral clearance (CL_{po}) was 32% lower ($P<0.01$) and the plasma elimination half-life ($t_{1/2}$) was 37% lower ($P< 0.02$) than in the normal controls. The amount of unchanged lamotrigine excreted in the urine was 30% greater in the Gilbert's subjects ($P < 0.01$) although this only amounted to 9.5% of the administered dose. Subjects with Gilbert's Syndrome have some impairment of lamotrigine elimination but this is unlikely to be clinically important.

Keywords lamotrigine pharmacokinetics glucuronidation Gilbert's Syndrome

Introduction

Lamotrigine is a new anticonvulsant (Binnie *et al.*, 1985) which is rapidly absorbed and has an elimination half-life of approximately 24 h (Cohen *et al.*, 1987). Administration for up to 7 days did not reveal any induction of hepatic microsomal enzymes in animals (Parsons & Miles, 1984) or man (Cohen *et al.*, 1987). It is metabolised mainly by glucuronidation. This conjugate is undetectable in plasma but appears in urine at a rate equal to the disappearance of lamotrigine from plasma (Cohen *et al.*, 1987) suggesting that glucuronidation is rate-limiting in drug elimination.

Gilbert's syndrome (idiopathic unconjugated hyperbilirubinaemia) (Gilbert & Lereboullet, 1901) is a common disorder of bilirubin metabolism. It is characterised by a raised unconjugated bilirubin in plasma with a normal conjugated bilirubin. The condition is benign and there are no associated abnormalities of liver function or histology. The hepatic clearance of bilirubin is impaired and the activity of the enzyme bilirubin uridine diphosphate glucuronyl transferase (UDPG) is decreased.

To determine whether glucuronidation of lamotrigine might be impaired in subjects with Gilbert's Syndrome, pharmacokinetic parameters and urinary excretion after a single therapeutic dose were compared with the values obtained in a group of normal volunteers studied earlier.

Methods

Amongst employees at Wellcome Research Laboratories who have participated in volunteer studies, a number of individuals have been identified with values of total bilirubin (almost all unconjugated) above the normal range for

Correspondence: Dr J. Posner, Human Pharmacology Unit, Wellcome Research laboratories, Langley Court, Beckenham, Kent BR3 3BS
* Present address: Centre for Human Drug Research, PO Box 9600, 2300 RC Leiden, The Netherlands

our laboratory (17 μmol l^{-1}). They are healthy with no other abnormality of liver function or evidence of haemolysis and were, therefore, presumed to have Gilbert's Syndrome. These employees were invited to participate in the proposed study which was approved by an external ethics committee. The volunteers gave written informed consent. A full medical screen confirmed that there were no clinically important abnormalities on medical history or examination, electrocardiogram, urinalysis, full blood count or plasma biochemistry including hepatic enzymes. The diagnosis of Gilbert's Syndrome was established by caloric restriction (Felsher *et al.*, 1970; Owens & Sherlock, 1973; Felsher, 1976; Gollan *et al.*, 1976). A blood sample was taken on two consecutive days after breakfast and after the second blood sample subjects were placed on a diet providing 400kCal over the next 24 h after which blood was taken again. The criteria for diagnosis were a total bilirubin (almost all indirect) consistently above the normal range when taking a normal diet and an increase of at least 10 μmol l^{-1} after caloric restriction for 24 h.

Seven subjects (2F, and 5M) fulfilled these criteria and volunteered to proceed with the study. None had ever been clinically jaundiced and all had normal conjugated bilirubin concentrations and plasma haptoglobin concentrations. Mean (± s.d.) total bilirubin increased in the subjects with Gilbert's Syndrome from 30 ± 8 (range 21–48) to 51 ± 14 (34–69) μmol l^{-1} ($P<0.001$) on caloric restriction. Conjugated bilirubin increased only slightly from 2.0 ± 1.5 to 2.8 ± 0.9 μmol l^{-1} ($P<0.05$). One of the Gilbert's subjects had already taken part in an earlier study of lamotrigine kinetics using an identical protocol (Cohen *et al.*, 1985). Her data were included in the analysis of the Gilbert's group and data from the remaining nine subjects (2F,7M) without Gilbert's Syndrome who had participated in the previous study served as the control. The average age of the Gilbert's group was 33 years (range 23–45) and their average weight was 73.5 kg (62–87). Corresponding values for the normal controls were 34 years (19–61) and 73.0 kg (62–87).

Subjects attended the laboratory after an overnight fast. A cannula was inserted into an antecubital vein under local anaesthesia and kept patent with heparinised saline. Blood samples (5 ml into lithium-heparin) were collected pre-drug and at the following times after ingestion of a capsule containing 120 mg lamotrigine: 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0 h. After removal of the cannula, subjects were transported home and subsequent samples were taken by venepuncture 24, 32, 48 and 72 h after dosing. Urine collections were commenced at the time of dosing in 2 h fractions during the first 12 h, then in 12 h collections up to 24 h and subsequently as 24 h collections until 168 h after dosing. Plasma, saliva and urine aliquots were stored at –70°C until analysis.

Plasma for bilirubin assay was separated immediately and samples were analysed taking care to minimise light exposure. Total bilirubin was analysed by a diazotide method (Jendrassik & Graf, 1938) using benzoate-caffeine accelerator and indirect bilirubin was analysed by diazotide without accelerator.

Plasma concentrations of lamotrigine were measured using an automated h.p.l.c. technique. All assays were performed in duplicate with a control to monitor interassay variation and a suitable range of 'spiked' standards. One ml of the internal standard BWA725C (3, 5-diamino-6-(2-methoxyphenyl)-1,2,4-triazine) in ethyl acetate (0.2 μg ml^{-1}) and 2 M sodium hydroxide were added to 200 μl of plasma samples, standards and controls followed by 8 ml ethyl acetate. After mixing and centrifugation the organic layer was separated and dried under nitrogen. The resulting residue was taken up in ethanol and transferred to micro-vials ready for injection. Using a flow-rate of 1.6–2.5 ml min^{-1} at ambient temperature, 20 μl were injected onto a 250 × 4.2 mm Zorbax sil column (Dupont), the eluant consisting of an ammonium hydroxide (sp.gr.0.88), ethanol, hexane mixture (0.25:20:80 by volume, respectively). The chromatographed components were detected at 306 nm with a u.v. detector. The ratios of peak areas of lamotrigine to internal standard were used to construct a calibration curve from the standards by the method of linear least squares regression and the lamotrigine concentration in the samples and controls determined. The lower limit of detection of the assay was 0.05 μg ml^{-1}; the coefficient of variation was less than 7% and the assay was linear throughout the range measured.

The glucuronide conjugate of lamotrigine in urine was measured by incubation of the sample in a pyrex tube with a crude β-glucuronidase extract (*Helix pomatia* GOA76, Sigma) and a pH 5 sodium acetate buffer overnight at 37°C. The tubes were then treated as for free lamotrigine, giving a total value from which the amount of hydrolysed conjugate could be calculated.

Plasma drug concentration-time profiles were fitted by a biexponential function (equation 1) using NONLIN (Metzler *et al.*, 1974). A weighting factor of 1/[concentration] was used to

minimise the difference in variance throughout the range of concentrations.

$$C = C'[e^{-k(t-t_{lag})} - e^{-k_a(t-t_{lag})}] \text{ equation 1}$$

where

C = plasma concentration at time t
C' = coefficient of the biexponential function
k_a = apparent first-order rate constant for absorption
k = first-order rate constant for elimination
t = time from drug administration
t_{lag} = lag time for absorption

The value of AUC was calculated from the integral of equation 1. Hence oral clearance was calculated from Dose/AUC. The elimination half-life was calculated from $0.693/k$.

Comparisons between pharmacokinetic parameters obtained from subjects with Gilbert's Syndrome and from the normal group were compared using Student's t-test.

Results

The mean pharmacokinetic parameters for lamotrigine obtained from the two groups of subjects are shown in Table 1. A single exponential term adequately described the elimination of lamotrigine from plasma. The fitted curves agreed well with observed plasma concentrations and showed a random spread of residuals. In the subjects with Gilbert's Syndrome the mean value of oral clearance was 32% lower ($P<0.01$) and the mean half-life was 37% longer ($P<0.02$) than in the controls. The mean maximum plasma concentration was not significantly different.

The cumulative excretion of lamotrigine and its conjugated metabolite is shown in Table 1. The total amount excreted over 168 h was 90.3 ± 9.4 mg (75.2% of the total administered dose) of which 11.4 ± 1.7 mg was unchanged lamotrigine (9.5% of the administered dose) and 79 mg was conjugated (66.0% of the administered dose). The total amount of lamotrigine excreted over 168 h was not significantly different in the two groups but the amount of unchanged lamotrigine was increased by 30% ($P<0.01$) in the Gilbert's group. The ratio of the amount excreted as unchanged lamotrigine to the amount excreted as conjugate was increased by 26% ($P=0.05$)

Discussion

This study has demonstrated an impairment of the excretion of lamotrigine in subjects with Gilbert's Syndrome. This benign condition occurs in 6% of the population (Owens & Evans, 1975). In our subjects the mean decrease in the oral clearance of lamotrigine was 32%. Therefore, on multiple dosing these subjects should have steady state plasma drug concentrations about 32% higher than normal subjects. The clinical implications of this are probably unimportant as lamotrigine seems to have a high therapeutic index. Some individuals with Gilbert's Syndrome present with jaundice and might be more severely affected but our volunteers had very similar values of baseline and fasting bilirubin as patients in the study of Owens & Evans (1975).

The longer $t_{½}$ of lamotrigine in our subjects supports the view that one or more steps in the glucuronidation process are rate-determining

Table 1 Mean ± s.d. pharmacokinetic parameters and urinary excretion of lamotrigine and its glucuronide in subjects with Gilbert's Syndrome and normal controls.

	Gilbert's Syndrome group (n = 7)	*Normal controls* (n = 9)	*Significance**
$t_{½}$ h	31.2 ± 7.4	22.8 ± 4.4	$P<0.02$
CL_{po} (ml min^{-1})	30.2 ± 7.7	44.2 ± 7.5	$P<0.01$
C_{max} (μg ml^{-1})	1.6 ± 0.2	1.5 ± 0.3	NS
t_{max} (h)	3.0 ± 1	3.0 ± 1	NS
Lamotrigine (mg excreted 168 h^{-1})	11.4 ± 1.7	8.8 ± 1.53	$P<0.01$
Glucuronides (mg excreted 168 h^{-1})	79.0 ± 10.2	75.6 ± 7.5	NS
Total recovery (mg 168 h^{-1})	90.3 ± 9.4	84.4 ± 8.06	NS
Lamotrigine/ glucuronide ratio	0.146 ± 0.035	0.116 ± 0.019	$P = 0.05$

* significance of differences by Student's t-test. NS = $P>0.05$

for the elimination of the drug. Further evidence for this is that patients on valproate, a drug that is also excreted by glucuronidation, demonstrate an increased lamotrigine half-life, suggesting that both drugs are competing for the same enzyme (Binnie *et al.*, 1985).

The possibility that some conjugated material might not be glucuronide but rather sulphate cannot be ruled out entirely in the present study. However, when compared with recoveries before incubation, the amount of free lamotrigine in urine from normal subjects was unchanged after incubation with the crude glucuronidase/sulphatase extract in the presence of the glucuronidase inhibitor β-1,4-saccharolactone, indicating that no conjugates other than glucuronide were present. Furthermore, incubation with a pure sulphatase preparation did not cause breakdown of conjugate. It seems unlikely that significant amounts of sulphate would be formed in the Gilbert's subjects when none is formed by normal individuals.

When lamotrigine is administered with anti-epileptic drugs which are enzyme inducers it is excreted more rapidly than when given alone; the half-life being reduced to approximately 12 h (Binnie *et al.*, 1985). The effect of enzyme induction on the slower metabolism of lamotrigine in Gilbert's Syndrome is unknown but enzyme induction by phenobarbitone has been used to normalise the plasma bilirubin levels in patients with Gilbert's Syndrome.

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References

Binnie, C. D., van Emde Boas, W., Land, G. S., Meijer, J. W. A., Overweg, J. & van Wieringen, A. (1985). Preliminary single-dose studies of a potential new anti-epileptic drug, lamotrigine (BW430C) in epileptic patients. *Br. J. clin. Pharmac.*, **20**, 285P.

Cohen, A. F., Posner, J. Land, G. & Winton, C. (1986). The pharmacokinetics of lamotrigine (BW430C), a potential anticonvulsant, in subjects with unconjugated hyperbilirubinaemia (Gilbert's Syndrome). *Proceedings of 3rd World Conference on Clinical Pharmacology and Therapeutics*, Abstract 906.

Cohen, A. F., Fowle, A. S. E., Land, G. S. & Bye, A. (1985). Pharmacokinetics in normal man of lamotrigine (BW430C), a new anticonvulsant. *Br. J. clin. Pharmac.*, **20**, 286P.

Cohen, A. F., Land, G. S., Breimer, D. D., Yuen, W. C., Winton, C. & Peck, A. W. (1987). Lamotrigine, a new anti-convulsant: pharmacokinetics in normal humans. *Clin. Pharmac. Ther.*, **42**, 535–541.

Felsher, B. F. (1976). Effects of changes in dietary components on the serum bilirubin in Gilbert's Syndrome. *Am. J. clin. Nutrition*, **29**, 705–709.

Felsher, B. F., Richard, D. & Redeker, A. G. (1970). The reciprocal relation between caloric intake and the degree of hyperbilirubinaemia in Gilbert's Syndrome. *New Engl. J. Med.*, **283**, 170–172.

Gilbert, A. & Lerebouillet, P. (1901). La Cholemie simple familiale *Semaine Medicale*, **21**, 241.

Gollan, J. L., Bateman, C. & Billing, B. H. (1976). Effect of dietary composition on the unconjugated hyperbilirubinaemia of Gilbert's Syndrome. *Gut*, **17**, 335–340.

Jendrassik, L. & Graf, P. (1938). Vereinfachte photometrische Methoden zur Bestimmung des Blutbilirubins. *Biochem Z.*, **297**, 81–89.

Metzler, D. M., Elfring, G. L. & McEwen, A. J. (1974). A package of computer programs for pharmacokinetic modelling. *Biomedics*, **30**, 562.

Owens, D. & Evans, J. (1975). Population studies on Gilbert's Syndrome. *J. med. Genetics*, **12**, 152–156.

Owens, D. & Sherlock, S. (1973). Diagnosis of Gilbert's Syndrome: Role of reduced Caloric intake test. *Br. med. J.* **3**, 559–563.

Parsons, D. N. & Miles, D. W. (1984). Metabolic studies with BW430C, a novel anticonvulsant. *Epilepsia*, **25**, 656.

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