Influence of stable, long-term treatment with phenobarbital on the activity of serum alanine aminotransferase and γ-glutamyltransferase

G. LIPPI, M. MONTAGNANA, G. L. SALVAGNO and G. C. GUIDI
Sezione di Chimica Clinica, Dipartimento di Scienze Morfologico-Biomediche, Università di Verona, Italy

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ABSTRACT

Phenobarbital, a long-acting barbiturate, is generally considered to be a fairly safe and effective drug; however, hepatotoxicity is an infrequent but potentially fatal adverse effect and there is little information on the serum activity of liver enzymes in patients on stable, long-term monotherapy. The serum activity of alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) are measured along with phenobarbital as part of the routine biochemical measurement in 128 consecutive adult out-patients on stable, long-term phenobarbital treatment. The control population consists of 2468 consecutive out-patients matched for age and gender. The patients on long-term phenobarbital therapy had significantly higher serum activities of ALT (27 IU/L versus 23 IU/L, $P<0.001$) and GGT (79 IU/L versus 24 IU /L, $P<0.001$). The prevalence of subjects with abnormal GGT values, but not ALT, was significantly higher than that in the control population. No significant differences were observed in either the mean activity or the prevalence of abnormal values of ALT or GGT between patients with suboptimal and therapeutic concentrations of the drug. These results suggest that chronic phenobarbital therapy may be associated with a clinically significant elevation of serum GGT activity. If confirmed, a specific GGT reference range should be adopted. Moreover, in those patients presenting with high serum GGT activity, it would not be necessary to reduce the dosage, discontinue the drug or change to a different anti-epileptic medication.

KEY WORDS: Alanine aminotransferase.
Enzymes.
gamma-Glutamyltransferase.
Liver.
Phenobarbital.

Introduction

Phenobarbital (5-ethyl-5-phenylbarbituric acid) is a long-acting barbiturate commonly used as the first-line drug to treat convulsions caused by tonic-clonic (grand mal), focal or psychomotor epileptic seizures, especially in developing countries, although it remains a popular choice in many industrialised countries. Phenobarbital has also been used for anxiety, insomnia and gall bladder dysfunction or blockage, and it is the drug of choice for treatment of febrile seizures and neonatal seizures in infants born to opiate- or barbiturate-addicted mothers.[1,2]

Phenobarbital has good anti-epileptic effect, but clinically significant untoward effects occur during long-term use. Additional problems include suboptimal response rates, several drug interactions, a narrow therapeutic index and significant dose-related adverse effects[3] such as hyperactivity, behavioural problems, sedation and even dementia.[4]

Approximately 25% of a phenobarbital dose is excreted unchanged in urine, whereas the majority of the active drug is metabolised in the liver to form inactive metabolites. Hydroxylation of phenobarbital is attributed to the cytochrome P450 (CYP) enzyme system, primarily CYP2C9, with minor contributions from CYP2C19 and CYP2E1. The typical therapeutic range is 15–40 mg/L, with critical levels reported for serum concentrations >65 mg/L.[1]

Although phenobarbital is generally considered to be a safe and effective drug, hepatotoxicity can be an infrequent, potentially severe outcome, as fatal adverse effects can develop in association with alcoholism.[5] Clinical studies have investigated the acceptability of phenobarbital and reported behavioural side-effects, especially in childhood, in developing countries,[6] and in the animal model.[7]

However, little information is available on the effects of this drug on biochemical markers of liver function in the adult population and whether or not liver enzymes traditionally used as markers of liver injury in serum biochemical profiles (e.g., γ-glutamyltransferase [GGT] and alanine aminotransferase [ALT]) can be modified by long-term therapy with phenobarbital remains unclear.[1,7–14] This aspect is significant clinically because if serum liver enzymes are elevated because of induction, there would be no indication to alter the anticonvulsant regimen. However, if elevations are due to early subclinical liver damage, discontinuation of the drug and initiation of alternative anticonvulsant therapy would be advisable to prevent the progress of liver damage that might result in liver failure.

The aim of this cross-sectional study is to determine the

Correspondence to: Professor Giuseppe Lippi
Sezione di Chimica Clinica, Dipartimento di Scienze Morfologico-Biomediche, Università degli Studi di Verona, Ospedale Policlinico G.B. Rossi, Piazzale Scuro, 10, 37134 – Verona, Italy
Email: ulippi@tin.it

Table 1. Serum alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) activities in adult out-patients on long-term, stable phenobarbital treatment, and control out-patients not taking phenobarbital or any other anti-epileptic drug.

	Control out-patients	Out-patient taking phenobarbital	*p*
n	2468	128	
Age (years)	56 (32–79)	54 (31–85)	0.148
Females	1204 (49%)	57 (45%)	0.395
GGT			
Mean (95% CI) IU/L	24 (7–139)	79 (22–559)	<0.001
Values >40 IU/L	524 (21%)	95 (74%)	<0.001
ALT			
Mean (95% CI) IU/L	23 (10–78)	27 (10–155)	<0.001
Values >40 IU/L	328 (13%)	24 (19%)	0.108

activity of two common serum liver-associated enzymes in adult patients on long-term, stable phenobarbital therapy, and compare the results with those from a reference control population.

Materials and methods

Over a one-year period (June 2006 to June 2007), 128 consecutive out-patients (age >30 years, M/F=71/57) receiving stable, long-term (>3 years) phenobarbital treatment as the single therapy for convulsions caused by tonic-clonic (grand mal; n=72), focal or psychomotor epileptic seizures (n=56) were included in the study. Liver enzymes and phenobarbital levels were measured as part of the routine biochemical profile requested by the general practitioner for routine patient monitoring. The mean age (95% confidence interval [CI]) of the patients was 54 years (31–85 years).

The control population consisted of 2468 consecutive out-patients (age >30 years, M/F=1264/1204) with a mean age (CI) of 56 years (32–79 years). All had been referred by the general practitioner for routine blood testing (including GGT and ALT) over the same one-year period. All the control subjects were in apparent good health and were not taking phenobarbital, other anti-epileptic drugs or hepatotoxic medication.

Venous blood was collected in the morning from fasting subjects. Assays for ALT (International Federation of Clinical Chemistry [IFCC] method with pyridoxal phosphate activation) and GGT (Szasz-Persijn method, employing L-γ-glutamyl-3-carboxy-4-nitroanilide) were performed on a Roche/Hitachi Modular System P (Roche Diagnostics, Mannheim, Germany). The upper value of the reference range for both GGT and ALT was 40 IU/L.

Serum phenobarbital was assayed by a quantitative homogenous particle-enhanced turbidimetric inhibition immunoassay (PETNIA) technique, which uses a latex particle-phenobarbital reagent and phenobarbital-specific monoclonal antibody, on a Dimension RxL (Dade Behring, Milton Keynes, UK). Phenobarbital present in the sample competes with the particles for the antibody, thereby decreasing the rate of aggregation measured by bichromatic turbidimetric readings at 340 and 700 nm. The rate of aggregation is inversely proportional to the concentration of the analyte in the sample.

The significance of differences between groups was assessed by the Mann-Whitney test (for continuous variables) and the χ^2 test (for categorical variables). Due to the non-Gaussian distribution, as verified by the Kolmogorov-Smirnov test, data were logarithmically transformed to improve normality prior to analyses. Statistical analyses were performed using the SPSS version 12.0 statistical package (SPSS, Chicago, IL) and statistical significance was set at $P<0.05$. Data are presented as mean (±95% CI) or percentages.

Results

The main characteristics of the study population, together with serum GGT and ALT activities, are shown in Table 1. The mean concentration of phenobarbital in patients taking the drug was 19 mg/L (95% CI: 8–40 mg/L). Thirty-five (27%) patients had subtherapeutic levels (<15 mg/L) and none had toxic levels (>65 mg/L).

The patients on long-term, stable phenobarbital monotherapy displayed significantly higher values for both ALT and GGT compared to the control subjects. However, although the mean serum GGT activity of patients taking phenobarbital was nearly three times higher than that of the control population (79 IU/L versus 24 IU /L, $P<0.001$), mean serum ALT activity was only marginally increased (27 IU/L versus 23 IU/L; $P<0.001$) and was within the upper limit of the reference range.

When stratifying the patients on phenobarbital therapy according to the suboptimal concentration of the drug (<15 mg/L), no significant differences were observed in mean serum ALT and GGT activities or in the prevalence of subjects with abnormal values for these liver enzymes (Table 2).

Discussion

Enzyme induction is the increased synthesis of the enzyme, resulting in increased amounts of the enzyme protein and its related activity. In 1912, phenobarbital became one of the first agents used against epilepsy and is now the most widely used anti-epileptic drug worldwide.

Phenobarbital induces the activity of several enzymes, including members of the CYP family, glucuronosyl

transferases and glutathione-S-transferases. Increased serum levels of liver-associated enzymes are also seen commonly in phenobarbital-treated animals with no clinical signs of liver disease.[1] Nevertheless, data on liver-associated enzymes in humans on stable, long-term phenobarbital monotherapy are limited and often contradictory.

It has been observed that phenobarbital treatment might resulted in significant increases in serum GGT activity in patients with Gilbert's syndrome.[8] Hirayanagi *et al*. also showed that a therapeutic dose of phenobarbital can cause significant elevation in serum GGT level.[9] Likewise, many results above the upper reference limit for serum GGT were obtained from patients who received phenobarbital as part of a multiple therapy, in combination with phenytoin, carbamazepine and valproate.[10]

In the animal model, long-term administration of phenobarbital was found to increase GGT activity in individual hyperplastic liver nodules.[11] Conversely, two independent studies showed that animals treated with phenobarbitone may show small increases in serum ALT activity and variable increases in alkaline phosphatase activity, but are unlikely to have alterations in GGT.[12,13] These findings are consistent with those of Huseby, who showed that animals treated with phenobarbital develop only moderate increases in GGT activity in serum and liver, whereas greater activities are found in bile.[14] Finally, Gaskill *et al*. reported that median liver homogenate ALT activity in phenobarbital-treated dogs was significantly lower than in controls, and thus does not support induction of hepatic ALT activity by the drug.[7]

The results of the present investigation suggest that patients on long-term, stable phenobarbital monotherapy might display a clinically negligible increase in serum ALT. The data also support a clinically significant association between raised serum GGT activity and phenobarbital therapy.

The originality of this investigation lies in the study design, as i) ALT and GGT was analysed simultaneously in patients on phenobarbital; ii) several confusing factors present in previous investigations could be ruled out (i.e., multiple therapy, combining phenobarbital with other anti-epileptic drugs); and iii) these results were obtained in humans rather than in an animal model. Therefore, these findings retain clinical significance and prompt further investigations to redefine the reference range of serum liver enzymes in patients on long-term therapy.

The substantial GGT elevation observed in the adult population, along with a modest increase of ALT activity, does not necessarily indicate hepatocellular damage and it is consistent with the hypothesis that elevation of serum GGT may result from hepatic microsomal enzyme induction. If this is confirmed, even in patients on long-term phenobarbital who have a high serum GGT activity, it would not be necessary to reduce the dosage, to discontinue the drug or change to a different anti-epileptic agent.[9] Conversely, in those patient who have levels that exceed the specific reference range, synergic effects due to alcoholism or additional pharmacological agents might be suspected and initiation of alternative anticonvulsant therapy would be warranted to prevent continued liver damage.

The authors are aware that the present study has limitations. The cross-sectional nature does not enable definitive conclusions to be draw and further prospective studies would help to identify phenobarbital-induced modifications of serum liver enzymes in humans. Furthermore, data could not be collected on alcohol consumption by patients on phenobarbital therapy or by the control population. Nevertheless, patients and controls were matched for ethnic origin, age, gender and prevailing environmental factors. Therefore, it is unlikely that the results were substantially biased by different dietary and lifestyle habits. □

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Table 2. Serum alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) activities in adult out-patients on long-term, stable phenobarbital treatment, stratified according to the serum concentration of the drug.

	Phenobarbital		
	<15 mg/L	≥15 mg/L	*P*
n	35	93	
Age (years)	55 (37–86)	54 (30–84)	0.498
Females	17 (49%)	40 (43%)	0.261
GGT			
Mean (95% CI) IU/L	81 (22–771)	78 (22–397)	0.063
Values >40 IU/L	24 (69%)	71 (76%)	0.067
ALT			
Mean (95% CI) IU/L	31 (12–163)	25 (9–122)	0.104
Values >40 IU/L	8 (23%)	16 (17%)	0.134

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