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Isoenzymes of alkaline phosphatase in epileptic patients receiving carbamazepine monotherapy

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Abstract

The effects of carbamazepine monotherapy were investigated in 20 female and 21 male epileptic patients to determine whether treatment would induce an increase in serum alkaline phosphatase (ALP) activity, a known effect of many anticonvulsant drugs. Serum total ALP activity was increased in nine out of the 41 patients (22%), serum bone ALP activity was increased in 10 (24%), and serum non-bone ALP activity was increased in three (7%). There was no significant difference when the mean of the patients' serum total ALP was compared with that of the controls.

Twenty per cent of the patients with increased serum bone ALP had normal serum total ALP, indicating that increased serum bone isoenzyme activity may precede an increase in the total enzyme activity. This should be considered when interpreting results of increased total ALP in epileptic patients.

An increase in the activity of the heterogenous group of enzymes collectively termed alkaline phosphatase (EC 3·1·3·1: orthophosphoric monoester, phosphohydrolase, alkaline optimum; ALP) in the serum of patients receiving treatment for epilepsy has been observed by several workers. Richens and Rowe conducted a survey of adult institutionalised epileptic patients, which showed an increase in the serum activity of total ALP.[1] Siest *et al* showed that antiepileptic drugs strongly increased the plasma ALP activity in adults (by 24% in men and 70% in women) and in children (by 56% in boys and 53% in girls).[2] Skillen and Pierides reported that serum total ALP was increased in seven out of 33 adult epileptic patients receiving anticonvulsant drug polytherapy.[3] The need, therefore, was to determine the extent of the role of carbamazepine (CBZ, Tegretol, CIBA Geigy). O'Hare *et al* had shown decreased serum calcium concentration and increased serum total ALP, both of which were significant, in epileptic patients receiving CBZ monotherapy.[4] Aldenhovel, in 1988, also reported increased serum ALP activity in 14% of patients receiving CBZ.[5] They did not show, however, that ALP isoenzymes were increased.

Our study was carried out to confirm the reported increase in serum total ALP activity in patients receiving CBZ monotherapy,[4,5] and also to determine, using a reliable precipitation technique, the actual source of the ALP isoenzyme that was increased.

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Methods

Forty one serum samples were selected from those sent to the Drugs Monitoring Laboratory of the National Hospital for Neurology and Neurosurgery, Queen Square, London, for routine estimation of anticonvulsant drug concentrations. Full clinical details of the patients whose sera were to be studied were obtained from their case notes. Only patients receiving CBZ and with no history or laboratory results suggestive of liver or bone disease were selected. They must have received CBZ monotherapy for a minimum period of two years at the time the samples were collected.

The 41 samples selected comprised 21 men aged 16–67 years (mean 38 years) and 20 women aged 21–66 years (mean 40 years). Samples used as the reference population were collected from the antecubital vein of 32 age-matched, healthy laboratory workers and staff from other departments in the hospital. Serum was separated from cells within two hours of collection and immediately stored at −20°C. The control group comprised 16 men and 16 women, aged 19 to 66 years (mean age 39 years).

MEASUREMENT OF TOTAL ALP

The serum total ALP was measured at 25°C according to the method of the German Society of Clinical Chemistry,[6] using p-nitrophenol phosphate as substrate and diethanolamine buffer (Merck, Darmstadt, Germany). The method was adapted to the Guildford 3500 discrete analyser.

PRECIPITATION AND CALCULATION OF BONE ALP ACTIVITY

The precipitation procedure and calculations were performed as described previously.[7–9] An aqueous solution of wheat germ lectin (Sigma Chemical Company, Poole, Dorset) was prepared by adding 5 ml distilled water to 25 mg of lyophilised powdered lectin. This gave a concentration of 5 g/l (139 μmol/l). To 1 ml of the aqueous lectin solution was added 40 μl of Triton X-100 (BDH Chemicals Ltd, Poole, Dorset), so that when mixed with an equal volume of plasma the final incubation concentration of Triton X-100 was 20 μl/ml. Fifty microlitres of lectin- Triton X-100 solution was mixed with 50 μl of serum and incubated for 30 minutes at 37°C. This solution was then transferred to a refrigerator and

kept at +4°C for two minutes before centrifugation at 2000 $\times g$ for 10 minutes. The supernatant was removed, ALP activity determined, and the measured value was referred to as non-bone ALP. Bone ALP activity was calculated by subtracting the corrected value of non-bone ALP from the total ALP activity, as described by Behr and Barnet.[9] Because the precipitation characteristics of the batch of wheat-germ lectin used were not checked, it was assumed for calculation purposes that there was a 100% precipitation of the bone fraction, and that the addition of Triton-X-100 would make precipitation of liver ALP fraction negligible. To calculate the total analytical recovery of isoenzyme activities, the precipitates were resuspended in 200 μl of isotonic saline and the ALP activity then determined. Summation of the isoenzyme activities of the precipitate and supernatant, followed by an adjustment for sample dilution, produced the value for total recovery of isoenzyme activity.

The significance of differences between the controls and patients receiving CBZ was tested using Student's t test. The significance level was set at $p < 0.05$.

Results

ANALYTICAL QUALITY CONTROL

Analytical recovery

To calculate the analytical recovery of total ALP activity from summation of activity in the precipitate and supernatant, 10 sera were examined. The values of total activity on direct measurement ranged between 70 IU/l and 240 IU/l (mean = 128; (SD) = 60·7). Summation of activities in the supernatant and precipitate measured separately and after adjustment for dilution gave a range of total ALP activity of 66–235 IU/l (mean 125, SD 60·0), which was equivalent to 98·4% recovery.

Precision studies

The between-batch precision (n = 20) of the precipitation method was determined by using a pooled serum with total ALP activity of 174 IU/l and mean bone ALP of 76 IU/l (range 63–96 IU/l). The coefficient of variation (CV) was 4·7% for between-batch precision. The within-batch precision (n = 8) was determined using the same pooled serum and the CV value was 2·3%.

External quality control

In addition to the above, the control samples from United Kingdom and Wellcome External Quality Assessment Programmes were both included in every batch of measurements. These were the samples used by the laboratory in which this research was carried out during their participation in the external quality control programme. The United Kingdom and Wellcome control samples gave CV values of 8·07% and 9·1%, respectively.

OBSERVATIONS AND DEDUCTIONS

The summation of total ALP, bone ALP, and non-bone ALP activities in the sera of controls and patients receiving CBZ monotherapy is shown in the table. The SD was generally higher in the patients than in the controls. The reference range for serum total ALP derived from the control group (mean ±2 SD) was 61–159 IU/l. Among the patients receiving CBZ monotherapy, five women and four men (nine out of 41 patients or 22%) had serum total ALP values above the upper limit of 159 IU/l. The mean serum total ALP of patients was higher than that of the controls, with values of 126 IU/l and 110 IU/l, respectively. The difference between the two means was not significant ($p = 0.05$).

The reference range for serum bone ALP derived from the control group (mean ±2 SD) was 24–96 IU/l.

Among the patients, six women and four men (10 out of 41 patients or 24%) had serum bone ALP activity above the upper reference limit of 96 IU/l. There was no significant difference when the mean for the controls was compared with that of the patients. Comparison of means for male controls with male patients and female controls with female patients did not show any significant differences ($p = 0.05$). Twenty per cent (two out of 10) of those patients with increased serum bone ALP had normal serum total ALP activity.

Three patients out of a total of 41 (about 7%) had increased serum non-bone ALP activity. The values of serum mean non-bone ALP activity were similar in both patients and controls.

Summary of total ALP, bone ALP, and non-bone ALP activities in sera of controls and patients receiving CBZ monotherapy

		Patients		
	Controls	*All*	*Women*	*Men*
Number of subjects (n)	32	41	20	21
Age in years				
Range	19–66	16–67	21–66	16–67
Mean ($\bar{x}$)	39	37	40	35
1SD	14·5	14·9	15·8	13·9
Total ALP (IU/l)				
Range	75–170	59–244	6–240	59–244
Mean ($\bar{x}$)	110	126	128	125
1SD	24·3	51·1	50·5	52·9
Bone ALP (IU/l)				
Range	36–118	74–208	14–182	25–208
Mean ($\bar{x}$)	60	76	73	79
1SD	18·2	47·7	49·6	48·8
Non-Bone ALP (IU/l)				
Range	30–120	12–110	17–110	12–98
Mean ($\bar{x}$)	50	50	55	46
1SD	21·4	26·9	27·9	25

Discussion

Biochemical osteomalacia, as indicated by decreased serum calcium concentration and increased serum ALP activity in patients receiving anticonvulsant drugs, has been observed by several investigators.[1 3 4 10 11] The cause of this osteomalacia is still not clear, although various mechanisms have been proposed.[3 4] CBZ is a potent anticonvulsant drug which is currently regarded as a first line treatment for generalised (tonic-clonic) and partial epilepsy.[12] The current view of monotherapy being preferred to polytherapy will

demand higher dosages of antiepileptic drugs, (such as CBZ), during which subtle metabolic effects may subsequently become more important.

In this investigation we have shown that increased serum total ALP in patients receiving CBZ monotherapy was mainly from bone rather than from liver and other sources. Nine patients out of a total of 41 (22%) had increased serum total ALP above the upper limit of the reference range (159 IU/l). Ten patients out of 41 (24%) had serum bone ALP above the upper limit (96 IU/l). This finding was similar to that of Richens and Rowe[1] in 1970, and Skillen and Pierides in 1976,[3] both of whom used electrophoresis to show that increased ALP in patients receiving anticonvulsant drugs was due mainly to the bone fraction. Moreover, these previous reports were based on patients receiving polytherapy. O'Hare *et al* studied 31 patients receiving CBZ monotherapy and found a significantly higher serum total ALP activity than in matched controls.[4] Although their result was similar to ours they did not identify the actual isoenzyme of ALP involved. Furthermore, in our results 20% of patients with increased serum bone ALP had normal serum total ALP. This finding shows that changes in the isoenzyme level of activity may precede a change in the serum total ALP. Earlier workers had suggested that patients on long term high dose single or combined CBZ treatment should have periodic biochemical checks for osteomalacia[4]; there may be a need to proceed to radiological and bone biopsy studies in selected cases.

In our report we have shown that wheat-germ lectin[7 8] may be used routinely to measure serum activity of the bone isoenzyme fraction of ALP in such patients. Measurement of bone ALP may provide a more sensitive indicator of osteomalacia than will measurement of total ALP alone, although further investigation is required. The use of wheat-germ lectin is also an improvement on most previous work involving measurement of the isoenzymes of ALP in patients receiving anticonvulsant drugs, which have relied on semiquantitative electrophoretic methods for estimation of the bone fraction.

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