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Alkaline phosphatase and its isoenzyme activity for the evaluation of bone metabolism in children receiving anticonvulsant monotherapy

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This study aimed to investigate whether carbamazepine, sodium valproate or phenobarbital as monotherapy in ambulatory epileptic children with adequate sun exposure have some effect on their bone metabolism based on the determination of total serum alkaline phosphatase (AP) levels and its bone isoenzyme activity. Blood samples were obtained from 118 epileptic children (37 on carbamazepine, 47 on sodium valproate and 34 on phenobarbital) and from corresponding healthy controls matched for age, gender and anthropometric parameters. AP and its liver, bone and intestinal isoenzyme levels, other common biochemical markers of bone and liver metabolism and drug levels were measured in the study participants. Patients on carbamazepine or phenobarbital had significantly elevated AP levels accompanied by increased bone and liver isoenzyme activity compared to controls. An increase of bone AP isoenzyme values, correlated with the duration of treatment ($r = 0.49$, $P = 0.002$), was found in children on sodium valproate without, however, a concomitant significant elevation of total AP values. We conclude that children who receive antiepileptic drugs as monotherapy, even when residing in a Mediterranean country with adequate sunlight, may have their bone metabolism affected as indicated by the elevated levels of bone AP isoenzyme. This isoenzyme, but not total AP values, could therefore be used as a marker for the selection of patients who would be benefited by a thorough evaluation of their bone metabolism profile.

Key words: alkaline phosphatase; alkaline phosphatase isoenzymes; anticonvulsant drugs; bone metabolism; epileptic children.

INTRODUCTION

An increase in the activity of serum enzymes, collectively termed alkaline phosphatase (AP), has been reported in patients receiving antiepileptic treatment[1–3]. However, AP isoenzyme activity has been evaluated only in adult patients on anticonvulsant monotherapy[4,5]. Several studies which assessed bone metabolism status in adults and children on antiepileptic drugs using other indicators than AP isoenzyme activity, such as biochemical markers of bone remodelling and/or calciotropic hormones, have shown rather controversial results[6–9]. The diversity of their findings could be attributed at least to some extent to the heterogeneity of population samples among different studies regarding the administered medications (monotherapy or combination therapy), the type of patients (ambulatory or institutionalized patients) or both. Furthermore, taking into account the well recognized association of sun exposure with vitamin D metabolism[10,11], findings derived from a population living in geographical areas with limited exposure to sunshine may not be applicable for inhabitants of sunny places.

In recent years, the assessment of bone mineral density by dual energy X-ray absorptiometry (DEXA) allowed the direct evaluation of bone mineralization in children on antiepileptic drugs. However, even

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these studies disclose rather conflicting results[12, 13] and the whole issue *of a possible link between antiepileptic drug administration and not clinically apparent osteomalacia* has therefore remained unresolved. This is still important as a possible undesirable side effect of anticonvulsant medication on bone metabolism could be prevented by the implementation of appropriate measures. However, prerequisite for such an intervention should be a sensitive and easily measured biochemical marker for the evaluation of bone metabolism alterations.

The objective of this study was to investigate whether AP and/or its bone isoenzyme levels are altered in children receiving as monotherapy carbamazepine, sodium valproate or phenobarbital and could therefore be used as markers for their bone metabolism monitoring. In order to evaluate whether a possible elevation of total AP levels is a reflection of liver or bone metabolism or both all the three main fractions of AP (bone, liver, intestinal) were measured and their relation with other commonly used biochemical markers of liver and bone metabolism was explored.

PATIENTS AND METHODS

The study population consisted of 118 ambulatory children, aged from 12 months to 14 years, treated for epilepsy with anticonvulsant monotherapy for a minimum of 6 months. All children were otherwise healthy and not receiving any other medication. None of the participants had ever received an antiepileptic drug other than the one which was given while recruited in the study. One hundred and eighteen clinically healthy children, based on history and clinical examination, matched for age, gender and anthropometric parameters (body weight and height), served as controls. Epileptic children were divided into three groups according to the antiepileptic medication as follows: group1: carbamazepine ($n = 37$, mean age $\pm$ SD $= 8.08 \pm 3.65$ years), group 2: sodium valproate ($n = 47$, age $\pm$ SD $= 8.13 \pm 3.9$ years) and group 3: phenobarbital ($n = 34$, mean age $\pm$ SD $= 2.4 \pm 1.6$ years) and each group corresponded to a matched for age, gender and anthropometric variables group of healthy controls (group 1c, group 2c and group 3c, respectively).

A blood sample was obtained after 12 hours fasting in early morning, between 8.00 and 10.00 a.m. Serum calcium (Ca), phosphorus (P), gamma-glutamyltransferase (γGT), alanine aminotransferase (SGOT), aspartate aminotransferase (SGPT), AP, AP bone, AP liver and AP intestinal isoenzyme levels were determined in all specimens, whereas drug levels were measured in epileptic children. An informed parental consent was provided and the study was approved by the Institutional Review Board.

Serum total AP was measured according to the method recommended by the Dutch Society of Clinical Chemistry, using commercially available kit (Roche, Germany) *and following the manufacturer's instructions*. The method was adapted to a Hitachi 704 discrete analyser. Serum bone (AP-B), liver (AP-L) and intestinal (AP-I) AP isoenzymes were separated by electrophoresis on agarose gel and *then visualized and quantified using the Hydragel ISO-PAL K20 kit (Sebia, France), following the manufacturer's recommendations.* Samples were tested in duplicate and the overall mean within-sample coefficient of variation for the assay was 8.9%. Serum Ca, P, γGT, SGOT, SGPT, and drug levels were measured according to the usual standard methods.

For the statistical analysis a P value <0.05 was considered as statistically significant.

RESULTS

Total AP, AP-B and AP-L isoenzyme levels were significantly higher among children receiving carbamazepine compared to the corresponding group of controls (Table 1). Total AP levels correlated significantly to both bone and liver AP enzymes ($r = 0.96$, $P < 0.001$; $r = 0.41$, $P = 0.013$, respectively). Ca, P, SGOT, SGPT did not show any significant difference whereas γGT was significantly higher in children on carbamazepine (mean value $\pm$ SD $= 23.7 \pm 9.0$ IU/l vs. 10.6 ± 5.9 IU/l, $P < 0.001$). Children on phenobarbital had significantly higher total AP and AP bone and liver isoenzyme levels compared to controls. Serum γGT levels were also significantly higher in the phenobarbital group compared to controls (mean value $\pm$SD $= 19 \pm 5.7$ IU/l vs. 8.9 ± 2.4 IU/l, $P = 0.001$, respectively), whereas the values of Ca, P, SGOT and SGPT did not show any significant difference. Children receiving sodium valproate had significantly elevated AP-B without any difference with respect to Ca, P, SGOT, SGPT and γGT. There was no correlation of drug levels with the total AP and its isoenzyme activity irrespective of the type of antiepileptic drug. Furthermore, there was no association of the duration of treatment with the AP and its isoenzyme activity within the groups of children who received carbamazepine or phenobarbital but there was a substantial and significant correlation of the duration of treatment with bone alkaline levels ($r = 0.49$, $P = 0.002$) in children on sodium valproate.

Table 1: Serum AP and its isoenzyme levels (mean values ± SD) in epileptic children receiving antiepileptic monotherapy and their corresponding controls.

Treatment (serum drug levels mean ± SD in mg l^{-1})	*n*	AP (IU/l)	*P*	AP-bone (IU/l)	*P*	AP-liver (IU/l)	*P*	AP-intestinal (IU/l)	*P*
Carbamazepine (6.8 ± 0.26)	37	233.3 ± 70.4	0.001	186.3 ± 64.2	0.001	33.9 ± 17.4	0.001	12.2 ± 7.8	NS
Controls	37	145.3 ± 54.0		86.2 ± 25.2		19.2 ± 11.5		15.3 ± 10.0	
Phenobarbital (15.2 ± 0.78)	34	227.0 ± 48.9	0.001	176.8 ± 46.0	0.02	36.5 ± 23.2	0.03	12.0 ± 7.4	NS
Controls	34	156.7 ± 64.5		138.0 ± 78.5		28.0 ± 23.1		4.9 ± 11.4	
Sodium valproate (59.1 ± 2.7)	47	162.3 ± 71.8	NS	127.9 ± 58.2	0.02	21.0 ± 12.7	NS	11.9 ± 12.7	NS
Controls	47	144.9 ± 53.3		87.5 ± 24.6		18.8 ± 11.1		15.0 ± 9.6	

P values derived from Student's *t*-test.

DISCUSSION

The issue of whether or not certain antiepileptic drugs affect the bone metabolism of the recipients remains rather controversial[1–9]. In this study we attempted to investigate whether AP and its isoenzyme activity indicate any alterations of bone metabolism in children on antiepileptic medications. *It was previously shown that biochemical indices of osteomalacia such as serum calcium and AP were related to bone mineral content measured by photon absorptiometry*[14]. We consider AP as the most appropriate marker for this purpose, as it is more sensitive compared to serum calcium levels, it can be easily measured and therefore any inferences from this study could be applicable in clinical practice. Although AP originates from different tissues, liver and bone isoenzymes are the most abundant parts of the total AP activity in the sera[15]. Therefore total AP levels mainly reflect hepatic and bone metabolism both of which may be affected by antiepileptic drugs. The determination of these isoenzymes makes it feasible to elucidate whether AP alterations are attributed to modification of liver or bone metabolism or both.

To the best of our knowledge this is the first study that evaluates AP isoenzyme activity in children on carbamazepine or sodium valproate as monotherapy. Similar studies have also been conducted by other investigators[16,17] in children, ambulatory or institutionalized, who, in contrast to our population, had received anticonvulsant medications in various combinations and not as monotherapy. Our study has the advantage of the homogeneity of the participating population within each group with respect to the type of administered medication. Although confounding could be raised as a concern for the interpretation of the results, the selection of controls matching for age, gender as well as anthropometric variables and the fact that they did not differ from cases with respect to sunshine exposure makes unlikely the possibility that the validity of our findings is subjected to the effect of any known confounder.

Our findings indicate that children on carbamazepine have elevated AP values due to both altered liver and bone function as both bone and liver alkaline isoenzymes were increased. Therefore AP itself cannot be used as a marker for routine monitoring of this risk as the elevation of AP may simply reflect the influence of carbamazepine on liver function. For this reason the determination of AP isoenzymes seems to be mandatory for the monitoring of bone metabolism alterations in patients on carbamazepine. It is beyond the objective and ability of this study to evaluate to what extent elevated bone AP levels correlate to a clinically important impact on bone metabolism. A recent study, however, using biochemical markers of bone formation and resorption suggests that carbamazepine administration may cause an increased bone turnover and not a reduced bone formation[18]. Altay *et al.*[12] also suggested that carbamazepine does not affect the bone mineral density of the recipients. However, as these results were derived from studies with a limited number of patients they do not constitute enough evidence for the clinician not to consider the possibility of vitamin D supplementation for some patients on carbamazepine.

The elevation of bone AP fraction in patients on sodium valproate which was not accompanied by a significant elevation of total AP suggests that sodium valproate administration does have an impact on bone mineral metabolism in ambulatory children and that normal total AP levels could not exclude the possibility that the respective bone fraction may be high. The increase of bone AP isoenzyme in this study group would have been rather expected as

decreased bone mineralization evaluated by DEXA or biochemical markers has been observed in children on sodium valproate even though treatment has been administered for a rather short period of time[13]. The fact that bone AP fraction correlated with duration of treatment in this group indicates that monitoring of these patients with markers of bone metabolism should be performed on a regular basis.

Children on phenobarbital in our study showed significantly elevated AP levels as well as bone and liver AP isoenzymes. Our findings are in accordance with those reported by Hahn *et al.*[19] who also showed a significant reduction in bone mass with a concomitant elevation of total AP, liver and bone AP in outpatient children who received phenobarbital and diphenylhydantoin either singly or in combination. However, in their analysis they treated the monotherapy group as a whole irrespective of the type of medication administered. Therefore although they clarified that no significant differences were observed between the phenobarbital and the diphenylhydantoin groups, it remains rather difficult to extract definite conclusions with respect to the effect of phenobarbital on bone metabolism out of that study.

In conclusion, AP bone isoenzyme activity, but not total serum AP levels, could be considered as a useful marker for the monitoring of bone metabolism alterations in children on antiepileptic drug monotherapy. Until more evidence is available it seems that periodic monitoring is necessary for patients on sodium valproate even when the initial evaluation reveals normal bone AP isoenzyme activity. *Further investigation with serum vitamin D metabolite concentrations and bone mineral density, where available, may be appropriate in patients with raised AP values in order to identify those who necessitate prophylactic vitamin D supplementation.*

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