

Mandibular bone density and calcium content affected by long-term anticonvulsant treatment in rats

E.M. Parara¹, P.B. Galanopoulou¹, G. Rallis², E. Vairaktaris³, C.P. Tesseromatis¹

¹Department of Pharmacology, Medical School, University of Athens, Greece;

²Department of Oral and Maxillofacial Surgery, General Hospital of Athens "KAT", Greece;

³Department of Oral and Maxillofacial Surgery, University of Athens Medical School, Greece

Abstract

Objective: Bone is perpetually absorbed and reformed, serving also to electrolyte homeostasis, mainly for calcium and phosphorus. Anticonvulsant medications are traditionally considered harmful to bone because of their interaction with the metabolism of vitamin D, due to hepatic enzyme induction. A study of the effect of anticonvulsant medications on mandibular bone quality was undertaken. **Materials and methods:** 24 Wistar rats in three groups received diphenylhydantoin or diazepam or placebo intraperitoneally (ip). Absolute bone weight, bone to body weight ratio, specific bone weight, absolute calcium concentration, calcium to mandibular bone weight ratio and mineral element concentration were examined after animal sacrifice, three months later. From the results it may be concluded that diazepam and diphenylhydantoin administration affect the mandibular bone density and calcium content in terms of absolute weight and specific weight. Mandibular calcium concentration was affected only by diphenylhydantoin treatment.

Keywords: Diphenylhydantoin, Diazepam, Mandible, Bone Mass

Introduction

Bone is a living structure, which following different stimuli, is under constant remodeling. Due to this perpetual absorption and formation, osseous tissue also serves as an aid for electrolyte homeostasis, mainly calcium and phosphorus¹. This is the main reason why bone exhibits great sensitivity to various substances, either pharmacological or not.

Anticonvulsant medications are traditionally considered harmful to bone² because of their interaction with the metabolism of vitamin D, due to hepatic enzyme induction³. In fact, antiepileptic drugs (AED) produce hypocalcemia and decrease biologically active vitamin D levels, leading to reduced bone mineral density. Furthermore, it is known that AED induce the cytochrome P450 enzyme system and accel-

erate the vitamin D metabolism due to conversion of 25-hydroxycalciferol to inactive polar metabolites⁴⁻⁷.

Epilepsy affects a large proportion of patients globally with a usually long-term required treatment, if not life long⁸. As patients' quality of life becomes increasingly important, issues such as disability or tendency to fractures turn into more and more contemporary⁹. The fact that most epileptic patients are diagnosed and treated during childhood or adolescence, when growth and maturation of the skeleton are not yet complete, is also significant¹⁰. This crucial side-effect of antiepileptic drugs may often be overlooked^{8,11}. However, the facial skeleton may even be affected by *in utero* exposure to anticonvulsant medication. Specific facial features of the condition can include telecanthus, broad nasal bridge, anteverted nares, shallow philtrum, thin upper lip, micrognathia, broad or tall forehead¹².

The consequences of diphenylhydantoin (DH) administration to facial bones could be compared to the effect of another pharmacological substance with similar metabolic pattern, such as diazepam. The effect of long-term administration of diazepam and DH on mandibular bone mass and calcium content was considered worth studying. Diazepam and DH are both metabolized in liver, by hydroxylation, and both eliminated with urine as glucuronid derivatives, hence

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Corresponding author: Eleni Parara, 16 Koimiseos Theotokou Street, 151 24 Athens, Greece

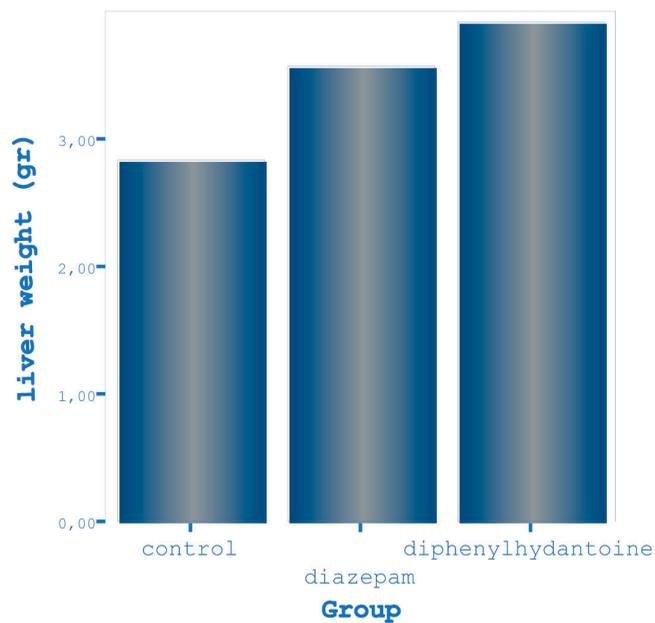
E-mail: eparara@dent.uoa.gr

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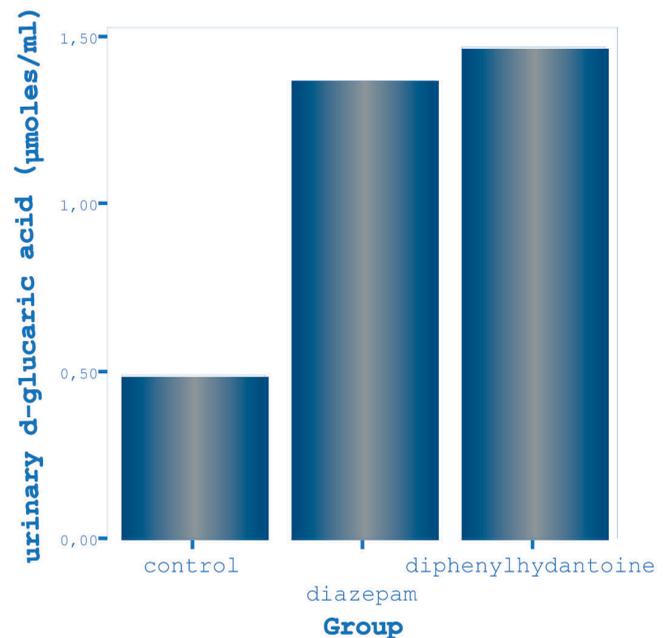
Parameter/Median values	Control	DH	D	p (Kruskal-Wallis)
Food intake (gr)	13.20	16.17	13.25	>0.05
Body Weight (gr)	265.80	240.67	233.88	>0.05
Liver weight (gr)	2.83	3.9175	3.57	<0.005
U D-glucuric A (μ mole/ml)	0.488	1.4675	1.37	<0.005
Absolute mandibular weight	0.966	.8263	.7844	<0.05
Bone to body weight ratio	.3640	.3588	.3444	>0.05
Specific mandibular weight	2.240	1.7275	1.6711	<0.05
Absolute calcium	11.1720	10.8863	12.9489	>0.05
Calcium to bone weight ratio	11.5340	13.3775	16.4411	=0.006
Ash to bone weight ratio	.5580	.6113	.6622	<0.005

DH: diphenylhydantoin, D: diazepam, gr: grammar, U D-glucuric A: Urinary D-glucuric acid excretion.

Table 1. Median values and level of significance for all parameters.



Graph 1. Liver weight by group (Kruskal-Wallis $p < 0.005$).



Graph 2. Urinary D-glucuric acid excretion by group (Kruskal-Wallis $p < 0.005$).

the latter can be utilised as biomarkers of the medication metabolism. Diazepam, a benzodiazepine usually administered in acute states of epilepsy (status epilepticus) is also deposited in bone tissues¹³. Furthermore diazepam in a previous study has been demonstrated to induce cytochrome P450 system in a similar way to DH¹⁴.

The aim of the study was: a) to investigate the influence of liver enzyme induction caused by long-term DH administration on mandibular bone density and b) to estimate the relative diazepam effects on bone density and compare them with those of DH.

Materials and methods

Twenty-four male Wistar rats aged 6-8 weeks and weighing 180 \pm 10 gr, divided into three groups were studied. Group A was allocated to receive DH at a daily dose of 70 mg/kg of body weight. The drug was suspended in 5% acacia oil. The second group received Diazepam at a daily dose of 10 mg/kg of body weight. The control group received only acacia oil 5%. All substances were administered intraperitoneally.

All animals were housed individually in plastic metabolic cages and had access to water and pulverized cereal rat food

ad libitum, which were measured daily as was daily urine discharge. Weighing took place every 10 days.

The animal motor activity was inspected for 4 hours daily with the Hugo Basil device, repeatedly before and after administration of the medication, in order to exclude reduced activity as a risk factor for reduced bone mass.

The animals were sacrificed on day 90. The liver was removed and weighed and the mandible was isolated from the surrounding tissues. After weighing and measuring, the mandible was burned at 600°C and the obtained ash was also weighed for evaluation of mineral bone substance.

Enzyme induction was evaluated with liver weight and D-glucaric acid contained in urine of the last 24 hours. D-glucaric acid was isolated and determined with the Marsh method¹⁵.

Absolute bone calcium, the calcium ratio to bone weight and bone volume and ash weight ratio to bone weight were indices of the quantity of calcium integrated in bone. Absolute bone weight, specific bone weight and relative bone weight are important factors for bone density estimation since low bone mass density (BMD) constitutes a significant risk factor for osteoporosis¹⁶.

Non-parametric statistical analysis methods for independent groups were used, i.e., Kruskal-Wallis and Mann-Whitney U test for statistical evaluation of the results.

Results

Food intake and final weight

Diazepam and DH administration did not seem to affect consumption of food, as it was similar for the three groups (Table 1). Final body weight was less for the two study groups than the control group, although not statistically significantly so (Table 1).

Total motor activity was similar between the experimental and control groups.

Hepatic enzyme induction

Hepatic enzyme induction was positively affected in both experimental groups. Liver weight was significantly higher between groups (Kruskal-Wallis $p=0.002$). Within groups, this result was maintained concerning comparison of the two experimental groups to the control group (Mann-Whitney $p=0.007$ for Control Vs Diazepam and $p=0.002$ for Control Vs DH). Urinary excreted D-glucaric acid was also statistically significantly higher between groups (Kruskal-Wallis $p=0.004$). This finding remained statistically significant for analysis within groups (Mann-Whitney $p=0.001$ for Control Vs Diazepam and $p=0.002$ for Control Vs DH) (Table 1, Graphs 1 and 2).

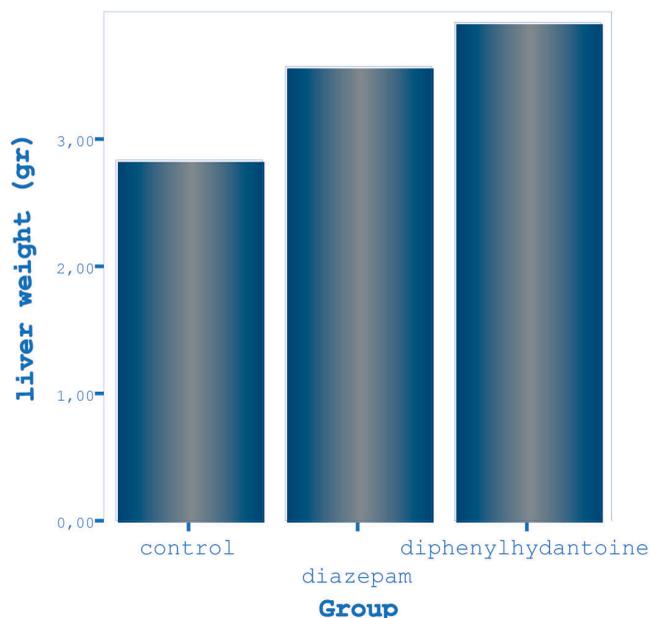
Mandibular indices

Absolute mandibular weight

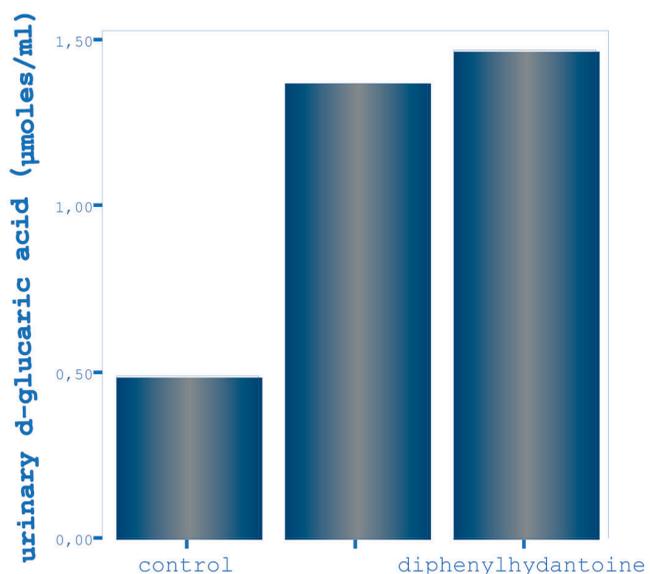
Absolute bone weight was statistically significantly lower for the two medication groups compared to control (Kruskal-

Group	N	Mean (gr)
control	8	13.20
diazepam	8	16.17
diphenylhydantoine	8	13.25
Total	24	

Table 2. Daily food intake for the three groups (Kruskal-Wallis $p>0.05$).



Graph 3. Absolute mandibular weight for the three groups (Kruskal-Wallis $p<0.05$).



Graph 4. Specific mandibular weight for the three groups (Kruskal-Wallis $p<0.05$).

Group	N	Minimum	Maximum	Mean	Std. Error
control	8	.35	.38	.3640	.006
diazepam	8	.28	.56	.3444	.03
Total	16				

Table 3. Bone to body weight ratio for control vs. diazepam (Mann-Whitney $p < 0.005$).

Group	N	Minimum	Maximum	Mean	Std. Error
control	8	.35	.38	.3640	.006
diphenylhydantoin	8	.29	.45	.3588	.03
Total	16				

Table 4. Bone to body weight ratio for control vs. diphenylhydantoin (Mann-Whitney $p > 0.05$).

Group	N	Minimum	Maximum	Mean	Std. Error
control	8	9.90	14.00	11.1720	.72
diazepam	8	10.65	15.73	12.9489	.51
diphenylhydantoin	8	9.48	15.40	10.8863	.69
Total	24				

Table 5. Absolute calcium for the three groups (Kruskal-Wallis $p = 0.031$).

Wallis between groups test $p < 0.05$, Table 1). This was observed for within groups comparison as well (Graph 3).

Bone to body weight

Concerning the ratio of bone to body weight, it did not differ significantly between groups. However, when analyzed within groups, the ratio was found to be significantly lower in the Diazepam group than the Control group (Tables 1, 3 and 4).

Specific bone weight

Specific bone weight was statistically significantly lower between groups (Table 1, $p = 0.006$) and this result remained so following statistical analysis within groups ($p \leq 0.006$). Graph 4 represents the mean specific mandibular weight for the three groups.

Mandibular calcium capacity indices

Absolute calcium

Calcium was found to be significantly less in the DH group compared to the Diazepam and Control groups. Statistical analysis revealed significance of the Kruskal-Wallis between Groups test ($p = 0.03$). The results can be seen at Table 1.

Calcium to bone weight ratio

The calcium to mandibular bone weight ratio was found to be significantly affected between groups (Table 1, Kruskal-Wallis $p = 0.006$). When statistical analysis was extended within

the groups, significantly lower ratio was found in the Control ($p = 0.001$) group compared to the Diazepam group (Table 6).

Ash to bone weight ratio

The ash to bone weight ratio was also significantly different between groups (Table 1, $p = 0.004$). Within groups, the ratio was higher for the Diazepam group when compared to the Control group (Table 7).

Discussion

Antiepileptic drugs present various adverse effects, including bone metabolism disruption and reduction of mineral capacity^{4,5,17-19}. However, according to a recent survey, less than 30% of neurologists are aware of such an association and routinely evaluate patients for bone disease^{11,20}. Each antiepileptic medication exerts different modifications to bone, thus resulting in decreased mineral density. The decrease in intestinal absorption of calcium, accelerated vitamin D metabolism, increased bone turnover, inhibition of osteocalcin and increased urinary loss of calcium and phosphorus due to renal tubular dysfunction, all contribute to the skeletal effect of AED. An increase of bone turnover might be the most important osteopenic effect of AED¹⁹. Bone turnover is a perpetual process that takes place in the skeleton and can be modified (accelerated or slowed down) by various conditions. An increase in the bone turnover rate causes defective mineralisation, because the balance between osteoid formation and mineralisation is disrupted²¹.

The above results support the hypothesis that the medications' effect is mediated by hepatic enzyme induction and subsequently increased vitamin D metabolism. Both drugs

Group	N	Minimum	Maximum	Mean	Std. Error
control	8	10.71	13.64	11.5340	.54
diazepam	8	13.73	20.54	16.4411	.8
diphenylhydantoin	8	9.63	19.75	13.3775	1.1
Total	24				

Table 6. Calcium to bone weight ratio for the three groups (Kruskal-Wallis $p=0.006$, Mann-Whitney within groups $p<0.005$).

Group	N	Minimum	Maximum	Mean	Std. Error
control	8	.55	.57	.5580	.004
diazepam	8	.61	.73	.6622	.01
diphenylhydantoin	8	.55	.66	.6113	.02
Total	24				

Table 7. Ash to bone weight ratio for the three groups (Kruskal-Wallis $p<0.005$, Mann-Whitney within groups $p<0.005$).

(diazepam and DH) caused statistically significantly higher liver enzyme induction compared to control. This was confirmed by the increase of liver weight, as well as increased D-glucaric acid urine excretion. Administration of DH leads to a greater induction of hepatic enzymes than diazepam, as has already been shown¹⁰, thereby increasing the metabolism of vitamin D and affecting bone mineral density. This is in agreement with the higher mean value of liver weight and excreted D-glucaric acid for the DH group, although this was not a statistically significant result in our study.

As for mandibular indices, absolute bone weight was significantly lower for the experimental groups compared to control, which is in agreement with the relevant literature^{4,7,8}. Concerning specific bone weight, the study groups presented statistically significantly lower mean values compared to control, which is justified, considering the effect of anticonvulsants on bone density^{4,7,8}.

The mandibular calcium capacity indices were also different between the two groups. Absolute calcium was significantly affected in the DH group. This is in accordance with the known effects of the drug⁴.

The ratio of calcium concentration to bone weight was lower for the control group than for the study groups. The denominator of bone weight, which was obviously higher for the control group, probably accounts for this result.

Concerning the ratio of ash to bone weight, which represents the mineral content of calcium phosphate, control was significantly low ranked. This may also be attributed to the higher bone weight noted in the control group.

These findings are not associated with differences in food intake as overall food consumption was similar in the three groups. Moreover, the mobility of the animals and final body weight did not show significant differences between groups.

Finally, our results seem to be related to induced hepatic enzymes rather than reduced calcium deposition, due to lack of exercise caused by the depressive effects of the studied drugs.

Conclusions

Mobility and food intake can represent confounding factors regarding evaluation of bone mass reduction. Since there were no differences in these parameters between groups, alterations of mandibular indices and calcium quantity values can be attributed to the administered experimental medications. Liver enzyme induction figures were statistically significantly higher in the experimental groups, signifying that the study medications were pharmacokinetically active.

Overall food intake was not different in the three groups. Total mobility of the animals and final body neither showed significant differences between groups. In conclusion, Diazepam and DH administration affected mandibular bone density in terms of absolute weight and specific weight. Calcium concentration was obviously also affected by diphenylhydantoin.

The results of the present study support the viewpoint that substances with liver enzyme induction properties may affect bone calcium concentrations with possible clinical implications regarding bone quality. Previous studies have shown a statistically significant relationship between bone mass reduction and periodontal disease²². Therefore, patients receiving anticonvulsant treatment should be considered as high risk for periodontitis occurrence. Callus formation following fractures may also be impaired in such patients, especially in the jaws, which, due to the close proximity to the oral cavity flora, are prone to infection.

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