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Impact of Some Older Generation Antiepileptics on Bone Remodeling

[1]Said Nadji, [2]Thamer Zaiter, [3]Sarra Zaidi, [3]Samah Boudouh, [3]Mohammed Taher Benmoussa and [2]Hanane Boukrous

[1]Laboratory of Toxicology, University Hospital of Batna, Route de Tazoult, Batna, Algeria
[2]Laboratory of Biochemistry, University Hospital of Batna, Route de Tazoult, Batna, Algeria
[3]Department of Pharmacy, Faculty of Medicine, University of Batna, Batna, Algeria

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Corresponding Author:
Said Nadji,
Laboratory of Toxicology,
University Hospital of Batna,
Route de Tazoult, Batna, Algeria

ABSTRACT

Older antiepileptics are commonly used for the treatment of epilepsy, in fact, drugs as Valproate, carbamazepine and phenobarbitalare hugely prescribed especially for children patients, the adverse effects of such medicines are miscellaneous, one of the effects is their impact on the bone tissue, which could have disastrous consequences as osteoporosis, fractures, etc. Through the present study patients following therapeutic drug monitoring for the three above mentioned drugs have been assessed for some biomarkers related to bone metabolism and bone remodeling. Statistical T.Test has been used to make comparison between subgroups representing some factors as the dose per day, the duration of the treatment and the used drug, which have been evaluated. The obtained results showed significant differences according to the treatment duration with higher impact related to longer treatment and the type of the drug used for the treatment. Furthermore, the study showed that the majority of the studied sample was beyond the normal biological levels. Finally, the study emphasizes the importance of the evaluation and the monitoring of the bone biomarkers following the prescription of antiepileptics treatment in order to prevent the occurrence of bone fragility or osteoporosis.

Key words: Antiepileptics, bone remodeling biomarkers, osteoporosis, epilepsy, bone metabolism, drug

INTRODUCTION

Bone tissue is a specialized mineralized connective tissue, characterized by its ability to renew itself. The stiffness and flexibility of the bone structure are maintained by the constant renewal of the matrix throughout life. This process allows repairing its constituent elements, thanks to the continuous activity of the bone cells[1].

Metabolic changes were associated with long term taking of antiepileptic drugs. One of these impacts may be related to bone metabolism by reducing Bone Mineral Density (BMD), mainly due to osteoporosis, which was classically defined as "a systemic disease of the skeleton featured by low bone mass and deterioration of bone architecture, resulting in increased bone fragility leading to fractures[2].

The diagnosis of osteoporosis is based, according to the WHO definition, on a measurement of the bone mass. This is evaluated by measuring the bone mineral content or Bone Mineral Density (BMD)[3].

A decrease in BMD of between 1 and 2.5 standard deviations from the mean value normally reported for that of young subjects defines osteopenia; a shift of BMD lower than 2.5 standard deviations defines osteoporosis[4,5].

Many antiepileptic drugs, mainly those of first and second generations could induce a decrease in bone mineral density (BMD), as several studies have reported[6,7].

This manifestation (BMD) inherent to some antiepileptic drugs was caused by their interference with the body metabolism (direct or indirect effect).

The different mechanisms are well described for old antiepileptic drugs, infact they can have a direct effect on the bone, or through the increase in vitamin D catabolism, which could be secondary to the activation of the hepatic cytochrome P450 (resulting in a transformation of 25-Hydroxy Vitamin D into inactive metabolites). This catabolism is mediated by the activation of the Pregnane X nuclear Receptor (PXR)[8].

As a consequence, the surge in the catabolism of vitamin D results in decreased calcium absorption and resulting in secondary hyperparathyroidism, increased bone resorption and accelerated bone loss (marked disorder of bone mineralization[9,10].

Clinically, the most severe form of this mechanism is Osteomalacia.

The conducted study aimed to understand the impact of three commonly used antiepileptic drugs: Valproate, Carbamazepine and phenobarbital, on bone tissue.

MATERIALS AND METHODS

This was a prospective descriptive study performed on patients treated with the above mentioned antiepileptics and being monitored at the Toxicology Laboratory of the University Hospital of Batna.

The study was based on the assay of the different biological parameters related to bone metabolism: calcium (Ca^{2+}), phosphorus (P), alkaline phosphatase (ALP), β-CrossLaps, parathormone (PTH), procollagen type 1 N-terminal propeptide (P1NP) and osteocalcin (Osteoc).

Micropipettes: The 100, 200, 500 and 1000 µL Sodium heparin tubes, COBAS INTEGRA 400 PLUS, COBAS 6000 C 501 unit, centrifuge Hettichzentrifugen universal 320R, freezer (-20°C) were used.

Collect and storage of the biological samples: A crucial condition before making the sampling was ensured that the steady- state is reached, namely, after a time corresponding to about five to seven times the elimination half-life of the drug from the beginning of treatment or the dose modification.

For the assay of valproate, carbamazepine and phenobarbital, blood samples were taken in the morning on an empty stomach. The collection of blood was made in heparinized tubes. Afterwards, the assay was performed on plasma (centrifugation at 15000 rpm for 10 min of the heparinized tubes).

Sample storage can be ensured in the freezer at -20°C for months, in the refrigerator at 2-8°C for 7 days or at room temperature for 24 h.

Table 1: The description of the Population Study

Factor	Frequency
Gender	Females: 25
	Males: 17
Age (years)	< 10: 64.28%
	[10-30] 28.75%
	[10-30] 2.38%
	>45: 4.76%

Overall sampling

The population study: The study was carried out on a sample of 42 patients undergoing antiepileptic treatment (Table 1) monitored during a period of 10 months from 01/10/2016 to 01/08/2017 in thelaboratory of toxicology of the university hospital of Batna, though; the biological parameters were assayed in the laboratory of biochemistry.

Inclusion criteria

- Patients on antiepileptic treatments, who undertake monitoring in the laboratory with completed records.

Exclusion criteria

- Patients who do not observe their treatment properly.
- Non-exploitable patients' records.

Studied parameters: The phosphocalcic balance: it assesses the levels of calcium and phosphorus in blood and the ratio calcium/phosphorus, hypercalcemia could be occurred due to different pathologies as for instance osteolysis, hypervitaminosis D and on the other hand, hypocalcemia could be due to vitamin D dependent rickets, idiopathic pseudo-hypoparathyroidism, hyperphosphoremia may be due to hypovitaminosis D, rickets, osteomalacia, while that hyperphosphoremia could be due to osteoporosis and hypervitaminosis D.

Parathyroid hormone test (PTH): PTH was formed in the parathyroid gland and was secreted into the blood stream. The evaluation of PTH directly determined the secretory activity of the parathyroid gland and the effect of calcium metabolism.

High levels of calcium could be a sign of hyperparathyroidism. A condition caused by overproduction of PTH. Meanwhile, that low levels of calcium could be a sign of hypoparathyroidism, or underproduction of PTH.

Osteocalcin assay: It is a non-collagenous protein of the bone matrix. It is a reflection of osteoblastic activity. It is therefore a marker of bone building. Osteocalcin levels were increased in metabolic bone diseases with increased bone or osteoid formation including osteoporosis, osteomalacia, rickets, hyperparathyroidism, on the other hand, decrease in osteocalcin was also observed in some disorders (e.g., hypoparathyroidism, hypothyroidism and growth hormone deficiency).

Alkaline phosphatase (ALP): It is an enzyme found in the bone, liver, intestine, placenta and kidneys. It is a marker of hepatic cholestasis. The assay of alkaline phosphatase was prescribed when the doctor suspects the presence of liver or bones diseases.

High levels may indicate an issue related to the bones such as rickets, Paget's disease, osteomalacia, bone cancer, or an overactive parathyroid gland.

β-CrossLaps/serum (CTx): A laboratory test to detect the C-terminal telopeptide (CTx, linear fragments of degradation) of collagen type 1. Collagen is the main component (90%) of the bone matrix. These fragments were released by bone resorption caused by osteoclast activity, Elevated levels of beta-C-terminal telopeptide (CTx) indicate increased bone resorption, in fact increased levels were associated with osteoporosis, osteopenia, Paget disease, hyperthyroidism and hyperparathyroidism.

Procollagen type 1 N-terminal Propeptide(P1NP): Laboratory test for structural fragments of type 1 collagen. Fragments are released by bone formation induced by osteoblasts activity[5], the used assay Elecsys® P1NP recognizes the primary and tertiary structure (total P1NP).

Statistical analysis: In this study evaluated the influence of the treatment duration, the dose per day and the used drug, in comparison between the different subgroups, in order to see if there were significant statistical differences and to assess the influence of these variables on bone metabolism.

The sample was divided into several subgroups according to the pre-defined distribution criteria for the statistical analysis. The statistical analysis was performed using the Microsoft Office Excel (2013), computerized statistical software. The results were presented as mean, standard deviation, median, min and max which represent the descriptive statistics.

The comparison of the means was carried out by the Student's "t" test for the different parameters, the difference was considered significant when $p<0.05$. After classifying the general sample according to the type of treatment, two sub-samples were obtained; one for patients under valproate treatment and the other was constituted by patients treated by carbamazepine and phenobarbital.

RESULTS AND DISCUSSION

Study of the sample under valproate: The statistical description of the population taking Valproate is described in Table 2.

From the results of the biochemical assay, the percentages of the normal values and those outside the physiological range are outlined and given in the Table 3.

It could be noted that, high percentages (>50%) of the population under Valproate Have ALP, osteocalcin, P1NP and β-crosslaps levels, beyond the normal range.

Study of the population under Valproate distributed according to the dose/day: The dose of 500 mg per day was chosen as the threshold, consequently, two subgroups were formed, one with dose less than 500 mg day^{-1}, the other with higher dose. The results showed a significant statistical t test ($p=0.03$) , for the comparison between the two subgroups for the parameter: Osteocalcin, with a mean of 50.73 and

Table 2: The statistical description of the population taking valproate

	Age (years)	Weight (kg)	Dose (mg day^{-1})	Duration (months)	ALP (UI L^{-1})	Ca^{+2} (mg L^{-1})	P (mg L^{-1})	PTH (Pmol L^{-1})	Osteoc (ng mL^{-1})	B-crosslaps (ng L^{-1})	P1NP (ng mL^{-1})
Mean	6.90	23.73	615.9	37.56	190.40	93.40	47.72	3.58	61.28	1.18	427.93
Median	5.25	20.00	500.0	22.50	193.00	90.00	48.90	3.29	57.49	1.10	356.80
Std. dev.	4.49	11.95	436.4	36.20	73.45	5.45	7.49	1.76	29.91	0.40	246.42
Min.	0.50	3.50	150.0	3.00	36.00	80.00	28.00	1.10	7.02	0.44	28.46
Max.	21.00	60.00	2000.0	156.00	321.00	100.00	63.00	9.75	143.00	1.94	1149.00

standard deviation of 46.35 for the subgroup <500 mg day^{-1} and mean of 69.48 and standard deviation of 62.62, on the other hand, all the other parameters show no significant statistical significance.

According to some studies[11-13], the osteocalcin levels within the subgroup under valproate treatment were significantly higher than those of the control group.

El wakkad *et al.*[14] found after an experimental study that oral administration of valproate to epileptic rats for 6 months resulted in a significant increase in ALP, osteocalcin and N-Telopeptides of Type I Collagen (NTx) compared to the control group.

Increases in the activating receptors of the NFkB ligand (RANKL) and TNFα; beside the increase in bone formation markers due to a higher osteoblasts activity or levels in order to compensate for the increased osteoclastic activity were observed[14].

A further study showed osteocalcin levels at the upper limit of the normal range which is somehow consistent with the results found in the present study[15].

Contrarily, Song et al reported that the levels of osteocalcin in children treated with valproate was lower compared to the control group[16].

Study of the population under valproate according to the duration of treatment: The threshold of 15-months duration was set to form the subgroups in order to make the comparison, 13 patients have treatment duration higher than 15 months and 19 patients less than 15 months.

Following the comparison of the means for each studied biochemical parameter of the two subpopulations, it can be noted that there is a significant statistical difference between the means of: P1NP, Calcium and alkaline phosphatase ($p<0.05$) (Table 4), whereas, there is no significant difference for the others. According to the study by Ayhan *et al.*[17]. there is a significant difference depending on the duration of valproate treatment with significant differences for PTH, phosphorus and alkaline phosphatase[17,18]. While, Sato *et al.*[12] found a significant increase in calcium and markers of adhesive bone resorption relational with the duration of treatment[12]. Other studies have shown significantly increased levels of Parathyroid hormone. In addition, an increase in serum alkaline phosphatase levels has been reported in several studies[17-19]. Babayigit *et al.*[20] studied healthy and epileptic children treated with valproate over a period of more than 1 year. It has been found that the mean concentration of serum alkaline phosphatase was significantly higher in the treated group compared to control one and this corroborates the results obtained in the present study. Similar alkaline phosphatase values have been observed in further studies[21,22]. High alkaline phosphatase values are explained by the effect of valproate on bone metabolism through osteoclasts stimulation.

Table 3: The percentages of normal and abnormal levels obtained for the studied parameters in the group under valproate

Parameters	<Normal	Normal	>Normal
Parathyroid hormone test	6%	91%	3%
Calcium	0	100%	0
Phosphorus	3%	91%	6%
Alkaline phosphatase	0%	22%	78%
Osteocalcin	3%	47%	50%
Procollagen type 1 N-terminal propeptide	0%	6%	94%
B-Cross laps	0%	3%	97%

Study of the population under the other assessed antiepileptic drugs: The biochemical markers results for the population treated with the antiepileptics: carbamazepine, phenobarbital, are summarized in the Table 5, while that the percentages of the patients taking carbamazepine and

Table 4: Comparison of patients under valproate according to the duration of the treatment

Parameter (</>15 months)	Mean	Median	Std dev	p (t-test)
Alkaline phosphatase <15 m	146.62	142	54.10	0.001
Alkaline phosphatase >15 m	220.31	223	70.82	
Calcium <15 m	91.53	90	3.76	0.03
Calcium >15 m	94.73	100	6.12	
Phosphorus <15 m	47.06	47.1	8.86	0.35
Phosphorus >15 m	48.16	49.7	6.62	
Parathyroid hormone test <15 m	2.9	2.59	1.27	0.02
Parathyroid hormone test >15 m	4.04	3.64	1.91	
Osteocalcin <15 m	59.46	57.12	31.76	0.39
Osteocalcin >15 m	62.52	57.86	29.40	
B-Crosslaps <15 m	1.10	1.03	0.40	0.20
B-Crosslaps >15 m	1.22	1.14	0.40	
Procollagen type 1 N-terminal propeptide <15 m	356.42	301.1	210.43	0.08
Procollagen type 1 N-terminal propeptide >15 m	476.85	435.7	262.38	

Table 5: The Statistical description of the population taking carbamazepine and phenobarbital

	Age (years)	Weight (kg)	Dose (mg day^{-1})	Duration (months)	ALP (UI L^{-1})	Ca^{+2} (mg L^{-1})	P (mg L^{-1})	PTH (Pmol L^{-1})	Osteoc (ng mL^{-1})	B-crosslaps (ng L^{-1})	P1NP (ng mL^{-1})
Mean	24.37	43.50	77.00	38.50	166.60	96.00	44.44	5.78	31.48	0.73	273.39
Median	15.00	27.50	90.00	84.00	185.00	90.00	39.90	5.09	23.94	0.75	85.54
Std. dev.	24.85	35.60	120.93	67.54	84.52	14.30	15.43	4.26	27.08	0.30	377.95
Min.	0.25	5.30	30.00	1.00	49.00	80.00	27.80	1.94	2.59	0.23	22.59
Max.	69.00	115.00	400.00	204.00	277.00	120.00	78.00	16.91	97.01	1.19	1200.00

Table 6: The percentages of normal and abnormal levels obtained for the studied parameters in the group under carbamazepine and phenobarbital

Parameters	<Normal	Normal	>Normal
Parathyroid hormone test	0%	80%	20%
Calcium	20%	70%	10%
Phosphorus	0%	90%	10%
Alkaline phosphatase	0%	30%	70%
Osteocalcin	10%	70%	20%
Procollagen type 1 N-terminal propeptide	0%	22%	78%
B-cross laps	0%	20%	80%

Table 7: Comparison between the group of population under valproate and the group of patients under carbamazepine and phenobarbital

Parameters	Means Valproate	Carb+ phenob	p
Alkaline phosphatase	190.41	166.60	0.218
Calcium	93.44	96.00	0.295
Phosphorus	47.72	44.44	0.265
Parathyroid hormone test	3.58	5.78	0.071
Osteocalcin	61.28	31.48	0.004
B-crosslaps	1.18	0.73	0.0005
Procollagen type 1 N-terminal propeptide	427.93	273.39	0.124

phenobarbital with normal and abnormal levels are grouped in the Table 6.

The comparison between the results obtained for the patients treated with Valproate and those treated by the other studied antiepileptics (phenobarbital, carbamazepine) is expressed as means and standard deviations with the value of the statistical significance (p) grouped in the Table 7.

From the obtained results, it was noted, that a significant statistical difference is uniquely observed for the parameters Osteocalcin and β-Crosslaps. This result could be influenced by the age difference between the two samples, indeed, the average age of the population under valproate is 6.9 years, while that is around 24 years old for, the population under the treatment by carbamazepine and phenobarbital, the duration of the treatment also could be an interfering factor, in fact the median of treatment duration for valproate sample is around 24 months, it is higher to pass around 88 months for carbamazepine/phenobarbital sample.

The high values of β-Cross laps in the population under treatment with valproate obtained in the present study are confirmed by the study of Sato *et al.*[12], where it has been found that concentrations of Osteocalcin (a marker of bone formation) and β cross laps (a marker of bone resorption) were higher for the population treated with valproate .

Tekgul *et al.*[23] found in a study that serum concentrations of calcium and phosphorus were significantly low ($p<0.05$) compared to the control population. Besides, it has been found, that the serum level of alkaline phosphatase and parathyroid hormone was significantly elevated ($p<0.05$). This study showed that all antiepileptic drugs (new and old) affect bone mineral status in children receiving treatment for more than 6 months, altering several biochemical markers (serum calcium, phosphorus, PAL and PTH) and radiological markers bone mineral density assessed using dual-energy X-ray absorptiometry (DXA)[23].

Antiepileptics such as carbamazepine and phenobarbital are known to be inducers of the enzyme cytochrome p-450 that can accelerate vitamin D metabolism. This effect may result in decreased intestinal calcium absorption, as a result, the onset of intermittent hypocalcemia may occur and leads to compensatory hyperparathyroidism and therefore, increases bone turnover and cortical bone loss. In severe cases, this could lead to a long-term loss of bone mass[24,25]. This hypothesis was consistent with some studies[26,27], but not with others[18,28,29].

The mechanisms by which carbamazepine causes such alterations of bone metabolism can be multiple, in addition to increasing the catabolism of vitamin D, it may influence metabolism by a direct effect on bone cell function[30] and the direct inhibition of intestinal calcium transport by a mechanism that is not mediated by vitamin D[31,32].

Elliott *et al.*[33] revealed that patients receiving valproate had less adverse effects on bone markers and enhanced bone density compared to those receiving phenobarbital or carbamazepine. While Hasaneen *et al.*[32] found a significant difference in biochemical markers (alkaline phosphatase, calcium, phosphorus, parathyroid hormone) and radiological markers (bone mineral density) between sample of patients under treatment of antiepileptics and a control sample[32,33].

CONCLUSION

It was concluded that the present study consisted of a biologically descriptive prospective analysis of patients on antiepileptic drugs. The obtained results allow attaining the following conclusions:

- Antiepileptic drugs may affect bone mineral density in patients receiving long-term treatment, modifying biochemical markers (mainly those related to bone remodeling: (Osteocalcin, P1NP,B-Crosslaps) that reflect their influence on bone remodeling.
- Antiepileptic drugs can leads to osteoporosis and rickets as well as increasing the risk of fracture.
- Some factors as the duration of the treatment and the type of drug may be crucial, with different effect on bone remodeling.

Due to that, regular monitoring of hypovitaminosis and of biochemical markers of bone remodeling is recommended

throughout the treatment period. In addition, prophylaxis with calcium and vitamin D may be administered for all patients. It is also recommended to undertake a larger scale study with long-term follow-up with complementary radiological markers.

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