Estrogen Supplementation for Bone Dematuration in Young Epileptic Man Treated with Anticonvulsant Therapy; A Case Report

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Abstract. We encountered a young man treated with anticonvulsant therapy who had greatly reduced bone mineral density. An 18-year-old man was admitted to our hospital for shoulder pain and further evaluation of decreased bone mineral density. He had been treated with anticonvulsants, including phenytoin, phenobarbital, valproic acid and zonisamide for seizures. Although testosterone was found within the normal range for adult men, the serum estrogen concentration was below the detection limit (<10 pg/ml) and his wrist epiphyses were not yet closed. After 10 months of treatment with the conjugated estrogen, both his height and weight showed improvement, while his bone mineral density and bone age were increased. These findings suggested that estrogen therapy had a significant effect on his skeletal growth and bone maturation in man. This is the first report showing the beneficial effect of estrogen supplementation in an epileptic man receiving treatment with anticonvulsants.

Key words: Estrogen, Bone maturation, Anticonvulsant, Aromatase

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OSTEOMALACIA is one of the major adverse events of anticonvulsant therapy, and 15% of patients treated with these drugs are reported to have reduced bone mineral density [1]. Although the reason why anticonvulsants cause skeletal demineralization has not been elucidated, one of the major hypotheses is that such derivatives have an inhibitory effect on the activation of vitamin D [2]. Patients with an estrogen receptor gene abnormality and aromatase deficiency have recently been reported, who did not have normal bone maturation or a normal pubertal growth spurt [3–5]. Epiphyseal closure was delayed in both sexes, resulting in a eunuchoid habitus, and sustained osteopenia [3–6]. We saw a young man treated with anticonvulsant therapy who had greatly reduced bone mineral density (BMD).

The serum estrogen concentration was below the detection limit (<10 pg/ml), although testosterone was within the normal range for adult men and his wrist epiphyses were not yet closed. We present the clinical course, manifestations and the therapeutic effect of estrogen in an epileptic man on long-term treatment with anticonvulsants.

Case report

An 18-year-old Japanese man was referred to our hospital for further evaluation of low BMD. He had been treated with anticonvulsants including phenytoin, phenobarbital, valproic acid and zonisamide since he was 3 years old. There was no family history of consanguineous marriage. He was born after a full term pregnancy to a primiparous woman and weighted 3.3 kg. His growth was uneventful before he was 8 years old. Physical examination revealed that his height was 160 cm, weight was 37.5 Kg, and BMI was 14.7 kg/m². He had an

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arm span of 163 cm and remarkable kyphosis (see Fig. 1). Gingival swelling and abnormal tooth formation were observed. There was no thyroid enlargement. The results of cardiovascular, respiratory, and abdominal examinations were all normal. The patient had male genitalia and bilaterally descended testes, each with a volume of 9.6 ml. He had Tanner stage 3 (13.9 ± 2.1 years) pubic hair and Tanner stage 3 external genitalia. Semen analysis was not done.

The results of the initial laboratory tests are shown in Table 1. Serum alkaline phosphatase (ALP) was increased to 700 IU/ml, whereas the serum levels of calcium and inorganic phosphorus were normal. Glucose tolerance, basal insulin, and lipids were all within the normal range. Results of hormonal measurements are listed in Table 2. Although the serum testosterone concentration was normal for an adult male, estradiol was found below the detection limit (<10 pg/ml). The serum dehydroepiandrosterone sulfate (DHEA-S) concentration was also low. Both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were within the normal range. The thyrotropin (TSH) concentration was slightly high and FT_4 was low. Pituitary functions stimulated by corticotropin releasing hormone,

Table 1. Results of standard laboratory tests

WBC	$3.08 \times 10^3/\mu\text{l}$	(3.0–10.0)
Neut	33.3%	(40–75)
Lymph	56.5%	(20–50)
RBC	$4.31 \times 10^6/\mu\text{l}$	(3.8–5.7)
Hb	13.3 g/dl	(12–18)
Ht	40.1%	(35–53)
PLT	$183 \times 10^3/\mu\text{l}$	(100–350)
T.P	7.4g/dl	(6.3–8.0)
Alb	4.4g/dl	(3.5–5.0)
GOT	17 U/l	(10–35)
GPT	11 U/l	(5–35)
LDH	151 U/l	(130–250)
ALP	700 U/l	(153–523)
CPK	96 U/l	(30–200)
T-CHO	174 mg/dl	(140–220)
TG	60 mg/dl	(55–150)
HDL-CHO	79 mg/dl	(35–80)
FBS	79 mg/dl	(60–90)
Na	137 mEq/l	(135–148)
K	4.1 mEq/l	(3.6–5.2)
Ca	8.9 mg/dl	(8.4–9.8)
IP	4.5 mg/dl	(3.0–4.7)

Fig. 1. Appearance of the patient.

Table 2. Results of endocrine tests

LH	5.5 mIU/ml	(0.6–6.2)
FSH	6.3 mIU/ml	(0.8–7.1)
PRL	4.7 ng/ml	(1.5–9.7)
GH	4.27 ng/ml	(<0.42)
IGF-1	100 ng/ml	(106–398)
ACTH	52 pg/ml	(9–52)
Cortisol	12.9 µg/dl	(4.0–18.3)
DHEA-S	87 ng/ml	(619–4060)
testosterone	589 ng/dl	(208–899)
f-testosterone	8.2 pg/ml	(14–40)
5 α dihydrotestosterone	0.65 ng/ml	(0.2–1.0)
17-ketosteroid (17KS)	2.8 mg/day	(4.6–18.0)
estradiol (E ₂)	<10.0 pg/ml	(19–43)
Sex hormone-binding globulin	130 nmol/l	(10–80)
TSH	7.0 µU/ml	(0.39–3.5)
FT ₄	0.59 ng/dl	(0.9–1.88)
anti-TPO antibody	<0.3 U/ml	(<0.3)
anti-Tg antibody	<0.3 U/ml	(<0.3)

TPO: Thyroid peroxidase Tg: Thyroglobulin

thyrotropin releasing hormone and growth hormone releasing hormone showed normal responses (data not shown). Bone metabolic markers are shown in Table 3. Urinary excretion of deoxypyridinoline and pyridinoline (measured by High performance liquid chromatography) as markers of bone resorption were

high, and the results of bone turnover tests were all consistent with high bone turnover. Markers related to bone formation such as osteocalcin were also high. Serum concentrations of anticonvulsants are shown in Table 4. Among the four medicines, only the serum concentration of phenobarbital was above the

Table 3. Results of biochemical measurements related to bone and calcium metabolism

osteocalcin	53 ng/ml	(2.5–13)
urine deoxypyridinoline	20 µmol/mol.CRE	(<6)
urine pyridinoline	138 µmol/mol.CRE	(10–25)
urine total hydroxyproline	258 µMol/24 h	(83–330)
intact PTH	27 pg/ml	(15–50)
calcitonin	<10 pg/ml	(47–85)
25-hydroxyvitamin D	13 ng/ml	(10–55)
1,25-dihydroxyvitamin D	58.3 pg/ml	(20–70)
24,25-dihydroxyvitamin D	<0.6 ng/ml	(<1)

Table 4. Serum concentrations of anticonvulsants on admission

		Normal therapeutic range	Dosage on admission
Phenytoin	19.1 µg/ml	(7–20)	225 mg/day
Phenobarbital	30.9 µg/ml	(15–30)	75 mg/day
Valproic acid	61.1 µg/ml	(50–100)	300 mg/day
Zonisamide	14.1 µg/ml	(10–40)	1200 mg/day

upper limit of the therapeutic range.

Skeletal radiographs revealed osteoporotic change without pseudofracture specific to osteomalacia. Radiographs of his wrists and hands revealed a bone age of 14.4 years (Fig. 2) and open epiphyses. The BMD of the lumbar spine (L2-L4) was 0.655 g/cm² (BMC: 24.61 g, area: 37.59 cm²) in dual energy X-ray absorptometry (DPX-L, X-Ray Bone Densitometer Version 1.3, Lunar Rad Co., WI, USA) [7] and the whole body BMD was 0.749 g/cm². Brain CT scan and MRI were normal. Diffuse high voltage slow waves were detected on the EEG. The ratio of radioactive iodine uptake(¹²³I) by the thyroid gland was 16.5% after 3 h. The thyroid volume deter-

Fig. 2. Radiographs of both hands and wrists show delayed bone age and open epiphyses.

mined by ultrasonography was 5.8 ml [8]. The Tanaka-Binet intelligence test gave a mental age of 6 years and 10 months and his intelligence quotient (IQ) was 40. His karyotype was normal male 46XY.

Since the very low serum estradiol level and unclosed epiphyses suggested loss of aromatase activity, we performed HCG challenge test, but, as shown in Fig. 3a), the serum E₂ level increased to 27.3 pg/ml when testosterone was significantly increased (1370 ng/dl) by stimulating with HCG.

His clinical course is shown in Fig. 4. After giving informed consent to patient and family, he was treated with an oral conjugated estrogen (Premalin[®]) in addition to L-thyroxine (L-T₄), in an attempt to promote bone maturation and increase bone mass. The doses of anticonvulsants, particularly phenytoin and phenobarbital, were tapered off and replaced by carbamazepine. Thereafter, his seizures became uncontrollable following the tapering off of the anticonvulsants.

We repeated the HCG challenge test during estrogen therapy. As shown in Fig. 3b), HCG-stimulated increase in the serum E₂ level was more prominent than before estrogen therapy. Nausea, fluid retention, hypertension, unusual headaches, weight gain, gynecomastia, and mood alteration were not observed during estrogen therapy. Ten months after estrogen treatment the serum estrogen level was normal, and twelve months after estrogen therapy his

a) Before supplementary treatment

b) After supplementary treatment

Fig. 3. HCG challenge test: 4000 IU/day of HCG was injected intramuscularly for three days. left side: before treatment, right side: after treatment.

Total BMD(g/cm²) 0.749
L2-L4 BMD(g/cm²) 0.655

0.755
0.658

0.799
0.765

Fig. 4. Clinical course of the patient.

height, weight, and arm span had increased up to 164.8 cm, 42.6 Kg, and 168 cm, respectively. Whole body BMD increased by 7% (0.799 g/cm²), and the L2-L4 BMD also increased by 16% (0.765 g/cm², BMC: 31.50 g, area: 36.29 cm²). The serum concentrations of osteocalcin and ALP were suppressed to 48 ng/ml and 538 IU/ml, respectively, but his epiphyses remained open 1 year after estrogen therapy.

Aromatase DNA analysis

Genomic DNA was extracted from circulating lymphocytes, and exons 9 and 10 of the aromatase gene were amplified by PCR. The primer sequences for the 2 coding exons of the gene were designed on the basis information on the intron-exon junction sequence. The forward primer sequence for exon 9 was 5'CCACAGGTGAGAGACATA3' and the reverse primer sequence was 5'GGGCTCCTTACATTCTTTGCAA3', whereas the primers for exon 10 were 5'CATAGGTTCTTATAGGTAC3', and 5'CCTTCTCTAGTGTTCAGACACC3', respectively.

PCR was carried out for 30 cycles; each cycle consisted of denaturation for 1 min at 94°C, annealing for 45 sec at 55°C, and extension for 1 min at 72°C. The DNA fragments thus obtained were sequenced directly (TAKARA Corp.). Comparison with the published sequence of the human CYP 19 gene indicated that he did not have the same aromatase gene mutants as have been previously reported, i.e. exon 9 and exon 10 point mutations [4, 5, 9, 10].

Discussion

The serum E₂ concentration of the present patient, whose bone age was 14.4 years with open epiphyses, was below the detection limit. We therefore suspected reduced aromatase activity, but the HCG challenge test demonstrated elevated estrogen level along with testosterone, indicating that aromatase activity still preserved, and aromatase DNA analysis did not suggest distinct gene mutation. Although the serum DHEA-S level and 17-concentration of ketosteroids in urine were low, testosterone was nor-

mal. These findings suggested that his adrenarchy was delayed.

Low vitamin D levels and abnormal calcium metabolism may be responsible for anticonvulsant-induced osteomalacia [1, 2]. In the present patient, however, serum levels of calcium, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D were all within the normal range. These data suggest that treatment with vitamin D could not be expected to have a sufficient effect on bone involvement. He was then treated with a conjugated estrogen and L-T₄, and both phenytoin and phenobarbital were simultaneously tapered off and discontinued. But because uncontrollable seizures occurred when his anticonvulsants were reduced, treatment with phenytoin and phenobarbital was started again. These anticonvulsants were absolutely necessary for the prevention of seizures. Ten months after treatment with the conjugated estrogen, his height and weight both showed improvement, and his BMD and bone age were also improved. These findings suggested that estrogen therapy had a beneficial effect on his skeletal growth and bone maturation. It was recently reported that anticonvulsants, such as phenytoin, clonazepam, and carbamazepine, can directly inhibit the activity of

cytochrome P-450 aromatase at therapeutic concentrations [11]. Similarly, aromatase activity in the present patient may have been decreased due to the direct action of phenytoin and phenobarbital. Estrogen is a sex hormone which plays an important role in growth and differentiation. Aromatase inhibitors were recently developed for the treatment of breast cancer and were shown to suppress bone mineralization and growth in male rats [12]. These data may also be relevant to humans. Similar decreases in bone mass and density were reported in hypogonadal males [13]. Since estrogen plays such a pivotal role in skeletal physiology, and men with constitutional delay of puberty have decreased bone mineralization as adults [14], it is possible that all patients with delayed puberty and patients treated with aromatase inhibitors are at risk of demineralization.

In conclusion, this case suggests that estrogen plays an important role in bone maturation and bone mineralization even in males, as in females. The present case report is the first to show the beneficial effect of estrogen supplementation in an epileptic man receiving treatment with anticonvulsants.

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