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INFLUENCE OF MODIFIED TRANDERMAL HORMONE REPLACEMENT THERAPY, INCLUDING MAGNESIUM, ON BONE FORMATION MARKERS WITH OSTEOARTHROSIS OF SPINE IN WOMEN

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Abstract

In a randomized study 50 women, aged 51.7±2.8 years, suffering from primary osteoarthrosis (OA), were divided into two, equal groups (I, II). The women were employed in garment industry in contract work system. They were working in compulsory, mainly standing position. The women complained of backache of the lumbar region continuing for the minimum 5 years.

During the study, bone mineral density (BMD) of the lumbar spine was assessed twice with the densitometry DEXA method (Lunar Corporation equipment). Before treatment, structural changes in the lumbosacral spine were revealed using a CT Simens Sonata Plus 4. One energy technique (SEQCT) was applied. Concentrations of bone-forming markers in serum were measured three times: before treatment and 3 and 12 months afterwards. The concentration of acid phosphatase in serum was assessed by the enzymatic method according to Hitachi. The concentrations of osteocalcin and procolagen were radioimmunologically assessed by means of DRG Company – sets and concentration basal prolactin (PRL) before treatment radioimmunoassy kits produced by bioMerieux.

In the first stage of the treatment, the women in the first group received placebo for three months. Slow Mag B6 was administered for three months to the women in the second group.

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In the second stage of the treatment, the women in both groups received 21-day therapeutic cycles of modified transdermal hormonal replacement therapy. Additionally, bromocriptine (2.5 mg per day) and Slow Mag B6 (160 mg per day) were administered orally. The cycles repeated at a 7-day interval. During the interval, withdrawal bleeding occurred.

The results were statistically assessed by means of computerized programme package Statistica PL, version 5. It was stated that in 60% of women suffering from primary OA the basal concentration of prolactin in serum in was elevated above 25 ng/ml; in 25% women it was on the border level, and in 15% of the patients it was below the lower limit of the normal values. The combined treatment in women suffering from OA caused increase in bone-forming markers and decrease in pathological resorption processes of mineralization of the vertebral bodies. After 12 months of the therapy, resorption in the lumbar spine was diminished compared to the initial values, before the treatment. These changes were significant in L3/L4 vertebral bodies ($p<0.05$).

Key words: Osteoarthrosis. bone mineral density, bone-forming markers, hyperprolactinemia, modified hormonal replacement therapy.

WPŁYW ZMODYFIKOWANEJ PRZEZSKÓRNEJ HORMONOTERAPII ZASTĘPCZEJ I MAGNEZU NA STĘŻENIA MARKERÓW TWORZENIA KOŚCI U KOBIET ZE ZMIANAMI ZWYRODNIENIOWYMI KRĘGOSŁUPA

Abstrakt

Badaniem objęto 50 kobiet w wieku 51,7±2,8 lat z pierwotną osteoartrozą (OA), podzielonych na 2 grupy wg listy randomizowanej, zatrudnionych w przemyśle włókienniczym, w systemie pracy akordowo-potokowej, w pozycji wymuszonej, które od 5 lat uskarżały się na bóle w okolicy kręgosłupa lędźwiowego. Gęstość mineralną trzonów kręgów lędźwiowych oceniono 2-krotnie densytometrem, firmy Lunar Corporation, metodą DEXA, natomiast zmiany strukturalne kręgosłupa L1/S tomografem firmy CT Siemens Sonata Plus 4 techniką pojedynczej energii (SEQCT).

Stężenia markerów tworzenia kości określono 3-krotnie: przed leczeniem, po 3 i 12 miesiącach leczenia. Stężenie kostnej fosfatazy zasadowej oznaczono enzymatycznie, osteokalcyny i prokolagenu radioimmunologicznie zestawami firmy DRG. Podstawową prolaktynę (PRL) oznaczono przed leczeniem radioimmunologicznie zestawem bioMerieux. Kobiety z grupy I w pierwszym etapie leczenia przez 3 miesiące otrzymywały placebo, a w grupie II doustnie Slow Mag B6 w dawce 160 mg/24 h. Natomiast w drugim etapie kobiety z obu grup otrzymywały w 21-dniowym cyklu terapeutycznym zmodyfikowaną, przezskórną hormonoterapię zastępczą, bromokryptynę doustnie w dawce 2,5 mg/24 h i Slow Mag B6 w dawce 160 mg/24 h z przerwą 7-dniową w celu wystąpienia krwawienia. Analizy statystyczne z uzyskanych wyników przeprowadzono za pomocą pakietu Statistica PL, wersja 5. Stężenie prolaktyny podstawowej u 60% kobiet z OA wynosiło powyżej normy 25 ng/ml, u 25% kobiet górna granica normy, a u 15% kobiet poniżej dolnej granicy normy.

Zastosowanie skojarzonego leczenia u kobiet z OA wywarło pobudzający wpływ nie tylko na stężenia markerów tworzenia kości, ale również na wzrost aktywności osteoklastów pobudzających proces resorpcji nadmiernej mineralizacji trzonów kręgowych, która w 12-miesięcznej obserwacji ulegała zmniejszeniu w stosunku do wartości wyjściowych ze znamiennością w kręgu L3 i L4 ($p<0,05$).

Słowa kluczowe: osteonrtroza, gęstość mineralna, markery tworzenia kości, hiperprolaktynemia, zmodyfikowana hormonoterapia zastępcza.

INTRODUCTION

Osteoarthrosis (OA) is connected with diminished metabolism of bones, bone formation and resorption and increasing mineral density (BMD). Osteroarthrosis is a very frequent disease in human population, leading to disability (Goggs et al. 2005) and associated with large costs of diagnosis and treatment (Annuon et al. 200, Szczepański 2000). Depending on its ethiopathogenesis, OA is divided into primary and secondary type. Primary OA is a genetic disease, which initially invades small joints of the upper limbs, and than major joints of the limbs. Secondary OA is a multifactorial disease, developing against the inflammatory (Deleo et al. 2001, Punki et al. 2005, Yoshihara et al. 2000, 2001) hormonal (Stanosz et al. 2000) and traumatic (Nielsen et al. 2008) background. Secondary OA is chronic and progressive. In the initial stages, arthritis and inflammatory reactions with inflammatory exudates are found. These early changes are dominated by inflammatory reactions connected with metaloproteinases (Yoshiihara et al. 2000) and cytokines (Raap et al. 2000). Despite causing social concern, osteoarthrosis is far less known than other chronic diseases, such as hypertension arterials, diabetes mellitus, osteoporosis or neoplasms. Although there is a wealth of reports on the etiopathogenesis and diagnosis of OA, no unambiguous recommendations for its treatment have been established.

OBJECTIVE

The aim of the study has been to analyse the influence of transdemal modified hormonal replacement therapy (HRT) and the application of magnesium and dopaminergic agent (bromocriptine) on the concentrations of bone alkaline phosphatase, osteoclacine, procolagene in serum and on the bone mineral density (BMD) of the lumbar spine in women in the early perimenopausal phase.

MATERIAL AND METHODS

The study compromised 50 women with primary osteoarthrosis aged 51.7 ± 2.8 divided into two, equal, randomized groups (control I and experimental II groups). There were no differences between the groups in the age, body mass index, parity, place of living, habits and working conditions or the duration of the postmenopausal period. The main complaints were the lumbar backache and paresthesis. Bone mineral density (BMD L_2-L_4), determined at baseline (before treatment) and at 12 months, was assessed

with a DEXA dual energy x-ray absorptiometry (Lunar Corporation DPX-IQ) scanner, which utilizes hydroxyapatite level in g/cm˛ as an expression of the degree of bone mineralization. The results of BMD L_2-L_4 were interpreted according to the WHO criteria. Before the treatment, structural changes in the lumbosacral spine were revealed using a CT Simens Sonate Plus 4. One energy technique (SEWCT) was applied. The concentrations of bone-forming markers: bone alkaline phosphatase, osteocalcine and procolagene in serum, were assessed. The concentration of bone alkaline phosphatase were assayed enzymatically (Rosałki et al. 1984). The concentrations of osteocalcine and procolagene were assessed radioimmunologically by means of a DRC company sets. The concentrations of basal levels of prolactin (PRL I) were measured by means of bioMerioux commercial sets. Statistical calculations were performed using Statistica PL Package, version 5 (Stanisz 1988). The women of the control group (I), were receiving placebo for months. The women of the study group (II) were administered a commercial, pharmaceutical therapeutic agent, i.e. Slow Mag B_6, in the daily dose 160 mg/24 h for 3 months. Afterwards, both groups underwent modified transdermal hormonal replacement therapy (HRT): micronized 17â-estradiol (molar mass 272,39 g/mol) in the form of patches (Systen, Janssen-Cilag, Switzerland) with subsequently increasing and decreasing doses (25, 50, 75 and 75 ug/dose), imitating the physiological serum concentrations of estrogens throughout the therapeutic cycle (Stanosz et al. 2007), with concomitant oral intermittent progesterone administration (molar mass 314.47 g/mol); (Lutein Firm Adamed, Poland) in the second phase of the therapeutic cycle in doses of 50 mg daily for six days and subsequently 100 mg daily for the next six days during 12 months. Moreover, independently of the stage of the therapeutic cycle, both groups received a commercial, pharmaceutical agent containing magnesium and vitamin B_6, called Slow Mag B_6, which was administered orally in the dose of 160 mg/24h, and a dopaminegic agent, bromocriptine (manufacturered by Polfa), in the dose 2.5 mg/24 h.

RESULTS

The results of the study are compiled in Tables 1, 2 and Figure 1. Table 1 presents concentrations of bone alkaline phosphate, osteocalcine, procolagene in serum before and after 12 months of the combined treatment in both groups (I, II). There were no differences in the concentrations of bone-forming markers between the groups before the treatment. In study group (group II), receiving magnesium, a statistically significant increase in bone alkaline phosphatase ($p < 0.05$) and procolagene ($p < 0.05$) occurred compared to women receiving placebo (group I). After 12 months of the combined treatment, in both groups (I, II) the concentrations of bone-forming markers in

Table 1

Concentrations of bone-forming markers before and after combined treatment

Groups	*n*	Before study			After 3 months of treatment			After 12 months of treatment		
		bone alkaline phosphatase (U L^{-1})	osteocalcine (mg ul^{-1})	procolagene (mg ml^{-1})	bone alkaline phosphatase (U L^{-1})	osteocalcine (mg ul^{-1})	procolagene (mg ml^{-1})	bone alkaline phosphatase (U L^{-1})	osteocalcine (mg ul^{-1})	procolagene (mg ml^{-1})
I	25	18.4 ±4.1	11.4 ±3.1	115.4 ±12.2	19.7 ±3.1	16.7 ±8.4	125.4 ±9.1	21.4 ±5.7	21.7** ±15.1	121.7* ±15.1
II	25	20.3 ±3.2	13.2 ±4.3	109.6 ±15.3	30.7* ±6.1	20.3 ±9.7	132.9* ±8.1	25.2 ±9.7	25.1** ±17.3	125.1* ±17.3

*$p<0.05$
**$p<0.01$

Table 2

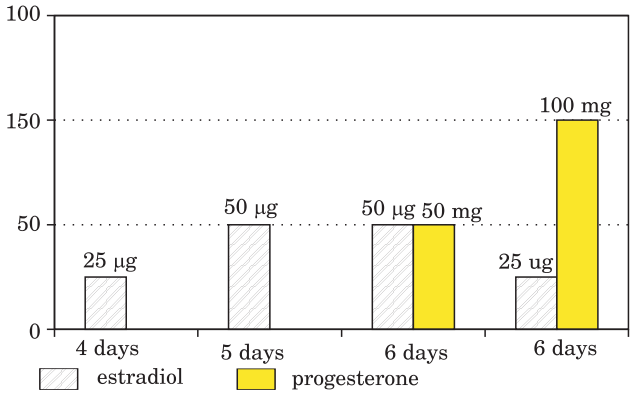
Bone mineral density of the lumbar spine L1-L4 (mg hydroxyapatite cm^{-2})

Groups	*n*	PRL (ng l^{-1})	Primary exam				Exams after 12 months of threat				Reference values
			L_1	L_2	L_3	L_4	L_1	L_2	L_3	L_4	
I	25	25.6 ±7.6	131 ±21.2	135 ±19.7	136 ±20.7	140 ±25.1	124 ±19.0	130 ±16.7	128* ±22.4	131.2* ±26.1	125 ±8.7
II	25	24.9 ±6.9	129.2 ±19.8	128.7 ±16.4	130.5 ±17.2	135.4 ±19.2	123.4 ±17.1	121.7 ±16.9	122.1* ±26.9	127.4* ±19.4	120.3 ±7.1

*$p<0.05$

serum were statistically higher in comparison with the initial values (Table 1). Table 2 contains results of the bone mineral density (BMD) of the lumbar spine L_1-L_4. There were no differences in the degree of mineralization of the lumbar spine between groups. However, after 12 months of the combined treatment, the degree of mineralization density decreased statistically significantly in vertebral bodies L_3 and L_4 ($p < 0.05$). Tomographic images of the lumbar sacral spine revealed in 15 women (30%) with OA the narrowing of intervertebral discs in L_1/ L_2 and L_2/L_3 with osteophytoses.

In 25 women (50%), herniae of annulus fibrosus of intervertebral discs without radiculalgia were found in the intervetebral spaces L_4/L_5 and L_5/S_1. In 10 women (20%), herniae of annulus fibrosus of intervertebral discs with compression of nerve roots causing radiculopathy were revealed in the intervetebral space L_5/S_1. Three months of Slow Mag B_6 administration in women of the study group caused significant diminishing of radiculopathy.



Pig. 1 The influence of modified transdermal hormone replacement in therapeutic cycle. Use of fluctuating doses hormones leads to obtaining physiological concentrations of estrogens and progesterone in serum

DISCUSSION

Magnesium is an intracellular bioelement essential for health. This macroelement is of great importance in physiological processes (ANNOUN et al. 2007, SZCZĘSNY 2002). Magnesium is an activator of many enzymes participating in energy processes, influencing concentration of other electrolytes by enhancing their capacity for assimilation (HUNTER et al. 2003). Children, elderly people, patients with hypertonia arterials, circulation insufficiency and the digestive system diseases are at risk of magnesium deficiency (ANNOUN et al. 2007). Administration of magnesium, in the present study, caused the activation of bone alkaline phosphatase (p 3< 0.05) in serum. In patients with OA, after this therapy, other metabolic processes occurring in bones were activated (IMADA et al. 2003, JIANG et al. 2008, TANIMOTO et al. 2004). The decreased metabolism in bones of patients with OA is caused not only by advanced age (SZCZEPAŃSKI 2000, SZCZĘSNY 2002), but also by enzymatic (YOSHIHARA et al. 2000), inflammatory (DELEO et al. 2001, PUNZI et al. 200) cytokines (RAAP et al. 2000, YOSHIHARA et al. 2004) and traumatic (NIELSEN et al. 2008) agents. Among hormonal factors involved in the development of OA, hyperprolactinemia, which is found in 86% of the studied women, seems very important (STANOSZ et al. 2000). Prolactin directly exerts inhibits osteoblasts and osteoclastic activity. Indirect influence of prolactin on bone metabolism, due to disturbances in the conversion of androgens into estrogens, has been demonstrated. The combined treatment discussed in this paper (magnesium plus dopaminegric agent) stimulated bone metabolism in women with OA, which was revealed as a significant increase in the concentration of bone alkaline phosphatase ($p < 0.05$) and osteocalcine ($p < 0.05$) in se-

rum. Moreover, after 12 months of the combined treatment the concentrations of bone-forming markers were significantly higher than before treatment. In women with spondyloarthrosis of the spine, an increase in BMD L_1-L_5 in comparison with reference values of the densitometer was found. It was caused not only by more intense mineralization of the vertebral bodies and ligaments (STANOSZ et al. 2000), but also by the mineralization of intervertebral discs (GARNELO et al. 2005, TANIMOTO et al. 2004, WANG et al. 2005) and the spinous processes (GOLDBERG 2000). Application of the combined treatment in women with OA demonstrated a favourable effect on the resorption process of vertebral bodies after 12 months of the treatment, accompanied by decreasing BMD of vertebral bodies compared to the initial values but, but significant results occurred only in vertebral bodies L_3 and L_4 ($P < 0.05$).

CONCLUSIONS

1. The stimulatory effect of the pharmacological therapeutic agent Slow Mag B_6 on bone formation processes occurs through significant increase of bone alkaline phosphatase and procolagene.

2. Frequent occurrence of hyperprolactonemia in women with osteoarthrosis suggests need to add a dopaminergic agent (bromocriptine) to the combined treatment of this chronic disease.

3. Administration of modified transdermal hormonal replacement therapy influences bone forming as well as bone resorption processes.

REFERENCES

ANNOUN N., GUERNE P.A. 2007. *Diagnosis and treatment of calcium pyrophosphate crystal-induced arthropathy.* Z. Rheumatol., 66(7): 576-8.

DELEO J.A., YEZIERSKI R.P. 2001. *The role of neuroinflammation and neuroimmune activation in persistent pain.* Pain, 90(1-2): 1-6.

GARNERO P., PETERFY C., ZAIM S., SCHOENHARTING M. 2005. *Bone marrow abnormalities on magnetic resonance imaging are associated with type II collagen degradation in knee osteoarthritis: a three-month longitudinal study.* Arthritis Rheum., 52(9): 822-9.

GOGGS R., VAUGHAN-THOMAS A., CLEGG P.D., CARTER S.D., INNES J.F., MOBASHERI A., SHAKIBAC M., SCHWAB W., BONDY C.A. 2005. *Nutraceutical therapies for degenerative joint diseases: a critical review.* Crit. Rev. Food Sci. Nutr.,45(3):145-64,

HUNTER D.J., HART D., SNIEDER H.. BETTICA P., SWAMINATHAN R., SPECTOR T.D. 2003. *Evidence of altered bone turnover, vitamin D and calcium regulation with knee osteoarthritis in female twins.* Rheumatology (Oxford), 42(11):1311-6.

IMADA M., TANIMOTO K., OHNO S., SASAKI A., SUGIYAMA H., TANNE K. 2003. *Changes in urinary bone resorption markers (pyridinoline, deoxypiridinoline) resulting from experimentally- induced osteoarthritis in the temporomandibular joint of rats.* Cranio, 21(1): 38-45.

JIANG L.S., ZHANG Z.M., JIANG S.D., CHEN W.H., DAI L.Y. 2008. *Differential bone metabolism between postmenopausal women with osteoarthritis and osteoporosis*. J. Bone Miner. Res., 23(4): 475-83.

GOLBERG M.,B. 2000. *The role of the chondrocyte in osteoarthritis.* Arthritis Rheum., 43 (9): 1916-26.

NIELSEN R.H., STOOP R,. LEEMING D.J., STOLINA M., QVIST P., CHRISTIANSEN C., KARSDAL M.A. 2008. *Evaluation of cartilage damage by measuring collagen degradation products in join extracts in a traumatic model of osteoarthritis.* Biomarkers, 13 (1): 79-87.

PUNZI L., OLIVIERO F., PLEBANI M. 2005. *New biochemical insights into the pathogenesis of osteoarthritis and the role of laboratory investigations in clinical assessment.* Crit. Rev. Clin. Lab. Sci., 42(4): 279-309.

RAAP T., JUSTEN H.P., MILLER L.E., CUTOLO M., SCHOLMERICH J., STRAUB R.H. 2000. *Neurotransmitter modulation of interleukin 6/Il-6/ and Il-8 secretion of synovial fibroblasts in patients with rheumatoid arthritis compared to osteoarthritis*. J. Rheumatol., 27 (11): 2558-65.

ROSALKI S.B., FOO A.Y. 1984. *Two new methods for separating and quantifying bone and live alkaline phosphatase isoenzymes in plasma.* Clin. Chem., 30 (7): 1182-6.

SZCZEPAŃSKI L. 2000. *Choroby zwyrodnieniowe stawów [Bone degenerative diseases].* Reumatologia., 38 (12): 87-95.

STANISZ A. 1988. *Przystępny kurs statystyki w oparciu o program Statistica PL na przykładach z medycyny, wersja 5 [Easy course in statistics based on Statistica PL software package and case studies in medicine, verison 5].* StatSoft. Kraków.

STANOSZ S., ŻOCHOWSKA E., KOZŁOWSKI A. 2000. *Stężenia prolaktyny i gęstość mineralna kości u kobiet ze zmianami zwyrodnieniowymi kręgosłupa [Concentrations of prolactine and bone mineral density in women with degenative changes in the spine].* Terapia, 1: 48-50.

STANOSZ S., ŻOCHOWSKA E., STANOSZ M. 2007. *Biochemiczne aspekty zmodyfikowanej, przezskórnej hormonoterapii zastępczej [Biochemical aspects of modified transdermal hormonal replacament therapy].* Ginekol. Pol., 78(12): 922-928.

SZCZĘSNY G.2002. *Patomechanizm powstawania zmian zwyrodnieniowych stawów*. Ortop. Traumatol. Rehab., 4 (2): 222-229.

TANIMOTO K., OHNO S., IMADA M., HONDA K., OHNO-NAKAHARA M., KAPILA S, TANNE K. 2004. *Utility of urinary pyridinoline and deoxcypyridynoline ratio for diagnosis of osteoarthritis at temporomandibular joint.* J. Oral Pathol. Med., 33 (42): 218-23

WANG Y., EBELING P.R., HANNA F., O' SULLIVAN R., CICUTTINI F.M. 2005. *Relationship between bone markers and knee cartilage volume in healthy men.* J. Rheumatol., 32 (11): 2200-4.

YOSHIHARA Y., NAKAMURA H., OBATA H., YAMADA H., HYAKAWA T., FUJIKAWA K., OKADA Y. 2000. *Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis.* Ann. Rheum. Dis., 59 (6): 455-61.

YOSHIHARA Y., TSUKAZAKI T., OSAKI M., NAKASHIMA M., HASUI K., SHINDO H. 2004. *Altered expression of inflammatory cytokines in primary osteoarthritis by human T lymphotropic virus type I retrovirus infection: a cross-sectional study.* Arthritis Res. Ther. 6 (4): R 347-54.

YONEHARA N., YOSHIMURA M. 2001. *Influence of painful chronic neuropathy on neurogenic inflammation.* Pain, 92 (1-2): 259-65.