Bone turnover markers in medicamentous and physiological hyperprolactinemia in female rats

Markeri koštanoj metabolizma u medikamentnoj i fiziološkoj hiperprolaktinemiji kod ženki pacova

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Abstract

Background/Aim. There is a lack of data on the effects of prolactin on calcium metabolism and bone turnover in hyperprolactinemia of various origins. The aim of this study was to compare the influence of medicamentous and physiological hyperprolactinemia on bone turnover in female rats. Methods. Experimental animals (18 weeks old, Wistar female rats) were divided as follows: the group P – 9 rats, 3 weeks pregnant; the group M3–10 rats that were intramuscularly administrated sulpirid (10 mg/kg) twice daily for 3 weeks, the group M6 – 10 rats that were intramuscularly administrated with sulpirid (10 mg/kg) twice daily for 6 weeks, and age matched nulliparous rats as the control group: 10 rats, 18-week-old (C1) and 7 rats, 24 weeks old (C2). Laboratory investigations included serum ionized calcium and phosphorus, urinary calcium and phosphorous excretion, osteocalcin and serum procollagen type 1 N-terminal propeptide (P1NP). Results. Experimental animals in the group P compared to the control group, displayed lower mean serum ionized calcium (0.5 ± 0.2 vs 1.12 ± 0.04 mmol/L; p < 0.05); increased urinary calcium (3.90 ± 0.46 vs 3.05 ± 0.58; p < 0.01) and significantly increased P1NP (489.22 ± 46.77 vs 361.9 ± 53.01 pg/mL; p < 0.001). Experimental animals in the group M3 had significantly decreased P1NP, compared to the control group. Prolongated medicamentous hyperprolactinemia (the group M6) induced increased serum ionized calcium (1.21 ± 0.03 vs 1.15 ± 0.02 mmol/L; p < 0.001); decreased serum phosphorus (1.70 ± 0.13 vs 1.89 ± 0.32 mmol/L; p < 0.001); decreased osteocalcin and P1NP. Conclusions. Physiological hyperprolactinemia does not have such harmful effect on bone metabolism as medicamentous hyperprolactinemia. Chronic medicamentous hyperprolactinemia produces lower serum levels of bone formation markers. Assessment of bone turnover markers in prolonged medicamentous hyperprolactinemia provides an opportunity for earlier diagnosis of bone metabolism disturbances and should be considered as mandatory.

Key words: hyperprolactinemia; pregnancy; sulpiride; rats; osteogenesis; biological markers; calcium; phosphorus; osteocalcin.

Apstrakt

Uvod/Cilj. Nema dovoljno podataka o efektnima prolaktnica na metabolizam kalcijuma i koštani promet kod hiperprolaktinemije različitog porekla. Cilj ovog rada bio je da uporedi uticaj medicamentne i fiziološke hiperprolaktinemije na koštani metabolizam kod ženki pacova. Metode. Eksperimentalne životinje (ženke pacova soja Wistar, stare 18 nedelja) podeljene su na sledene grupe: grupa P – devet pacova, 3 nedelje trudno; grupa M3–10 pacova, kojima je tokom treće sezone trudno administrirao sulpirid (10 mg/kg); grupa M6 – 10 pacova kojima je tokom šest sedam nedelja, davan intramuskularno sulpirid (10 mg/kg); grupa M6 – 10 pacova, kojima je tokom šest sedam nedelja, davan intramuskularno sulpirid (10 mg/kg); grupa M6 – 10 pacova, kojima je tokom šest sedam nedelja, davan intramuskularno sulpirid (10 mg/kg); grupa M6 – 10 pacova, kojima je tokom šest sedam nedelja, davan intramuskularno sulpirid (10 mg/kg); grupa M6 – 10 pacova, kojima je tokom šest sedam nedelja, davan intramuskularno sulpirid (10 mg/kg). rezultati. U poredenju sa kontrolnom grupom eksperimentalne životinje u grupi P imale su sniženj jonican kalcijum u serumu (0,5 ± 0,2 vs 1,12 ± 0,04 mmol/L; p < 0,01), povećane fosfatne fosfate u serumu (2,42 ± 0,46 vs 2,05 ± 0,58 mmol/L; p < 0,01) i značajno povišen P1NP (489,22 ± 46,77 vs 361,9 ± 53,01 pg/mL; p < 0,001). Značajan pad P1NP zabeležen je u eksperimentalnoj grupi M3 u poredenju sa kontrolnom grupom. Prolongirana medicamentna hiperprolaktinemija u grupi M6 dovela je do porasta jonican kalcijuma u serumu (1,21 ± 0,03 vs 1,15 ± 0,02 mmol/L; p < 0,001), pada fosfata (1,70 ± 0,13 vs 1,89 ± 0,32 mmol/L; p < 0,001).

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Introduction

Hyperprolactinemia (HP) is a common hypothalamic-pituitary axis disorder. This “abnormal laboratory value” may be caused by any process interfering with dopamine synthesis, its transport to the pituitary gland or its action on lactotroph dopamine receptors. The complexity of various etiologies, HP could be divided into 4 categories: physiological, pathological, medicamentous and functional HP.

The most frequent clinical symptoms of HP, regardless of its origin, are galactorrhea, oligo- or amenorrhea and sterility in women and impotence, libido loss and gynecomastia in men. In last few decades, there is growing evidence of decreased bone mineral density (BMD) and increased activity of bone turnover markers caused by HP. Prolactin (PRL)-secreting pituitary tumors in people and rats are associ-
ated with osteopenia. Antipsychotic-induced HP can cause osteoporosis and increased risk of hip fracture. Antipsychotic-induced HP can cause osteoporosis and increased risk of hip fracture. Pregnancy and prolonged lactation, conditions with physiological HP, can lead to a significant bone loss. Although rapid mineralizing neonatal skeleton (during pregnancy) and higher calcium demand for milk production (during lactation) places significant stress on maternal calcium homeostasis, bone loss is usually recovered after weaning. Longitudinal studies have shown that there is no detrimental effect of parity and prolonged breast-feeding on long-term bone health.

Although physiological and medicamentous HP have different final effects on skeletal system, there is a lack of data regarding the effects of PRL on calcium metabolism and bone turnover in HP of various origins. The aim of this experimental study was: 1) to determine if there was a difference in calcium metabolism during pregnancy (physiological HP) and in sulpirid-induced HP (medicamentous HP); 2) to compare the influence of medicamentous and physiological HP on bone turnover markers; 3) and to reveal a possible effect of prolonged medicamentous HP on calcium metabolism and bone turnover markers.

Methods

Animals

Pregnant and age matched nulliparous Wistar female rats (18 weeks old) were obtained from the Animal Laboratory Centre Torlak, Institute for Medical Research, Military Medical Academy, Belgrade, Serbia. Experimental study was conducted in Biomedical Research Center, Medical Faculty, University of Niš, Serbia. The weight of experimental animals ranged 290–340 g. They were housed under a 12 : 12 h light-dark cycle (lights on at 06 h) and fed standard chow and water. Room temperature was 23–25°C with average humidity of 50–60%. The study was approved by the Ethical Committee of the Medical Faculty, University of Niš, Serbia.

Experimental design

Experimental animals were divided into the following groups: 9 rats, 3 week pregnant (P – physiological HP during pregnancy; gestation period in Wistar rats 19–22 days); 10 rats with intramuscularly administrated sulpirid (10 mg/kg) twice daily for 3 weeks (M3 – medicamentous HP); 10 rats with intramuscularly administrated sulpirid (10 mg/kg) twice daily for 6 weeks (M6 – medicamentous HP). Since bone growth and calcium accretion are normally age dependent, we used age matched nulliparous rats as control groups: 10 rats, 18 weeks old (C1), and 7 rats, 24 weeks old (C2). All rats in each group (pregnant, medicamentous treated and controls) were sacrificed on the same day.

Laboratory investigations

In order to confirm HP serum PRL levels were measured in all experimental groups and compared with controls. PRL concentration was measured using enzyme-linked immunosorbent assay kit for PRL. The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of PRL in rat serum, plasma and other biological fluids (manufactured by Usenq, Life Science Inc.). All experimental animals were analyzed for serum ionized calcium and urinary calcium, inorganic phosphorus and urinary phosphate. All rats, in each group, were kept in single rat metabolic cages, 24 h before they were sacrificed, in order to collect 24 h urine for calciuresis and phosphorus diuresis. Rats were anesthetized with intramuscular injection of 10% ketamine hydrochloride (0.3 mL per animal). Blood samples until exsanguination were taken by puncture of left myocardial ventricle through midline thoracoabdominal incision. Mineral assays were done by the following methods: serum ionized calcium by potentiometric method; urinary calcium by photometric colour test (Beckman Coulter, OLYMPUS analyzer); serum and urine phosphate concentration by photometric UV test (Beckman Coulter, OLYMPUS analyzer); The bone turnover markers studied were: osteocalcin (OC) and serum procollagen type 1 N-terminal propeptide (P1NP). The methods used for bone turnover markers were: OC by electrochemiluminescence immunoassay (N-MID Osteocalcin, Cobas, Roche) and P1NP was measured using enzyme-linked immunosorbent assay kit for P1NP (Usenq, Life Science Inc.).

Statistical analysis

Data were analyzed using SPSS (version 15.0). Continuous (measurable) parameters were presented with mean values (x) and standard deviation (SD), median (md), maximum (max) and minimum (min) values. The Shapiro-Wilk test was used to determine normality of parameters distribution. Differences were tested by Student's t-test for independent samples if the distribution of parameters was normal and Mann-Whitney U-test was used if parameters distribution was deviated. We used Student's t-test for dependent samples (normal distribution) and Wilcoxon test (deviated distribution) to test statistical significance between continuous parameter values at the beginning and the end of the study.

Results

Changes in prolactin concentration, calcium metabolism and bone turnover markers in physiological hyperprolactinemia

PRL concentrations were significantly higher during the third week of pregnancy (P), compared with C1 (181.80 ± 29.65 vs 105.38 ± 28.34 pg/mL; p < 0.001) (Table 1).

Urinary calcium and phosphorus excretion (measured as daily total calcium and daily phosphorus excretion) significantly increased during pregnancy compared to the control group (urinary calcium 3.90 ± 0.46 mmol/L/24 h vs 3.05 ± 0.58 mmol/L/24 h; p < 0.01; urinary phosphorus 141.15 ± 20.65 mmol/L/24 h vs 45.54 ± 7.99 mmol/L/24 h, p < 0.001) (Table 2).

Changes in prolactin concentration, calcium metabolism and bone turnover markers in medicamentous hyperprolactinemia

Significantly increased PRL levels in sulpirid-treated rats, compared to age matched controls, confirmed the state of medicamentous HP (M3: 182.03 ± 57.80 vs 105.38 ± 28.34 pg/mL; p < 0.001; M6: 148.92 ± 20.46 vs 112.01 ± 11.92 pg/mL; p < 0.001). Even though lower PRL concentration were verified in M6 in comparison with M3, decrease was not significant (148.92 ± 20.46 vs 182.03 ± 57.80 pg/mL, p > 0.05).

Table 1

<table>
<thead>
<tr>
<th>Concentration of prolactin, osteocalcin and P1NP in experimental groups</th>
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<td>Experimental groups</td>
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<tr>
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<tr>
<td>C1</td>
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<td>M3</td>
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OC concentrations were significantly lower during pregnancy compared to C1 (9.01 ± 1.09 ng/mL vs 17.50 ± 2.76 ng/mL, p < 0.001) (Table 1).

In medicamentous HP (M3), serum calcium levels were higher, with no significant difference compared to C1 (1.15 ± 0.04 vs 1.12 ± 0.04 mmol/L) but serum calcium levels

Table 2

<table>
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<th>Calcium and phosphorous serum concentrations and 24-h urine excretion values</th>
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<td>Parameter</td>
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<tr>
<td>Serum ionized calcium (mmol/L), x ± SD</td>
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<td>Urine calcium excretion (mmol/24h), x ± SD</td>
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<td>Urine phosphorous (mmol/24h) excretion (x ± SD)</td>
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P1NP concentrations were significantly higher in physiological HP (P) in comparison to that in the C1 group (489.22 ± 46.77 pg/mL vs 361 ± 53.01 pg/mL, p < 0.001) (Table 1).

Serum ionized calcium concentrations were significantly decreased in pregnancy (0.5 ± 0.2 mmol/L vs 1.12 ± 0.04 mmol/L, p < 0.001) followed by significantly increased phosphorus compared to C1 (2.42 ± 0.46 mmol/L vs 2.05 ± 0.19 mmol/L; p < 0.05) (Table 2).

mL). With prolongation of medicamentous HP (M6) calcium concentrations continued to rise and serum phosphorus levels to fall. After 6 weeks of sulpirid provoked HP, serum calcium concentrations were significantly increased in comparison with C2 (1.21 ± 0.03 mmol/L vs. 1.15 ± 0.02 mmol/L, \( p < 0.001 \)). There was no significant difference in serum phosphorus concentrations with a prolongation of sulpirid treatment (1.70 ± 0.13 mmol/L vs. 1.89 ± 0.32 mmol/L, \( p > 0.05 \)).

In M3 rats calciesis was significantly increased compared to the control group (4.31 ± 1.11 mmol/24 h vs. 3.05 ± 0.58 mmol/24 h, \( p < 0.01 \)) while phosphorus diuresis was not significantly changed (50.58 ± 9.77 mmol/24 h vs. 45.54 ± 7.99 mmol/24 h, \( p > 0.05 \)).

With longer duration of medicamentous HP (M6) there were no significant changes in urinary calcium and phosphorus excretion compared to C2 (calciesis: 2.88 ± 0.6 mmol/24 h vs. 3.37 ± 0.87 mmol/24 h; phosphorus diuresis: 53.93 ± 14.05 mmol/24 h vs. 55.03 ± 20.37 mmol/24 h).

OC concentrations were decreased in sulpirid-treated rats compared to age matched control groups (M3: 15.28 ± 2.51 ng/mL vs. 17.50 ± 2.76 ng/mL; M6: 13.55 ± 3.42 ng/mL vs. 16.18 ± 2.0 ng/mL) but without statistical significance.

P1NP concentration in M3 compared to C1 was significantly decreased (309.60 pg/mL ± 36.74 vs. 361.90 ± 53.01 pg/mL, \( p < 0.05 \)) and even more in comparison with physiological HP (309.60 ± 36.74 pg/mL vs. 489.22 ± 46.77 pg/mL; \( p < 0.001 \)). Although the tendency of further P1NP decrease was noticed with longer duration of medicamentous HP, no significant difference was verified (291.70 ± 71.03 pg/mL, \( p > 0.05 \)).

Discussion

Our study results confirmed the expected PRL increase during pregnancy. The results of mineral analyses and bone turnover markers conducted in this experimental group could be considered as representative for physiological HP.

Calcium homeostasis during pregnancy is changed due to elevated fetus demand for calcium and maternal adaptations. Adaptation mechanisms include increase in intestinal calcium absorption, decrease in urinary calcium excretion or mobilization of maternal bone mineral.

A decrease in total serum calcium concentration during pregnancy has already been reported \(^{19, 20}\) and usually considered as a consequence of hemodilution and decreased serum albumin \(^{19, 21, 22}\). In order to avoid low calcium concentration due to dilutional effect, we measured serum ionized calcium level. Our study results confirmed a significant decrease in ionized calcium, which is in the contrast with previously reported results of unchanged ionized calcium throughout gestation \(^{23, 24}\) and more consistent with data from several animal models, reporting fall of ionized calcium in late pregnancy \(^{25, 26}\). Rapid fetus growing in late pregnancy may exceed the maternal capacity to maintain a normal serum calcium level and result in decreased ionized calcium.

Inorganic phosphorus is very often considered to be a passive companion of calcium fluxes. Studies which evaluate phosphate balance during physiological HP (as pregnancy) are less common than calcium studies. Serum phosphate levels are usually reported as normal throughout pregnancy in humans and animals \(^{19, 21}\). Our study results showed significantly increased serum phosphorus during pregnancy. It is consistent with decreased serum ionized calcium in our experimental study, increased parathyroid hormone during rat pregnancy, reported in previous animal models \(^{21}\) and a fact that dietary phosphorus is absorbed almost twice as efficiently as dietary calcium \(^{27}\).

The increase of urinary calcium excretion during pregnancy is consistent with previous reports \(^{19, 20}\). It is considered as a consequence of increased calcium absorption and elevation in glomerular filtration rate (GFR) during pregnancy, which together exceed the reabsorptive capacity of the kidney \(^{19, 21}\).

Changes in urinary phosphorus excretion, during pregnancy, could be also due to increased dietary intake in late pregnancy, increased absorption and increased GFR during pregnancy.

OC fulfils all three of the following criteria for reliable bone turnover marker: it is osteoblast-produced protein, its increase correlates with increased bone formation, and it has fast response to changes in skeletal homeostasis. A decline in serum OC during pregnancy, in this study is consistent with the findings of previous reports \(^{19, 20, 28, 29}\). Decreased OC in pregnancy may be related to hemodilution, fetal contribution \(^{20}\), increased renal degradation secondary to increased GFR \(^{29}\) or lacking of normal values during pregnancy \(^{22}\).

P1NP together with carboxy terminal propeptide (P1CP) are a part of the process in which type I procollagen is transformed into type I collagen. Since type I collagen constitutes 90% of bone proteins, it may be considered as very valuable and precise marker of bone formation. Our study results are consistent with limited, previously reported data, showing low P1CP and P1NP concentration in the first trimester \(^{28, 30}\) with the tendency to rise above normal in the late pregnancy \(^{30, 31}\). Higher osteoblastic activity, in physiological HP, could explain faster recovery of bone loss after pregnancy and lactation.

Increased PRL levels in sulpirid-treated rats were confirmed in our study. Therefore, the results of mineral analyses and bone turnover markers, conducted in these experimental groups, could be considered as representative for medicamentous HP. With sulpirid treatment prolongation slightly decreased PRL concentrations were verified. Data from the literature usually cover the issue of different sulpiride effects, according to the low or high dosages \(^{32}\). Lower concentration of dopamine antagonist (sulpiride) can block presynaptic dopamine (D) 2 receptors, leading to decreased dopamine synthesis and release. Lactotrophs are released of dopamine inhibition and hyperprolactinemia occurs. Higher sulpiride concentrations are needed to block postsynaptic D2 receptors \(^{33}\). There are no literature data showing different effect of sulpiride with longer treatment duration. A possible explanation for decreased PRL concentration with prolongation of sulpiride treatment could be up-regulation of D2 receptors in lactotrophs after longer blocking sulpiride effect or postreceptors downstream of cAMP/calcium signalling which is necessary for PRL release \(^{34}\).

Studies conducted to reveal a connection between medicamentous HP and skeletal system are often based on parameters of bone mineral density and biochemical turnover markers. There are less available data about HP influence on calcium and phosphorus levels. Our study results, showing no significant changes in serum ionized calcium and phosphorus, during a 3-week sulpirid-provoked HP, are consistent with limited previously reported data. Even though there are growing evidences that prolonged medicamentous HP can lead to decreased bone mineral density, there are still missing data about calcium and phosphorus changes during those conditions. Our study results revealed a significant calcium increase and phosphorus decrease during longer medicamentous HP. Hypercalcemia in prolonged medicamentous HP could be a result of increased calcium absorption in upper intestine (absorptive hypercalcaemia), increased net bone resorption (remodelling hypercalcaemia) or increased tubular calcium reabsorption (tubular reabsorptive hypercalcaemia). In last decade, many experimental studies confirmed very important and direct PRL role in regulating intestinal calcium absorption. All of these studies are based on physiological HP. There are no literature data showing that medicamentous HP also leads to increased intestinal calcium absorption. The findings of PRL receptor mRNA expression in osteosarcoma cell lines, cultured calvaria osteoblasts and in tibia, femur and vertebrae in normal adult rats suggested bones as possible direct targets of PRL. It is still uncertain whether hypercalcaemia in prolonged medicamentous HP could be considered as a consequence of direct PRL influence on bones.

Renal tubular dysfunction resulting in excess calcium loss, caused by sulpirid is not so far reported. A significant fall of urinary calcium excretion in prolonged medicamentous HP was not previously reported, to our knowledge, and could be a result of some still unknown mechanisms, switched on to prevent further calcium loss.

Different studies conducted in women with major depressive disorder, with or without borderline personality disorder, before psychotropic medication, or treated with antidepressant, found increased OC. Our study results, presenting lower OC, but still in normal referent range, are more consistent with data provided in schizophrenic patients with antipsychotic treatment.

There are no previously reported data, to our knowledge, about changes in P1NP in medicamentous HP. Our results, for the first time show statistical significant decrease of this osteoblastic marker in sulpirid-induced HP. Prolonged medicamentous HP leads to further fall of P1NP, reflecting poor osteoblastic activity.

Even though OC and P1NP are bone formation markers their serum levels reflect different aspects of osteoblastic activity. Osteocalcin is mostly produced during the mineralization phase, while procollagen peptides are mostly produced by proliferating osteoblasts.

Limitation of this study is a lack of biochemical markers of bone resorption. Comparison of bone resorption/formation rates could also be very important data about bone remodelling in physiological and medicamentous hyperprolactinemia.

Conclusion

We demonstrated herein that ionized calcium concentrations were significantly different in physiological and medicamentous hyperprolactinemia (decreased in late pregnancy and increased in sulpirid-induced hyperprolactinemia). Quite opposite influence of physiological and medicamentous hyperprolactinemia on bone formation marker procollagen type 1 N-terminal propeptide, revealed an increased osteoblastic activity in pregnancy and decreased bone formation in sulpirid-provoked hyperprolactinemia. These results provide a possible explanation why pregnancy does not determine such harmful effect on bone metabolism, while medicamentous hyperprolactinemia leads to decreased bone mineral density. The present experimental data of further procollagen type 1 N-terminal propeptide decrease in prolonged medicamentous hyperprolactinemia, provide information on dynamic, time-dependent and origin-dependent osteoregulatory roles of prolactin.

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