Effects of raloxifene on development of the methotrexate-induced changes in bone mechanical properties of male rats

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Abstract: Methotrexate, a cytostatic and immunosuppressive drug, has been reported to deteriorate the osseous system. Raloxifene, a selective estrogen receptor modulator, is used in the prevention and treatment of postmenopausal osteoporosis. There is a lack of data on possible ways of preventing the unwanted skeletal effects of prolonged immunosuppressive therapy. The aim of the present study was to investigate the effects of raloxifene on mechanical properties of the femur in male rats administered methotrexate. The experiments were carried out on mature male Wistar rats, which were divided into 6 groups: controls, rats administered raloxifene hydrochloride (5 mg/kg po), rats administered methotrexate (0.5 mg/kg po or im), and rats administered raloxifene hydrochloride (5 mg/kg po) plus methotrexate (0.5 mg/kg po or im). Raloxifene was administered for 28 days and methotrexate was administered for the first 10 days of the experiment. After 28 days of drug administration, mechanical parameters of the whole femur were determined: the extrinsic stiffness, the ultimate load and the breaking load, deformation caused by the ultimate and breaking loads, and the load causing fracture of the femoral neck. Additionally, the mass of isolated femurs and their mineral and calcium content were determined. Intragastrically or intramuscularly administered methotrexate impaired the endurance of the tested bones. Administration of raloxifene alone had no significant effects on the mechanical parameters of the femur. Administration of raloxifene resulted in a reduction of the adverse changes in the osseous system induced by methotrexate. Concluding, the experiments demonstrated a protective action of raloxifene against the effects of methotrexate on the osseous system in male rats.

Key words: femur, male rats, methotrexate, raloxifene

Introduction

The damaging effect of such drugs as glucocorticosteroids [4, 16], heparin [12] and anticonvulsants [11] on the osseous system has been well recognized. A significant loss of bone mass is also observed as a result of prolonged administration of cytostatic drugs [18, 20, 22, 23]. Cytostatics at lower doses are used as immunosuppressants. Administration of cytostatics at immunosuppressive doses may also lead to significant disorders in bone remodeling processes. Due to the fact that prolonged administration of immunosuppressive drugs in clinical practice is necessary, the development of methods aimed at minimizing their adverse effects becomes important.
There is a lack of data concerning the possible ways of preventing the effects of prolonged immunosuppressive drug therapy on the skeletal system. Methotrexate is an example of a cytostatic drug used as an immunosuppressant. Methotrexate, a purine antimetabolite, is a competitive inhibitor of dihydrofolate reductase. It is commonly used as a long-term small-dose therapy of psoriatic and rheumatoid arthritis. There are inconsistent reports on the effects of methotrexate at immunosuppressive doses on the bone loss [5, 8, 15, 17].

In the present study, we examined the effects of methotrexate on the skeletal system. Since in our previous study [6] we observed a profound effect of methotrexate on the rat skeletal system after intramuscular, but not oral administration (1 mg/kg for 5 days, repeated after a 10-day interval), we decided to administer the drug at 0.5 mg/kg daily both im and po.

Raloxifene, a selective estrogen receptor modulator (SERM), commonly administered in the osteoporosis to prevent osteoporotic fractures in postmenopausal women, was selected to prevent bone mass loss after methotrexate administration. A significant improvement of osseous tissue parameters in women after raloxifene administration suggested that this drug may be tested for treatment of osteoporosis in men [13, 14]. In fact, in our previous study, we demonstrated that in male rats raloxifene exerted very similar effects to those exerted in ovariectomized female rats [13]. As hardly any data are available in literature on the influence of raloxifene on male osseous system in conditions of experimental osteopenia, we decided to perform the tests on male rats.

Materials and Methods

The experiments were carried out on mature male Wistar rats (4-month-old), which were administered raloxifene, methotrexate or raloxifene and methotrexate. Raloxifene hydrochloride (Evista, Eli Lilly, GB) was administered intragastrically at 5 mg/kg for 28 days, methotrexate (Methotrexate, Lachema, CZ) was administered intragastrically (po) or intramuscularly (im) at 0.5 mg/kg for the first 10 days of the experiment.

The rats were divided into 6 groups (n = 7): 1) control rats, 2) rats administered raloxifene hydrochloride at 5 mg/kg po for 28 days, 3) rats administered methotrexate at 0.5 mg/kg po for the first 10 days of the experiment, 4) rats administered raloxifene hydrochloride at 5 mg/kg po for 28 days and methotrexate at 0.5 mg/kg po for the first 10 days of the experiment, 5) rats administered methotrexate at 0.5 mg/kg im for the first 10 days of the experiment, 6) rats administered raloxifene hydrochloride at 5 mg/kg po for 28 days and methotrexate at 0.5 mg/kg im for the first 10 days of the experiment.

After 28 days of the experiment, the rats were sacrificed and left and right femurs were isolated. The tests on mechanical properties of the isolated left femurs included the determination of the extrinsic stiffness [N/mm], the ultimate load (maximal load sustained by the femur) and the breaking load (the load at which the diaphysis was fractured) [N], as well as the determination of the deformation caused by the ultimate and breaking load [mm], using a three-point bending test. The load which resulted in the right femoral neck fracture was also determined, using a compression test. The set used for testing of bone mechanical properties was previously described [24].

Additionally, the mass of isolated left femurs was determined by weighing after prior removal of soft tissues, and the mineral content in the left femurs was determined by weighing 48 h after mineralization in a muffle furnace at 640°C. Calcium content was determined in the mineralized left femurs by means of a colorimetric method using Pointe-180 Plus biochemical analyzer.

The results are presented as the arithmetic means ± SEM. One-way ANOVA followed by post-hoc Duncan’s test was used for estimation of statistical significance. When one-way ANOVA could not be used because of the lack of homogeneity of variance, Kruskal-Wallis ANOVA, followed by Mann-Whitney U test was used.

The experiments on animals were carried out with approval of the Local Ethics Commission, Katowice.

Results

The results of the determination of the mass, mineral and calcium content in isolated femoral bones and body mass of the examined animals are presented in Table 1.
The body mass after administration of methotrexate po or ip did not differ statistically significantly from the control rats. Administration of raloxifene caused a decrease in body mass gain in comparison with the control rats. The rats which received methotrexate and raloxifene had body mass significantly decreased in comparison with the control rats and similarly to that of rats receiving raloxifene alone.

When compared to the controls, methotrexate administered either po or im resulted in a decrease in the femur mass (by 7.32% and 10.50%, respectively) and mineral content (statistically significantly, by 9.43% and 13.81%, respectively), while the calcium content remained unchanged. However, the femur mass was not different from that of the control group when determined per 100 g of body mass; similarly, the mineral content of the femurs was not different per 100 mg of bone mass. Rats which were administered raloxifene displayed an insignificant decrease in the femur mass (by 5.07% and 5.29%, respectively) in comparison with the results obtained for the respective group administered methotrexate only (po or im); however, the mass per 100 g of body mass was statistically significantly increased (by 9.35%) in the rats receiving methotrexate po and raloxifene, and significantly increased in the rats receiving methotrexate im and raloxifene (by 7.98%). A statistically significant increase was also observed in the mineral content per 100 mg of bone mass in the group administered methotrexate po and raloxifene (by 6.73%), in comparison with the group receiving methotrexate po.

The results of mechanical tests are presented in Table 2.

Administration of methotrexate po or im resulted in insignificantly decreased values of extrinsic stiffness (by 5.07% and 5.29%, respectively) in comparison with the control group. After po administration, methotrexate decreased the ultimate load (statistically significantly, by 13.65%) and the breaking load (by 9.62%). The deformation caused by the ultimate load was greater by 6.80%. Similar results were observed after im administration of methotrexate. Administration of methotrexate and raloxifene concurrently displayed no significant changes in the mass of femurs when compared to the results obtained for the respective group administered methotrexate only (po or im); however, the mass per 100 g of body mass was statistically significantly increased (by 9.35%) in the rats receiving methotrexate po and raloxifene, and insignificantly increased in the rats receiving methotrexate im and raloxifene (by 7.98%). A statistically significant increase was also observed in the mineral content per 100 mg of bone mass in the group administered methotrexate po and raloxifene (by 6.73%), in comparison with the group receiving methotrexate po.
metrotrexate po or im statistically significantly weakened also the strength of the femoral neck. The values causing fracture of the femoral neck were smaller by 8.03% and by 17.11%, respectively.

Administration of raloxifene did not significantly affect the mechanical properties of the femur in comparison with the control group.

Administration of raloxifene to the rats receiving metrotrexate po or im resulted in the increases of the values of the ultimate and breaking load in comparison with the rats receiving metrotrexate only (statistically insignificant). The administration of raloxifene in groups receiving metrotrexate resulted also in an increased value of the load necessary to fracture the femoral neck, by 7.60% and 11.54% (statistically significantly), respectively.

Discussion

The tests of the mechanical properties of bones in laboratory animals constitute an important source of information on the changes in the osseous system which may occur in prolonged immobility, long-term drug administration or action of specific nutritional, metabolic, hormonal and environmental factors. In the present study, mechanical properties of the whole femurs and the femoral neck in rats treated with metrotrexate were examined.

The results of the determinations indicated that intragastric or intramuscular administration of metrotrexate deteriorated the mechanical properties of the whole femur in the investigated rats (the ultimate and breaking load). The slight decrease in extrinsic stiffness was a parameter which was also indicative of deteriorated mechanical properties of the femurs after metrotrexate administration. The femoral neck strength was significantly weakened after metrotrexate administered in both ways.

The observed deterioration of mechanical properties of the femur probably reflects the structural changes induced by the drug administration. The mass of mineral substances in the femur was significantly decreased after administration of metrotrexate, and the mass of the femur tended to decrease. However, no changes were observed in the proportions of the femur mass to the body mass and the femur mineral content to the mass of the bone.

Literature data indicate that the administration of metrotrexate at higher doses in oncology is connected with the loss of bone mass, development of osteopenia and an increased susceptibility to fractures [18, 20, 22, 23]. The reports on the use of metrotrexate at lower doses, such as those used in immunsuppres-
sion, are more controversial. No negative effects of methotrexate administration at small doses on bone density, metabolic turnover indexes and histomorphometric bone formation parameters were demonstrated in humans [5, 8]. It was, however, demonstrated that in patients who were administered methotrexate with prednisolone, the bone loss was more substantial than in those treated with prednisolone alone [5]. On the other hand, the experimental tests carried out on animals showed significant osteopenia, which was connected with the decreased activity of osteoblasts and the intensified resorption processes, after prolonged (16 weeks, once a week) administration of methotrexate at 3 mg/kg [17]. In our previous study, methotrexate administered intramuscularly at higher doses (1 mg/kg or 5 mg/kg) with intervals, inhibited the formation and mineralization of bone matrix and impaired bone mechanical properties in male rats [6]. Other tests demonstrated a decrease in the rate of bone formation after methotrexate administration at 0.75 mg/kg for a short time (5 days), with no effect on the osteoblast number and surface, which, according to the authors, indicates that the mechanism of the methotrexate-induced osteopenia is related to the decreased osteoblast activity [15]. In the present study, methotrexate was used at a dose of 0.5 mg/kg daily (that is 3.5 mg/kg/week). However, after 9 days of administration, the rats in groups receiving methotrexate began to die, and the methotrexate administration was stopped after 10 days of the experiment. It seems that the main reason for the diagnosed deterioration of mechanical properties of the femurs is the inhibition of bone formation and mineralization by methotrexate, which may indirectly lead to the domination of osteoclastic bone resorption. This observation is in compliance with the above-mentioned results of Friedlaender et al. [15]. The dose of methotrexate administered in our experiment (0.5 mg/kg) was similar to that used by those authors, although the time of administration was twice as long (10 days).

Raloxifene displays a number of protective actions preventing the loss of osseous tissue and the decrease in bone endurance due to estrogen deficiency in females, which was acknowledged in numerous experimental studies on animals, and in clinical tests [7]. The use of raloxifene in osteoporosis in men is increasingly considered [13, 14].

Cellular mechanisms of bone mass loss in men are not well recognized. The mineral density of bones in men may depend on the level of endogenous estrogens which are created as the result of androgen transformation caused by aromatase [1]. A substantial increase in estradiol level in blood serum, observed after testosterone treatment in osteoporotic men with normal gonadal function, may be responsible for the observed reduction of bone resorption [2]. In addition, cases of intensified osteoporosis have been reported in men displaying aromatase deficiency and mutation of estrogen receptor leading to its inactivation [3, 19]. It is supposed that cells in bone remodeling units in men may be more susceptible to estrogens than in women [21]. We observed that raloxifene induced similar effects in the skeletal system of male rats to those observed in the ovariectomized female rats [13]. Thus, the use of selective estrogen receptor modulators, including raloxifene, which within the osseous and cardiovascular systems imitate the estrogen action, in the treatment of osteoporosis in men has its theoretical justification. The effects of raloxifene on bone strength have not been studied in men. We decided to determine the effects of raloxifene on the osseous system in healthy mature male rats under experimental conditions by assessing the mechanical properties of the femurs, and to establish its usefulness in preventing methotrexate-induced changes in the osseous system of rats.

The animal models demonstrated that raloxifene had a beneficial effect on the osseous tissue in ovariectomized rats when administered at 1–10 mg/kg for 4 months [10]. However, raloxifene administered at 3 mg/kg for 5 weeks also prevented the cancellous osteopenia [9]. In the present study, we used the raloxifene in male rats at a dose of 5 mg/kg daily for 4 weeks.

The results of the present study indicated that raloxifene alone had no influence on the examined bone parameters other than a statistically significant increase in the femur mass per 100 g of body mass, and an increase in the mineral content in the femur per 100 mg of the bone in male rats. The values for the femoral diaphysis deformation at the ultimate load and at the moment of fracture, as well as the ultimate load endured by the bone resulting in the femoral diaphysis or neck fracture were not different from the values obtained for the controls. Although raloxifene did not affect the bone mechanical parameters in control rats, its administration to animals which were concurrently administered methotrexate intragastrically or intramuscularly resulted in a reduction of adverse changes induced by methotrexate. Both the ultimate and breaking load values of
the whole femur, as well as the load resulting in the femoral neck fracture were increased.

The improvement in the mechanical properties of the femur after administration of raloxifene in the animals treated with methotrexate may result from the stimulation of bone mineralization by raloxifene, as the increased ratio of the femur mass to the body mass, and increases in the mineral content and the ratio of the bone mineral content to bone mass were observed.

In conclusion, our study demonstrated a protective action of raloxifene (5 mg/kg po) against the adverse effect of methotrexate on the osseous system in male rats. The results of the present study indicate the potential to use raloxifene in the treatment of skeletal disorders not only in female, but also in male subjects.

Acknowledgment:
This study was supported by grant No.2P05000326 from The Ministry of Science and Informatization, Poland.

References: