Pharmacological Reports
2012, 64, 386–395
ISSN 1734-1140

Effects of thalidomide on the development of bone damage caused by prednisolone in rats

Ilona Kaczmarczyk-Sedlak[1], Maria Zych[1], Katarzyna Rotko[2], Lech Sedlak[1]

[1]Department of Pharmacognosy and Phytochemistry, [2]Department of Pharmacology, Medical University of Silesia, Jagiellońska 4, PL 41-200 Sosnowiec, Poland

Correspondence: Ilona Kaczmarczyk-Sedlak, e-mail: farmafit@sum.edu.pl

Abstract:
Background: The methods used in treatment of osteoporosis induced by glucocorticosteroids are not effective enough. There is a need for new drugs which could be useful in counteracting the influence of glucocorticosteroids on osseous tissue.
The aim of the present study was to investigate the effects of thalidomide on the development of osteoporosis induced by glucocorticoid (prednisolone) in rats.
Methods: The experiments were carried out on 3-month-old male Wistar rats. The animals were divided into 4 groups: I – control rats; II – prednisolone (10 mg/kg *po*); III – prednisolone (10 mg/kg *po*) + thalidomide (15 mg/kg *po*); IV – prednisolone (10 mg/kg *po*) + thalidomide (60 mg/kg *po*). The drugs were administered for 3 weeks.
The body mass gain, bone mass in the tibia, femur and L-4 vertebra, histomorphometric parameters of the tibia (width of osteoid, diaphysis transverse growth, area of the transverse cross-sectional of the bone marrow cavity and the cortical bone) and the femur (width of trabeculae, width of epiphyseal cartilage, diaphysis transverse growth, area of the transverse cross-sectional of the bone marrow cavity and the cortical bone) were studied.
Results: Prednisolone induced osteoporotic skeletal changes in mature male rats (decreases in the bone mass, the width of the periosteal and endosteal osteoid, the transverse cross-sectional area of the cortical bone, the width of trabeculae, and the diaphysis transverse growth were observed).
Thalidomide administered at a dose of 15 mg/kg *po* inhibited the development of changes in macrometric and histomorphometric parameters induced by prednisolone in the skeletal system of rats.
Conclusion: The results may constitute indirect evidence for possible clinical trials conducted in order to define the possibility to apply thalidomide in treatment of bone diseases in humans.

Key words:
bone, glucocorticosteroids, osteoporosis, prednisolone, rat, thalidomide

Introduction

Prolonged high-dose glucocorticoid therapy is the most common cause of secondary osteoporosis, also called 'glucocorticoid-induced osteoporosis'. Glucocorticoids suppress bone formation, increase bone resorption and cause negative calcium balance and high risk of fractures [18, 21, 24, 26]. At the same time, glucocorticoids may cause suppression of gonad activity and inhibit the anabolic impact of sex hormones on the osseous tissue [37].

Similar disorders in the osseous tissue to the ones observed in patients with post-steroidal osteoporosis may be induced in rats as a consequence of administering prednisolone. The method is used in preclinical tri-

als and in experimental pharmacology in order to assess the influence of drugs on disorders in remodeling of osseous tissue induced with glucocorticoids [44].

Currently, in treatment of glucocorticoid-induced osteoporosis calcium and vitamin D supplementation, antiresorptive drugs (mainly bisphosphonates) and, recently, anabolic agents (teriparatid) are used [3, 24]. However, new possibilities of pharmacotherapy in osteoporosis induced by glucocorticoids are being searched for. The purpose of this work was to examine the influence of thalidomide on the processes taking place in osseous tissue in rats in which poststeroidal osteoporosis was induced as a consequence of administering prednisolone.

The basis for the suppositions that thalidomide will influence the osseous system in rats in the experimental model of osteoporosis induced by prednisolone are the results obtained earlier, which indicate the positive impact of the drug in the case of experimental osteoporosis induced by bilateral ovariectomy [15] and in rats with *osteomyelitis aseptica* [13].

Thalidomide and its analogues (lenalidomide, pomalidomide) belong to the group of immunomodulatory drugs (IMiDs) having anti-tumor, anti-inflammatory, anti-angiogenic properties in multiple myeloma. The studies within the last years also indicate the positive effect of IMiDs in osteolitic changes in bones of patients suffering from multiple myeloma. In the treatment of multiple myeloma IMiDs are used together with glucocorticoids [36, 43, 45].

Materials and Methods

The experiments were carried out on 3-month-old male Wistar rats, fed a standard diet *ad libitum*. Permission for the experiments on animals was granted by the Local Ethics Commitee, Katowice, Poland.

The animals were divided into 4 groups (n = 7).

I. Control group;
II. Prednisolone 10 mg/kg *po* daily;
III. Prednisolone 10 mg/kg *po* daily + thalidomide 15 mg/kg *po* daily;
IV. Prednisolone 10 mg/kg *po* daily + thalidomide 60 mg/kg *po* daily.

Prednisolone and thalidomide were administered to the rats once a day for 3 weeks. On the first and the last day of the experiment, the animals were given tetracycline hydrochloride (20 mg/kg *ip*) in order to mark the calcification front [11].

After 3 weeks of the experiment, the animals were sacrificed. The right and left femoral and tibial bones and L-4 vertebra were prepared. In the isolated bones, the mass and the macrometric parameters were determined (length, diameter of the diaphysis at the midlength and diameter of the epiphysis) with the use of electronic caliper.

The femoral and tibial bones were used to prepare histological specimens. The histological specimens were prepared and measured as described above [10]. From the tibial bone, transverse cross-sections were made, perpendicularly to the long axis, starting from the point where fibula grows into it. Three tibial slices were obtained by cutting. From the femoral bone, a longitudinal section of the distal epiphysis was made, in the medial part and plane. The sections were ground on the tarnished glass. The first preparation from the tibia was remained unstained. The rest of the preparations (2^{nd} and 3^{rd} tibial cross-section slices together with the longitudinal section slice of the femoral distal epiphysis) were stained.

In the histological specimens, the following histomorphometric parameters were assessed: the width of the osteoid of the tibial bone on the side of the *periosteum* and on the side of the marrow cavity (periosteal and endosteal osteoid), the width of the trabeculae in the distal femoral epiphysis and metaphysis, and the width of epiphyseal cartilage in the distal femoral epiphysis. Histomorphometric measurements of bones comprised also the assessment of the area of the transverse cross-sectional of the cortical part of the diaphysis and the area of the transverse cross-sectional of the marrow cavity of the femoral and tibial bone applying by lanameter (magnification: 50×). In the histomorphometric measurement, the tetracycline method was applied in order to assess the diaphysis transverse growth of the tibia on the side of the *periosteum* and on the side of the marrow cavity (periosteal and endosteal bone growth) [11].

The histomorphometrical measurements were carried out with the use of an Optiphot 2 microscope connected with an RGB camera and a personal computer (software: Lucia G 4.51, Laboratory Imaging), with final magnifications of 200× and 500×.

The results are presented in tables as the arithmetic mean values (± SEM). The results obtained in the prednisolone group (group II) were compared to those of the control rats (group I) with the use of Student's