Regulation of bone metabolism in immunosuppressant (FK506)-treated rats

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Abstract After organ transplantation, severe osteoporosis is occasionally seen, and the use of immunosuppressants is thought to be one of the causes of such osteoporosis. In the present study, we investigated the effects of FK506 monotherapy on bones and determined the mechanism of onset of osteoporosis, both by assessing chronological changes in bone metabolism and by identifying factors that facilitate bone resorption. In 8-week-old male Sprague-Dawley rats, FK506 (1 mg/kg) was injected intraperitoneally every day for 5 weeks (FK506-treated group), and for comparison, physiological saline was administered in the same manner in a control group of rats. Serum and urine samples were collected at weeks 0, 1, 3, and 5 of administration. The femur and tibia were collected within 24 h of the final administration. When compared to the control group, findings on three-dimensional micro-computed tomography of the femur for the FK506-treated group showed a significant decrease in trabecular bone volume. The level of serum osteocalcin in the FK506-treated group at week 1 of administration was significantly higher than the control. Throughout the administration period, the sum of urinary pyridinoline (PYD) and deoxypyridinoline (Dpd) was significantly higher in the FK506-treated group. Of the various bone resorption factors tested, the level of serum parathyroid hormone (PTH) in the FK506-treated group was significantly higher than the control at week 3 of administration. The results of the present study confirmed that FK506 monotherapy in rats induced high-turnover osteoporosis. Soon after the start of FK506 administration, bone formation and resorption were elevated, and PTH appeared to have been involved in the maintenance of the elevated bone resorption.

Key words osteoporosis · bone metabolism marker · immunosuppressant

Introduction

Recent advances in organ transplantation have been marked, and are mostly attributable to advances in transplantation techniques and immunosuppressants. To prevent organ rejection, lifelong use of immunosuppressants is essential for patients who undergo organ transplantation, and only a small proportion of these patients are weaned off immunosuppressants after a long period of time [1]. Cyclosporin A and FK506 are two common immunosuppressants that are clinically used today. The results of past studies have shown that the immunosuppressive effects of these agents are based on the inhibition of T-cell differentiation and proliferation. These agents bind to immunophilin (cyclophilin A and FK506-binding protein [FKBP]), and the resulting complex suppresses the activity of calcineurin, which is a calmodulin-dependent protein phosphatase. Consequently, the production of cytokines, including interleukin-2 (IL-2), by sensitized T cells, and the subsequent T-cell activation, is suppressed [2,3]. However, cyclosporin A and FK506 not only suppress the immune system but also induce many adverse reactions, including nephropathy [4–6], and this can lead to the onset of osteoporosis following organ transplantation [7,8].

Bones are constantly being renewed through osteoblast-induced bone formation and osteoclast-induced bone resorption. Osteoporosis is characterized by an increase in bone resorption and a decrease in bone formation. Following the transplantation of an organ such as the liver, heart, or kidney, patients occasionally develop severe osteoporosis. This adverse reaction is recognized as a clinically important problem, and there have been reports on onset factors, risk factors, and prevention [9–12]. However, because various factors are involved in the onset of osteoporosis following organ transplantation, no general consensus has been reached. Numerous studies in rats have been conducted...
to investigate the effects of cyclosporin A or FK506 monotherapy on bones, and it has been clarified that cyclosporin A or FK506 monotherapy causes osteoporosis [13–17]. The results of studies on bone morphology, blood biochemistry, and histology have shown that the osteoporosis associated with continuous immunosuppressant administration is attributable to increased bone resorption caused by an increase in the number of osteoclasts. We also reported that when FK506 was continuously administered to rats for a sufficient length of time, osteoporosis could be induced. Furthermore, we assessed bone morphology using soft X-rays and micro-three-dimensional (3D) computed tomography (CT), and revealed that, histologically, osteoporosis was attributable to an increase in the number of osteoclasts [18]. From the viewpoint of bone formation, levels of serum osteocalcin have been measured, but the focus of osteoporosis studies has largely been on bone resorption, and there has not been a study of the cause of the osteoporosis associated with FK506 administration based on bone formation and resorption. Also, none of the studies have followed rats with FK506-induced osteoporosis for periods as long as 5 weeks and analyzed the general kinetics.

In recent years, the usefulness of bone metabolism markers in assessing the activities of osteoblasts and osteoclasts has been reported, and these markers have often been used to assess the disease state of osteoporosis, select drugs, and evaluate therapy efficacy. With bone metabolism markers, both bone formation and resorption can be assessed, and, because the measurement of these bone metabolism markers is a noninvasive procedure, their levels can be measured frequently in order to determine chronological changes. It is clinically useful to ascertain the chronological changes in osteoblast and osteoclast activities when we wish to identify the mechanism of onset of osteoporosis associated with organ transplantation. In the present study, in an attempt to identify both the cause of osteoporosis following organ transplantation and a means to prevent it, we conducted an experimental study by continuously administering FK506 to rats. In the 5-week administration period, we were able to confirm the onset of osteoporosis, clarify the bone dynamics, in terms of bone formation and resorption, using bone metabolism markers, and identify the cause of osteoporosis by analyzing various data.

Materials and methods

Animal procedures

Twelve 8-week-old male Sprague-Dawley rats (Japan SLC, Shizuoka, Japan), each weighing approximately 267 ± 6.6 g, were used in this study. All rats were housed under similar conditions, at 21°C, under a 12-h light/12-h dark cycle and maintained on a diet of CRF-1 (Oriental Yeast, Tokyo, Japan), containing 1.27% calcium, 0.84% phosphorus, and 0.25% magnesium, and tap water ad libitum.

Drugs

Prograf (Fujisawa Pharmaceutical, Osaka, Japan), a solution containing 5 mg FK506/ml, was diluted in physiological saline to obtain a final concentration of 1 mg/ml.

Experimental protocols

After acclimatization for a week, rats were randomly divided into two groups and were intraperitoneally injected with either FK506, at a dose of 1 mg/kg per day (FK506-treated group; n = 7) or physiological saline (control group; n = 5) for the duration of the 5-week experiment. All rats were weighed at weekly intervals and bled at weeks 0, 1, 3, and 5, under diethyl ether anesthesia (Wako Pure Chemical Industries, Osaka, Japan). Blood was collected by subclavian venous puncture, except in week 5, when samples were obtained from the abdominal aorta at the time the animals were killed. Blood samples were centrifuged and the serum stored at −80°C until assayed. Urine was collected in metabolic cages, during an 18-h period, overnight in weeks 0, 1, 3, and 5 after the start of the experiment. Urine samples were centrifuged and stored at −80°C until assayed. While they were under diethyl ether anesthesia, all rats were killed in week 5, 24 h after the last administration of either FK506 or physiological saline. Femurs and tibiae were removed from each rat and were used for analysis.

Analysis of bone weights and evaluation of femurs by 3D-micro CT

The left femurs and tibiae were used to determine dry (dehydrated) weights after being dried in a convection oven at 60°C for 10 days. The right femurs were used for 3D reconstruction by means of micro-CT (µCT 20; Hitachi Medical, Tokyo, Japan). Each specimen was held in a cylindrical sample holder and then 200 slices were analyzed within the area determined through a pre-scout view. Each acquired image was reconstructed to produce a 3D image with an extended marching cube algorithm for 3D observation on display. The trabecular bone volume fraction (bone volume [BV] / tissue volume [TV] %) and trabecular separation (TbS) were calculated with an MCT system software package (Hitachi Medical, Tokyo, Japan).