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Original article

Additive effect of zoledronic acid and alfacalcidol in the treatment of disuse osteoporosis in rats

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ABSTRACT

Objectives: Disuse by bed rest, limb immobilization or space flight causes rapid bone loss. We conducted the present study to investigate the therapeutic effects of zoledronic acid (ZOL), alone and in combination with alfacalcidol (ALF) in a rat model of disuse osteoporosis.
Methods: In the present study, 3-month-old male Wistar rats had their right hind-limb immobilized (RHLI) for 10 weeks to induce osteopenia, then were divided into four groups: 1 – RHLI positive control; 2 – RHLI plus ZOL (50 μg/kg, i.v. single dose); 3 – RHLI plus ALF (0.5 μg/kg, oral gauge daily); 4 – RHLI plus ALF (0.5 μg/kg, oral gauge daily) plus ZOL (50 μg/kg, i.v. single dose) for another 10 weeks. One group of non-immobilized rats was used as negative control. At the end of the treatment, the femurs were removed and tested for bone porosity, bone mechanical properties, and bone dry and ash weight.
Results: Combination therapy with ZOL plus ALF was more effective in decreasing bone porosity than each drug administered as monotherapy in RHLI rats. With respect to improvement in the mechanical strength of the femoral mid-shaft, the combination treatment of ZOL plus ALF was more effective than each drug administered as a monotherapy. Moreover, combination therapy using ZOL plus ALF was more effective in improving dry bone and ash weight, than single-drug therapy using ZOL or ALF in RHLI rats.
Conclusions: These data suggest that combination therapy with ZOL plus ALF represents a potentially useful therapeutic option for the treatment of disuse osteoporosis.

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Efeito combinado do ácido zoledrônico e do alfacalcidol no tratamento da osteoporose por desuso em ratos

Palavras-chave:
Osteoporose por desuso
Estudo com ratos
Ácido zoledrônico
Alfacalcidol

RESUMO

Objetivos: O desuso pelo repouso no leito, pela imobilização de membros ou por missões espaciais provoca a perda óssea rápida. Fez-se este estudo para investigar os efeitos terapêuticos do ácido zoledrônico (ZOL), isoladamente e em combinação ao alfacalcidol (ALF), em um modelo de rato com osteoporose por desuso.

Métodos: Ratos Wistar machos de três meses foram submetidos à imobilização da pata traseira direita (IPTD) por 10 semanas para induzir a osteopenia; em seguida, foram divididos em quatro grupos: 1 – IPTD para controle positivo; 2 – IPTD mais ZOL (50 μg/kg, dose única intravenosa); 3 – IPTD mais ALF (0,5 μg/kg, via oral diariamente); 4 – IPTD mais ALF (0,5 μg/kg, via oral diariamente) mais ZOL (50 μg/kg, dose única intravenosa) por outras 10 semanas. Um grupo de ratos não imobilizados foi usado como controle negativo. No fim do tratamento, os fêmures foram removidos e testaram-se a porosidade do osso e suas propriedades mecânicas, além do peso seco e das cinzas do osso.

Resultados: A terapia combinada com ZOL mais ALF foi mais eficaz em reduzir a porosidade do osso do que a monoterapia com um dos fármacos administrado isoladamente em ratos submetidos à IPTD. No que diz respeito à melhoria da resistência mecânica da diáfise femoral média, o tratamento combinado com ZOL mais ALF foi mais eficaz do que a monoterapia com um dos fármacos administrado isoladamente. Além disso, a terapia combinada com ZOL mais ALF foi mais eficaz na melhoria do peso seco e das cinzas do osso do que a monoterapia com ZOL ou ALF em ratos submetidos à IPTD.

Conclusões: Esses dados sugerem que a terapia combinada com ZOL mais ALF representa uma opção terapêutica potencialmente útil para o tratamento da osteoporose por desuso.

Introduction

Mechanical loading is essential for the normal functioning of bone tissue.[1] Maintenance of skeletal integrity, bone mass and bone formation in weight-bearing limbs are dependent on gravity.[2] Skeletal unloading induced by prolonged cast or splint fixation, stress protection secondary to plate fixation of fractures, incapacitation due to chronic illness or spinal cord injury, or weightlessness associated with orbital space flight causes a decrease in bone mass in both human and animal models.[2,3] Immobilization (disuse) osteoporosis causes net bone loss as a result of an imbalance between bone resorption and bone formation.[3] Hence, we have to keep in mind that bone loss due to prolonged immobilization increases the susceptibility to fractures in patients with spinal cord injuries, elderly requiring bed rest and astronauts during long space missions. Therefore, it is very essential to select optimal treatment for effective management of disuse osteoporosis.

Bisphosphonates inhibit bone resorption as they are selectively incorporated into osteoclasts and interfere with the resorptive action of osteoclasts.[4] Zoledronic acid (ZOL) is a third generation nitrogen containing bisphosphonate, and is widely used for postmenopausal and glucocorticoid-induced osteoporosis in humans.[5–8] The effect of a single treatment of ZOL for immobilization-induced osteoporosis has been shown in animal studies.[9,10] Although anti-resorptive agents such as bisphosphonates are effective in reducing bone loss, they are not able to induce formation of a new bone.[7,10]

Alfacalcidol (1 alpha-hydroxy Vitamin D_3 – ALF) is a synthetic Vitamin D analog, a calcium regulating hormone, and is frequently used in several countries to treat osteoporosis.[9,11] ALF reduces parathyroid hormone levels, as a result of both increased calcium absorption and an inhibition of the proliferation of the parathyroid gland, and also decreases the release of pro-inflammatory cytokines, which contribute to osteoclast activation. Moreover, ALF stimulates the formation and action of osteoblasts, leading to increased bone formation.[12–15] It has been demonstrated previously that the administration of ALF diminished the effect of immobilization in the development of osteoporosis.[9,16]

ZOL and ALF are commercially available in India. ZOL is known to inhibit osteoclast-mediated bone resorption,[9,12,17] while ALF exerts both anabolic and anti-resorptive effects on the skeleton.[13,18,19] A combination of two different drugs is believed to be a more effective treatment than a single treatment for osteoporosis; the combination of bisphosphonate and a bone anabolic drug has been used clinically for postmenopausal osteoporosis.[20,21] As immobilization-induced bone loss involves both increased bone resorption and decreased bone formation,[3] it seems obvious to target the immobilization-induced bone loss with a combined anti-resorptive and bone anabolic treatment regimen, such as ZOL and ALF. The effects of a combined ZOL and ALF treatment have previously been studied in ovariectomized rats,[17] whereas this treatment regimen has not previously been investigated in rat model of disuse osteoporosis. Consequently, the aim of the present study was to investigate the efficacy of a bone anabolic agent ALF, a bone anti-resorptive