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焦磷酸鈣添加焦磷酸鈉於治療骨質疏鬆症之研究

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焦磷酸鈣添加焦磷酸鈉於治療骨質疏鬆症之研究

A Study of Bioactive Ceramic $Ca_2P_2O_7$ with $Na_4P_2O_7{\cdot}10H_2O$ Addition on Treatment of Osteoporosis

計畫編號：NSC 91-2320-B-002 -142

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主持人：台大醫學院骨科　孫瑞昇醫師

一、中文摘要

骨質疏鬆症的臨床症狀是身體骨組織質量的減少和顯微結構的破壞而導致骨折危險性的增加。停經後由於抑鈣素和動情激素分泌的減少是造成骨質疏鬆症的主要危險因子，導致骨質流失大於形成速率。治療停經後骨質疏鬆的新藥-福善美(FOSAMAX®)氨基雙磷酸鹽類藥物，在近年來扮演的角色越顯重要。本研究中我們將評估福善美與 SDCP 焦磷酸鹽對骨質疏鬆的效果，我們也試著去闡明其作用機制。

研究中以福善美與 SDCP 焦磷酸鹽於 1ml 的去離子水中為懸浮態的口服藥物，探討兩個月大的 W.S.母老鼠，經由人工雙側卵巢切除或是假開刀，切除卵巢的母鼠一個月後產生骨質疏鬆，此時分為六組開始口服給藥，第一組給予福善美每天 1.0 毫克；第二組、第三組、第四組分別給予 SDCP 每天 1.0 毫克、0.5 毫克、0.25 毫克；第五組給予生理食鹽水為安慰劑；第六組為不治療的控制組。動物分開飼養於溫、溼度、給水、食物、藥物控制的環境下在 1、2 個月後犧牲。以組織學觀察評估骨骺端骨小樑數目與結構。取右側肱骨、尺骨、橈骨、股骨、脛骨作骨灰燼的骨密度評估。動物犧牲時以心臟穿刺抽血，血液經離心後，利用血清生化分析法做血清鈣、磷離子、鹼性磷酸腖(ALP)、副甲狀腺素(PTH)、麩胺醯基轉胜酶或轉移酶(GOT、GPT)、澱粉酶(AMY)、肌酸酐(CRE)的濃度作為評估骨重塑速率的參考。

口服福善美與SDCP焦磷酸鹽一個月後並不會造成血清生化上之變化。人工雙側卵巢切除後，海綿骨之骨小樑明顯變薄且失去其連貫性。而口服福善美與SDCP焦磷酸鹽後，海綿骨之骨小樑明顯變後且其失去其連貫性之骨小樑有明顯恢復之現象。

經由此實驗，SDCP 焦磷酸鹽對骨質疏鬆症之正向關係可以得到釐清。而 SDCP 焦磷酸鹽是否具有骨質疏鬆症臨床使用的潛力也將進一步予以確定。

關鍵詞：骨質疏鬆、福善美、**SDCP** 焦磷酸鹽、血清、組織學觀察。

Abstract

Osteoporosis is a disease with clinical symptoms of loss of bone mass and deterioration of microarchitecture on body skeleton, which results in an increased risk of fractures. The loss of calcitonin and estrogen production in menopause is the major

risk factor for osteoporosis, causing increased skeletal resorption and relatively decreased bone formation. Bisphosphonates have recently gained an increasing role in the management of osteoporosis. The aminobisphosphonate, Fosamax® (alendronate sodium, MSD) has recently been introduced as a new agent for the treatment of postmenopausal osteoporosis. The purpose of this study is to evaluate the effect of Fosamax® and SDCP on osteoporosis. We also try to elucidate the mechanisms of Fosamax® and SDCP on osteoporosis.

In the study, Fosamax® and SDCP were dissolved in 1ml distilled water as suspension for oral administration. Two-month-old sham-operated and OVX female W.S. rats were used as experimental animals. After one mouth of OVX, the animals were started to feed with Fosamax®, SDCP and placebo. The test animals were divided into 6 groups. The animals of the 1st group were treated by Fosamax® via oral administration with a daily dose of 1.0mg. The animals for the 2nd, 3rd, and 4th group were orally administrated by SDCP with a daily dose of 1.0mg, 0.5mg, and 0.25mg, respectively. The normal saline was used as placebo for the animal of 5th group. The 6th group was controlled group without any treatment. All the animals were caged separately with air condition, auto-washing, and humidity control. After administrated all kinds of drug for a period of time, the animals were scarified. The trabecula number and structure around the epiphyseal area were examined by histological observation. The righ humerus, ulna, radius, femur, and tibia were harvested to evaluate BMD by the method of bone ash. Serum alkaline phosphonatase (ALP) and parathyroid hormone (PTH) assay were measured to evaluate the bone turnover rate.

After ingestion of either alendronate or sintered dicalcium pyrophosphate for one month, most of the biochemical parameters did not show any significant change. After ovariectomy, there is significant thinning and disconnection of trabeculae in the lumbar vertebrae when compared with the shamed operated normal control. After ingestion of the alendronate and/or sintered dicalcium pyrophosphate, the trabeculation of lumbar vertebrae showed thickening of trabeculae with restoration of interconnection.

Thus, sintered dicalcium pyrophosphate can increase bone mass in the ovariectomized rat. The possible clinical application of SDCP in the osteoporosis will be established.

Keywords: **osteoporosis, Fosamax®, SDCP, histological observation**

INTRODUCTION

Osteoporosis is a major health-care problem of ageing communities. Postmenopausal osteoporosis is a common disorder characterized by an increase in bone resorption relative to bone formation, generally in conjunction with an increased rate of bone

turnover. The progressive decrease in bone mass leads to an increased susceptibility to fractures, which result in substantial morbidity and mortality. The ultimate goal of pharmacologic treatment in osteoporosis is to reduce the risk of fractures by increasing bone mass of normal quality. Sintered dicalcium pyrophosphate is a synthetic compound that has been proved to be quite biocompatible to bone tissue in the animal model (Lin et al. 1995). Later, sintered dicalcium pyrophosphate was shown to be more biocompatible than hydroxyapatite (Sun et al. 1997). As a pyrophosphate analogs, sintered dicalcium pyrophosphate should be useful in clinical setting characterized by abnormal bone resorption such as osteoporosis. In this study, we use ovariectomized rat model is to evaluate the effects of sintered dicalcium pyrophosphate on the prevention of osteoporosis.

MATERIALS AND METHODS

Thirty-six female rats weighing between 250-350 g were used in this study. They were randomly divided into six groups. In Group A, the negative normal control group, sham operation without ovariectomy was performed. The Group B was positive osteoporotic control group. The rats in Groups B to F received bilateral ovariectomy via bilateral retroperitoneal approaches. In the Groups A and B, starch were given; while in the group C, Alendronate sodium 10 mg/day was given. In the Groups D to F, sintered dicalcium pyrophosphate (Sun et al. 1997-b) 2.5 mg, 5 mg, and 10 mg/day were given, separately. The animals were sacrificed at 4 weeks after treatment. For all rats, whole blood samples were obtained for the biochemical study of bone metabolism including alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase (GOT/GPT), amylase, creatinine, calcium, inorganic phosphorus content and parathyroid hormone. Long bone of four limbs and lumbar spine were harvested immediately. The long bones of four limbs were trimmed of soft tissue, the weight of bone ashes of each long bone was measured for further analysis. The histomorphologic study of cancellous bone was made at the lumbar vertebrae.

RESULTS

After ingestion of either alendronate or sintered dicalcium pyrophosphate for one month, most of the biochemical parameters in serum including aspartate aminotransferase and alanine aminotransferase (GOT/GPT), amylase, creatinine, calcium, inorganic phosphorus did not show any significant change. After ovariectomy, the alkaline phosphatase titer in the serum increased from 167.5 U/L in the normal rats to 355.0 U/L in the ovariectomized rats; ingestion of alendronate did not affect the increase in alkaline phosphatase titer by ovariectomy, but the ingestion of sintered pyrophosphate did decrease the alkaline phosphatase titer in the ovariectomized rats. After ovariectomy, the parathyroid hormone in the serum did not showed any statistical change when compared to that of the normal rats; ingestion of

alendronate or sintered pyrophosphate increased the parathyroid hormone titer in the ovariectomized rats. After ovariectomy, there is significant thinning and disconnection of trabeculae in the lumbar vertebrae when compared with the shamed operated normal control. After ingestion of the alendronate and/or sintered dicalcium pyrophosphate, the trabeculation of lumbar vertebrae showed thickening of trabeculae with restoration of interconnection. When compared with the ovariectomized rats, ingestion of 10 mg/day alendronate increased the bone ashes, 1.44%, 7.84%, 1.38% and 0.01% in the bone ashes of arm, forearm, femur and tibia separately; while ingestion of 10 mg/day sintered dicalcium pyrophosphate increased the bone ashes, 6.65%, 13.1%, 10.36% and 6.21% in the bone ashes of arm, forearm, femur and tibia separately.

DISCUSSION

The ingestion of either alendronate or sintered dicalcium pyrophosphate did not have any deleterious effect on the liver, kidney and pancrease, since there is no significant changes in the biochemical parameters such as aspartate aminotransferase and alanine aminotransferase, amylase, creatinine, calcium, and inorganic phosphorus. The alendronate is a potent inhibitor of bone resorption. To maintain the calcium and phosphorus homeostasis, the ovariectomized rats must secret more parathyroid hormone to achieve this goal. This is the reason why the ingestion of alendronate or sintered pyrophosphate did significantly increase the parathyroid hormone titer in the ovariectomized rats. After ovariectomy, the ingestion of alendronate increased the bone mineral contents in the long bones; while the effects were even better after ingestion of sintered dicalcium pyrophosphate. Like bisphosphonates, the fine mechanism or mechanisms by which sintered dicalcium pyrophosphates act on bone resorption are not known; however, they seem to be due either to direct inhibition of osteoclasts, cells that may be derived from granulocyte-macrophage colony forming units, or to an indirect effect on secretion of soluble osteoclast-activating factors by osteoblasts. However, this hypothesis needs to be evaluated in the further study.

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