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Original Research Article

Effect of co-administration of alendronate and allan sodium phosphate for the management of osteoporosis

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Abstract

Purpose: *To study the therapeutic effect of combination of alendronate and allan sodium phosphate on osteoporosis.*
Methods: *Patients diagnosed with osteoporosis (100) were randomly assigned to control and study groups (50 subjects/group). The control group received conventional calcium treatment (calcium carbonate D3) after surgery. They consisted of 21 males and 29 females aged 62 to 78 years. The observation group consisted of 22 males and 28 females aged 63 to 77 years (mean age = 68.90 ± 6.43 years), and were treated with sodium alendronate and allan sodium phosphate. Bone mineral density (BMD), bone pain score, clinical effectiveness and levels of calcium, phosphorus, osteocalcin, collagen N-terminal peptide (NTX) and urinary calcium/creatinine were determined using ELISA kits.*
Results: *After 1 year of treatment, lumbar vertebral and femoral neck BMD significantly increased in both groups. The study group had significantly higher BMD than the controls ($p < 0.05$). Blood calcium levels increased significantly after 1 year in both groups. The treatment led to a higher level of osteocalcin in the study group than in the control group ($p < 0.05$). However, the treatment brought about significantly lower NTX level in the study group than in controls.*
Conclusion: *The results indicate that combined administration of sodium alendronate and allan sodium phosphate is effective for the treatment of osteoporosis.*

Keywords: *Osteoporosis, Allan sodium phosphate, Alendronate, Apoptosis-related factors, Calcium*

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INTRODUCTION

In China, the incidence of osteoporosis is on the increase probably due to aging population, and the disease is ranked fourth in the country [1]. Osteoporosis is a degenerative disease of the bone caused by destruction of the microstructure of the bone and increase in bone fragility [2]. The human skeleton is in a continual process of metabolic renewal. The cause of osteoporosis is an imbalance in skeletal metabolism which leads to distortion of the homeostasis between skeletal growth and bone resorption [3].

The osteoblasts in bone tissue are involved in bone formation, while osteoclasts break down bone tissue to liberate calcium. Thus, osteoporosis results from reduced osteoblast activity and increased osteoclast activity [4].

Cytochrome C (Cyt C), Apaf-1 and caspase-9 are markers of apoptosis in bone tissue, which can reflect the degree of destruction of bone cells and the severity of osteoporosis, and can also be used as prognostic reference [5]. Alendronate is a side-chain nitrogenous bisphosphonate often employed for treating osteoporosis. Its pharmacological action is based on the inhibition of the function of osteoclasts and promotion of their apoptosis. It has been reported that allan phosphate promotes the proliferation and transformation of osteoblasts [6]. This study was aimed at investigating the clinical effect of combination of alendronate and allan sodium phosphate in the treatment of osteoporosis.

EXPERIMENTAL

Materials and chemicals

Calcium carbonate D3 tablets (H10950029) were obtained from Wyeth Pharmaceutical Co., Ltd, China; alendronate sodium (J20130085) was product of Merck Sharp and Dohme, Italy. Dual-energy X-ray absorptiometer was purchased from Lunar, USA. Biochemical analyzer was purchased from Beckman, USA. Urinary calcium analyzer was product of BIORAD, USA, while osteocalcin and NTX ELISA kits were products of Beijing Yinghua Company, China.

This study received approval from the Ethical Committee of our Hospital (approval no. 20189708), and was carried out in line with Helsinki Declaration of 1964 as amended in 1996 [7].

General information on patients

A total of 100 patients diagnosed with osteoporosis were recruited over a 2-year period for this study. The patients were assigned to two groups: control and study (50 patients/ group). The control group received conventional calcium treatment after surgery. They consisted of 21 males and 29 females aged 62 to 78 years (mean age = 68.51 ± 6.62 years). The study group received sodium alendronate and allan soduim phosphate. The study group comprised 22 men and 28 women (mean age = 68.90 ± 6.43 years).

Inclusion and exclusion criteria

The included patients were: (1) patients whose conditions conformed with the "Chinese osteoporotic fracture diagnosis and treatment guidelines and "primary osteoporosis diagnosis and treatment guidelines"; (2) patients aged 60 years and above; (3) patients whose lumbar spine BMD as measured using dual energy X-ray absorptiometry had *t*-score < - 2.5; (4) patients with no history of fracture; (5) subjects who were not on glucocorticoids, sex hormones, anti-osteoporotic drugs and other bone metabolism drugs six months before commencement of the study; (6) patients who had no heart, lung, liver, kidney, blood system, and endocrine diseases; and (7) patients who signed written informed consent with their family members.

The excluded patients were: (1) those who had tumors, endocrine diseases, thyroid disease, adrenal disease, diabetes and other diseases of bone metabolism; (2) patients with congenital skeletal hereditary diseases; (3) patients who were on drugs that affect bone metabolism six months before commencement of the study. (4) Patients with mental illness and senile dementia; and (5) patients who did not sign written informed consent with their family members.

Treatment regimen

For the control group, a tablet of calcium carbonate D3 was given to each subject twice a day. The study group received alendronate sodium and allan sodium phosphate once a week (70 mg each time) 2 weeks after the operation of metal fixation fracture. The two groups of patients received treatment for 1 year.

Therapeutic indices

The bone mineral density (BMD) of the lumbar vertebrae (L2 to L4) and femoral neck bone were determined 1 year before commencement of treatment, and 1 year after completion of treatment using DPX-1 dual-energy x-ray absorptiometer. The measurement was done in triplicate and the mean value calculated. Bone pain score was based on the patient's self-described degree of pain which was calibrated using Changhai pain rating scale according to Zhang's report [8]. Clinical effectiveness was derived from bone pain score and BMD improvement before and after treatment as indicated in Eqs 1 and 2, where *IR* is improvement range; *Pt* is pre-treatment; *At* is after treatment; *Bt* is before treatment; *TE* is total effectiveness; *Se* is significant effect; *E* is effective, and *Tn* is total number of cases.

$$IR = \{(Pt - At)/Bt\}100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$TE = \{(Se + E)/Tn\}100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

The criteria for assessing effectiveness are shown in Table 1.

Table 1: Criteria for evaluation of effectiveness

Grade	Standard required
Significant effect	Increased BMD which improved by more than 10 % or bone pain improvement by more than 50 %
Effective	Increased BMD which improved by 5 - 10 % or bone pain improvement by 30 - 50 %
Ineffective	Increased BMD which improved by less than 5 % or bone pain improvement by less than 30 %

Determination of bone metabolism indices

Venous peripheral blood (5 ml) was taken from patients in both groups and used for the determination of the serum levels of calcium, phosphorus and creatinine using biochemical analyzer. Serum osteocalcin (BGP) and collagen N-terminal peptide (NTX) were measured using ELISA kits, while the level of urinary calcium ion was determined in the urine of patients collected at two time points.

Determination of levels of apoptotic factors

A year after surgery, bone samples of patients were collected from their metal fixtures, cut into bits, washed with sterile saline, and filled into a cryotube which were frozen in liquid nitrogen at -80 ℃. The bone tissue under liquid nitrogen was ground, and the protein content extracted for determination of apoptotic factors (Cyt C, Apaf-1 and caspase-9) using Western Blotting.

Measurement of safety

Adverse reactions (gastrointestinal reaction, routine blood, abnormal liver and kidney functions) were recorded.

Statistical analysis

Data are expressed as mean ± SD, and were analyzed with SPSS (version 18.0). Groups were compared using Student *t*-test and Chi-square test. Statistical significance was fixed at $p < 0.05$.

RESULTS

General information

There were no significant differences in the demographic data and duration of disease between the two groups of patients (Table 2).

BMD and bone pain

Treatment for 1 year resulted in increases in the lumbar vertebral and femoral neck BMDs in both groups. The BMD of patients in the observation group was significantly higher than in that in the control group ($p < 0.05$). The observation group bone pain score was lower than that of the control group ($p < 0.05$; Table 3).

Table 2: Profile of the patients (N = 50, %)

Group	Sex		Educational level			Body mass index (kg m^{-2})	Course (years)	Age (years)
	Male	*female*	*University or above*	*High school*	*Junior high school and below*			
Control	21 (42.00)	29 (58.00)	10 (20.00)	21 (42.00)	19 (38.00)	24.03 ± 4.05	5.03 ± 2.25	68.51 ± 6.62
Study	22 (44.00)	28 (56.00)	9 (18.00)	19 (38.00)	22 (44.00)	23.99 ± 4.06	4.97 ± 2.51	68.70 ± 6.53
χ^2	0.041			0.372		0.049	0.252	0.146
P	0.840			0.830		0.961	0.802	0.885

Table 3: Bone mineral density (BMD)

Group	Time point	Lumbar vertebral BMD (g/cm^2)	Femoral neck BMD (g/cm^2)	Bone pain score
Control	Pre-treatment	0.67 ± 0.08	0.54 ± 0.06	6.35 ± 1.45
	After 1-year of treatment	0.71 ± 0.08*	0.59 ± 0.07*	3.44 ± 1.34*
Study	Pre-treatment	0.67 ± 0.07	0.55 ± 0.06	6.33 ± 1.32
	After 1-year of treatment	0.76 ± 0.08*$^{\#}$	0.65 ± 0.05*$^{\#}$	1.67 ± 1.55*$^{\#}$

*$P < 0.05$, relative to value before treatment; $^{\#}p < 0.05$, relative to control group.

Clinical effectiveness

Total effectiveness in the control group (76.00 %) was inferior to that of the observation group (92.00 %) ($p < 0.05$; Table 4).

Levels of calcium, phosphorus, osteocalcin, NTX and urinary calcium/creatinine

Treatment for 1 year led to significant enhancement in blood calcium in both groups, and an increase in the observation group osteocalcin to a level significantly higher than that of the control group ($p < 0.05$). However, the treatment decreased observation group NTX to a level significantly lower than that of control group patients. In addition, the 1-year treatment significantly decreased urinary calcium/creatinine in observation group patients but the calcium/creatinine level was higher than the corresponding control group value ($p < 0.05$; Table 5).

Bone tissue apoptosis-related factors

The pre- and post-treatment levels of Cyt C, Apaf-1 and caspase-9 in the control group were comparable ($p > 0.05$). However, there were significant reductions in their post-treatment values in patients in the observation group, to levels lower than their corresponding control group levels ($p < 0.05$; Table 6).

Safety

There were no cases of routine blood, liver and kidney dysfunction in the two groups of patients. Although 3 cases of gastrointestinal reactions were seen in the observation group, adverse reactions in the two groups were comparable ($p = 0.243$).

DISCUSSION

Osteoporosis is common among the elderly and postmenopausal women. Nearly 70 million people aged over 50 years in China suffer from varied degrees of osteoporosis, while the incidents of total osteoporosis in people aged 60 years and above is close to 40 % [9]. Osteoporosis does not present early symptoms and so people do not take treatment serious until they are diagnosed with fracture. The human skeleton is in a continual process of metabolic renewal.

Table 4: Clinical effectiveness (n, %)

Group	Significantly effective	Effective	Ineffective	Total effectiveness
Study	25 (50.00)	21 (42.00)	4 (8.00)	46 (92.00)
Control	16 (32.00)	22 (44.00)	12 (24.00)	38 (76.00)
χ^2	-	-	-	4.762
p	-	-	-	0.029

Table 5: Levels of bone metabolic indices before and after treatment

Group	Time point	Blood calcium (mmol/l)	Blood phosphorus (mmol/l)	Blood osteocalcin (µg/l)	Collagen N-terminal peptide (µg/l)	Urinary calcium/ creatinine
Control	Before treatment	1.94 ±0.12	1.01 ± 0.26	12.13 ± 1.21	25.32 ± 2.21	0.20 ± 0.04
	After 1-year of treatment	2.36±0.21*	1.03 ± 0.23	11.91 ± 1.24	25.41 ± 2.34	0.19 ± 0.05
Study	Before treatment	1.96 ± 0.14	1.02 ± 0.28	12.16 ± 1.25	25.22 ± 2.47	0.19 ± 0.06
	After 1-year of treatment	2.39±0.25*	1.04 ± 0.27	14.62± 1.27*#	20.33 ± 1.67*#	0.13 ± 0.05*#

*$P < 0.05$, relative to pre-treatment level; # $p < 0.05$, relative to control

Table 6: Changes in apoptotic factors

Group	Time point	Cyt C	Apaf-1	Caspase-9
Control	Pre-treatment	0.67 ± 0.21	0.51 ± 0.24	0.79 ± 0.34
	Post-1year treatment	0.66 ± 0.31	0.53 ± 0.26	0.76 ± 0.32
Study	Pre-treatment	0.65 ± 0.22	0.52 ± 0.27	0.78 ± 0.31
	Post-1-year treatment	0.4± 0.25*#	0.42±0.28*#	0.32±0.23*#

*$P < 0.05$, relative to level before treatment; #$p < 0.05$, relative to control group

Some authors have suggested that 10 % of the human skeleton is renewed within a year.

Due to the imbalance between skeletal metabolism and skeletal reconstruction, the balance between skeletal growth and bone resorption is usually disrupted, leading to osteoporosis [10]. Bone resorption is carried out by osteoclasts. Once formed, osteoclasts are activated by signal molecules which cause them to release inflammatory factors, cytokines and digestive enzymes into bone tissue, thus inducing apoptosis of bone cells, and destruction of bone microstructure. However, bone formation requires the involvement of osteoblasts [11]. When osteoblasts receive activation signals, they synthesize and release collagen and calcium-binding proteins into bone tissues, thus bringing about an increase in the formation and mineralization of bone microstructure.

Decreases in the number and activity of osteoblasts and increased osteoclast activity are two important factors that cause apoptosis of bone tissue and destruction of bone microstructure [12]. The traditional remedy for osteoporosis (calcium therapy) has been replaced by other treatment strategies. In the present study, lumbar vertebral BMD, femoral neck bone density, and bone pain scores were significantly improved in both groups of patients following 1 year of therapy. The patients in the observation group had better improvement than the control of patients, an indication that combination of alendronate and allan sodium phosphate may significantly increase BMD, and also reduce bone pain.

A significant increase in the levels of blood calcium was observed in the two groups after 1-year of treatment. In the observation patients, the level of osteocalcin was markedly increased, NTX was significantly reduced, while urinary calcium to creatinine ratio was elevated.
Osteocalcin (BGP) reflects the activity of osteoblasts, NTX reflects the activity of osteoclasts, while the ratio of urinary calcium to creatinine reflects extent of resorptive activity in bone. The results of this study are in agreement with those previously reported [13,14], and suggest that a combination of alendronate sodium and allan sodium phosphate may reduce bone resorption and the activity of osteoclasts, and promote proliferation and differentiation of osteoblasts at low concentrations.

Cytochrome (Cyt C), Apaf-1 and caspase-9 are apoptosis-related factors released by mitochondria in a series of cascade reactions after receiving apoptotic signals from upstream pathways, and they are markers of apoptosis [5]. The levels of these three factors in bone tissue can reflect the degree of apoptosis in bone cells and the trend of subsequent bone destruction. In this study, treatment with calcium did not elicit any significant differences in the levels of Cyt C, Apaf-1 and caspase-9. However, treatment with a combination of sodium alendronate and allan sodium phosphate significantly reduced their levels. This appears to suggest that sodium alendronate may inhibit the activity of osteoclasts and prevent apoptosis of bone tissue cells.

Limitations of the study

The main limitation of this study is the small sample size and a single race/population used.

CONCLUSION

The combination of sodium alendronate and allan sodium phosphate is effective in the treatment of osteoporosis. The effectiveness of this combined therapy occurs through a mechanism involving improvements in BMD, NTX, Cyt C, Apaf-1 and caspase-9.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Fei Hu conceived and designed the study, Jialian Chen, Xiong Peng, Fei Hu collected and analysed the data, while Jialian Chen wrote the manuscript.

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