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今井　剛，西永　正典，松下　健二

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今井　剛[1)]，西永　正典[3)]，松下　健二[2)]

[1)] 国立長寿医療センター研究所老化制御研究部
[2)] 同口腔疾患研究部
[3)] 高知大学医学部老年病科
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Association of Number of Teeth with Cognitive Function in the Elderly

Takeshi IMAI[1)], Masanori NISHINAGA[3)], Kenji MATSUSHITA[2)]

Department of Aging Intervention[1)], Department of Oral Disease Research[2)], National Institute for Longevity Science, National Center for Geriatrics and Gerontology, Aichi, Japan
[3)] Department of Medicine and Geriatrics, Kochi Medical School, Kochi, Japan

Abstract

It has been reported that number of teeth is associated with cognitive function in elderly populations with dementia. However, little is known about this association in an ordinary elderly population. We evaluated this relationship in a Japanese population of elderly people aged from 65 to 92 years (n = 345; 122 males and 223 females) residing in Kahoku-chou (now Kami City) in Kochi Prefecture of Japan. Dental examinations were performed all subjects with the Mini-Metal State Examination (MMSE) and Kohs task test for assessing cognitive function. Associations were not found between number of residual teeth and MMSE in total subjects or in males or females. However, associations were found between number of residual teeth and Kohs score in males. These results suggest that cognitive functions, especially, motor cognition, may be associated with number of teeth in ordinary elderly males.

Key words: cognitive function, dementia, teeth, oral health, elderly, longevity

緒　言

高齢者における認知機能の低下やうつ状態は，高齢者の生活の質（Quality of Life; QOL）を著しく低下することが指摘されており，今後我が国において高齢化社会が進行するにあたって，認知機能の改善，認知機能低下の予防対策は急務である．

最近の疫学調査の結果から，自分の歯でよくかむことは高齢者の栄養状態の維持に重要であるばかりでなく，全身状態にも良い影響を及ぼしていることが解明されつつある．我が国においては，1989年に厚生省（現厚生労働省）が“80歳で自分の歯を20本保持しよう”という8020運動が提唱され，自分の歯を残す意義について検証が始まった．厚生労働省も1997年度から厚生科学研究事業の1テーマとして“高齢者の口腔保健と全身的な健康状態の関係についての総合研究”を課題に掲げ，福岡県，

Corresponding author: Kenji Matsushita, DDS, PhD
Department of Oral Disease Research[1)],National Institute for Longevity Science, National Center for Geriatrics and Gerontology36-3 Gengo, Morioka-cho, Obu, Aichi 474-8511, Japan
E-mail: kmatsu30@nils.go.jp

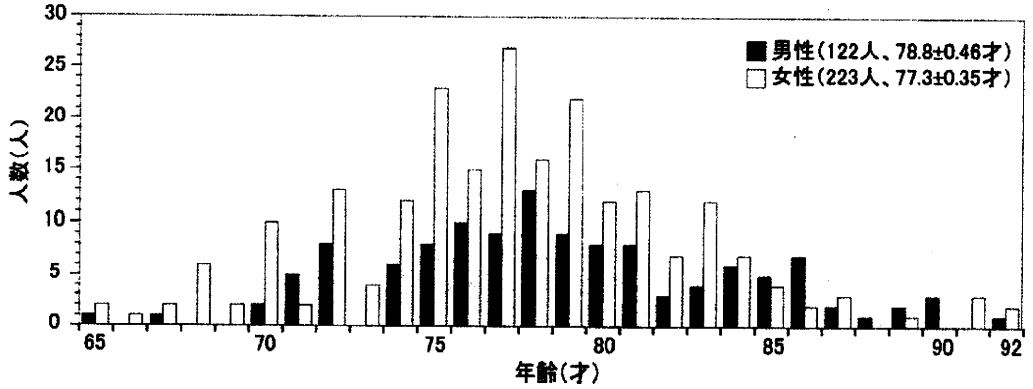

図1．被験者の年齢分布．65歳から92歳までの男性（122人，平均年齢78.8±0.46才），女性（223人，平均年齢77.3±0.35才）について，横軸に年齢を，縦軸にその人数を示した．

愛知県，新潟県などで疫学研究が実施された．それらの研究の結果から，歯・咀嚼機能とQOL，咀嚼機能と日常生活動作（Activities of Daily Living; ADL），咀嚼機能と運動能力，咀嚼機能と認知能力等との関連性が明らかになった[1]．歯の喪失がAlzheimer病（AD）発症の危険因子の一つであるとの報告があり，ADでは残存指数が少ないことが指摘されている[2,3]．また，咬合力と認知能力との正の相関も指摘されてきた[4]．簡易認知機能検査法である Mini-Mental State Examination (MMSE)[5]を用いた，福岡県において1998年に始まった疫学調査の結果では，60歳および65歳住民の残存歯数とMMSEとの間に有意な正の相関が認められている[6]．以上の結果は，残存歯数と認知能に関連性があることを示唆している．

本研究では，高知県香北町において1991年から2001年にかけて行われた高齢者の長期縦断疫学調査の調査結果をもとに[7-23]，高齢者の残存歯数と認知機能，特に動作性認知機能との関連性について検討した．

対象および方法

本研究は，1991年から2001年にかけて高知県香北町において行われた縦断的検診事業「香北町健康長寿研究」(KAHOKU LONGITUDINAL AGING STUDY; "KALS")の調査結果の内1994年度に行われたデータを統計学的に解析したものである[7-23]．

対象者は，同町在住の65歳以上の老年者1,488名のうち，残存歯数検査および簡易板Kohsテスト，MMSEテスト等の認知機能テストを受けた被験者で，低酸素性脳症，脳卒中後遺症等については除外した345名（男：女＝122：223）である（図1）．平均年齢＋標準誤差は男性78.8±0.46才で，女性は77.3±0.35才である．特に，簡易版Kohs立方体テストに関しては，今回は時間を短縮し効率を上げるために，原版の課題1，2，4，7，10，11，14の7題を選択して実施した（47点満点）[7]．なお，KALSにおける簡易版Kohsテストでも認知機能を評価できることが既に確認されている[7,10,12]．

統計学的解析にあたっては，分散分析（analysis of variance; ANOVA）を用いて解析を行った．有意差のあるものに関してはFisher's exact testを用い，またp値をSTATVIEWを用いて計算した．有意水準は$p<0.05$とした．図2から図4の棒グラフには平均値と標準誤差を示した．

結　果

1．残存歯数と年齢，性差との関係

図2に，年齢と残存歯数の関係を示した．65歳から92歳の被験者を4つの群（A群：65-74才，B群：75-80才，C群：80-85才，D群：86-92才）にわけ，男女別に残存歯数の平均値を算出した．その結果，男性においてはA群（65-74才）とD群（86-92才）に有意な差がみられた（$p=0.0080$）．女性においてはA群（65-74才）に対してC群（81-85才）及びD群（86-92才）との間に有意差がみられた（共に$p<0.0001$）．

更には，1993年度の厚生労働省口腔疾患実態調査のデータ（70～74才：14.41本，75～79才：9.01本，80～84才：7.41本）[24]と高知県香北町における高齢者の歯の残存歯数のデータと本調査による高知県香北町のデータ（70～74才：8.49本，75～79才：6.69本，80～84才：3.64本）を比較すると，前期高齢者，後期高齢者とも全国平均を下回っていた．

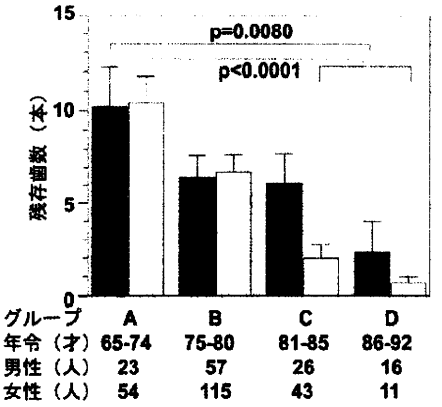

図2．性別高齢者年齢と残存歯数の関係．年齢別に4群（A, B, C, D）に分けて，各群における残存歯数の平均値および標準誤差を示した．A群：65-74才（男性23人，女性54人），B群：75-80才（男性57人，女性115人），C群：81-85才（男性26人，女性43人），D群：86-92才（男性16人，女性11人）．A群男性に比べてD群男性は有意に低く（p=0.0080），A群女性（レーン2）に比べてC，D群の女性（レーン8，10）は共に有意に低かった（p<0.0001）．

2．残存歯数と認知機能の関係

次に，認知機能と残存歯数との関連性について，MMSEと簡易版Kohs立方体テスト等と残存歯数との関連を解析した（図3）．図2の結果より加齢により（男性D群，女性CおよびD群）平均残存歯数が有意に下がるため，男性・女性ともに有意差がみられない65歳から80歳に設定した（男性80人，平均年齢75.8±0.37才，女性169人，平均年齢75.2±0.27才）．歯がない男性は32人（平均年齢76.9±0.45才），歯がある男性は48人（平均年齢75.1±0.48才），歯がない女性は80人（平均年齢76.2±0.35才），歯がある女性は89人（平均年齢74.4±0.36才）であった．歯のある男性の平均歯数は12.5±1.44本で，歯のある女性の平均歯数は15.2±1.07本であった．

歯の有無と簡易版Kohsスコアとの関連性を調べた結果，男性にのみ有意差が認められ，歯のある65-80歳の男性は歯のない男性に比べKohsスコアが有意に高かった（p=0.0252）．一方，女性に関しては歯の有無と簡易版Kohsスコアとの間には有意な差は認められなかった（p=0.1008）（図3A）．また，男性群において年齢，MMSEスコアと歯の有無との関連を調べた結果，歯のある男性と歯のない男性の平均年齢には有意差がみられないこと（図3B，各々p=0.1190）さらにはMMSEスコアと歯の有無との間にも有意な相関関係は認められなかった（0.1039）．従って，高齢者の男性において歯の有無と簡易板Kohsスコアとの間に特異的に有意差が見られることが明らかになった．

最後に各個人の数値の相関を解析した（図4）．MMSEと残存歯数（図4B）との間ならびに年齢と残存歯数（図4C）との間には有意な相関はみられなかった．同様の解析をKohsスコアについても行った（図4A）．その結果，図3と同様，歯のない群に比べて歯のある群はKohsスコアが高い傾向にあったが，残存歯数との間に有意な相関関係は認められなかった．

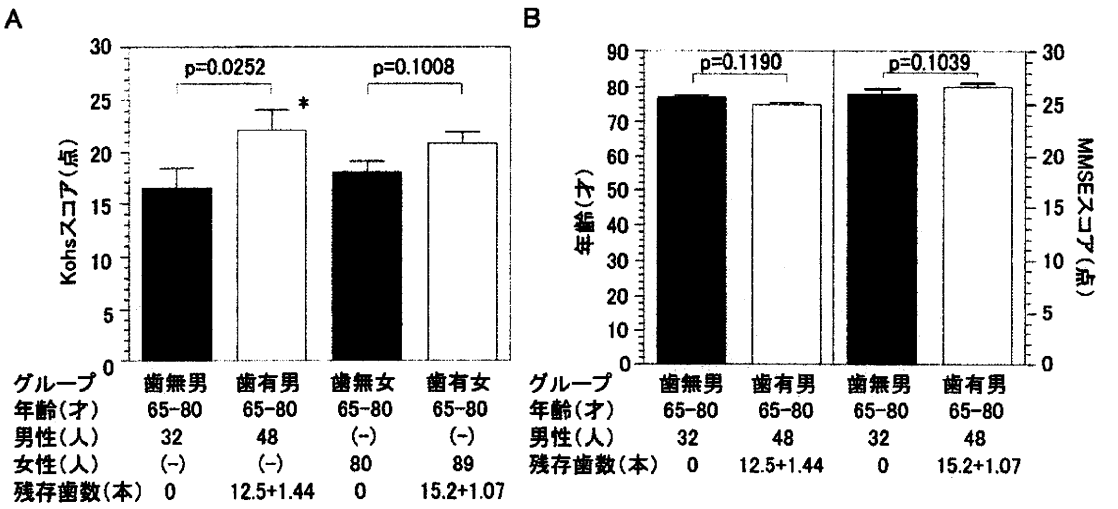

図3．認知機能と歯の有無の関係．65-80歳の男女について簡易版Kohsテスト（パネルA），年齢およびMMSEテスト（パネルB）を行った．歯のない群とある群に分けて，その平均値および標準偏差を表した（歯のない男性32人，歯のある男性48人で残存歯数12.5±1.44本，歯のない女性80人，歯のある女性89人で残存歯数15.2±1.07本）．A）Kohsスコアに関しては男性のみ歯の有無で有意差がみられた（*，p=0.0252）．女性はみられなかった（p=0.1008）．B）年齢およびMMSEスコアに関しては有意差がみられなかった（各々p=0.1190, 0.1039）．

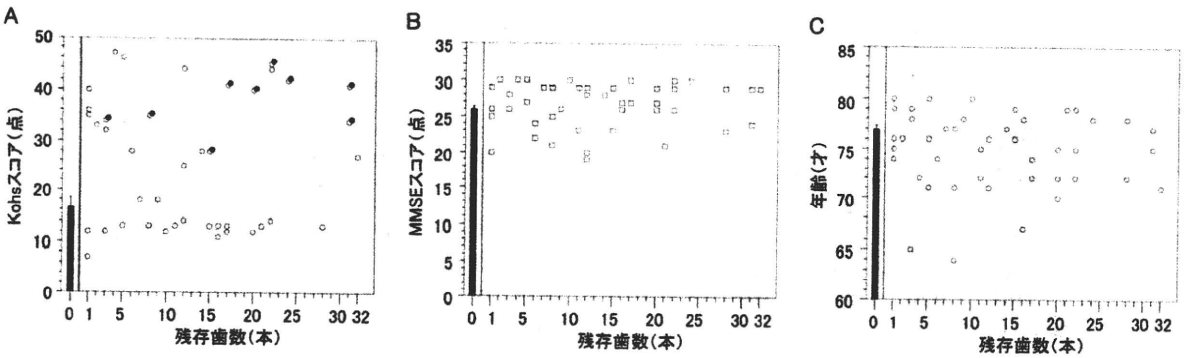

図4．認知機能と残存歯数の関係．65-80歳の男女についてKohsテスト（パネルA），MMSEテスト（パネルB），年齢（パネルC）を行った．残存歯数とKohsスコアをプロットし，両者の相関関係を示した．特に歯のない群の値は棒グラフにすることで歯のある群と差別化した．

考　察

高齢者の歯の残存歯数に関しては，1993年度の厚生労働省口腔疾患実態調査のデータと本調査による高知県香北町のデータを比較すると，前期高齢者，後期高齢者ともに全国平均を大きく下回っており，高齢者口腔の健康状態は良好とは言いがたいことが判明した．同地域におけるオーラルヘルスプロモーションの推進が必要である可能性が考えられた．

認知機能を調べるために，香北町の調査ではMMSE，長谷川式簡易知能評価スケール改訂版（Revised Hasegawa Dementia Scale: HDSR），簡易版Kohs立方体テスト等を行っている．本研究では，MMSEスコアあるいはKohsスコアと残存歯数の関連性について検討した．その結果，残存歯の有無とMMSEスコアとの間には相関はみられなかったものの，残存歯の有無とKohsスコアとの間には有意な相関が認められた．MMSEは，記憶，見当識，計算能力などを質問形式で行うもので，総合的な認知機能を評価することができる．これまでも，健全歯数あるいは残存歯数とMMSEスコアとの有意な相関が示されている[6,26,27]．本研究においても歯の健康状態を考慮して関連性を検討すれば，関連性が浮き彫りになった可能性が考えられるが，本調査では齲蝕の程度について解析がなされていなかったため，相関関係を明らかにすることができなかった．一方，Kohs 立方体テストの結果と健全歯数あるいは残存歯数等との関連性を調べた報告はこれまでにない．Kohs立方体テストは，4種の色に塗り分けられた約3cm立方の積み木を4個から16個使い，指示された図版の模様と同じ模様になるように積み木を組み合わせていくものであるため，空間および運動認知機能に特化した試験であるといえる．本研究の結果から，高齢の男性において歯の有無は空間や運動認知機能に影響を及ぼす可能性が示唆された．一般に，認知機能は男性の方が女性より高いことが知られており，これは男性の方が高度な教育を受けていることによることが推察されているが，今回男性に限定して歯の有無と認知機能に相関がみられたことは非常に興味深い．また，咬合関係や咀嚼能力等との関係も非常に興味のあるところであり，今後別の疫学調査において検討していきたいと考えている．

結　論

高齢者の残存歯の有無は高齢者の空間認知機能および運動性認知機能と相関があることが明らかになった．

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ORIGINAL ARTICLE

High level of serum undercarboxylated osteocalcin in patients with incident fractures during bisphosphonate treatment

Masataka Shiraki · Yasushi Yamazaki · Yumiko Shiraki · Takayuki Hosoi · Naoko Tsugawa · Toshio Okano

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Abstract To evaluate the possible interaction of metabolic effects in the mevalonate pathway between amino-bisphosphonates (amino-BP) and vitamin K, the serum level of undercarboxylated osteocalcin (ucOC) was measured in amino-BP users in relationship to incident fracture occurrence. Osteoporotic patients (mean age, 70.7 ± 9.1 years; $n = 231$) treated with alendronate or risedronate were followed for 3.4 ± 2.1 years, and observations regarding the presence or absence of incident fractures in their vertebrae were made based on vertebral X-ray films every year. During the observation period, new fractures were found in a total of 71 patients (incident vertebral fracture, $n = 61$; the remaining 10 patients had long bone fractures). The baseline data of the patients with incident fractures indicated that incident fractures are more likely to occur in older patients who have a higher number of prevalent vertebral fractures and lower baseline lumbar bone mineral density (LBMD) as compared to patients without incident fractures. There was no significant difference in the changes of LBMD and urinary excretion of NTX after treatment. On the other hand, the serum level of ucOC in patients with incident fractures and with amino-BP treatment was significantly higher (2.75 ± 0.19 ng/ml) than that in patients without incident fractures and with amino-BP treatment (2.28 ± 0.13 ng/ml) ($P = 0.038$). These results indicate that older age, a greater number of prevalent fractures and higher ucOC levels, and lower LBMD are risks for incident fractures despite use of amino-BP. The time-dependent incident fracture rate was higher in accordance with an increase in the number of risk items ($P < 0.001$ in log-rank and Wilcoxon tests). In conclusion, measurement of undercarboxylated osteocalcin may be useful for assessing fracture risk in patients receiving amino-BP treatment.

Keywords Undercarboxylated osteocalcin (ucOC) · Bisphosphonates treatment · Osteoporosis · Incident fractures · Bone mineral density

M. Shiraki (✉) · Y. Yamazaki · Y. Shiraki
Research Institute and Practice for Involutional Diseases, 1610-1 Meisei, Misato, Azumino, Nagano 299-8101, Japan
e-mail: ripid@fc4.so-net.ne.jp

T. Hosoi
Department of Clinical Research and Development, National Center for Geriatrics and Gerontology, Aichi, Japan

N. Tsugawa · T. Okano
Department of Hygienic Sciences, Kobe Pharmaceutical University, Hyogo, Japan

Introduction

Recent progress in key pathogenesis of osteoporosis has focused on bone resorption through increased osteoclastic activity. Bisphosphonates specifically inhibit osteoclastic activity through inhibition of the mevalonate pathway [1], achieving a decrease in bone turnover followed by an increase in secondary mineralization of bone. Such actions of bisphosphonates are connected to prevention of bone fractures in osteoporosis [2] because bisphosphonates turn the negative bone balance positive. Although bisphosphonate has been established as a first-line drug for preventing fractures in osteoporosis, complete inhibition of new fractures in osteoporosis has not been achieved [3–5]. This failure may be partly explained by the concept that complex pathogenesis of osteoporosis and the reduction in bone turnover or increase in bone density induced by bisphosphonates may not be sufficient to achieve thorough inhibition of incident fractures. In fact, deficiencies of many

nutrients such as vitamin D, calcium, and vitamin K have accounted for possible risk factors of incident fractures in osteoporosis [6, 7]. Among these nutrients, vitamin K deficiency or insufficiency has been consistently reported as a risk factor for osteoporotic fractures [7–9]. Vitamin K is thought to maintain bone strength through gamma-carboxylation of matrix glutamic acid residues of protein. In vitamin K insufficiency or deficiency, a small amount of undercarboxylated osteocalcin (ucOC) is released from the osteoblasts into circulation. Thus, the serum concentration of ucOC has been recognized as being a sensitive marker of vitamin K deficiency in bone. Serum ucOC decreases significantly after menatetrenone (vitamin K_2) [10, 11] or vitamin K_1 [12] treatment, suggesting that vitamin K homologues may improve bone osteocalcin content and may be linked to reduction of the incident fracture rate. Furthermore, Okano et al. [13] reported that phylloquinone (vitamin K_1) can be converted to menaquinone (vitamin K_2) in various cells, including osteoblasts, through geranylgeranylation in the side chain, and that menaquinone 4 was considered to be an active form of vitamin K because menaquinone 4 was reported to bind to nuclear receptor SXR [14]. It is possible that this metabolic process of vitamin K activation may be inhibited by bisphosphonates as a result of inhibition of geranylgeranylation of protein through reduction of farnesyl diphosphate (FPP) synthase activity [1, 2]. Therefore, there may be a close relationship between the effect of bisphosphonates on cell function and vitamin K activation in the same cell. However, until now, there have been no data regarding the relationship between the state of vitamin K and the effect of bisphosphonates on fracture prevention. In this study, the authors attempted to investigate preliminarily whether the state of vitamin K in bone modulates the effect of bisphosphonates on fracture prevention.

Materials and methods

Subjects

Ambulatory postmenopausal women more than 45 years old with primary osteoporosis and undergoing amino-bisphosphonates (amino-BP) treatment during the period from January 2000 to June 2008 were eligible for participation in the study. Exclusion criteria consisted of endocrine disorders such as hyperthyroidism or hyperparathyroidism, a history of extensive gastrointestinal surgery or chronic renal failure, and current use of medications known to result in secondary osteoporosis. The patients were participants in a Nagano cohort study, and therefore baseline examinations such as bone density measurement and measurements of serum levels of calcium, phosphate, and urinary excretion of N-telopeptide of type I collagen (NTX) had been performed for baseline data with informed consent. Baseline X-ray examinations to confirm the presence or absence of preexisting fractures were also performed at the time that the patients registered for the Nagano cohort study. The period for conducting follow-up observations of each participant was calculated as the time from their inclusion into the study up to their death, minus 1 year after the occurrence of incident fractures or to the end of June 2009, whichever occurred first. Follow-up was conducted on all the subjects in this study for more than 1 year.

Intervention

Study subjects were started on amino-BP treatment, either alendronate 5 mg/day orally or risedronate 2.5 mg/day orally by Japanese dosage regulation. Alternatively, some patients received equivalent weekly doses of alendronate (35 mg/week) or risedronate (17.5 mg/week). Patients were continued on amino-BP treatment for the duration of their participation in the study. Vitamin K_2 administration was prohibited in the participants. All the patients were treated with amino-BP alone during the entire observation period.

Bone mineral density (BMD) measurements

Lumbar spine bone mineral density (LBMD) was measured by dual-energy X-ray absorptiometry (DXA) using a Lunar DPX-L or DPX-IQ (Lunar Corporation, Madison, WI, USA). The interassay variance of LBMD in the laboratory was 0.5 ± 0.5% [coefficient of variation (CV) ± SD] [15]. To guard against machine drift, a quality assurance test was carried out for every measurement. The baseline value of LBMD was used to diagnose osteoporosis, and measurements of LBMD were repeated every 6 months. The value of the last observation was used as the value of LBMD after the treatment.

Detection of prevalent and incident vertebral fractures

Prevalent and incident vertebral fractures were diagnosed by a semiquantitative visual method using lateral thoracolumbar spine radiographs in accordance with the method described by Genant et al. [16]. To detect incident vertebral fractures, spine radiographs were routinely taken at 1-year intervals, and additional X-rays were taken whenever the subjects complained of symptoms suggestive of new clinical vertebral fractures. Both new clinical and morphometric fractures were counted as incident vertebral fractures. Incident long bone fractures were identified from medical records or confirmed using X-ray films. Although incident clinical (symptomatic) fractures in vertebrae or

other parts of the bone structure were easily recognized when they occurred, morphometric vertebral fractures were sometimes difficult to detect clinically. Because the exact timing when a morphometric incident vertebral fracture occurred could not be determined for some of the patients with such fractures, the time of the spinal radiograph showing the fractures was considered as being the time of the fracture. Fractures induced by major trauma were excluded from the analysis; namely, fractures induced by a fall from standing height were categorized as incident fractures, but fractures induced by a fall from a point higher than body height were excluded.

Diagnosis of osteoporosis

Diagnosis of osteoporosis was made in accordance with the osteoporosis diagnostic criteria (2000 version) proposed by the Japanese Society for Bone and Mineral Research [17]. Osteoporosis is diagnosed as the presence of fragility fractures in any bone lesion in a person with a BMD less than 80% (–1.63 SD) of the BMD of the young adult mean (YAM). Osteoporosis is also diagnosed when the LBMD is less than 70% (–2.45 SD) of the BMD of a YAM, even if the person has no prevalent fragility fractures.

Biochemical indices

Nonfasting serum and urine samples were collected as baseline data at the time of enrollment. Routine biochemical data including serum levels of calcium and phosphate were analyzed immediately using an autoanalyzer. Urinary N-terminal telopeptides of type I collagen (NTX) were measured with an enzyme-linked immunosorbent assay (ELISA) kit (Osteomark Ostex, Princeton, NJ, USA), and the value of NTX was standardized by the concentration of creatinine in the same urine sample. Urine samples were collected during the second voiding of the day. Urinary NTX was measured before and at the end of the observation.

Measurement of undercarboxylated osteocalcin

Serum level of undercarboxylated osteocalcin (ucOC) was measured using a new electrochemiluminescence immunoassay (Sanko Junyaku, Ibaraki, Japan) [8]. Because ucOC measurements were not available when the study began, serum level of ucOC could not be measured at baseline for any of the participants, but during treatment values could be obtained for all participants. Measurement of ucOC in patients without incident fracture was conducted at the end of the observations. On the other hand, for patients with incident fractures, serum samples were taken 1 or more years after the occurrence of incident fractures to determine ucOC.

Ethical considerations

The study protocol was reviewed by the ethical committee of the Research Institute and Practice for Involutional Diseases (RIPID), and comprehensive written informed consent was obtained from all study subjects.

Statistical analysis

In the descriptive analysis of the baseline characteristics, numerical data are expressed as mean ± SD. Comparisons of baseline characteristics between subjects with and without incident fractures were performed using two levels of one-way analysis of variance (ANOVA). Comparisons between the values before and after treatment were based on a paired t test. To assess confounding effects of the risks, stepwise multiple regression analysis was used. After confirmation of independent risks for incident fractures in amino-BP users, secondary analyses were carried out: the sum of the existing risk factors in individual subjects was calculated, and the patients were categorized by the calculated number of risks. Subsequently, time-dependent incident fracture rates were analyzed using a Kaplan–Meier plot. Here, the number of patients with high ucOC in each category of risk was tested by Pearson's Chi-square test. The level of significance was set at less than 0.05 (Table 1).

Results

Demography of the subjects

From among the patients visiting the outpatient care unit of Research Institute and Practice for Involutional Diseases, a total of 269 patients with osteoporosis were recruited for this study. Of these patients, 38 were excluded from the study because of lack of baseline data or missing follow-up data. The remaining 231 patients were followed for 1 or more years and were adapted to the following analyses. The mean ± SD age of participants was 70.0 ± 9.1 years old, and 140 subjects (60.6%) had prevalent fractures. The average observation period was 3.4 ± 2.1 years, with the longest observation period being 9 years. After bisphosphonate treatment, urinary excretion of NTX decreased significantly, from 55.2 to 30.0 nM/mM Cr (45.7% of the baseline; $P < 0.0001$ in paired t test), and LBMD increased significantly, from 0.774 to 0.844 g/cm^2 (+9.0% increase from baseline; $P < 0.0001$ in paired t test).

Table 1 Comparison of baseline data and data at end of observation of subjects

Item	Baseline	End of observation
Age (years)	70.7 ± 9.1	74.1 ± 8.9*
Body weight (kg)	49.1 ± 7.4	47.5 ± 7.6
Body height (cm)	149.2 ± 6.3	148.2 ± 5.8
Serum Ca (mg/dl)	9.19 ± 0.41	9.24 ± 0.43
Serum Pi (mg/dl)	3.49 ± 0.46	3.50 ± 0.50
NTX (nM/mM Cr)	55.2 ± 30.7	30.0 ± 19.3*
Initial bone mineral density (BMD) (g/cm^2)	0.774 ± 0.129	0.844 ± 0.144*

Values are expressed as mean ± SD. *$P < 0.0001$ versus baseline in paired t test

Table 2 Fracture outcomes during the observation

Site of fractures	Baseline	Incident fractures
None	208	160
Vertebrae	117	61
Colles	9	4
Hip	5	2
Other sites	9	4

Multiple prevalent fractures in multiple bone sites were observed in 14 cases; incident fractures were counted as the first incident fracture

Prevalent and incident fractures in the participants

A total of 154 sites of prevalent fractures were counted in 140 patients, indicating that 14 cases had multiple prevalent fractures. Incident fractures were observed in 71 cases during the observation period, and the most prominent fracture site was the vertebral body, with both morphometric and clinical symptomatic fractures (Table 2).

Baseline data of patients with and without incident fractures

To screen for risks for incident fractures in amino-BP users, comparisons were made on baseline data between patients with incident fractures and those without incident fractures during the observation period. Table 3 shows the comparison of baseline data between patients with and without incident fractures. Patients with incident fractures during amino-BP treatment were characterized by older age and a lower initial lumbar BMD as compared to the patients without incident fractures. The number of prevalent vertebral fractures in the patients with incident fractures was higher than that of the patients without incident fractures, suggesting that incident fractures may occur in more severe cases of osteoporosis even during bisphosphonate treatment.

Bone outcomes after treatment in patients with and without incident fractures

Follow-ups were conducted on all the patients treated with amino-BP in the form of LBMD, urinary NTX, serum levels of calcium and phosphate, and measurement of ucOC at the end of the observation period. Comparisons were made in the values obtained between patients with and without incident fractures to determine what kinds of changes occurred in bone parameters after the treatment in association with incident fractures (Table 4).

Among the various outcomes related to bone metabolism, only serum level of ucOC was significantly higher in the patients with incident fractures than in those without, suggesting that vitamin K deficiency in bone may exist in the patients with incident fractures.

Stepwise regression analysis for the risk of future fractures in amino-BP users

From the primary analyses, the baseline age, LBMD, number of prevalent vertebral fractures, and ucOC after treatment were considered to be risks for fracture susceptibility in bisphosphonate users. To exclude confounding factors, multiple stepwise regression analysis among these risks was performed. The four risks just mentioned were recognized as independent risks for incident fractures in bisphosphonates users (Table 5).

Logistic regression analysis and receiver operating characteristic (ROC) analysis for each risk to evaluate the risk assessment of each patient (Fig. 1)

To evaluate the time-dependent fracture rate, secondary analyses using a Kaplan–Meier plot analysis were performed. After deciding the cutoff value for each risk using ROC analysis, the sum of the risks (0–4) was calculated for each participant. The cutoff values for each risk were as follows: 75 years or older for age, 0.763 g/cm^2 or less for LBMD, two or more for number of prevalent vertebral fractures, and 2.6 ng/ml or more for ucOC. The patients were divided into five categories in accordance with the presence of risks. Group 0 consisted of patients without any risk ($n = 31$), group 1 consisted of patients with one risk ($n = 67$), group 2 consisted of patients with two risks ($n = 75$), group 3 consisted of patients with three risks ($n = 47$), and group 4 consisted of patients with four risks ($n = 11$). Groups 0, 1, 2, 3, and 4 consisted of 2, 11, 20, 24,

Table 3 Baseline data in the patients with or without incident fractures during amino-bisphosphonates (amino-BP) treatment

Items	Incident fracture (–)	Incident fracture (+)	*P*
Number of cases	160	71	–
Age (years)	69.0 ± 0.7	74.3 ± 1.1	<0.0001
BMI (kg/m^2)	21.8 ± 0.2	22.3 ± 0.4	0.272
Serum Ca (mg/dl)	9.23 ± 0.03	9.14 ± 0.05	0.060
Serum P (mg/dl)	3.53 ± 0.04	3.41 ± 0.05	0.083
NTX (nM/mM Cr)	54.1 ± 2.5	57.2 ± 3.7	0.448
Initial BMD (g/cm^2)	0.788 ± 0.010	0.743 ± 0.015	0.0157
Number of prevalent vertebral fractures	1.09 ± 0.16	2.58 ± 0.25	<0.0001

The baseline data were compared between the patients with or without incident fractures, retrospectively. A total of 71 patients had new fractures during amino-BP treatment. The patients with incident fractures were of higher age, with a greater number of prevalent vertebral fractures and lower lumbar bone mineral density (LBMD) at baseline. Data are expressed as mean ± SE. Statistical analysis was made by analysis of variance (ANOVA)

Table 4 Bone-related outcomes at the end of observation in the patients with or without incident fractures

Outcomes	Incident fracture (–)	Incident fracture (+)	*P*
LBMD (g/cm^2)	0.854 ± 0.011	0.823 ± 0.017	0.131
Ca (mg/dl)	9.26 ± 0.03	9.16 ± 0.05	0.091
P (mg/dl)	3.54 ± 0.04	3.43 ± 0.06	0.140
NTX (nM/mM Cr)	28.6 ± 1.5	33.2 ± 2.3	0.104
ucOC (ng/ml)	2.28 ± 0.13	2.75 ± 0.19	0.038

Data listed in Table 4 were obtained at the end of the observations. In a case having incident fracture, serum and urinary samples were taken 1 or more years after the occurrence of incident fracture or 1 or more years after the recognition of morphometric fracture (2.6 ± 0.6 years after the recognition of incident fracture). Data are expressed as mean ± SE. ucOC, undercarboxylated osteocalcin

Table 5 Stepwise regression analyses of the risks for fracture susceptibility in amino-BP users

Risk	χ^2	*P*	R^2
Number of prevalent vertebral fracture	21.999	0.0000	0.0772
Age (years)	9.1164	0.0025	0.1092
ucOC (ng/ml)	4.4385	0.0351	0.1247
Baseline LBMD (g/cm^2)	3.8923	0.0485	0.1384

Stepwise regression analysis was performed among the four risks, which may relate to having incident fractures during amino-BP treatment. All four risks were significantly associated with incident fractures

and 9 patients with incident fractures, respectively. The Kaplan–Meier plot indicated a significantly higher susceptibility of incident fractures in accordance with an increasing number of risks (see Fig. 1). Furthermore, the number of patients with high ucOC (>2.6 ng/ml) in each category is shown in Table 6. The percentages for the patients with high ucOC were significantly increased in

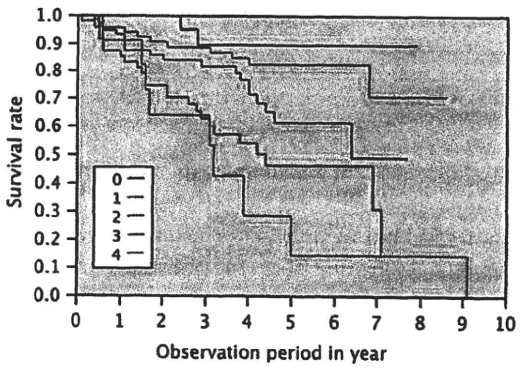

Fig. 1 Kaplan–Meier plot of the categorized patients under amino-bisphosphonate (amino-BP) treatment in accordance with the number of fracture risks (0–4). The lower survival rate from incident fractures was observed in accordance with the number of fracture risk in patients with amino-BP-treated osteoporosis ($P < 0.001$ in log-rank and Wilcoxon test)

accordance with increase in number of risks ($P < 0.0001$), providing further proof that high ucOC was an independent risk factor for incident fractures in osteoporosis during bisphosphonates treatment.

Discussion

In this study, traditional risk factors, such as older age, low BMD, and the presence of preexisting fractures [18], for incident fractures in osteoporosis were also recognized as risks for incident fractures in bisphosphonate users. However, no significant contribution of incident fractures to changes in LBMD and bone resorption markers was seen, although such changes considerably favored fracture prevention; that is, LBMD increased by about 11% and 8% for patients with and without incident fractures, respectively, and urinary excretion of NTX decreased by about 60% and

Table 6 Rate of patients with high ucOC in five risk categories

Risk category	ucOC < 2.6 ng/ml	ucOC ≥ 2.6 ng/ml	Percent (%)
0	31	0	0
1	48	19	28.4
2	46	29	38.7
3	19	28	59.6
4	0	11	100

The trend for the rate of patients with high ucOC was apparently increased in accordance with increase in number of risks. $\chi^2 = 49.1$ and $P < 0.0001$ by Peason's Chi-square test

50% for patients with and without incident fractures, respectively. These changes in LBMD and urinary NTX were not significantly different between patients with and without incident fractures. These changes in parameters were in good agreement with previous reports obtained from Japanese postmenopausal osteoporosis [19]. Therefore, the biological effects of bisphosphonates on bone density and resorption were identical regardless of the occurrence of new fractures during treatment. Previous reports have indicated that increase in BMD and decrease in bone markers were not strong predictors for future fractures in bisphosphonates users [20–24].

On the other hand, the serum level of ucOC of fractured patients was significantly higher than that of patients without any new fractures. Secondary analysis using stepwise regression analysis revealed that the level of ucOC in amino-BP users was a significant independent risk for incident fractures (Table 5). Because ucOC was measured after an incident fracture occurred, it was thought that the difference in ucOC levels between patients with and without incident fracture may have reflected the occurrence of a fracture but not a vitamin K deficiency in bone. However, this possibility is unlikely because urinary excretion of NTX did not differ between these two groups, indicating that there was no significant difference in bone resorption. It has been recently reported that the biological action of amino-BP and activation of vitamin K have a point of contact, that is, the geranylgeranylation of molecules. Amino-BP inhibits this process [1, 2] and, in contrast, vitamin K activation requires geranylgeranylation of the side chain [13]. Therefore, it is possible that bisphosphonates may interfere with vitamin K activation in bone cells, especially in patients with preexisting vitamin K deficiency. This hypothesis prompted the authors to measure ucOC in patients treated with amino-BP. Results indicate that patients with incident fractures during amino-BP treatment may be lacking vitamin K in bone cell levels consequent to a higher level of ucOC. However, Hirano et al. [24] reported that amino-BP treatment caused a decrease in ucOC level but resulted in no change in carboxylated osteocalcin (cOC), suggesting that amino-BP did not decrease the carboxylation of osteocalcin. So, further clarification is required whether amino-BP treatment affects carboxylation on osteocalcin in both patients with or without vitamin K deficiency in a prospective study design. This is the first report that suggests the usefulness of measuring ucOC in amino-BP users in terms of fracture prediction. Because ucOC decreased after vitamin K_2 treatment [11], concurrent use of vitamin K_2 with amino-BP may be effective in preventing the occurrence of new fractures in patients with higher ucOC levels during treatment with only amino-BP.

The limitations of this study were as follows. First, the present results must be evaluated as a prospective study design because the present study was a retrospective study. Second, a larger number of participants is necessary to increase statistical power. Third, direct evidence that shows that bisphosphonates inhibit vitamin K activation in bone cells is required in vitro, and we have to measure serum carboxylated osteocalcin level together with ucOC to assess gamma-carboxylation during amino-BP treatment in vivo. Last, because other risk factors for fracture such as the physical activity of the participants was not evaluated in the present study, those factors related to increased susceptibility to fractures should be incorporated into the model in future.

In conclusion, it is useful to evaluate the serum level of ucOC in patients with amino-BP to predict future fracture occurrence.

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ORIGINAL ARTICLE

Design of a pragmatic approach to evaluate the effectiveness of concurrent treatment for the prevention of osteoporotic fractures

Rationale, aims and organization of a Japanese Osteoporosis Intervention Trial (JOINT) initiated by the Research Group of Adequate Treatment of Osteoporosis (A-TOP)

Masataka Shiraki · Tatsuhiko Kuroda · Nobuaki Miyakawa · Naohito Fujinawa · Kazumasa Tanzawa · Akiko Ishizuka · Shiro Tanaka · Yukari Tanaka · Takayuki Hosoi · Eiji Itoi · Shigeto Morimoto · Akira Itabashi · Toshitsugu Sugimoto · Toshihiko Yamashita · Itsuo Gorai · Satoshi Mori · Hideaki Kishimoto · Hideki Mizunuma · Naoto Endo · Yoshiki Nishizawa · Kunio Takaoka · Yasuo Ohashi · Hiroaki Ohta · Masao Fukunaga · Toshitaka Nakamura · Hajime Orimo

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Abstract The aim of osteoporosis treatment is to prevent future fractures. Although concurrent treatment has been used very frequently for osteoporosis in clinical practice, there are no data on accurate and verified effectiveness of concurrent treatment for fracture prevention in patients with osteoporosis. To clarify the clinical usefulness of concurrent treatment, the Japan Osteoporosis Society has authorized the establishment of the A-TOP (Adequate Treatment of Osteoporosis) research group. The objective of this research is to establish a design for a clinical trial to prove whether concurrent treatment using both alfacalcidol (1-alpha-hydroxycholecalciferol) and alendronate is more effective as compared to treatment using alendronate alone

For the A-TOP (Adequate Treatment of Osteoporosis) Research Group.

M. Shiraki (✉)
Department of Internal Medicine, Research Institute and Practice for Involutional Diseases, 1610-1 Meisei, Misato, Azumino, Nagano 399-8101, Japan
e-mail: ripid@fc4.so-net.ne.jp

T. Kuroda · N. Miyakawa
Public Health Research Foundation, Tokyo, Japan

N. Fujinawa
I'cros Co., Ltd., Tokyo, Japan

K. Tanzawa
ING Corporation, Tokyo, Japan

A. Ishizuka
Japan Clinical Research Support Unit, Tokyo, Japan

S. Tanaka
Division of Clinical Trial Design and Management, Translational Research Center, Kyoto University, Kyoto, Japan

Y. Tanaka
Department of Epidemiology and Biostatistics, School of Health Sciences and Nursing, The University of Tokyo, Tokyo, Japan

T. Hosoi
Department of Advanced Medicine, National Center for Geriatrics and Gerontology, Aichi, Japan

E. Itoi
Department of Orthopaedic Surgery, Tohoku University School of Medicine, Sendai, Japan

S. Morimoto
Geriatric Medicine, Kanazawa Medical University, Ishikawa, Japan

A. Itabashi
Saitama Center for Bone Research, Kubojima Clinic, Saitama, Japan

T. Sugimoto
Department of Endocrinology and Metabolism, Faculty of Medicine, Shimane University, Shimane, Japan

T. Yamashita
Department of Orthopaedic Surgery, Sapporo Medical University School of Medicine, Sapporo, Japan

I. Gorai
Department of Obstetrics and Gynecology, International University of Health and Welfare Atami Hospital, Shizuoka, Japan

S. Mori
Bone and Joint Surgery, Seirei Hamamatsu General Hospital, Shizuoka, Japan

in terms of fracture prevention. The present study was named JOINT (Japanese Osteoporosis Intervention Trial) and is based on a method using national, prospective, randomized, open-labeled, blinded endpoints focusing on postmenopausal osteoporosis with a high risk for fracture. The patients were mainly selected by practitioners and allocated randomly by a central registration system into two groups, of which one received 5 mg/day of alendronate alone, and the other received 1 μg/day of 1-alpha-hydroxycholecalciferol (alfacalcidol) in addition to the alendronate. The endpoints focused primarily on fracture prevention, and the patients' quality of life (QOL) and change in body height, as well as adherence and the adverse events of the treatments were evaluated secondarily. To obtain sufficient statistical power in the events during a 2-year observation period, the patients who are expected to have higher risk were selected to participate in this study, and it was decided that the final plan would involve 890 patients per group (two-sided alpha = 0.05, power = 0.8). Data collection began in November 2003. Correspondence regarding the registration of the investigator and the progress of the study was conducted through a web system from the Public Health Research Foundation to practitioners.

Keywords Alendronate · Alfacalcidol · Concurrent treatment · Fracture prevention · Osteoporosis

Introduction

Osteoporosis, which is characterized by compromised bone strength and increased susceptibility to fractures, which lead to deterioration in the QOL and increased mortality, is a national burden on an aging society [1, 2]. However, recent studies indicate that treatment with a parathyroid hormone, bisphosphonates or a selective estrogen receptor modulator (SERM) [3–9] may decrease the risk of fractures in patients with osteoporosis.

Although bisphosphonate treatment currently represents the most powerful form of treatment available for fracture prevention in osteoporotic patients, it has not succeeded in completely preventing osteoporotic fractures [3–5, 7–9]. Therefore, concurrent treatment of osteoporosis has been frequently used by Japanese practitioners without any concrete evidence regarding fracture reduction. Since the concept of evidence-based medicine (EBM) has been introduced to clinical practice since the 1990s [10], the Japan Osteoporosis Society and the Japanese Society of Bone Mineral Research have edited the clinical guideline for treatment of osteoporosis (Chief editor: Hajime Orimo [11]). However, the writers recognized that there was a lack of evidence in the effectiveness of concurrent treatment of osteoporosis. Furthermore, it was expected that the patients who visit clinics have varying degrees of risk of fracture, which may differ from the degree of those who participated in development trials for bisphosphonates. This possibility would make it easier to obtain pragmatic evidence in general clinical practice.

Starting in 2000, the Japan Osteoporosis Society had planned to investigate the effectiveness of treatment of osteoporosis in order to provide evidence to general practitioners. Before constructing evidence, some feasibility studies were required to confirm the consensus in the diagnosis of incident fractures among the researchers and to elucidate the risk of future fractures in the patient population. In addition to these efforts in the field of osteoporosis, the Japanese government also established an

H. Kishimoto
Department of Orthopedic Surgery, San-in Rosai Hospital, Tottori, Japan

H. Mizunuma
Department of Obstetrics and Gynecology, Hirosaki University School of Medicine, Aomori, Japan

N. Endo
Division of Orthopedic Surgery, Niigata University Medical and Dental Hospital, Niigata, Japan

Y. Nishizawa
Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan

K. Takaoka
Department of Orthopaedic Surgery, Osaka City University Medical School, Osaka, Japan

Y. Ohashi
Department of Biostatistics, School of Public Health, The University of Tokyo, Tokyo, Japan

H. Ohta
Department of Obstetric and Gynecology, Tokyo Women's Medical University, Tokyo, Japan

M. Fukunaga
Department of Nuclear Medicine, Kawasaki Medical School, Okayama, Japan

T. Nakamura
Department of Orthopedic Surgery, University of Occupational and Environmental Health School of Medicine, Kokura, Japan

H. Orimo
Health Science University, Yamanashi, Japan

ethical guideline for clinical trials [12], and the International Committee of Medical Journal Editors launched a clinical trial registry [13]. Such kinds of progress in the circumstances of clinical trials have enabled for investigator-initiated clinical trials in general practice.

The Adequate Treatment of Osteoporosis (A-TOP) study group was established in 2000 [11] in affiliation with the Japan Osteoporosis Society and organized a team for clinical trial management. The team consisted of clinical investigators (planning and analysis), foundation (funding) managers, officers from non-profit organization (data management) and several companies (data collection). This was the first joint team to create the post-making evidence for osteoporosis. In November 2003, A-TOP initiated a randomized clinical trial referred to as the Japanese Osteoporosis Intervention Trial (JOINT). The purpose of JOINT was to confirm the clinical significance of concurrent use of osteoporotic drugs. The first protocol, named JOINT-01, was initiated in 2002, but was suspended the following year due to a change in drug labeling. A second protocol, named JOINT-02, was established to clarify the effect of adding 1-alpha-hydroxycholecalciferol (alfacalcidol) to alendronate (ALN), using the incident fracture rate as the primary endpoint. In this paper, the rationale, organization and study design of JOINT-02 are introduced.

Rationale and aims

In 2002, the Japan Society of Osteoporosis sent a letter to randomly selected practitioners and enclosed a questionnaire regarding whether concurrent treatment using bisphosphonate and another drug was being utilized to treat osteoporosis. Surprisingly, 87.8% (79/90 practitioner) of the doctors who responded did have experience using concurrent treatment [14]. The most frequent drugs used in concurrent treatment with amino-bisphosphonate were alfacalcidol (93.7%), followed by calcitonin (50.6%), as there were expectations for these drugs to exhibit more potent inhibition of fracture occurrence or more significant increase in BMD, even though there was no apparent evidence. In addition to the lack of evidence related to fracture prevention, the safety profile of concurrent treatment had not been evaluated. Thus, evaluations of the effectiveness and safety of concurrent treatment were urgently required. Etidronate [15] and ALN [8, 9] were used as the drugs to confirm anti-fracture effectiveness in comparison to alfacalcidol in Japanese osteoporotic patients. However, these clinical trials were carried out at specific institutions and were initiated by experts in accordance with tight regulations. As a result, there may have been differences in the selected treatment and in the backgrounds of the patients between treatments conducted at these institutions and those conducted in general practice. In addition, the adherence of the treatment is expected to be lower in general practice than in institutions with experts who are committed to developmental trials. Thus, pragmatic study is urgently needed to evaluate whether bisphosphonates are effective to the same extent at the level of general practitioners as compared to the prior study (Phase III study).

Feasibility studies

The Japan Osteoporosis Society started discussions to execute a national clinical trial for obtaining evidence regarding the effectiveness of concurrent treatment in 2000. An executive committee of A-TOP was organized in 2002 and planned on forming a consensus regarding judgment standards for pre-existing fractures and incident vertebral fractures [16]. Morphometric criteria for incident fractures combined with a semi-quantitative assessment were thought to provide useful information on the study of clinical osteoporosis, especially for international comparisons. Next, to assume the number of participants in the clinical trial, the incident fracture rate and the risk of incident fracture were analyzed in the patient population, and the number of participants with sufficient statistical power [17] was calculated. Bone resorption marker was an independent risk factor for incident vertebral fractures in Japanese women. When the newly discovered risk factor was incorporated into the inclusion criteria in addition to conventional selection criteria such as age, prevalent fractures and bone mineral density, a reduction of about 40% in the estimated sample size was achieved. Thus, measurement of bone resorption markers is useful in reducing the sample size and the observation period in fracture-prevention studies carried out for developing drugs used to treat osteoporosis.

Materials and methods

Study design

Objective

JOINT was the first national, prospective, randomized, multicenter, open-labeled, blinded endpoints, controlled trial for osteoporosis made up mainly of practitioners of investigators in Japan. The objective of JOINT-02 was to clarify additive efficacy in terms of fracture prevention and safety, QOL and adherence in simultaneous use of alfacalcidol and ALN.

Subjects, intervention and endpoints

Confirmations regarding the patients were made by practitioners based on the inclusion and exclusion criteria (Table 1) after obtaining written informed consent. The

Table 1 Inclusion and exclusion criteria

Inclusion criteria
Postmenopausal osteoporosis[a]
Over 70 years old
Ambulatory patients who do not require any help
Able to answer QOL questionnaire
Corresponds to more than one of A-TOP's risk factors for fracture[b]
Exclusion criteria
Metabolic bone diseases other than osteoporosis[c]
Contraindication to the drugs (ALN or alfacalcidol)
Dysfunction in communication of intentions
Severe degenerative deformation of vertebra
Abnormal heart function
Abnormal hepatic function
Abnormal kidney function
Treatment of osteoporosis by bisphosphonate within 6 months prior to the present study

These thresholds were decided by risk analysis by the A-TOP research group

[a] Over 1 year after menopause

[b] Pre-existing vertebral fracture number ≥ 1; BMD $\leq$ Young Adult Mean -3 SD; Urinary DPD ≥ 7.6 nmol/mmol Cr; or NTX $\geq$ 54.3 nmol BCE/mmol Cr

[c] Hyperparathyroidism and hyperthyroidism were excluded

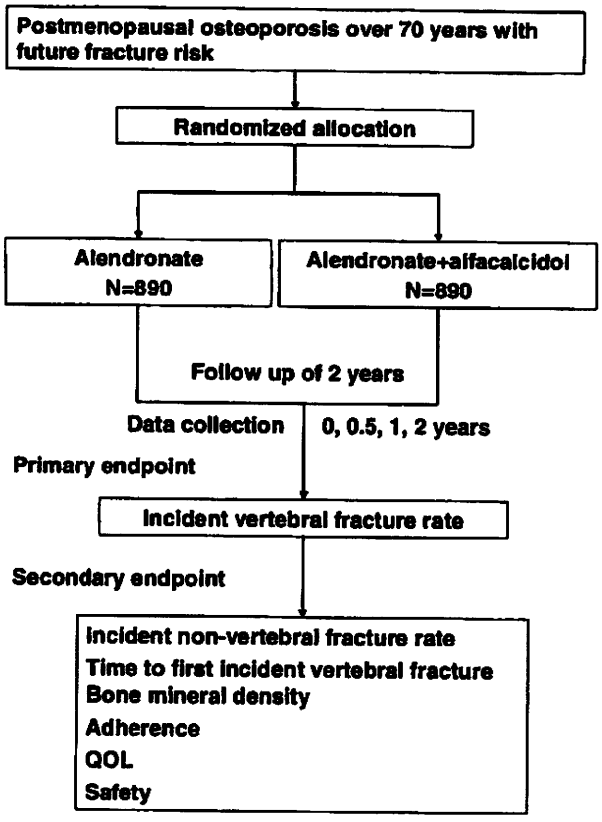

Fig. 1 Study design and outcomes

participants were selected by the practitioners and registered by Japan Clinical Research Support Unit (JCRSU), and then randomly allocated with a modified minimization method using age, number of pre-existing vertebral fracture number, bone mineral density (BMD) and value of bone metabolic marker into the group to be administered only ALN or the group that was to be administered both ALN and alfacalcidol. Registration and allocation of the participants were carried out on the Internet. After initiation of the assigned treatment, clinical data were collected at intervals of half a year for 2 years by I'cros Co., Ltd., through their visiting data collection service. Data were input to the database using a web system developed by ING Corporation. The primary endpoint was to compare the incident vertebral fracture rate between the intervention arms. The secondary endpoints were to compare the differences in the time to first incident vertebral fracture, non-vertebral fracture rate, bone mineral density, adherence, QOL and safety (Fig. 1). In addition, sub-group analyses categorized by baseline characteristics such as age, body mass index (BMI), serum 25-hydroxyl vitamin D levels, the number of pre-existing vertebral fractures and fracture grade were candidate factors. If participants wanted to change the designated treatment because of side effects or occurrence of fractures, they were permitted to do so, and follow-up observations were continued. Please see Table 1 and Fig. 1.

Sample size

Assumptions regarding the fracture rate in the ALN group were made based on a paper by Kushida et al. (Phase III trial for alendronate), in which it is reported that there was a 12.2% fracture rate during observations conducted over 2 years [8]. Since there are not much data on concurrent use of ALN and alfacalcidol [18], the authors' expectations were such that the effects of ALN would be added to those of alfacalcidol and that the hazard ratio of the alfacalcidol combined arm to ALN alone would be 0.64 [19]. The sample size was then estimated to be 890 cases per arm (two-sided alpha = 0.05, power = 0.8), taking account of a dropout rate of 10% referring to the value of the prior clinical trial of fracture intervention [4].

Fracture evaluation

X-ray films of conventional lateral radiographs of lumbar and thoracic vertebrae were taken and collected by I'cros Co., Ltd. After masking the patient's information, two

independent readers (orthopedist, TN and radiologist, MF) simultaneously reviewed films from T4 to L4 in chronological sequence and graded vertebral fracture based on a semi-quantitative method [20]. Before the start of the study, these two observers held meetings to make adjustments between their own criteria for grading vertebral fractures. When the diagnosis of pre-existing fractures made by the reviewers differed from those made by the practitioners, the reviewers' diagnosis was adopted preferentially. If inconsistencies arose between the readers in diagnosing pre-existing and incident vertebral fractures, the two readers negotiated between themselves to reach a consensus. Incident bone fractures other than those of the vertebrae were comprehended from the chart, and the occurrence of fractures was confirmed based on X-ray films or a record of the operation.

Clinical data

BMD at the lumbar vertebrae, hip (proximal femur), distal radius (dual energy X-ray absorptiometry) or left-sided second metacalpal bone (microdensitometry) was measured at baseline and at 6-month intervals for 2 years at each institute. The data of BMD obtained from the different machines and from different bone sites were calculated as the percentage change in each time point from the baseline value. The statistical difference in change of BMD between the group that received combined treatment and the group that was administered only ALN was compared based on each set of data for BMD for the different bone sites. Body height was measured at baseline, 12 and 24 months. Bone turnover markers (urinary type I collagen cross-linked N-telopeptide or urinary excretion of deoxypyridinoline) were measured at baseline and again 6 months after initiating treatment. QOL was assessed by using self-administered questionnaires (JOQOL and EQ-5D) at baseline and at 6, 12 and 24 months after initiating treatment [21]. Serum samples were sent to the central laboratory (SRL Co., Japan), and 25-hydroxyl vitamin D concentrations were measured. Other routine biochemical examinations were carried out at baseline and at 2 years after initiating treatment in order to estimate biochemical adverse events. All adverse events were reported to JCRSU, coded by MedDRA, and categorized as either "known" or "unknown." If an unknown adverse effect occurred, it was reported to the investigator and ethical committee.

Ethics and registration

Ethical issues regarding protocol were reviewed by the ethical committee for JOINT under the Declaration of Helsinki (Dr. Rikushi Morita, Chairman). If it turned out that a patient was at a disadvantage under observation, the ethical committee was given permission to stop the protocol. This study was registered at UMIN-CTR (University Hospital Medical Information Network—Clinical Trial Registry) with the number C000000001.

Statistical analysis

Analysis of the intent to treat principle was applied to the statistical analysis. Efficacy analysis uses a full analysis set (FAS), and all of the enrolled patients are applied to the analysis except for patients without efficacy data, patients who do not correspond to inclusion criteria and patients who do not receive treatment. The PPS (protocol per set) group is defined as consisting of patients without any serious protocol violation.

Recruitment of the practitioner

The explanatory meeting of the protocol and registration was held in all of Japan. The executive members of the A-TOP research group were responsible for the presentation of the protocol and for the recruitment of study institutions and practitioners. The A-TOP committee created the WEB site on the Internet so that the registration of the study could be executed directly. I'cros Co., Ltd., was also involved as a collaborator in calling practitioners.

Results and discussion

Several principles had to be considered for acceptance of concurrent treatment: firstly, the concurrent treatment should be clinically and statistically significantly more effective than the basic treatment; secondly, the concurrent treatment should be of the same level of safety as single treatment; thirdly, the concurrent treatment should be cost-effective. Although these principles should be evaluated before adopting concurrent treatment, the authors have applied concurrent treatment to osteoporosis widely, without any background evidence. Among these principles, the authors have decided to evaluate the first two issues, effectiveness and safety, in the present study. Since this type of evaluation is absolutely required by a clinician, a researcher-initiative study was considered as being the most suitable type of evaluation. This was the reason why the authors decided to use a researcher-initiative clinical trial in determining the effectiveness of concurrent treatment for osteoporosis. The JOINT-02 protocol was the first randomized, controlled trial conducted nationwide for osteoporosis in Japan initiated by researchers, and its scale was also the largest ever. It was therefore necessary for various organizations to collaborate together, and there

have been no previous reports on how to manage the PROBE trial in Japan. This is why we wanted to report the design of JOINT-02. In this paper, we have presented the organization of the A-TOP research group and execution of the JOINT 02 protocol. This is because we believe that this report should help a researcher who is willing to build a new nationwide investigation that is constructed by an organization of clinical research work.

Since the primary aim of JOINT-02 was to determine whether concurrent treatment using ALN and alfacalcidol is superior to treatment using ALN alone in terms of fracture prevention, true ("hard") endpoints such as vertebral fractures or long bone fractures were selected as the primary endpoint. The diagnosis of whether vertebral fractures were present or not on the X-ray films was made by two independent reviewers who did not have any information about the patient; when the judgment of fractures was split between the two reviewers, the reviewers negotiated with each other. Identifying vertebral fractures is more difficult than identifying long bone fractures due to some cases of new vertebral fractures not showing clinical symptoms and the shape of the vertebral body making it difficult at times to recognize whether there is a fracture. Therefore, to avoid misdiagnosis, diagnosis of pre-existing and incident vertebral fractures was made by two different observers. In cases where there was a discrepancy in the diagnosis of vertebral fractures between the reviewers and the practitioner, which occurred with regard to pre-existing fractures, the reviewers' judgment was given priority over that of the practitioner.

Surrogate ("soft") endpoints such as change in BMD or bone turnover markers were considered to be inadequate as primary endpoints in making conclusive statements regarding the efficacy of concurrent treatment. In this type of study, the soft endpoint (BMD or biomarkers) will connect dropout bias less effectively than the case-selected hard endpoint, because such markers are not able to be made blind to the clinicians. Furthermore, previous studies indicate that changes in BMD or bone markers do not predict future fractures [22–24].

In recent literature, it has been reported that poor adherence of bisphosphonates leads to a decline in the beneficial effects of this drug on bone [25–28]. It is expected that in contrast to prior developmental trials, this current study may have a higher dropout rate, since in a researcher-initiative study, the registered practitioner is not forced to maintain adherence very strictly. As a result, adherence in the present study may resemble the actual circumstances of adherence to bisphosphonate treatment by a general practitioner. It will be interesting to determine whether adherence in this study modifies fracture prevention by alendronate.

Although a careful plan for this study has been set up, the results will be applied to osteoporosis patients with the same background as the present study population, but not adapted to the entire osteoporosis population. Despite this limitation, we believe that the results will give us very important information regarding the concurrent treatment of osteoporosis.

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