J Musculoskelet Neuronal Interact 2013; 13(4):454-463

Original Article

Hylonome

Acetylcholinesterase inhibitors and healing of hip fracture in Alzheimer's disease patients: a retrospective cohort study

H. Eimar[1,‡], A. Perez Lara[2,4‡], I. Tamimi[3], P. Márquez Sánchez[2], I. Gormaz Talavera[3], F. Rojas Tomba[3], T. García de la Oliva[3], F. Tamimi[1]

[1]Faculty of Dentistry, McGill University, Montreal, Quebec, H3A 0C7, Canada; [2]Radiology Department, Hospital Regional Universitario Carlos Haya, Malaga, 29002, Spain; [3]Traumatology and Orthopedic Surgery Department, Hospital Regional Universitario Carlos Haya, Malaga, 29017, Spain; [4]Hospital Punta de Europa, Algeciras, Cádiz, 11207, Spain; [‡]The first two authors contributed equally to this work

Abstract

Objectives: This study was designed to assess effects of cholinergic stimulation using acetylcholinesterase inhibitors (AChEIs), a group of drugs that stimulate cholinergic receptors and are used to treat Alzheimer's disease (AD), on healing of hip fractures. **Methods:** A retrospective cohort study was performed using 46-female AD patients, aged above 75 years, who sustained hip fractures. Study analyses included the first 6-months after hip fracture fixation procedure. Presence of AChEIs was used as predictor variable. Other variables that could affect study outcomes: age, body mass index (BMI), mental state or type of hip fracture, were also included. Radiographic union at fracture site (Hammer index), bone quality (Singh index) and fracture healing complications were recorded as study outcomes. The collected data was analyzed by student's-t, Mann-Whitney-U and chi-square tests. **Results:** No significant differences in age, BMI, mental state or type of hip fracture were observed between AChEIs-users and nonusers. However, AChEIs-users had better radiographic union at the fracture site (relative risk (RR),2.7; 95%confidence interval (CI),0.9-7.8), better bone quality (RR,2.0; 95%CI,1.2-3.3) and fewer healing complications (RR,0.8; 95%CI,0.7-1.0) than nonusers. **Conclusion:** In elderly female patients with AD, the use of AChEIs might be associated with an enhanced fracture healing and minimized complications.

Keywords: Fracture Healing, Bone, Acetylcholine, Acetylcholinesterase Inhibitors, Parasympathetic Nervous System

Introduction

The central nervous system affects bone remodeling through the adrenergic and cholinergic branches of the autonomous nervous system[1-4]. Adrenergic activity has been associated with bone resorption and drugs that inhibit this activity have been found to increase bone accrual, reduce the risk of hip fractures and accelerate fracture healing[5,6]. In contrast, cholinergic activity has been recently found to have a positive effect on bone accrual[1,7-10], however, this physiological mechanism has never been explored as potential therapy for bone diseases such as osteoporosis and fracture healing.

One way of stimulating cholinergic activity is by administration of cholinergic agonists such as acetylcholinesterase inhibitors (AChEIs)[11]. AChEIs are a group of drugs that cause stimulation of cholinergic receptors by inhibiting the action of acetylcholinesterase; thus, increasing the levels of acetylcholine in the synaptic space. These drugs are widely used to treat Alzheimer's disease (AD) and other forms of dementia since the mid-1990s[12].

Alzheimer's disease (AD) is characterized by a degradation of the hypothalamus, a brain structure that encloses cholinergic components[13]. Surprisingly, AD patients suffer from a low bone mineral density that is highly correlated with the cholinergic degradation of the hypothalamus[14,15]. Due to the reduced bone density, it has been shown that AD patients are more prone to bone fractures, particularly hip fractures, compared

The authors have no conflict of interest.

Corresponding author: Faleh Tamimi, Faculty of Dentistry, McGill University, 3640 University Street, Montreal, Quebec H3A 0C7, Canada
E-mail: faleh.tamimimarino@mcgill.ca

Edited by: J. Rittweger
Accepted 13 October 2013

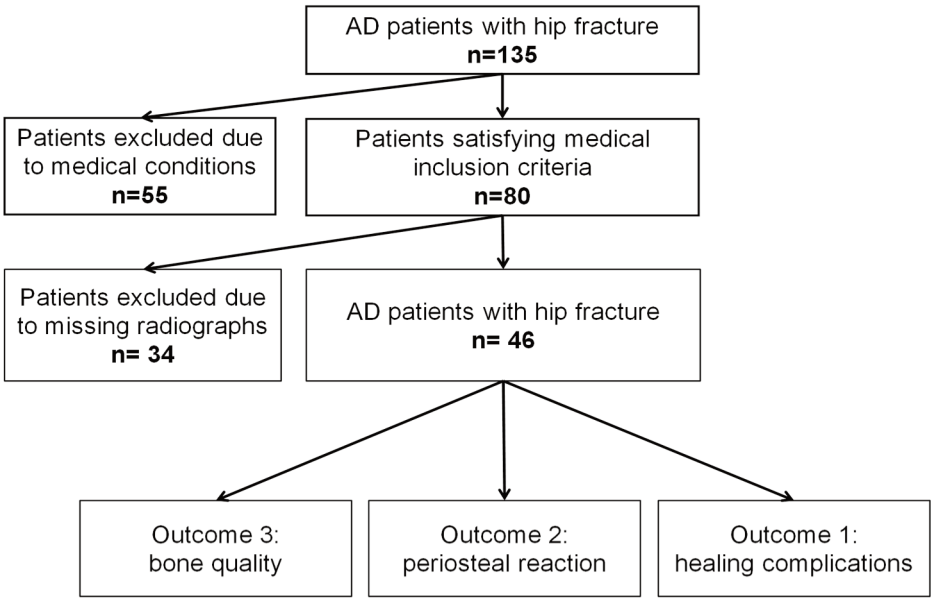

Figure 1. Flow chart describing the selection process of the participants in our cohort study.

with the rest of population[16-18]. Healing of hip fractures in AD patients is usually slow and often results in delayed-union, non-union, need for re-intervention, or even death[19]. Previously, we have demonstrated that AD patients who are receiving AChEIs, are associated with lower risk of hip fracture[9]. However, the potential effect of these drugs on fracture healing has not been explored yet.

We hypothesize that the administration of AChEIs may have a beneficial effect on bone regeneration that could accelerate fracture healing. In order to test this hypothesis, we have designed a *retrospective cohort* study in which we compared the healing of hip fractures in a group of AD patients under AChEI treatment with another group of AD patients receiving no AChEIs. In this paper, we provide evidence for a potential approach to enhance fracture healing and reduce clinical complications, through stimulation of cholinergic activity by AChEIs.

Materials and methods

Study design

Approval from the ethical committee at Carlos Haya Hospital, in Malaga, Spain, was obtained to carry out a retrospective cohort study on AD patients who sustained hip fracture injuries at the same hospital. Patients records were identified in the computerized database of the Department of Traumatology, and the original hardcopy files were retrieved for manual examination. The overall study period was 8 years, between January 1, 2004 and December 31, 2012. All the hip fractures in AD patients that occurred within the study period were reviewed.

Patients, who were female, aged between 75 and 95 years at the date of the fracture, were included in this study. Our study did not include male patients since the number of male patients who satisfied our inclusion and exclusion criteria was too low. Accordingly, our study excluded male patients to control the number of parameters (variables) that could affect the study outcomes. Patients who were smokers or previously diagnosed with any of the following diseases, which are known to substantially affect bone metabolism and fracture healing, were excluded: osteomalacia, paget disease, Vitamin D deficiency, hyperthyroidism, Cancer [excluding non-melanoma skin cancer], Alcoholism, patients on corticosteroids, patients on anti-epileptic drugs, patients on bisphosphonates. Patients were also excluded if their five-week postsurgical radiographs were missing.

Hip fractures were defined according to the International Classification of Diseases (Tenth Revision, Codes 72.0-72.2) as a fracture of the proximal femur ranging from the femoral neck (intracapsular) to the subtrochanteric (extracapsular) region. The analyses included the first six months after operation.

A total of 135 AD patients who suffered hip fractures were identified in our health care area during the study period; among these patients 80 fulfilled our medical inclusion criteria (Figure 1). Due to missing radiographs, 34 patients were excluded; and therefore, 46 patients were included in our final assessment model (Figure 1).

Study variables

The following parameters which may affect the study outcomes were retrieved from the patients' files, computerized records and standardized questionnaires: patient age, body mass index (BMI), Charlson comorbidity score (CCS) and the grade of AD according to the clinical dementia rating scale (CDR)[20-23]. In addition, information about type of hip fracture: intracapsular or extracapsular fractures were retrieved. The presence of AChEIs was considered as the predictor variable.

Study outcomes

Degree of calcification and bone quality

Five weeks-postsurgical plain pelvic radiographs (antero-posterior), taken as part of the routine follow-up, showing the fractured hip and non-fractured hip of the AD patients were retrieved. All of the retrieved radiographs were performed at our department using a digital conventional hip x-rays machine (General Electric Corporation, Milwaukee, WI) with an adjusted X-ray exposure to match the anatomy of patient being examined. The retrieved radiographs were examined by two experienced musculoskeletal radiologists blinded to the patient treatment group. Inter-observer agreement among the two radiologists was significantly high ($\varkappa$=0.824; 95%CI=0.691-0.957; p<0.001).

The following parameters were retrieved from the radiographs: the degree of calcification (radiographic union) at the fracture site and the bone quality at the non-fracture site.

Degree of radiographic union at the fracture site was assessed following the criteria proposed by Hammer et al[24]. Hammer et al classified the degree of radiographic union of bone fracture into 5 grades; from grade 1 (corresponding to a complete calcification) to grade 5 (corresponding to a fracture without any evidence of calcification), based on the quality of the bridging callus and the presence or absence of a fracture line (Hammer union index)[24].

Bone quality at the non-fracture site was assessed following the criteria proposed by Singh et al[25]. Singh et al classified bone quality into 6 grades; from grade 1 (corresponding to a poor bone quality) to grade 6 (corresponding to a normal bone quality), based on the trabeculae morphology and distribution at the proximal femur (Singh index)[25].

Healing complication

Fracture healing complications that occurred within 6 months from the date of hip fracture were retrieved from the patients' files. The investigated complications included the presence of infection, delayed-union, new hip fractures and surgical re-intervention.

Statistical analyses

Inter-observer agreements for the evaluations of the degree of bone calcification (radiographic union) of the fracture site and bone quality using radiographs were done by Kappa Test ($\varkappa$). The value ranged from +1, with perfect agreement, to -1, which corresponds to absolute disagreement.

Clinical outcomes (degree of fracture calcification, bone quality and fracture healing complications) as a function of patients' characteristics (age, BMI, CCS, CDS and type of hip fracture) were tested using unpaired Student T-test and Mann-Whitney U test. AD Patients' characteristics and clinical outcomes as a function of the presence of AChEIs treatment were tested using unpaired Student T-test and chi-square test. Ratios of the probabilities (relative risk) of the study outcomes were presented with 95% confidence intervals (CIs), and accompanied *p* values. Values of *p* were two-sided and considered statistically significant if less than 0.05.

Sample sizes calculation to reject the null hypothesis, that states the study outcomes in AChEIs users and non-users are equal, were conducted using the chi-square test based on the following inputs: (i) expected fair radiographic union at the fracture site (hammer index ≤3) at the 5th-week in 100% of AChEIs users versus 60% in nonusers (controls)[26]; (ii) type I error probability (α) equals to 0.05. It was determined that at least fifteen patients in each group would be needed to reach a power of 80%. In addition, Post-hoc power calculations (following Fleiss test), to reject our null hypothesis, were conducted based on the following inputs: (i) number of included AChEIs users; (ii) ratio between AChEIs nonusers to AChEIs users; and (iii) probabilities of each outcome in AChEIs users and non-users[27].

Results

Study outcomes distribution in AD patients

Degree of radiographic union (calcification)

Analyses of the retrieved radiographs demonstrated that 3 hip fractures had grade 2, 11 hip fractures had grade 3, 10 hip fractures had grade 4 and 11 hip fractures had grade 5 on fracture healing scale (Hammer union index). Degree of calcification of the fracture site could not be evaluated in 11 radiographs since the hip fracture was treated with a hip hemiarthroplasty.

Due to the small number of hip fractures in each grade, we categorized them into 2 groups: hip fractures that showed grade 3 or less (fair healing) and hip fractures that showed higher than grade 3 (poor healing) on fracture healing scale. The distribution of these two groups as function of AD patients' characteristics is shown in Table 1. AD patients who showed calcification of the fracture site of grade 3 or lower did not have significant differences in age, BMI, mental state or type of hip fracture compared to those who had degree of calcification higher than grade 3 (Table 1). Moreover, degree of radiographic union at the fracture site was not significantly related to bone quality measured at the non-fracture hip (RR,1.5; 95%CI, 0.9-2.7; p=0.129).

Degree of bone quality

Analyses of the retrieved radiographs demonstrated that 10 non-fracture hips had grade 2, 19 non-fracture hips had grade 3, 10 non-fracture hips had grade 4, 3 non-fracture hips had grade 5 based on bone quality scale (Singh index). Bone quality could not be evaluated in 4 radiographs due to the poor quality of radiographs showing the non-fracture hip.

Due to the small number of non-fracture hips in each grade, we categorized them into 2 groups: non-fracture hips that showed grade 3 or less (poor bone quality) and non-fracture hips that showed higher than grade 3 (fair bone quality) on bone quality scale. The distribution of these two groups as function of AD patients' characteristics is shown in Table 1. AD patients who showed bone quality higher than grade 3 at

Characteristics	Fracture healing index[c]			Bone quality index[f]			Healing complication		
	≤3 (n=14)[d] (fair)	>3 (n=21)[d] (poor)	p-value[e]	>3 (n=13)[g] (fair)	≤3 (n=29)[g] (poor)	p-value[e]	Yes (n=4)	No (n=42)	p-value[h]
Age, years[a]	82.2±4.2	83.4±2.3	0.337	83.0±3.4	82.9±3.5	0.906	81.8±2.2	83.2±3.7	0.460
BMI[a,b]	26.8±4.6	25.4±3.9	0.359	27.4±5.6	24.9±3.5	0.081	22±1.6	25.9±4.3	0.076
CDR[a]	1.8±0.7	2.2±0.8	0.118	1.8±.7	2.0±0.8	0.193	2.2±0.7	2.0±0.7	0.608
CCS[a]	2.3±1.3	2.4±1.6	0.407	1.9±1.0	2.2±1.2	0.418	1.8±0.5	2.3±1.4	0.407
Type of hip fracture									
Extracapsular	12	15	-	-	-	-	1 (25%)	26	-
Intracapsular	2	6	0.424	-	-	-	3 (75%)	16	0.152

Abbreviation: AD: Alzheimer's disease; AChEIs: acetylcholinesterase inhibitors; BMI: body mass index; CDR: clinical dementia rating scale; CCS: Charlson comorbidity score.
[a] Values are expressed as the mean and standard deviation.
[b] Measured as weight in kilograms divided by the square of height in meters.
[c] Measured based on criteria proposed by Hammer et al.[18]
[d] Missing data: degree of fracture healing could not be evaluated for 11 patients since their hip fractures were treated with a hip hemiarthroplasty.
[e] Calculated with Student's t-test for independent samples.
[f] Measured at the non-fracture hip based on criteria proposed by Singh et al.[19]
[g] Missing data: Singh index could not be evaluated for 4 patients since the radiographs showing the non-fractured hip were poor.
[h] Calculated with Mann-Whitney U test for independent samples.
p-value was calculated by chi-square test.

Table 1. AD patients' characteristics as a function of the study outcomes.

Characteristics	Users of AChEIs (n=24)	Nonusers of AChEIs (n=22)	p-value
Age, years[a]	82.9±3.6	83.2±3.6	0.807[b]
BMI[a,d]	26.1±4.3	24.5±3.9	0.102[b]
CDR[a]	1.8±0.7	2.3±0.7	0.069[b]
CCS[a]	2.5±1.3	2.0±1.1	0.170[b]
Type of hip fracture			
Extracapsular	15 (63%)	12 (55%)	-
Intracapsular	9 (37%)	10 (45%)	0.584[c]

Abbreviation: AD: Alzheimer's disease; AChEIs: acetylcholinesterase inhibitors; BMI: body mass index; CDR: clinical dementia rating scale; CCS: Charlson comorbidity score.
[a] Values are expressed as the mean and standard deviation.
[b] p-value was calculated by Student's t-test for independent samples.
[c] p-value was calculated by chi-square test.
[d] Measured as weight in kilograms divided by the square of height in meters.

Table 2. AD patients' characteristics as a function of AChEIs treatment.

the non-fracture site did not have significant differences in age, BMI, mental state or type of hip fracture compared with those who had bone quality of grade 3 or less (Table 1).

Healing complications

The distribution of healing complications observed at the fracture site as function of AD patients' characteristics is shown in Table 1. AD patients with fracture healing complications did not have significant differences in age, BMI or mental state compared with those without complications (Table 1). However, all of the reported complications at the fracture site were observed in patients who had poor bone qualities (bone quality index ≤3) (RR,1.5; 95%CI, 1.2-1.9; p=0.159).

Three, out of four complications, were observed in patients who suffered from intracapsular hip fractures and the forth complication was in a patient who suffered from an extracapsular hip fracture (RR=0.8; 95%CI, 0.7-1.0; Power=0.56; p=0.152; Table 1). The reported complications were 2 cases of post-surgical infections, 1 case of new hip fracture at a nearby site and 1 delayed-union case. Both infection cases occurred in patients who were previously treated with a hip hemiarthoplasty. These cases who suffered from infections were retreated

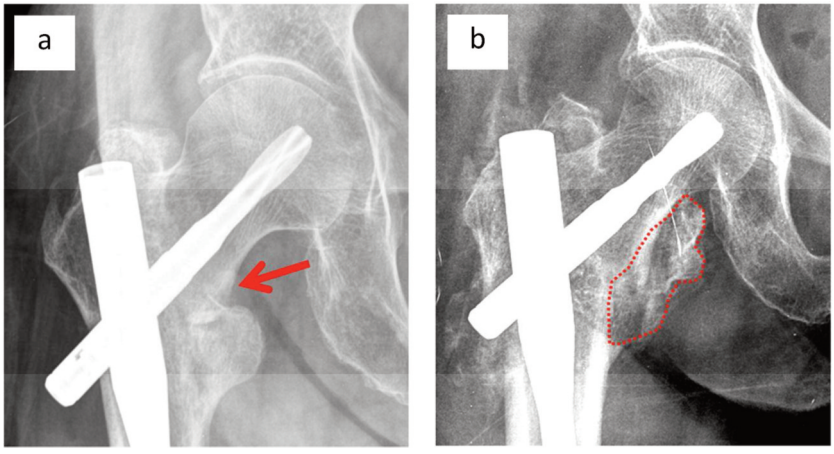

Figure 2. Frontal radiograph of two trochanteric hip fractures fixed with a proximal femoral nail antirotation (PFN-A, Synthes®, Solothurn, Switzerland) in an AD patient not receiving AChEIs (a) and another one receiving AChEIs treatment (b), respectively. Radiographs were taken 5 weeks after the initial intervention. It can be observed that there is a large callus formation in the fracture of the patient treated with AChEIs (area within red-dot-line), but there is no periosteal reaction in the fracture of the patient not treated with AChEIs (red arrow).

with Gridlestone osteotomy, however, one of them died 4 months following the operation, while second patient survived for more than 5 years. Patient with the delayed-union of the hip fracture was retreated with Gridlestone osteotomy; due to high risk of surgical complications and low mobility demand.

AChEIs distribution in AD patients and study outcomes

Among the patients included, 24 were AChEIs users and 22 were non-users. Out of 24 AChEIs users, 10 patients had been treated with rivastigmine 3-12 mg/day for 12-48 months, 7 patients had been treated with donepezil 5-10 mg/day for 12-36 months and 7 patients had been treated with galantamine 6-24 mg/day for 12-48 months prior to enrolment.

The distribution of the presence of AChEIs in the study group as a function of age, BMI, CDR, CCS and type of hip fracture is shown in Table 2. Users of AChEIs did not have significant differences in age, BMI, mental state or type of hip fracture compared with AChEIs non-users (Table 2).

Interestingly enough, users of AChEIs were associated with a better degree of calcification at the fracture site (fracture healing index ≤3) and a better bone quality (bone quality index >3) than nonusers (Figure 2 and Table 3). Moreover, patients using AChEIs had no complications following hip fracture fixation than nonusers.

Discussion

In this study we provided the first clinical evidence for the previously unexplored potential role of cholinergic stimulation on fracture healing. Based on our cohort study, AD patients receiving AChEIs such as rivastigmine, donepezil or galantamine expressed accelerated radiographic union (calcification) at the fracture site, better bone quality and less postoperative comorbidity compared to AChEIs-nonusers.

Healing of hip fractures in elderly, such as AD patients, is slow and usually requires a long-term immobilization of the fracture ends leading to greater morbidity and mortality[28-33]. Accordingly, several studies were conducted to find new therapeutic approaches to accelerate bone regeneration and fracture healing[34-36]. Nowadays, the only approved drugs that are commonly prescribed to improve fracture healing are parathyroid hormone (PTH) analogs[34,37]. However, even though PTH analogs are successful therapies for fracture healing, their use is limited due to the high cost and side effects[38]. In our study, we show that AChEIs might accelerate the radiographic union at the fracture sites, which may enhance the healing process. Accordingly, future *in vivo* and longitudinal clinical studies should be conducted in order to investigate the positive effect of AChEIs on bone quality and fracture healing.

AChEIs accelerate calcification at the fracture site

In this study, healing of hip fractures was evaluated radiologically, by two radiologists who showed a high level of agreement, following criteria proposed by Hammer et al[24]. This method has been previously used to evaluate radiographic union of the fracture in several clinical studies[32,33] with a moderate overall general agreement[39].

In this study, AD patients who showed a high degree of calcification of their hip fractures (grade 3 or less on fracture healing index) had similar demographic characteristics to those patients who showed a poor calcification degree (higher than grade 3 on fracture healing index). Moreover, our study illustrated that types of hip fracture (intracapsular and extracapsular) or quality of bone (assessed by Singh index) did not significantly influence the radiographic union of the hip fracture. However, our results indicated that most hip fractures showing higher degree of calcification or radiographic union

Characteristics	Users of AChEIs (n=24)	Nonusers of AChEIs (n=22)	Crude RR (95%CI)	*Power*	*p-value*[e]
Fracture healing index[a]					
>3 (poor)	9 (38%)	12 (56%)	-		-
≤3 (Fair)	11 (46%)	3 (14%)	2.7 (0.9-7.8)	0.56	0.036
Missing[b]	4 (16%)	7 (32%)	0.9 (0.5-1.6)	0.05	1.000
Bone quality indexc					
≤ 3 (Poor)	9 (37%)	20 (91%)	-		-
>3 (Fair)	11 (46%)	2 (9%)	2.0 (1.2-3.3)	0.92	0.001
Missing[d]	4 (16%)	0 (0%)	0.31 (0.2-0.5)	0.04	0.018
Healing complication					
No	24 (100%)	18 (82%)	-		-
Yes	0 (0%)	4 (18%)	0.8 (0.7-1.0)	0.56	0.029

Values are mean (95% confidence interval).
Abbreviation: AD: Alzheimer's disease; AChEIs: acetylcholinesterase inhibitors; RR: Relative Risk.
[a] Measured based on criteria proposed by Hammer et al.[19]
[b] Missing data: degree of fracture healing could not be evaluated for 4 AChEIs users and 7 AChEIs nonusers since their hip fractures were treated with a hip hemiarthroplasty.
[c] Measured based on criteria proposed by Singh et al.[20]
[d] Missing data: Singh index could not be evaluated for 4 AChEIs users since the radiographs showing the non-fractured hip were poor.
[e] p-value was calculated by chi-square test.

Table 3. Study outcomes as a function of AChEIs treatment.

were observed in AD patients receiving AChEIs (Table 3), indicating that more bone regeneration might have occurred in these patients. These findings represent the first clinical evidence demonstrating that administration of AChEIs might be associated with an enhanced hip fracture healing. However, future research will have to be performed in order to assess whether the administration of these drugs are also associated with faster stability of the fracture site.

AChEIs favor bone mass

This study investigated the bone quality at non-fracture sites using Singh index as described by Sernbo et al[40]. This method has been previously used to evaluate bone mineral density in clinical studies, and it has been shown to have a good correlation with results obtained from Dual-energy X-ray Absorptiometry (DXA), the most popular tool to measure bone mineral density[41-45].

In this study, AD patients with bone quality higher than grade 3 based on bone quality index had similar patients' characteristic to those with bone quality of grade 3 or below. However, our results indicated that AChEIs users had better bone quality (bone quality index >3) than non-users. These results are aligned with our previous clinical study, in which it has been shown that users of AChEIs were associated with a lower risk of hip fracture[9], and it might open a new insight to treat bone diseases such as osteoporosis and minimize bone fractures.

Singh index refers to the architectural quality of trabecular network which is affected mainly by bone resorption rather than bone formation. The higher Singh index score observed in AChEIs users compared to nonusers might be related to the fact that these drugs would suppress bone resorption rate by promoting osteoclasts (bone-resorbing cells) apoptosis[f], independently or in addition to their potential role in promoting the proliferation of osteoblasts (bone-forming cells)[4]. However, future research will have to be performed to confirm these hypotheses.

AChEIs minimize healing complications

Healing complications following hip fractures in elderly patients are common. A metaanalysis study reported that healing complications might occur in 49% of patients who suffered hip fractures[46]. Hip fracture typically results in a 1.8-year decrease in life expectancy[31,32]. Most of deaths due to hip fractures occur within the first 3-6 months following the event[31,32]. The remaining mortality beyond the initial 6 months is associated with institutionalization and new deficits that are only indirectly related to the fracture itself[31,32].

In this study, we detected four healing complications that occurred within 6 months after the hip fracture fixation procedure. Healing complications were not associated with patient's age, gender, BMI, or with their mental status. Interestingly, our study illustrated that all complications at the fracture site were observed in patients who were not AChEIs users, indicating a potential positive effect of AChEIs on minimizing the fracture healing complications. However, healing complications were also found in patients who showed poor bone quality as assessed by Singh index. Accordingly, the observed complications at the fracture sites might not be solely dedicated to the un-usage of AChEIs.

The mechanism by which AChEIs accelerate fracture healing

In this study, we investigated the effect cholinergic stimulation through the use AChEIs on bone mass. We included

three AChEIs: rivastigmine, donepezil and galantamine, that are capable to stimulate cholinergic receptors: nicotinic and muscarinic, located peripherally and within the central nervous system[47]. Cholinergic activity is known to favor bone mass either indirectly through suppression of the adrenergic activity and/or directly through suppression of osteoclasts proliferation[1,7]. Also, substantial observations pointed toward a possible direct up-regulation of osteoblasts by cholinergic activity[4,48,49].

Limitations and future directions

One limitation of this study was the small number of patients included. Despite this, we found that there is a strong association with a good power between radiographic union of hip fractures, bone quality and healing complications in one hand and the use of AChEIs on the other hand. Another limitation in our study was the use of X-rays radiography to assess the effect of AChEIs on bone healing and bone quality. Analysis of radiographs is subjective; however, it was conducted by two radiologists who were blinded to the treatment patient group, and yet showed a high inter-observer agreement among their results.

One more limitation in this study is that Hummer index was applied to assess the radiographic union of hip fractures from radiographs recorded in an antero-posterior view. Ideally, in order to assess the degree of radiographic union of bone fracture using Hammer index, two radiographs (antero-posterior and lateral radiographs) taken from perpendicular positions is recommended[24]. Another limitation present in our study is that the radiographic union assessment of the fracture site did not include 4 AChEIs users and 7 AChEIs non-users, since their hip fractures were fixed by prostheses. However, those missing participants did not significantly affect the results of radiographic union of the fracture site (Table 3). We also could not assess the bone quality for other 4 AChEIs users due to the poor quality of radiographs showing the non-fracture sites (Table 3). However, comparing the characteristics, radiographic union of the fracture site and fracture healing complications of these excluded AChEIs users versus the rest of participants were not significant (Table Supplementary 2), indicating a small potential effect of these patients on bone quality results reported in this study.

An additional limitation of this study is that the relationship of rivastigmine, donepezil and galantamine dosages and durations with the study outcomes could not be assessed for statistical significance due to the small number of participants who used these drugs. In Table supplementary 1, we assessed the association of each of the included AChEI with the study outcomes.

One more limitation in this study is the missing information about the recovery process that patients received after the hip fracture fixation procedure which might affect the study outcomes. A further limitation in this study was the missing information about the criteria used by physicians to prescribe AChEIs for AD patients[50]. It has been found that physicians are less likely to prescribe AChEIs for patients with high number of comorbidities[50], which could affect our results. Nevertheless, the comorbidity grades, based on CCS, of the AD patients included in our study were not associated with the study outcomes: fracture healing, bone quality and healing complication.

For all of these reasons, future large-scale prospective randomized clinical trials, adjusted to all possible variables that might influence study outcomes, will have to be performed in order to confirm the findings of this study. Moreover, *in vivo* and clinical studies assessing biochemical bone markers, bone density measurements, and bone microarchitecture will have to be performed to explore the effect of AChEIs on bone structure and mineral content.

Conclusion

To the best of our knowledge this is the first clinical evidence demonstrating that the administration of AChEIs might be associated with a faster calcification (radiographic union) of hip fracture and a better bone quality in female, aged between 75 and 95 years AD patients. These findings might help identify new therapeutic approaches to accelerate bone regeneration and fracture healing, although additional clinical studies are needed to confirm these potentially important findings.

References

1. Shi Y, Oury F, Yadav VK, Wess J, Liu XS, Guo XE, Murshed M, Karsenty G. Signaling through the M3 Muscarinic Receptor Favors Bone Mass Accrual by Decreasing Sympathetic Activity. Cell Metab 2010;3:231-238.
2. Walker LM, Preston MR, Magnay JL, Thomas PB, El Haj AJ. Nicotinic regulation of c-fos and osteopontin expression in human-derived osteoblast-like cells and human trabecular bone organ culture. Bone 2001;6:603-8.
3. Hoogduijn MJ, Cheng A, Genever PG. Functional nicotinic and muscarinic receptors on mesenchymal stem cells. Stem Cells Dev 2009;1:103-12.
4. En-Nosse M, Hartmann S, Trinkaus K, Alt V, Stigler B, Heiss C, Kilian O, Schnettler R, Lips KS. Expression of non-neuronal cholinergic system in osteoblast-like cells and its involvement in osteogenesis. Cell Tissue Res 2009;2:203-215.
5. Minkowitz B, Boskey AL, Lane JM, Pearlman HS, Vigorita VJ. Effects of propranolol on bone metabolism in the rat. J Orthop Res 1991;6:869-75.
6. Schlienger RG, Kraenzlin ME, Jick SS, Meier CR. Use of beta-blockers and risk of fractures. Jama-J Am Med Assoc 2004;11:1326-1332.
7. Bajayo A, Bar A, Denes A, Bachar M, Kram V, Attar-Namdar M, Zallone A, Kovacs KJ, Yirmiya R, Bab I. Skeletal parasympathetic innervation communicates central IL-1 signals regulating bone mass accrual. Proc Natl Acad Sci U S A 2012;38:15455-60.
8. Kliemann K, Kneffel M, Bergen I, Kampschulte M, Langheinrich AC, Duerselen L, Ignatius A, Kilian O, Schnettler R, Lips KS. Quantitative analyses of bone

composition in acetylcholine receptor M3R and alpha7 knockout mice. Life sciences 2012;21-22:997-1002.
9. Tamimi I, Ojea T, Sanchez-Siles JM, Rojas F, Martin I, Gormaz I, Perez A, Dawid-Milner MS, Mendez L, Tamimi F. Acetylcholinesterase inhibitors and the risk of hip fracture in Alzheimer's disease patients: A case-control study. J Bone Miner Res 2012;7:1518-1527.
10. Eimar H, Tamimi I, Murshed M, Tamimi F. Cholinergic regulation of bone. J Musculoskelet Neuronal Interact 2013;2:124-32.
11. Pepeu G, Giovannini MG. Cholinesterase inhibitors and beyond. Curr Alzheimer Res 2009;2:86-96.
12. Massoud F, Gauthier S. Update on the pharmacological treatment of Alzheimer's disease. Curr Neuropharmacol 2010;1:69-80.
13. Callen DJ, Black SE, Gao F, Caldwell CB, Szalai JP. Beyond the hippocampus: MRI volumetry confirms widespread limbic atrophy in AD. Neurology 2001;9:1669-74.
14. Loskutova N, Honea RA, Brooks WM, Burns JM. Reduced limbic and hypothalamic volumes correlate with bone density in early Alzheimer's disease. J Alzheimers Dis 2010;1:313-22.
15. Loskutova N, Honea RA, Vidoni ED, Brooks WM, Burns JM. Bone density and brain atrophy in early Alzheimer's disease. J Alzheimers Dis 2009;4:777-85.
16. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. Lancet 2011;9770:1019-31.
17. Sato Y, Kanoko T, Satoh K, Iwamoto J. Risk factors for hip fracture among elderly patients with Alzheimer's disease. J Neurol Sci 2004;2:107-112.
18. Buchner DM, Larson EB. Falls and fractures in patients with Alzheimer-type dementia. Jama 1987;11:1492-5.
19. Baker NL, Cook MN, Arrighi HM, Bullock R. Hip fracture risk and subsequent mortality among Alzheimer's disease patients in the United Kingdom, 1988-2007. Age and ageing 2011;1:49-54.
20. Busato A, Roder C, Herren S, Eggli S. Influence of high BMI on functional outcome after total hip arthroplasty. Obes Surg 2008;5:595-600.
21. Nandy S, Parsons S, Cryer C, Underwood M, Rashbrook E, Carter Y, Eldridge S, Close J, Skelton D, Taylor S, Feder G, Group obotFPPS. Development and preliminary examination of the predictive validity of the Falls Risk Assessment Tool (FRAT) for use in primary care. Journal of Public Health 2004;2:138-143.
22. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology 1993;11:2412-4.
23. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;5:373-83.
24. Hammer RR, Hammerby S, Lindholm B. Accuracy of radiologic assessment of tibial shaft fracture union in humans. Clin Orthop Relat Res 1985;199:233-8.
25. Singh M, Nagrath AR, Maini PS. Changes in trabecular pattern of the upper end of the femur as an index of osteoporosis. The Journal of Bone & Joint Surgery 1970; 3:457-467.
26. Peichl P, Holzer LA, Maier R, Holzer G. Parathyroid hormone 1-84 accelerates fracture-healing in pubic bones of elderly osteoporotic women. J Bone Joint Surg Am 2011;17:1583-7.
27. JL F: Statistical Methods for Rates and Proportions. pp. 38-46. Edited, 38-46, NewYork, John Wiley, 1981.
28. Gruber R, Koch H, Doll BA, Tegtmeier F, Einhorn TA, Hollinger JO. Fracture healing in the elderly patient. Exp Gerontol 2006;11:1080-93.
29. Wolinsky FD, Fitzgerald JF, Stump TE. The effect of hip fracture on mortality, hospitalization, and functional status: a prospective study. Am J Public Health 1997;3:398-403.
30. Bentler SE, Liu L, Obrizan M, Cook EA, Wright KB, Geweke JF, Chrischilles EA, Pavlik CE, Wallace RB, Ohsfeldt RL, Jones MP, Rosenthal GE, Wolinsky FD. The aftermath of hip fracture: discharge placement, functional status change, and mortality. Am J Epidemiol 2009; 10:1290-9.
31. Kanis JA, Oden A, Johnell O, De Laet C, Jonsson B, Oglesby AK. The components of excess mortality after hip fracture. Bone 2003;5:468-73.
32. Braithwaite RS, Col NF, Wong JB. Estimating hip fracture morbidity, mortality and costs. J Am Geriatr Soc 2003;3: 364-70.
33. Abou-Setta AM, Beaupre LA, Rashiq S, Dryden DM, Hamm MP, Sadowski CA, Menon MR, Majumdar SR, Wilson DM, Karkhaneh M, Mousavi SS, Wong K, Tjosvold L, Jones CA. Comparative effectiveness of pain management interventions for hip fracture: a systematic review. Ann Intern Med 2011;4:234-45.
34. Aspenberg P, Genant HK, Johansson T, Nino AJ, See K, Krohn K, Garcia-Hernandez PA, Recknor CP, Einhorn TA, Dalsky GP, Mitlak BH, Fierlinger A, Lakshmanan MC. Teriparatide for acceleration of fracture repair in humans: a prospective, randomized, double-blind study of 102 postmenopausal women with distal radial fractures. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2010;2:404-14.
35. Kristiansen TK, Ryaby JP, McCabe J, Frey JJ, Roe LR. Accelerated Healing of Distal Radial Fractures with the Use of Specific, Low-Intensity Ultrasound. A Multicenter, Prospective, Randomized, Double-Blind, Placebo-Controlled Study*. The Journal of Bone & Joint Surgery 1997;7:961-73.
36. Wolf S, Janousek A, Pfeil J, Veith W, Haas F, Duda G, Claes L. The effects of external mechanical stimulation on the healing of diaphyseal osteotomies fixed by flexible external fixation. Clin Biomech (Bristol, Avon) 1998; 4-5:359-364.
37. Ellegaard M, Jorgensen NR, Schwarz P. Parathyroid hormone and bone healing. Calcif Tissue Int 2010;1:1-13.
38. Hodsman A, Papaioannou A, Cranney A, Canada O, Comm CG, Parath WGSR. Clinical practice guidelines

for the use of parathyroid hormone in the treatment of osteoporosis. Can Med Assoc J 2006;1:48-51.
39. Whelan DB, Bhandari M, McKee MD, Guyatt GH, Kreder HJ, Stephen D, Schemitsch EH. Interobserver and intraobserver variation in the assessment of the healing of tibial fractures after intramedullary fixation. J Bone Joint Surg Br 2002;1:15-8.
40. Sernbo I, Johnell O. Changes in Bone Mass and Fracture Type in Patients with Hip-Fractures - a Comparison between the 1950s and the 1980s in Malmo, Sweden. Clin Orthop Relat Res 1989;238:139-147.
41. Wachter NJ, Augat P, Hoellen IP, Krischak GD, Sarkar MR, Mentzel M, Kinzl L, Claes L. Predictive value of Singh index and bone mineral density measured by quantitative computed tomography in determining the local cancellous bone quality of the proximal femur. Clin Biomech (Bristol, Avon) 2001;3:257-62.
42. Krischak GD, Augat P, Wachter NJ, Kinzl L, Claes LE. Predictive value of bone mineral density and Singh index for the *in vitro* mechanical properties of cancellous bone in the femoral head. Clin Biomech (Bristol, Avon) 1999; 5:346-51.
43. Karlsson KM, Sernbo I, Obrant KJ, Redlund Johnell I, Johnell O. Femoral neck geometry and radiographic signs of osteoporosis as predictors of hip fracture. Bone 1996; 4:327-330.
44. Masud T, Jawed S, Doyle DV, Spector TD. A Population Study of the Screening Potential of Assessment of Trabecular Pattern of the Femoral-Neck (Singh Index) - the Chingford Study. Brit J Radiol 1995;808:389-393.
45. D'Amelio P, Rossi P, Isaia G, Lollino N, Castoldi F, Girardo M, Dettoni F, Sattin F, Delise M, Bignardi C. Bone mineral density and singh index predict bone mechanical properties of human femur. Connect Tissue Res 2008; 2:99-104.
46. Lu-Yao GL, Keller RB, Littenberg B, Wennberg JE. Outcomes after displaced fractures of the femoral neck. A meta-analysis of one hundred and six published reports. J Bone Joint Surg Am 1994;1:15-25.
47. Weinstock M, Razin M, Chorev M, Enz A. Pharmacological Evaluation of Phenyl-Carbamates as Cns-Selective Acetylcholinesterase Inhibitors. J Neural Transm-Supp 1994;43:219-225.
48. Sato T, Abe T, Chida D, Nakamoto N, Hori N, Kokabu S, Sakata Y, Tomaru Y, Iwata T, Usui M, Aiko K, Yoda T. Functional role of acetylcholine and the expression of cholinergic receptors and components in osteoblasts. FEBS Lett 2010;4:817-24.
49. Liu PS, Chen YY, Feng CK, Lin YH, Yu TC. Muscarinic acetylcholine receptors present in human osteoblast and bone tissue. Eur J Pharmacol 2011;1:34-40.
50. Hoffmann F, van den Bussche H, Wiese B, Schon G, Koller D, Eisele M, Glaeske G, Scherer M, Kaduszkiewicz H. Impact of geriatric comorbidity and polypharmacy on cholinesterase inhibitors prescribing in dementia. BMC Psychiatry 2011:190.

Supplementary Tables

Characteristics	Fracture healing index[a,b]				Bone quality index[c,d]				Healing complication			
	≤3 (n=14)	>3 (n=21)	RR (95%)	P[e]	>3 (n=13)	≤3 (n=29)	RR (95%)	P[e]	Yes (n=4)	No (n=42)	RR (95%)	P[e]
No Drug	3	12	-	-	2	20	-	-	4	18	-	-
Rivastigmine	4	4	1.6 (0.8-3.3)	0.156	4	4	1.8 (0.9-3.7)	0.013	0	10	0.8 (0.7-1.0)	0.148
Donepezil	4	2	2.4 (0.8-7.7)	0.064	4	2	2.7 (0.9-8.5)	0.002	0	7	0.8 (0.7-1.0)	0.224
Galantamine	3	3	1.6 (0.7-3.7)	0.198	3	3	1.8 (0.8-4.1)	0.020	0	7	0.8 (0.7-1.0)	0.224

Abbreviation: AChEIs: acetylcholinesterase inhibitors; RR: Relative Risk.
[a] Measured based on criteria proposed by Hammer et al.[19]
[b] Missing data: degree of calcification at the fracture site could not be evaluated for 7 AChEIs nonusers, 2 Rivastigmine, 1 donepezil and 1 neostigmine users since their hip fractures were treated.
[c] Measured based on criteria proposed by Singh et al.20
[d] Missing data: Singh index could not be evaluated for 2 Rivastigmine, 1 donepezil and 1 neostigmine users since the radiographs showing the non-fractured hip were poor.
[e] p-value was calculated by chi-square test.

Table Supplementary 1. Study outcomes as a function of AChEIs treatment (no treatment, Rivastigmine, Donepezil and Galantamine).

	Patients with missing information	Rest of the patients	P
Age, years[a]	84.5±5.4	82.9±3.5	0.405[d]
BMI[a,b]	27.8±4.4	25.3±4.2	0.266[d]
CDR[a]	2.5±0.6	2.0±0.7	0.224[d]
CCS[a]	3.6±2.2	2.2±1.6	0.018[d]
Fracture healing[c]			
Poor	3	11	-
Fair complication	1	20	0.193[e]
No	4	38	-
Yes	0	4	0.686[e]

Abbreviation: BMI: body mass index; CDR: clinical dementia rating scale; CCS: Charlson comorbidity score.
[a] Values are expressed as the mean and standard deviation.
[b] Measured as weight in kilograms divided by the square of height in meters.
[c] Measured based on criteria proposed by Hammer et al.[18]
[d] p-value was Calculated with Mann-Whitney U test for independent samples.
[e] p-value was calculated by chi-square test.

Table Supplementary 2. Characteristics of patients who had their bone quality not evaluated (missing data) vs. Characteristics of rest of patients who had their bone quality assessed.