and the maximal isometric strength was determined as the peak torque (Nm) in the data analysis.

One-legged standing (OLS) test

The OLS test is a commonly used balance assessment of postural stability. For the OLS test, we asked participants to look straight ahead at a dot 50 cm in front of them, then to stand on their preferred leg with their eyes open and hands down alongside the trunk. OLS balance was measured as the length of time (0–60 s) participants were able to stand on one leg. The better of the two trials was used for statistical analysis.

Walking speed

WS was measured using a 5-m walking test. The participants' usual WS was measured over an 11-m straight and level path. The time taken (in seconds) to pass the 5-m mark on the path was used as the participant's score. A 3-m approach was allowed before the starting marker, and an additional 3 m of space was provided after the end marker of the 5-m path to ensure a usual walking pace throughout the task. Participants were instructed to walk the 11-m path at their usual walking pace. The time to complete the 5-m walking test was measured once and was used to calculate walking speed (m/min).

Falls follow-up

Fall frequency during the 12-month follow-up period was measured with two face-to-face interviews at 6 months and 12 months after baseline measurements. A fall was defined as "an unexpected event in which the person comes to rest on the ground, floor, or lower level" [27]. In this study, 'fallers' were defined as people who had at least one fall during the 12-month follow-up period [28].

Magnetic resonance imaging (MRI) procedure

Magnetic resonance imaging (MRI) was performed using a 1.5-T system (Magnetom Avanto, Siemens, Germany). Three-dimensional volumetric acquisition of a T1-weighted gradient-echo sequence was then used to produce a gapless series of thin sagittal sections using a magnetization preparation rapid-acquisition gradient-echo sequence (repetition time, 1,700 ms; echo time, 4.0 ms; flip angle 15°, acquisition matrix 256 × 256, 1.3-mm slice thickness). Tissue segmentation, regulation, registration, and normalization were conducted in the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/), which is incorporated in the SPM8 software (http://www.fil.ion.ucl.ac.uk/spm/), running on MATLAB R2010a (Mathworks). Diffeomorphic Anatomical Registration using Exponentiated Lie algebra (DARTEL) [29] was conducted for the image analysis. The normalized images were transformed into Montreal Neurological Institute space. The gray matter images were then smoothened using a Gaussian kernel of 12-mm full-width at half-maximum.

Statistical analysis

For baseline comparisons, basic characteristics and physical performance tests including knee-extension strength, OLS, and WS were compared between fallers and non-fallers using *t*-tests. Chi-square tests for differences in proportions were used to compare differences in sex and history of falling in the past year at baseline between the faller and non-faller groups. To describe variations in different physical performance factors related to falls, multivariate logistic regression analyses were performed to reveal the physical performance factors independently related to falls during the 12-month follow-up after adjusting for age, sex, body mass index (kg/m^2), and history of falling in the past year at baseline. We calculated the odds ratios (OR) with 95% confidence intervals (CI). These statistical analyses were calculated using SPSS for Windows version 19.0 (SPSS Inc., Chicago, IL).

In the voxel-based morphometry (VBM) analysis, data preprocessing and analysis was performed with the VBM8 toolbox, which is incorporated in the SPM8 software. VBM [30] was used to examine differences in baseline gray matter volume between fallers and non-fallers. We used unpaired t-tests in SPM8 to identify the locations of smaller gray matter volume in fallers compared to non-fallers during the 12-month follow-up period using MRI data at baseline. Age and sex were included as covariates. The statistical threshold selected for these analyses was $P < .001$ (uncorrected), with an extent threshold of 100 voxels.

Results

The characteristics and physical performance tests at baseline are presented in Table 1. Over the 12-month follow-up period, 11 of the 42 participants (26.2%) experienced at least one fall. Fallers exhibited poorer one-legged standing time ($p < .01$) and slower walking speed ($p < .01$) compared with non-fallers. In addition, the faller group had a significantly higher rate of fall history at baseline compared with the non-faller group ($p < .01$). In the multivariate logistic regression, OLS time (sec) (OR [95% CI]: 0.89 [0.81, 0.98], $p = .02$) was associated with a significantly lower rate of falls during the 12-month follow-up after adjusting for age, sex, body mass index, and history of falling in the past year at baseline. There was no statistical evidence of associations between falls and knee-extension strength (Nm) (1.02 [0.96, 1.08], $p = .59$) and walking speed (m/min) (0.91 [0.81, 1.03], $p = .13$) (Table 2).

Table 1 Comparison of characteristics and physical performance tests between non-fallers and fallers at baseline

	Total (n = 42)	Non-fallers (n = 31)	Fallers (n = 11)	P-value
Age, years	75.6 ± 6.3	75.2 ± 6.5	76.8 ± 5.9	0.462
Female, n (%)	18 (42.9)	12 (38.7)	6 (54.4)	0.362
History of falling in the past year, n (%)	13 (31.0)	6 (19.4)	7 (63.6)	0.006
Knee-extension strength, Nm	60.5 ± 26.8	63.4 ± 23.3	52.3 ± 34.7	0.242
One-legged standing time, sec	32.3 ± 24.2	38.9 ± 22.3	13.8 ± 19.7	0.002
Walking speed, m/m	66.7 ± 12.6	70.0 ± 11.8	57.5 ± 10.4	0.004
Mini-mental state examination, score	26.3 ± 2.7	26.6 ± 2.0	25.5 ± 3.9	0.112

The gray matter density profiles used for examining differences between fallers and non-fallers at baseline are shown in Figure 1. VBM analysis revealed that fallers exhibited lower gray matter density compared with non-fallers in the bilateral middle frontal gyrus and superior frontal gyrus (Table 3). These regions correspond to the premotor cortex and supplementary motor area.

Discussion

The present study examined whether baseline physical performance and gray matter volume are related to falls during a 12-month follow-up period in community-dwelling older adults with MCI. Our results indicated that older adults with MCI exhibiting poor balance had a greater risk of falls during the 12-month follow-up period, while adjusting for age, sex, body mass index, and history of falling at baseline. In addition, baseline lower gray matter volume in the middle frontal gyrus and superior frontal gyrus was associated with the occurrence of subsequent falls. To our knowledge, this is the first study to examine the association between lower gray matter density and risk of falls in older adults with MCI.

Problems with gait and balance have been reported to have the strongest association with falling [2,31]. Slower walking speed has been found to be an independent predictor of falling [32,33]. Poor balance represented by increased postural sway and gait asymmetry has been reported to approximately triple the risk of falling [2]. A previous systematic review and meta-analysis provided a summary estimate for falls due to balance impairment at a relative risk of 1.42 [34]. Therefore, an assessment of balance and gait for older adults, particularly those without a history of falling, has been recommended [35]. Moreover, cognitive impairment has been associated with the risk of falls as well as deficits of physical function [2]. A recent systematic review and meta-analysis confirmed that cognitive deficits detected in clinical assessment are associated with an increased fall risk in community and institution-dwelling older adults [36]. A number of studies have examined the risk of falls in older adults with dementia [37]. However, little research has focused on individuals with MCI. MCI is increasingly recognized as a substantial clinical problem in older populations [38], so it is important to determine risk factors for falling among older individuals with MCI, and to develop effective fall-prevention strategies. A previous study showed that older women with MCI demonstrated a greater number of risk factors for falling compared with older women without MCI [14]. The results of the present study indicate that poor balance assessed by one-legged standing time predicts falls in people with MCI prospectively over 12 months. Although fallers exhibited slower walking speed compared with non-fallers, walking speed was not associated with the occurrence of subsequent falls after adjusting for age, sex, body mass index, and history of falling at baseline. There was no difference in the extension strength between fallers and non-fallers. The results of this study indicate that poor balance is the important factor related to an increased risk of falling among people with MCI. Muscle weakness and problems with mobility had been considered to be the important contributors to the risk of falling in older people [5], and there are presumably some relationships. In study cohorts including older people with MCI and similar lower muscle strength, like the present study, poor balance may have a greater impact on increased risk of falling

Table 2 Multivariate logistic regression summary for physical performance on falls (n = 42)

Variables	Odds ratio	95% confidence intervals	*p* Value
Knee-extension strength, Nm	1.017	0.957-1.080	0.588
One-legged standing time, sec	0.891	0.809-0.981	0.019
Walking speed, m/m	0.911	0.806-1.029	0.133

Notes: Age, sex, body mass index (kg/m^2) and history of falling in the past year at baseline were included as covariates.

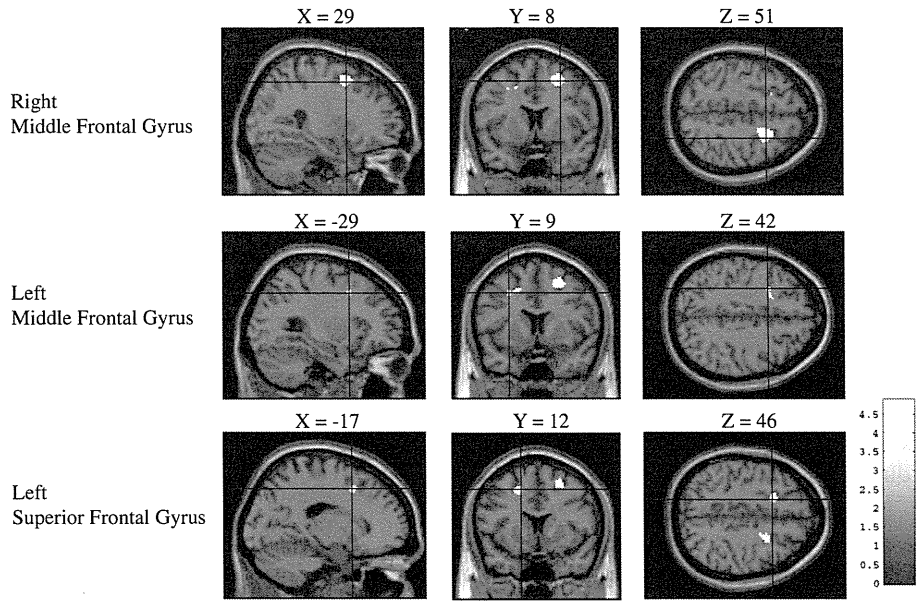

Figure 1 Gray matter density in fallers versus non-fallers. Regions of gray matter reduction in fallers compared to non-fallers ($p < 0.001$, uncorrected). Fallers exhibited a greater reduction of gray matter loss in the bilateral middle frontal gyrus and superior frontal gyrus.

than walking performance. Certainly, poor balance could be one of the predictors of walking decline among older people [39]. Balance ability may be an important dimension of physical functioning to predict the occurrence of subsequent falls among older people with MCI, as well as those with intact cognition. The present study has advantages including the examination of occurrence of subsequent falls during a 12-month follow-up period and neuroimaging assessments in older adults with MCI. However, our sample was not large, and selection bias may affect the results of the relationships between physical performance and occurrence of subsequent falls. Therefore, future studies with larger numbers of MCI subjects and a longitudinal design are needed to add evidence to the present results.

Unlike previous investigations, the current study included MRI scanning and a follow-up assessment of falls in community-dwelling older adults with MCI. The results provide the first evidence that lower gray matter volume in the middle and superior frontal gyrus is related to the occurrence of subsequent falls among older adults with MCI. Age-related changes in the brain may contribute to the subtle onset of motor disturbances in older people. Previous brain-imaging studies of older adults have reported that age-related changes in the brain, such as lower global brain volume, WMH, and microbleeds, are associated with clinical measures of poor balance and slow gait [40-43]. The association between MRI-detected lower brain volume and falls in older adults with MCI has not been examined longitudinally. In the present study, fallers exhibited decreased gray matter density compared with non-fallers in the bilateral middle frontal gyrus and superior frontal gyrus corresponding to premotor cortex and supplementary motor area. These particular regions are likely to play an important role in predicting fall-risk because the middle frontal gyrus is involved in controlling behavior with spatial and sensory guidance.

Growing evidence suggests that brain function is associated with physical function, as confirmed by neuroimaging techniques. Structural changes of the brain in older people are reported to be related to physical performance, such as gait dysfunction [44,45], postural instability [24], and lack of cardiorespiratory fitness [46].

Table 3 VBM results including age and sex as covariates

Location	Cluster size (K)	Peak T	Z score	P (uncorrected)	MNI coordinates		
					X	Y	Z
Right middle frontal gyrus	594	4.87	4.27	< 0.001	29	8	51
Left middle frontal gyrus	165	4.35	3.90	< 0.001	−29	9	42
Left superior frontal gyrus		4.78	4.20	< 0.001	−17	12	46

Note: *VBM* voxel-based morphometry.

Activation in the frontal cortex, including the premotor cortex and the supplementary motor areas, have been reported to increase during human gait by studies using near-infrared spectroscopic imaging [47-50]. Previous studies have reported that lower brain volume in the prefrontal areas is associated with slower gait in high-functioning or cognitively normal older adults [23,40,51]. Other neuroimaging studies have indicated that gait requires complex visuo-sensorimotor coordination, and is associated with activation of the medial frontoparietal region, e.g. the primary sensory and motor areas, supplementary motor area, lateral premotor cortex, cingulate cortex, superior parietal lobule, precuneus, and the infratentorial region including the dorsal region [52-54]. The middle frontal gyrus is involved in motor output and the direct control of behavior, as well as planning, spatial guidance, and sensory guidance of movement [55]. Lower gray matter volume in the premotor cortex and supplementary motor area may be risk factors for falls in older adults. Falls often occur when older individuals attempt to avoid an obstacle in their path, requiring the control of behavior and the planning of movement under sensory guidance. The premotor cortex and supplementary motor area may play an important role in preventing falls when spatial and sensory guidance are required for movement.

Several limitations of the current study should be noted. First, fall experience during the 12-month follow-up period were confirmed with two face-to-face interviews at 6-months and 12-months after baseline, while previous studies have reported that monthly fall diaries and follow-up telephone calls provide more accurate measures of fall frequency [56,57]. Second, participants who had at least one fall during the 12-month follow-up period were categorized as fallers in this study. A previous study reported that single fallers are more similar to nonfallers than to recurrent fallers on a range of medical, physical, and psychological risk factors [58]. Other studies defined fallers as people who had at least one injurious or two non-injurious falls [17,59]. In addition, our MRI scans were performed using a 1.5-T system with relatively low resolution. We performed the VBM analysis to identify the locations of group differences in gray matter volume. Therefore, we consider that our results cannot provide evidence for whether the effects of physical performance are independent of the gray matter volume or whether the latter confounds the association between the former and the fall risk. Although it is unclear whether lower gray matter volume is related to poor balance in older adults with MCI, the current study revealed that poor balance and lower gray matter volume in the middle frontal gyrus and superior frontal gyrus were associated with falls. To clarify these points, we consider that future studies including larger numbers of subjects and countable data for structural changes in the brain (e.g., described volumes in cubic millimeters) are needed.

Conclusions

The current findings indicate that poor balance predicts falls over a 12-month period, and that lower gray matter volume in the middle frontal gyrus and superior frontal gyrus was associated with falls in older adults with MCI. Maintaining physical function, especially balance, and brain structural changes through many sorts of prevention strategies in the early stage of cognitive decline may contribute to decreasing the risk of falls in older adults with MCI.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HM has made substantial contributions to conception and design, subject recruitment, analysis and interpretation of data, and writing the manuscript. HS has made substantial contributions to conception and design, subject recruitment, interpretation of data, and writing the manuscript. TD has made substantial contributions to subject recruitment, acquisition of data, interpretation of data, and manuscript preparation. HP has made substantial contributions to conception and design, interpretation of data, and writing the manuscript. DY contributed subject recruitment and manuscript preparation. KU and KT contributed subject recruitment and acquisition of data. TLA has been involved in drafting the manuscript or revising it critically for important intellectual content. TS has made substantial contributions to conception and design and writing the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This study was supported in part by a grant from the Japanese Ministry of Health, Labour and Welfare (Project for optimizing long-term care, B-3) and a Grant-in-Aid for JSPS Fellows (23–9862) from the Japan Society for the Promotion of Science.

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Received: 6 November 2012 Accepted: 2 August 2013
Published: 5 August 2013

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doi:10.1186/1471-2377-13-102
Cite this article as: Makizako *et al.*: Poor balance and lower gray matter volume predict falls in older adults with mild cognitive impairment. *BMC Neurology* 2013 **13**:102.

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Dement Geriatr Cogn Disord Extra 2013;3:223–232
DOI: 10.1159/000354189
Published online: August 16, 2013

1664–5464/13/0031–0223$38.00/0
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Original Research Article

Six-Minute Walking Distance Correlated with Memory and Brain Volume in Older Adults with Mild Cognitive Impairment: A Voxel-Based Morphometry Study

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Key Words
Exercise capacity · Logical memory · Visual memory · Brain atrophy · Fitness · Walking · Cognitive impairment

Abstract
Background/Aims: High fitness levels play an important role in maintaining memory function and delaying the progression of structural brain changes in older people at risk of developing dementia. However, it is unclear which specific regions of the brain volume are associated with exercise capacity. We investigated whether exercise capacity, determined by a 6-min walking distance (6MWD), is associated with measures of logical and visual memory and where gray matter regions correlate with exercise capacity in older adults with mild cognitive impairment (MCI). ***Methods:*** Ninety-one community-dwelling older adults with MCI completed a 6-min walking test, structural magnetic resonance imaging scanning, and memory tests. The Wechsler Memory Scale-Revised Logical Memory and Rey-Osterrieth Complex Figure Tests were used to assess logical and visual memory, respectively. ***Results:*** The logical and visual memory tests were positively correlated with the 6MWD ($p < 0.01$). Poor performance in the 6MWD was correlated with a reduced cerebral gray matter volume in the left middle temporal gyrus, middle occipital gyrus, and hippocampus in older adults with MCI. ***Conclusions:*** These results suggest that a better 6MWD performance may be related to better memory function and the maintenance of gray matter volume in older adults with MCI.

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Dement Geriatr Cogn Disord Extra 2013;3:223–232
DOI: 10.1159/000354189

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Introduction

Mild cognitive impairment (MCI) is a heterogeneous condition associated with the transitional phase between normal cognitive aging and dementia [1]. Progression rates to dementia and Alzheimer's disease (AD) for individuals with MCI have been reported as being in the range of 6–25% per year [2]. MCI may be the optimum stage at which to intervene with preventive therapies.

Increased physical activity and higher aerobic fitness levels, defined as cardiorespiratory fitness, have been associated with the maintenance of cognitive function and a decreased risk for developing dementia [3, 4]. Recent randomized controlled trials (RCTs) of aerobic exercise for healthy older adults provided evidence that participation in exercise programs involving aerobic exercise leads to an improvement in cognitive function [5] and a greater brain volume in specific regions, e.g. in the prefrontal cortex [6] and hippocampus [7]. Previous cross-sectional studies have suggested that higher fitness levels associated with greater brain volumes in these regions were characteristic among healthy older adults [8, 9]. Some longitudinal studies have shown supportive results of the assumption that a greater physical activity predicts a stable cognitive function [10, 11] and gray matter volume [12].

Physical activity and exercise interventions can have a positive effect on cognitive function in older adults and even in those in the MCI stage [13, 14]. In addition, a recently proposed RCT will examine the effects of a moderate physical activity program on delaying the progression of structural brain changes in older adults with MCI [15]. These studies suggest that a higher exercise capacity plays an important role in maintaining cognitive function and delaying structural brain changes in MCI. However, it is unclear which specific brain regions are associated with exercise capacity performance in older adults with MCI.

We investigated whether a 6-min walking distance (6MWD), to be established as exercise capacity performance, is associated with measures of gray matter volume in older adults with MCI. The 6-min walking test (6MWT) is useful for predicting the maximal oxygen uptake related to cardiorespiratory fitness [16] and is easily administered in clinical settings [17]. The relationship between a 6MWD and memory performance was also examined in this study. A decline in memory performance represents a typical clinical sign of AD and can be observed 10 years prior to the expected symptom onset of AD [18]. In addition, poor memory performance and a lower gray matter volume in the medial temporal area, including the hippocampus, could predict progression to AD in older individuals with MCI [19, 20]. Maintaining exercise capacity may be related to a better memory performance and less brain atrophy in MCI subjects, and this positive relation may contribute to decreasing the risk of progression to AD. However, few studies have reported associations between fitness performance and memory performance in MCI subjects. We hypothesized that a better exercise capacity performance would correlate with a better memory performance and a greater brain volume among MCI subjects. A high exercise capacity may be sustained by a physically active lifestyle; this is potentially an important pathway for maintaining a healthy brain, both in terms of size and reduced damage.

Participants and Methods

Participants

Subjects in this study were recruited from our volunteer databases (n = 1,543), which included elderly individuals (≥65 years old). Participants had to be community-dwelling adults aged ≥65 years. Furthermore, all participants were required to meet the definition of MCI based on the Petersen criteria (not normal cognitive function for age, not demented, and

Dement Geriatr Cogn Disord Extra 2013;3:223–232
DOI: 10.1159/000354189

Table 1. Demographic and health characteristics (n = 91)

Age, years	74.2±6.3
Female gender	47 (51.6)
BMI	23.2±3.2
Diagnosis	
Hypertension	40 (44.0)
Diabetes mellitus	8 (8.8)
Medication, ≥3	33 (36.3)
Mental status	
GDS, points	3.6±3.1
MMSE, points	27.0±1.9
Physical status	
Instrumental self-maintenance[a], points	4.9±0.3
Walking speed, m/s	1.1±0.3

Values are mean ± SD or number (percentage). GDS = Geriatric Depression Scale.

[a] The Tokyo Metropolitan Institute of Gerontology Index of Competence subscale (0–5).

essentially normal functional activities) [21]. A total of 528 potential participants exhibiting a Clinical Dementia Rating score of 0.5 or a subjective memory complaint were enrolled in the first eligibility assessment. Of these, 135 participants underwent the second eligibility assessment, including neuropsychological tests, physical performance tests, face-to-face interviews, and magnetic resonance imaging (MRI) scans. The inclusion criteria required that the participants were ≥65 years old, lived independently in the community (i.e., had no impairment of activities of daily living), were Japanese speaking with sufficient hearing and visual acuity to participate in the examinations, and had general cognitive function (Mini-Mental State Examination [22]) scores between 24 and 30. Exclusion criteria were a history of major psychiatric illness (e.g. schizophrenia or bipolar disorder), other serious neurological or musculoskeletal diagnoses, and clinical depression (Geriatric Depression Scale [23] score ≥10). In addition, we excluded 9 participants who could not perform the physical performance tests and did not meet satisfactory requirements for the MRI scan. Finally, 91 participants complied with the inclusion criteria, and their data were analyzed in the present study. This study was approved by the Ethics Committee of the National Center for Geriatrics and Gerontology, and all participants provided written informed consent. Table 1 summarizes the characteristics of the participants.

Logical and Visual Memory

Logical and visual memory performances were in a standardized format and were administered by licensed, well-trained clinical speech therapists.

The Wechsler Memory Scale-Revised (WMS-R) Logical Memory (LM) [24] was used to assess logical memory. The WMS-R LM subtest requires the examiner to read aloud two short stories to the participant, each with 25 content units. In this study, stories from the Japanese version of the WMS-R LM test were used. After each story, the participant was asked to repeat the story immediately as close to verbatim as possible (immediate recall, Logical Memory-I). The recall was recorded verbatim and scored later according to the manual guidelines. After a 30-min delay, the examiner asked the subject to repeat each of the two stories once again for the delayed recall measure (delayed recall, Logical Memory-II).

The Rey-Osterrieth Complex Figure Test (ROCFT) [25] was used to assess visual memory. The ROCFT is a widely used instrument for assessing visual memory. The participants were

requested to copy the ROCFT figure and reproduce it immediately and again after a 30-min delay. They were not informed that they would be asked to recall the figure. The participants were allowed as much time as they needed for both copy and recall. During the retention interval, unrelated tests (e.g. Mini-Mental State Examination) were administered. The drawings were scored based on a 36-point scoring system.

Six-Minute Walking Test

We used the 6MWT to quantitatively measure participants' exercise capacity. The 6MWT measures the maximum distance that a person can walk in 6 min. The 6MWT is a modification of the 12-min walk/run test originally developed by Cooper [26] and is commonly used as an assessment of exercise capacity. The 6MWT is useful for predicting the maximal oxygen uptake related to cardiorespiratory fitness and is easily administered in clinical settings [17]. The 6MWT was assessed by licensed, well-trained physical therapists. The participants were instructed to walk from one end of a 10-meter course to the other and back again as many times as possible in 6 min, while under the supervision of a physical therapist. After each minute, participants were informed of the time elapsed and were given standardized encouragement. The distance (meters) walked in 6 min was recorded.

MRI Procedure

MRI was performed using a 1.5-tesla system (Magnetom Avanto; Siemens, Germany). Three-dimensional volumetric acquisition of a T1-weighted gradient-echo sequence was then used to produce a gapless series of thin sagittal sections using a magnetization preparation rapid-acquisition gradient-echo sequence (repetition time, 1,700 ms; echo time, 4.0 ms; flip angle, 15°; acquisition matrix, 256 × 256; slice thickness, 1.25 mm). Tissue segmentation, regulation, registration, and normalization were conducted in the voxel-based morphometry (VBM) 8 toolbox (http://dbm.neuro.uni-jena.de/vbm/), which is incorporated in the SPM8 software (http://www.fil.ion.ucl.ac.uk/spm/), running on MATLAB R2010a (Mathworks). Diffeomorphic Anatomical Registration using Exponentiated Lie Algebra (DARTEL) [27] was conducted for the image analysis. The normalized images were transformed into the Montreal Neurological Institute (MNI) space. The gray matter images were then smoothened using a Gaussian kernel of 12 mm full width at half maximum.

Statistical and VBM Analyses

We calculated Pearson correlation coefficients, assessing simple relationships between memory tests and the 6MWD. We used linear regression analyses to assess independent relationships between the variables, while controlling for age and sex to minimize the confounding influence of age-related changes in exercise capacity and memory performance. Standardized beta values were calculated. These statistical analyses were performed using SPSS for Windows, version 19.0. Statistical significance was set at 0.05 for these analyses.

In the VBM analysis, data preprocessing and analysis was performed with the VBM8 toolbox, which is incorporated in the SPM8 software. VBM [28] was applied to determine regions where gray matter density showed a positive correlation with exercise capacity assessed by the 6MWT. We performed a multiple regression analysis on the smoothed gray matter images in SPM8. Age and sex were included in the model as covariates. The statistical threshold was set to $p < 0.05$, corrected for multiple comparisons across the reduced search volume using the family-wise error rate (FWE), with an extent threshold of 40 voxels. The detection of labeled regions from coordinates in the results was conducted using the SPM Anatomy Toolbox [29].

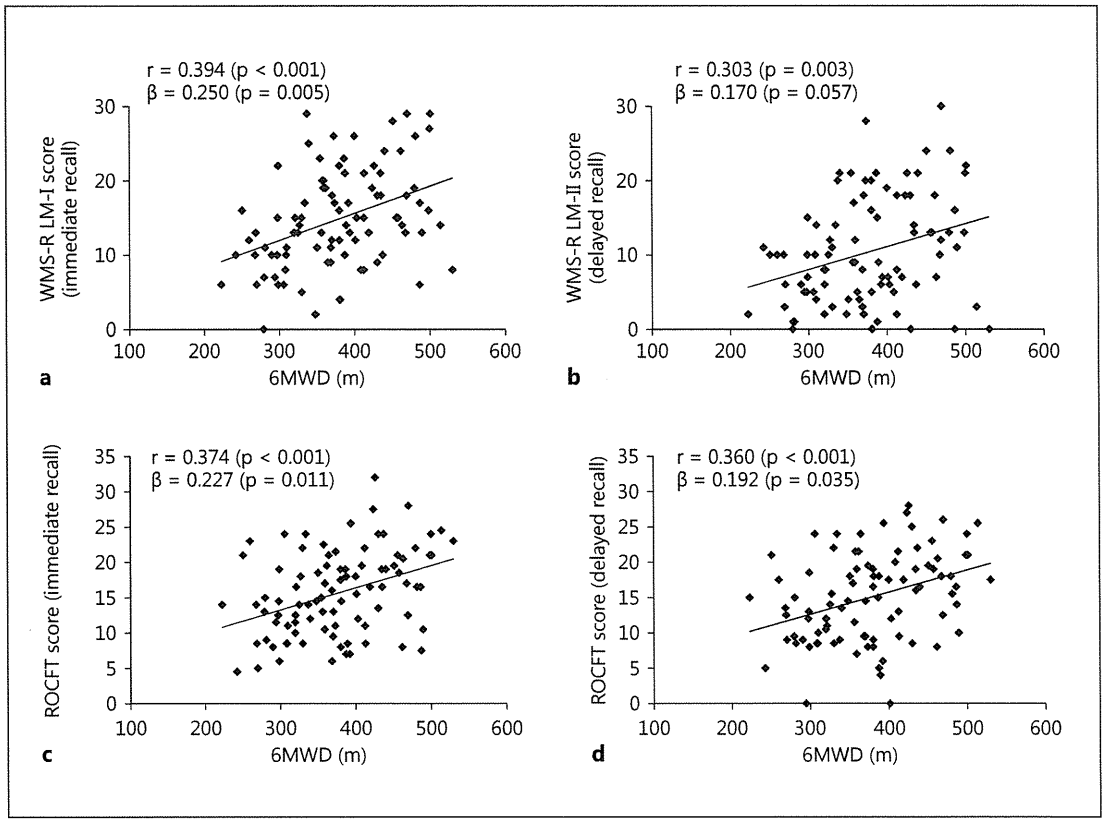

Fig. 1. Correlations between 6MWDs and memory performance tests. Pearson correlation coefficients (r) and standardized beta values (controlling for age and sex) are presented. **a** WMS-R LM-I (immediate recall). **b** WMS-R LM-II (delayed recall). **c** ROCFT (immediate recall). **d** ROCFT (delayed recall).

Results

Simple correlations were examined between the 6MWD and memory tests (fig. 1). Higher scores in all memory tests were significantly associated with a better performance on the 6MWT (WMS-R LM-I, $r = 0.394$, $p < 0.001$; WMS-R LM-II, $r = 0.303$, $p = 0.003$; ROCFT (immediate), $r = 0.374$, $p < 0.001$; ROCFT (delay), $r = 0.360$, $p < 0.001$). Although the relationship between the WMS-R LM-II and 6MWT was not statistically significant when the linear regression model was adjusted for age and sex (WMS-R LM-II, $\beta = 0.170$, $p = 0.057$), the other three memory tests were associated with the 6MWT even after controlling for age and sex [WMS-R LM-I, $\beta = 0.250$, $p = 0.005$; ROCFT (immediate), $\beta = 0.227$, $p = 0.011$; ROCFT (delay), $\beta = 0.192$, $p = 0.035$].

Using multiple regression analysis in SPM8, we examined regions where gray matter density showed a positive correlation with exercise capacity. After adjusting for age and sex, gray matter density in the left middle temporal gyrus, middle occipital gyrus, and hippocampus showed positive correlations with the 6MWD (FWE, $p < 0.05$) (fig. 2). For the MNI coordinates, cluster size, peak F values, and Z values, please refer to table 2. Figure 3 shows the highly linear relationship between 6MWD and adjusted gray matter density in the left hippocampus.

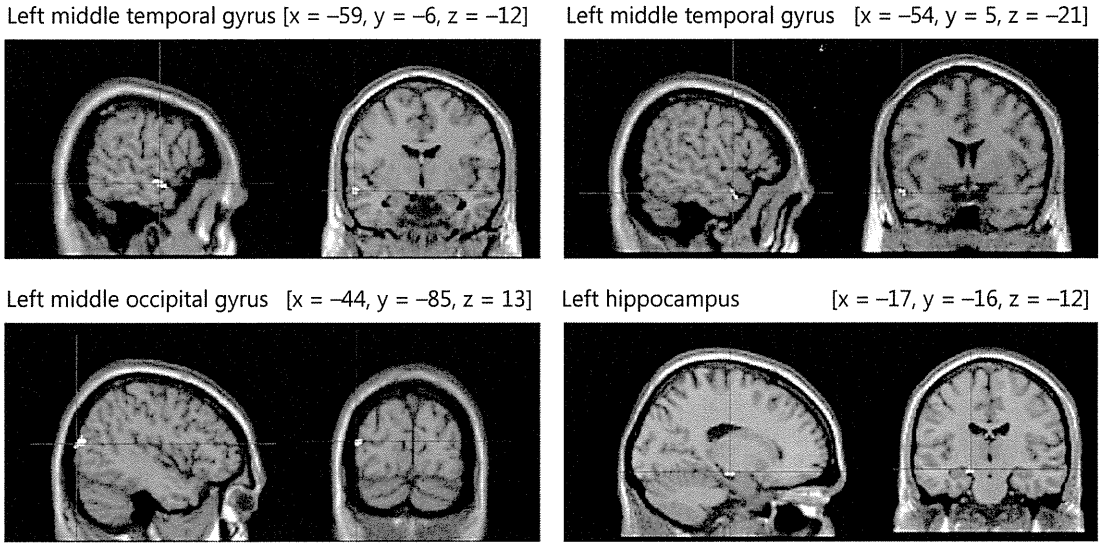

Fig. 2. Brain regions showing an association between a better performance in the 6MWT and a greater gray matter volume. After adjusting for age and sex, gray matter density in the left middle temporal gyrus, middle occipital gyrus, and hippocampus showed positive correlations with the 6MWD.

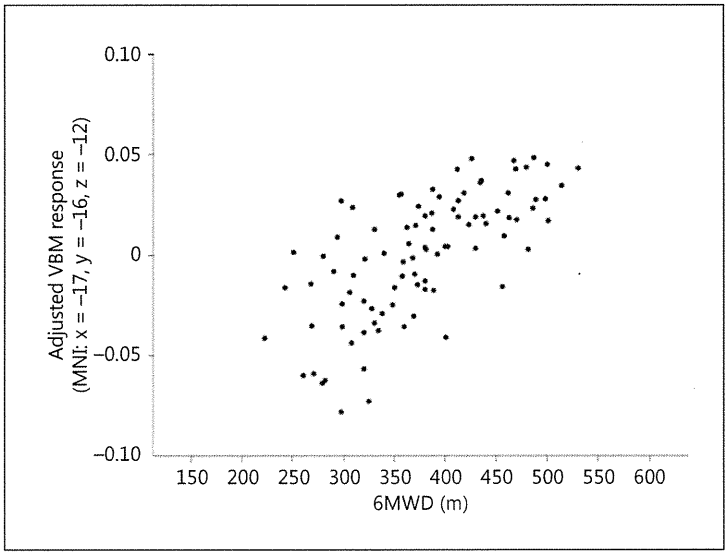

Fig. 3. Correlation between VBM response in the left hippocampus peak voxel (adjusted for effects of age and sex) and the 6MWD.

Discussion

We confirmed that memory performance was significantly positively associated with exercise capacity as assessed by a 6MWD in older adults with MCI. After adjusting for age and sex, gray matter density in the left middle temporal gyrus, middle occipital gyrus, and hippocampus showed positive correlations with exercise capacity.

Previous epidemiological studies in aging populations have suggested beneficial effects of increased physical activity on brain health and function [30, 31]. In a cross-sectional study of 75 healthy older individuals, a positive association between physical activity and memory performance was reported [32]. An interventional study among older adults indicated a

Table 2. VBM results of a 6MWD and volume regions of interest after adjusting for age and sex

Location	Cluster size, K	Peak F	Z-score	FWE, p	MNI coordinates, mm		
					x-axis	y-axis	z-axis
Left middle temporal gyrus	79	32.81	5.13	0.004	−59	−6	−12
	27	27.58	4.74	0.024	−54	5	−21
Left middle occipital gyrus	105	28.87	4.84	0.016	−44	−85	13
Left hippocampus	46	29.54	4.89	0.013	−17	−16	−12

correlation between an increase of total physical activity and improved episodic memory after low- and medium-intensity physical training [33]. Pereira et al. [34] demonstrated that verbal memory performance was improved after completion of a 3-month aerobic exercise regime among adults aged 21–45 years. This improvement in verbal memory performance positively correlated with an improvement of the participants' cardiovascular fitness level and with the cerebral blood volume in the dentate gyrus of the hippocampus. These results support the present study, indicating associations between a greater 6MWD and a better memory function among older adults with MCI.

One advantage of the present results is the indication of the association between exercise capacity performance and gray matter volumes using MRI data among MCI subjects. In a large cross-sectional study of elderly subjects without dementia, physical fitness was highly and significantly associated with hippocampal volumes [8]. Another cross-sectional study also indicated that increased cardiorespiratory fitness was associated with a better preservation of gray matter volumes, particularly in the medial temporal lobes, including the hippocampus and parahippocampal gyrus [35]. Moreover, recent RCTs of aerobic exercise for older adults provided evidence for positive associations between aerobic exercise and greater brain volumes in specific regions. An RCT in a large cohort of older adults documented significantly larger hippocampal volumes after 1 year of aerobic exercise compared with the control intervention of simple stretching and toning [7]. The results of this study also confirmed that an increased exercise capacity performance was associated with greater brain volumes in specific regions, including the left middle temporal gyrus, middle occipital gyrus, and hippocampus even after adjusting for age and sex among MCI subjects.

A previous study using VBM analysis revealed that there was a significantly greater gray matter loss in converters from MCI to probable AD relative to nonconverters in the hippocampal area, inferior and middle temporal gyrus, posterior cingulate, and precuneus [36]. In a longitudinal study where individuals in late adulthood were followed up for 9 years, a greater physical activity predicted greater volumes of the frontal, occipital, entorhinal, and hippocampal regions [12]. Gray matter volumes in the medial temporal lobe, including the entorhinal, parahippocampal, and hippocampal regions, may contribute to the prediction of subsequent cognitive decline and conversion from MCI to AD [37], and may be important for maintaining memory function [38]. We demonstrated linear relationships between VBM response in the left hippocampus peak voxel and the 6MWD in figure 3. This association may indicate protective effects of exercise capacity on cognitive decline in older adults with MCI.

Recent interventional studies suggested that physical activity and aerobic exercise have beneficial effects on memory function. These effects are possibly mediated by gray matter volume and neurotrophic factors, especially brain-derived neurotrophic factor (BDNF) [7, 33], which is highly concentrated in the hippocampus [39] and is important for synaptic plasticity [40]. In a previous study including young adult males, both acute and chronic exercise improved medial temporal lobe function concomitant with increased concentrations of BDNF

in the serum. This suggests a possible functional role for this neurotrophic factor in exercise-induced cognitive enhancement [41]. Exercise has consistently been shown to enhance learning and persistently upregulate expression of BDNF in the hippocampus of rodent models [42, 43]. These previous results may support the present findings that exercise capacity is related to brain volume including the medial temporal lobe. However, this study did not provide evidence of mechanisms for protective effects of aerobic fitness on brain volume through neurotrophic factors. Future studies are needed to provide insight into how mechanisms that increase fitness may enhance cognition, especially memory, and prevent age-related structural brain changes.

Several possible limitations should be considered when interpreting our findings. We are conscious of the limitations of our cross-sectional design. Longitudinal and interventional studies should be designed to clarify the relationship between exercise capacity and cognitive function among MCI subjects. In addition, we recognize that there is important information regarding the effect of exercise capacity on the conversion rate from MCI to AD. Our results indicate that a higher exercise capacity may be related to a better memory function and a greater gray matter volume in several brain regions. This has been found in other studies including healthy older adults [44] or AD patients [35]. However, in the present and previous studies, different methods of assessment were used to identify fitness levels. Previous studies that examined the relationships between aerobic fitness and brain volume used the measurement of peak oxygen consumption [35, 44]. We assessed participants' exercise capacity with the 6MWT. This measure is widely used in clinical settings to identify exercise capacity and is associated with peak oxygen consumption in older adults. We did not include data from healthy older persons and patients with AD in the present study. Additional neurological analyses that include data from healthy older adults and AD patients are needed to determine the relationships between exercise capacity and brain changes in AD-related processes. Although a previous neuroimaging study suggested that the apolipoprotein Eε 4 genotype in MCI might be associated with structural changes typically found in the early stages of AD [45], our data did not consider the effects of genetic factors, such as the presence of the apolipoprotein E risk allele.

In conclusion, a higher exercise capacity measured by the 6MWT is associated with a better memory function and a greater gray matter density, including the left middle temporal gyrus, middle occipital gyrus, and hippocampus in older adults with MCI. To strengthen our findings, future studies are required to examine the effects of intervention on exercise capacity and the related change in brain volume in the specific regions and memory function among MCI subjects.

Acknowledgments

We would like to thank the Obu City Office for their assistance with participant recruitment. We would also like to thank the technical staff from the Department of Radiology, National Hospital for Geriatric Medicine, National Center for Geriatrics and Gerontology for MRI data acquisition. This work was supported by a grant from the Japanese Ministry of Health, Labour and Welfare (Project for optimizing long-term care; B-3) to T.S. and a grant-in-aid for JSPS Fellows (23-9862) from the Japan Society for the Promotion of Science to H.M.

Disclosure Statement

There are no conflicts of interest.

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Ishijima et al. Arthritis Research & Therapy 2014, 16:R18
http://arthritis-research.com/content/16/1/R18

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RESEARCH ARTICLE Open Access

Intra-articular hyaluronic acid injection versus oral non-steroidal anti-inflammatory drug for the treatment of knee osteoarthritis: a multi-center, randomized, open-label, non-inferiority trial

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Abstract

Introduction: While many of the commonly used conservative treatments for knee osteoarthritis (OA) have been recognized to be effective, there is still insufficient evidence available. Among the pharmacological treatments for knee OA, oral non-steroidal anti-inflammatory drugs (NSAIDs) act rapidly and are recommended for the management of OA. However, frequent and serious adverse effects of NSAIDs have been recognized. Intra-articular injections of hyaluronic acid (IA-HA) for the treatment of knee OA have been shown to reduce pain and improve joint function. However, there has been no qualified direct comparison study of the efficacy and safety between IA-HA and NSAIDs for patients with knee OA. The aim of this study was to clarify the efficacy and safety of early-phase IA-HA in comparison to those of NSAIDs for patients with knee OA.

Methods: This multicenter, randomized, open-label, parallel-group, non-inferiority comparison study with an oral NSAID involved a total of 200 patients with knee OA. An independent, computer-generated randomization sequence was used to randomly assign patients in a 1:1 ratio to NSAIDs three times per day for five weeks (n = 100) or IA-HA once a week for five weeks (n = 100). The primary endpoint was the percentage change in the patient-oriented outcome measure for knee OA, the Japanese Knee Osteoarthritis Measure (JKOM) score. All patients were questioned regarding any adverse events during treatment. The full analysis set (FAS) was used for analysis. The margin of non-inferiority was 10%.

Results: The analyses of primary endpoint included 98 patients in the IA-HA group and 86 patients in the NSAID group. The difference in the percentage changes of the JKOM score between the two intervention arms (IA-HA; -34.7% ($P<0.001$), NSAID; -32.2% ($P<0.001$)) was −2.5% (95% confidence interval (CI): -14.0 to 9.1), indicating IA-HA was not inferior to NSAID. The frequency of both withdrawal and adverse events in the IA-HA group were significantly lower than those in the NSAID group ($P = 0.026$ and 0.004, respectively).

Conclusions: The early efficacy of IA-HA is suggested to be not inferior to that of NSAIDs, and that the safety of the early phase of IA-HA is superior to that of NSAIDs for patients with knee OA.

Trial registration: UMIN Clinical Trials Registry (UMIN-CTR), UMIN000001026.

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Introduction

Osteoarthritis (OA) is an increasingly important public-health problem [1]. The total societal cost of the treatment of OA has been estimated to increase worldwide because of its dramatic growth in morbidity [2]. The current treatment for knee OA consists of conservative treatment, such as exercise, physical therapy, pharmacological agents and, in some cases, surgical treatment [3,4]. While many of the commonly used conservative treatments have been recognized to be effective [5], there is still insufficient evidence available.

Among the pharmacological treatments for knee OA, oral non-steroidal anti-inflammatory drugs (NSAIDs) act rapidly and are recommended for the management of OA, although frequent and serious adverse effects of NSAIDs have been recognized [5]. Hyaluronic acid (HA) is a natural constituent of joint fluid. Intra-articular injections of HA (IA-HA) for the treatment of knee OA have been shown to reduce the pain and improve joint function [5-7]. Although IA-HA is also recommended, it acts relatively slowly and there was considerable heterogeneity in the outcomes between trials [8-11]. In addition, there has been no qualified direct comparison study of efficacy and safety between IA-HA and NSAIDs for patients with knee OA.

The aim of this multicenter, randomized, parallel-group, open-label, non-inferiority trial was to compare the early efficacy and safety of IA-HA and NSAIDs in patients with knee OA.

Methods

Study design and participants

The trial was planned by the Cartilage Metabolism Research Group, consisting mainly of Japanese orthopedists, to clarify the early efficacy and safety of IA-HA (high molecular weight 2,700 kDa HA, Chugai Pharmaceutical Co. Ltd., Tokyo, Japan) in comparison to an NSAID (loxoprofen sodium, Daiichi Sankyo Pharmaceuticals Co. Ltd., Tokyo, Japan), in a multicenter, randomized, open-label, parallel-group, non-inferiority trial. The protocol was reviewed and approved by the ethics committee of Juntendo University, Tokyo, Japan, and was also reviewed by the institutional review board of each participating institution. This study was undertaken at 20 hospitals throughout Japan between February, 2008 and December, 2010 (see Acknowledgements), in accordance with the Declaration of Helsinki, and with the Ethical Guidelines for Clinical Studies of the Japanese Ministry of Health, Labor, and Welfare. This trial was registered at UMIN-CTR [12], UMIN000001026.

Subjects

All patients provided written informed consent before enrollment in this trial. The inclusion criteria for the present study included (1) subjects who were able to walk with painful knee OA and fulfilled the criteria for knee OA of the medial femorotibial joint as defined by the American College of Rheumatology (ACR) [13], (2) the age of the subjects ranged from 50 to 80, (3) female subjects were required to be postmenopausal, and (4) all subjects had radiographic knee OA with Kellgren-Lawrence (K/L) grade 1 to 3 [14] evaluated by the weight-bearing anteroposterior X-rays of the tibiofemoral joint using the bilateral standing extended view.

The exclusion criteria included (1) patients who had received either an oral, topical or intra-articular steroid during the four weeks before the study, (2) patients who had received IA-HA within four weeks before the study, (3) patients who had received either an oral, topical or suppository NSAID within two weeks before the study, (4) patients who had secondary knee OA, (5) patients with patellofemoral OA with a K/L grade of 3 or higher, (6) patients with severe OA (K/L grade 3 or higher) in a location other than the knee joint, (7) patients with rheumatoid arthritis, (8) patients who had received joint replacement surgery in either knee or/and a hip, (9) patients who had allergies to either HA or NSAIDs, (10) patients who had either hematological, cardiac, hepatic or renal disorders, (11) patients who had experienced an asthma attack induced by NSAIDs, and (12) patients whom the physician recognized as not suitable for enrollment in the study for other reasons.

Randomization and masking

A centralized, computer-generated randomization was conducted to randomly assign patients in a 1:1 ratio to the IA-HA or NSAID groups. Investigators were masked to assignment before, but not after, randomization. The website for patient registration and randomization was prepared and controlled by the coordinating data center (Gunma University, Maebashi, Japan). The blocked randomization was stratified by the participating medical center and the K/L grade of knee OA.

Treatment procedures

A total of 200 patients with symptomatic knee OA were registered from 20 hospitals and randomized for treatment with the NSAID or IA-HA, as described above. For patients treated with the NSAID, they received three daily 60 mg NSAID tablets (total 180 mg)/day, one after each meal, for five weeks. Additional use of gastro-protective drugs, such as a proton pump inhibitor (PPI), in combination with the NSAID was allowed for those in the NSAID group. For patients treated with IA-HA, an intra-articular injection of high-molecular-weight 2,700 kDa HA (25 mg) was administered into the affected joint five times, at weekly intervals in the morning. Concomitant use of other drugs for the treatment of OA and drugs that affect bone and cartilage metabolism were not allowed during the trial.

Outcome measures for the assessment of efficacy and safety

The patients were evaluated for their (1) baseline characteristics, (2) radiographic analysis of the knee, (3) compliance with the treatment, (4) clinical manifestations, and (5) safety.

Evaluation of the response to treatment (efficacy)

Pain was evaluated by a visual analog scale (VAS, 0 to 100). The clinical manifestations were evaluated by the Japanese Knee Osteoarthritis Measure (JKOM) score [15]. The JKOM is a patient-based, self-answered evaluation score that includes four subcategories: pain and stiffness (0 to 32), activities of daily living (0 to 40), social activities (0 to 20), and general health conditions (0 to 8) with 100 points as the maximum score. The JKOM score is higher in patients with more pain and physical disability, and this evaluation modality is considered to have sufficient reliability and validity for studies of the clinical outcomes of Japanese subjects with knee OA [15]. The measure has also been shown to have reliability and validity by means of statistical evaluation and comparison with other health-related scales, such as the Western Ontario and McMaster Universities Arthritis Index (WOMAC) and the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) [15].

The primary endpoint was to compare the percentage change from baseline in the JKOM score at five weeks. The secondary endpoint was to compare the percentage change from baseline in the pain VAS score.

The definition of a response to treatment was made following the criteria defined by the Outcome Measures in Rheumatology Clinical Trials (OMERACT) and Osteoarthritis Research Society International (OARSI) [16]. This measure consists of both absolute and relative changes in scales, including both pain and function, to evaluate the affected knee. Relative change means the percentage of change during the study (final minus baseline over baseline × 100), whereas absolute change indicates the absolute change during the study (final minus baseline on an interval scale of 0 to 100). Before assessing patients based on this scale, we partly modified it for this study by using the JKOM score, as already reported [17,18]. The response was defined as relief of joint pain or improvement in function (at least 50% reduction of the score) and a decrease of at least 20 mm on the VAS, or clinical improvement meeting at least two of the following three conditions: a decrease in joint pain of at least 20% and at least 10 mm on the VAS; an improvement in function of at least 20% and a decrease of at least 4 points from a total 40 points (equal to an absolute change of 10%) on the JKOM functional subcategory scale; and a decrease in the patient's global assessment score by at least 20% and at least 10 points from a total of 100 on the total JKOM scale.

Assessment of adverse events induced according to the treatment modality (safety)

Safety was monitored by recording all adverse events, evaluating the laboratory data and assessing vital signs. This was performed for all participants in both groups at each weekly visit.

Statistical analysis

Sample size determination

The trial was designed to establish whether the symptom-modified effect of IA-HA was non-inferior to that of NSAID (Δ10%). The sample size of this non-inferiority trial was calculated to require a total of 194 patients (97 per treatment group) based on the results of our pilot study with a 5% dropout rate, 10% non-inferiority margin, 27% standard deviation (SD), 5% one-sided alpha level, and power = 0.8 (pilot study: Toshitaka Nakamura, unpublished data, 2007). The 10% margin was set as the smallest value that would be clinically important, assuming a reduction of 30% in the mean percentage change of JKOM score in patients with both IA-HA and NSAID treatment and a reduction of 10% those receiving a placebo treatment.

Data analysis

The primary statistical analyses of efficacy and safety were performed on the full analysis set (FAS), which included all patients treated at least once. For the primary endpoint of the study, a two-sided 95% confidence interval (CI) for the group difference 'test treatment minus reference treatment' was calculated for the percentage change from baseline in the JKOM score as non-inferiority analysis. The non-inferiority of the test treatment was confirmed if the upper limit of the CI was ≤ margin of non-inferiority delta (10%). For the secondary endpoint, the group difference and its 95% CI was calculated for the percentage change from baseline in the VAS pain score.

Quantitative variables were described using the mean, standard deviation and range. The efficacy of treatment was examined by a paired *t* test for both JKOM score and pain VAS score. A multiple logistic regression analysis was used to estimate odds ratios and their 95% CIs between the IA-HA and NSAID treatments in models adjusted for age, K/L grade, body mass index (BMI) and the participating medical centers.

All analyses were performed using the SAS System Release 9.1 software program (SAS Institute, Cary, NC, USA). The registration number of this trial is UMIN000001026, and information on the trial can be found online at [12].

Results

Patient baseline characteristics

A flow chart of this trial is shown in Figure 1. When 200 patients were enrolled, half (100) of the patients were

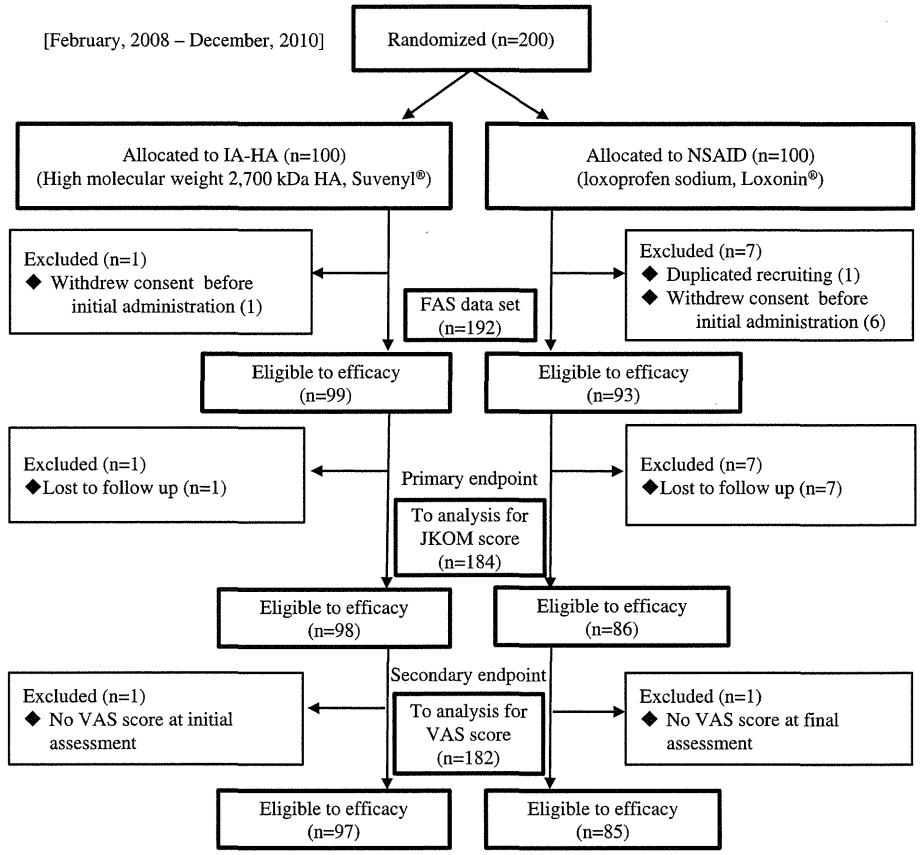

Figure 1 A flow chart of the study. FAS, full analysis set; HA, hyaluronic acid; IA-HA, intra-articular injections of HA; ITT, intention-to-treat; JKOM, Japanese Knee Osteoarthritis Measure (JKOM); NSAID, non-steroidal anti-inflammatory drug; VAS; visual analog scale.

randomly allocated into the NSAID group, and the other half allocated into the IA-HA group. Two patients in the IA-HA group and 14 patients in the NSAID group were excluded; therefore, the remaining 184 patients were included in the analyses of the primary endpoint.

The baseline patient characteristics are shown in Table 1. No significant statistical differences between the baseline characteristics of both groups were found.

Efficacy analyses (primary and secondary endpoints)

For the primary endpoint analysis, the JKOM score of the patients in both the NSAID group and in the IA-HA group was significantly reduced by the treatment ($P<0.001$, Table 2), and the percentage change from baseline in the JKOM score for the two groups was −32.2% and −34.7%, respectively. The difference in the percentage changes of the JKOM score between the two intervention arms (primary endpoint) was −2.5% (95% CI: -14.0 to 9.1%).

In a multiple regression analysis performed taking into consideration the factors considered to stratify the study design, the difference in the primary endpoint between the two intervention arms was also less than 10% (data not shown). These results demonstrate that the IA-HA treatment was non-inferior to the NSAID treatment for the percentage reduction in the clinical symptoms evaluated by the JKOM.

For the secondary endpoint analysis, the pain VAS score of the patients in the NSAID group was significantly

Table 1 Baseline characteristics of the patients in the study

		IA-HA (n = 99)	NSAID (n = 93)
Age (y)	Mean (SD)	68.2 (7.1)	68.5 (7.0)
Gender	Male	27	22
	Female	72	71
BMI	Mean (SD)	23.8 (3.4)	24.4 (3.6)
K/L grade	1	16	15
	2	48	50
	3	35	28
JKOM score (Min:0 - Max:100)	Mean (SD)	33.8 (15.8)	31.6 (14.1)
Pain VAS (Min:0 - Max:100)	Mean (SD)	60.3 (22.4)	55.1 (21.9)

IA, intra-articular; HA, hyaluronic acid; NSAID, non-steroidal anti-inflammatory drug; BMI, body mass index; K/L, Kellgren-Laurence grade; JKOM, Japanese Knee Osteoarthritis Measure; VAS, visual analog scale.