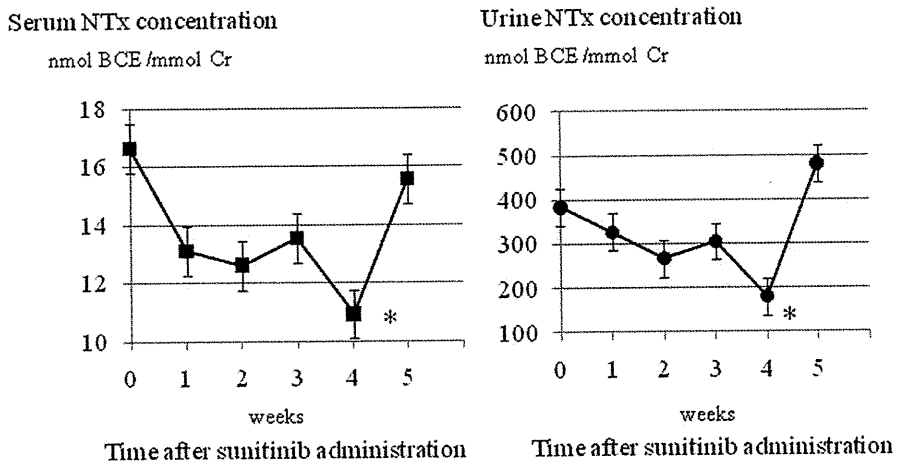

Figure 3. Alteration of bone resorption markers in sunitinib-treated patients with metastatic RCC. Serum and urine levels of NTx 28 days after oral administration of sunitinib were significantly lower than initial levels (*$p < 0.01$). Characteristics of the 16 sunitinib-treated patients are shown in Table 1.

transforming growth factor beta, fibroblast growth factor, insulin-like growth factors I and II, PDGF and bone morphogenetic proteins.[17] When osteoclasts absorb bone by secreting protons and proteases, these growth factors are released and they provide fertile ground for the growth of cancer cells. Therefore, osteoclasts are a suitable therapeutic target in the treatment of bone metastases. In this study, there were significantly fewer TRAP-positive osteoclasts in the mice treated with sunitinib than in those treated with vehicle control (Fig. 1*e*). This observation is consistent with previous reports.[10,18] Zwolak *et al.* reported that treatment with sunitinib decreased the percentage of active osteoclasts to 45.6% ± 5.8% compared with the percentage in untreated tumor-bearing mice (79.4% ± 8.6%), suggesting that sunitinib treatment (40 mg/kg/day) may inhibit osteoclast maturation.[18] Murray *et al.* reported that sunitinib inhibited osteoclast development and function mediated by M-CSF, which is one of the differentiating factors for osteoclasts and is a target tyrosine kinase of sunitinib, both *in vitro* and *in vivo*.[10] Our clinical observation of decreases in serum and urine NTx is also in line with these reports (Fig. 3).

NTx is a degradation product of Type I collagen and is often used as a marker of bone resorption both in serum and urine. Some clinical studies have suggested that levels of NTx correlate with the presence and extent of bone metastases, prognosis and response to treatment.[19,20] Although our data did not show an association between the reduction rate of NTx and the efficacy of sunitinib, further investigation is necessary to clarify this association, especially in bone metastatic lesions.

During the completion of this manuscript, we found that ACHN originated from papillary renal cancer in a 22-year-old patient (Reference [21] and by personal communication from Dr. Ernest Borden). Recent studies have suggested the possible clinical efficacy of sunitinib for patients with clear and non-clear cell cancer.[22,23] However, there are no prospective Phase 2 or Phase 3 studies clarifying this question. We therefore have to wait for the results of a large prospective study on the use of sunitinib for non-clear cell cancer. Since bone is the second most common site of metastases for RCC, we reported an indirect mechanism that may partly help to elucidate the reasons for the clinical efficacy of sunitinib.

Mesenchymal-epithelial transition factor (MET) and fumarate hydratase (FH) are considered to be the genes responsible for Type 1 and Type 2 papillary RCC, respectively.[24,25] MET, which is a proto-oncogene, encodes a tyrosine kinase membrane receptor, and activation of MET can indirectly promote angiogenesis and tumor growth through overexpression of VEGF.[26,27] FH is an enzyme in the mitochondrial tricarboxylic acid (TCA) cycle. Loss of FH leads to a state of pseudohypoxia through overexpression of hypoxia-inducible factor (HIF), resulting in an increase in downstream targets, including VEGF.[26,28] Therefore, activation of MET and loss of FH, which are considered to be responsible for Type 1 and Type 2 papillary RCC, lead to angiogenesis. Clinically, Ljungberg *et al.* demonstrated that the mRNA levels of VEGF, VEGF-receptor Type 1 and VEGF-receptor Type 2 above the median were related to adverse survival in papillary RCC.[29] Therefore, it is relevant to measure VEGF in a clear or non-clear cell RCC model.

To elucidate whether sunitinib has any other indirect effects, we measured the concentrations of VEGF and M-CSF. However, we found no significant difference between the two groups in the serum concentrations of these growth factors. This observation is consistent with previous findings. Ebos *et al.* reported a significant increase in the serum VEGF level on administration of 60–120 mg/kg sunitinib.[30] While it has been shown that sunitinib is a multikinase inhibitor that

inhibits Class III and Class V RTKs, including PDGF receptors, VEGF receptors, KIT and FLT3, with low nanomolar potency,[30] other growth factor-mediated signals might be inhibited by sunitinib. Further investigation is necessary to clarify the precise mechanism of action of sunitinib and its clinical efficacy against bone metastases.

Conclusion

In conclusion, we demonstrated that oral administration of a clinically achievable dose of sunitinib prevented the growth of RCC bone metastases *in vivo*. Because RCC cell lines are resistant to clinically and preclinically achievable plasma concentrations *in vitro*, prevention of osteoclast activity and/or maturation is one of the mechanisms of growth inhibition in metastatic bone lesions. Our study supports the use of sunitinib as an initial treatment for RCC patients with bone metastasis.

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Using a Smartphone while walking: a measure of dual-tasking ability as a falls risk assessment tool

Sir—Falls are relatively common among older people; 30% of 65-year-old community-dwelling adults experience at least one fall per year. Of these falls, 6% result in fractures [1, 2]. Falls typically occur during locomotion; therefore, previous studies have focused on identifying the changes in locomotor performance which occur with increasing age [3, 4].

In every-day life, locomotion typically occurs under complicated circumstances with cognitive attention focused on other tasks. Lundin-Olsson *et al.* [5] reported a novel method for predicting falls based on the dual-task (DT) performance of subjects. In recent years, numerous studies have evaluated DT walking in elderly people. However, Beauchet *et al.* [6] reported that reliable conclusions cannot be drawn from the prediction of falls based on DT results due to the lack of standardisation in DT paradigms. We considered that the two main limitations of the previous research using DT protocols [7–12] were: (i) insufficient evaluation of the performance of the secondary task and (ii) the lack of standardisation of the DT protocols.

The aim of the present study was to evaluate the use of a Smartphone-based application for assessing dual-tasking ability as a tool for predicting the risk of falls in a community-dwelling elderly population.

Methods

Participants

Participants for this study were recruited through advertisements placed in local newspapers. A total of 318 community-dwelling older individuals (mean age, 78.9 [7.3] years) participated in this study. The exclusion criteria ensured that none of the participants had any indications of the following clinical conditions: (i) serious visual impairment, (ii) inability to ambulate independently (those individuals requiring the assistance of a walking frame were excluded), (iii) a score of <7 on the Rapid Dementia Screening Test [13], (iv) symptomatic cardiovascular disease, (v) Parkinson's disease or stroke, (vi) peripheral neuropathy of the lower extremities or (vii) severe arthritis. Written informed consent was obtained from each subject in accordance with the guidelines approved by the Kyoto University Graduate School of Medicine and the Declaration of Human Rights, Helsinki, 1975. Each participant was categorised as either a high-risk (HR) or low-risk (LR) elderly individual on the basis of whether they had experienced at least one fall within the past year (self-reported). A fall was defined as any event that led to unplanned, unexpected contact with a supporting surface during walking. On the basis of this classification, the participants were divided into HR ($n = 90$) and LR ($n = 228$) groups (Table 1).

Smartphone data collection

The Android-based Smartphone Android Dev Phone 2 (HTC Corp., Taiwan) was used as a measurement device. Android is a popular operating system for Smartphones. The phone is lightweight (123 g with battery) and has

Table 1. Characteristics of the participants in the GR and LR groups

Characteristic	HR ($n = 90$) Mean (SD)	LR ($n = 228$) Mean (SD)	*P*-value	Effect size	95%CI
Age	79.1 (7.4)	78.8 (7.2)	0.733	0.04	−1.41 to 2.00
Height, cm	153.3 (6.7)	153.6 (6.4)	0.703	0.05	−1.81 to 1.22
Weight, (kg)	53.7 (10.2)	54.2 (9.8)	0.695	0.05	−2.78 to 1.86
Gender, female, *n* (%)	62 (56.3%)	146 (64.0%)	0.435		
ST walking time, s	13.3 (5.3)	11.6 (4.3)	0.001*	0.34	0.70 to 2.87
DT walking time, s	27.8 (28.2)	20.9 (22.9)	0.019*	0.24	1.14 to 12.67
ST android, score	39.7 (14.5)	36.9 (12.5)	0.081	−0.23	−0.35 to 6.09
DT android, score	29.5 (12.4)	34.8 (9.5)	<0.001*	0.56	−7.78 to −2.84
DT time lag, %	52.2 (35.1)	39.4 (32.4)	0.001*	0.36	5.00 to 20.49
DT point lag, %	34.8 (46.4)	3.8 (34.1)	<0.001*	0.67	21.89 to 39.97
DT total lag, %	86.9 (52.6)	42.9 (47.7)	<0.001*	0.84	32.51 to 55.54
TUG, s	15.8 (12.6)	11.8 (5.3)	0.000*	0.32	2.01 to 6.03
One leg standing, s	7.5 (12.8)	10.4 (11.4)	0.046*	0.25	−5.74 to −0.05
FR, cm	21.5 (8.2)	24.5 (9.5)	0.006*	0.32	−5.12 to −0.89
Five-chair stand test, s	12.9 (6.9)	10.7 (4.5)	0.001*	0.34	1.03 to 3.59

*Indicates statistical significance, Student's *t*-test, $P < 0.05$.
CI, confidence interval; DT, dual-task; ST, single task; DT time lag (%) = 100 * (DT walking time − ST walking time)/ST walking time. DT point lag (%) = 100 * (DT Android score − ST Android score)/ST Android score. DT total lag (%) = DT point lag + DT time lag. TUG, time up and go.

triaxial accelerometers. The use of Android-based applications is advantageous because they are free to develop, offer flexible design options, and can be easily and rapidly distributed over the Internet. The author (K.O.) developed an Android application (RollingBall) for the assessment of fall risk (available for download at http://www.kuhp.kyoto-u.ac.jp/~kazuya/RollingBall.apk) in which a small blue ball (1.5 cm in diameter) is moved on a large white circle (4 cm in diameter) by tilting the phone. The inclination of the phone is determined by the triaxial accelerometers (Figure 1). The Android application also calculates a score based on coordinate data of the ball on the circle; higher scores indicate that the blue ball is nearer to the centre of the circle. The application was based on the 'walking while carrying a ball on a tray' task, previously demonstrated to be a good predictor of falling (Yamada M., unpublished data).

Participants used the application in single- (ST) and dual-task conditions. In the ST condition, participants used the application for 15 s while stationary (ST Android test). The instructions were as follows: 'Using your left hand (or the hand without a cane), please control the Smartphone to keep the blue ball in the centre of the white circle'. The score calculated by the application was recorded as a variable. In the DT condition, the participants walked 15 m at a comfortable speed while using the Android application. The participants were instructed as follows: (i) They should walk at a comfortable speed while positioning and maintaining the blue ball at the centre of the white circle with the left hand (or the hand without a cane). (ii) It was not necessary to constantly look at the Smartphone screen. (iii) The exercise should be performed safely to ensure that no accidents, such as falls, occurred. The score calculated by the application and the time taken to walk 15 m were recorded as variables. Before the tests were carried out, a trained evaluator gave standardised verbal instructions regarding the test procedure and a visual demonstration of the tests. The test–retest reliability, determined using the inter-class correlation coefficient (ICC [1.1]). was 0.976. The tests were performed in a random order. The score under each condition was calculated as an average of the scores obtained from the two trials. The reduction in performance due to walking, the DT lag, was calculated as follows for both the application score (DT point lag) and

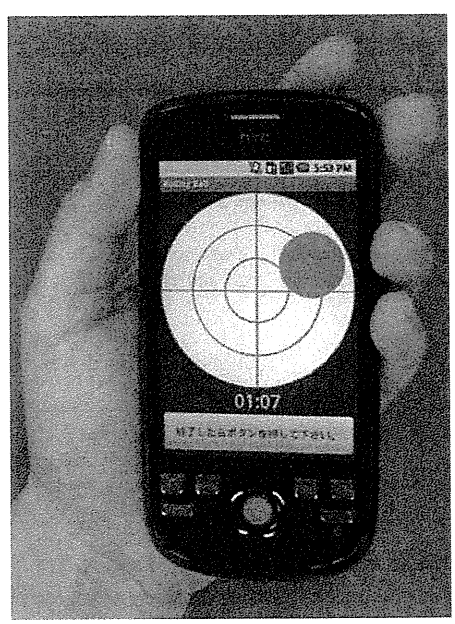

Figure 1. The developed Android application allowed users to control the position of a small blue circle (1.5 cm in diameter) on a large white circle (4 cm in diameter). The score was automatically calculated on the basis of the coordinate tracking data of the blue circle.

walking time (DT time lag) variables [14]:

$$\text{DT lag}(\%) = 100 * \frac{(\text{DT condition} - \text{ST condition})}{\text{ST condition}}$$

The DT total lag was then calculated using the following equation:

$$\text{DT total lag } (\%) = \text{DT point lag} + \text{DT time lag}$$

Data collection for other physical performance tests

In addition to DT walking, the participants were subjected to five other physical performance tests that are widely used to identify HR elderly adults: 10 m walk under an ST condition (ST walking) [15], timed up and go (TUG) test [16], functional reach (FR) [17], one-leg stand [18] and five-chair stand tests [19]. The tests were performed in random order. For each performance task, the participants performed two trials and an average score was calculated.

Statistical analysis

Differences in the physical performance variables between the HR and LR groups were analysed using a Student's *t*-test. To compare physical performance in the two groups, effect sizes were determined. The effect size was calculated as: (HR mean − LR mean)/standard deviation. The relationship between the scores from the Smartphone test and the five previously validated tests was assessed using Pearson's correlation coefficient. All data analysis was carried out in the Statistical Package for Social Science (Windows version 11.0). A *P*-value of <0.05 was considered statistically significant for all analyses.

Results

Descriptive statistics for patient characteristics in the two fall risk groups are summarised in Table 1. Participants in the HR and LR groups were comparable and well-matched in terms of their age, height, weight and gender. With the exception of the ST Android, DT walking time, one-leg standing and FR test results ($P > 0.05$), all physical performance tests demonstrated that the elderly participants in the LR group had significantly better scores than those in the HR group. The largest effect size was the DT total lag in all physical performance tests. The results for DT total lag were weakly, but significantly, correlated with those for ST walking time ($r = 0.267$, $P < 0.001$) and those for the TUG ($r = 0.194$, $P = 0.001$), one-leg standing ($r = -0.195$, $P = 0.001$), FR ($r = -0.202$, $P < 0.001$) and five-chair stand ($r = 0.161$, $P = 0.005$) tests.

Discussion

This is the first study to examine the use of a Smartphone device for DT-based fall risk assessment. The present findings support the conclusion of previous experimental studies that measurement of changes in gait while dual tasking is an effective tool in the clinical assessment of fall risk [7–12]. Several characteristics of the Smartphone application developed here are considered to contribute to increasing the demands on the attention of HR elderly participants during DT walking. First, the application represents a simple manual task (i.e. maintaining a small circle in a central position on a large circle) that participants can easily understand and perform. Second, the application provides the ability to measure performance in both the principal and secondary tasks. This constitutes an improvement over previous DT-related reports, which did not sufficiently evaluate the participants' performance in secondary tasks [7–12]. Changes in physical performance during dual tasking are considered to be due to the competing demands for the participant's attention required to successfully complete both tasks [20, 21]. Therefore, performance in both the principal and secondary tasks needs to be evaluated. The results for DT total lag weakly correlated with those from previously validated physical performance tests. Our results reveal that the Smartphone test evaluates the risk of falls by using a different parameter from that used in previously validated physical performance tests.

In addition to the benefits of the developed Smartphone application as a clinical assessment tool, we assessed whether this application could be used as tool for public health promotion. The Smartphone application has a number of advantages over conventional DT-based fall risk assessment tests. First, it is able to measure performance in both principal and secondary tasks. Second, because the application is downloadable from the Internet, it can be readily accessed and distributed throughout the world. Third, the simplicity and portability of the application permits self-assessment of fall risk by concerned individuals in non-clinical settings. However, there is a serious limitation in this study. The developed Smartphone application could not predict falling in older adults as this study was based on the participants having experienced falls in the previous year.

Key points

- A Smartphone-based application was used to assess dual-tasking ability as a measure of the risk of falls.
- The results for DT total lag weakly correlated with those for previously validated physical performance tests.
- This is the first study to examine the use of a Smartphone device for the assessment of the risk of falling.

Conflicts of interest

None declared.

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Long-term effect on mortality of a multicomponent cognitive behavioural group intervention to reduce fear of falling in older adults: a randomised controlled trial

SIR—Fear of falling and avoidance of activity due to fear of falling are common in older people. Prevalence rates for fear of falling in community-living older persons range from 20 to 60% [1–8] and for avoidance of activity due to fear of falling from 15 to 55% [1, 6, 7, 9–11]. Fear of falling and related avoidance of activity may lead to adverse consequences, like functional decline [8, 12, 13], restriction of social participation [9], decreased quality of life [2, 8, 12], increased risk of falling [8, 10, 13] and institutionalisation [12]. Indeed, fear of falling is suggested to be a potential health problem of equal importance to a fall [12].

Several interventions showed to reduce fear of falling or to improve confidence regarding performing activities without falling [14]. Particularly two multicomponent cognitive behavioural group interventions explicitly aimed at reducing excessive fall-related fear and unnecessary avoidance of activity showed beneficial outcomes in randomised controlled trials in community-living older people [15, 16]. In the first study confidence in performing activities without falling and mobility range were improved directly and 12 months after a multicomponent cognitive

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Effect of resistance training on physical performance and fear of falling in elderly with different levels of physical well-being

SIR—Several factors are involved in the maintenance of activities of daily living (ADL) in older adults. Skeletal muscle mass and strength are important factors for maintaining independence and quality of life in elderly. Several recent cross-sectional studies have shown the associations of muscle strength with physical fitness and disability [1, 2]. Loss of muscle mass (sarcopenia) is prevalent in older adults [3] and represents an impaired state of health with mobility disorders, increased risk of falls and fractures, impaired ability to perform ADL, disabilities and loss of independence [4–6].

Fear of falling is common in older adults. The prevalence varies from 21 to 85%, is higher in women than in men, and increases with age [7]. The risk factors of fear of falling are shown to be physical frailty [8], perception of poor health [9], obesity, cognitive impairment, depression, poor balance [10] and history of at least one fall [7].

Resistance training is an effective intervention to improve the physical function in older adults by increasing strength and physical performance [11]. However, it is still controversial whether resistance training is effective for all levels of elderly people. For example, we reported that decreased muscle power is a reliable predictor of falls only in frail elderly [12].

We hypothesised, therefore, that there is a differential effect of resistance training on physical performance according to the level of physical well-being. The aim of this study was to compare the effects of resistance training on skeletal muscle mass, physical performance and fear of falling in robust and frail elderly.

Methods

Participants

Participants were recruited by an advertisement in a local press. We used the following criteria to screen participants in an initial interview: aged ≥65 years, community dwelling, has visited a primary care physician within the previous 3 years, score of ≥8 by Rapid Dementia Screening Test [13], able to walk independently, willing to participate in group exercise classes for at least 6 months, access to transportation and no regular exercise in the previous 12 months.

We also used the interview to exclude participants based on the following exclusion criteria: severe cardiac, pulmonary, or musculoskeletal disorders, pathologies associated with an increased risk of falls (i.e. Parkinson's disease or stroke) and use of psychotropic drugs. We obtained written informed consent from each participant in accordance with the guidelines approved by the Kyoto University Graduate School of Medicine and the Declaration of Human Rights, Helsinki, 1975.

Frailty definition

The frailty classification was based on a composite of previous work. The Timed Up and Go (TUG) is a simple test developed to screen basic mobility performance and has been shown to be significantly associated with ADL in frail older adults [14]. It has been reported that elderly with a TUG score greater than 13.5 s can have an increased risk of falling [15]. Frailty was defined as a TUG score >13.5 s. Based on key components of the screening examination (TUG score greater than 13.5 s), 159 elderly adults were classified as the frail group, whereas 178 elderly adults were classified as the robust group because they had a TUG score of ≤13.5 s.

Resistance training

All participants underwent resistance training sessions twice a week for 50 weeks. All participants performed the seated row, leg press, leg curl and leg extension exercises on resistance-training machines. Training loads were chosen using the 10-repetition maximum (10-RM, the maximal weight that can be lifted 10 times). Participants used the 10-RM for 3 sets of 10 repetitions for each machine exercise. Participants were required to adjust the training weight to ensure failure at the 10-RM. It took approximately 1 h to finish all sessions, with 15-min warm-up at the beginning and 10-min cool-down stretch at the end.

Bioelectrical impedance analysis measurement

A bioelectrical impedance data acquisition system (Physion MD; Physion Co. Ltd, Kyoto, Japan) was used to determine

the bioelectrical impedance of the right upper and lower limbs [16]. This system applies a constant current of 800 mA at 50 kHz through the body. Participants lay supine with their arms and legs extended and relaxed during bioelectrical impedance measurement. Leg lean mass (LLM) per whole-body weight was used for the analysis.

Measurement of physical performance

All participants underwent five measurements upon entry into the study (pre-test), which included 10-m walk test, TUG test, single leg standing (SLS), functional reach (FR) and 5-chair stand. The order of performing these tests was random. For each performance task, the participants performed two trials, and an average score was calculated from these two trials. All baseline and pre-test measurements were completed prior to randomisation.

Measurement of fear of falling

Falls Efficacy Scale (FES) [17] is the most frequently used surrogate measure for fear of falling in older adults. The reliability and validity of FES have been previously reported [17]. FES was measured at baseline and at 12 months. FES is based on the operational definition of fear as 'low perceived self-confidence at avoiding falls during essential, relatively nonhazardous activities'. Briefly, participants were asked how concerned they were about the possibility of falling while performing 10 different activities on a 4-category scale from 1 (not at all concerned) to 4 (very concerned). If participants indicated that they did not perform or were unable to perform the activity, they were encouraged to respond hypothetically. FES emphasises mainly indoor, home-based activities.

Required sample size

We designed the effect size of the current study to be 0.4. With a significance level of 0.05, a power of 80%, and a moderate effect size (0.4), a minimum of 100 participants were needed in both the intervention and control groups. Accounting for a potential 20% attrition rate, a total of 240 participants were recruited for this study, which was deemed large enough to detect statistically significant differences.

Statistical analysis

We analysed the effects of resistance training on all outcome measures using a mixed 2 (group: robust and frail groups) × 2 (time: pre-intervention, post-intervention) ANOVA. A 0.05 type 1 error rate was chosen *a priori* to indicate statistical significance. A *post hoc* paired *t*-test for within-group comparisons was performed to compare each dependent variable. The Bonferroni procedure was used to adjust the type 1 error rate of each analysis to 0.025 (0.05/2) as an indication of statistical significance to guarantee an overall type 1 error rate of 0.05. Data were entered and analysed using the Statistical Package for Social Science (Windows version 18.0).

Results

We screened 412 elderly and enrolled 337 (81.8%) who met the inclusion criteria for the trial and agreed to participate (Figure 1A). Most of the elderly who did not meet the inclusion criteria ($n = 66$) were excluded because they had exercised regularly for 6 months prior to the screening. Nine people who might have been eligible for the study declined after telephone screening. Of the 337 individuals who were enrolled in this study, 307 (91.1%) completed the 12-month

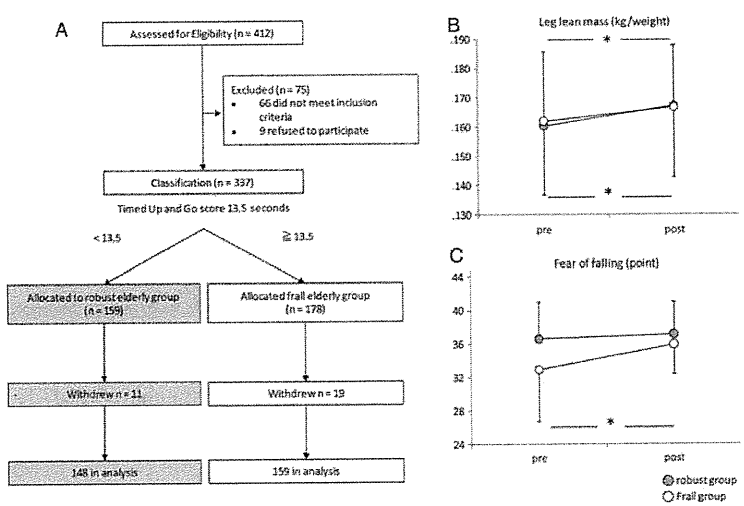

Figure 1. (A) Flow chart showing the disposition of participants throughout the trial. (B) LLM after resistance training in the robust and frail groups was significantly increased from baseline ($P < 0.05$). (C) The frail group had significantly greater improvements in fear of falling ($P < 0.025$).

intervention along with the second interview and the tests at the end of the study. Among them 148 in the robust group (93%) and 159 in the frail group (89%) completed the study.

All 100 scheduled intervention sessions were completed. The median relative adherence was 92% (25–75th percentile, 85–95%) for the robust group and 92% (85–95%) for the frail group. No health problems, such as cardiovascular and musculoskeletal complications, occurred during the training sessions or testing. Minor problems were observed in both groups such as aching muscles after the first training session and fatigue. All the problems were managed easily by adjustment of the intervention and were improved during subsequent interventions.

Effect of the resistance training on outcome measures

LLM after resistance training in the robust and frail groups was significantly increased from the baseline ($P < 0.05$) (Table 1, Figure 1B). Pre- and post-intervention group statistics and group × time interactions are summarised in Table 1. A statistically significant group × time interaction was observed for TUG, FR and fear of falling ($P < 0.05$) (Figure 1C). Bonferroni-corrected paired-sample t-tests demonstrated a significant effect of the resistance training on TUG, FR and fear of falling in the frail group ($P < 0.025$).

Discussion

In this study, we showed that LLM was improved by the resistance training programme in both groups. However, the effect on physical function was limited to frail elderly defined by TUG. The role of muscle strength on physical function is supported by numerous cross-sectional studies that have shown a strong association between low muscle strength and decreased mobility in elderly [18]. On the

Table 1. Functional fitness items by group at pre- and post-intervention

	Robust group ($n = 148$)		E/S	P-value[a]	Frail group ($n = 159$)		E/S	P-value[a]	P-value[b]	F-value 1. Time effect 2. Group × Time
	Mean	SD			mean	SD				
Age, years	75.4	7.7			76.1	8.3			0.440	
Height, cm	157.7	10.1			156.7	9.1			0.266	
Weight, kg	58.2	11.1			56.8	10.9			0.280	
Gender, female n (%)	74 (50.0%)				82 (51.5%)				0.436	
Fall incidence, n (%)	48 (32.4%)				77 (48.4%)				0.003	
Leg lean mass, kg/weight										
Pre	0.160	0.024	0.39	<0.001	0.162	0.024	0.27	0.002	0.448	32.1**
Post	0.167	0.024			0.167	0.021				1.1
Percent change, %	0.05	0.09			0.04	0.11				
Walking time, s										
Pre	10.0	1.9	0.11	0.294	16.1	3.8	0.16	0.130	0.017	1.1
Post	10.2	2.1			15.5	4.1				3.6
Percent change, %	0.3	15.5			−7.7	27.5				
Timed up and go test, sec										
Pre	9.9	1.8	0.09	0.374	17.4	3.0	0.32	0.004	0.002	6.1*
Post	10.1	2.5			16.1	3.9				10.5**
Percent change, %	0.9	18.1			−14.5	37.6				
One leg standing, s										
Pre	9.8	11.8	0.06	0.567	1.7	1.9	0.16	0.160	0.987	0.1
post	9.2	13.9			2.6	5.4				1.4
Percent change, %	−47.3	173.4			46.8	248.3				
Functional reach, cm										
Pre	23.5	5.9	0.01	0.948	18.0	5.6	0.46	<0.001	0.029	7.5**
Post	23.4	5.9			20.9	6.8				8.0**
Percent change, %	−7.2	46.4			23.6	48.1				
Five chair stand, s										
Pre	11.2	3.2	0.07	0.498	16.8	5.2	0.17	0.144	0.004	1.6
Post	11.5	4.7			15.1	8.6				3.1
Percent change, %	5.0	31.3			−29.9	72.8				
Fear of falling, points										
Pre	36.6	4.4	0.18	0.081	32.9	6.2	0.51	<0.001	<0.001	26.2**
Post	37.1	3.9			35.9	3.5				15.4**
Percent change, %	1.5	7.3			12.9	23.3				

E/S, effect size.
[a]As calculated by comparing pre- and post-intervention.
[b]As calculated by group comparison.
*$P < 0.05$.
**$P < 0.01$.

other hand, muscle strength does not depend solely on muscle mass, and the relationship between strength and mass is not linear [19]. Rantanen *et al.* reported that the relationship between muscle strength and physical disability in older adults is non-linear [20]. The discrepancy between these results may stem from the heterogeneity of subjects. In this study, we stratified subjects into robust and fail elderly groups. In frail elderly, the 50-week resistance training programme was effective for the improvement of LLM and physical performance. In contrast, there was no correlation between the change in LLM and physical performance in robust elderly undergoing the resistance training programme. These results suggested that our resistance training programme is not effective for the improvement of physical performance in robust elderly. Furthermore, resistance training improved muscle strength, but did not improve physical performance in the relatively healthy elderly [21]. On the other hand, in frail elderly, improvements in leg power, independent of strength, appear to make an important contribution to clinically meaningful improvements in physical performance [22].

Resistance training improved balance function, such as FR in frail elderly. Improved balance function with resistance training is hypothesised to occur by reduced motor-unit discharge variability [23]. However, SLS was not improved. These results suggested that balance improvement after power training may be explained, in part, by adaptations in force control. However, resistance training *per se* is not effective for balance function. For the improvement of balance function, it is useful to add not only the resistance training but also balance training, such as Tai Chi Chuan [24].

In addition to improving physical performance, the resistance training programme was effective for decreasing fear of falling, but only in the frail group. It is considered important to reduce fear of falling by targeting downstream factors such as physical functioning [25] or predictors of those factors [26]. Thus, our study has an important implication for the reduction in fear of falling in frail elderly.

There are several limitations to this study that warrant mention. First, although we used only TUG to define frailty, TUG may not be enough to define frailty. For example, the short physical performance battery evaluates balance, gait, strength and endurance by examining an individual's ability [27]. It has been recently recommended by an international working group to use a functional outcome measure in clinical trials in frail older adults [28]. Second, we did not measure muscle force. The relationship between LLM and muscle strength is still unclear and needs to be addressed in future studies. Third, no follow-up was conducted. Evidence regarding the long-term effect of exercise on fall prevention is limited, and, therefore, this issue also needs to be addressed. Finally, a control group was lacking. The participants in both groups may have had higher motivation and interest in health issues than the general elderly population.

This is the first study to demonstrate that the effects of a resistance training programme on physical performance differed according to the level of physical well-being. Future work should determine whether tailor-made interventions can effectively improve physical function in both robust and frail elderly.

Key points

- The current trial compared the effects of resistance training between robust and frail elderly on skeletal muscle mass, physical performance and fear of falling.
- Skeletal muscle mass after resistance training was significantly increased from the baseline in both groups.
- The resistance training programme was more effective for the improvement of physical performance and fear of falling in frail elderly than in robust elderly.

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Conflicts of interest

None declared.

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Transient ischaemic attack, vascular risk factors and cognitive impairment: a case–controlled study

SIR—Cognitive impairment, especially difficulties with temporal orientation and verbal recall, is associated with the increasing number and severity of vascular risk factors (VRFs) such as hypertension and diabetes [1–3] which can result in an associated impairment of the cerebral microcirculation causing white matter volume changes linked to large artery stiffness [4, 5]. These cognitive deficits can be detected by using simple standard screening tools [6] such as the Mini Mental State Examination [7], Montreal Cognitive Assessment (MoCA) [8] and the DemTec [9], and have been shown to be related to the development of both subclinical (mild) or established vascular disorders [7–12].

However, our understanding of the relation between transient ischaemic attacks (TIAs) and cognitive status is incomplete. We hypothesised that subjects with newly diagnosed TIA would have evidence of an associated mild cognitive impairment; this being a manifestation of the same pathological process underlying the pathogenesis of the vascular event being initiated and accelerated by VRFs. The aims of the current study were, therefore, (i) to examine whether patients with first ever TIA and no history of stroke have evidence of cognitive impairment and, if so, whether the extent of the impairment was greater than expected compared with an age-, sex-matched control populations without VRFs and (ii) to determine which VRFs are associated with cognitive impairment.

Methodology

We conducted a case–controlled study between August and November 2008 in a University Hospital in UK (catchment population 750,000). Cases were defined as those patients with first ever TIA aged ≥45 years, assessed in a

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RESEARCH ARTICLE Open Access

Prostaglandin E2 receptor type 2-selective agonist prevents the degeneration of articular cartilage in rabbit knees with traumatic instability

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Abstract

Introduction: Osteoarthritis (OA) is a common cause of disability in older adults. We have previously reported that an agonist for subtypes EP2 of the prostaglandin E2 receptor (an EP2 agonist) promotes the regeneration of chondral and osteochondral defects. The purpose of the current study is to analyze the effect of this agonist on articular cartilage in a model of traumatic degeneration.

Methods: The model of traumatic degeneration was established through transection of the anterior cruciate ligament and partial resection of the medial meniscus of the rabbits. Rabbits were divided into 5 groups; G-S (sham operation), G-C (no further treatment), G-0, G-80, and G-400 (single intra-articular administration of gelatin hydrogel containing 0, 80, and 400 μg of the specific EP2 agonist, ONO-8815Ly, respectively). Degeneration of the articular cartilage was evaluated at 2 or 12 weeks after the operation.

Results: ONO-8815Ly prevented cartilage degeneration at 2 weeks, which was associated with the inhibition of matrix metalloproteinase-13 (MMP-13) expression. The effect of ONO-8815Ly failed to last, and no effects were observed at 12 weeks after the operation.

Conclusions: Stimulation of prostaglandin E2 (PGE2) via EP2 prevents degeneration of the articular cartilage during the early stages. With a system to deliver it long term, the EP2 agonist could be a new therapeutic tool for OA.

Keywords: prostaglandin E2, EP_2, ONO-8815Ly, osteoarthritis, ACLMT

Introduction

Osteoarthritis (OA) is the single most common cause of disability in older adults [1]. It is a complex process involving a combination of cartilage degradation, repair, and inflammation. However, its pathogenesis is not yet fully understood [2]. Articular cartilage is composed of chondrocytes, and an extensive extracellular matrix (ECM). The major ECM components are type II collagen and aggrecan. In normal cartilage, catabolic and anabolic activities are in dynamic equilibrium. Chondrocytes can produce several catabolic cytokines such as IL-1 and TNF-α, which in turn induce the production of proteinases including matrix metalloproteinases (MMPs) and disintegrin-like and metalloproteinase with thrombospondin, that lead to the destruction of the matrix network [3,4]. Among the MMPs, MMP-13 (collagenase 3) plays a particularly important role in causing OA [5]. Indeed, transgenic mice carrying an inducible human *MMP-13* gene develop pathological changes similar to those observed in human OA patients, when the transgene is expressed in articular cartilages of postnatal mice [6]. Moreover, inhibitors of MMP-13 prevent the degradation of articular cartilage [5,7]. Chondrocytes also produce anabolic cytokines such as the bone morphogenetic protein family members and insulin-like growth factor-1 (IGF-1), which induce the synthesis of collagen and initiate the proliferation of chondrocytes [3]. A disruption of

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the equilibrium between the catabolic and anabolic activities results in catastrophic damage to the articular cartilage, ultimately inducing the pathological condition known as OA.

Prostanoids, including prostaglandin (PG) D2, PGE1, PGE2, PGF2α, prostacyclin (PGI2), and thromboxane A2, are lipid mediators produced in a sequence of cyclooxygenase (COX) -1, -2-catalyzed reactions [8]. The role of PGE2 in the development of OA is controversial. Some reports point to an important role in inflammation [9]. Pro-inflammatory signaling mediators such as IL-1 and TNF-α induce the synthesis of PGE2 by promoting the expression or activities of COX-2 and microsomal PGE synthase-1 [10]. PGE2 then promotes IL-1 expression as part of a positive feedback mechanism, degrades the cartilage ECM [4,10-13], and finally induces apoptosis of chondrocytes [3]. Other reports insist that PGE2 opposes the effect of IL-1 [14] and stimulates the gene expression of type II collagen [3,15]. In addition, PGE2 stimulates the synthesis of proteoglycan and collagen through the expression of an IGF-1-binding protein [16,17]. PGE2 works through four isoforms of the EP receptor, EP1 to EP4. Previously, we considered that the controversy could result from differences in the mode of action and tissue distribution of each receptor [18]. Using an EP2 selective agonist, we showed that EP2 receptor-mediated PGE2 signaling enhances the growth of chondrocytes [18,19] and promotes the regeneration of articular cartilage in rabbits with cartilage defects [19].

In the current study, we investigate the effect of an EP2 agonist on articular cartilage in a rabbit model of traumatic degeneration.

Materials and methods

Materials

Microspheres loaded with a selective EP2 agonist, ONO-8815Ly (lysine salt) [20], were prepared by the emulsion-solvent evaporation method [19,21]. Briefly, ONO-8815Ly and polylactic-co-glycolic acid (PLGA) were mixed to form a water/oil emulsion, and added to the outer water phase containing polyvinyl alcohol under stirring with a turbine-shaped mixer at 5000 rpm to obtain a water/oil/water emulsion. PLGA microspheres that did not contain ONO-8815Ly in its free form were recovered by centrifugation and lyophilized to remove residual organic solvent and water. Then, a gelatin aqueous solution (20%, w/w) was poured into the microsphere suspension to form a gel. For the crosslink reaction, a glutaraldehyde aqueous solution (12.5 mg/ml) was poured into the microsphere suspension. Small cylinder-shaped gelatin hydrogels (4 mm in diameter and 2 mm in thickness) containing ONO-8815Ly (0, 80, or 400 μg of ONO-8815/gel) were obtained by hollowing out the gelatin hydrogel sheet. Diffusion kinetics analyses showed that ONO-8815Ly is gradually released from the microsphere over a period of seven days *in vitro* (Figure 1).

Animal model for traumatic degeneration

Four-month-old female Japanese white rabbits (weighing approximately 3 kg) were used. Traumatic degeneration was induced as described for the anterior cruciate ligament and menisectomy transection (ACLMT) model [22]. Operations were performed under general anesthesia, and a skin incision was made on the medial side of the patella. Soft tissues and articular capsules were cut to expose the knee joints. The anterior cruciate ligament was transected at the attachment to the tibia in the knee-flexed position, and the anterior horn of the medial meniscus was resected. The articular capsule and skin were sutured in layers with 4-0 nylon sutures. After the operation, rabbits were allowed to move freely. Preliminary experiments revealed that osteoarthritic changes were observed in this model at as early as two weeks after operation (data not shown).

Treatments with the EP2-agonist

A total of 64 animals were randomly assigned to five groups: G-S (sham operation), G-C (no further treatment), G-0, G-80, and G-400 (single intra-articular administration of gelatin hydrogel containing 0, 80, and 400 μg of ONO-8815Ly, respectively). Sham-operated rabbits (G-S; n = 4) received no further treatment, and were sacrificed either 2 (n = 2) or 12 weeks (n = 2) after the operation.

The ACLMT surgery was performed on both the knees of each of the remaining 60 rabbits to avoid any unequal bearing of weight due to pain on one side. No further treatment was performed in animals of the

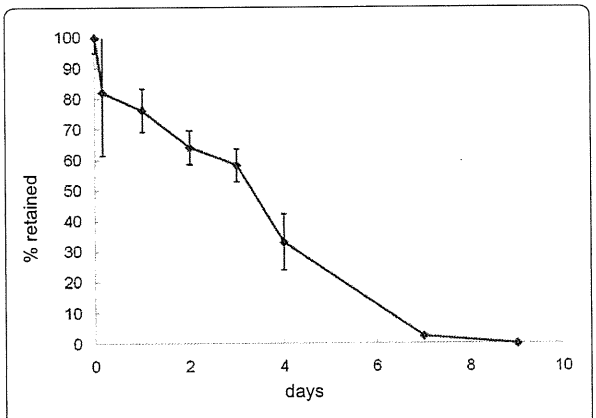

Figure 1 The diffusion kinetics of ONO-8815Ly from microspheres *in vitro*. Microspheres loading ONO-8815Ly were soaked in PBS, and the amount of retained ONO-8815Ly at each time point was measured by high-performance liquid chromatography and calculated as the ratio to the initial amount (n = 5).

control group (G-C; n = 12). In the treatment groups, no further treatment was performed on the right knee, but a gelatin hydrogel cylinder containing ONO-8815Ly (G-0, G-80, and G-400; n = 16 per group) was placed on the fatty pad of the left knee at the time of operation. Rabbits were sacrificed two weeks (G-C, n = 6; G-0, G-80, and G-400, n = 10 per group) or 12 weeks (n = 6 per group) after the operation. All the experiments with animals were approved by the institutional animal research committee, and performed according to the Guidelines for Animal Experiments of Kyoto University.

Histological examination

Rabbits were sacrificed 2 or 12 weeks after surgery, and the distal femur and proximal tibia of the left side of each animal were resected, fixed at 4°C overnight in a 10% formalin solution, and decalcified in formic acid for three days. After neutralization by 10% sodium sulfate for 24 hours, the samples were embedded in paraffin. Serial sections were prepared in the coronal plane through the middle of the femoral and tibia condyles, and one section from each sample was used for each of the histological analyses. In every section, the entire cartilage portion in full depth was evaluated. The specimens were stained with safranin O/Fast Green or H&E using standard procedures. The histological grade of cartilage degeneration was evaluated using the modified Mankin's scoring system [23], which was adopted as the original system [24] for the evaluation of the rabbit model. All the results shown herein represent the combined scoring data of two researchers.

Immunohistochemical analyses

Immunohistochemical examination was performed as follows. In brief, after deparaffinization, sections were incubated with 0.3% hydrogen peroxide for 30 minutes. Then, sections were treated with proteinase K for two minutes (proliferating cell nuclear antigen [PCNA] staining) or with hyaluronidase for 60 minutes (MMP staining), after which they were incubated with the following primary antibodies: mouse anti-human PCNA monoclonal antibody (1:100; Dako, Glostrup, Denmark), mouse anti-human MMP-13 monoclonal antibody (1:20; AnaSpec Inc., San Jose, CA, USA), or mouse anti-rabbit MMP-3 monoclonal antibody (1:50; Daiichi Fine Chemical Co. Toyama, Japan). All antibody dilutions were made in PBS. After an overnight reaction with the primary antibody at 4°C, sections were incubated with horseradish peroxidase-conjugated anti- mouse IgG (Vector Laboratories, Southfield, MI, USA) at room temperature for 30 minutes. Signals were visualized with 3, 3'-diaminobenzidine tetrahydrochloride, and nuclei were counterstained with hematoxylin. The percentage of PCNA-, MMP-13-, and MMP-3-positive cells in the cartilage was calculated by methods similar to those described above. Results of histological and immunohistochemical analyses were evaluated by two observers who were blinded to the identity of each sample.

Primary chondrocyte cultures

Primary culture of chondrocytes was performed using articular cartilage tissues harvested from non-treated rabbits (NRC cells) or ACLMT-operated rabbits (ORC cells). Briefly, thinly sliced cartilage tissues were incubated with collagenase (4 mg/ml; Sigma Aldrich, St. Louis, MO, USA) in DMEM for 12 hours. Cells were then collected by centrifugation, seeded into type I collagen-coated dish (Corning International K.K.,Tokyo, Japan), and cultured with DMEM containing 10% FBS supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2/95% air. Chondrocytes were grown in monolayer cultures, and were passaged when reaching confluence. Cells at the second passage were used for the assay. ONO-AE1-259-01, a selective agonist of EP2, was used to stimulate EP2 signaling in the presence or absence of IL-1β (Sigma Aldrich, St. Louis, MO, USA).

Real-time PCR

Total RNA was extracted from cultured cells using the RNeasy kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. All reverse transcription reactions were performed with an RT-PCR kit using 1 μg of total RNA with a Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) for conversion into cDNA. The mRNA expression levels of *MMP-13* and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) were quantified by real-time PCR using SYBR Green (Applied Biosystems, Foster City, CA, USA) and the ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). All reactions were run in triplicate, and the amount of PCR product of each gene was calculated using the standard curve method and normalized to *GAPDH* levels, which were used as an internal control. Using the ratio obtained for the untreated sample as a standard (1.0), the relative ratio of the treated samples was presented as the relative expression levels of the *MMP-13* gene. Sequences of primers used in this experiment were as follows: 5'-aggagcatggcgacttctac-3' and 5'-taaaacagctccgcatcaa-3' (*MMP-13*) and 5'-gctctccagaacatcactcctgcc-3' and 5'-cgttgtcataccaggaaatgagct-3' (*GAPDH*).

Statistical analysis

The statistical analyses were performed using the Statcel2 software (The publisher OMS Ltd., Saitama, Japan). The results are shown as the mean ± standard deviation (SD). The Kruskal-Wallis test was performed for screening

purposes, and the Steel-Dwass method for multiple comparisons was used if there was a significant difference between samples. A P value less than 0.05 was considered to be significant.

Results

Therapeutic effect of ONO-8815Ly in the early stages of degeneration

At two weeks after the operation, articular cartilages in medial condyles of G-C (Figure 2a, b) and G-0 (Figure 2a, c) showed severe degenerative findings such as surface irregularity including clefts and reactive changes such as clonal proliferation of chondrocytes. The intensity of safranin O staining was reduced in G-C (Figure 2a, g) and G-0 (Figure 2a, h). The grade of degenerative findings was less prominent in sections of G-S (Figure 2a, a), G-80 (Figure 2a, d) and G-400 (Figure 2a, e) than in those of G-C or G-0. Safranin O staining was stronger in sections of G-80 (Figure 2a, i) and G-400 (Figure 2a, j). Similar findings were observed in sections prepared from lateral femoral condyles. The degenerative changes were less prominent and the safranin O staining was stronger in sections of G-S (Figure 2b a and 2f), G-80 (Figure 2b, d and 2i) and G-400 (Figure 2b, e and 2j) than in those of G-C (Figure 2b, b and 2g) or G-0 (Figure 2b, c and 2h).

Histological grade was evaluated using a modified Mankin's scoring system [23,24]. The grades of medial condyle in each sample were scored and mean values were compared (Figure 2c). Scores were significantly better for G-80 than for G-0. The effect of ONO-8815Ly was more prominent in lateral condyles, and both G-80 and G-400 showed much better scores than G-C or G-0 (Figure 2d).

Similar findings were observed in medial (Figure 3a) and lateral (Figure 3b) condyles of tibiae. The degenerative changes were less prominent and the safranin O staining was stronger in sections of ONO-8815Ly-treated groups (G-80 and G-400) than in those of non-treated groups (G-C and G-0). The effect of ONO-8815Ly was similar between G-0 and G-80 in medial condyles (Figure 3c), whereas G-80 and G-400 showed better values than G-C or G-0 in lateral condyles (Figure 3d). These results suggested that ONO-8815Ly prevents degenerative change in articular cartilages during the early stages.

Therapeutic effect of ONO-8815Ly in the late stages of degeneration

Similar analyses were performed using sections prepared at 12 weeks after surgery. In the case of femoral condyles, no improvements of cartilage degeneration were observed in sections of ONO-8815Ly-treated groups (G-80 or G-400) (Figure 4a, d and 4e) and the staining of safranin O also showed no difference (Figure 4a, i and 4j). Similar results were obtained in lateral condyles of femora (Figure 4b). In agreement, there was no significant difference in Mankin's score in the analyses of medial (Figure 4c) or lateral (Figure 4d) condyles of femora.

Similar results were obtained in the tibiae. Neither medial nor lateral condyles showed better histological features by the treatment with ONO-8815Ly, and the Mankin's score showed no improvements (data not shown).

These results suggested that the effect of ONO-8815Ly failed to last, at least when using this drug delivery system.

Growth promoting effect of ONO-8815Ly

The proliferating activity of chondrocytes was evaluated by PCNA staining (Figure 5). The proportion of PCNA-positive cells in femoral (Figures 5a and 5b) and in tibial (Figures 5c and 5d) condyles at two weeks after operation were similar among all groups, suggesting that the improvement of cartilage degeneration by the EP2 agonist was not due to the acceleration of cell proliferation.

EP2- selective agonist inhibits the expression of MMP-13 in ACLMT

MMP-3 and MMP-13 are major proteases degrading the ECM. The expression of these enzymes was analyzed by immunohistochemistry using samples prepared at two weeks after the operation. For MMP-3, there were no significant differences in staining intensity or number of positive cells between any of the groups (Figure 6). For MMP-13, however, significant differences were observed (Figure 7). The staining of MMP-13 was much stronger in G-C and G-0 (Figure 7a, b and 7c) than in G-S, G-80, or G-400 (Figure 7a, a, d, and 7e). The proportion of MMP-13-positive cells was significantly lower in sections of G-80 and G-400 than in sections of G-C or G-0 (Figure 7b). Similar results were obtained for the intensity (Figure 7a, f, i, and 7j) and the ratio of MMP-13-positive cells (Figure 7c) in the analyses of lateral condyles.

EP2-selective agonist inhibits IL-1β-induced *MMP-13* mRNA expression

To confirm the effect of EP2 agonist on MMP-13 expression, the expression of the *MMP-13* gene by primary cultured chondrocytes was evaluated by quantitative real-time PCR (Figure 8). The expression levels of *MMP-13* were similar in NRC and ORC cells under basal culture conditions. Similarly, EP2 agonist treatment showed no significant effects on *MMP-13* levels on either cells. When NRC and ORC cells were treated with IL-1β (50 pg/ml), the expression levels of *MMP-13* mRNA were significantly increased in both cells. IL-1β-induced expression of MMP-13 mRNA in ORC cells was reduced by

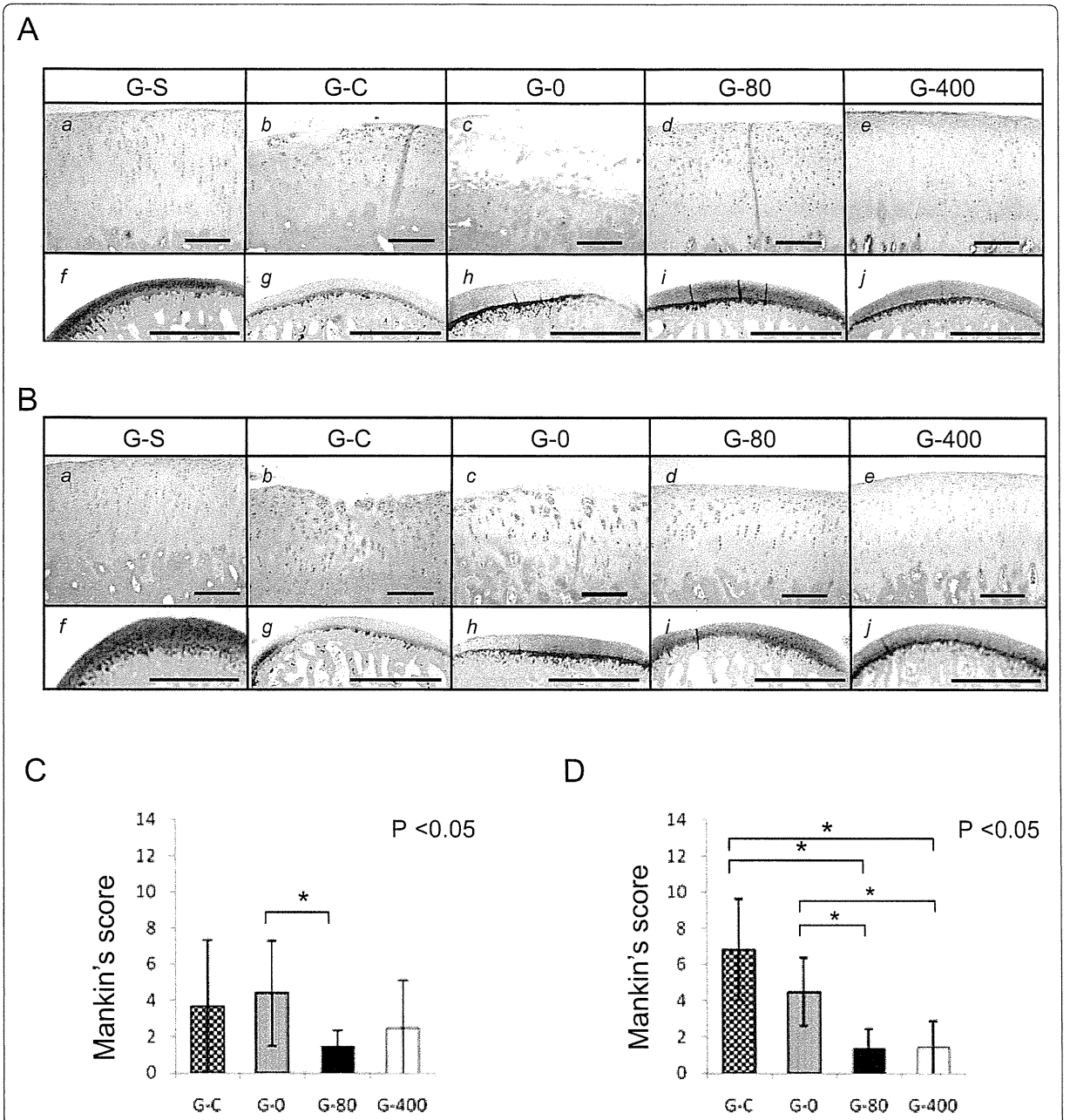

Figure 2 Effect of ONO-8815Ly in the early stages of OA (femur). Histological findings and grade of degeneration, scored using Mankin's system, in (**a**) medial and (**b**) lateral femoral condyles at two weeks after the operation. Sections were prepared from samples of (*a* and *f*) G-S, (*b* and *g*) G-C, (*c* and *h*) G-0, (*d* and *i*) G-80, and (*e* and *j*) G-400, and stained with (*a* to *e*) H&E or (*f* to *j*) safranin-O. Magnification, 200× (H&E) and 40× (safranin-O). Scale bar, 200 μm (HE) and 2.0 mm (safranin-O). *$P < 0.05$. Values are the mean ± standard deviation. OA, osteoarthritis.

co-treatment with the EP2 agonist in a dose-dependent manner, and the maximum reduction was 37% at 1 μM of EP2 agonist. In the case of NRC cells, the maximum reduction (27%) was observed at the concentration of 0.1 μM.

Discussion

The effect of PGE2 on the progression of OA is still a matter of debate. In some reports, PGE2 was shown to destroy articular cartilage by degrading cartilage ECM [12,13]. It has also been reported to down-regulate the

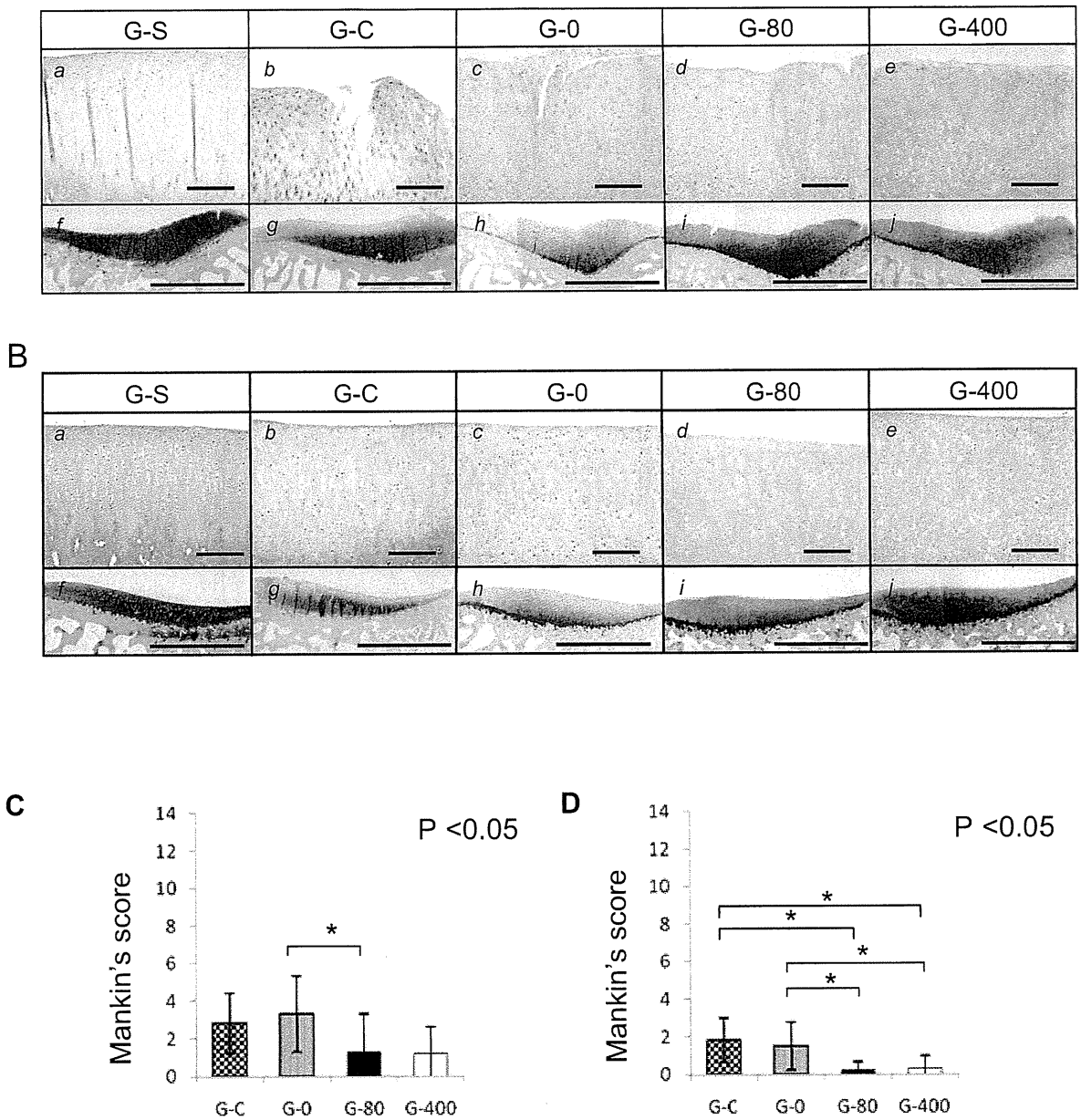

Figure 3 Effect of ONO-8815Ly in the early stages of OA (tibia). Histological findings and grade of degeneration, scored using Mankin's system, in (**a**) medial and (**b**) lateral tibial condyles at two weeks after the operation. Sections were prepared from samples of (*a* and *f*) G-S, (*b* and *g*) G-C, (*c* and *h*) G-0, (*d* and *i*) G-80, and (*e* and *j*) G-400, and stained with (*a* to *e*) H&E or (*f* to *j*) safranin-O. Magnification, 200× (H&E) and 40× (safranin-O). Scale bar, 200 μm (H&E) and 2.0 mm (safranin-O). *$P < 0.05$. Values are the mean ± standard deviation. OA, osteoarthritis.

production of IL-6 by IL-1α and IL-1β via EP2/EP4 receptors [25,26]. PGE2 at very low concentrations inhibits the production of IL-1β, TNF-α, and MMP-13 in the articular cartilages of OA patients [27]. In the current study, the production of MMP-13 was decreased by an EP2 agonist (Figures 7 and 8), which is consistent with the *in vitro* data described in a recent report [28]. Continuous administration of non-steroidal anti-inflammatory drugs to patients with OA exacerbates OA [29,30]. These contradictory results

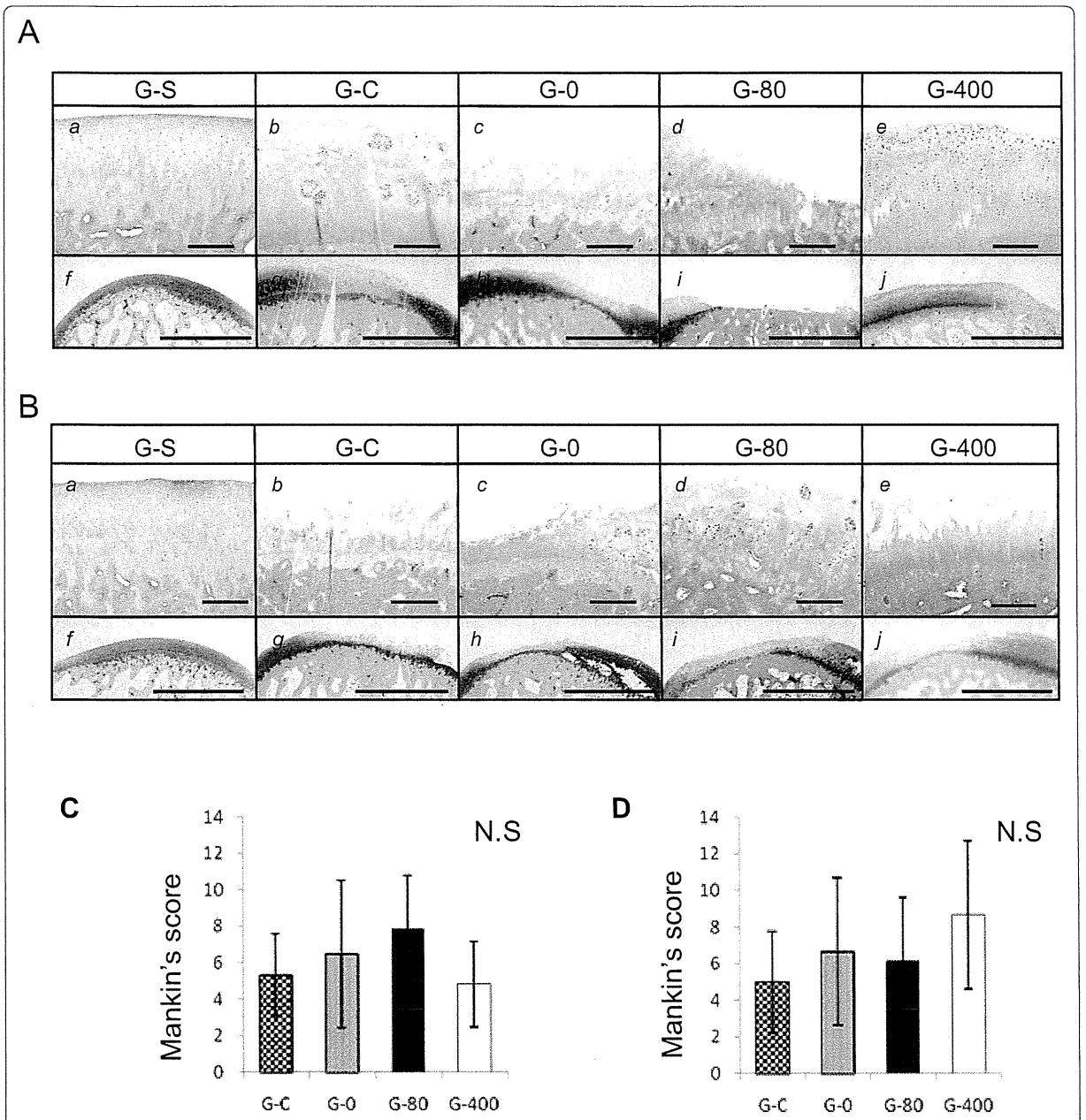

Figure 4 Effect of ONO-8815Ly in the late stages of degeneration (femur). Histological findings of **(a)** medial and **(b)** lateral femoral condyles at 12 weeks after the operation. Sections were prepared from samples of (*a* and *f*) G-S, (*b* and *g*) G-C, (*c* and *h*) G-0, (*d* and *i*) G-80, and (*e* and *j*) G-400, and stained with (*a* to *e*) H&E or (*f* to *j*) safranin-O. Magnification, 200× (H&E) and 40× (safranin-O). Scale bar, 200 μm (H&E) and 2.0 mm (safranin-O). Grade of degeneration scored using Mankin's system in **(c)** medial and **(d)** lateral femoral condyles at 12 weeks after the operation. Values are the mean ± standard deviation. N.S, not significant.

may be due to the differences in the experimental dose of PGE2 agonist used, or due to the pleiotropic effects of PGE2 through different types of receptors (EP1 to EP4). Therefore, analyses should be conducted with agonists specific for each type of receptor. IL-1β-induced expression of *MMP-13* mRNA was reduced by EP2 signaling both in NRC and ORC cells *in vitro* (Figure 8). Moreover, IL-1β-induced expression of *MMP-13* mRNA was reduced in ORC cells, but not in NRC cells, in a dose-dependent manner, that is, *MMP-*

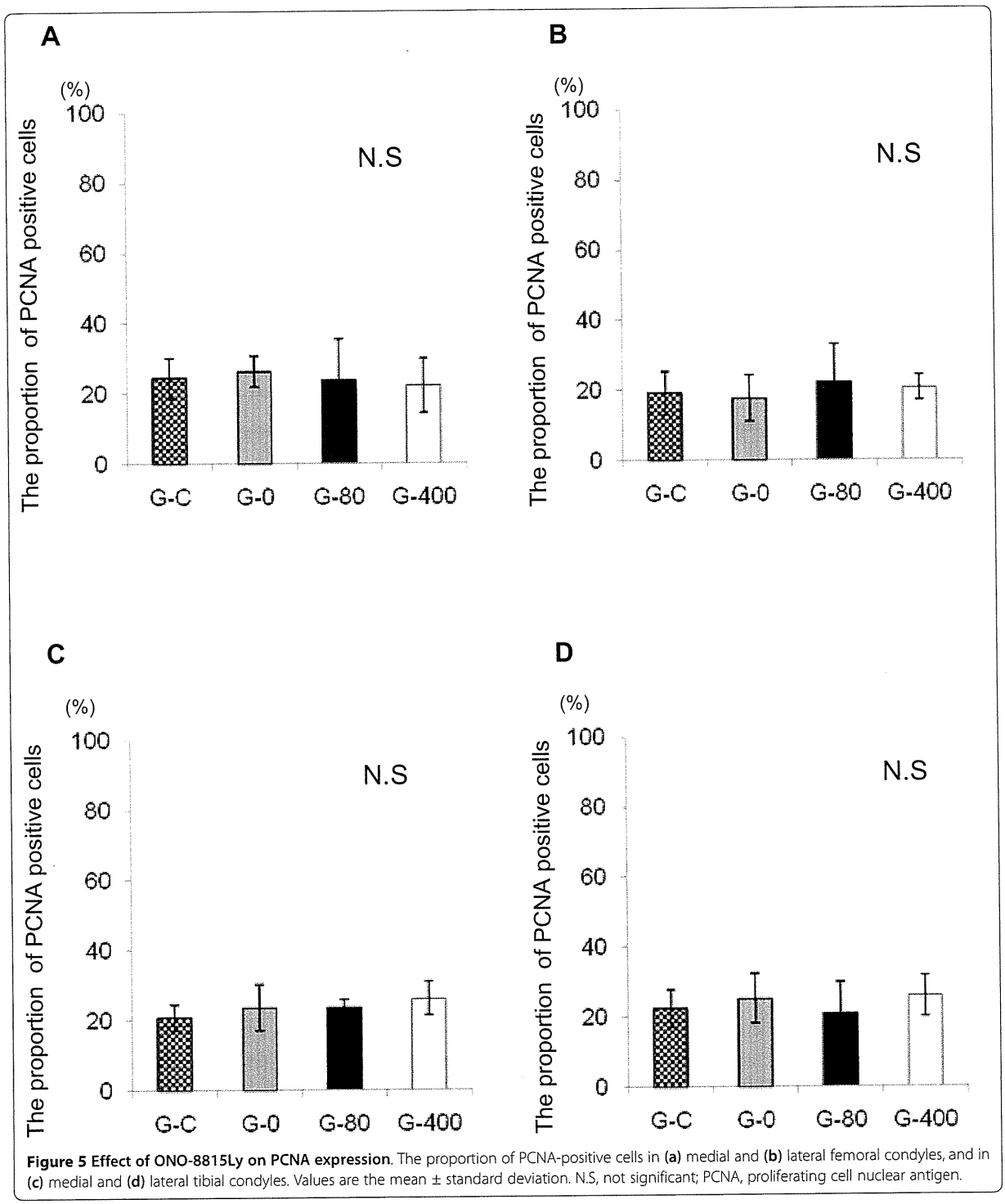

Figure 5 Effect of ONO-8815Ly on PCNA expression. The proportion of PCNA-positive cells in **(a)** medial and **(b)** lateral femoral condyles, and in **(c)** medial and **(d)** lateral tibial condyles. Values are the mean ± standard deviation. N.S, not significant; PCNA, proliferating cell nuclear antigen.

13 expression was higher in the presence of 1 μM of ONO-AE-259-01 than in the presence of 0.1 μM of ONO-AE-259-01 (Figure 8). An EP2 agonist acts as an anti-inflammatory drug at low doses, but if the concentration exceeds 1 μM, the anti-inflammatory effect may become weak (Figure 8). In fact, some authors have reported that excess EP2 agonists may act rather as inflammatory-inductive drugs.