

**Figure 3.** Histologic analysis of the ankle joints of the same rat in the M+/E+ group on day 21. **A** and **B**, Staining with hematoxylin and eosin (H&E). **C**, **D**, **E**, and **F**, Staining with tartrate-resistant acid phosphate (TRAP). **G** and **H**, Staining with toluidine blue (TB). The electroporation procedure was applied to the left ankle joint only (EP+) (**A**, **C**, **E**, and **G**). No inflammatory synovial tissue erosion into subchondral bone was observed with application of electroporation (**A**) compared with MTX only (**B**). Inflamed synovium infiltrated with lymphocytes was found to contain abundant osteoclastic multinucleated cells on TRAP staining (**D** and **F**). However, there was no difference in metachromasia of articular cartilage in the left and right hind paws. **E**, Higher-magnification view of the boxed area in **C**. **F**, Higher-magnification view of the boxed area in **D**. Bo = subchondral bone; Ca = cartilage; Pa = pannus; S = synovial tissue (see Figure 1 for other definitions).

significantly and consistently increased from day 11 until the end of the experiment. However, in the M+/E+ group, swelling of the left hind paw was significantly suppressed on days 14, 18, and 21 (Figure 1B) when compared with the 3 control groups (M+/E-, M-/E+, and M-/E-). The gross appearance of the hind paws is shown in Figures 2A and B. Thus, application of electrical pulses appeared to prevent the hind paw joints from progressing to advanced AIA. The degree of swelling differed significantly between the left (electrically

treated [EP+]) and right (EP-) paws of the same rat in the M+/E+ group (Figure 1C).

**Radiologic evaluation of bones and joints.** Radiologic analysis revealed that the hind paw joints were severely damaged in the M-/E-, M-/E+, and M+/E- groups at 21 days after injection of the adjuvant. Therefore, at a dose of 0.125 mg/kg body weight, MTX did not prevent the joint damage (Figure 2D) or local swelling (Figure 2B) caused by progression of arthritis. In contrast, the radiologic damage score was significantly lower in the electrically treated left (EP+) hind paws in the M+/E+ group (Figures 2A and C and Table 1).

**Histologic analyses.** In the M+/E+ group, the histologic scores were significantly lower in the left hind paws (EP+) than in the right hind paws (EP-) (Figures 3A and B and Table 1). Inflamed synovial tissues with abundant lymphocytes were observed to erode into subchondral bone (Figure 3B). In sections of these joints, the population of TRAP-positive multinucleated osteoclastic cells was significantly lower in the bones of the left hind paw (EP+) than in those of the right hind paw (EP-) (Figures 3C, D, E, and F and Table 1). Toluidine blue staining revealed no degenerative changes of cartilage tissue, including irregularity of articular surface, disorganization of tidemark, and alternation of metachromasia, in either hind paw (Figures 3G and H).

## DISCUSSION

These results indicate positive effects of pulsed electrical stimulation for attenuating arthritis by enhancing the antiarthritic effect of MTX. We believe that this is attributable to micropores created by the electrical pulses in the cytoplasmic membranes of cells in the synovium or other inflamed cells. The subsequent passive influx of MTX into the cells would attenuate the inflammatory responses that led to the AIA, although this study did not provide direct evidence of MTX influx. In this preliminary study, we could not identify the cells targeted by electrochemotherapy, and MTX-negative synovial cells, inflammatory cells, or both, may be targets for the drug.

The effects of electrical fields on living cells have been investigated since the 1960s, and high-voltage electrical pulses have been reported to generate transient and reversible pores in cell membranes. This phenomenon has been termed electroporation and is currently used to transfer genes or drugs into cells (6). Electrochemotherapy involves electroporation with drugs, and this methodology is used for the treatment of malignant tumors (5-9). The use of electrochemotherapy to introduce anticancer drugs into malignant tumors has been reported, e.g., bleomycin

for melanoma, basal cell carcinoma, Kaposi's sarcoma, squamous cell carcinoma (6), or chondrosarcoma (15). However, electrochemotherapy with MTX for the treatment of RA has not been reported, although the less-permeable character of MTX and its use as a DMARD in RA would make it an ideal candidate for this approach. Because the effect of pulsed electrical stimulation is expected only at the local site, this method might be applicable for an isolated joint with arthritis that is refractory to systemic chemotherapy or in the early stages of RA involving a limited number of joints without significant joint-destructive changes.

Clinical application of this therapy should not affect normal tissues. Using TUNEL staining, we did not observe any difference in the number of apoptotic cells between the M+/E+ and M+/E- groups (data not shown). We also confirmed in the pilot study that electrical pulses, used under the same conditions as those used in this experiment, did not influence the normal tissues of inbred 9-week-old male Lewis rats. In this pilot study, no inflammatory reactions were observed on histologic examination of the area treated with the electrical pulses, suggesting that electroporation under these conditions did not cause any damage to normal tissue, including cartilage, bone, muscle, and blood vessels (results not shown). However, the clinical application of electrochemotherapy requires further study, including the dose of MTX and the parameters of the electrical pulses.

This experimental study is limited in 2 key areas. First, electrochemotherapy was not applied to joints with established arthritis, and the effect of electrochemotherapy was estimated based on the progression of arthritis. This differs from the clinical situation, in which, as indicated previously (10,11), the inflammatory phase in this AIA model is self-limiting. Therefore, the efficacy of electrochemotherapy for the treatment of established chronic arthritis is difficult to determine in this model. Second, optimization of the application of pulsed electrical current may not be sufficient to obtain maximum delivery of MTX into cells and to achieve maximal antiinflammatory effect in RA. The conditions that enable the efficacy of electrical stimulation in electrochemotherapy may be quite different from the condi-

tions used in the clinical treatment of malignancies that were reference sources for the present study. The potential value of electrochemotherapy for the treatment of RA has been illustrated by these studies, and further work is required to optimize electrochemotherapy to control disease in joints with RA refractory to treatment.

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## Potential risk factors for prolonged recovery following whiplash injury

Received: 31 May 2003  
Revised: 16 January 2004  
Accepted: 4 March 2004  
Published online: 25 May 2004  
© Springer-Verlag 2004

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**Abstract** A retrospective analysis of insurance data was made of 600 individuals claiming compensation for whiplash following motor vehicle accidents. Three hundred randomly selected claimants who had settled their injury claims within 9 months of the accident were compared with 300 who had settled more than 24 months after the accident. We compared the two groups to identify possible risk factors for prolonged recovery, for which settlement time greater than 24 months was a marker. Variables considered included demographic factors, type of collision, degree of vehicle damage, workers compensation, prior claim or neck disability, treatment and time to settlement. Consulting a solicitor was associated with a highly significant, four-fold increase of late settlement of the claim. A concurrent workers' compensation claim, prior neck disability and undergoing physiotherapy

or chiropractic treatment were weakly associated with late settlement. The degree of damage to the vehicle (as indicated by cost of repairs) was not a significant predictor of late settlement. Late settlement may be the direct effect of legal intervention, independent of the severity of the injury. Whilst the financial benefit to the claimant of consulting a solicitor is apparent, the benefit of prolonged disability is not. It may be to the advantage of both insurers and claimants if those likely to proceed to late settlement could be recognised early and their claims settled expeditiously.

**Keywords** Whiplash · Neck injury · Motor vehicle accident · Compensation claim · Legal representation

### Introduction

Whiplash is a common injury. In South Australia approximately 4,000 claims for whiplash, at a cost of the order of Australian \$50 million, are made annually (for a population of 1.5 million). There is some evidence that the incidence of this condition has increased in recent decades, although it appears unrelated to increased seat belt use [11].

In an extensive review, the Quebec Task Force (QTF) on whiplash-associated disorders has noted that cases are usually self-limited, with a median time to recovery –

measured by time to the end of disability compensation – of 31 days. However, a significant fraction exhibited prolonged disability: 10% of the cases studied in the Quebec cohort study were still unable to resume normal activity 200 days post-injury [8]. In a review of studies published since the QTF report, considerable variation has been found in the duration and extent of recovery. Important sources of variation have been the outcome measures used (e.g., settlement of claim, return to work, persistence of symptoms) and the type of insurance system (e.g., tort or no-fault) [3].

It is unclear if protracted disability from whiplash is related to the degree of trauma. In a 1996 review of whi-

plash, Stovner concluded that evidence for a causal link between trauma and chronic symptoms was sparse [9]. Two studies published since have shown predictive effects of some collision factors (e.g., collisions that are not rear-end) but have failed to show any association between crash severity and prognosis [5, 7].

Unexplained prolonged disability and lack of evidence on effective treatment have led to conflicting opinions on the role of psychological factors and litigation. Some studies in countries with differing insurance-payment systems have found evidence that psychosocial and legal issues may increase morbidity following whiplash injury [4, 6]. However, a randomised double-blinded study in Australia has shown a beneficial effect of radiofrequency neurotomy on chronic whiplash patients. This finding led the authors to propose that psychological effects are likely to be a consequence, rather than the cause, of chronic pain following whiplash and that the most likely cause of pain is post-traumatic dysfunction of the cervical zygapophyseal joints [12]. This study attempts to identify risk factors that may predispose to prolonged disability following whiplash injury.

## Materials and methods

Records of whiplash claims filed over the period 1993–1996 were obtained with personal identifiers deleted. This data set was divided into two sub-files: (i) claims settled within 9 months of injury and (ii) claims settled more than 24 months after the injury. Subjects with radiological damage to the cervical spine, neurological deficit and/or significant associated injuries were excluded. Three hundred anonymous records were randomly selected from each sub-file.

A series of univariate analyses was conducted for the relationship between late (>24 months) settlement and the following potential predictors: age, sex, occupation, position in the vehicle, type of collision, prior or concurrent workers' compensation claim, prior neck disability, cost of vehicle repair, whether a solicitor was consulted and cost of treatment. The 600 claims were classified into those with and without the potential risk factor, and the relationship between the risk factors and late settlement was estimated as a risk ratio as follows:

Risk ratio = proportion of subjects with risk factor whose claim was settled after 24 months, divided by the proportion of subjects without the risk factor whose claim was settled after 24 months.

Since one-half of the subjects were selected from the "late settlement" category, the expected proportion of subjects with any risk factor who had a late settlement, in the absence of any association between the factor and late settlement, would be one-half, and the risk ratio equal to one. A risk ratio significantly greater than one would therefore suggest that the risk factor increased the risk of late settlement. The statistical significance of the risk ratio was estimated using a chi-square test. In cases where there were more than two categories of predictor variable (e.g., occupation, mode of injury), a chi-square test for homogeneity was applied to determine whether the distribution differed significantly from the expected value.

A comparison was also made of the distribution of certain variables in the groups in the "early settlement" and "late settlement" categories, using non-parametric analyses. Variables found in the univariate analyses to be significantly related to late settlement were entered into a log binomial model to estimate the role of the variables after adjustment for mutual confounding.

## Results

### Gender

Of the 600 claimants, 63.5% (381) were female, significantly greater than the proportion of males. Fifty-three percent of the women settled their claims after 24 months, compared with 46% of the men. However, the excess of women with late settlement was not statistically significant (risk ratio=1.15, Table 1).

### Age

The proportion of claims settled early for each age stratum is shown in Table 2. In most age strata the proportion of subjects whose settlement was late was close to the expected value of 50%. The exception was subjects aged 65 years and over, of whom only 28% settled late. The latter accounts for the  $\chi^2$  value for homogeneity of 13.0, *df* (degrees of freedom)=4,  $p=0.01$ . There is no obvious trend away from late settlement for age, and non-parametric testing confirmed that age was not a significant predictor for prolonged settlement.

### Occupation

The proportion of late claims by occupation is shown in Table 3. There was no significant association with late settlement in any occupational category ( $p=0.62$ ).

### Type of collision

There was significant variation in the proportion of claims settled late between different types of accident ( $\chi^2=17.3$ ,

**Table 1** Settlement time by gender

Sex	Early settlement (<9 months)	Late settlement (>24 months)	Total
Male	119	100	219
Female	181	200	381

Risk ratio for late settlement (F/M)=1.15, NS

**Table 2** Settlement time by age

Age range (years)	Early settlement (<9 months)	Late settlement (>24 months)	% with late settlement
0–24	70	61	47
25–44	151	147	49
45–54	36	61	63
55–64	22	23	51
65+	21	8	28

**Table 3** Settlement time by occupation

Occupation	Early settlement (<9 months)	Late settlement (>24 months)	% with late settlement
Blue collar	79	70	47
White collar	112	126	53
HD	43	37	46
Unemployed	23	21	48
Pensioner	28	24	46
Student	15	22	59

$\chi^2=0.62$ , NS.

**Table 4** Settlement time by type of collision

Mode of injury	Early settlement (<9 months)	Late settlement (>24 months)	% with late settlement
Rear hit	146	152	51
Front hit	7	28	80
Rear and front hit	43	39	48
Side hit	92	76	45
Rollover	12	5	29

$df=4$ ,  $p=0.002$ ). Eighty percent of subjects who had experienced a front-end collision had a late settlement. Only 29% of rollovers had a late settlement, although the number of accidents in this category was small. For rear-end, side-impact and chain collisions the proportion of late settlements was close to the expected value of 50% (Table 4).

#### Position in vehicle

Of the claimants, 423 were drivers, and 177 were passengers, of whom 145 were in the front seat. None of the positions in the vehicle was predictive of early or late settlement of claim.

#### Workers' compensation

Only 58 of the 600 claims were subject to workers' compensation, of which 46 (79%) had a late settlement, compared with 52% for non-workers' compensation cases. Thus workers' compensation cases were significantly more likely to have a late settlement (risk ratio=1.5,  $p=0.001$ ). Thirty-five claimants had had a prior workers' compensation claim, but there was no significant association with a history of a prior workers' compensation claim (54% late settlement for those with a previous claim vs 50% with no previous claim). Of employed subjects, the median time off work for those who settled within 9 months was 5 days, compared with 4 days for those who settled late (Kruskal-Wallis  $\chi^2=0.02$ , NS).

**Table 5** Settlement time by cost of repairs

Cost of repairs	Early settlement (<9 months)	Late settlement (>24 months)	% with late settlement
<\$1000	64	54	46
\$1000-2500	85	82	49
>\$2,500	86	92	52
Written off	65	72	53

#### Prior neck disability

Of the 131 subjects who had a history of neck disability, 58% settled late, compared with 48% for those with no prior neck disability. A history of neck disability was thus predictive of late settlement (risk ratio=1.2,  $\chi^2=4.3$ ,  $p=0.04$ ).

#### Damage to vehicle

The cost of repairs as an index of vehicle damage was not a predictor of late settlement. As shown in Table 5, there was no trend towards late settlement with increasing cost of repairs, nor was having the vehicle written off associated with late settlement. Whether the vehicle was driveable after the accident was not a significant predictor of late settlement.

#### Seeking medical attention on the day of accident

Of the 155 subjects attending a hospital on the day of the accident, 58% settled late compared with 47% of the other subjects. Thus, attending hospital on the day of the accident is a weak but statistically significant predictor of late settlement (risk ratio=1.23,  $\chi^2=5.4$ ,  $p=0.02$ ). However, a non-hospital medical consultation on the day of the accident had an opposite association. Of the 144 subjects who saw a doctor other than in a hospital on the day of the accident, 41% settled late, compared with 50% of subjects who were not seen at all on the day of the accident, although the effect was not statistically significant (risk ratio=0.69,  $\chi^2=3.3$ ,  $p=0.07$ ). Overall, attendance at either a hospital or a medical practitioner's rooms on the day of the accident had no association with late settlement (50% late settlement irrespective of attendance).

#### Attending a physiotherapist or chiropractor

Four hundred seventy subjects attended a physiotherapist some time between the accident and settlement. Those who attended were more likely to settle late (54% vs 32%, risk ratio=1.7,  $\chi^2=20.8$ ,  $p=0.001$ ); but for these subjects, a risk of late settlement was not associated with the length of time between the accident and first consultation. The median time until the physiotherapist was seen was one

week for both those who settled early and those who settled late. At some time between the accident and settlement, 102 subjects attended a chiropractor. Those who attended were more likely to settle late (63% vs 47%, risk ratio=1.3,  $\chi^2=8.0$ ,  $p=0.005$ ). Of the subjects who attended a chiropractor, there was a greater time before the first consultation in those who settled late. The median time until the chiropractor was seen was 1 week in those who settled early and 8 weeks in those who settled late.

#### Consulting a solicitor

Of the 344 subjects settling their claim through a solicitor, 75% settled late, compared with only 17% of those who settled directly with the insurer. Thus there was a highly significant association between consulting a solicitor and likelihood of a late settlement (risk ratio=14.6,  $\chi^2=197$ ,  $p=0.001$ ).

#### Total cost

The median total claim cost for the 300 subjects who settled early was Australian \$3,907, and for the 300 who settled late the median cost was \$19,457. The difference was significant ( $p=0.001$ ).

#### Multivariate analysis

The following variables were entered into a log binomial model: nature of collision (front end, rear end, etc.), making workers' compensation claim, prior neck disability, attending a physiotherapist, attending a chiropractor and consulting a solicitor. As shown in Table 6, there was an elevated risk of late settlement associated with making a worker's compensation claim and prior neck disability. However, the elevation was small in each case (1.15 and 1.14, respectively) and of only marginal statistical significance. Attending either a chiropractor or physiotherapist also accounted for increased risk of late settlement. The increases were small (1.16 and 1.30, respectively) but

statistically significant. On the other hand, consulting a solicitor was associated with over a 4-fold increase in risk of late settlement, an increase which was highly significant.

#### Discussion

Factors identified as presenting a risk of late settlement were front-end collisions, claims involving workers' compensation, history of prior neck disability, undergoing physiotherapy or chiropractic treatment and consulting a solicitor. By far the strongest association was consultation with a solicitor. The degree of damage to the vehicle (as indicated by cost of repairs) was not a significant predictor. Other factors not predictive of prolonged settlement were a history of prior workers' compensation claim, the period off work, occupational category, whether the subject was the driver or a passenger, and early presentation for medical attention.

The association of front-end collision and late settlement is similar to findings of a recent study of Quebec motor vehicle crashes, in which front and side collisions were found to predict delayed recovery [10]. The Quebec Task Force excludes front-end impact from its definition of whiplash [8]. It is plausible that the distinct dynamics of front-end collisions will yield prognostic markers that differ from those of whiplash injury. However, our multivariate analysis eliminated the nature of the collision as a significant predictor of late settlement: correlation analysis showed that this was not due to collinearity between the type of collision and the other variables.

The association of prior neck disability with late settlement is plausible and consistent with findings from other studies [2]. However, multivariate analysis showed this factor to be only weakly predictive of late settlement. Injury subject to a workers' compensation claim was similarly identified in the initial analysis as predictive of late settlement but found in the multivariate analysis to be only weakly predictive. Correlation analysis showed that this was not due to collinearity with consulting a solicitor (i.e., there was no association between having a work-related motor vehicle injury and consulting a solicitor).

A critical question is whether the duration of disability from whiplash injury is related to the severity of injury. The available data did not provide a direct measure of injury severity. The only available index was the cost of vehicle repair. Since the degree of damage to the vehicle and severity of injury are both related to the amount of energy transfer, some correlation is to be expected. Similarly, a delay in settlement beyond 2 years – the outcome variable used in this study – is not necessarily synonymous with prolonged disability. However, a correlation is likely. Cassidy et al. have reported a strong association between intensity of neck pain and level of physical functioning, on the one hand, and time to closure of the claim for whiplash injury, on the other [1]. Our finding of a lack of as-

**Table 6** Results of multivariate analysis (log binomial model) of possible determinants of late settlement

	Relative risk estimate	95% confidence interval	<i>p</i> -value ( $\chi^2$ )
Mode of injury	0.98	0.94–1.02	0.33
Workers comp claim	1.15	0.99–1.34	0.08
Prior neck disability	1.14	1.00–1.29	0.06
Attended chiropractor	1.16	1.03–1.29	0.01
Attended physiotherapist	1.30	1.05–1.63	0.02
Consulted solicitor	4.13	3.11–5.48	<0.0001

sociation between crash severity and prognosis is supported by other recently published studies [5, 7]. On the basis of the indirect measures of both injury severity and duration of disability, these results fail to show any relationship between severity of injury and recovery time.

The strong association between consulting a solicitor and late settlement may be interpreted in two ways: (1) the more severe injury cases may consult a solicitor, with the late settlement resulting from prolonged disability due in turn to the severity of injury; or (2) consultation with the solicitor may be a direct cause of prolonged settlement, independent of the severity of injury. Since our findings, albeit based on indirect measures, showed no association between injury severity and duration of disability, we suggest that the late settlement and increased cost of the claim may be the direct effect of legal intervention and independent of the severity of the injury. Whilst the financial benefit to the claimant of consulting a solicitor is apparent, the benefit of prolonged disability is not. It may be to the advantage of both insurers and claimants if those

likely to proceed to late settlement could be recognised early and their claims settled expeditiously.

As factors indicative of greater trauma were not predictive of prolonged settlement, we hypothesise that psychosocial factors are more important determinants of outcome. Accordingly, we are now undertaking a prospective study of whiplash injuries, to measure the influence of psychological, social, physical and emotional well-being on the duration of disability.

As discussed above, there are other dimensions of recovery in addition to settlement of injury claim. These include the return to work, need for continuing treatment and ability to perform activities of daily living. Our prospective study employs a variety of such measures of outcome.

**Acknowledgements** Data for this study were provided by the State Government Insurance Commission of South Australia. Drs Abraham, Eckerwall and Nakamura were recipients of grants from Sofamor Danek International. Dr Eckerwall was also supported by the Swedish Society of Medicine. We also thank Dr J Mehta and Ms A. O'Riordan for their assistance.

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# ENDOSCOPIC SPINAL SURGERY

-RECENT ADVANCES IN THE FIELD OF SPINAL SURGERY

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*Endoscopic spinal surgery is one of the recent armamentarium popularized into the medical sciences for the treatment of spine ailments.*

*Initially in thoracic spine, it was used by the cardiothoracic surgeons for the treatment of lesions in the thoracic cavity. A decreased post-operative morbidity and reduced hospital stay made it increasingly popular amongst the patients as well as the surgeons. Its main indications in thorax include the anterior release for scoliosis, resection of herniated thoracic disc, resection of intrathoracic tumor etc.*

*In the lumbar spine, it has been used for the excision of the lumbar discs and the fusion of the lumbar spine.*

*Nowadays, posterior endoscopic disc excision is one of the commonest indications for which this technique is being used.*

## INTRODUCTION

**I**n recent decades, advances in surgical equipment and refinement of surgical techniques have steadily increased the use of endoscopy in all fields of surgery. These advances are based on the development of optical instruments such as scopes, cameras monitors etc. and have been fostered by the desire of patients to undergo minimally invasive surgery.

In the field of spinal surgery, Obenchain reported the first case of laparoscopic lumbar discectomy, following which Mack et al described video-assisted thoracoscopic (VAT) surgery. Endoscopic surgery has since become one option of surgical treatment for spinal disorders.

## THORACOSCOPIC SPINAL SURGERY

In the beginning of the 1990s, thoracoscopic procedures were extensively utilized by cardiothoracic surgeons in the treatment of lesions that involved the thoracic cavity. Following development of equipment and refinement of the technique, the first reports dealing with endoscopic surgery for the thoracic spine were published in early 1990s. Comparisons between thoracoscopy and open thoracotomy have demonstrated that use of



endoscopic techniques decreases postoperative pain, improves shoulder girdle function and decreases morbidity, while reducing blood loss, time required in an ICU and overall length of hospital stay.

The indications for thoracoscopic surgery include biopsy, anterior release for scoliosis, resection of thoracic disc herniation, excision of tumors originating from nerves and reconstruction following vertebrectomy.

### **ANTERIOR RELEASE FOR SCOLIOSIS**

Nearly ten reports have been published on the results of thoracoscopic anterior release for spinal deformity, based on video-assisted thoracoscopic (VAT) surgery and appears best indicated for treatment of moderate curvature (in the range of 55-75 degrees). In a comparative study by Newton et al, VATS technique yielded the same results as open procedures in achieving spinal flexibility, as assessed by average percentage of correction and same results were reported in two further studies. In addition, the rate of complications of the VATS technique was reported to be 18% and almost the same as for the open method.

These preliminary reports enabled correction of scoliosis with newly designed instrumentation under thoracoscopic observation. The operative scar resulting from this surgical procedure is along the midaxillary line. The operative scar resulting from trochar insertion can thus be under the arm, enabling good cosmetic results.

### **RESECTION OF THORACIC DISC HERNIATION**

Resection of thoracic disc herniation is also a good indication for thoracoscopic surgery. Several reports have documented encouraging early results of use of the VATS procedure for thoracic disc herniation. Even over long-term follow-up,

endoscopic discectomy yielded results equivalent to those of the classical open technique.

### **RESECTION OF INTRATHORACIC TUMOR**

Resection of para-vertebral tumor in the dumbbell-shaped thoracic cord tumor is also a good indication for thoracoscopic surgery. This type of surgery makes use of a combined posterior and anterior approach. First, a standard posterior approach is used to perform hemilaminectomy of the thoracic spine and of the medial facetectomy. The intraspinal and foraminal tumor component can usually be resected with a posterior approach. The anterior part of the tumor can then be approached under thoracoscopic observation. Excellent results have thus far been reported with use of this method.

### **VERTEBRECTOMY, RECONSTRUCTION AND INSTRUMENTATION**

Conditions of the anterior column resulting from trauma or infection can be excised, reconstructed or stabilized using anterior instrumentation devices. The beneficial use of the endoscope for such procedures is in the approach to the upper thoracic spine (T2-T4) and the thoracolumbar junction (T11-L2). In the classical open method, the approach to this region is not easy without disinsertion of the scapula or minimal disinsertion of the diaphragm, which necessitates complex reconstructions.

### **LAPAROSCOPIC LUMBAR SPINAL SURGERY**

Laparoscopic discectomy was first described in 1991. This case report of a young male patient with an L5/S1 herniation, in whom surgery was performed by the transperitoneal route with simultaneous endoscopic, video and fluoroscopic guidance, demonstrated the relative ease of access to this disc and in this instance, the procedure could be performed on day care surgery basis. The same group of authors further described their technique

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and reported 15 cases of laparoscopic discectomy in 1995. They subsequently switched onto a retroperitoneal approach, which they considered easier and safer, especially at L4/5 and above this level.

**Laparoscopic lumbar fusion** in humans was first reported in 1995. In that report, complications in 100 endoscopic spinal surgeries including 22 of laparoscopic lumbar fusion were described. The next report concerned a series of six patients, five of whom had successful L5/S1 laparoscopic fusion with bone dowels (metal pins) and in one of whom the endoscopic approach had to be abandoned because of iliac vein laceration. Two further studies reported were of 17 and 34 cases respectively of laparoscopic spinal fusion and each described the approach to the L4/5 as well as the L5/S1-disc.

Recently, due to complications (of laparoscopic discectomy) such as postoperative intra-abdominal adhesions, retrograde ejaculation and great vessel injury, the retroperitoneal endoscopic approach has begun to be utilized. The disadvantage of this approach is the need to retract the psoas muscle, which contains several peripheral nerves. Nakamura et al subsequently described a method of retracting this muscle easily and intermittently. In order to make this approach easier, retroperitoneal endoscopically-assisted mini-laparotomy has been utilized for anterior lumbar interbody fusion. This approach has been reported to have less morbidity than completely closed endoscopic surgery.

## **POSTERIOR ENDOSCOPIC DISCECTOMY**

In 1975, percutaneous lumbar discectomy with posterolateral approach was first reported. Subsequently, this technique evolved to include the use of automated disc removal devices, spinal endoscopy and lasers. However, indications

for these procedures have generally been limited to contain lumbar disc herniations, because lumbar radiculopathies (pain in the nerve root) secondary to large free-fragment disc pathology and any type of bony compression of the nerve root are still specific contraindications to percutaneous lumbar discectomy.

In the early 1980s, following the introduction of the technique and instrumentation described by Casper, there was progressive spread of use of microscopes for disc herniation surgery. This has permitted a less invasive approach than the open one, with more rapid postoperative recovery.

Some surgeons have attempted to combine the less invasive microsurgical technique via the traditional midline posterior approach with modern endoscopic technology. Foley and Smith developed a new system for endoscopic posterior discectomy using a tubular retractor. This technique has the same goal as conventional open lumbar discectomy under endoscopic visualization through a small tubular retractor. With this method of true endoscopic surgery, it is possible to successfully remove the disc and/or remove bone lesions compressing a nerve root, as in open approaches, but with a small skin incision and less disruption of the fascia and the paraspinous muscle, reducing postoperative pain. For these reasons, this procedure has greatly decreased the average hospital stay for routine lumbar discectomy.

Since Foley's report, the procedure has been widely utilized for lumbar discectomy. Its relative lack of invasiveness compared with conventional open discectomy has been demonstrated. Recently, resection of recurrent disc herniation and decompression for lumbar spinal canal stenosis has also become an indication for this surgical procedure.

## **CLINICAL FOCUS**

- ★ Endoscopic spinal surgery is one of the recent advancements and is minimally invasive.
- ★ Its main advantages are reduced post-operative morbidity, cosmetically superior results and reduced hospital stay.
- ★ Commonly being used for disc surgery and anterior release for scoliosis.
- ★ Endoscopic spinal surgery has been recently extended for use in recurrent disc excision and lumbar canal stenosis.

## **CONCLUSION**

Endoscopic surgery is being commonly used for the treatment of spine problems. It is being used both for thoracic as well as for lumbar spine. Small incisions, short hospital stay and less postoperative pain has led to reduced morbidity to the patients.

The benefits like small scars (cosmetically better) and early return to work are making it more patient-

friendly. But a long learning curve and adequate training are mandatory to give satisfactory results.

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## **ABSTRACTS**

### **MINIMAL ACCESS SPINAL TECHNOLOGIES : STATE-OF-THE-ART, INDICATIONS AND TECHNIQUES**

Minimal access spinal technologies aim primarily at minimizing the trauma associated with surgical exposure of the spine. They owe their existence mainly to recent progress in optical and imaging devices and to the development of instrumentations specifically designed for insertion via minimally invasive approaches.

No published scientific studies have proved that minimally invasive techniques are superior over standard techniques. However, patients benefit from the decreased postoperative pain, shorter hospital stay and expedited return to normal activities.

Finally, minimal access spinal technologies are evolving at a fast pace. Progress is being made in defining the indications and assessable results have been obtained for a number of lesions.

**SOURCE :** Assaker R. et al Neurosurgery Department, Roger Salengro Teaching Hospital, Lille, France. Joint Bone Spine. 2004 Nov;71(6):459-69.

### **LOW-DOSE ASPIRIN IN GENERAL PRACTICE**

Until now, there has been confusing evidence as to whether general practitioners should recommend aspirin to patients to reduce the risk of heart attack and other cardiovascular events. Maria Carla Roncaglioni and colleagues used the setting of general practice to carry out a randomised controlled trial to investigate low-dose aspirin in the prevention of cardiovascular events and also looked at a possible role for vitamin E, which is known to prevent oxidative damage.

Low-dose aspirin given by general practitioners in addition to treatment of specific risk factors was found to contribute a beneficial preventive effect. The results for vitamin E, however, were not conclusive. Walter W Rosser discusses the difficulties in carrying out such trials in a general practice setting, but says that general practitioners should now have the confidence to recommend low doses of aspirin for primary prevention.

**SOURCE :** The Lancet, 2001; 357:84, 89.

Bone 37 (2005) 555 – 562

## Augmentation of bone morphogenetic protein-induced bone mass by local delivery of a prostaglandin E EP4 receptor agonist

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Reprinted from BONE, Vol. 37, No. 4, October 2005  
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## Augmentation of bone morphogenetic protein-induced bone mass by local delivery of a prostaglandin E EP4 receptor agonist

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Received 14 October 2004; revised 9 April 2005; accepted 29 April 2005  
Available online 18 July 2005

### Abstract

Recombinant human bone morphogenetic protein (rhBMP) is viewed as a therapeutic cytokine because of its ability to induce bone. However, the high doses of rhBMP required for bone induction in humans remain a major hurdle for the therapeutic application of this protein. The development of a methodology that would effectively overcome the weak responsiveness to human BMP is highly desired. In the present study, we investigate the ability of a prostaglandin E EP4 receptor selective agonist (EP4A) to augment the bone-inducing ability of BMP in a biodegradable delivery system. A block copolymer composed of poly-D,L-lactic acid with random insertion of *p*-dioxanone and polyethylene glycol (PLA–DX–PEG, polymer) was used as the delivery system. Polymer discs containing rhBMP-2 and EP4A were implanted into the left dorsal muscle pouch of mice to examine the dose-dependent effects of EP4A. Fifty mice were divided into 5 groups based on the contents of rhBMP and EP4 in the polymer (group 1; BMP 5 µg EP4A 0 µg, group 2; BMP 5 µg EP4 3 µg, group 3; BMP 5 µg EP4 30 µg, group 4; BMP 5 µg EP4 300 µg, group 5; BMP 0 µg EP4 30 µg, *n* = 10 each). All implants were harvested, examined radiologically, and processed for histological analysis 3 weeks after surgery. On dual-energy X-ray absorptiometry (DXA) analysis, the bone mineral content (BMC) of the ossicles was 6.52 ± 0.80 (mg), 9.36 ± 1.89, 14.21 ± 1.27, and 18.75 ± 2.31 in groups 1, 2, 3, and 4 respectively. In terms of BMC, the values of groups 3 and 4 were significantly higher than those of group 1. The mean BMC value of group 4 was approximately 3 times higher than that of group 1. No significant difference in body weight was noted among the groups during the experimental period. In summary, the presence of a prostaglandin E EP4 receptor selective agonist in the carrier polymer enhanced the bone-inducing capacity of rhBMP-2 with no apparent systemic adverse effects. © 2005 Elsevier Inc. All rights reserved.

**Keywords:** Bone morphogenetic proteins; Bone metabolism; Bone volume; Bone mineral density; Biomaterials

### Introduction

Bone has an inherent regenerating potential, and damaged bone or fractures are repaired by local new bone (callus) formation in a period of several weeks after an injury. The regenerating potential of bone has been attributed to factors or molecules with the biological capacity to induce mesenchymal cells to differentiate into bone- or cartilage-forming cells (osteoblasts and chondrocytes) and thereby form the callus. Bone morphogenetic proteins (BMPs) were originally isolated on the basis of their ability to induce

ectopic cartilage and bone formation via an endochondral cascade when implanted in experimental animals [1]. Because of the specific biological activity of BMPs and the successful generation of synthetic BMPs by DNA recombination, there is tremendous interest in using these proteins for bone repair and reconstructive surgery in a clinical setting [2]. However, 2 problems need to be addressed before we can witness the widespread clinical use of rhBMPs. One issue involves the use of a carrier material that has adequate safety and efficacy for BMP delivery. Currently, bovine collagen is used clinically as a carrier for rhBMPs, but use of this material comes with the risk of contracting bovine spongiform encephalopathy (BSE) or Creutzfeldt–Jacob disease (CJD). These diseases are

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potentially transmitted by prion proteins through cattle-derived foods and implant materials. Another problem is the high dose of rhBMP required for clinical efficacy in human patients. For example, to achieve a single level of spinal fusion, several to 10 mg of rhBMP are required. This results in the high cost and limited use of BMP as a substitute for bone autograft. Large doses of BMP may also increase the risk of potential adverse events in patients [3–6].

To address the issue of finding a suitable carrier, we have developed new biodegradable synthetic polymers that work effectively to deliver rhBMP and elicit new bone formation consistently at the implanted sites. The combination of rhBMP-2 and the polymers has enabled the successful regeneration of critical-size bone defects in experimental animals [7–10].

To improve the performance of rhBMP, we have sought agents to reinforce the bone-inducing activity of the protein and increase the induced bone mass. To this end, we have examined phosphodiesterase (PDE) inhibitors (pentoxifylline, rolipram) and a compound (ONO-4819), which is a prostaglandin (PG) EP4 receptor selective agonist (EP4A) [11–13]. PGE produced by cells of the osteoblastic lineage has been implicated as a regulator of bone metabolism through stimulation of either bone formation or resorption [14–16]. Exogenously applied PGE, either systemically or locally, also has enhanced bone formation in *in vivo* experimental models [17–19]. These biological effects of PGE are mediated through PGE receptors, which have been classified into 4 sub-types, EP1 through EP4. These EP receptors are encoded by distinct genes and are expressed in a tissue-specific manner [20–25]. In general, PGE mediated via EP1 increases intracellular  $Ca^{2+}$  concentration, EP2 and EP4 increase cAMP, and EP3 reduces cAMP and modulates down-stream signaling [25]. Knockout mouse studies have revealed that EP4 is the major receptor that mediates the PGE<sub>2</sub>-induced anabolic action in bone [26–30]. Systemic administration of an EP4 agonist (ONO-4819) enhanced new bone formation in mice, and an EP4 antagonist suppressed the increase in trabecular bone volume induced by PGE<sub>2</sub> [13,30–33]. In our previous study, the systemic administration of these drugs by daily injection for 1 week during the initial phase of BMP-induced bone formation led to a significant augmentation of ossicle mass [13]. These results suggest that the efficient local release of these activators for BMPs could induce augmented bone formation without adverse effects due to high dose and long-term administration. Therefore, we examined the effects of adding a low dose of ONO-4819 to the BMP delivery system on new bone formation.

## Materials and methods

### Drugs/chemicals/materials

The prostanoid receptor EP4-selective agonist (ONO-4819), methyl 7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(3*S*)-3-hydroxy-4-(*m*-methoxymethylphenyl)-1-butenyl]-5-oxocyclopentyl]-5-thiaheptanoate (Patent Cooperation Treaty publish No. WO 00/03980), was obtained from Ono Pharmaceutical (Osaka, Japan) and dissolved in phosphate-buffered saline prior to use.

rhBMP-2 was produced by the Genetics Institute (Cambridge, MA) and donated to us through Yamanouchi Pharmaceutical Co. (Tokyo, Japan). The rhBMP-2 was supplied in a buffer solution (5 mmol/l glutamic acid, 2.5% glycine, 0.5% sucrose, and 0.01% Tween-80) at a concentration of 1 µg/µl after filter sterilization.

Poly-D,L-lactic acid-*p*-dioxanone-polyethylene glycol block copolymer (PLA-DX-PEG) (MW; 9800, PLA/DX/PEG molar ratio; LA/Dx/E0 = 43/14/43) was synthesized and provided to us by Taki Chemicals Co. (Kakogawa, Japan). The structural formula of the polymer is shown in Fig. 1. The polymer has a sticky gel-like character at room temperature and turns into a soft gel at 50°C. The physicochemical characteristics and the efficacy of this polymer as a carrier material for rhBMP-2 have been described by our group in previous reports [9,10]. The minimal optimal content of rhBMP-2 required to induce new bone formation was approximately 1 µg in 20 mg of the polymer (0.005%) in mice, 0.02% in rabbits, and 0.04% in dogs based on our previous experimental data [8,10,34].

### Animals

One hundred and ten closed colony male ICR mice (4-weeks old; Nippon SLC, Hamamatsu, Japan) were housed and acclimated in cages with free access to food and water for 1 week. Experiments were carried out in strict accordance with the Institutional *Guidelines for the Care and Use of Laboratory Animals* of Osaka City University.

### Preparation of PLA-DX-PEG polymer implants containing rhBMP-2 and ONO-4819

To prepare a single implant, 30 mg of the PLA-DX-PEG polymer was softened by heating to 37°C, mixed with an aliquot of either the rhBMP-2 solution (0.5 µg/5

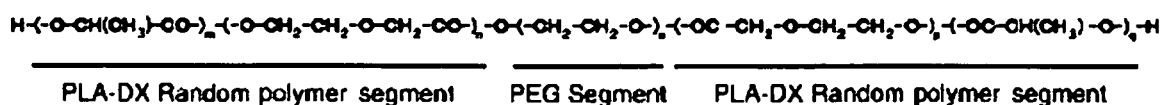


Fig. 1. Structural formula of PLA-DX-PEG polymer. Structural formula of the poly-D, L-lactic acid with random insertion of *p*-dioxanone and polyethylene glycol block copolymer (PLA-DX-PEG). The subscripts *m*, *n*, *o*, *p*, and *q* represent variable numbers of these units.

$\mu\text{l}$  or 5  $\mu\text{g}/5 \mu\text{l}$ ) or rhBMP-2 and ONO-4819 solution (3  $\mu\text{g}/3 \mu\text{l}$ , 30  $\mu\text{g}/3 \mu\text{l}$ , 300  $\mu\text{g}/3 \mu\text{l}$ ) and then fabricated into a disc (6 mm diameter, Fig. 2). In summary, 0, 3, 30, or 300  $\mu\text{g}$  of ONO-4819 was mixed with the polymer plus 5  $\mu\text{g}$  of rhBMP-2 and implanted into mice in each group (5 mice in each group and 1 implant/mouse). To examine the effects of ONO-4819 alone, 30  $\mu\text{g}/8 \mu\text{l}$  was added to the polymer without rhBMP-2. All procedures were carried out under sterile conditions. The implants were stored at  $-40^{\circ}\text{C}$  in a freezer until required for implantation.

### Experimental design

To examine the dose-dependent effects of the EP4 receptor agonist on ectopically induced bone formation by rhBMP-2, 50 mice were divided into 5 groups (10 mice per group). The mice were anesthetized by diethyl-ether gas inhalation, and the PLA–DX–PEG polymer discs prepared as described above were surgically implanted into the left dorsal muscle pouches (one pellet per animal) of the mice. In group 5, polymer discs containing 30  $\mu\text{g}$  of ONO-4819, but no rhBMP-2, were implanted in the same manner.

1. 5  $\mu\text{g}$  of rhBMP-2 per animal
2. 5  $\mu\text{g}$  of rhBMP-2 and 3  $\mu\text{g}$  ONO-4819 per animal
3. 5  $\mu\text{g}$  of rhBMP-2 and 30  $\mu\text{g}$  of ONO-4819 per animal
4. 5  $\mu\text{g}$  of rhBMP-2 and 300  $\mu\text{g}$  of ONO-4819 per animal
5. 30  $\mu\text{g}$  of ONO-4819 per animal

At 1, 2, and 3 weeks after surgery, the body weight of each mouse was measured and recorded. Three weeks after surgery, the mice were sacrificed, and the implants were harvested and processed for histological analysis following morphological and radiological examination.

### Radiological and histological analyses for rhBMP-2 induced ectopic bone

All harvested tissues were radiographed with a soft X-ray apparatus (Sofron Co., Ltd., Tokyo, Japan). The bone mineral content (BMC) (milligrams per ossicle) of each ossicle was measured by dual-energy X-ray absorptiometry (DXA) using a bone mineral analyzer (DCS-600EX, Aloka Co., Tokyo). The ossicles or tissue mass from each group was then fixed in neutralized 10% formalin, decalcified with K-CX (Fujisawa Pharmaceutical Co., Ltd. Japan), dehydrated in gradient ethanol series, and embedded in paraffin wax. Sections of 3  $\mu\text{m}$  thickness were cut, stained with hematoxylin–eosin, and observed under a light microscope.

### Bone metabolic markers in mice

To investigate the anabolic effects of ONO-4819 on systemic bone metabolism, an additional 60 mice were divided into 3 groups as follows: sham-operated mice that received sham operation and lacking implants (10 mice per group), group 1: 5  $\mu\text{g}$  of rhBMP-2 per animal (5 mice per group) and group 3; 5  $\mu\text{g}$  of rhBMP-2 and 30  $\mu\text{g}$  of ONO-4819 per animal (5 mice per group). Blood samples were collected from mice of each group at 1, 2, and 3 weeks. The samples were stored at  $-80^{\circ}\text{C}$  until biochemical analysis. Serum osteocalcin was measured by immunoradiometric assay (IRMA) using a commercial kit (Immutopics, Inc. San Clemente, CA) according to the manufacturer's instructions. Total alkaline phosphatase (ALP) activity, calcium (Ca), and phosphate (P) in serum were also measured in each group with commercially available kits.

### Statistical analysis

Data are presented as mean  $\pm$  SE. The degree of significance was determined by post hoc testing using the



Fig. 2. PLA–DX–PEG polymer disc. Photograph of 6-mm-diameter PLA–DX–PEG polymer disc. The polymer has a hard sticky gel-like property at room temperature and softens when heated to  $50^{\circ}\text{C}$ .

Bonferroni method. An associated probability ( $P$  value) of  $<0.05$  was considered significant.

## Results

### Body weight changes in animals

In our previous experiments, mice that received systemic injection of an excessive dose (100  $\mu\text{g}/\text{kg}$ ) of ONO-4819 every 8 h for 3 weeks showed a significant decline in body weight gain. In the current experiments, no significant difference in body weight gain was noted among the groups that received implants with or without local release of ONO-4819 (Fig. 3).

### Radiological and histological evaluations

Pieces of hard tissue were harvested from the implantation sites of mice from groups 1, 2, 3, and 4 at 3 weeks after implantation. In group 5 (ONO-4819, 30  $\mu\text{g}$  without BMP-2), no evidence of hard tissue formation was found at the implantation sites. On soft X-ray radiograms, the calcified samples retrieved from the mice revealed a trabecular network encased within a shell-shaped bone layer (Fig. 4). Histological sections of these samples showed normal characteristics of bone with trabeculae and hematopoietic marrow in the inter-trabecular space, findings that were also common to ossicles from groups 1, 2, 3, and 4. (Fig. 5) Radiological images indicated that the ossicles from group 3 (rhBMP-2, 5  $\mu\text{g}$  + ONO-4819, 30  $\mu\text{g}$ ) and 4 (rhBMP-2, 5  $\mu\text{g}$  + ONO-4819, 300  $\mu\text{g}$ ) were larger than those observed from control group 1 (rhBMP-2, 5  $\mu\text{g}$  without ONO-4819).

On DXA analysis, the bone mineral content (BMC) of the ossicles containing ONO-4819 increased in a dose-dependent manner (3, 30, and 300  $\mu\text{g}$  groups were  $9.36 \pm 1.89$  mg,  $14.21 \pm 1.27$  mg, and  $18.75 \pm 2.31$  mg, respectively) Ossicles from group 1 mice (without ONO-4819) had a BMC of  $6.52 \pm 0.80$  mg. In terms of BMC, the values of groups 3 and 4 were significantly higher than those of group 1. The mean BMC value of group 4 (BMP-2, 5  $\mu\text{g}$  + ONO-4819, 300  $\mu\text{g}$ ) ossicles was approximately 3 times higher than that of the control group (Fig. 6).

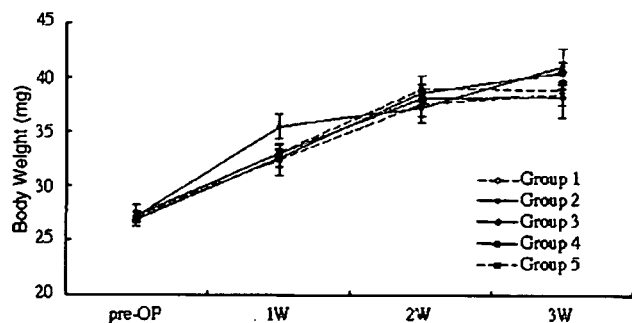


Fig. 3. Body weight. No significant difference in body weight was noted among the groups with implants with or without ONO-4819.

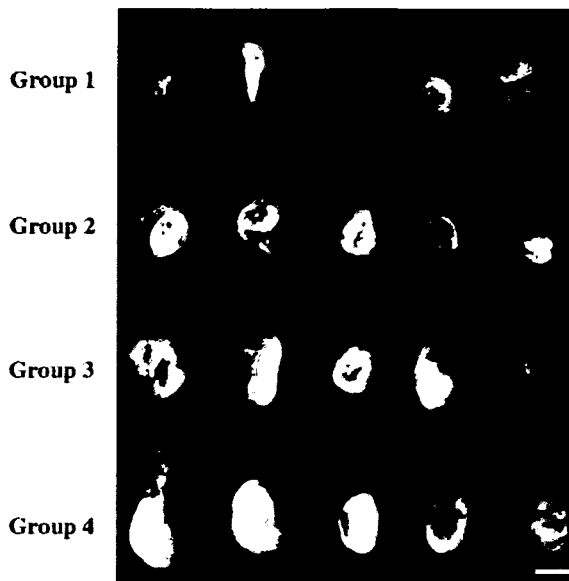


Fig. 4. Radiographic findings. Soft X-ray photograph of the ossicles harvested at 3 weeks after implantation (bar = 5 mm). A typical implant from each group is shown (groups 1, 2, 3, and 4). Both the radio-opaque areas and radiological densities of the ossicles on the radiogram were larger in groups 3 and 4 than in control group 1.

### Serum osteocalcin and ALP activity assay

At 1 week, both serum osteocalcin ( $299.8 \pm 24.4$  ng/ml) and ALP activity ( $495.2 \pm 32.0$  IU/l) levels significantly increased in group 3 compared to the sham-operated animals (osteocalcin  $208.6 \pm 25.6$  ng/ml, ALP activity  $356.0 \pm 39.8$  IU/l). At 2 weeks, serum ALP activity ( $439.0 \pm 76.8$  IU/l) levels had increased significantly when compared to the sham-operated animals (ALP activity  $313.2 \pm 12.1$  IU/l) (Fig. 7A). However, there were no significant differences among the groups at 3 weeks after implantation (Fig. 7B). In addition, there was no significant increase in serum calcium and phosphate level among them at any time point (data not shown). No significant changes in serum osteocalcin and ALP levels from the baseline were recorded in the groups that received implants containing ONO-4819.

## Discussion

Based on these data, EP4A was examined for its ability to enhance BMP-induced bone formation and improve rhBMP-2 performance. In our previous study, systemic subcutaneous injections of the EP4A (ONO-4819) for 3 weeks increased bone mass induced by rhBMP-2 and caused a decline in body weight gain in the experimental animals [13]. To achieve the anabolic action and avoid the systemic adverse effect, low doses of the drug were added to the degradable polymer carrying the rhBMP-2 and implanted into the host mice. In this study, in a very encouraging response, ONO-4819 significantly increased the BMP-induced bone mass in dose-dependent manner



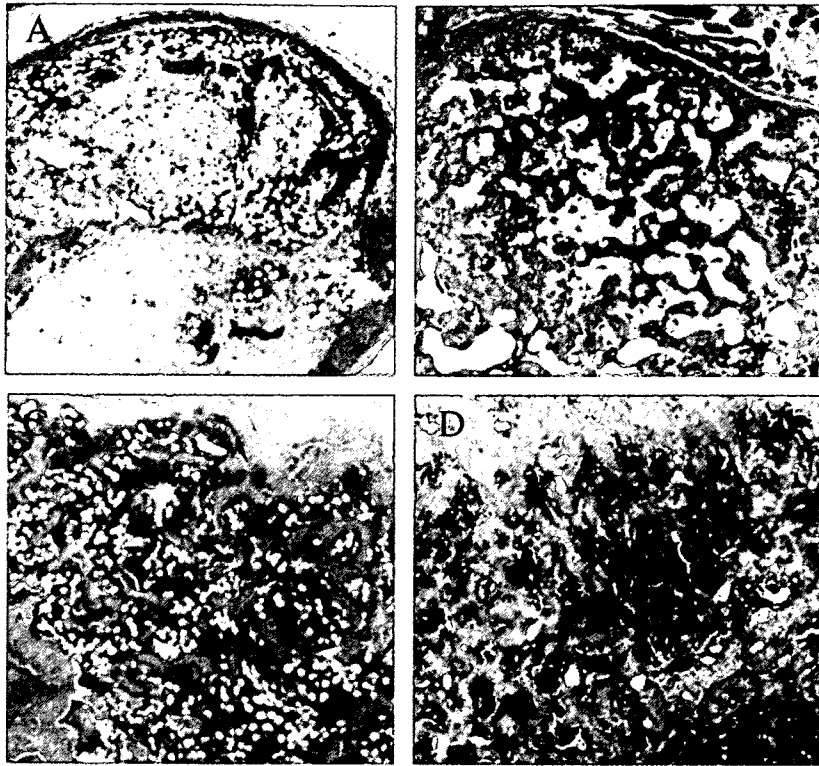


Fig. 5. Histology. Histological sections of the ossicles at 3 weeks after implantation are shown (hematoxylin–eosin stain; original magnification  $\times 40$ ). (A) group 1: 5  $\mu\text{g}$  of rhBMP-2, (B) group 2: 5  $\mu\text{g}$  of rhBMP-2 and 3  $\mu\text{g}$  of ONO-4819, (C) group 3: 5  $\mu\text{g}$  of rhBMP-2 and 30  $\mu\text{g}$  of ONO-4819, (D) group 4: 5  $\mu\text{g}$  of rhBMP-2 and 300  $\mu\text{g}$  of ONO-4819. New bone formation with hematopoietic marrow and bony trabeculae was visible in the rhBMP-2-induced ossicles. In groups 3 and 4, there were visible increases in the number and thickness of bony trabeculae when compared to the ossicles from group 1.

without significant body weight loss. The total dose of ONO-4819 required for a doubling of the BMP-induced bone mass was reduced when compared to the dose required using consecutive systemic administration (3 injections/day for 3 weeks) of the drug.

Enhanced bone formation by systemic administration of the EP4A over an experimental period of 3 weeks was essentially reproduced by the local release of the agent over the first week following implantation. This is the period when young mesenchymal cells most likely migrate, proliferate, and infiltrate the BMP/polymer composite implants before new bone formation gets underway

[7,9,10]. It is possible that these young mesenchymal cells were responsible for the bone formation enhanced by EP4A. Therefore, a low dose of the EP4A, ONO-4819, delivered locally and concurrently with rhBMP enhanced new bone formation and significantly increased bone mass. The effective period of local release of the EP4A is not greater than 2 weeks based on the degradation rate of the polymer [9,10]. Therefore, one possible explanation for the bone mass increased by EP4A is that EP4A works first in osteoblast precursors with a potential for chondro-osseous differentiation in the early phase of the bone-forming reaction. In the previous study, due to identifying the time phase when ONO-4819 exerts its pharmacological effects, EP4A was systemically administered for 1 week over pre (–1–0 week), initial (0–1 week), middle (1–2 week), or late (2–3 week) phase, respectively. The anabolic effects of EP4A were seen in mice that received EP4A exclusively in the initial phase. This result might also indicate that EP4A and BMP work cooperatively to stimulate osteoblastic differentiation in its early stage at the interface to the BMP-retaining pellets. Previous *in vitro* studies support our consideration. Suda et al. reported that EP2/EP4 seems to be involved in osteoblastic differentiation, and EP1/EP3 is likely to be associated with their proliferation [35]. Weinreb et al. described that PGE<sub>2</sub> stimulates osteoblastic differentiation through an anabolic effect in rat bone marrow cultures mediated by activation of EP4, probably

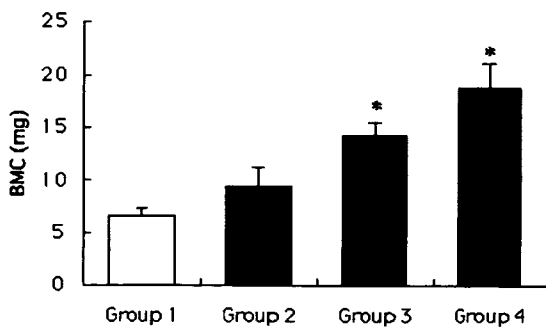


Fig. 6. Bone mineral content. The bone mineral content (BMC) of the ossicles at 3 weeks after implantation. BMC of ossicles was dose-dependently higher in groups 2, 3, and 4 than those in the group 1. Data expressed as mean  $\pm$  SE. \*Significantly different from controls ( $P < 0.05$ ).

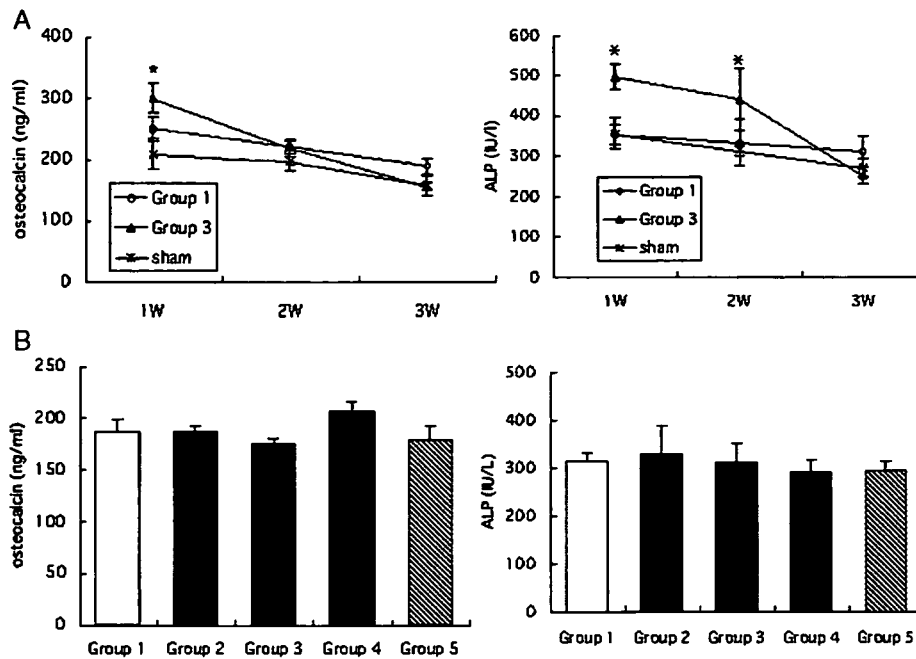


Fig. 7. Serum osteocalcin and ALP. Serum osteocalcin and ALP levels. (A) Serum osteocalcin and total ALP activity from group 3 with rhBMP-2 and ONO-4819 pellets were significantly increased compared to the sham group at 1 week. Total ALP activity from group 3 with rhBMP-2 and ONO-4819 pellets was significantly increased compared to the sham group at 2 weeks. (B) There were no significant differences in serum osteocalcin and ALP levels among the groups at 3 weeks after implantation.

by recruiting noncommitted osteogenic precursors [36,37]. Yoshida et al. described that PGE<sub>2</sub> induced the expression of core-binding factor alpha-1 (Runx2/Cbfa1) and enhanced the formation of mineralized nodules in a culture of bone marrow cells from wild-type mice, both of which were absent in a culture of cells from EP4 knockout mice. EP4 activation increased the number of Runx2 positive cells [30]. EP4 exerts this effect by inducing osteoblast differentiation. On the other hand, several studies indicate that EP4 is essential for PGE<sub>2</sub>-induced bone resorption. Suzawa et al. described that, in mouse calvaria cultures, EP4A markedly stimulated bone resorption, and in calvaria culture from EP4 knockout mouse, a marked reduction in bone resorption to PGE<sub>2</sub> was found. EP4A induced cAMP production and the expression of osteoclast differentiation factor mRNA in osteoblastic cells [27]. Stimulation of osteoclastogenesis in cocultures of osteoblasts and spleen cells in response to PGE<sub>2</sub> is markedly decreased when the osteoblasts are derived from cells lacking the EP4 receptor [26–29]. These in vitro studies indicate that PGE<sub>2</sub>-EP4 signaling works first in osteoblast precursors to induce osteoblast for bone formation and then works in mature osteoblasts to induce osteoclasts on newly formed bone. Further studies are required to elucidate the detailed mechanism of action of the EP4 receptor agonist in in vitro systems using less differentiated osteogenic cells.

The anabolic effect of PGE<sub>2</sub> on bone was exhibited through the activation of EP2 or EP4 and consequent elevation of intracellular cAMP level [23]. In this respect, the action of an EP4 agonist may be similar to that of PTH,

PDE-4, which also promotes bone formation and intercellular cAMP accumulation. Daily subcutaneous injection of parathyroid hormones (PTH) is known to enhance systemic bone formation, and daily systemic injection of phosphodiesterase-4 (PDE-4)-selective inhibitor, rolipram, can enhance BMP-2-dependent ectopic new bone formation in mice [11,38]. Although the detailed mechanisms of cAMP signal on bone formation have been unclear, these results might indicate that cAMP functionally has a key role in the regulation of the BMP action in osteoblast differentiation, and further studies are required.

Another possible mechanism of the anabolic effect of EP4A on the BMP-induced bone formation comes from studies involving cyclooxygenase-2 (COX-2). Zhang et al. showed the complementary effect of BMP-2 in a bone marrow cell culture from COX-2 knockout mice and suggested that BMP-2 is a target gene for PGE<sub>2</sub>-induced bone formation [39]. Chikazu et al. reported that BMP-2 transcriptionally induces COX-2 expression, which in turn regulates, via the Runx2 binding site, production of PGE<sub>2</sub> and promotion of osteoblastic differentiation [40]. These results indicate that BMP and PGE<sub>2</sub> might have complementary or cooperative anabolic effects on mesenchymal cells to stimulate the early phase of osteoblastic differentiation.

Potent bone anabolic activity of EP4A is expected from clinical application for fractures and bone defects in patients. Development of a more effective way of exposing responding cells and tissues to EP4A is likely to be needed for cost effectiveness, clinical efficacy, and long-term safety.

In cases with a longer fracture healing time, such as in humans, a carrier might be necessary for the sustained release of EP4A to be effective. The property of this polymer would allow retention of rhBMP-2 for a period that is significant to elicit new bone formation and thereby provide a scaffold for further bone growth. Retention of the proteins at the implantation site for a sufficient period to promote progenitor cell migration and cell proliferation has been shown to enhance osteoinductive activity. Our results show that local administration of ONO-4819 using PLA–DX–PEG polymer can mimic the local bone anabolic effect of PGE<sub>2</sub> without an excessive dose. The ability to deliver a molecule so that it will induce a specific biologic effect is critical to the success of pharmacological agent therapy.

In conclusion, a new EP4 receptor agonist compound (ONO-4819) can enhance the bone-inducing activity of rhBMP-2 when administered using a local polymer-based carrier with no apparent systemic adverse effects. This compound may be a useful tool for enhancing the performance of rhBMP-2. This could have a significant impact on the costs associated with using this therapeutic cytokine for bone regeneration and repair in clinical practice. Further safety checks are required before ONO-4819 can be used for this purpose.

#### Acknowledgments

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Project Grant No. 12137203) and by grants from the Ministry of Health, Labor and Welfare of Japan.

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