たものであり，サービスの基本は身体障害を有する高齢者に対する身体ケアに置いている。現在，約150万人といわれている認知症高齢者数は，2015年には約250万人に増加すると予測されていることから制度の軸足を「認知症ケア」にも置くことが求められる。このために高齢者の尊厳の保持といった観点から，環境変化の影響を受けやすい認知症高齢者の特性に配慮して小規模・多機能型サービスの創設や，早期の診断・対応から始まる継続的な地域支援体制の整備および虐待防止のための権利擁護システムの充実等が必要となる。また，高齢者独居世帯や高齢者夫婦のみ世帯において介護が必要となっても，できる限り住み慣れた地域で人生を送ることができるような地域ケア体制を整備していくことが求められる。これを実現するためには夜間や緊急時の対応も視野に置いたケア体制の充実や地域における総合的なマネジメント体制の整備を進めるとともに，これを支える地域での基盤整備の必要がある。

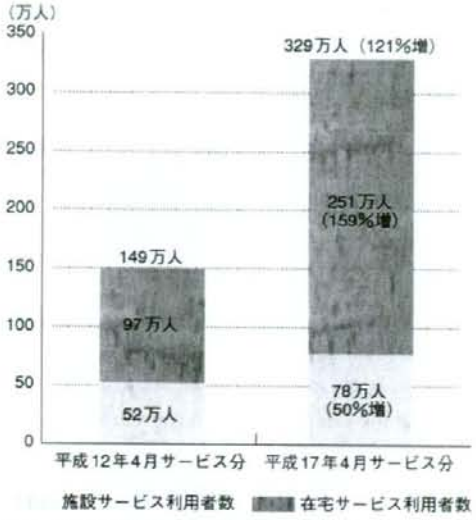

図1　サービス利用者数の推移（厚生労働省資料より）
過去5年間で介護保険の利用者は149万人から329万人へと倍増した。

3. 介護保険法の改正点

3.1. 介護予防の導入

今回の介護保険法の改正の大きなポイントが介護予防の導入にある。介護予防とは，単に高齢者の運動機能や栄養状態といった個々の要素の改善だけを目指すものではない。WHOの生活機能分類International Classification of Functioning, Disability and Health[2]の考え方を土台として（図4），これら心身機能の改善や環境調整などを通じて，個々の高齢者の生活行為（活動レベル）や参加（役割レベル）の向上をもたらし，1人ひとりの生き甲斐や自己実現のための取り組みを支援して，生活の質（QOL）の向上をめざすものである。これにより，国民の健康寿命を出来る限り延伸するとともに，真に喜ぶに値する長寿社会を創成することを目指している。

介護予防の具体的なサービスは軽度の要介護者が対象となる「新予防給付」と要介護認定では認定されなかった方や地域の虚弱高齢者を対象として市町村が主体となって行われる「地域支援事業」の2つが大きな柱となる。これまでの介護保険のサービスについても，要介護高齢者の生活の自立に役立っているかという観点から見直しを行い，足りないものを補う介護サービスから，適切なケアマネジメントにより，高齢者の尊厳のある自立した生活を支える介護へと転換を図ることにある。

これまでの介護保険制度の要介護認定において，要支援に該当する高齢者と要介護1の中で認知症の程度が重くサービス

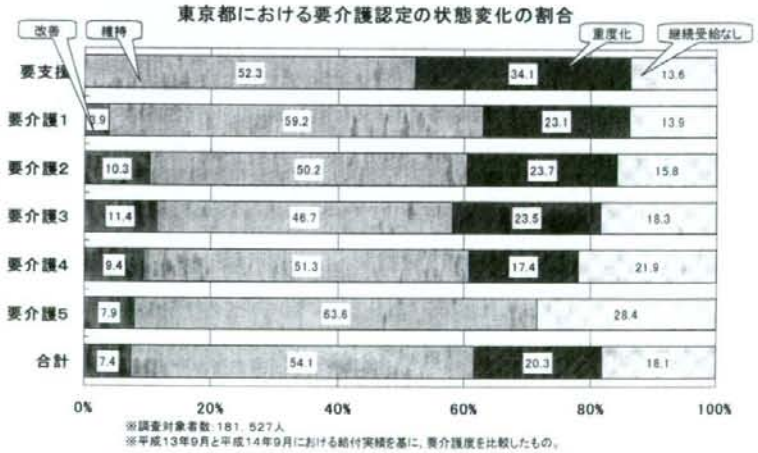

図2　東京都における要介護認定の状態変化の割合（厚生労働省資料より）
要支援など軽度の要介護者において1年度に要介護度が重度化した高齢者が少なくない。

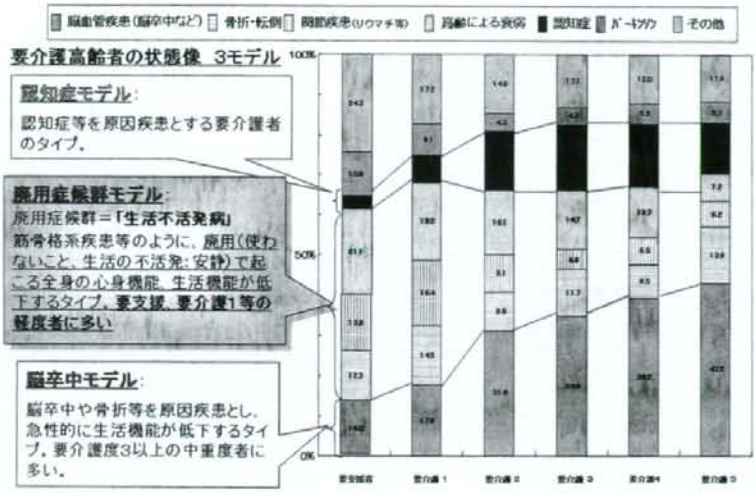

図3. 要介護に陥った原因疾患（厚生労働省資料より）
要介護状態に陥った原因疾患は要支援といった軽度の要介護者については，廃用症候群に該当する疾患の割合が多い。

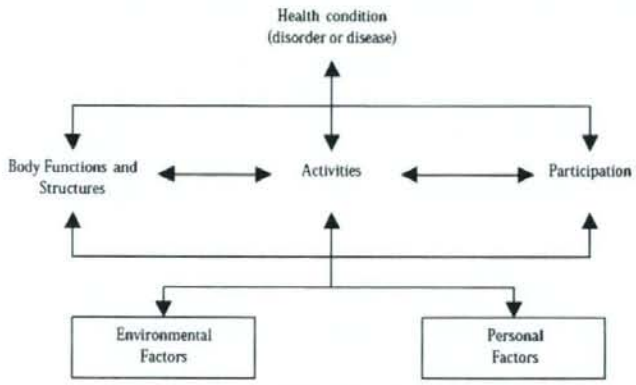

図4. 国際生活機能分類における各要因の概念図

を理解できない場合，心身の状態が不安定な場合を除いた高齢者を新たな要支援1，2と再分類し，新予防給付の対象者となった。また，要介護認定での非該当者や自立した生活を送っていても要介護高齢者の予備軍である虚弱な高齢者に対しても，「地域支援事業」による介護予防プログラムを受けることが可能となった。地域支援事業は保険者である各市町村の責任で行うことになっている。

これらの新予防給付と地域支援事業については，市町村に新たに設けられる「地域包括支援センター」においてケアマネジメントが行われることが大きな特徴である。これらのサービスの評価等にも市町村が積極的に関わることにより，より効率的なサービスが行われることが期待される。

介護予防の導入にあたっては，これまでの国内外の文献の精査による既存の研究の検討や，長寿科学総合研究事業における研究結果や未来志向プロジェクトにおける先駆的な取り組み，さらには厚生労働省が行った介護予防市町村モデル事業などの結果を踏まえ検討されてきた。適切なケアマネジメントを導入することや，既存の在宅及び通所サービスの評価・見直しを行った上で，新たに追加すべきサービスとして，以下のものが導入された。

新たな要支援者を対象に行われる新予防給付には，運動器の機能向上，栄養改善，口腔機能の向上が導入された。このほかに認知症，うつ，閉じこもりなどの対応についても，主として幅広い集団に対してサービスを実施することが有効と考えられることから，地域支援事業において実施することとなった。これらのプログラムについては各分野の専門家によって構成される研究班において検討が行われた[3]。そのなかで，特に地域支援事業については，対象者の把握と選定が大切であり，これ

まで市町村などのプログラムに参加できなかったような，真にサービスが必要な高齢者をいかに把握し，実際のサービスにつなげるかということが重要である。対象者はこれまで通りの本人や家族からの1) 当事者ルート，2) 民生委員や老人クラブなどを通じた住民ルート，3) 商工会や農協や各種サークルなどを通じた民間ルート，4) 介護予防に関する検診な保健活動による行政ルート，5) 医療機関を通じたルートなどを通じて様々なチャンネルを通じてハイリスクの高齢者を把握する必要がある。その上で市町村が主体となって創設される「地域包括支援センター」での適切なケアマネジメントに基づき，以下のような具体的なプログラムを本人の積極的な選択と同意の基に行うこととなった。

3.2. 介護予防の内容

口腔機能の向上

高齢者の日常生活において楽しみの第1位は食事であり，おいしく，楽しく，安全な食生活は高齢者が健康で生き生きとした生活を送る上で欠かすことの出来ないものであるとされる[4]。自分でおいしく食べられることは，脱水や低栄養の予防にもつながる。要介護度が重度化するにつれて嚥下性肺炎を起こす危険性が高くなると言われているが，口腔ケアが嚥下性肺炎の予防に対して重要な役割を担うことが証明されてきており[5]，また，サンプルサイズは少ないものの，インフルエンザ感染についてもウイルスが感染する際の開裂に必要なトリプシン様プロテアーゼを減少させることから，口腔ケアがインフルエンザの予防にも効果がある可能性も示唆されている[6]。これらのことから，口腔ケアの重要性について，嚥下性肺炎を初めとした肺炎の予防といった観点から重要であることについては根拠が整いつつある[7]。この介護予防プログラムにおいては，様々な原因疾患や高齢化により引き起こされる軽度の摂食・嚥下障害を有する高齢者に対して必ずしも専門家でないスタッフにより，健口体操を始めとしたプログラムを行うこととなっている。摂食・嚥下訓練についての適応は，その時の状態だけでなく，脳血管障害によるものであれば，その障害部位，発病からの日数や年齢などによって影響を受けることから，これらの要因を総合的に判断し訓練の対象として適切かどうか判断される。介護予防により実際どのような高齢者を対象として行えば有効であるのかについてのデータなどについては今後，更なる研究が必要であると考えられる。また，何故，このような口腔機能の向上のプログラムが軽度の要介護者のみに実施され，重度の要介護者について必ずしも実施される体制にないことなどもこれからの課題となろう。しかしながら，これまで広く認知されているとは言い難かった口腔ケアの重要性について，この介護予防への導入を機会として，様々な分野に波及していくことが考えられる。

運動器の機能向上

高齢による衰弱あるいは転倒などの明確な疾病ではないが，加齢に伴う生活機能の低下については，身体や精神の活動低下が背景にあると考えられ，これまで不可逆的なものと考えられてきたが，ここ十数年余りの間に虚弱な高齢者においても運動器の機能向上がもたらされることが明らかになって以来，国内外の数多くの研究によって運動器の機能低下の改善や予防が可能であることが明らかになった[8]。マシンを使ったトレーニング以外にも，弾力性のあるバンドをもちいたもの[9]，ダンベルをもちいたもの，あるいは太極拳をふくめたバランストレーニングにより転倒予防や運動機能の改善に役立つことが分かってきている[10]。これらの知見を基として，高齢者の個々に応じたメニュにより，より適切なプログラムを行うことと，運動負荷を軽負荷のものから段階的に高めていくコンディショニング期間を設けるとともに，その後，筋力の向上をねらった筋力向上期間，さらに最終的に利用者のニーズを反映させた機能的トレーニング期間へと3ヶ月程度を1周期としている。

低栄養対策

入院患者や虚弱高齢者における低栄養を予防することの重要性は次第に認識されるようになってきており，医療施設においてもNST (nutrition support team) などの取り組みにより特に経口からの栄養摂取の重要性が認識されてきている。低栄養状態にある高齢者に対して食事によって適正なタンパク質，エネルギーの摂取を行うことによって栄養状態が改善し，身体機能の改善が行われることは，メタアナリシス等の解析で示されている[11]。介護保険を利用する高齢者においては，通所介護を利用している高齢者においては約1割で低栄養対策が必要とする報告もある[12]。我が国での栄養に関連した対策というのは，これまでの栄養指導として生活習慣病の予防及び重症化予防を主な目的として，塩分制限や脂質の制限など食べる楽しみを制限する指導になりがちであったが，介護予防の観点からは，食べる楽しみを重視し，食べることにより低栄養状態を予防・改善し，高齢者の生活機能を維持・向上させることが必要である。低栄養状態の改善のためには，単に食事を提供するのではなく，個別の計画に基づいた栄養素等の摂取と食事についての適切な相談が有効であることが明らかにされており，他職種協働による，双方的コミュニケーションを重視し行うことが適当である。

3.3. 介護予防の導入以外の改正点

・施設給付の見直し

食費と居住費については在宅と施設の利用者負担の公平性，介護保険給付と年金給付の調整といった観点から，低所得者に対して低所得者の区分の見直しなどを行った上で，介護老人福祉施設，介護老人保健施設及び介護療養型医療施設の施設において光熱費に該当する居住費，食費を保険給付の対象外とし，2005年10月から入所者の自己負担となった。

・新たなサービス体系の確立・居住系サービスの充実・地域包括ケア体制の整備

高齢者のなかで認知症の方や独居の方が増加しており，これらの方々のこれらかのケアを考えた時には住み慣れた地域で

なじみの関係の中で生活していくことが重要である。このために，小中学校区など自宅の近くに通いのサービスを中心として希望に応じて訪問サービスや泊まりを組み合わせてサービス提供する小規模多機能型居宅介護や24時間安心して生活できる体制を整備するため夜間対応型訪問介護といった地域密着サービスが創設された。これまでの居住系サービスについても一定の居住水準等を満たす高齢者専用賃貸住宅などにも特定施設としての対象を拡大するなどサービスの充実を行うこととなった。また，地域包括支援センターの設置等によって，要介護状態になっても高齢者のニーズ地域包括ケア体制の充実を目指すこととなった。

4. おわりに

介護保険制度がより効率的かつ有効な制度となるべく施行後5年後の改正が行われた。この中で，介護予防のプログラムとして口腔ケアがプログラムに取り込まれたことは，歯科関係者としては非常に喜ばしいことである。実際のプログラムの実施には主に現行の介護スタッフが主体となり，歯科医師の関わり方は必ずしも明確ではなく，歯科治療を行っている場合には介護保険での介護予防としての報酬を算定できないなど，介護保険制度と歯科医療との関わり方について新たな関係構築が必要となってくると考えられる。このような状況の中で，在宅であろうと介護施設入所者であろうと高齢者にとって必要な口腔ケア，摂食・嚥下訓練，歯科治療といったサービスの提供体制を医療制度，介護保険制度の両者の中でどのようにシステムとして整備していけるかが今後重要となる。

内容要旨：2000年に施行された介護保険制度は我が国に欠かせない社会保障制度の一つとなってきているが，過去5年間でサービス給付やそれに伴う費用が急増し，今後も増加していくことが予想される。高齢者の自立の支援のために，より効率的で有効な制度とするために様々な制度改正が行われることとなった。

中でも，要支援者に対する「新予防給付」と要支援まで至らない虚弱高齢者を対象とした「地域支援事業」等が介護予防として新たに導入された。その中のプログラムの一つの重要な柱として「口腔機能の向上」が全ての市町村において施行されることとなった。プログラム対象者の選定，関わるスタッフの専門性や歯科医療との関わりなど多くの課題が残されており，これらを解決しながら，よりよいプログラムに改善していく必要がある。

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著者プロフィール

小坂　健（おさか　けん）

昭和39年7月生まれで，長野県立伊那北高等学校卒業後，平成2年に本学医学部を卒業し，循環器内科医として働いた後，設立されたばかりの東京大学大学院の国際保健学教室で本学出身の梅内教授に師事，修士課程でネパールやヴェトナムでの保健医療の研究のあと，博士課程の途中で，国立感染症研究所（旧予防医学研究所）感染症情報センターの設立に際して呼ばれて研究員，その後主任研究官になり，内閣府食品安全委員会の微生物部門，ウイルス部門の専門委員などを務めた。又，平成13年にはハーバード大学公衆衛生大学院の客員研究員（タケミフェロー）として留学し国際保健やバイオテロ対策の研究を行った。平成16年からは行政に関わり，厚生労働省老人保健課の課長補佐として，介護予防をはじめとした介護保険制度の改正，ガン対策や認知症対策の推進などに尽力し，平成17年7月より現職。

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平成 7年 3月　同　修了
平成 7年 4月　東京大学大学院医学系研究科・衛生学教室博士課程入学
平成 9年 3月　同　中途退学（国立感染症研究所就職のため）

職歴
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ORIGINAL PAPER

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Comparison of age-dependent expression of aggrecan and ADAMTSs in mandibular condylar cartilage, tibial growth plate, and articular cartilage in rats

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Abstract A disintegrin and metalloproteinase with thrombospondin motif (adamalysin–thrombospondins, ADAMTS) degrades aggrecan, one of the major extracellular matrix (ECM) components in cartilage. Mandibular condylar cartilage differs from primary cartilage, such as articular and growth plate cartilage, in its metabolism of ECM, proliferation, and differentiation. Mandibular condylar cartilage acts as both articular and growth plate cartilage in the growing period, while it remains as articular cartilage after growth. We hypothesized that functional and ECM differences between condylar and primary cartilages give rise to differences in gene expression patterns and levels of aggrecan and ADAMTS-1, -4, and -5 during growth and aging. We employed in situ hybridization and semiquantitative RT-PCR to identify mRNA expression for these molecules in condylar cartilage and primary cartilages during growth and aging. All of the ADAMTSs presented characteristic, age-dependent expression patterns and levels among the cartilages tested in this study. ADAMTS-5 mainly contributed to ECM metabolism in growth plate and condylar cartilage during growth. ADAMTS-1 and ADAMTS-4 may be involved in ECM turn over in articular cartilage. The results of the present study reveal that ECM metabolism and expression of related proteolytic enzymes in primary and secondary cartilages may be differentially regulated during growth and aging.

Keywords ADAMTS · Aggrecan · Mandibular condylar cartilage · Articular cartilage · Growth plate · Growth · Aging

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Introduction

Aggrecanase is a member of the metalloproteinase family which degrades a major cartilaginous extracellular matrix (ECM) component, aggrecan (Abbaszade et al. 1999; Arner et al. 1999; Tortorella et al. 1999; Caterson et al. 2000; Tang 2001; Arner 2002). Three members of the adamalysin–thrombospondins (ADAMTSs), ADAMTS-1, -4 (aggrecanase-1), and -5 (aggrecanase-2), are capable of cleaving an aggrecan molecule (Abbaszade et al. 1999; Tortorella et al. 1999; Kuno et al. 2000) at its specific sites, in a different manner from matrix metalloproteinases (MMPs), another large family of ECM degrading enzymes. Together with MMPs, ADAMTSs play a role in cartilage ECM metabolism during the development of cartilage and progression of joint diseases (Lohmander et al. 1993; Fosang et al. 1996; Lark et al. 1997; Caterson et al. 2000; Sandy and Verscharen 2001). Most studies have focused on the activity of aggrecanases, especially their production and activation under disease conditions, such as inflammatory responses and joint diseases, whereas their physiological expression patterns have not been determined during growth and aging.

Synovial joints are classified into two types, primary joints, such as the knee, and secondary joints, such as the temporomandibular joint (TMJ) (Ten Cate 1994). In

primary joints, primary articular and growth plate cartilages function separately for articulation and growth, respectively, whereas mandibular condylar cartilage performs both of these functions during growing period. ECM components and cellular organization of mandibular condylar cartilage, as a secondary cartilage, are different from those of primary cartilages as demonstrated previously (Silbermann et al. 1987; Luder et al. 1988; Mizoguchi et al. 1992). While mandibular condylar cartilage has been shown to have five distinct layers, primary cartilages, including growth plate and articular cartilage, is composed of four layers during the growth period (Luder et al. 1988; Mizoguchi et al. 1992). Primary cartilage cells express both type II collagen and aggrecan, cartilage-specific ECM components; however, cells in the upper two layers of mandibular condylar cartilage do not express either of these molecules (Mizoguchi et al. 1992; Takahashi et al. 1996; Shibata et al. 2001). Proliferating cells in growth plate and articular cartilage are well-differentiated chondrocytes, but those in mandibular condylar cartilage are not (Mizoguchi et al. 1992). Thus, cell proliferation and matrix synthesis in mandibular condylar cartilage are regulated differently from those of primary cartilages. In addition, during the growth, development, and maturation of the synovial joints, growth plate cartilage disappears by the end of the growth period. Similar to articular cartilage, which remains in the epiphysis of long bones, mandibular condylar cartilage becomes articular cartilage by losing hypertrophic chondrocytes after growth. Therefore, it can be considered that ECM metabolism in primary and mandibular condylar cartilage is differently regulated.

In this study, we examined the hypothesis that functional and ECM differences between condylar and primary cartilage give rise to differences in gene expression patterns and levels of ADAMTS-1, -4, and -5 during growth and aging. To test this hypothesis we used semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR) and in situ hybridization (ISH) using a newly identified rat ADAMTS-5 mRNA and subcloned aggrecan, ADAMTS-1 and ADAMTS-4.

Materials and methods

Experimental animals and tissue preparation

Male Wistar rats 4, 8, 16, and 32 weeks old were used in this study. Five animals for each age group were perfused via the ascending aorta with 4% paraformaldehyde and 0.5% glutaraldehyde in phosphate-buffed saline (PBS), pH 7.4, under pentobarbital anesthesia as described previously (Sasano et al. 1996). Procedures for tissue preparation were basically identical to our previous report (Bae et al. 2003). After the animals were perfused, TMJs and knee joints were dissected, further fixed in the same fixatives, and decalcified in 10% ethylene diamine tetra-acetic acid (EDTA). They were dehydrated, embedded in paraffin, and 8-μm-thick sagittal sections were cut for ISH analysis under RNase-free conditions and hematoxylin and eosin (H&E) staining. Animal experiments were conducted under the approval of the Animal Care and Use Committee of Tohoku University, Japan.

Cloning aggrecan and ADAMTSs and generating cRNA probes

Reverse transcriptase polymerase chain reaction-based cloning was employed to obtain partial or full clones of each molecule. Based on the homology between mouse and human ADAMTS-5 cDNA sequences, degenerate amplimers were designed as shown in Table 1. For other molecules, amplimers were designed based on the cDNA sequences of rats shown in Table 1 and in our previous study (Nakamura et al. 2005). PCR conditions used in the present study are also summarized in Table 1. The cDNA fragments obtained were subcloned into pCRII-TOPO vector (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. Nucleic acid sequences of ADAMTS-5 were analyzed by the dye-terminating method using ALF express II sequencer (Amersham Pharmacia Biotech, Buckinghamshire, UK) and the other fragments were analyzed by Takara (Osaka, Japan) to confirm the nucleic acid sequences. To create Dig-labeled riboprobes for sense and antisense fragments, the plasmids were linearized and transcribed by using Sp6 or T7 RNA polymerases (Stratagene, La Jolla, CA, USA) into cRNA as indicated in Table 1.

In situ hybridization

The protocol for ISH has been described previously (Ohtani et al. 1992; Sasano et al. 1996; Zhu et al. 2001). Sections were deparaffinized, rehydrated, and immersed in 0.2 N HCl for 20 min at room temperature, then incubated with 20 μg/ml proteinase K (Roche Diagnostics, Indianapolis, IN, USA) at 37°C for 30 min. After sections were dehydrated in ethanol, they were hybridized with approximately 400 ng/ml riboprobes at 45°C for 16 h. Sections were incubated with 20 μg/ml RNase A (Sigma, St Louis, MO, USA) in 1× SSC (saline–sodium citrate buffer) during stringent wash in 2× SSC and 1× SSC at 45°C. After sections were incubated with anti-Dig alkaline phosphatase-conjugated antibody (Roche Diagnostics) at 4°C overnight in a moisture chamber, signals were visualized and nuclear counterstaining was performed using methyl green. Sections were mounted and observed under light microscopy.

Semiquantitative RT-PCR

Gene expression of aggrecan and ADAMTS-1, -4, and -5 were semiquantified by RT-PCR in three types of cartilage. Bilateral mandibular condylar, growth plate, and articular cartilages were dissected from 4-week-old and adult male Wistar rats killed by ether anesthesia.

Table 1 Conditions for cloning, creating riboprobes and semi-quantitative RT-PCR

Gene name and accession no.	Nucleic acid sequences of amplimers		PCR annealing temperature(°C)	Product length (bp)	Cycle number for semi-quantitative RT-PCR	Restriction enzymes for		RNA polymerase
Aggrecan NM_022190	Upstream	GTTAGTGGAGGGCGTGAC	55	634	32 cycles	Anti-sense	*Eco*RV	Sp6
	Downstream	CTTGGCTGTTTCTGCTGTT				Sense	*Bam*HI	T7
ADAMTS-1 NM_024400	Upstream-1	GTTGGGAAAGGAAAGCAGA	68	1,123	–	Anti-sense	*Eco*RV	Sp6
	Downstream-1	AGGGTTGTGGCAGGAAATA				Sense	*Bam*HI	T7
	Upstream-2	GGCGGAGGACGAAGAGT	62	445	48 cycles	–	–	–
	Downstream-2	GGAAGCGAGGAGTAGCAAC						
ADAMTS-4 XM_237904	Upstream	CTACAACCACCGAACCGAC	60	602	48 cycles	Anti-sense	*Spe*I	T7
	Downstream	TGCCAGCCACCAGAACTT				Sense	*Eco*RV	Sp6
ADAMTS-5 AF142099 NM_011782	Upstream-1	ATGCKNCTYGRNTGGGC	60	1,395	–	NA	NA	NA
	Downstream-1	ACCGTCATCCAGAAATTC				NA	NA	NA
	Upstream-2	GATCTCTAGAATCATTCATG TGACACCCTG	60	1,685	–	Anti-sense	*Spe*I	Sp6
	Downstream-2	GATCTCTAGAAACCACAGGCT AACATTTC				Sense	*Xho*I	T7
	Upstream-3	GGCTGTGGTGTGCTGTG	58	758	48 cycles	–	–	–
	Downstream-3	CTGGTCTTTGGCTTTGAAC						
GAPDH MN_017008	Upstream	TGTTTGTGATG GTGTGAA	56	485	30 cycles	–	–	–
	Downstream	ATGGGAGTTGCTGTTGAG						

Mandibular condyles and tibial epiphysis were removed from mandibular bone and tibia, respectively. Cartilaginous tissues in the articular surfaces of mandibular condyle and tibia were removed carefully under dissection microscope in ice-cold PBS by using fine scalpels. After articular cartilage was obtained, bone tissue in secondary ossification center and cartilaginous tissue fragments of remained articular cartilage were carefully removed, then growth plates were separated from tibial epiphysis of 4-week-old rats. The specimens were frozen in liquid nitrogen and homogenized in lysis buffer to isolate total RNA using the RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Standardized 200 ng amounts of total RNA were reverse transcribed before PCR amplification. PCR conditions and number of reaction cycles were empirically determined by drawing amplification curves at each annealing temperature for each molecule (Table 1). Optical density from each amplified product separated on 2% agarose gel was digitized and measured using NIH imaging software (National Institutes of Health, Bethesda, MD, USA), and relative gene expression levels were semi-quantified against glyceraldehyde-3-phosphate dehydrogenase. Expression levels were statistically analyzed by Scheffe's test.

Results

Cloning rat ADAMTS-5

The deduced amino acid sequence of rat ADAMTS-5 is shown in Fig. 1, aligned with human and mouse ADAMTS-5. The coding sequence of rat ADAMTS-5 mRNA consists of 2,787 bp (GenBank Accession No. AY382879), which generates 928 amino acid residues. Rat ADAMTS-5 mRNA had 93.6 and 84.9% homology with that of mice and humans, respectively. The amino acid sequence of rat ADAMTS-5 showed 96.2 and 90.3% homology to that of mice and humans, respectively. Rat ADAMTS-5 protein lacked two amino acid residues in the metalloproteinase domain at Asp^{326} and Thr^{327} when compared to mouse ADAMTS-5. Pre- and pro-domains were less conserved in rats when compared to mice or humans. The domain structure of ADAMTS-5 in rats was identical to that in other species with a metalloproteinase domain including a catalytic domain, two thrombospondine-1 motifs, and a disintegrin-like motif.

Histological observation (Figs. 2, 3)

During the growth period, mandibular condylar cartilage consisted of five cell layers: a fibrous layer with fibroblasts embedded in the fibrous connective tissue, a proliferative cell layer with undifferentiated and proliferating polygonal cells, a transitional cell layer with flattened cells without cytosolic lipid droplets, a mature cell layer, and a hypertrophic cell layer (Fig. 2a, b). Tibial

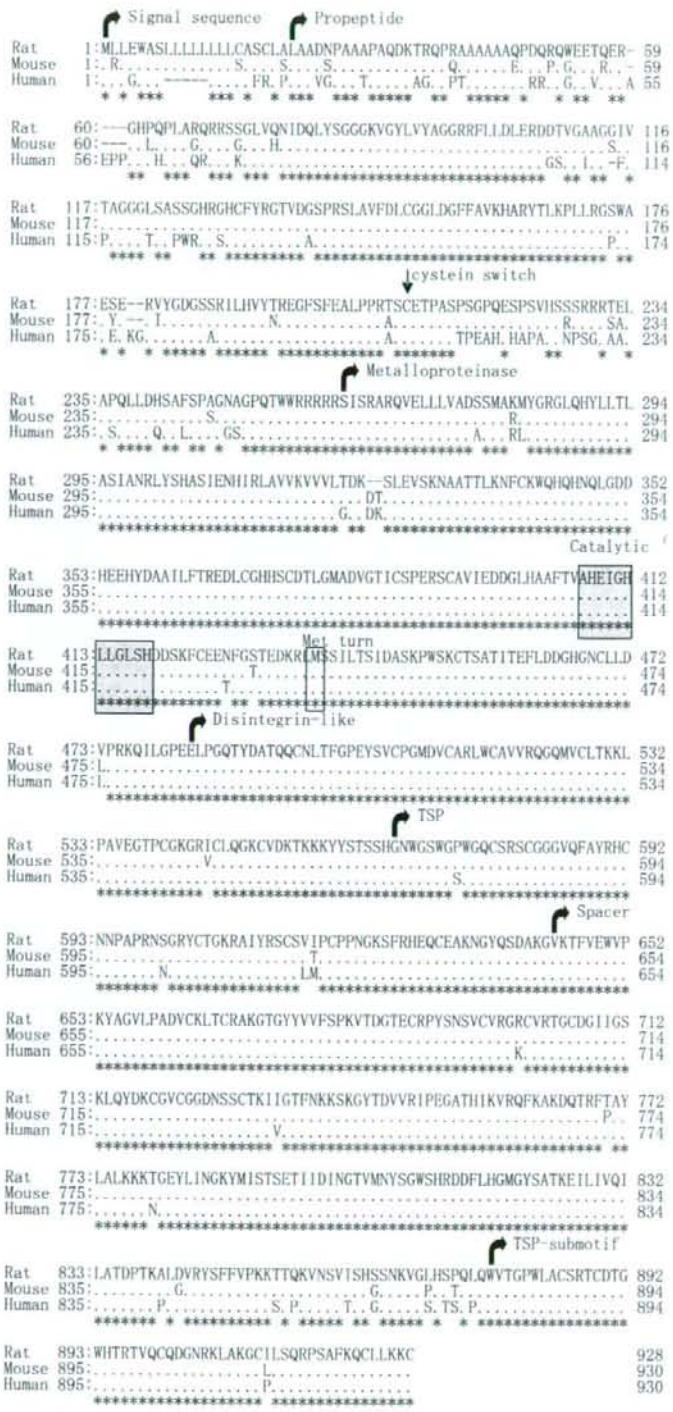

Fig. 1 The amino acid sequence deduced from the ADAMTS-5 cDNA is shown aligned with those of human and mouse ADAMTS-5. Residues matching the consensus sequence of the three proteins are *dotted* in the human and mouse sequences. The conserved zinc-binding motif and "Met turn" are *boxed*. *TSP* thrombospondin motif

epiphyseal growth plate cartilage and articular cartilage consisted of four layers: a resting cell layer with chondrocytes, which was the smallest in size of all the layers; a proliferative cell layer with flattened, proliferating cells; a mature cell layer containing ovoid-shaped well-differentiated chondrocytes with lipid drops; and a hypertrophic

cell layer consisting of enlarged cells with disorganized cytosolic structures during the growth period (Fig. 3a, b). Mandibular condylar cartilage showed characteristics of growth plate cartilage with a hypertrophic cell layer at the lower border involved in endochondral bone formation during growth. In contrast, mandibular condylar cartilage at 16 and 32 weeks of age mainly consisted of three layers: a resting cell layer, a proliferating cell layer, and a mature cell layer (Fig. 2c, d), and closely resembled articular cartilage lacking the hypertrophic cell layer seen in younger animals (Fig. 3c). The uppermost layer of aged mandibular condylar cartilage was a fibrous layer with elongated fibroblasts embedded in fibrous connective tissue, the second layer was a proliferating cell layer consisting of small proliferating cells, and the lower layer was a mature cell layer with well-differentiated chondrocytes, including some hypertrophic cells (Fig. 2c, d). Tibial articular cartilage consisted of three layers at 32 weeks of age (Fig. 3c).

Gene expression patterns (Figs. 4, 5, 6)

Age 4 and 8 weeks (Figs. 4, 5)

At 4 and 8 weeks of age, positive hybridization signals for aggrecan were observed in mature and hypertrophic chondrocytes in condylar, articular, and the growth plate cartilage (Figs. 4a, e, 5a, e). While condylar cartilage did not show positive signals for aggrecan in the upper three layers (Fig. 4a, e), a positive signal was observed in the resting and proliferating cell layers in both articular and growth plate cartilage (Fig. 5a, e). ADAMTS-1

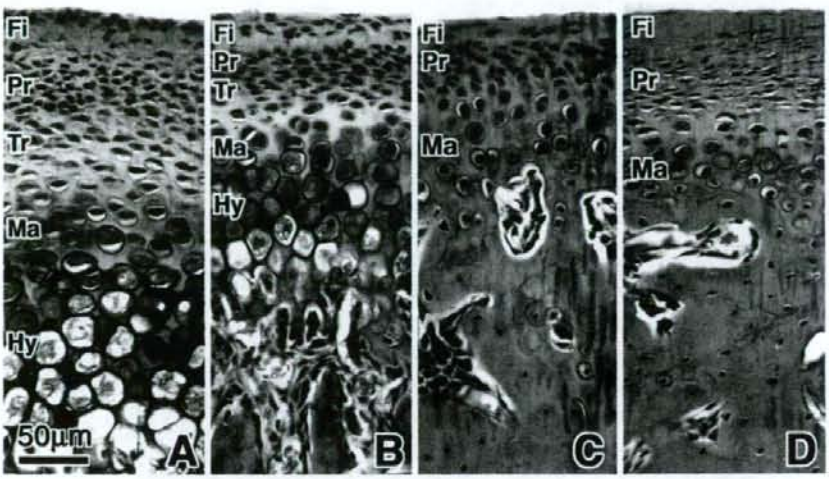

Fig. 2 Sagittal sections of mandibular condylar cartilage stained with H&E from 4-week-old (**a**), 8-week-old (**b**), 16-week-old (**c**), and 32-week-old (**d**) rats. *Fi* fibrous layer; *Pr* proliferative cell layer; *Tr* transitional cell layer; *Ma* mature cell layer; *Hy* hypertrophic cell layer. *Scale bar* 50 μm; original magnification: ×40

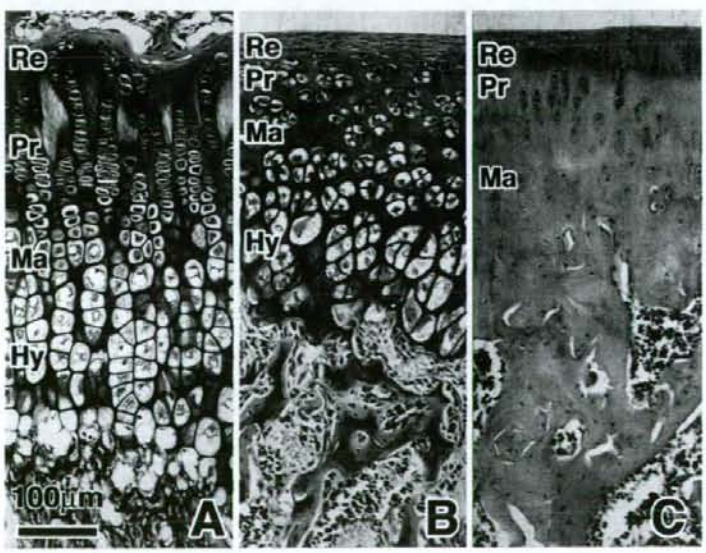

Fig. 3 Sagittal sections of growth plate and articular cartilage stained with H&E. Growth plate cartilage from 4-week-old rats (**a**) and articular cartilage from 4-week-old (**b**), and 32-week-old (**c**) rats. *Re* resting cell layer; *Pr* proliferative cell layer; *Ma* mature cell layer; *Hy* hypertrophic cell layer. *Scale bar* 100 μm; original magnification: ×20

expression was observed in all of the cell layers in the three types of cartilage during growth (Figs. 4b, f, 5b, f). In condylar cartilage, mature chondrocytes showed the strongest hybridization signals to ADAMTS-1 compared to the other layers at ages 4 and 8 weeks (Fig. 4b, f). The cells in the transitional cell layer and below were positive for ADAMTS-4 in condylar cartilage, with the strongest expression in hypertrophic chondrocytes (Fig. 4c, g). Cells in all four layers showed positive signals for ADAMTS-4 in growing articular cartilage at 4 weeks (Fig. 5g), while cells in the proliferating cell layer were negative in growth plate cartilage (Fig. 5c). ADAMTS-5 showed positive hybridization signals localized in mature and hypertrophic chondrocytes in both condylar (Fig. 4d, h) and growth plate cartilage (Fig. 5d), while it was observed in all cell layers in articular cartilage at 4 weeks of age (Fig. 5h). During the growth period, the expression domain of aggrecan covered that of ADAMTS-5, while other ADAMTSs had a greater expression domain than that of aggrecan, especially ADAMTS-1, which was expressed ubiquitously in all types of cartilage during the growth period. In addition, all types of ADAMTSs were expressed in all four layers of articular cartilage during growth.

Age 16 and 32 weeks (Fig. 6)

After growth was completed and the hypertrophic cell layer disappeared, positive hybridization signals for aggrecan were localized in mature chondrocytes of condylar and articular cartilage (Fig. 6a, e, i). With aging, aggrecan expression was maintained in the well-differentiated chondrocytes in the mature cell layers. However, the strength of the hybridization signal decreased with age. The hybridization signal for ADAMTS-1 in the fibrous layer, which was positive at 8 weeks (Fig. 4b, f), was negative at 16 and 32 weeks (Fig. 6b, f). Consequently, the area negative for ADAMTS-1 in condylar cartilage expanded from the fibrous layer to the proliferating cell layer by 32 weeks (Fig. 6b, f). The hybridization signal for ADAMTS-1 remained in all of the cell layers in articular cartilage at 32 weeks of age (Fig. 6j). ADAMTS-4 was

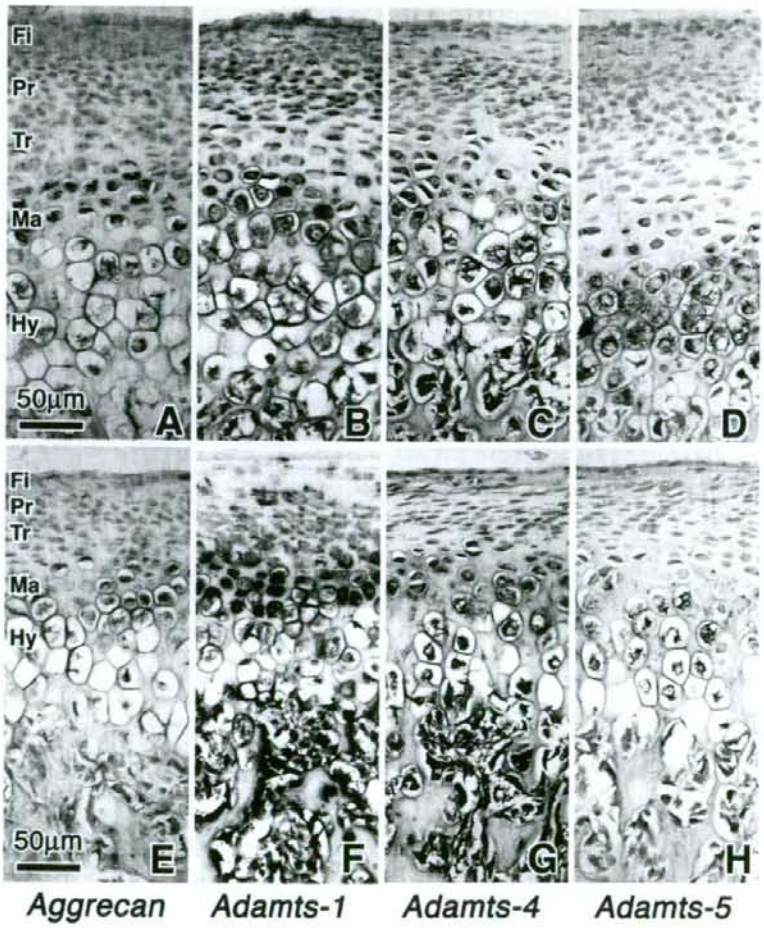

Fig. 4 In situ hybridization analysis for aggrecan (**a**, **e**), ADAMTS-1 (**b**, **f**), ADAMTS-4 (**c**, **g**), and ADAMTS-5 (**d**, **h**) of sagittal sections of mandibular condylar cartilage from 4-week-old (**a**–**d**) and 8-week-old (**e**–**h**) rats. *Fi* fibrous layer; *Pr* proliferative cell layer; *Tr* transitional cell layer; *Ma* mature cell layer; *Hy* hypertrophic cell layer. Brown-purple staining in the cytosol is a positive hybridization signal. *Scale bar* 50 μm; original magnification: ×40

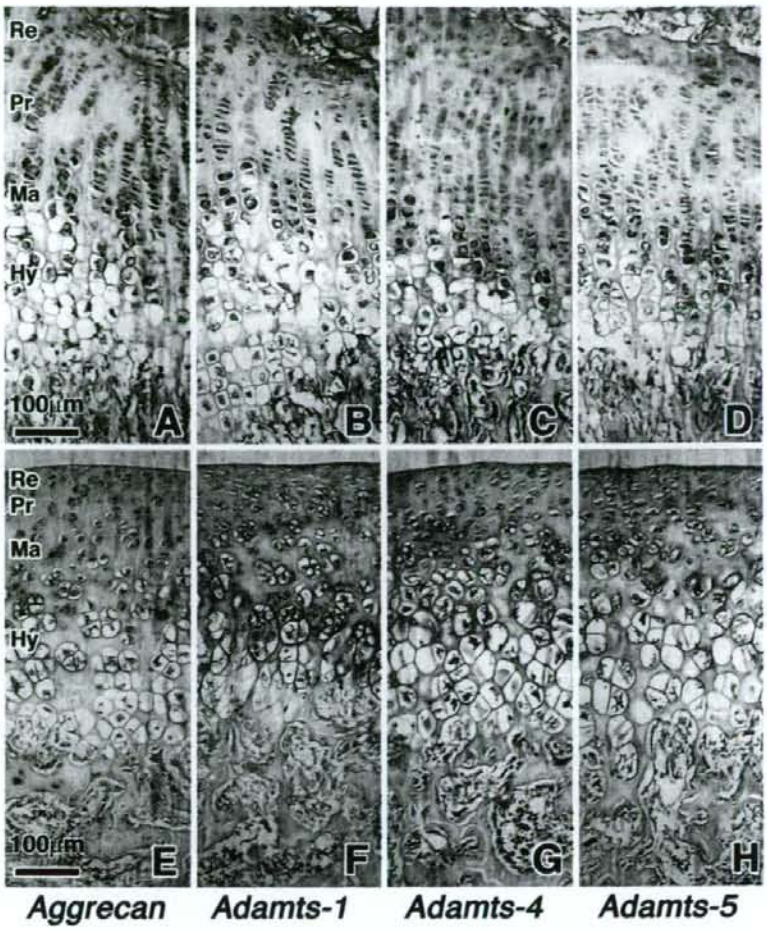

Fig. 5 In situ hybridization analysis for aggrecan (**a**, **e**), ADAMTS-1 (**b**, **f**), ADAMTS-4 (**c**, **g**), and ADAMTS-5 (**d**, **h**) of sagittal sections of growth plate (**a**–**d**) and articular cartilage (**e**–**h**). *Re* resting cell layer; *Pr* proliferative cell layer; *Ma* mature cell layer; *Hy* hypertrophic cell layer. Brown-purple staining in the cytosol is a positive hybridization signal. *Scale bar* 100 μm; original magnification: ×20

localized in mature chondrocytes at 16 weeks of age (Fig. 6c) and remained so at 32 weeks of age in condylar cartilage (Fig. 6g). It was only localized in mature chondrocytes in articular cartilage (Fig. 6k). ADAMTS-5 was expressed in the lower part of the mature cell layers in condylar cartilage at 16 and 32 weeks (Fig. 6d, h), while it was not expressed in aged articular cartilage (Figs. 6l, 7).

Gene expression levels

The expression level of all ADAMTSs examined was maintained during aging in mandibular condylar cartilage, whereas that of ADAMTS-4 and -5 decreased in articular cartilage during aging (Fig. 7a, b). ADAMTS-4 expression in growth plate cartilage was significantly lower than that in articular cartilage at the same age (Fig. 7a, b). Gene expression levels of aggrecan and ADAMTS-1 were similar in all types of cartilage and at all ages examined, while slightly, but not significantly, less expression of ADAMTS-1 was observed in aged mandibular condylar cartilage (Fig. 7a, b).

Discussion

ADAMTS-5 is also known as aggrecanase-2, which cleaves aggrecan, one of the cartilage-specific macromolecules (Doege et al. 1991). While all three ADAMTS genes examined have been identified in mice, cattle, and humans, this is the first time that ADAMTS-5 has been identified in rats. Rat ADAMTS-5 conserved all of the domains seen in mice and humans (Abbaszade et al. 1999). Since rat ADAMTS-5 lacks two amino acid residues in the metalloproteinase domain and the catalytic domain is conserved completely when compared to humans and mice, aggrecanase activity of ADAMTS-5 may differ in rats from other species.

The age-dependent changes in aggrecan expression in condylar cartilage were similar to those of type II collagen demonstrated previously (Ohashi et al. 1997; Bae et al. 2003); expression and localization of type II collagen becomes restricted to mature cell layers as aging progresses. In addition, the expression pattern of versican is

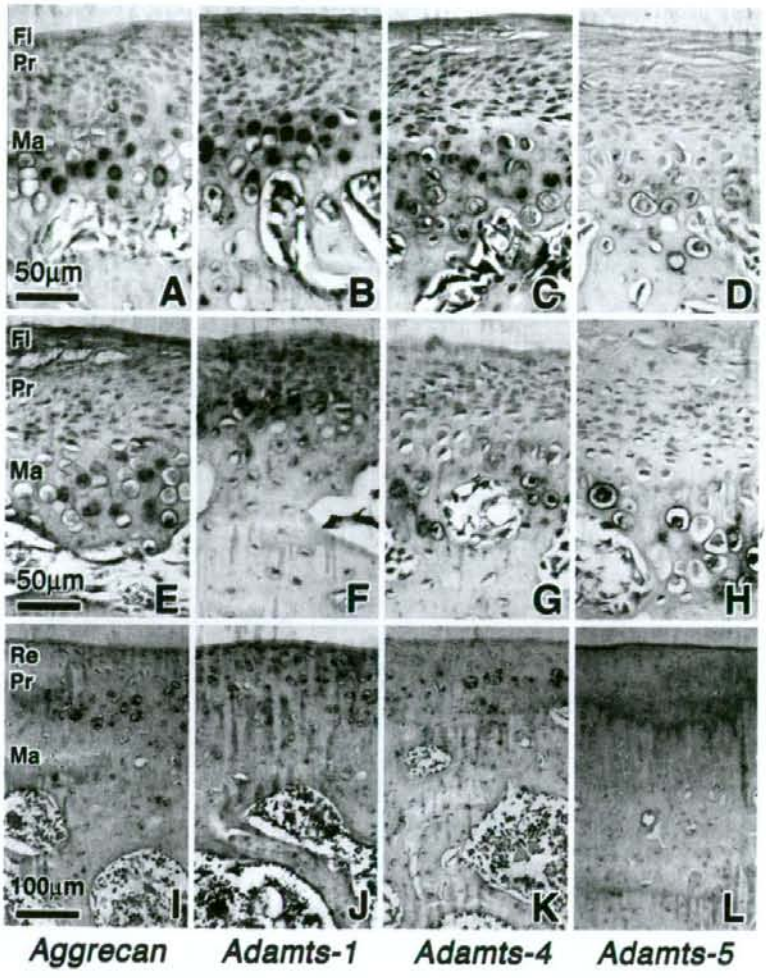

Fig. 6 In situ hybridization analysis for aggrecan (**a**, **e**, **i**), ADAMTS-1 (**b**, **f**, **j**), ADAMTS-4 (**c**, **g**, **k**), and ADAMTS-5 (**d**, **h**, **l**) of sagittal sections of mandibular condylar cartilage from 16-week-old (**a–d**) and 32-week-old (**e–h**) rats and articular cartilage from 32-week-old (**i–l**) rats. *Re* resting cell layer; *Fi* fibrous layer; *Pr* proliferative cell layer; *Ma* mature cell layer. Brown-purple staining in the cytosol is a positive hybridization signal. *Scale bar* in **a–h** 50 μm and **i–l** 100 μm; original magnification: ×40 (**a–h**) and ×20 (**i–l**)

regulated differently in condylar and primary cartilage during growth (Shibata et al. 2001). While versican is co-expressed with aggrecan in primary cartilage, its expression is restricted to the fibrous layer of condylar cartilage, while aggrecan is expressed in the mature and hypertrophic cell layer during embryonic growth. Several researchers have investigated the substrate specificity of the three ADAMTSs: ADAMTS-1 cleaves aggrecan and versican (Kuno et al. 2000; Sandy and Verscharen 2001); ADAMTS-4 cleaves aggrecan, brevican, and versican (Matthews et al. 2000; Nakamura et al. 2000; Tortorella et al. 2000; Sandy and Verscharen 2001; Sztrolovics et al. 2002); and ADAMTS-5 cleaves aggrecan (Abbaszade et al. 1999; Arner 2002). Since ADAMTS-1 is the only ADAMTS among those examined in the present study that is expressed in the fibrous layer of condylar cartilage, it may contribute to versican metabolism in this layer. Thus, specific expression patterns of each ADAMTS may reflect the expression of their specific substrates during endochondral ossification.

Tibial growth plate and mandibular condyle are sites of endochondral bone formation. Chondrocytes in the growth plate and condylar cartilage deposit cartilaginous ECM components such as aggrecan and type II collagen to provide a template that is subsequently replaced by bone tissue. Cell volume increases as chondrocytes differentiate during this process (Luder et al. 1988). Besides the expression of ADAMTSs, chondrocytes produce several types of MMP during growth (Bae et al. 2003; Gepstein et al. 2003). Therefore, once deposited, ECM components such as type II collagen and aggrecan are degraded by aggrecanases in combination with MMPs. ADAMTS-5, mainly expressed in mature and hypertrophic chondrocytes in both condylar and growth plate cartilage, may provide space for expanding chondrocytes during terminal differentiation. In addition, recent studies have demonstrated that targeted

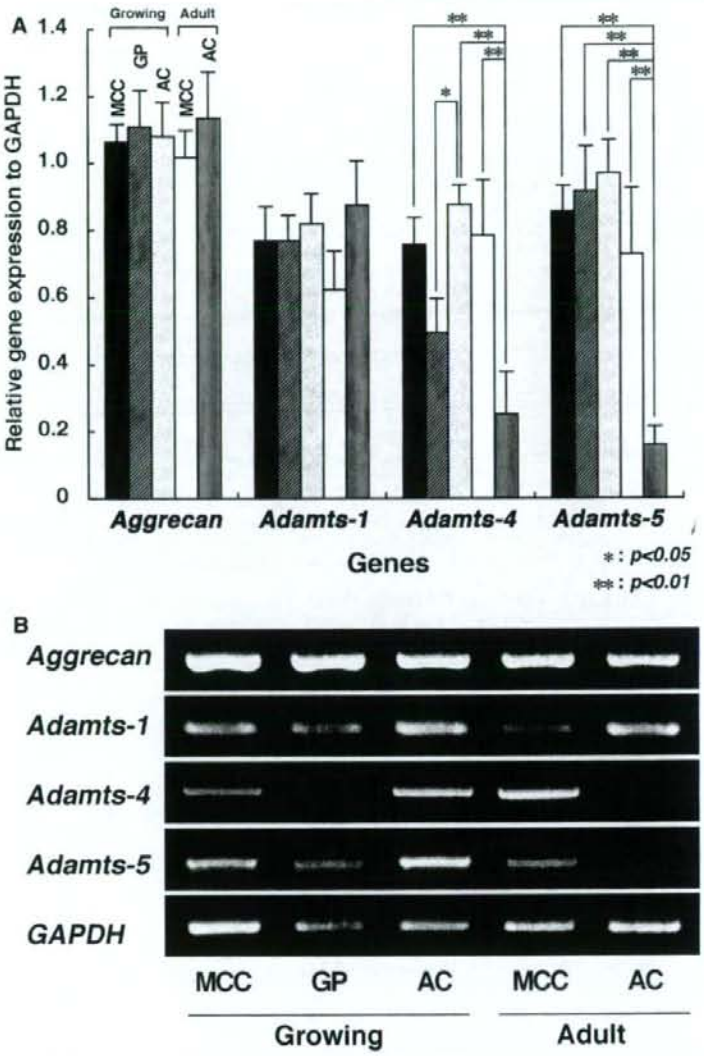

Fig. 7 Graph indicating the results of semiquantitative RT-PCR (**a**) and representative images of agarose gel electrophoresis (**b**) ($n=3$). *MCC* mandibular condylar cartilage (*closed bar* growing; *open bar* adult); *GP* growth plate cartilage (*oblique stripe bar*); and *AC* articular cartilage (*shaded bar* growing; *vertical stripe bar* adult). $*P<0.05$ and $**P<0.01$

disruption of active ADAMTS-5, but not ADAMTS-4, inhibits experimentally induced inflammatory degeneration of cartilage (Glasson et al. 2005; Stanton et al. 2005) in growing mice. Therefore, it may be that ADAMTS-5 is a major aggrecanolytic enzyme contributing not only to such pathological processes, but also to physiological degradation of ECM molecules during the growth period.

After growth, all ADAMTSs expressed in the mature chondrocytes of condylar cartilage may play a role in the physiological turnover of aggrecan in order to maintain cartilage tissue. However, ADAMTS-1 and ADAMTS-4, but not ADAMTS-5, could contribute to the physiological turnover of aggrecan in aged articular cartilage. Therefore, ECM remodeling in aged mandibular condylar cartilage could be regulated differently from that in articular cartilage.

In summary, ADAMTS-5 appears to contribute mainly to degradation of ECM molecules such as aggrecan in growth plate and condylar cartilage, depending upon its ECM composition and cellular organization during growth. In conclusion, the results of the present study reveal that ECM metabolism by ADAMTSs and expression of ADAMTSs in primary and secondary cartilage may be differentially regulated during growth and aging, depending upon the functional differences in different types of cartilage.

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RESEARCH REPORTS
Biological

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Clodronate Inhibits PGE_2 Production in Compressed Periodontal Ligament Cells

ABSTRACT

Periodontal ligament (PDL) cells play an essential role in orthodontic tooth movement. We recently reported that clodronate, a non-N-containing bisphosphonate, strongly inhibited tooth movement in rats, and thus could be a useful adjunct for orthodontic treatment. However, it is not clear how clodronate affects the responses of PDL cells to orthodontic force. In this study, we hypothesized that clodronate prevents the mechanical stress-induced production of prostaglandin E_2 (PGE_2), interleukin-1β (IL-1β), and nitric oxide (NO) in human PDL cells. A compressive stimulus caused a striking increase in PGE_2 production, while the responses of IL-1β and NO were less marked. Clodronate concentration dependently inhibited the stress-induced production of PGE_2. Clodronate also strongly inhibited stress-induced gene expression for COX-2 and RANKL. These results suggest that the inhibitory effects of clodronate on tooth movement and osteoclasts may be due, at least in part, to the inhibition of COX-2-dependent PGE_2 production and RANKL expression in PDL cells.

KEY WORDS: clodronate, periodontal ligament cell, mechanical stress, prostaglandin E_2.

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INTRODUCTION

Periodontal ligament cells play an essential role in orthodontic tooth movement, and mechanically induced bone resorption is known to be a rate-limiting step (Rygh, 1987; Igarashi *et al.*, 1994). Recently, we demonstrated that clodronate, a non-N-containing bisphosphonate that has been used to treat various metabolic bone diseases associated with excessive bone resorption (Plosker and Goa, 1994; Fleisch, 2000), strongly inhibited bone resorption induced by orthodontic mechanical stress and tooth movement in rats, suggesting that it could be a useful adjunct for orthodontic treatment (Liu *et al.*, 2004). A previous study has demonstrated the direct inhibitory action of clodronate on osteoclastic bone resorption, *i.e.*, clodronate induces apoptosis of osteoclasts through incorporation into the cells (Frith *et al.*, 2001). Although the number of osteoclasts on the pressure side of the periodontal ligament decreased in clodronate-treated animals, the mechanism of action in this process has not yet been determined. The purpose of the present study was to clarify how clodronate affects the responses of periodontal ligament cells to orthodontic force, especially those leading to bone resorption. In this study, we hypothesized that clodronate prevents the mechanical stress-induced production of prostaglandin E_2 (PGE_2), interleukin 1β (IL-1β), and nitric oxide (NO) in cultured human periodontal ligament cells, which are known to play important roles in the bone-resorptive responses to orthodontic mechanical stimulation (Yamasaki *et al.*, 1980; Chumbley and Tuncay, 1986; Saito *et al.*, 1991; Zhou *et al.*, 1997; Alhashimi *et al.*, 2001; Iwasaki *et al.*, 2001; Hayashi *et al.*, 2002; Shirazi *et al.*, 2002).

MATERIALS & METHODS

The protocol for the experiment was approved by the Research Ethics Committee of Tohoku University Graduate School of Dentistry, and informed consent was obtained from all patients.

Drug

Clodronate (dichloromethylene bisphosphonate disodium salts) was obtained from Procter & Gamble Pharmaceuticals' Woods Corners Laboratories (Norwich, NY, USA).

Compression of Primary Human Periodontal Ligament Cells

Primary periodontal ligament cells were derived from human tooth roots extracted for orthodontic treatment. Donors were healthy young adults of both sexes (from 20 to 34 yrs old), free of periodontal disease. The cells were cultured in α-MEM supplemented with 10% FBS, antibiotics, and 1×10^{-8} M 1α,25-dihydroxyvitamin D_3 (Duphar, Amsterdam, Netherlands) at 37°C in an atmosphere of 5% CO_2 in humidified air. The medium was changed every 5 days, and the cells underwent from 4 to 8 passages until use.

For the experiment, periodontal ligament cells were seeded on 35-mm wells in a six-well plate at a density of 3×10^5 cells/dish and cultured until they were confluent. They were then transferred to 2 mL of fresh medium that contained a specific concentration of clodronate and cultured for an additional 24 hrs. After

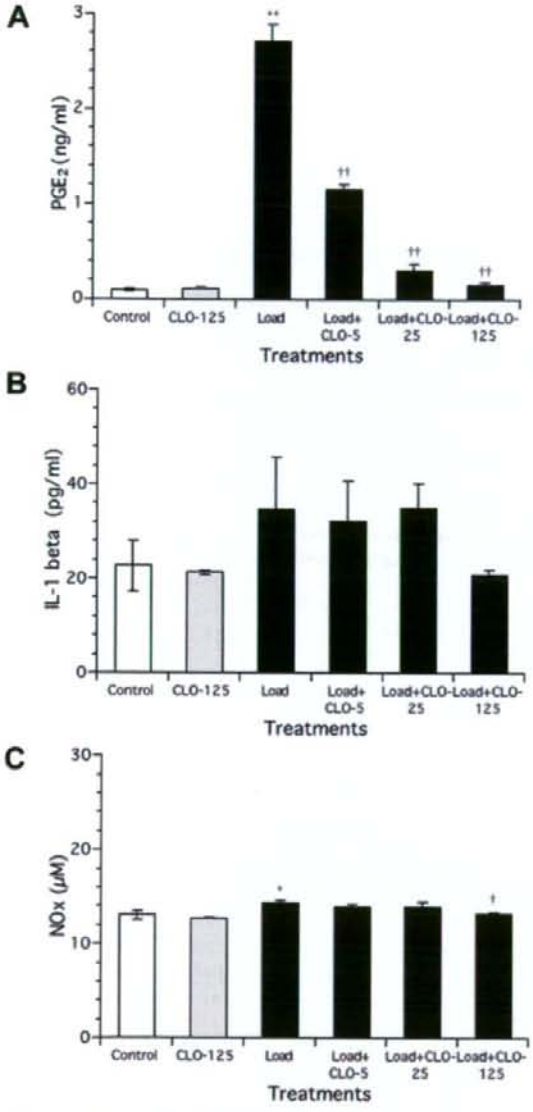

Figure 1. Effects of clodronate on prostaglandin E_2 (PGE_2) **(A)**, interleukin 1β (IL-1β) **(B)**, and nitric oxide (NO) **(C)** production in periodontal ligament cells induced by compressive mechanical stress. Each column and bar represent the mean ± SEM (n = 3). *Significant increase *vs.* control ($P < 0.05$). **Significant increase *vs.* control ($P < 0.01$). †$P < 0.05$ compared with load. ††$P < 0.01$ compared with load. CLO: clodronate (5, 25, 125 μM).

the pre-culture, the cells were continuously compressed according to the method described previously (Kanzaki *et al.*, 2002). Briefly, compressive force was applied directly to periodontal ligament cells by the placement of a custom-made glass cylinder (diameter, 30.3 mm; height, 14.8 mm; thickness, 2.0 mm) that contained lead granules over a confluent cell layer in the well. We adjusted the force magnitude by adding or reducing the granules. In the present study, the cells were subjected to 2.0 g/cm^2 of compressive force for 48 hrs. After the experiment, total RNA was extracted from each culture with the use of the QuickPrep Total RNA Extraction Kit (Pharmacia Biotech, Uppsala, Sweden). The culture medium was also withdrawn and stored at -20°C for determination of PGE_2, IL-1β, and NO. The concentrations of PGE_2 and IL-1β were measured with respective specific enzyme immunoassay kits (for PGE_2, RPN222, Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK; for IL-1β, QLB00, R&D Systems, Inc., Minneapolis, MN, USA).**(AQ)** We evaluated NO production by measuring nitrite and nitrate concentrations in the medium using the HPLC-Griess method (Ohta *et al.*, 1994).

Since responsiveness of cultured human periodontal ligament cells varies depending on their sources, the experiment was repeated, and each single experiment was performed with cells from a different subject.

Semi-quantitative Reverse-transcription Polymerase Chain-reaction (RT-PCR) Assays for Cyclo-oxygenase-2 (COX-2) and Receptor Activator Nuclear Factor κB Ligand (RANKL) Gene Expression

We reverse-transcribed extracted RNA to synthesize cDNA using You-Prime First Strand Beads (Pharmacia Biotech) and Oligo $(dT)_{15}$ primer (Promega, Madison, WI, USA). First-strand cDNA was then subjected to PCR amplification with gene-specific PCR primers. The primers used in this study were: 5′-AGC AGA GAA AGC GAT GGT-3′ (forward) and 5′-GGG TAT GAG AAC TTG GGA TT-3′ (reverse) for RANKL, 5′-AAC CCA CTC CAA ACA CAG-3′ (forward) and 5′-CTG GCC CTC GCT TAT GAT CT-3′ (reverse) for COX-2, and 5′-ATG AGG ATC CTC ACC GAG CGC GGC TAC AGC-3′ (forward) and 5′-ACA CCA CTG TGT TGG CGT ACA GGT CTT TGC-3′ (reverse) for β-actin. PCR was performed with a KOD Dash DNA Polymerase Kit (Toyobo Co., Ltd.; LDP-101, Tokyo, Japan). Annealing temperatures were 58°C for RANKL, 51°C for COX-2, and 58°C for β-actin. Numbers of PCR cycles were 42-44 for RANKL, 32-33 for COX-2, and 27 for β-actin. The PCR products were subjected to electrophoresis and stained with ethidium bromide. The relative intensities of the gel bands were measured with the use of Scion Image Analysis software (Scion Co., MD).**(AQ)** The method has been described in detail previously (Kanzaki *et al.*, 2002).

Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA), followed by Fisher's PLSD test. $P < 0.05$ was considered a significant difference.

RESULTS

Clodronate showed different effects on PGE_2, IL-1β, and NO production in periodontal ligament cells induced by compressive mechanical stress (Fig. 1). The compression of cells at 2.0 g/cm^2 for 48 hrs caused nearly a 30-fold increase in PGE_2 release (Fig. 1A), while the increase was not significant for IL-1β (Fig. 1B) and only minimal for NO (Fig. 1C). Clodronate (5, 25, 125 μM) concentration-dependently inhibited the mechanical stress-induced increase in PGE_2 production in periodontal ligament cells (Fig. 1A). The inhibitory effect of clodronate on NO production was significant only at the highest concentration (125 μM) (Fig. 1C).

The application of compressive force to periodontal ligament cells also caused a more than two-fold increase in

mRNA expression for both COX-2 and RANKL (Figs. 2A, 2B). Clodronate (5, 25, 125 μM) significantly inhibited these responses (Fig. 2B).

Although the responsiveness of periodontal ligament cells to compression varied between and among experiments (individuals), the inhibitory effects of clodronate on stress-induced PGE_2, COX-2, and RANKL were reproducible (Table).

DISCUSSION

Clodronate is a non-N-containing bisphosphonate that possesses potential anti-inflammatory activity as well as anti-bone-resorptive activity (Österman *et al.*, 1995; Richards *et al.*, 2001). It has been shown that clodronate inhibits the production of pro-inflammatory molecules, including IL-1β (Pennanen *et al.*, 1995; Makkõnen *et al.*, 1999), NO (Makkõnen *et al.*, 1996; 1999), and PGE_2 (Felix *et al.*, 1981; Igarashi *et al.*, 1997) in macrophages and/or osteoblastic cells.

The present results clearly demonstrated that clodronate could also prevent the mechanical stress-induced production of PGE_2 by periodontal ligament cells, which is one of the most important signaling molecules in the responses of periodontal ligament to orthodontic force (Yamasaki *et al.*, 1980; Saito *et al.*, 1991; Kanzaki *et al.*, 2002). The compressive stimulus caused a striking increase in PGE_2 production, while responses were less marked for IL-1β and NO. Clodronate significantly inhibited the mechanical stress-induced production of PGE_2 in a concentration-dependent manner. Furthermore, clodronate strongly inhibited stress-induced gene expression for COX-2 and RANKL.

Prostaglandins have been shown to play a crucial role in osteoclast formation induced by orthodontic mechanical stress (Yamasaki *et al.*, 1980; Sandy and Harris, 1984; Zhou *et al.*, 1997). Recently, Kanzaki *et al.* (2002) demonstrated that compressive force stimulates osteoclastogenesis in the co-culture of peripheral blood mononuclear cells with periodontal ligament cells, by increasing the expression of RANKL in periodontal ligament cells. RANKL is known to be an essential factor in the differentiation and activation of osteoclasts (Suda *et al.*, 1999). It has also been demonstrated that this increase in RANKL expression paralleled that in COX-2 expression and was dependent on PGE_2 production (Kanzaki *et al.*, 2002). Clodronate inhibited all of these responses in compressed periodontal ligament cells, suggesting that it may have decreased RANKL expression in these cells by inhibiting the COX-2-dependent production of PGE_2. At present, the mechanism by which clodronate inhibits COX-2 expression in periodontal ligament cells is not known. Although NO and IL-1 have been shown to induce COX-2 in osteoblastic cells (Buttery *et al.*, 2002; Pilbeam *et al.*, 2002), their involvement is not likely, since the effects of mechanical stress with or without clodronate on the production of these molecules were only minimal or insignificant.

In our previous *in vivo* study, the number of osteoclasts on the pressure side of the periodontal ligament decreased in clodronate-injected animals (Liu *et al.*, 2004), indicating that clodronate may have either inhibited the recruitment of osteoclasts, promoted osteoclast apoptosis, or both (Rogers *et al.*, 2000). The present *in vitro* results suggest that clodronate may have impaired the ability of periodontal ligament cells to support osteoclast formation by decreasing RANKL expression. It is also possible that the decreased expression of RANKL promoted osteoclast apoptosis, and hence decreased the number of osteoclasts, since RANKL has been shown to act as a survival factor and to prevent apoptosis of osteoclasts (Lacey *et al.*, 2000). Osteoclast apoptosis has been considered to be a major

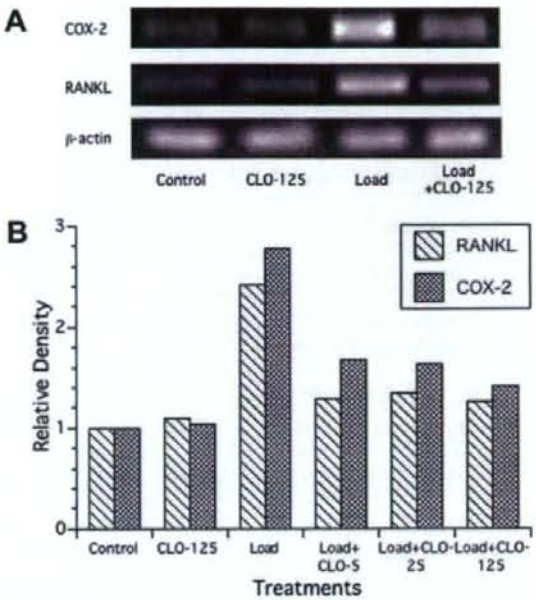

Figure 2. Effect of clodronate on gene expression for cyclo-oxygenase-2 (COX-2) and receptor activator nuclear factor κB ligand (RANKL) in compressed periodontal ligament cells. **(A)** RT-PCR for COX-2, RANKL, and β-actin. CLO: 125 μM. **(B)** Relative expression of RANKL mRNA and COX-2 mRNA determined by densitometric analysis. Values were corrected for β-actin mRNA expression. Representative results of 1 of 3 independent experiments are shown. CLO: clodronate (5, 25, 125 μM).

Table. Comparison of the Effects of Clodronate on Stress-induced Prostaglandin E_2 (PGE_2), Cyclo-oxygenase-2 (COX-2), and Receptor Activator Nuclear Factor κB Ligand (RANKL) in Human Periodontal Ligament Cells among Experiments (individuals)

		Treatments		
Variables	Experiment[a]	Control	Load	Load ± Clodronate (25 μM)
PGE_2 (ng/mL)	1	0.09 ± 0.02	2.70 ± 0.18	0.29 ± 0.07
	2	0.12 ± 0.01	0.45 ± 0.04	0.20 ± 0.05
COX-2 mRNA (Relative expression)	1	1.00	2.78	1.64
	2	1.00	3.32	2.07
RANKL mRNA (Relative expression)	1	1.00	2.42	1.34
	2	1.00	3.21	2.19

[a] Each experiment was performed with cells from a different individual.

mechanism of action for the inhibition of bone resorption by this bisphosphonate (Halasy-Nagy *et al.*, 2001). Frith *et al.* (2001) demonstrated that clodronate is incorporated into osteoclasts and metabolized to adenosine 5′-(β, γ-dichloromethylene) triphosphate, which may induce apoptosis in these cells. In addition to the formation of this ATP analogue, the inhibition of RANKL expression in supporting cells like periodontal ligament cells might also be involved in the induction of apoptosis in osteoclasts.

In conclusion, the present results suggest that the inhibitory effects of clodronate on orthodontic tooth movement and osteoclasts may be due in part to the inhibition of COX-2-dependent PGE_2 production, which leads to decreased RANKL expression in periodontal ligament cells subjected to orthodontic mechanical stress.

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Magnetic motion capture system using *LC* resonant magnetic marker composed of Ni–Zn ferrite core

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We have proposed a magnetic motion capture system using an *LC* resonant magnetic marker. The proposed system is composed of an exciting coil, an *LC* marker, and a 5×5-matrix search coil array (25 search coils). The *LC* marker is small and has a minimal circuit with no battery and can be driven wirelessly by the action of electromagnetic induction. It consists of a Ni–Zn ferrite core (3 mmϕ×10 mm) with a wound coil and a chip capacitor, forming an *LC* series circuit with a resonant frequency of 186 kHz. The relative position accuracy of the system is less than 1 mm within the area of 100 mm^3 up to 150 mm from the search coil array. Compared with dc magnetic systems, the proposed system is applicable for precision motion capture in optically isolated spaces without magnetic shielding because the system is not greatly influenced by earth field noise. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2171927]

I. INTRODUCTION

Effective methods for accurately detecting the motion of unseen objects, such as in an optically shielded space, are strongly required by the medical field for applications such as radiotherapy or endoscopic examinations. In such cases, particularly for measurements within a human body, the applied marker must be small and free from electric wiring. In addition, the location and orientation of the marker must be known exactly during the measurement. Magnetic motion capture systems are believed to satisfy these requirements. There have been several investigations into determining the position of a magnetic object by measuring the magnetic field of the object.[1–6] However, conventional systems require a comparatively large-sized magnetic object as a marker or the marker must contain electric wiring in order to obtain a high signal-to-noise (SN) ratio for the magnetic signal from the marker. To address this, we have proposed and developed a magnetic motion capture system using a magnetically coupled *LC* resonant marker.[7–9] The small-sized marker uses a soft ferrite core with a coil, representing a minimal *LC* circuit with no battery, driven wirelessly by electromagnetic induction. The magnetic signal of the marker is detected by a matrix-designed search coil array. Our proposed system allows the approximate orientation of the marker and the position of the marker to be determined accurate to within 1 mm under limited conditions.[9] In this paper, we examine the accuracy of the proposed system in detecting the position and orientation of the marker over a wide area in order to expand the detectable space.

II. SYSTEM COMPONENTS AND THEORY

Figure 1 shows a schematic diagram of the motion capture system. The system is composed of the measurement equipment and a coil assembly, consisting of a driving coil, an *LC* marker, and a pickup coil array. As shown in the figure, we use the right-handed coordinate system. Figure 2 shows a photograph of the coil system and the *LC* marker. The marker consists of a Ni–Zn ferrite core (3 mm in diameter and 10 mm long) with 335 turns of wound coil and a chip capacitor (680 pF), representing an *LC* series circuit designed for a resonant frequency of 186 kHz. The search coil array consists of 25 coils placed at intervals of 45 mm on an acryl board, configuring a matrix layout. Each coil is made of 100 turns of polyester enameled copper wire (PEW) around an acryl bobbin of 25 mm in diameter. A sinusoidal excitation of 22 V was applied to the driving coil (ten turns of PEW around an acryl coil 210-mm square) and the marker was strongly excited at its resonant frequency by electromagnetic induction. In this paper, the square-shaped driving coil is adopted to improve the SN ratio for pickup coils placed around the four corners. As a result, the SN ratio increased up to 15% for these coils.

The induction field of the marker is used to determine the position and orientation of the marker. However, the induced voltage detected at the pickup coils includes both the induction of the exciting field and the marker field, as they

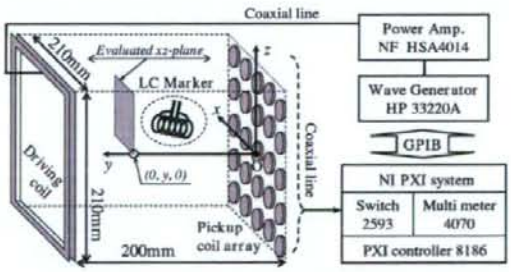

FIG. 1. Schematic diagram for the proposed motion capture system.

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