Localization of Cathepsin K in Bovine Odontoclasts during Deciduous Tooth Resorption

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Abstract. Cathepsin K is a cysteine proteinase, which is abundantly and selectively expressed in osteoclasts. It is believed to play an important role in the proteolysis of bone resorption by osteoclasts. The objectives of this study were to investigate the association of cathepsin K in the physiological root resorption of deciduous teeth and to identify the cathepsin K-producing cells in deciduous root resorption. RT-PCR and Northern blot analysis of the total RNAs extracted from bovine active and resting root-resorbing tissues, which lie between the root of deciduous tooth and its permanent successor, were performed. The active root-resorbing tissue, which has a high population of odontoclasts on its surface that is attached to resorbing root surface, showed an extremely high expression of cathepsin K in comparison with the resting root-resorbing tissue. By in situ hybridization, cathepsin K mRNA was highly and selectively expressed in multinucleated odontoclasts that aligned along the surface of the tissue and apposed to the resorbing root surface of the deciduous tooth. Western blot analysis of the active root-resorbing tissue was used to characterize the anti-cathepsin K antibody. A band of 27 kDa, corresponding with the predicted size for mature cathepsin K, was demonstrated. Immunohistochemistry confirmed the specific localization of cathepsin K protein to the odontoclasts. These results demonstrate that odontoclasts in the deciduous root resorption express cathepsin K mRNA and protein that may participate in the proteolysis of root resorption of the deciduous tooth.

Key words: Cathepsin K — Odontoclast — Root resorption — Deciduous tooth — In situ hybridization — Immunohistochemistry

Root-resorbing tissue is a granulation tissue lying between the root of the deciduous tooth and its permanent successor [1–3]. The pattern of deciduous tooth resorption is not continuous, but alternates with periods of repair [2, 4, 5]. Therefore, there are two phases of root-resorbing tissues: the active phase and the resting phase [2, 3]. The active root-resorbing tissue looks red because of an abundant supply of blood vessels but the resting tissue looks white. The red root-resorbing tissue has a number of odontoclasts aligned along its surface attached to the resorbing root. In contrast, the white tissue has few odontoclasts on its surface [2, 3]. The active root-resorbing tissue was found to have bone-resorbing activity when incubated on bone slices [2] and collagenolytic activity when cultured on collagen gel [1]. These biochemical data clearly indicate that the red root-resorbing tissue is responsible for the root resorption of the deciduous tooth [1, 2].

It is well-established that cathepsin K, a member of the papain family of cysteine proteinases, is highly and selectively expressed within the osteoclasts [6–9]. Cathepsin K activity is necessary for bone resorption as demonstrated by deficient bone resorption in pycnodysostotic patients, a rare hereditary disease due to a cathepsin K mutation [10, 11], in the cathepsin K knockout mice [12], and in the tissue cultures performed in the presence of cathepsin K antisense oligonucleotides [13]. Type I collagen is one of the most abundant molecules in the organic matrix of dentin as well as bone. The degradation of this collagen is a major event during bone and root resorption. It has been reported that cathepsin K alone can both depolymerized collagen fibers and cleave triple helices at multiple points [14] and it also has gelatinolytic activity [6]. However, the role of cathepsin K in root resorption still remains to be clarified.

To determine the implication of cathepsin K in the degradation of the organic matrix of deciduous teeth during root resorption, we attempted to describe the cathepsin K expression in bovine root-resorbing tissue by using the reverse transcription (RT)-polymerase chain reaction (PCR) and Northern blot analysis, we also used in situ hybridization and immunohistochemistry to detect which cells produce this enzyme.
Materials and Methods

Tissue Isolation and RNA Extraction

We obtained fresh mandibles of about 1-year-old calves and removed the root-resorbing tissue from between the root of the lower deciduous incisor that was being resorbed and the developing permanent tooth germ. Total RNA was extracted from both the white and red root-resorbing tissues using the single step guanidine-phenol-chloroform method [15].

RT-PCR

Total RNA (5 μg) was reverse-transcribed into first-strand complementary DNA (cDNA) using the Superscript™ Pre-amplification System (Gibco BRL, Rockville, MD). The cDNA was used for amplification by PCR. The reactions were denatured at 94°C for 40 sec, annealed at 55°C for 40 sec, and extended at 72°C for 50 sec for 30 cycles. The cathepsin K cDNA was amplified with the sense primer 5'-GGT TGG TTC CTG TTG GCC TTT-3' and antisense primer 5'-TGC TCT CTT CAG GGC TTT CTC-3', yielding a 310 bp PCR-product. The primers for bovine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are as follows: 5'-ACC ACA GTC CAT GCC ATC AC-3' for sense and 5'-TCC ACC ACC CTG TTG CTG TA-3' for antisense. The 452 bp GAPDH cDNA fragments were obtained. Following amplification, 10 μl aliquots of primer-specific amplification products were analyzed on 5% polyacrylamide gel electrophoresis and ethidium bromide staining.

Northern Blot Analysis

Twenty micrograms of total RNA from both the white and red root-resorbing tissues were denatured and separated by formaldehyde-agarose gel electrophoresis. The RNA samples were then electrophoretically transferred onto Zeta-Probe Blotting Membranes (Bio-Rad Laboratories, Hercules, CA). Cathepsin K and GAPDH probes were prepared from PCR products that were purified with a Qiaquick PCR Purification Kit (Qiagen GmbH, Hilden). The probes were radioabeled with [α-32P]dCTP using a Ready To Go™ DNA Labeling Kit (Pharmacia Biotech, Uppsala, Sweden). The hybridization was performed for 1 hour at 68°C in the ExpressHyb hybridization solution (Clontech Laboratories, Palo Alto, CA). After hybridization, the membrane was washed according to the manufacturer’s specifications. The bound probes were detected by autoradiography.

Preparation of tissue sections. Deciduous teeth and root-resorbing tissues were obtained by a previously described method [3]. Briefly, resorbing deciduous incisors were extracted from fresh mandibles of about 1-year-old calves. The exposed root-resorbing tissues (only in the active phase), lying on permanent tooth germs, were then carefully removed. Both the extracted teeth and the root-resorbing tissues were fixed in 4% paraformaldehyde in PBS (pH 7.4). After fixation, some of the extracted teeth were decalcified in Morse’s solution (22.5% sodium citrate) for 50 min; in prewarmed 0.1× SSC for 1 hour at 65°C; in prewarmed 0.1× SSC for 1 hour at 65°C. Diogoxigenin detection was carried out using the DIG Nucleic Acid Detection kit (Boehringer Mannheim) according to the manufacturer’s instructions.

Enzyme Histochemical Staining for Tartrate-Resistant Acid Phosphatase

Histochemical demonstration of TRAP activity was performed according to the method of Burstone [18]. Sections were incubated with 0.1 M acetate buffer (pH 5.2) containing naphthol AS-MX phosphate (Sigma, St. Louis, MO) as the substrate, Fast Red Violet LB salt (Sigma) for the color reaction, and 50 mM L-(-) tartrate acid.

Antibody

A mouse monoclonal IgG1 antibody generated by immunizing mice with a peptide corresponding to amino acids 182-195 (SEDAYPYVQGESE) of human cathepsin K (Oncogene Research Products, Cambridge, MA) was used in this study.

Western Blot Analysis

Bovine active root-resorbing tissues were cut into small pieces and homogenized in a solution of 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.5% Nonidet®P40 [Ethylphenolpoly (ethyl-ene glycolether)] (BDH Chemicals Ltd., Poole, England), and 1 mM phenyl-methyl-sulfonyl fluoride (PMSF) (Sigma). The mixtures were centrifuged, and the supernatants were mixed with sample buffer (2% sodium dodecyl sulfate (SDS), 100 mM dithiothreitol, 60 mM Tris, pH 6.8, 0.01% bromophenol blue) and boiled for 5 min. Samples were subsequently fractioned in SDS-polyacrylamide gel electrophoresis using 10% gel and transferred to the Immuno-Blot PVDF Membrane (Bio-Rad Laboratories). The membrane was incubated with the mouse antixatepsin K antibody for 2 hours at room temperature and followed by horseradish peroxidase-conjugated rabbit anti-