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Matrix metalloproteinase inhibition impairs the processing, formation and mineralization of dental tissues during mouse molar development

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Abstract

Organotypic cultures of embryonic mouse tooth germs were used to investigate the developmental expression and roles of MMPs in the formation and mineralization of dentin and enamel matrices. The spatially and temporally regulated expression of MMP-2, MMP-9 and MMP-20 in developing mouse tooth germs in vivo was maintained in culture. The inhibition of metalloproteinases activity by marimastat altered the morphogenesis and mineralization of the tooth germs associated with an inhibition of the activation of both MMP-20 and MMP-2. MMP inhibition deregulated the molecular processing of two major dental matrix proteins, amelogenin and dentin sialoprotein (DSP). This coincided with their accumulation and the loss of their normal distribution within the extracellular matrix, resulting in a defective mineralization of dentin and enamel matrices. These findings demonstrate the critical role of MMPs in the processing and maturation of the dental matrix.

Keywords: Tooth; Dentin; Enamel; Dentin sialoprotein (DSP); Amelogenin; MMPs; Marimastat

Introduction

Matrix metalloproteinases (MMPs) have been implicated in the physiological and pathological extracellular remodelling of tissues and in invasive and migratory events both in vivo and in culture. They have also been implicated in embryonic development and morphogenesis, although fewer studies have addressed their roles in tooth development. Odontogenesis involves morphological changes including spatial organization and tissue specific differentiation, which requires the synthesis and organization of the extracellular matrix (ECM), which later mineralizes. The dental crown is composed of enamel and dentin consisting of proteins secreted by ameloblasts and odontoblasts, respectively. These highly specialized cells produce matrices which form a microenvironment that triggers the deposition of hydroxyapatite (HA). Forming enamel is mainly composed of amelogenins, which account for 90% of the enamel matrix. These low molecular weight proteins, which are rapidly proteolytically processed, are thought to regulate crystal growth and orientation [1,2]. The extracellular matrix of the dentin is composed of collagen fibers and non-collagenous proteins (NCPs) such as the dentin sialoprotein (DSP) and dentin phosphoprotein (DPP), both products of the same dentin sialophosphoprotein (DSPP) gene which are involved in the regulation of mineralization.

Developing tooth tissues have previously been evaluated for the expression of several MMPs, but only a few of these studies were concerned with dental matrix formation occurring at the later stages of development [3,4]. In situ

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hybridization and immunostaining revealed that MMP-2 is widely expressed in the dental mesenchyme and in the basement membrane of mouse molar germs [5]. It was suggested that MMP-2 plays a role in basement membrane degradation, thus allowing physical communication between odontoblasts and ameloblasts. At a later stage of development, its expression in dental tissues was restricted to differentiated odontoblasts while its main activator MT1-MMP was expressed on the cell surfaces of both ameloblasts and odontoblasts [6]. MMP-9, on the other hand, was intensely and transiently expressed in the early dental mesenchyme surrounding the invaginating tooth bud, but its expression seems to diminish at the later stages [7,8]. Immunohistochemical studies of the forming rat incisor also revealed an uneven distribution of both MMP-2 and MMP-9, with the highest levels identified at the dentino–enamel junction and coincided with the lowest level of TIMP-1 and TIMP-2 [4]. MMP-20 cleaves amelogenin to produce fragments commonly observed in vivo [9]. It may also process other enamel proteins and is thought to regulate enamel mineralization. It is mainly expressed in ameloblasts, but a transient expression was also found in odontoblasts, although not in dentin [10]. In both ameloblasts and odontoblasts, the expression of amelogenin preceded that of MMP-20, suggesting a developmentally controlled regulation [10,11]. These studies on the role of MMPs during the early stages of tooth development were largely based on their pattern of expression and localization and more direct evidence for their involvement and role in the formation and mineralization of enamel and dentin matrices is lacking. The use of MMPs inhibitors in various culture systems have helped to evaluate their role in cell invasion, migration and development and contributed to our appreciation of the possible roles they may play in vivo [12,13]. Developed by the British Biotech Pharmaceuticals, marimastat (BB-2516) is one of the most studied MMP inhibitors both in vitro and in vivo preclinical and clinical studies. It is a collagen mimicking peptide with a hydroxamate group which inhibits MMP activity by chelating the active site zinc ion and exhibits a potent inhibitory activity towards a wide range of MMPs [14,15].

In this study, we used as a model an organotypic culture of embryonic mouse tooth germs to investigate the developmental expression and implication of three MMPs, MMP-2, MMP-9 and MMP-20, at stages of terminal morphogenesis and cell differentiation of tooth development. This model allows the visualization of the onset of matrix apposition and early mineralization, which result in the development of dentin and enamel after 10 days in culture. These germ explants have the advantage of being accessible to a targeted experimental perturbation. Using the broad spectrum MMP inhibitor marimastat, we demonstrate an alteration of enamel and dentin formation, as well as an inhibition of mineralization, associated with an inhibition of the activation of both MMP-20 and MMP-2. We also describe the consequences of this MMP deregulation on the precise processing of two principal dental matrix proteins, amelogenin and dentin sialoprotein, which may be considered as characteristics for amelogenesis and dentinogenesis, respectively.

Materials and methods

Mice

Timed pregnant Swiss mice (Charles River, Lyon France) were mated between 8 am and 10 am on day 0. Mice with vaginal plugs were killed at the 18th day of gestation by cervical dislocation, and the mandibular first molar tooth germs were dissected from the embryos under a stereo-microscope in cold Hank's buffer. They were processed for protein extraction or explanted into culture.

Antibodies

The anti-MMP-20 polyclonal Ab, developed in rabbit and directed against a synthetic peptide corresponding to the hinge region of the metalloproteinase, was from Sigma (St. Louis, MO, USA). Monoclonal antibodies for MMP-2 (CA 801) and polyclonal antibodies for MMP-9 were produced in the laboratory of R. Fridman [16,17]. Polyclonal antibody for DSP was kindly given by W.T. Butler (University of Texas, Houston, USA), and the polyclonal antibody for amelogenin is a gift of BIORA (Malmoe, Sweden). Anti-fibronectin polyclonal antibody was from Chemicon International (Temecula, CA). Goat anti-mouse IgG–peroxidase conjugated and swine anti-rabbit IgG-peroxidase conjugated were from Dako (Glostrup, Denmark).

Germs explant culture

Mandibular first molar tooth germs from E18 embryos were cultured in semi-solid medium, BGJb (Gibco-In Vitrogen, Carlobad, CA, USA), supplemented with 20% of fetal bovine serum (P Pierce, Rockford, IL, USA), 20 mg/ml L-Glutamine, 275 μg/ml sodium ascorbate (Merck KG aA, Darmstadt, Germany) and an antibiotic solution (Gibco-In Vitrogen, Carlobad, CA, USA) containing penicillin (10.000 U/ml) and streptomycin (10 mg/ml) diluted to a final concentration of 1% in an specific atmosphere rich in oxygen (50% O_2, 5% CO_2, 45% N_2) at 37°C. The medium was filled with 0.5% agar (Bacto Agar, Difcolab, Detroit, MI, USA) and changed at 2, 4 and 7 days. The cultures were stopped at day 10, when the control germs form a 15- to 25-μm-thick dentin layer and an aprismatic enamel layer about 5 μm thick [18]. These measurements were carried out on micrographs of sections observed with the light microscope, after correction for the magnification used. For time course experiments, cultures were also stopped at 2, 4 and 6 days. For the MMP inhibition experiments, a stock solution of