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Inhibition of Matrix Metalloproteinase-9 Activity by Zinc Lactate and Zinc Acetate

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Abstract

Background/Aim: Matrix metalloproteinase-9 (MMP-9) is considered to be implicated in progression of dental diseases such as dentin caries and erosion. In this study, the effects of some metal salts composed of any of Mg^{2+}, Ca^{2+}, Zn^{2+} and either lactate ion or acetate ion on MMP-9 activity were examined.

Materials and Methods: Enzymatic activity of MMP-9 was measured by using a MMP-9 Colorimetric Drug Discovery Kit. Gelatinolysis by MMP-9 was revealed by gelatin zymography using a Gelatin-Zymography Kit.

Results: Enzymatic activity of MMP-9 was found to be inhibited by two zinc salts, zinc lactate (Zn-Lac) and zinc acetate (Zn-Ac). Effect of each zinc salt was concentration-dependent. These two compounds had comparable inhibitory effects, and enzymatic activities of MMP-9 were reduced by approximately 90% at 1 mM. Furthermore, both zinc salts down-regulated gelatinolysis.

Conclusion: These findings suggest that application of Zn-Lac and Zn-Ac may become a new strategy for preventing progression of dentin caries and erosion.

Keywords: Matrix metalloproteinase-9, Inhibition, Enzymatic activity, Gelatinolysis, Zinc lactate, Zinc acetate, Dentin caries and erosion

Introduction

Matrix metalloproteinases (MMPs) are a family of structurally related zinc-dependent endopeptidases that mediate the degradation of extracellular matrix [1,2]. MMPs are implicated in many biological and pathologic processes, such as tumor invasion and metastasis, wound healing, angiogenesis, inflammation [3–6]. MMPs are also known to be involved in several dental diseases such as caries, pulpitis, and periodontitis [7–9]. MMPs have been classified into subgroups such as collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs, and other MMPs. Gelatinases consist of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9), that are known to be active in the degradation of denatured fibrillary collagens, type IV collagen that constitutes basement membrane, and several other components of extracellular matrix [10–13]. MMP-9 is known to be involved in progression of tumor invasion and metastasis, several neurological diseases and inflammatory processes, rheumatoid arthritis [14–16]. Besides, in oral environment, MMP-9 is considered to be implicated in progression of dental diseases such as dentin caries and erosion [17]. Previous studies have revealed that MMP-9 can promote the destruction of dentin collagen in carious lesions and have suggested MMP-9 plays an important role in caries progression [18–20]. Therefore, regulation of MMP-9 activity is of current interest in clinical dentistry.

Numerous organic compounds classified into some types of compounds such as arylamide, hydroxamate, arylsulfonyl acetamide and quinoxaline have been developed as MMP-9 inhibitor mainly for the purpose of prevention of tumor invasion and metastasis [21]. In clinical dentistry, several types of metal inorganic salts such as NaF, $[Ag(NH_3)_2]F$, $SnCl_2$, $CuSO_4$, $HgSO_4$, $ZnSO_4$ have been found to exhibit inhibitory effect on MMP-9 activity [19,22–26]. But there have been few reports about the effects of metal salts on organic acids.

In the present study, I therefore examined the effects of some metal salts composed of any of Mg^{2+}, Ca^{2+}, Zn^{2+} and either lactate ion or acetate ion on MMP-9 activity.

Materials and Methods

Chemicals

Magnesium lactate (Mg-Lac) trihydrate, zinc lactate (Zn-Lac) trihydrate, and zinc acetate (Zn-Ac) dihydrate were purchased from Fujifilm Wako Pure Chemical Co. (Tokyo, Japan) and calcium lactate (Ca-Lac) pentahydrate was from Nacalai Tesque, Inc. (Tokyo, Japan). Recombinant human MMP-9 (rhMMP-9) (proenzyme, 92 kDa) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Measurement of enzymatic activity

Enzymatic activity of MMP-9 was measured by using a MMP-9 Colorimetric Drug Discovery Kit (Enzo Life Science, Plymouth, PA, USA). Measurement procedure was performed according to the manufacturer's recommendation. Briefly, MMP-9 enzyme was incubated with a test compound in assay buffer for 30 min at 37°C, then a chromogenic substrate peptide (Acetyl-Pro-Leu-Gly-[2-mercapto-4-methylpentanoyl]-Leu-Gly-OC_2H_5) was added. Absorbance at 405 nm was measured continuously for 30 min in a microplate reader. Enzymatic activity was evaluated based on cleavage of the chromogenic substrate peptide.

Gelatin zymography

Gelatinolysis by MMP-9 was revealed by gelatin zymography using a Gelatin-Zymography Kit (Cosmo Bio type) (Cosmo Bio Co., Ltd., Tokyo, Japan). Precast polyacrylamide gel blended with gelatin, sample preparation buffer, electrophoresis buffer, washing buffer, reaction buffer and staining solution were provided in the kit. Zymography procedure was performed according to the manufacturer's recommendation. Briefly, rhMMP-9 was diluted in phosphate-buffered saline, and resulting rhMMP-9 solution was mixed with equal volume of sample preparation buffer, then electrophoresed on precast gel at 15 mA constant current. The loaded amount of MMP-9 was 1 ng per lane. Gels were cut into strips, each containing one lane of rhMMP-9, and washed. Each gel strip was incubated separately in reaction buffer containing a test compound at 37°C for 24 hrs. Gel strips were stained in staining solution for 60 min. After gel strips were destained, gelatinolytic activity was detected as clear bands in the background of uniform staining.

Statistical analysis

Data are presented as the mean ± S.D. Statistical analysis was performed using one-way analysis of variance (ANOVA) to compare all specific group means so as to detect overall differences, followed by Student's *t*-test to determine whether difference between any two specific groups is statistically significant. A p value of less than 0.05 was considered statistically significant.

Results

I first examined the effects of Mg-Lac, Ca-Lac and Zn-Lac on enzymatic activity of MMP-9. As shown in **Figure 1**, 1 mM Zn-Lac was found to exhibit potent inhibitory effect on enzymatic activity of MMP-9, whereas Mg-Lac and Ca-Lac did not have any significant effect at same concentration.

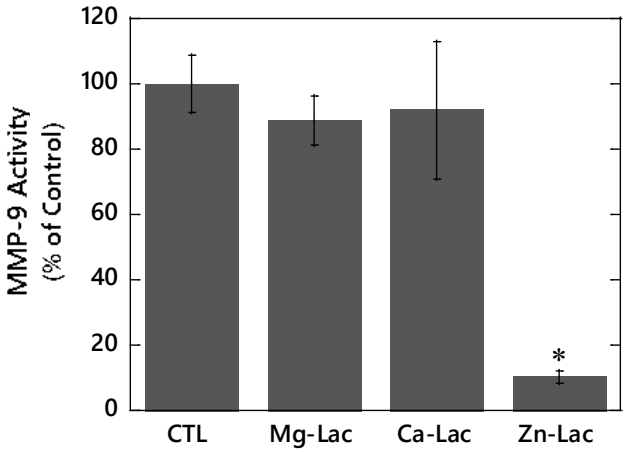

Figure 1. Effects of Mg-Lac, Ca-Lac, and Zn-Lac on enzymatic activity of MMP-9. Measurement of enzymatic activity of MMP-9 was conducted in the absence (control) or presence of 1 mM Mg-Lac, 1 mM Ca-Lac, or 1 mM Zn-Lac by using a MMP-9 Colorimetric Drug Discovery Kit as described under "Materials and Methods". Data are expressed as the mean ± S.D. of triplicate samples (n = 3). *: $p < 0.01$ compared with control. CTL: Control; Mg-Lac: Magnesium Lactate; Ca-Lac: Calcium Lactate; Zn-Lac: Zinc Lactate; MMP-9: Matrix Metalloproteinase-9.

This result implied that some other zinc salts could possibly inhibit enzymatic activity of MMP-9, subsequently the effect of another zinc salt, zinc acetate (Zn-Ac) was also examined.

As shown in **Figure 2**, each of Zn-Lac and Zn-Ac reduced enzymatic activity of MMP-9 in a concentration-dependent manner between 0.001 mM and 1 mM. The two compounds had comparable inhibitory effects and enzymatic activities of MMP-9 were reduced by approximately 90% at 1 mM.

Effects of Zn-Lac and Zn-Ac on gelatinolysis by MMP-9 were demonstrated using gelatin zymography. As shown in **Figure 3**, a band migrated at 92 kDa that corresponded to pro-MMP-9 enzyme was diminished by treatment with 0.1 mM Zn-Lac or 0.1 mM Zn-Ac, thus both of Zn-Lac and Zn-Ac clearly down-regulated the gelatinolysis by rhMMP-9.

Discussion

Activation of MMPs is known to be involved in several dental diseases such as caries, pulpitis, and periodontitis [7–9]. Among MMPs, MMP-9 has emerged as one of the important therapeutic targets in caries treatment. Previous studies have revealed that MMP-9 can promote the destruction of dentin collagen in carious lesions, therefore plays an important role in caries progression [18–20]. In dental carious lesions, MMP-9 is known to be the predominant MMP, and is found to be much activated [17]. Dentinal fluid samples from teeth that possessed carious lesions had significantly higher MMP-9 levels than those from clinically healthy teeth [18].

Zinc has been widely used in clinical dentistry [27]. It is an important component of dental materials such as dental

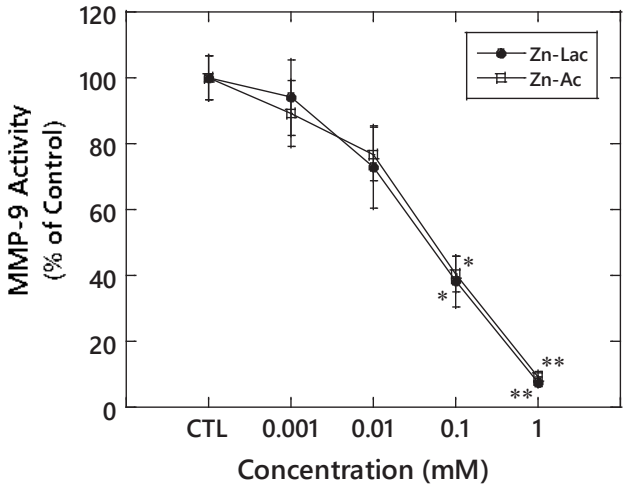

Figure 2. Concentration response of the effects of Zn-Lac and Zn-Ac on enzymatic activity of MMP-9. Measurement of enzymatic activity of MMP-9 was conducted in the absence (control) or presence of indicated concentration of Zn-Lac or Zn-Ac by using a MMP-9 Colorimetric Drug Discovery Kit as described under "Materials and Methods". Data are expressed as the mean ± S.D. of triplicate samples ($n = 3$). *: $p < 0.05$ and **: $p < 0.01$ compared with control. CTL: Control; Zn-Lac: Zinc Lactate; Zn-Ac: Zinc Acetate; MMP-9: Matrix Metalloproteinase-9.

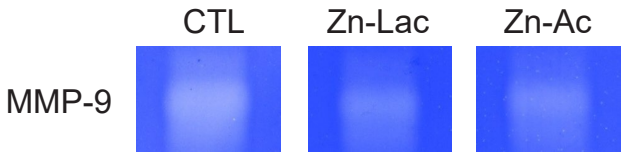

Figure 3. Effects of 0.1 mM Zn-Lac and 0.1 mM Zn-Ac on gelatinolysis by MMP-9. Gelatin zymography was conducted using a Gelatin-Zymography Kit as described under "Materials and Methods". After electrophoresis of rhMMP-9, gels were cut into strips, each containing one lane of rhMMP-9. Each gel strip was subjected to gelatinolytic reaction in the absence (control) or presence of 0.1 mM Zn-Lac or 0.1 mM Zn-Ac. CTL: Control; Zn-Lac: Zinc Lactate; Zn-Ac: Zinc Acetate; MMP-9: Matrix Metalloproteinase-9.

cements and restorative materials, and it is also included in toothpaste and mouthwash as an active ingredient. Zinc in excess was found to reduce MMP-mediated collagen degradation, was thereby expected to prevent progression of dentin caries and erosion [28].

In the present study, enzymatic activity of MMP-9 was found to be potently inhibited by two zinc salts of organic acids, Zn-Lac and Zn-Ac. Furthermore, gelatinolysis was attenuated by these zinc salts as demonstrated by gelatin zymography. The decrease in gelatinolysis is conceivably mediated by inhibition of enzymatic activity of MMP-9. These two zinc salts might have been useful in preventive dentistry. Zn-Lac has been used as a component of toothpaste and mouth rinse, whereas Zn-Ac has been used as plaque-inhibiting agent [29,30].

The precise mechanism underlying MMP-9 inactivation by Zn-Lac and Zn-Ac remains to be clarified. It is possible to speculate that Zn^{2+} binds to specific sites to induce conformational changes, thereby inactivating the enzyme. The mechanism by which carboxypeptidase A, a zinc-dependent metalloproteinase, is inhibited by zinc has been well studied [31]. A second Zn^{2+} binds to the active site of the enzyme and forms $ZnOH^{+}$ complex that bridges the catalytic Zn^{2+} to a side chain of the active site, resulting in inactivation of the enzyme. Similar mechanisms might work in the inhibition of MMP-9 by Zn-Lac and Zn-Ac.

There are limitations to the present study. For measurement of enzymatic activity of MMP-9, synthetic substrate peptide was used. Although it is of interest to explore whether matrix degradation in dentin specimens could also be inhibited by Zn-Lac and Zn-Ac, dentin substrate experiments have not yet been carried out. Furthermore, the effects of Zn-Lac and Zn-Ac have not been validated *in vivo*. Studies on the effects of Zn-Lac and Zn-Ac *in vivo* are certainly necessary for future clinical application of Zn-Lac and Zn-Ac for the treatment of dentin carious lesion.

Application of Zn-Lac and Zn-Ac may become a new strategy for preventing progression of dentin caries and erosion.

Conclusion

Enzymatic activity of MMP-9 was found to be potently inhibited by two zinc salts of organic s, Zn-Lac and Zn-Ac. Furthermore, gelatinolysis was attenuated by these zinc salts. The decrease in gelatinolysis is conceivably mediated by inhibition of enzymatic activity of MMP-9. Application of Zn-Lac and Zn-Ac may become a new strategy for preventing progression of dentin caries and erosion.

Conflicts of Interest

The author has no conflict of interest to declare.

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