JOURNAL OF DENTISTRY 42 (2014) 329–335

Available online at www.sciencedirect.com

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The inhibitory effects of silver diamine fluorides on cysteine cathepsins☆

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ARTICLE INFO

Article history:
Received 2 October 2013
Received in revised form
22 November 2013
Accepted 26 November 2013

Keywords:
Cysteine cathepsins
Matrix metalloproteinases
Arrest caries
Dentine
Silver diamine fluoride

ABSTRACT

Aim: The expression of cysteine cathepsins in human carious dentine suggests that this enzyme contributes to the collagen degradation in caries progress. This study investigated whether silver diamine fluoride (SDF) inhibited the activity of cysteine cathepsins.
Methods: Three commercial SDF solutions with concentrations at 38%, 30% and 12% were studied. Two fluoride solutions with the same fluoride ion (F^-) concentrations as the 38% and 12% SDF solutions, and 2 silver solutions with the same silver ion (Ag^+) concentrations as the 38% and 12% SDF solutions were prepared. Five samples of each experimental solution were used to study their inhibitory effect on two cathepsins (B and K) using cathepsin assay kits. Positive control contained assay buffer and cathepsins dilution was used to calculate the percentage inhibition (difference between the mean readings of the test solution and control solution divided by that of the control group).
Results: The percentage inhibition of 38%, 30% and 12% SDF on cathepsin B were 92.0%, 91.5% and 90.3%, respectively ($p < 0.001$); on cathepsin K were 80.6%, 78.5% and 77.9%, respectively ($p < 0.001$). Ag^+ exhibited the inhibitory effect against both cathepsin B and K with or without the presence of F^- ($p < 0.01$). The solutions containing Ag^+ have significantly higher inhibitory effect than the solutions containing F^- only ($p < 0.01$).
Conclusion: According to this study, SDF solution at all 3 tested concentrations significantly inhibited the activity of cathepsin B and K.

1. Introduction

A recent review concluded that silver diamine fluoride (SDF) is a safe, effective, efficient, and equitable caries-preventive agent that appears to meet the criteria of the WHO Millennium Goals and the US Institute.[1] Clinical trials showed that SDF prevented and arrested coronal caries in primary teeth[2,3] and root caries in permanent teeth.[4] Numerous laboratory studies have found that SDF is effective in increasing microhardness[5–7] and the mineral content of dentine caries.[6,8,9] Apart from the demineralisation of hydroxyapatite, caries progression also involves significant degradation of dentine collagen.[10] Mammalian collagenolytic enzymes such as matrix metalloproteinases (MMPs) were found to play a crucial role in collagen degradation.[11] In addition, cysteine cathepsins (or cathepsins)

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0300-5712/$ – see front matter
http://dx.doi.org/10.1016/j.jdent.2013.11.018

were identified in the degradation of extracellular matrix components.[12,13] Tersariol et al.,[12] reported that cathepsin activities were associated with MMPs activities in dentine. Scaffa et al.,[14] suggested that cathepsins may also be responsible for the collagen degradation in caries lesions. Hence, the inhibition of MMPs and cathepsins may contribute to caries arrest.

Cathepsins are proteolytic enzymes that can be identified in dentine caries and human pulp. They are members of the C1 family of papain-like enzymes, which are the largest and the best-characterised family of cysteine peptidases. Cathepsins can extracellularly degrade type I collagen and proteoglycans, which are the main components of the dentine organic structure. Cathepsins are active and stable in acidic environments and mostly unstable at neutral acidity.[15] The autocatalytic activation of cathepsins is substantially accelerated in the presence of anionic polysaccharides.[16]

Cathepsins are differentiated by their structure, catalytic mechanism, and the proteins they cleave. Cathepsin B can be found in human carious dentine and saliva. Its activity varies in relation to the depth and age of carious lesions, while remaining stable in saliva.[13] Cathepsin B cleaves in the non-helical telopeptide extensions of collagens.[17] Cathepsin K can catabolize collagen and break down dentine. It cleaves the collagen at the triple helical region.[15] These properties suggest that cathepsins may play crucial roles in collagen breakdown in dentine caries lesions.

We found that SDF strongly inhibited the proteolytic activity of MMP-2, MMP-8, and MMP-9, which are three of the main MMPs that take part in collagen degradation,[18] our resent study also demonstrated that SDF prevented dentine collagen degradation from bacterial collagenase challenge.[19] To serve as an effective caries arresting agent, SDF needs to function as a broad-spectrum inhibitor that restrains other dentinal proteolytic enzymes. Unlike the MMPs first discovered in dentine pathologies over 15 years ago, cathepsins were recently detected in dentine.[12,13,15] However, no studies have examined the effectiveness of SDF on cathepsins. Thus, the aim of this study was to investigate the inhibitory effects of 3 common, commercially available SDF solutions at different concentrations on cysteine cathepsins. Freshly prepared silver nitrate ($AgNO_3$) and sodium fluoride (NaF) solutions were used for comparison. Two null hypotheses are tested: firstly, there is no difference in inhibitory effect on cysteine cathepsins by SDF, $AgNO_3$ and NaF solutions; and secondly, there is no difference in inhibitory effect on cysteine cathepsins by 38%, 30%, and 12% SDF solutions.

2. Methods and materials

2.1. Reagents

Commercially available SDF solutions at concentrations of 12% (Cariostop, Biodinamica, Brazil), 30% (Cariostop, Biodinamica, Brazil), and 38% (Saforide, Toyo Seiyaku Kasei, Japan) were selected for this in vitro study. Freshly silver and fluoride solutions were prepared, which contained equivalent concentrations of silver ions (Ag^+) and fluoride ions (F^-) as the 38% and 12% solutions of SDF, respectively. The 3 commercially available SDF solutions had high pH values (pH = 12–13), which could have affected the cysteine cathepsin activity. Therefore, 3 control groups of SDF at 38%, 30%, and 12% buffered with 10% nitric acid (HNO_3) to lower the acidity (pH = 9) were prepared. Five samples of each experimental solution were used in this study. The 10 experimental solutions assessed were numbered groups 1–10, as shown in Table 1.

2.2. Inhibition of cathepsin enzymatic activity by the experimental solutions

We used human recombinant cathepsins and Sensolyte cathepsin assay kits (cathepsins B and K) from AnaSpec Inc. (San Jose, CA, USA). The Sensolyte cathepsin assay kit is a homogeneous assay that can be used to detect enzyme activity and for screening cathepsin inhibitors. The cathepsin B enzyme was diluted 1000-fold in a dithiothreitol (DTT)-containing assay buffer, while the cathepsin K enzyme was pro-incubated with an equivalent amount of activation buffer for 40 min at room temperature before being diluted 300-fold in a DTT-containing assay buffer. The enzyme diluents were prepared immediately before use according to the protocol suggested by the manufacturer. Then, 40 μl diluted cathepsin enzyme and 10 μl experimental solution were added to each well of a black fluorometric 96-well microtiter plate (Fisher Scientific, Gainesville, FL, USA). The wells of the microtiter plate also received 50 μl of diluted cathepsin substrate solution (dilution factor: 1:100) to give a total volume in each well of 100 μl. Thus, the concentration of the experimental solution was diluted 10 times during the reaction. Namely, the

Table 1 – Fluoride and silver content and acidity (pH) of the 10 experimental solutions.

Group	Product	Chemicals	F^- (ppm)	Ag^+ (ppm)	pH
1	Saforide 38%	$Ag(NH_3)_2F$	44,800	255,000	13
2	Buffered saforide	$Ag(NH_3)_2F + HNO_3$	44,800	255,000	9
3	Cariestop 30%	$Ag(NH_3)_2F$	35,400	200,000	12
4	Buffered cariestop 30%	$Ag(NH_3)_2F + HNO_3$	35,400	200,000	9
5	Cariestop 12%	$Ag(NH_3)_2F$	14,150	80,000	12
6	Buffered cariestop 12%	$Ag(NH_3)_2F + HNO_3$	14,150	80,000	9
7	Silver solution A	$AgNO_3$	–	255,000	10
8	Silver solution B	$AgNO_3$	–	80,000	10
9	Fluoride solution A	NaF + KF[a]	44,800	–	9
10	Fluoride solution B	NaF	14,150	–	9

[a] Potassium fluoride was added to 4% NaF to form a solution with 44,800 ppm fluoride ions.