

Cerium Chloride Reduces Enamel Lesion Initiation and Progression in vitro

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Key Words

Cerium chloride · Enamel · Fluoride

Abstract

Aim: Determination of the potential of cerium chloride to reduce artificial carious mineral loss and lesion depth progression. **Methods:** A total of 160 enamel samples were prepared from 40 bovine lower central incisors. Crowns were sectioned into four pieces, embedded in acrylic resin, ground flat and allocated to eight groups (S1–S4 and D1–D4; n = 20). Specimens of groups D1–D4 were stored (for 7 days) in a demineralizing buffer solution to induce caries-like lesions. Afterwards, samples were treated for 30 s with one of the following solutions: placebo (S1 and D1), amine fluoride (S2 and D2), cerium chloride (S3 and D3) and a combination of fluoride and cerium chloride (S4 and D4). After another 7 (D1–D4) or 14 (S1–S4) days in demineralizing buffer solution, integrated mineral loss and lesion depth were determined by transversal microradiography and compared by Scheffé's post hoc tests. **Results:** In groups S1–S4, the highest values for integrated mineral loss and lesion depth were observed for group S1 (placebo), the lowest values for group S4. The results in groups S2–S4 were not significantly different. In groups D1–D4, the highest values for integrated mineral loss and lesion depth were observed for group D1 (placebo), the lowest values in groups D3 and D4. In group D2, integrated mineral loss and lesion depth were significantly lower as

compared to D1, but significantly higher compared to groups D3 and D4. **Conclusion:** Cerium chloride and its combination with fluoride are able to significantly reduce carious mineral loss and the progression of lesion depth.

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It is evident that the overall reduction in the prevalence of dental hard tissue loss due to caries can be mainly attributed to the use of fluoride in different forms and different application regimens [Carvalho et al., 2001; Aleksejuniene et al., 2004; Griffin et al., 2007], as fluorides promote remineralization and inhibit the demineralization of dental hard tissues under cariogenic conditions [Luoma et al., 1989].

However, local and systemic side effects such as dental fluorosis [Fejerskov et al., 1994; Carvalho et al., 2001], skeletal fluorosis [Sato and Niwa, 1996] and toxicity could also be observed after the use of fluorides in high concentration [Forsman, 1977]. Therefore, the search for alternative materials for caries prevention is still warranted.

Already in 1999, Zhang et al. [1999] tested the use of lanthanide solutions and their combination with sodium fluoride solutions for the prevention of carious-like lesion development of root cementum to test possible alternative caries-preventive compounds. They showed that the protective effects of the different lanthanide solutions

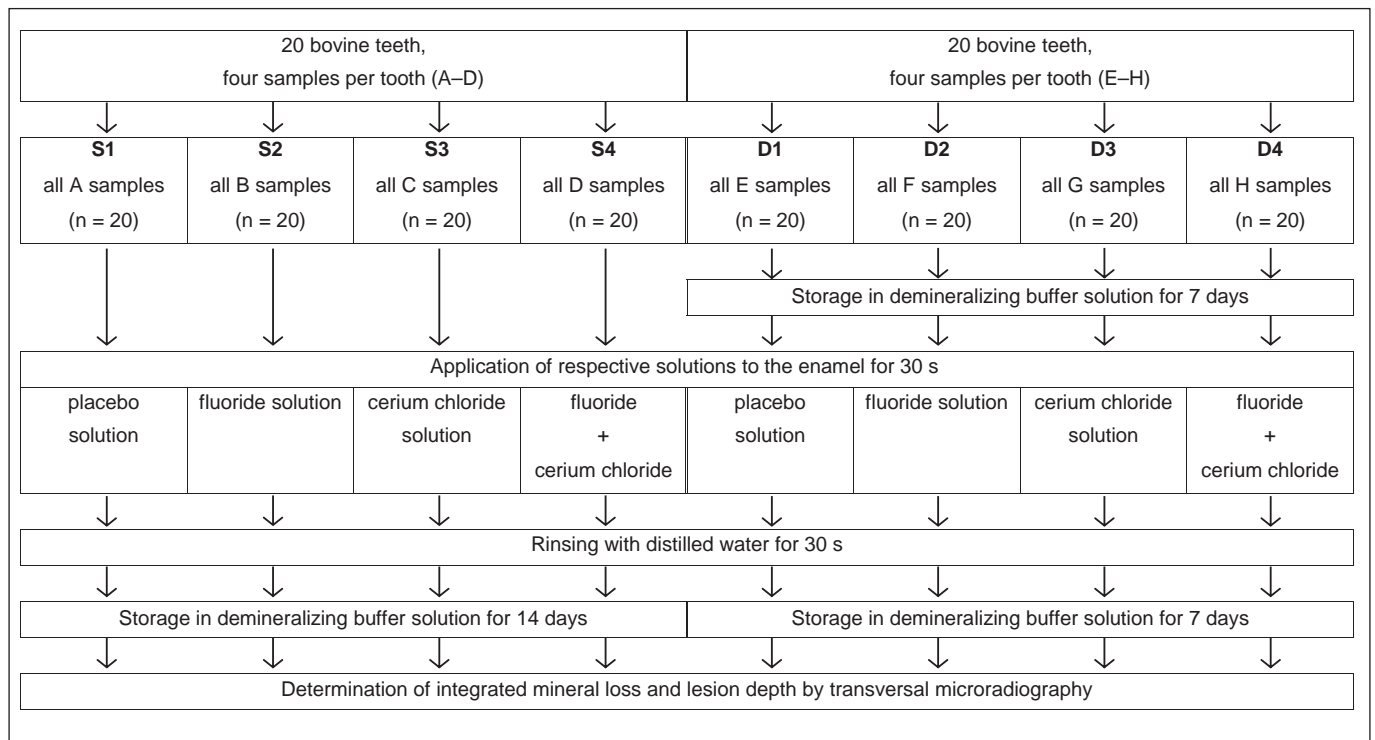


Fig. 1. Experimental procedure.

were more or less comparable with those of fluoride solutions [Zhang et al., 1999]. Among these compounds, cerium chloride and its combined application with amine fluoride have shown a significant anti-erosive potential [Wegehaupt et al., 2010, 2011].

The rare earth elements lanthanum and cerium showed lower toxicity than fluoride and a weaker tendency to accumulate in the liver, kidney and brain [Shimomura et al., 1980]. A recent study showed a stimulating effect of cerium chloride on fibroblasts, but a depressing influence on osteoblasts, which could be compensated for by adding rhBMP-2 [Schmidlin et al., 2012].

To the authors' best knowledge, there has been no study reporting the use of lanthanide solutions and combinations of lanthanides with fluoride to prevent carious-like lesion formation in enamel.

Therefore, this study aimed to determine the potential of cerium chloride to reduce artificial carious mineral loss and lesion depth progression when applied on sound and pre-demineralized enamel. The null hypothesis was that the application of cerium chloride would result in a comparable mineral loss and lesion depth progression as amine fluoride.

Materials and Methods

The experimental procedure is presented in figure 1.

Sample Preparation

For this study 160 enamel samples were prepared from 40 bovine lower central incisors. The teeth were free from defects and/or cracks on X-ray examination and were sectioned at the cementum-enamel junction with a water-cooled diamond disc. The pulp tissue was removed with endodontic files. Crowns were sectioned into four pieces and marked in order to identify respective samples of one tooth during the whole treatment process. The enamel blocks were embedded in acrylic resin (Palavit G, Kulzer, Wehrheim, Germany) and the enamel surface was ground with abrasive paper (800, 1,000, 1,200, 2,400 and 4,000 grit; Waterproof Silicon Carbide Paper, Struers, Erkrath, Germany). During these grinding steps the outermost 200 µm of enamel was removed. This enamel loss was measured with a micrometer (Mitutoyo, Tokyo, Japan). For teeth 1–20 one piece each was randomly allocated to one of four groups (sound specimens S1–S4) and for teeth 21–40 one piece each to one of four groups (demineralized specimens D1–D4, n = 20 per group). The samples were stored under moist conditions until used.

Samples of groups D1–D4 were stored at 37°C in a demineralizing buffer solution to induce artificial caries lesions. The demineralizing buffer solution was prepared according to the equation given by Buskes et al. [1985]. The solution was renewed every 2

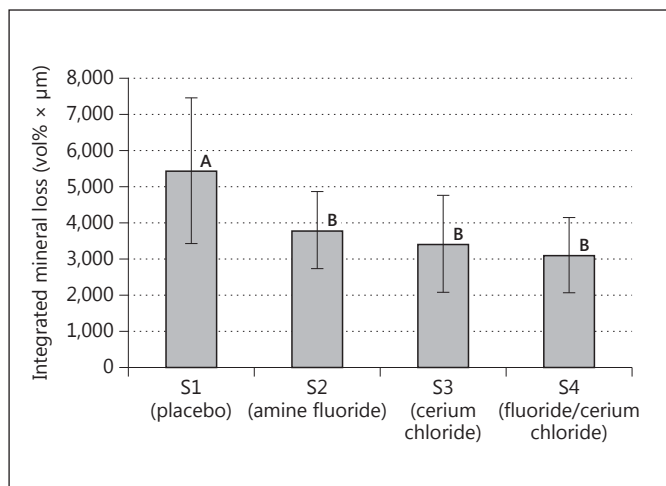


Fig. 2. Integrated mineral loss (mean ± SD) in groups S1–S4 (previously sound samples). Groups not statistically significantly different are marked with identical capital letters.

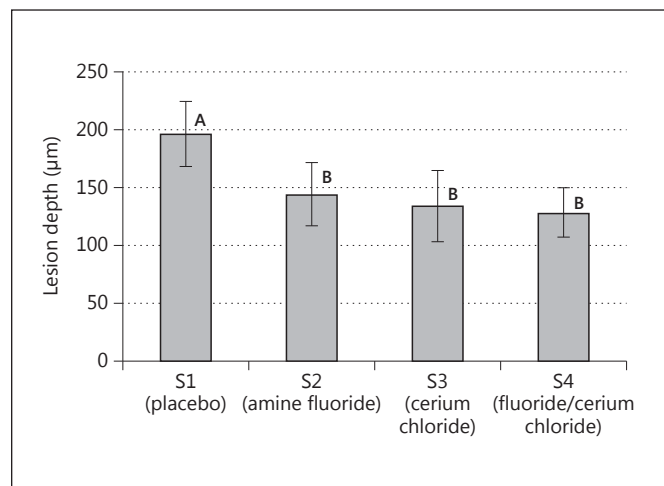


Fig. 3. Lesion depth (mean ± SD) in groups S1–S4 (previously sound samples). Groups not statistically significantly different are marked with identical capital letters.

days and kept under constant agitation. After 7 days, the samples of groups D1–D4 were removed from the demineralizing buffer solution and rinsed with distilled water. All samples were stored at 100% humidity until treatment.

Preparation of Treatment Solutions and Allocation

The placebo solution for groups S1 and D1 was prepared by mixing 0.10 g of sodium benzoate with 99.90 g of distilled water. For groups S2 and D2, a commercially available amine (olafur and dactaflur) fluoride solution (10,000 ppm F; Elmex fluid, GABA International AG, Therwil, Switzerland) was used. The cerium solution of groups S3 and D3 was composed of 10.00 g of Cer(III) chloride, 0.10 g of sodium benzoate and 89.90 g of distilled water (pH 4.94). For groups S4 and D4 no special solution was prepared. Groups S4 and D4 were treated with a combination of the solutions used for groups S2 and D2 and S3 and D3.

These solutions (groups D1–D3 and S1–S3) or combinations of solutions (groups D4 and S4) were then applied to the enamel for 30 s under constant agitation. Afterwards, samples were rinsed with distilled water to remove exceeding solutions for another 30 s. In general, samples treated with the combination application were first treated with Elmex fluid and then cerium chloride was immediately applied according to the methods published previously [Wegehaupt et al., 2010].

Samples of groups D1–D4 were then stored in the demineralizing buffer solution for another 7 days, while the samples of groups S1–S4 were stored in the demineralizing buffer solution for 14 days.

Determination of Integrated Mineral Loss and Lesion Depth

For the determination of integrated mineral loss and lesion depth a slice was cut perpendicular to the enamel surface of the samples and ground to a thickness of 80–100 μm. The measurement of integrated mineral loss and lesion depth was performed by transversal microradiography following a standard protocol [Magalhaes et al., 2009].

Data Presentation and Statistical Analysis

Data were coded in Excel and analyzed with SPSS version 16. For data presentation, mean values and standard deviations of integrated mineral loss and lesion depth were calculated.

Data analysis was performed using ANOVA and Scheffé's post hoc tests to compare the mineral loss and lesion depth within groups S1–S4 and D1–D4. Significance level was set at 95%.

Results

Mineral Loss and Lesion Depth in Previously Sound Enamel (S1–S4)

Integrated mineral loss and lesion depth in groups S1–S4 are illustrated in figures 2 and 3, respectively. Furthermore, the p values of the comparisons within groups S1–S4 are presented in table 1.

The samples of the group treated with the placebo solution (group S1) showed the highest values for mineral loss and lesion depth (5,440 ± 2,027 and 196 ± 28 μm).

The lowest levels of integrated mineral loss (3,101 ± 1,059 vol% × μm) and lesion depth (128 ± 21 μm) were observed for samples treated with the combination of fluoride and cerium chloride (group S4), which were statistically not different from samples treated with the amine fluoride solution (group S2; 3,784 ± 1,071 vol% × μm and 144 ± 27 μm) and the cerium chloride solution only (group S3; 3,409 ± 1,337 vol% × μm and 134 ± 31 μm).

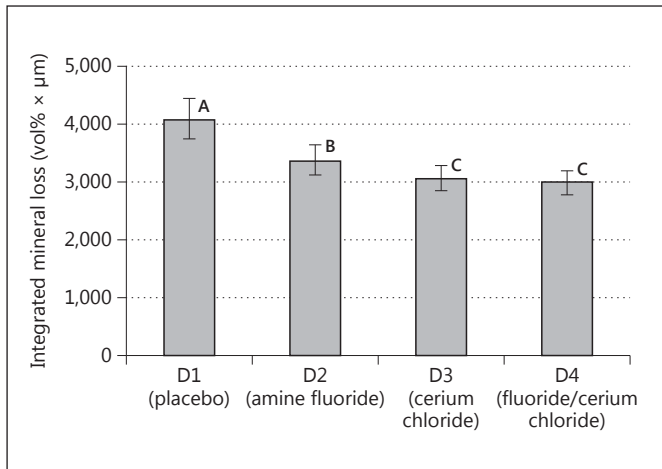


Fig. 4. Integrated mineral loss (mean ± SD) in groups D1–D4 (pre-demineralized enamel samples). Groups not statistically significantly different are marked with identical capital letters.

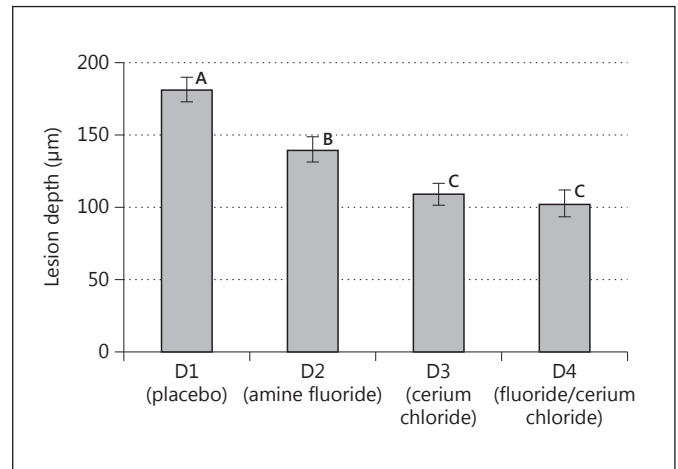


Fig. 5. Lesion depth (mean ± SD) in groups D1–D4 (pre-demineralized enamel samples). Groups not statistically significantly different are marked with identical capital letters.

Table 1. Statistical significance (p values) of the comparison among groups S1–S4 for mineral loss and lesion depth

Comparisons	For mineral loss	For lesion depth
S1 vs. S2	0.0060*	<0.0001*
S1 vs. S3	0.0004*	<0.0001*
S1 vs. S4	<0.0001*	<0.0001*
S2 vs. S3	0.8754	0.7177
S2 vs. S4	0.5187	0.3406
S3 vs. S4	0.9261	0.9260

Significant differences are marked with an asterisk.

Table 2. Statistical significance (p values) of the comparison among groups D1–D4 for mineral loss and lesion depth

Comparisons	For mineral loss	For lesion depth
D1 vs. D2	<0.0001*	<0.0001*
D1 vs. D3	<0.0001*	<0.0001*
D1 vs. D4	<0.0001*	<0.0001*
D2 vs. D3	0.0039*	<0.0001*
D2 vs. D4	0.0002*	<0.0001*
D3 vs. D4	0.8461	0.1397

Significant differences are marked with an asterisk.

Mineral Loss and Lesion Depth in Previously Pre-Demineralized Enamel (D1–D4)

Integrated mineral loss and lesion depth in groups D1–D4 are presented in figures 4 and 5, respectively. Furthermore, the p values of the comparisons between groups D1–D4 are presented in table 2.

The highest values for integrated mineral loss and lesion depth were observed again for placebo group D1 (4,098 ± 346 vol% × μm and 181 ± 9 μm, respectively).

The lowest levels of integrated mineral loss and lesion depth were observed for samples treated with the combination of fluoride and cerium chloride (D4; 2,993 ± 209 vol% × μm and 103 ± 9 μm) and with cerium chloride alone (D3; 3,068 ± 209 vol% × μm and 109 ± 7 μm), reaching statistical significance. Following treatment with

amine fluoride (group D2), the values for integrated mineral loss (3,384 ± 260 vol% × μm) and lesion depth (140 ± 9 μm) were significantly lower as compared to the placebo group (D1), but significantly higher compared to groups D3 and D4 (p < 0.05).

Discussion

In this laboratory study, cerium chloride and its combination with fluoride were able to significantly reduce mineral loss and the progression of lesion depth. However, results must be interpreted with caution as there are some relevant shortcomings.

One potential point of discussion is the choice of a xenogeneic substrate. All enamel samples were prepared from bovine lower incisors. Several other studies concerning artificial caries lesion formation also used bovine enamel [Wiegand et al., 2005; Magalhaes et al., 2009]. The main advantage of using bovine incisors is that it is easier to obtain a sufficient number of sound bovine teeth than human teeth [Oesterle et al., 1998]. Furthermore, bovine teeth, in contrast to human teeth, have less caries and no fluoride application history that might influence artificial caries demineralization or have an influence on the interaction of enamel with fluorides or other chemical substances that are applied. Therefore, optimal standardized comparisons can be made. Finally, it is possible to gain more than one sample from one tooth and therefore to reduce potential differences in baseline properties in different groups.

Another limitation of the present study might be that no remineralization was performed, like storage in artificial or human saliva, to simulate the clinical situation [Lippert and Hara, 2012; Patil et al., 2012]. We assume that using a remineralization solution or human saliva might result in lower values for mineral loss and lesion depth in the same time period, but should not fundamentally change the findings of the present study.

The hypothesis of the present study that the application of cerium chloride would result in equal artificial caries mineral loss and lesion depth progression as the application of amine fluoride solution was confirmed for previously sound samples, but not for pre-demineralized samples. In the latter, the application of cerium chloride and the combination of cerium solution and fluoride solution led to a significantly smaller artificial caries mineral loss and less lesion depth progression. This is consistent with findings by Zhang et al. [1999], who also showed a more favourable protective effect with the combination of cerium solution and fluoride solution in root cementum as compared to the sole use of fluoride solution, although this result was not significant.

Significantly stronger inhibition of demineralization for cerium alone and its combined application compared with the fluoride-only application were only found on pre-demineralized samples and not for the previously sound substrate, which might be attributed to differences in the chemical interaction and an increased uptake of substances into the substrate due to a more porous surface in the pre-demineralized samples. Due to this finding it might be concluded that cerium and its combined application with fluoride are more effective on affected tissues than fluoride only. Also, on previously sound sub-

strate a better protective effect was observed for cerium and its combined application, although these findings were not statistically significant.

In general, the protective effect of cerium against artificial carious mineral loss and lesion depth development/progression can be attributed to changes in the crystal structure of hydroxyapatite and its derivate after cerium application. The atomic radii of calcium and cerium are similar (180 vs. 185 pm, respectively), while the electric charge valence of cerium (1.12 on the Pauling scale is higher than that of calcium, 1.00 on the Pauling scale). Thus a replacement of calcium by cerium in hydroxyapatite is imaginable [Zhang et al., 1999]. This replacement of calcium by cerium in hydroxyapatite has recently been confirmed by EDS analysis and EDS mapping performed by Wegehaupt et al. [2010, 2011]. As the ionic radii and the electric charge valence influence the stability of apatite [Kiss et al., 1990], hydroxyapatite with calcium replaced by cerium has a more stable crystal structure due to the higher electric charge valence of cerium.

Despite the limitations of this study, we conclude that cerium chloride and its combination with fluoride are able to reduce mineral loss and lesion depth development/progression under artificial caries conditions. On both substrates, i.e. sound and pre-demineralized enamel, the protective effect of the combination of cerium solution with fluoride solution was tentatively better than that of cerium solution and fluoride solution alone. On the basis of this finding, further studies investigating different ratios of cerium chloride and fluoride should be performed to optimize the protective effect against artificial caries mineral loss and lesion depth progression/development.

Disclosure Statement

The authors report no conflicts of interest or any source of funding. The authors alone are responsible for the contents and the writing of the paper.

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