Cerium chloride reduces enamel lesion initiation and progression in vitro

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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. © 2013 S. Karger AG, Basel.

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Cerium chloride reduces enamel lesion initiation and progression in vitro

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Running title: Caries protection by cerium

Key words: Enamel, caries, cerium chloride, fluoride

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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 in four pieces, embedded in acrylic resin, ground flat and allocated to eight groups (S1–S4 and D1–D4; n = 20).

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Results: Within groups S1-S4, the statistically highest $\Delta Z$ and ld was observed for group S1 (placebo). Lowest values ($\Delta Z$ and ld) were observed for group S4. The results among groups S2-S4 were not significantly different.

Within groups D1-D4, the statistically highest $\Delta Z$ and ld was observed for group D1 (placebo). Lowest values ($\Delta Z$ and ld) were observed in groups D3 and D4. In group D2 $\Delta Z$ and ld was 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 lesion depth development.
Introduction

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 demineralization of dental hard tissues under cariogenic conditions [Luoma et al., 1989]. However, also negative 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 be observed after the use of fluorides in high concentration [Forsman, 1977]. Therefore, alternative materials for caries prevention are still warranted and open for experimental scrutiny.

Already in 1999, Zhang and co-workers [1999] tested the use of lanthanides solutions and their combinations 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 lanthanides solutions 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; Wegehaupt et al., 2011]. The rare earth elements (lanthanum and cerium) showed a lower toxicity than fluoride and a lower 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 by adding rhBMP-2 [Schmidlin et al., 2012].

To the author’s best knowledge, there has been no study reporting the use of lanthanides 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 diagram 1.

Sample preparation
For this study 160 enamel samples were prepared from forty bovine lower central incisors. The teeth were free of defects and/or cracks when trans-illuminated 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 in 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, 1000, 1200, 2400 and 4000 grit; Water Proof Silicon Carbide Paper, Struers, Erkrath, Germany). During these grinding steps the outermost 200 µm of enamel were removed. This enamel loss was controlled with a micrometer (Mitutoyo, Tokyo, Japan). One piece per tooth 1 – 20 was randomly allocated to one of four groups (sound specimens S1 – S4) and one piece per tooth 21 – 40 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 following the formulation given by Buskes et al. [Buskes et al., 1985]. The solution was renewed every 2 days and kept under constant motion. 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 their treatment.

Preparation of treatment solutions and allocation
The placebo solution for groups S1 and D1 was prepared by admixing 0.10 g sodium benzoate with 99.90 g distilled water. For groups S2 and D2, a commercially available amine (Olaflur and Dectaflur) 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 Cer(III)chlorid, 0.10 g sodium benzoate and 89.90 g distilled water (pH 4.94). For groups S4 and D4 no special solution was prepared. The 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 solution combinations (groups D4 and S4) were then applied on the enamel for 30 s under constant motion. 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 the integrated mineral loss ($\Delta Z$) and lesion depth (ld) a slice was cut perpendicular to the enamel surface of the samples and ground to a thickness of 80–100 µm. The measurement of the integrated mineral loss ($\Delta Z$) and lesion depth (ld) was performed by transversal microradiography (TMR) following a standard protocol [Magalhaes et al., 2009].

Data presentation and statistical analysis

Data were coded in EXCEL and analysed 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 Scheffe’s post hoc tests to compare the mineral loss and lesion depth within the groups S1 – S4 and D1 – D4. Significance level was set at 95%.

Results

Mineral loss and lesion depth in previous sound enamel (S1 – S4)

The integrated mineral loss and lesion depth in groups S1 – S4 are illustrated in Figures 1 and 2, 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 statistically highest mineral loss and lesion depth ($5440 \pm 2027$ vol%µm and $196 \pm 28$ µm).
The lowest integrated mineral loss (3101 ± 1059 vol%µm) and lesion depth (128 ± 21 µm) was observed for samples treated with the combined fluoride/cerium chloride (group S4), which was statistically not different to samples treated with the amine fluoride solution (group S2; 3784 ± 1071 vol%µm and 144 ± 27 µm) and the cerium chloride solution only (group S3; 3409 ± 1337 vol%µm and 134 ± 31 µm).

Mineral loss and lesion depth in previous pre-demineralised enamel (D1 – D4)

The integrated mineral loss and lesion depth in groups D1 – D4 are presented in Figures 3 and 4, respectively. Furthermore, the p-values of the comparisons within groups D1 – D4 are presented in Table 2.

The statistically highest values for mineral loss (ΔZ) and lesion depth (ld) were observed again for the placebo group D1 (4098 ± 346 vol%µm and 181 ± 9 µm, respectively).

The significantly lowest ΔZ and ld was observed for samples treated with the combined fluoride/cerium chloride (D4; 2993 ± 209 vol%µm and 103 ± 9 µm) and cerium chloride (D3; 3068 ± 209 vol%µm and 109 ± 7 µm). Following treatment with amine fluoride (group D2) integrated mineral loss (3384 ± 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 was able to significantly reduce mineral loss and lesion depth development. However, results must be interpreted with caution as there are some relevant shortcomings.

One potential point of discussion is the choice of a xenogenic substrate. All enamel samples were prepared from bovine lower incisors. Different other studies concerning artificial caries lesion formation have also used bovine enamel [Wiegand et al., 2005; Magalhaes et al., 2009]. A 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 or no fluoride application history that might influence the later artificial caries demineralization or have an influence on the interaction of enamel with fluorides or other applied chemical substances. 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 the different groups.

Another limitation of the present study might be that no remineralisation 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 remineralisation solution or human saliva might result in lower values for the mineral loss and the 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 results in equal artificial caries mineral loss and lesion depth progression as the amine fluoride solution, has been proved correct as for previous sound samples, but has to be rejected for pre-demineralized samples. In the latter, application of cerium chloride and the combination cerium solution and fluoride solution led to a significantly lower artificial caries mineral loss and lesion depth progression. This is in accordance to findings by Zhang and co-workers [1999], who also showed a more favourable protective effect with the combination 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 combinatory application compared with 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 the 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 combinatory application with fluoride is more effective on affected tissues than fluoride only. Also, on previous sound substrate a better protective effect was observed for cerium and its combinatory 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 pm vs 185 pm, respectively), while the electric charge valence of cerium (1.12 (Pauling-scale)) is higher than that of calcium (1.00 (Pauling-scale)). Thus a replacement of calcium by cerium in the hydroxyapatite is imaginable [Zhang et al., 1999]. This replacement of calcium by cerium in
hydroxyapatite has been confirmed recently by the 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] the hydroxyapatite with calcium replaced by cerium has a more stable crystal structure due to the bigger electric charge valence of the cerium.

Within the limitation 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 cerium solution with fluoride solution was tentatively better than that of cerium solution and fluoride solution alone. Due to this finding, further studies investigating different ratios of cerium chloride and fluoride should be performed to optimise the protective effect against artificial caries mineral loss and lesion depth progression/development.
References:


Table legends:

Tab. 1: p-values of the comparisons within groups S1 – S4 for mineral loss and lesion depth. Significant differences are marked with *.

Tab. 2: p-values of the comparisons within groups D1 – D4 for mineral loss and lesion depth. Significant differences are marked with *.
<table>
<thead>
<tr>
<th>Comparisons</th>
<th>For mineral loss</th>
<th>For lesion depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 vs. S2</td>
<td>0.0060 *</td>
<td>&lt;0.0001 *</td>
</tr>
<tr>
<td>S1 vs. S3</td>
<td>0.0004 *</td>
<td>&lt;0.0001 *</td>
</tr>
<tr>
<td>S1 vs. S4</td>
<td>&lt;0.0001 *</td>
<td>&lt;0.0001 *</td>
</tr>
<tr>
<td>S2 vs. S3</td>
<td>0.8754</td>
<td>0.7177</td>
</tr>
<tr>
<td>S2 vs. S4</td>
<td>0.5187</td>
<td>0.3406</td>
</tr>
<tr>
<td>S3 vs. S4</td>
<td>0.9261</td>
<td>0.9260</td>
</tr>
</tbody>
</table>

Tab. 1: p-values of the comparisons within groups S1 – S4 for mineral loss and lesion depth. Significant differences are marked with *. 
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

Tab. 2: p-values of the comparisons within groups D1 – D4 for mineral loss and lesion depth. Significant differences are marked with *. 
20 bovine teeth, four samples per tooth (A–D)  
20 bovine teeth, four samples per tooth (E–H)

S1 all samples A  
(n = 20)

S2 all samples B  
(n = 20)

S3 all samples C  
(n = 20)

S4 all samples D  
(n = 20)

D1 all samples E  
(n = 20)

D2 all samples F  
(n = 20)

D3 all samples G  
(n = 20)

D4 all samples H  
(n = 20)

Storage for 7 d in demineralizing buffer solution

Application of respective solutions on the enamel for 30 s

placebo solution

fluoride solution

cerium chloride solution

fluoride + cerium chloride

placebo solution

fluoride solution

cerium chloride solution

fluoride + cerium chloride

Rinsing with distilled water for 30 s

Storage for 14 d in demineralizing buffer solution  
Storage for 7 d in demineralizing buffer solution

Determination of integrated mineral loss (ΔZ) and lesion depth (ld) by transversal microradiography (TMR)

Dia 1.:  
Experimental procedure
Fig 1.: Integrated mineral loss ($\Delta Z$) (mean ± SD) in groups S1–S4 (previous sound samples).

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

Groups not statistically significantly different are marked with identical capital letters.
Fig 3.: Integrated mineral loss (ΔZ) (mean ± SD) in groups D1–D4 (pre-demineralised enamel samples).

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

Fig 4.: Groups not statistically significantly different are marked with identical capital letters.