

Comparison of the Microhardness of Surface Enamel Exposed to Anti-asthmatic Inhalants

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ABSTRACT

Objective: To determine the effect of anti-asthmatic inhalers salbutamol and budesonide on the surface microhardness of bovine tooth enamel.

Materials and methods: The study was experimental, prospective, longitudinal, and comparative. The sample consisted of permanent mandibular incisors, which were prepared in ($n = 90$) blocks of dental enamel of size 3×3 mm and 2 mm thick, separated into 6 groups of 15 specimens each in sterile bottles properly labeled and contained in artificial saliva at 37°C. Three measurements (baseline, 5 days, and 10 days) were performed after immersion to determine the microhardness using a Vickers microdurometer programmed to apply a load of 100 gm for 15 seconds.

Results: It was observed that the enamel surface microhardness decreased after 5 and 10 days, after being in contact with the anti-asthmatic inhalers based on salbutamol and budesonide. In addition, it was evidenced that there is a greater decrease in the superficial microhardness of the enamel when comparing the values at the beginning and after 10 days; likewise, the reduction in the microhardness of enamel exposed to budesonide was greater (120.8 kg/mm²) compared to salbutamol (112.3 kg/mm²) ($p < 0.001$).

Conclusion: The two anti-asthmatic inhalers studied decreased superficial enamel microhardness, with the budesonide-based inhaler having a greater erosive effect.

Clinical significance: This research allowed us to know the values of the microhardness of the superficial enamel after being exposed to different anti-asthmatic inhalers that are indicated in daily clinical practice. Therefore, it is important to evaluate this microhardness since the use of different inhalers is very prevalent.

Keywords: Enamel surface, Inhalers, Microhardness.

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INTRODUCTION

Enamel is the hard tissue that covers the crown of the tooth, it is the hardest biological part of the organism. Therefore, it can resist fracture during masticatory stress. The hardness of the enamel is due to its composition; however, some areas of the enamel may be more prone to penetration.^{1,2}

According to the WHO, asthma affected an estimated 262 million people in 2019. Asthma is a chronic inflammatory disease affecting the airways. Clinically it presents with recurrent episodes of wheezing, coughing, and shortness of breath among other symptoms especially at night and in the morning. Asthma is more likely in patients with a history of allergies and rhinitis (hay fever). Environmental contamination and dust mites are considered as potential factors. In addition, when used in excess, the chemical composition of inhalers could have adverse effects such as dental erosion, changes in pH, and salivary fluid.³⁻⁵

Oral administration of anti-asthmatic drugs requires the need for greater doses which could produce greater adverse effects such as tremors and tachycardia. Therefore, the inhalation route is recommended because it produces fewer side effects, and the onset of action is faster. The use of pressurized aerosol or measuring dose by inhalation is recommended, with the active substance being administered as an aerosol. The maneuver consists of exhaling completely and initiating a slow deep inspiration of a few seconds while the inhaler is discharged. Salbutamol is a medication indicated as a treatment for respiratory tract conditions such as bronchitis. Budesonide is also indicated for respiratory

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distress. Although both drugs do not have the same components, Salbutamol acts as a bronchodilator while Budesonide acts as a corticosteroid.⁶⁻⁹

Direct lesions to the tooth enamel can be caused by a reduction in salivary flow following prolonged use of acidic medications such as anti-asthmatics in the oral cavity, especially considering the frequency of intake (3–4 times a day) and use at night. However, only a few studies on the possible effects of certain medications have been performed to date.^{1,3,5,6}



Fig. 1: Freshly extracted bovine permanent mandibular incisor teeth

Therefore, the aim of this study was to determine the *in vitro* effect of anti-asthmatic inhalers salbutamol and budesonide on the superficial microhardness of tooth enamel.

MATERIALS AND METHODS

Study Design

This study was carried out in the Laboratory of the Faculty of Dentistry of the UNFV in January 2018. The study was experimental *in vitro*, comparative, longitudinal, and prospective. The unit of analysis was formed by bovine mandibular incisors. The sample size was calculated using the means comparison formula using the Stata® V.15.0 software, for which an α of 0.05 and a β of 0.8 were used, determining an ($n = 90$) enamel specimens of dental pieces.

Inclusion criteria were bovine permanent mandibular incisor teeth recently extracted and in good condition. On the other hand, the exclusion criteria were teeth with anomalies in the dental enamel and/or with fractures or dental caries.

The groups were divided into 6 subgroups of 15 specimens each as follows:

Group I: Bovine teeth submitted to Salbutamol, Ventolin Inhal® (Baseline)

Group II: Bovine teeth submitted to Salbutamol, Ventolin Inhal® (Day 5)

Group III: Bovine teeth submitted to Salbutamol, Ventolin Inhal® (Day 10)

Group IV: Bovine teeth submitted to Budesonide, Neumocort (Baseline)

Group V: Bovine teeth submitted to Budesonide, Neumocort (Day 5)

Group VI: Bovine teeth submitted to Budesonide, Neumocort (Day 10)

Preparation of Specimens

Recently extracted bovine permanent mandibular incisors were selected and placed in an isotonic physiological solution to maintain hydration, and then stored at 37°C (Fig. 1). The incisors were cut into 90 blocks of 3 × 3 mm and 2 mm thick tooth enamel using 0.25 μ m diamond disks on the vestibular faces. The transparent acrylic specimens were fabricated using a circumferential mold of 1 cm diameter by 1 cm thickness. The samples were constructed with dimensions of 3 × 3 mm in height and 2 mm in thickness, then these were placed in the solutions to be evaluated for 5 and 10 days, respectively. The samples were only removed to subsequently evaluate the surface microhardness of the enamel after 5 and 10 days (group S: Salbutamol and group B: Budesonide).

Demineralization

Demineralization was performed with a solution based on 50 mL/L of acetic acid, 3 mmol/L of calcium, and 3 mmol/L of phosphate with a pH of 4.5. Then the neutral solution based on calcium and phosphate was applied. Finally, each sample was immersed for 30 minutes twice a day. Between each immersion, each sample was rinsed with distilled water for approximately 2–3 minutes.⁹ This procedure was performed twice daily for 7 days. Then, on the 5th and 10th day, the surface microhardness of the samples was measured again following the same method applied for the initial measurement.

Surface Microhardness

The specimens were separated into groups, each in sterile bottles properly labeled containing artificial saliva and at 37°C. The data were collected before exposing the specimens to the anti-asthmatic inhalers through the Vickers Microhardness test (25 gm load for 10 seconds),⁹ measuring the initial superficial microhardness of the enamel in the laboratory. These dental enamel specimens showed no cracks or fracture lines when viewed under a 400-magnification microscope incorporated into the microdurometer. Three initial measurements were performed to determine the average of the initial microhardness using the laboratory's LG HV-100 microdurometer. Three measurements were made according to ISO 28399-2011 and recorded as values in kg/mm².

Statistical Analysis

The database was developed in Excel, and the statistical analysis of the data was performed with the Stata® V15.0 statistical software. Then to decide the application of the type of statistical test that we will use, the difference in means was contrasted, and the Shapiro–Wilk normality test was applied for each of the 6 groups. Therefore, it was decided to use parametric tests (Student's *t*) for related samples, ANOVA, and Bonferroni as a *post hoc* test, establishing a level of significance of $p < 0.05$.

RESULTS

In (Fig. 2), the tooth enamel exposed to the Salbutamol group in the three stages (baseline, day 5, and day 10) had a greater surface microhardness of 316 ± 41.6 , 223.1 ± 37.5 , and 203 ± 34.4 kg/mm², respectively. However, the enamel microhardness was lower in bovine teeth that were exposed to Budesonide in the three evaluation times (baseline, day 5, and day 10), because they had values of 270 ± 37.2 , 169.7 ± 25.0 , and 149.2 ± 24.8 kg/mm², respectively.

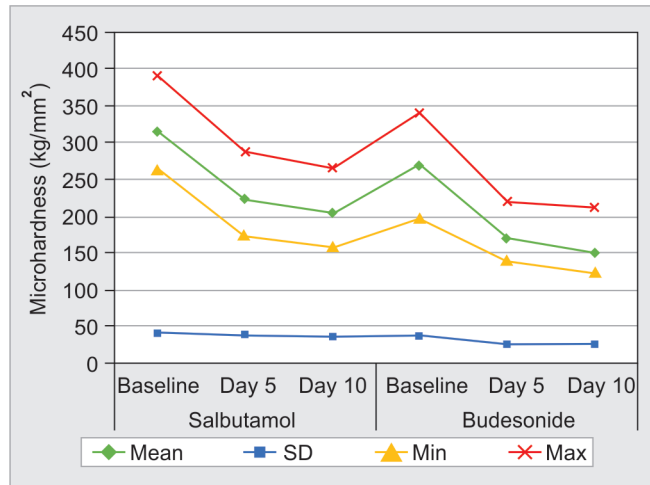


Fig. 2: In vitro comparison of enamel surface microhardness

Table 1: Effect of antiasthmatics on enamel microhardness according to time

Group	Time	Mean	SD	Min	Max	p*	p**	p***
Salbutamol	Baseline	316.0	41.6	264.7	391.2			<0.001
	Day 5	223.1	37.5	172.5	286.9	>0.05	<0.001	<0.001
	Day 10	203.7	34.4	155.2	265			<0.001
Budesonide	Baseline	270.0	37.2	197.0	340.0			<0.001
	Day 5	169.7	25.0	137.7	219.1	>0.05	<0.001	<0.001
	Day 10	149.2	24.8	122.9	211.0			<0.001

Significance level ($p < 0.05$); *Shapiro-Wilk test; all groups presented normal distribution, so it was decided to use parametric tests in inferential statistics; **ANOVA test; ***Bonferroni (*post hoc* test)

When evaluating the normal distribution of the data, it was evident that all groups presented homogeneity because a $p > 0.05$ was obtained. Significant differences were observed on comparing the superficial enamel microhardness at baseline and at days 5 and 10 ($p < 0.001$). Similarly, the teeth exposed to budesonide showed a p -value of < 0.001 for the 3 evaluation times (Table 1).

Finally, the inference of the present study according to the results obtained was that the Bonferroni *post hoc* test showed statistically significant differences on comparing the 6 groups exposed to both salbutamol and budesonide ($p < 0.001$) (Table 1).

DISCUSSION

Usually, oral liquid drugs and aerosols are prescribed more frequently in clinical activity, however, these usually contain certain additives such as sucrose, fructose, and/or glucose, which can exacerbate the bacterial fermentation process by releasing acids and consequently lowering the pH of the dental biofilm, increasing the risk of ionic degradation of hydroxyapatite crystals, thereby increasing the risk of caries.¹⁰⁻¹² The effects of this type of medication are described in some studies in which Salbutamol, which has a pH of 3.6, was evaluated and found to favor enamel dissolution. Likewise, in our study, it was observed that the enamel surface exposed to this drug also presented a decrease in the superficial microhardness of the enamel. This erosive effect found corroborates previous *in vitro* studies, which showed that acidic medications can reduce the microhardness of tooth enamel.^{1,3,6,9,12} This potential erosive effect of salbutamol sulfate can be explained by low pH, presence of citric acid, high titratable

acidity, low-capacity damping, and presence of ethyl alcohol in its formulation.^{1,12}

Because liquid oral medications and chronic diseases are generally recommended, however, they are consumed daily for prolonged periods. Acids are commonly used as buffering agents to maintain chemical stability, and thus improve the presentation of flavor. *In vitro* studies have shown that acidic medications can reduce the hardness of enamel because they influence the roughness of enamel, however, little is known about the effect of oral medications on dental surfaces under erosive conditions.^{3,13,14}

Several studies observed a substantial loss of enamel with the use of certain medications with characteristics that could increase their erosive potential (low endogenous pH and high titratable acidity), probably due to the presence of citric acid.^{4,6} For example, in some studies the immersion of enamel into a neutral solution for 21 hours a day was not enough to avoid demineralization by two 30-minute immersions in the drugs studied. These findings are like those of the study by Costa et al.,⁶ which showed a significant decrease in the primary enamel surface after a pH cycle and 3 minutes of 5 dives in an antihistamine syrup. In addition, nocturnal use of the antihistamine Claritin D[®] showed a significant change in surface microhardness, like that found in the present study and others.^{4,6,15-17}

Bovine enamel specimens were used in the present study because of their similarity to human enamel and easy acquisition. It was found that the group exposed to Salbutamol had a greater microhardness surface at baseline and at days 5 and 10 of 316 ± 41.6 , 223.1 ± 37.5 , and 203 ± 34.4 kg/mm². Similarly, in the study by Scatena et al.¹ microscopy image showed

erosion of enamel surfaces exposed to the drug, as well as a significant increase in roughness and tissue loss and a significant decrease in microhardness ($p = 0.0325$). Lastly, regarding dentin surfaces, salbutamol sulfate was morphologically found to have an erosive effect *in situ* on primary dentin. Our results coincide with those described by Costa et al.⁶ who found that the mean hardness values obtained after the use of this drug were significantly lower than the initial values. On the other hand, Valinoti et al.³ reported that the drug Dimetapp® (*Brompheniramine*) showed the highest amount of erosive patterns, while Klaricid® (*Clarithromycin*) presented *in vitro* protection against acid attacks, perhaps due to its mineral content and viscosity. Furthermore, the study by Farag et al.¹⁰ described an association between the absence of asthma and the severity of dental erosion ($p = 0.03$) compared with non-asthmatic patients. Finally, according to the study by da Costa et al.,⁶ the action of a pediatric drug like budesonide with low pH and high acidity in the primary enamel could induce erosions. In agreement with other studies,^{18–21} our results also showed that anti-asthmatic drugs modify the morphology and/or resistance of the enamel.

The main limitation of this research was the difficulty in reproducing the clinical simulations because the design was experimental *in vitro*;²² however, some situations in the oral cavity involving the chronic use of medications such as antihistamines, which may be highly acidic, can also reduce salivary flow. The type of teeth is also an important limitation since only bovine incisors were used in an *in vitro* study. Another major limitation was that only two types of anti-asthmatic medications were evaluated due to logistical resources, which may leave a certain gap in the understanding of the behavior of tooth enamel with other asthma medications. Nonetheless, this study included two antiasthmatic drugs presenting fewer adverse effects compared to others, and the results described may be useful for both adult and child populations receiving dental care.

Finally, further studies over a longer study period are needed to evaluate the effects of anti-asthmatic inhalers of similar properties to those used in our study on enamel microhardness. Moreover, future *in situ* studies would provide more real-life results considering the biological factors present in the mouth. In addition, mouthwash might be used after the application of the inhaler, to decrease the acidity of the enamel surface and to determine whether this might prevent a significant loss of enamel surface microhardness. This can generate stress in the patients affecting the occlusion.²³

CONCLUSION

In summary, the limitations of this *in vitro* study concluded that both salbutamol and budesonide inhalers at 5 and 10 days of use decreased enamel surface microhardness, however, the budesonide-based inhaler had the greatest erosive effect. Therefore, care must be taken at the time of use, since the erosive effect is directly proportional to the advance of time.

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REFERENCES

- Scatena C, de Mesquita-Guimarães KSF, Galafassi D, et al. Effects of a potentially erosive antiasthmatic medicine on the enamel and dentin of primary teeth: an *in situ* study. *Microsc Res Tech* 2018;81(9): 1077–1083. DOI: 10.1002/jemt.23074.
- Ho S-W, Lue K-H, Ku M-S. Allergic rhinitis, rather than asthma, might be associated with dental caries, periodontitis, and other oral diseases in adults. *PeerJ* 2019;7:e7643. DOI: 10.7717/peerj.7643.
- Valinoti AC, Pierro VSDS, Da Silva EM, et al. *In vitro* alterations in dental enamel exposed to acidic medicines. *Int J Paediatr Dent* 2011;21(2):141–150. DOI: 10.1111/j.1365-263X.2010.01104.x.
- Hatipoğlu Ö, Pertek Hatipoğlu F. Association between asthma and caries-related salivary factors: a meta-analysis. *J Asthma* 2022;59(1):38–53. DOI: 10.1080/02770903.2020.1826045.
- Gomes RNS, Bhattacharjee TT, Carvalho LFCS, et al. ATR-FTIR spectroscopy and μ -EDXRF spectrometry monitoring of enamel erosion caused by medicaments used in the treatment of respiratory diseases. *Microsc Res Tech* 2018;81(2):220–227. DOI: 10.1002/jemt.22970.
- Costa CC, Almeida IC, Costa Filho LC. Erosive effect of an antihistamine-containing syrup on primary enamel and its reduction by fluoride dentifrice. *Int J Paediatr Dent* 2006;16(3):174–180. DOI: 10.1111/j.1365-263X.2006.00713.x.
- Eversole SL, Saunders-Burkhardt K, Faller RV. Erosion prevention potential of an over-the-counter stabilized SnF2 dentifrice compared to 5000 ppm F prescription-strength products. *J Clin Dent* 2015;26(2):44–49. PMID: 26349125.
- Chellai P, Sivasdas G, Chintu S, et al. Effect of anti-asthmatic drugs on dental health: a comparative study. *J Pharm Bioallied Sci* 2016;8 (Suppl 1):S77–S80. DOI: 10.4103/0975-7406.191973.
- Soares DN, Valinoti AC, Pierro VSS, et al. Cross-sectional microhardness of bovine enamel subjected to three paediatric liquid oral medicines: an *in vitro* study. *Eur Arch Paediatr Dent* 2012;13(5):261–265. DOI: 10.1007/BF03262882.
- Farag ZHA, Awooda EM. Dental erosion and dentin hypersensitivity among adult asthmatics and non-asthmatics hospital-based: a preliminary study. *Open Dent J* 2016;10:587–593. DOI: 10.2174/1874210601610010587.
- Arafa A, Aldahlawi S, Fathi A. Assessment of the oral health status of asthmatic children. *Eur J Dent* 2017;11(3):357–363. DOI: 10.4103/ejd.ejd_65_17.
- Scatena C, Galafassi D, Gomes-Silva JM, et al. *In vitro* erosive effect of pediatric medicines on deciduous tooth enamel. *Braz Dent J* 2014;25(1):22–27. DOI: 10.1590/0103-6440201302344.
- Bozejac BV, Stojšin I, Đuric M, et al. Impact of inhalation therapy on the incidence of carious lesions in patients with asthma and COPD. *J Appl Oral Sci* 2017;25(5):506–514. DOI: 10.1590/1678-7757-2016-0147.
- Friesen LR, Bohaty B, Onikul R, et al. Is histologic esophagitis associated with dental erosion: a cross-sectional observational study? *BMC Oral Health* 2017;17(1):116. DOI: 10.1186/s12903-017-0408-z.
- Alexandria AK, Meckelburg Nde A, Puetter UT, et al. Do pediatric medicines induce topographic changes in dental enamel? *Braz Oral Res* 2016;30:S1806.
- Tootla R, Kotru G, Connolly MA, et al. Asthma inhalers and subsurface enamel demineralisation: an *in situ* pilot study. *Eur J Paediatr Dent* 2005;6(3):139–143. PMID: 16216094.
- Godara N, Khullar M, Godara R, et al. Evaluation of cariogenic potential of dry powder inhalers: a case-control study. *Lung India* 2013;30(2):113–116. DOI: 10.4103/0970-2113.110418.
- Boskabady M, Nematollahi H, Boskabady MH. Effect of inhaled medication and inhalation technique on dental caries in asthmatic patients. *Iran Red Crescent Med J* 2012;14(12):816–821. DOI: 10.5812/ircmj.4658.
- Tootla R, Toumba KJ, Duggal MS. An evaluation of the acidogenic potential of asthma inhalers. *Arch Oral Biol* 2004;49(4):275–283. DOI: 10.1016/j.archoralbio.2003.11.006.
- Hagedorn C, Kässner F, Banik N, et al. Influence of salmeterol/fluticasone via single versus separate inhalers on exacerbations

- in severe/very severe COPD. *Respir Med* 2013;107(4):542–549. DOI: 10.1016/j.rmed.2012.12.020.
21. Lussi A, Carvalho TS. Analyses of the erosive effect of dietary substances and medications on deciduous teeth. *PLoS One* 2015;10(12):e0143957. DOI: 10.1371/journal.pone.0143957.
22. Pirca K, Balbín-Sedano G, Romero-Tapia P, et al. Remineralizing effect of casein phosphopeptide-amorphous calcium phosphate and sodium fluoride on artificial tooth enamel erosion: an *in vitro* study. *J Contemp Dent Pract* 2019;20(11):1254–1259. PMID: 31892675.
23. Marín M, Rodríguez Y, Gamboa E, et al. Level of work stress and factors associated with bruxism in the military crew of the Peruvian Air Force. *Med J Armed Forces India* 2019;75(3):297–302. DOI: 10.1016/j.mjafi.2019.01.001.