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New animal model of extrinsic dental erosion-Erosive effect on the mouse molar teeth

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ABSTRACT

Objective: Consumption of acidic food and drinks is considered as important risk factor for development of dental erosion. There are several *in vitro* and *in situ* studies focusing on the risk indicators and preventive treatment, however, the need for a standardized animal model has been emphasised for many years. The aim was to establish an animal model of extrinsic dental erosion, which may serve as a standard for future studies to improve our understanding of the erosion.

Design: Two acidic drinks, sports drink and cola drink, were given to young mice for six weeks. Experimental and control (water) molars and incisors were dissected out and observed by scanning electron microscopy (SEM). Mandibular first molars were subsequently ground transversely and observed again by SEM. The tooth height and enamel thickness were measured on the SEM images.

Results: The lingual surface of the mandibular molars was most eroded after consumption of acidic drinks. The cola drink exhibited higher erosive effect on mandibular molars compared to sports drink. The lingual tooth height, compared to control, was about 34% and 18% lower in the cola drink and sports drink molars, respectively. Compared to the control molars, the lingual enamel was about 23% thinner in the sports drink molars and totally eroded on the certain lingual areas of the cola drink molars.

Conclusions: This new animal model of extrinsic dental erosion and the presented method with ground molars observed in SEM are suitable for further studies, which will gain deeper insights into the erosive disease.

1. Introduction

Several chemical and mechanical impacts contribute to the wear of the dentition throughout life. The manifestation of dental erosion, acid induced dental substance loss, has generally been accepted to be a multifactorial condition caused by various extrinsic and intrinsic acid sources (Lussi & Carvalho, 2014). There are indications that the prevalence of erosive tooth wear is increasing, especially in younger people, partly due to a change in nutritional habits and lifestyle (Jaeggi & Lussi, 2014; Mulic, Vidnes-Kopperud, Skaare, Tveit, & Young, 2012). A recent review and meta-analysis estimated the prevalence among children and adolescents to be on average 30% (Salas, Nascimento, Huysmans, & Demarco, 2015). Extrinsic acids are mainly acidic drinks and food. Therefore, individuals consuming such products frequently are at risk for this type of dental hard tissue destruction. However, clinicians are still observing that dental erosion may occur or be absent regardless of these factors. The reason for that is still elusive, but it has been suggested that the individual`s susceptibility to dental erosion is influenced by genetic variation (Chadwick et al., 2005; Sovik, Skudutyte-Rysstad, Tveit, Sandvik, & Mulic, 2015; Uhlen, Stenhagen et al., 2016), as well as by factors in the oral environment (Chadwick et al., 2005; Uhlen, Mulic, Holme, Tveit, & Stenhagen, 2016).

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Both the salivary flow rate and the composition of saliva are important factors that could have impact on its protective properties, and saliva has been considered as the most important biological factor in the prevention of dental erosion (Buzalaf, Hannas, & Kato, 2012; Hara & Zero, 2014). It has been suggested that the flow rate may be the best clinical indicator of the protective properties of saliva (Tenovuo, 1997). As far as we know, there are only a few studies investigating the association of low salivary flow rate and the occurrence of dental erosions (Aldosari et al., 2018; Jarvinen, Rytomaa, & Heinonen, 1991; Jensdottir, Buchwald, Nauntofte, Hansen, & Bardow, 2013; Johansson, Norring, Unell, & Johansson, 2012; Mulic, Tveit, Songe, Sivertsen, & Skaare, 2012). Furthermore, different fluoride treatments in high concentrations are recommended as part of preventive treatment for individuals with risk for dental erosion. Conventional fluorides offer some, but limited protection against erosion (Magalhaes, Wiegand, Rios, Buzalaf, & Lussi, 2011). Therefore, the interest has grown into fluoride compounds containing polyvalent metal cations such as stannous fluoride (SnF_2) and titanium tetrafluoride (TiF_4). These agents have shown a protective, anti-erosion effect *in situ* (Schlueter, Klimek, & Ganss, 2009; Stenhagen, Hove, Holme, & Tveit, 2013). It has been concluded that tin-containing fluoride product might provide the best protection (Magalhaes et al., 2011).

Although there are a number of *in vitro* and *in situ* studies focusing on the risk indicators and preventive treatment of dental erosion, there are only few studies that have investigated the influence of certain risk indicators related to dental erosion in animal models (Aldosari et al., 2018; Sorvari & Kiviranta, 1988; Sorvari, Kiviranta, & Luoma, 1988; Sorvari, 1989; Sorvari, Pelttari, & Meurman, 1996). Standardized *in vivo* models, which compared to *in vitro* and *in situ* studies without the saliva and soft tissue interactions, are suitable for further studies that may gain deeper insights into the salivary influence on development of dental erosive lesions. An advantage of an animal model, compared with human studies, is that the experimental procedures may be performed under more controlled conditions. Furthermore, human *in vivo* experiments are considered as unethical because of the irreversible loss of dental hard tissues. In the animal models used previously, the methods with limited possibility to study the details did not allow registration of small erosive lesions and their depths (Higo et al., 2009; Sorvari & Kiviranta, 1988).

The need for a standardized animal model for studying dental erosion has been emphasised for many years (Curzon & Hefferren, 2001). A new animal model where dental lesions of different severity can be created and analysed with sensitive methods is therefore warranted. The aim of the present study was to create an animal model of extrinsic dental erosion that will improve our understanding of erosive dental disease and serve as an appropriate model for future studies. For this purpose, experimental dental erosion was induced in mouse, and the erosive effect of products containing both citric (sports drink) and phosphoric (cola drink) acid on their dentition was studied in detail. We hypothesise that acidic drinks induce dental erosion in mouse teeth, and that comprehensive measurements of enamel loss and reduction in the tooth height may be recorded by SEM.

2. Materials and methods

2.1. Animal model

Ninety phenotypical, young female mice (CD-1 strain, 7 weeks old, 30 ± 5 g body wt) were selected for the study. Prior to experimental use, the animals were given standard laboratory fodder and water *ad libitum*, and they were maintained on a 12 h light: dark cycle, at 21 °C with a relative humidity of 65%. The animals were kept in accordance with Norwegian regulation and legislation (Norwegian Regulation on Animal Experimentation of 2015 based on EU directive on the Protection of Animals used for Scientific Purposes 2010/63/EU and Norwegian Animal Welfare Act of 2009). The experiment was approved by Norwegian Food Safety Authority (FOTS ID 12710).

Before the experimental erosive procedures, the wire cages with solid bottom and bedding were prepared in order to reduce the wear of the dentition by attrition. The cages were carefully inspected before the mice were transferred into them. Any hard objects such as wooden sticks and plastic wheels were removed from the cages, and the animals were only supplied with paper boxes and paper ribbons as a part of environment enrichment. All cages were replaced two times per week, and the animals were monitored daily. Moreover, in order to reduce attrition of the teeth during the experiment, the standard laboratory fodder was softened prior to feeding. Fifty pieces of Teklad Global 18% Protein Rodent Diet (Envigo Teklad, Madison, WI, USA) were soaked with 165 ml of cold tap water, sealed in a plastic bag and left for softening overnight.

The mice were randomly distributed into three experimental groups, which were provided with distilled water (control), Red Bull sugar free sports drink (citric acid, pH = 3.39), and Coca Cola drink (phosphoric acid, pH = 2.27), respectively. Each group (n = 30 animals), was further divided into triplicate subgroups, *i.e.* ten animals per cage. Two 250 ml bottles with drinks were placed into each cage, and the bottles were replaced three times per week. Prior to the experiment, the changes in pH of both sports drink and cola drink were monitored over a period of three days, and the results showed no changes in pH. During the experiment, all animals were provided with softened laboratory fodder and drinks *ad libitum*, and the consumption of drinks in each cage was recorded. After the experimental period of six weeks, all animals were sacrificed by cervical dislocation, and their heads were fixed in 70% ethanol. All animals were weighted at the start and at the end of the experiment.

2.2. Scanning electron microscopy

The maxillary and mandibular molars and incisors were dissected out and fixed in 70% ethanol. The isolated teeth were thoroughly cleaned by dissection and by gentle brushing under running tap water. The specimens were air-dried overnight and mounted on brass cylinders with cyanoacrylate glue, sputter-coated with 30 nm platinum and observed in a Philips XL30 ESEM (Philips, FEI, Netherlands) operated at 12 kV.

The jaw segments containing all three molars were thereafter embedded in Epon and ground transversely. The grinding was performed under a stereo-microscope using grits 800 and 1200 3 M waterproof silicone carbide paper (3 M, St. Paul, MN, USA) in a specially designed apparatus (Risnes, 1985). The ground surfaces were then polished by grinding the specimens against the backside of the 3 M waterproof silicone carbide paper with 0.05 µm particle size alumina powder (Buehler Micropolish, Buehler, Lake Bluff, IL, USA) in water. After careful brushing under running tap water and removal of excess water, the teeth were etched for 45 s in 1% nitric acid, air-dried overnight, sputter-coated with 30 nm platinum and observed in scanning electron microscopy (SEM). For the transversely ground molars the whole procedure (grinding, polishing, etching, air-drying, sputter-coating, and observing in SEM) was repeated, creating two transversely ground planes for observation. The first plane (T1) was positioned on the mesial aspect of buccal cusp B2 and lingual cusp L2 where the tip of the cusps exhibited enamel-free areas (Lyngstadaas, Moinichen, & Risnes, 1998). The subsequent plane (T2) was ground further in distal direction ending on the distal aspect of buccal cusp B2 and lingual cusp L2 where the tip of the cusps was covered with enamel. The T1 and T2 planes were positioned in an area where the occurrence of dental erosions on the first molars were noted when the whole teeth were observed in the SEM.

2.3. Measurements and statistical analysis

SEM images of the transversely ground and etched plane T1 were