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Combined effect of amoxicillin and sodium fluoride on the structure of developing mouse enamel *in vitro*

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ARTICLE INFO

Article history:
Accepted 12 March 2013

Keywords:
Enamel
NaF
Amoxicillin
Enamel hypomineralization

ABSTRACT

Objective: Excess fluoride intake during tooth development is known to cause dental fluorosis. It has also been suggested that amoxicillin use in early childhood is associated with enamel hypomineralization. The aim was to investigate separate and combined effects of sodium fluoride (NaF) and amoxicillin on enamel formation *in vitro*.

Design: Mandibular molar tooth germs of E18 mouse embryos were cultured for 10 days in a medium containing NaF (10, 12 or 15 μM) and/or amoxicillin (0.5, 1, 2 or 3.6 mg/mL) or sodium clavulanate (0.07 mg/mL) alone or in combination with 0.5 mg/mL of amoxicillin. Morphological changes were studied from the whole tooth photographs and histological tissue sections with light microscope.

Results: Only with the highest concentrations of NaF or amoxicillin alone the extent of enamel in the first molars measured as the vertical enamel height/crown height ratio was reduced ($p < 0.01$, $p < 0.001$, respectively). At lower concentrations, combination of NaF (12 μM) and amoxicillin (2 mg/mL) significantly reduced enamel extent compared with the controls ($p < 0.001$). Histologically, the ameloblasts were still columnar but poorly organized and the nascent enamel was often non-homogeneous. Enamel formation was not seen in any second molars exposed to 12 μM NaF and 2 mg/mL of amoxicillin (or higher concentrations) compared with the presence of enamel in half of the controls ($p < 0.001$).

Conclusions: Amoxicillin and NaF dose dependently affect developing enamel of mouse molars *in vitro* and the effects are potentiative. The clinical significance of the results remains to be studied.

1. Introduction

Tooth development is regulated by a sequence of inductive and reciprocal interactions between epithelial and mesenchymal cells. The development proceeds through different stages, and can be divided into morphogenesis, dental cell differentiation and secretion, and mineralization of tooth specific matrices.[1] Enamel is formed by epithelial ameloblasts. They undergo three major stages: secretory, transition and maturation stages. At the secretory stage, ameloblasts secrete the enamel matrix. Almost immediately when the enamel matrix

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0003–9969/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.archoralbio.2013.03.007

is laid down a small amount of calcium phosphate crystals is formed. Once the full thickness of enamel has been deposited at a certain site, the secretory ameloblasts transform through a short transitional stage into maturation stage ameloblasts. At this stage, final degradation of enamel matrix proteins takes place and the mineralization is completed.[2,3]

Tooth development is strictly genetically controlled but susceptible to environmental disturbances. Basically, a systemic factor that disturbs the function of ameloblasts during the secretory stage generates abnormally thin, or hypoplastic enamel. Disturbances during the transitional and maturation stages of amelogenesis result in pathologically soft, hypomineralized enamel of normal thickness.

In humans, excessive fluoride ingestion during tooth development can cause enamel fluorosis, leading to increased porosity of mature enamel. Amoxicillin use has been suggested to be associated with Molar-incisor hypomineralization (MIH) and fluorosis-like defects.[4–7] MIH is a condition where the quality of the enamel is impaired. It affects 1–4 of the first permanent molars and commonly also one or several permanent incisors.[8] The prevalence of MIH varies markedly; figures from 2.8 to 44% have been reported.[9]

In experimental studies both fluoride and amoxicillin have been found to disturb dental hard tissue formation. Enamel hypomineralization has been observed in different mouse strains exposed to fluoride.[10,11] Fluoride has also been shown to impair enamel matrix secretion and mineralization, and dentin mineralization *in vitro*.[12] Amoxicillin, in turn, has been found to affect amelogenesis in cultured mouse molars[6] and dentin mineralization in rat incisors *in vivo*.[13] The severity of the defects caused by amoxicillin[6] or fluoride[10] is dose dependent.

In children, the stage of development of the dentition is dependent on the age and therefore, susceptibility of different teeth or tooth groups to developmental disturbances at different times varies. For the most part, the crowns of human permanent incisors and first molars mineralize during the first years of life and therefore they are at greatest risk for developmental enamel defects, such as fluorosis and MIH, during that period.[14,15] Children get low levels of fluoride mainly by the use of fluoride toothpaste and during the first year also from infant formulas diluted to water containing fluoride.[16] In early childhood, a high proportion of children get amoxicillin for the treatment of acute otitis media or other common childhood infections.[17]

The aim of the present study was to investigate experimentally if simultaneous exposure to amoxicillin and NaF has potentiative effects on developing enamel *in vitro*. For this purpose, E18 mouse embryonic teeth were cultured in the presence of each agent alone or together at different concentrations. At the start of culture, the stage of mouse first molar development (E18) approximately corresponds to that of human first permanent molar development slightly before birth. Morphological changes were studied from whole tooth photographs and histological tissue sections with light microscope.

2. Materials and methods

2.1. Animals and teeth

The embryonic age of mice (NMRI × NMRI) was set according to the day of the vaginal plug, which was designated day 0 (E0). The mice were anaesthetized with CO_2 and killed by cervical dislocation on embryonic day 18 (E18). The use of animals has been approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Science of the University of Helsinki.

2.2. Organ culture

Mandibular molar tooth regions from E18 embryos (N = 176) were dissected under a stereomicroscope and transferred to a Trowell type organ culture as described by Thesleff and Sahlberg.[18] The basal medium was Dulbecco's modified Eagle's medium (D-MEM; Gibco BRL, Paisley, Scotland) supplemented with 10% foetal calf serum (Gibco BRL, Paisley, Scotland) and 100 μg/mL of ascorbic acid (Sigma, St. Louis, MO). The culture medium was changed every 2–3 days.

2.3. Exposure of tooth explants to NaF and/or amoxicillin

Stock solutions of amoxicillin (4 mg/mL in DMEM, ICN Biomedicals Inc., Aurora, Ohio) and NaF (4 mM in H_2O) were prepared. Mandibular first and second molar tooth germs were cultured for 10 days with NaF (10, 12 or 15 μM) or amoxicillin (0.5, 1, 2 or 3.6 mg/mL) alone or in combination. Additionally, tooth germs were cultured with sodium clavulanate alone (0.07 mg/mL) or in combination with 0.5 mg/mL of amoxicillin. Sodium clavulanate is commonly used in combination with amoxicillin to overcome resistance to bacterial β-lactamase, which inactivates most β-lactam antibiotics. The concentrations of NaF (12 and 15 μM) had earlier been found to be the lowest with barely detectable effects if any.[19] The concentrations of amoxicillin were virtually the same as in our earlier study.[6] The concentration of sodium clavulanate was defined as 1:7 of the amoxicillin concentration, which is a common proportion. As in the earlier study, no other antibiotic or fungicide was present throughout the experiment. Control teeth were cultured without NaF, amoxicillin or clavulanate. The explants were cultured at 37° C in 5% CO_2 in humidified air for 10 days. A total of 12 experiments with various concentrations of the agents were performed. The experiments included 176 mouse embryos and 348 teeth with 5–11 teeth in each exposure group.

2.4. Examination of enamel formation

After 10 days of culture, the explants were inspected stereomicroscopically and photographed. From the images, the vertical heights of the enamel and the tooth crown were measured on the mesial surface of the first molar by means of analySIS 3.00 (Soft Imaging System GmbH, Münster, Germany). The extent of enamel in the first molars was defined as the ratio of the enamel height to the crown height.