ARCHIVES OF ORAL BIOLOGY 59 (2014) 400–406

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/aob

The effect of high temperature on the development of mouse dental enamel *in vitro*

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ARTICLE INFO

Article history:
Accepted 16 January 2014

Keywords:
Dental enamel
Amelogenesis
Culture temperature
Mice

ABSTRACT

Objective: Our aim was to study the effect of high temperature (39 °C) corresponding to fever on the development of enamel in cultured mouse molars.
Design: For morphological studies mandibular molar blocks from E18 mice were cultured for 11 days. After three days at 37 °C the teeth were exposed to 39 °C for three or five days and returned to 37 °C. At the end of culture, the tooth explants were photographed. The heights of the enamel and the crown of the first molars were measured and the enamel/crown height ratio was calculated. The ratios were compared between the groups using the Mann–Whitney test. To analyze gene expression in ameloblasts and odontoblasts with RT-qPCR and *in situ* hybridization, part of the test explants were cultured for three days at 37 °C and then five days at 39 °C. Control explants were kept at 37 °C for 11 or eight days.
Results: The enamel/crown height ratio of the first molars cultured for five days at 39 °C was smaller than that of the unexposed ($P < 0.001$). Tooth morphology did not differ between controls and exposed teeth. In qPCR-analysis, *dentine sialophosphoprotein* showed clearly decreased expression. *In situ* hybridization showed no dentine sialophosphoprotein expression in preameloblasts. The expressions of bone morphogenic protein 4, *matrix metalloproteinase 20*, *dentine matrix protein 1*, *amelogenin* and *osteocalcin* showed a trend of downregulation.
Conclusions: High culturing temperature interferes with enamel formation of mouse molars and alters the expression of some genes essential for normal enamel development.

1. Introduction

The dental enamel is susceptible to environmental and genetic disturbances that exert their influences during the development of the tooth. Inductive interactions between epithelial and mesenchymal cells initiate the development and differentiation of the dentine forming odontoblasts and the enamel producing ameloblasts. Once the full thickness of enamel has been deposited in a certain area, the secretory ameloblasts transform through a short transitional phase into maturation stage ameloblasts. Shortly after enamel secretion has started, proteinases originating from the ameloblasts begin to degrade enamel proteins as mineralization proceeds throughout the secretory and maturation stages.

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0003–9969/$ – see front matter
http://dx.doi.org/10.1016/j.archoralbio.2014.01.005

The vulnerability of ameloblasts to environmental disturbances is thought to be highest at the transitional and early maturation stages.[1] If the matrix production is disturbed, enamel thickness and/or extent will be reduced, whereas problems of the mineralization may cause qualitative changes, such as hypomineralization. Also the size of teeth can be influenced by environmental poisons and drugs as shown in our previous studies.[2,3]

Fever is known to help recover from disease. However, clinical and experimental studies suggest that fever can impair tooth development. There is evidence that childhood illnesses such as pneumonia and otitis media, which are often accompanied by fever, are risk factors for developmental enamel defects.[4–6] It has been shown that fever affects the development of dentine in the continuously growing incisors of rats[7,8] and recently it was found that fever has an effect on the development of rat incisor enamel.[9] By using an exogenous pyrogen, turpentine, to rise the body temperature, Tung and coworkers detected radiolucency in the part of the enamel that had formed during the fever, suggesting abnormal development and defective mineralization.[9]

It has been found that the expressions of approximately 100 genes are affected by high temperature.[10] About half of them are heat shock proteins whose expression levels increase during heat stress and many of which play important roles in cell physiology. Hyperthermia is also known to act as a teratogen during gestation. Various congenital defects caused by hyperthermia depend on the timing and duration of exposure. The development of the craniofacies and central nervous system is commonly disturbed, leading to clefts and malformed teeth.[11,12]

The aim of this study was to investigate if an elevated temperature (39 °C) corresponding to fever *in vivo* influences the development of 18 days old embryonic mouse molars *in vitro*. In particular, we were interested in whether and how heat stress affects the expression of genes which are essential in tooth development, especially enamel formation. Our null-hypothesis was that a high temperature itself does not interfere with normal tooth development.

2. Materials and methods

2.1. Animals and tooth culture

The use of animals was approved by the Institutional Animal Care and Use Committee of the University of Helsinki. Pregnant mice (NMRI × NMRI) were anesthetized in CO_2 and killed by cervical dislocation on embryonic day 18 (E18) and the embryos were removed.

Culture was set up according to Thesleff et Sahlberg.[13] In short, mandibular molar blocks from E18 embryos were prepared under a microscope in Dulbecco's phosphate buffered saline (D-PBS). The dissected teeth were moved onto polycarbonate nuclepore filters (pore size 0.1 μm; Corning Inc., New York, USA) supported by a stainless steel grid. The incubator was humidified and its CO_2 concentration was 5%. The media were changed every other or every third day and the growth of teeth was monitored.

The culture medium was Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL, Paisley, Scotland) supplemented with 10% foetal calf serum (FCS; Gibco BRL), 100 μg/mL ascorbic acid (Sigma, St. Louis, MO, USA) and 2 IU/mL penicillin–streptomycin.

The dissected teeth to be studied morphologically were divided into three groups. The control teeth were cultured for 11 days at 37 °C. In the exposure groups, the teeth were first cultured for three days at 37 °C. Depending on the group, the teeth were then cultured for either three days at 39 °C and finally five days at 37 °C or for five days at 39 °C and finally three days at 37 °C, to let any morphological changes to become evident. The temperatures and exposure times were chosen according to the *in vivo* study by Tung et al.[9]

For the gene expression studies, the explants were cultured for three days at 37 °C and five days at 39 °C. The experiments were finished after eight days of culture to assay the immediate effects of high temperature without further culturing at 37 °C.

2.2. Experimental material

We dissected a total of 222 mandibular molar blocks. Stereomicroscopic images for the measurements of tooth size and enamel and crown heights were taken from 66 cultured explants. Nine distinct cultures (three for each group) forming three separate experiments were performed. Each culture dish contained from 7 to 10 teeth. Histological sections were made from 30 explants. 156 blocks were cultured for gene expression analysis.

2.3. Preparation of the explants for histological examination and in situ hybridization

After 11 days of culture the teeth were photographed under a stereomicroscope and fixed with 4% paraformaldehyde (PFA; Sigma) in PBS at 4 °C for 24 h. The explants were demineralized in EDTA–PFA-solution (EDTA 0.33 mol/L, 2% PFA) for two weeks, dehydrated through an ascending ethanol series and treated with xylene. Finally the tissues were embedded in paraffin and serially sectioned at 7 μm. For histological examination with a light microscope, sections were deparaffinized, rehydrated, stained with haematoxylin and eosin (HE) and mounted with Mountex (Histolab Products AB, Göteborg, Sweden).

2.4. Measurements of enamel and crown heights

Enamel and crown heights were measured from the stereomicroscopic images and the ratios of enamel height to crown height were calculated. The height measurements were performed from the mesial cusp of the first molar (Fig. 1A). The measurements were made by means of analySIS 3.00 (Soft Imaging System GmbH, Münster, Germany). The differences in enamel/crown height ratios between the exposed groups and the corresponding control group were tested using Mann–Whitney test (SPSS, Chicago, IL, USA). The probability value of <0.05 was considered significant.