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Short Report

Quantitative X-ray microanalytical and histochemical patterns of calcium and phosphorus in enamel in human amelogenesis imperfecta

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ABSTRACT We used scanning electron microscopy (SEM) and quantitative X-ray microanalysis, with the peak-to-local background ratio method and microcrystalline calcium and phosphrous salts as standards, to establish quantitative pattern of biomineralization (Ca/P ratio) in enamel of incisors and molars from patients with a clinical and genetical diagnosis of amelogenesis imperfecta (AI). No significant differences were observed between the Ca/P ratio of AI and normal enamel teeth.

Introduction

The use of scanning electron microscopy (SEM) with electron-probe X-ray microanalysis (EPMA) to examine mineralized tissues is a productive tool for the histochemical study of both morphological features and the chemical elements that take part in the biomineralization process. It is only in recent years that a quantitative approach has been developed to investigate mineralized tissues with the peak-to-local-background (P/B) ratio method, using crystals of inorganic salts as standards. The purpose of this study was to establish the quantitative pattern of biomineralization (Ca/P ratio) using this approach in altered prism enamel of incisors and molars from patients with a clinical and genetic diagnosis of amelogenesis imperfecta (AI).

Materials and Methods

Ten specimens with AI (5 incisors and 5 molars) and ten from normal incisors and molars were immersed in Freon 22-cooled liquid nitrogen. The samples were transferred to a Polaron E 5350 freeze-drying apparatus and dried at –80ºC for 24 h. This was followed by carbon-coating and examination in a Philips XL30 microscope with an EDAX DX-4 microanalytical system. The operating conditions were: operating voltage = 15 kV; spot size= 500 nm; tilt angle = 35º; take-off angle = 61.34º; count rate = 1200 cps; live time = 50 s. Ten analyses were done for each tooth. Spectra were collected by pin-point electron beam at x 40 000. Standards of Ca and P salts were processed in an identical manner and used for quantitative analyses (Campos *et al.*, 1992, Campos *et al.*, 1994; López-Escámez and Campos, 1993, Sánchez-Quevedo *et al.*, 1998). The elemental weight fraction of each salt standard was calculated as reported in previous publications (Warley, 1997). Carbon-coated specimens were gold-coated after EPMA analysis, and were examined in a Philips XL-30 SEM and photographed.

Results

Quantitative histochemical data expressed as weight fraction and determined in enamel with altered prisms (Fig. 1) showed that Ca concentration in teeth with AI was 32.63 ± 3.81 in enamel of incisors and 34.25 ± 4.42 in molars. The concentration of P in teeth with AI was 18.10 ± 3.25 in incisors and 18.63 ± 2.78 in molars. Weight fraction in control incisors was Ca 29.93 ± 1.85 and P 16.28 ± 2.28; the corresponding figures for control molars were Ca 34.04 ± 3.00 y P 18.25 ± 2.19. The Ca/P ratio was 1.80 and 1.84 in incisors and molars respectively in teeth with AI, and 1.84 and 1.86 in control incisors and molars.

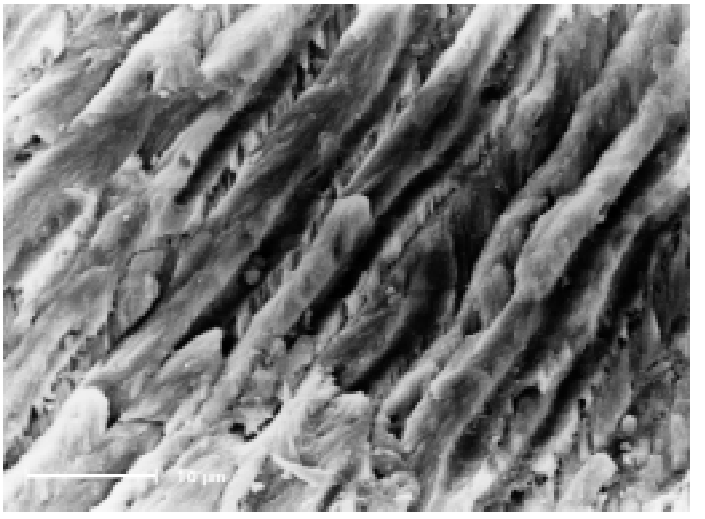

Fig. 1. Scanning electron microscopy. *Enamel with altered prisms.*

Conclusions

Microprobe analysis revealed no significant differences between the Ca/P ratio in the enamel of teeth with AI and normal teeth.

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This results indicate that, as suggested by Wright (1991), AI and normal teeth are probably similar at least in in primary composition (i.e., apatite) regardless of the location of the tooth in the dental arch and regardless of whether structural alterations in the prisms are detected.

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References

CAMPOS, A. LOPEZ-ESCAMEZ, J.A. CAÑIZARES, F.J. and CRESPO, P.V. (1992). Electron probe X-ray microanalysis of Ca and K distributions in the otholithic membrane. *Micron and Microscopica Acta* 23: 349-350.

CAMPOS, A. LOPEZ-ESCAMEZ, J.A. CRESPO, P.V. CAÑIZARES, F.J. and BAEYENS, J.M. (1994). Gentamicin ototoxicity in otoconia: quantitative electron probe X-ray microanalysis. *Acta Otolaryngol. (Stockh.)* 114: 8-23.

LOPEZ-ESCAMEZ, J.A. CRESPO, P.V. CAÑIZARES, F. and CAMPOS, A. (1993). Standards for quantification of element in the otolithic membrane by electron probe x-ray microanalysis: calibration curves and electron beam sensitivity. *J. Micros.* 171: 215-222.

SÁNCHEZ-QUEVEDO, M.C. NIETO-ALBANO, O.H. GARCIA, J.M. GOMEZ DE FERRARIS, M.E. and CAMPOS, A. (1998). Electron probe microanalysis of permanent human enamel and dentine. A methodological and quantitative study. *Histol. Histopathol.* 13: 109-113.

WARLEY, A. (1997). *X-ray microanalysis for biologists.* Portland Press, London.

WRIGHT, J.T. ROBINSON, C. and SHORE, R. (1991). Characterization of the enamel ultrastructure and mineral content in hypoplastic amelogenesis imperfecta. *Oral Surg. Oral Med. Oral Pathol.* 72: 594-601.