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The Effect of X-Ray Irradiation on the Demineralization of Bovine Dental Enamel

A Constant Composition Study

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Key Words. Constant composition · Demineralization · Enamel · Radiation caries · Radiotherapy

Abstract. In this study the effect of X-ray irradiation on demineralization of bovine dental enamel in vitro was investigated. Enamel specimens were irradiated with 72 Gy and subsequently demineralized (140 h) under reproducible constant composition conditions at pH = 5 in the presence of methylhydroxydiphosphonate (MHDP). Microhardness measurements after demineralization without MHDP showed significant differences ($p < 0.001$) between irradiated and nonirradiated enamel specimens; no significant differences were found in the presence of MHDP. Quantitative microradiography showed that both mineral loss and lesion depth were significantly lower ($p < 0.001$) for the irradiated enamel specimens compared with the nonirradiated ones. Surface layer formation was observed in irradiated enamel demineralized in a solution without MHDP. It was shown that X-ray irradiation decreased the enamel acid solubility in vitro.

In patients receiving irradiation therapy for malignancies of the head and neck region a highly destructive form of dental caries is frequently observed [Del Regato, 1939; Frank et al., 1965]. In general a pronounced hyposalivation is considered the most important etiological factor. The reduction in salivary flow results in a loss of the protective properties of saliva, a decrease of the pH of saliva and a quantitative and qualitative shift in oral microflora. This shift in microflora is accompanied by a change in the pattern of food consumption to frequent, nondetergent, high-carbohydrate meals [Del Regato, 1939; Frank et al., 1965; Brown et al., 1975, 1976; Shannon et al., 1977, 1978; Cowman et al., 1983].

X-ray irradiation could also change the enamel susceptibility to acid dissolution by affecting the enamel structure. Results from the literature are contradictory [Poyton, 1968; Jervøe, 1970; Wieman et al., 1972; Walker, 1975; Zach, 1976; Shannon et al., 1978; Joyston-Bechal, 1985]. The reason for the contradictory data may be the variable concentration conditions under which the demineralization studies on irradiated dental enamel were achieved. For this reason these studies are incomparable and nonreproducible.

In this study a constant composition technique [Buskes et al., 1985] was chosen for demineralization of enamel for three main reasons: (1) During artificial lesion formation the composition of the demineralization solution remains constant, providing a reproducible method; (2) the constant composition renders a constant driving force for demineralization, and (3) the liquid flowing across the enamel specimens imitates the constant salivary flow in the oral situation.

For practical reasons the demineralization process was investigated in a solution containing methylhydroxydiphosphonate (MHDP), which inhibited demineralization and induced the formation of subsurface lesions in vitro [Francis, 1969; Featherstone et al., 1978, 1979]. Also demineralization of irradiated enamel in the absence of MHDP was performed to exclude possible effects of MHDP on the surface layer formation [Featherstone et al., 1978].

The aim of this in vitro study was to investigate the effect of X-ray irradiation on demineralization of bovine dental enamel under constant composition conditions.

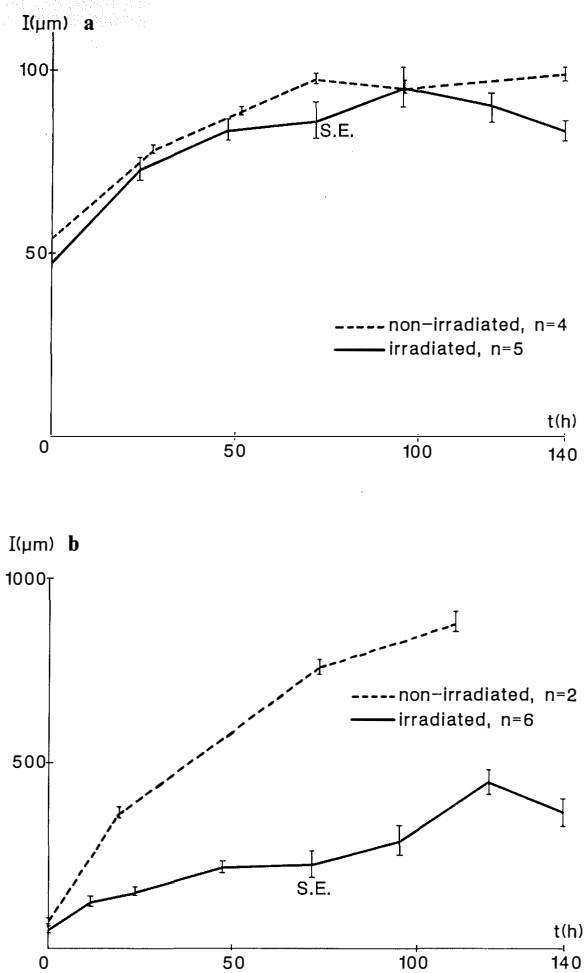

Fig. 1. Microhardness indentation length (I) as a function of demineralization time for the irradiated and control specimens in the presence of 6 μ*M* MHDP **(a)** and without MHDP **(b)**. Each value is the average of n enamel specimens. Ten indentations were made on each specimen. SE = Standard error.

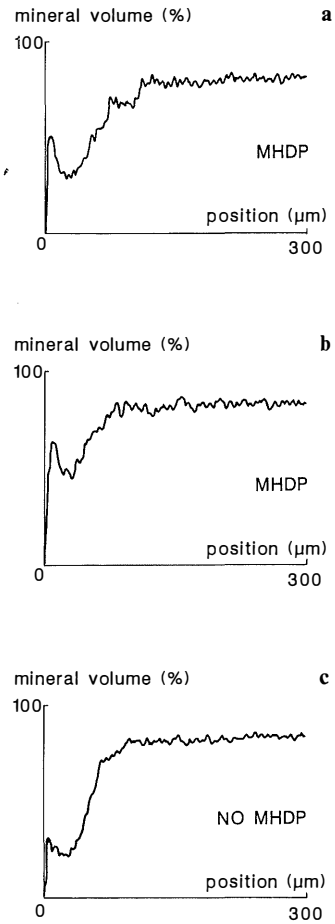

Fig. 2. a Typical microradiogram of a control specimen after demineralization (140 h) in the presence of MHDP (6 μ*M*). **b** Typical microradiogram of an irradiated enamel specimen after demineralization (140 h) in the presence of MHDP (6 μ*M*). **c** Typical microradiogram of an irradiated enamel specimen after demineralization (60 h) without MHDP.

Materials and Methods

Enamel Specimens Preparation

Labial enamel surfaces of freshly extracted mature bovine incisors were partially ground flat (Siawat grid 600) and cut in rectangles by means of a water-cooled diamond saw. After carefully checking for the presence of preparation damage or lesions, the enamel specimens were embedded in polymethylmethacrylate (de Trey) and polished with grinding paper (Siawat grid 800). Subsequently, they were ultrasonically cleaned in tap water for 10 min.

The embedded specimens (n = 56) were divided at random into four groups. Two groups were irradiated, two others served as non-irradiated control groups.

X-Ray Irradiation

To approach oral circumstances during irradiation, the embedded enamel specimens were immerged in an open glass container under 2 cm of water and the irradiation was carried out fractionally. All samples were irradiated twice daily with 2 Gy, during a period of 18 days. The overall dose was 72 Gy (Linac, 8 MeV photon irradiation, source to specimen distance 100 cm; field size 15 × 15 cm).

After irradiation all control and irradiated enamel surfaces were partially covered with nail varnish in order to preserve an internal control area.

Demineralization

The irradiated and control specimens were demineralized as described by Buskes et al. [1985]. Artificial lesions were produced by means of an acidic solution of 10 liters containing 3 m*M* $CaCl_2 \cdot 2H_2O$, 3 m*M* KH_2PO_4 and 50 m*M* CH_3COOH. The solution was buffered by adding 48 ml 10 *M* KOH; the pH of the solution

was 5. A trace of thymol (Reinst) was added as a fungistat. All chemicals used were of pA purity from Merck.

One group of control and one group of irradiated specimens were demineralized in the same acidic solution containing supplementary 6 μ*M* MHDP (Procter & Gamble). The specimens of the other two groups were demineralized in the acid solution without MHDP.

Hardness Measurements

To check changes caused by demineralization in the outer enamel surface, microhardness measurements were performed with a Leitz miniload hardness tester with a Knoop diamond at a load of 50 g. Ten indentations were made perpendicular to the enamel surface (a) before irradiation, (b) immediately after irradiation (t = 0 h) and (c) longitudinally during demineralization (t = 24, 48, 72, 96, 120 and 140 h).

Microradiography

The microradiographic method of de Josseling de Jong and ten Bosch [1985a, b] was applied to determine the type of lesion, its depth and the mineral loss ΔZ. The lesion depth is defined as the distance from the enamel surface to the point where the mineral volume differs more than 5% from that of sound enamel. After 48 and 96 h and at the end of each experiment (140 h) microradiograms were made. For this purpose the enamel specimens were sectioned with a water-cooled diamond saw in two or three slices of 600-μm thickness. The slices were polished on grinding paper (Siawat grid 800) to a thickness of 80 ± 10 μm measured with a micrometer gauge. Microradiograms were made on photographic film (Kodak SO-253) with a Cu-Kα source (Philips X-ray diffractometer PW 1730). After development the film was scanned on a microdensitometer (Leitz MPV) connected to a microcomputer (Apple II^+). Each microradiogram was scanned at three different spots. Two tracings were chosen at random in the demineralized area, one was made in the nondemineralized control area.

X-Ray Diffraction

To investigate possible changes of the crystalline structure of enamel caused by irradiation, an X-ray diffraction study was performed. For this purpose labial enamel of 10 caries-free bovine incisors was pulverized. The powder was divided into two equal portions of which diffractograms were made by using an X-ray diffractometer with filtered Cu-Kα radiation (Philips X-ray diffractometer PW 1730). Subsequently, without changing the orientation of the enamel granules, a single dose irradiation was achieved (Orthovolt, 125 kV, 23 mA, total dose 72 Gy) and the diffraction study repeated.

Scanning Electron Microscopy

From each labial enamel surface of 3 mature bovine incisors, which were prepared as before ('enamel specimens preparation'), two blocks were cut out. From the resulting six blocks, three were irradiated (Orthovolt, 125 kV, 23 mA, total dose 72 Gy), the three others were controls. Of all blocks SEM pictures were taken to determine changes at the crystallite level. For this purpose the enamel specimens were broken and cleaned with freon. Subsequently a thin Au-layer (approximately 12.5 nm) was sputtered on the broken specimens. With a Jeol-35C Scanning Electron Microscope, operated at 25 kV, scanning micrographs were taken at various magnifications.

Table I. Mineral loss and lesion depth of the irradiated and nonirradiated enamel specimens after various demineralization periods (mean ± SD)

Time, h	X-ray	ΔZ, $kg \cdot m^{-2}$	l_d, μm	n
48	+	0.029 ± 0.020	30 ± 15	16
	−	0.047 ± 0.007	42 ± 3	15
96	+	0.050 ± 0.030	53 ± 21	11
	−	-	-	
140	+	0.044 ± 0.030	51 ± 27	25
	−	0.093 ± 0.018	90 ± 15	25

ΔZ = Mineral loss; l_d = lesion depth; + = irradiated; − = nonirradiated. Demineralization was performed in a solution as described under Materials and Methods containing supplementary 6 μ*M* MHDP; n denotes the number of tracings.

Results

In figure 1 the results of the microhardness tests are given. Specimens demineralized in an acid solution containing MHDP showed no significant differences. The microhardness data for irradiated and control enamel specimens demineralized in an acid solution without MHDP were significantly different ($p < 0.001$, t test).

Lesion depth and mineral loss estimated microradiographically after 48, 96 and 140 h demineralization are compiled in table I. For the irradiated specimens compared with the control ones mineral loss and lesion depth after 48 and 140 h were significantly different ($p < 0.001$, t test). After 140 h of demineralization the following relation was calculated:

$$\Delta Z \text{ irradiated enamel} \simeq \tfrac{1}{2} \Delta Z \text{ nonirradiated enamel.}$$

Although less pronounced, a similar relation for the lesion depth was obtained.

Typical microradiograms of irradiated and control specimens after demineralization with or without MHDP are shown in figure 2. After demineralization

of irradiated and control enamel specimens in a demineralization solution without MHDP the created lesions had weakened most specimens in such a way that sectioning for microradiography was impossible without damaging. In all irradiated enamel slices which were not damaged by sectioning a surface layer was observed (fig. 2c). X-ray diffractograms made before and immediately after irradiation were identical. No differences for the characteristic reflections were observed. SEM pictures of irradiated enamel compared with the controls did not show structural differences.

Discussion

Obviously there is much controversy in the literature about the effect of X-rays on the acid solubility of dental enamel. From most studies it is difficult to draw an exact parallel, because the circumstances of demineralization are different. Wiemann et al. [1972], Walker [1975] and Shannon et al. [1978] demineralized in small volumes of liquid so the calcium and phosphate concentrations increased with time. Joyston-Bechal [1985] used an artificial gelatin gel, but gel systems also contain many impurities. The constant composition method used in this study resulted in negligible calcium ($<2.5\%$), phosphate ($<1.5\%$) and pH (<0.04) variations, through which comparison of the demineralization behavior between irradiated and nonirradiated enamel was possible.

From the microradiographic data (type of lesion, mineral loss and lesion depth) it is apparent that the acid solubility of dental enamel is reduced by X-ray irradiation. Considering these data it is remarkable that in case of demineralization of irradiated enamel specimens in a solution without MHDP surface layer formation was observed. To our best knowledge surface layer formation in nonirradiated enamel demineralized under similar conditions has not been published before. Because hardness is a qualitative parameter for the outer enamel region, the presence of this surface layer might also explain the significant differences in indentation length between these two groups of specimens.

The surface layer formation as well as the differences in mineral loss and lesion depth might consequently be caused by irradiation effects. In this study neither differences in X-ray diffractograms nor in SEM pictures could be observed. According to our findings and the results reported by Zach [1976] it is not the inorganic phase which is responsible for the altered behavior of enamel after irradiation at a therapeutic level. However, when increasing the total dose far beyond this level (10,000 Gy) Jervøe [1970] found in an X-ray diffraction study irradiation-induced changes in the crystalline structure of human enamel. A better explanation for the alterations observed at a therapeutic level might be that irradiation of enamel causes changes in the organic matrix resulting in changed diffusion properties. A study concerning the latter is in progress now.

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References

Brown LR, Dreizen S, Handler S, et al: Effect of radiation-induced xerostomia on human oral microflora. J Dent Res 1975;54: 740–750.

Brown LR, Dreizen S, Handler S: Effects of selected caries preventive regimens on microbial changes following irradiation induced xerostomia in cancer patients; in Stiles HM, Loesche WJ, O'Brien TC (eds): Microbial Aspects of Dental Caries. Washington, Information Retrieval, 1976, vol 1.

Buskes JAKM, Christoffersen J, Arends J: Lesion formation and lesion remineralization in enamel under constant composition conditions. Caries Res 1985;19:490–496.

Cowman RA, Baron SS, Glassman, AH, et al: Changes in protein composition of saliva from radiation-induced xerostomia patients and its effect on growth of oral streptococci. J Dent Res 1983;62:336–340.

Del Regato JA: Dental lesions observed after roentgen therapy in cancer of the buccal cavity, pharynx and larynx. Am J Roentgenol 1939;42:404–410.

Featherstone JDB, Duncan JF, Cutress TW: Surface layer phenomena in in vitro early caries-like lesions of human tooth enamel. Arch Oral Biol 1978;23:397–404.

Featherstone JDB, Duncan JF, Cutress TW: A mechanism for dental caries based on enamel processes and diffusion phenomena during in vitro caries simulation on human tooth enamel. Arch Oral Biol 1979;24:101–112.

Francis MD: The inhibition of calcium hydroxyapatite crystal growth by polyphosphonates and polyphosphates. Calc Tiss Res 1969;3:151–162.

Frank RM, Herdly J, Philippe E: Acquired dental defects and salivary gland lesions after irradiation for carcinoma. J Am Dent Assoc 1965;70:868–883.

Jervøe P: X-ray diffraction investigation on the effect of experimental and in situ radiation on mature human teeth. A preliminary report. Acta Odontol Scand 1970;28:623–631.

de Josseling de Jong E, ten Bosch JJ: Error analysis of the microradiographic determination of mineral content in mineralized tissue slices. Phys Med Biol 1985a;30:1067–1075.

de Josseling de Jong E, ten Bosch JJ: Measurement and optimalization of the microradiographic method and its subsystems. SPIE Proc 1985b;492:485–492.

Joyston-Bechal S: The effect of X-radiation on the susceptibility of enamel to an artificial caries-like attack in vitro. J. Dent 1985; 13:41–44.

Poyton HG: The effects of radiation on teeth. Oral Surg 1968;26: 639–646.

Shannon IL, Starcke EN, Wescott WB: Effect of radiotherapy on whole saliva flow. J. Dent Res 1977;56:693.

Shannon IL, Wescott WB, Starcke EN, et al: Laboratory study of cobalt-60-irradiated human dental enamel. J Oral Med 1978; 33:23–27.

Walker R: Direct effect of radiation on the solubility of human teeth in vitro. J Dent Res 1975;54:901.

Wieman MR, Davis MK, Besic FC: Effect of X-radiation on enamel solubility. J Dent Res 1972;51:868.

Zach GA: X-ray diffraction and calcium-phosphorus analysis of irradiated human teeth. J Dent Res 1976;55:907–909.

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