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Effect of X-Ray Irradiation on the Permeability of Bovine Dental Enamel

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Abstract. To estimate permeability properties of bovine dental enamel, complex impedance measurements and radioisotope diffusion experiments were carried out before and after X-ray irradiation (single dose, 72 Gy) of enamel specimens. Neither impedance measurements nor diffusion experiments showed significant changes in permeability.

Introduction

Bovine dental enamel irradiated at a therapeutic level (2-Gy doses, twice daily, to a total of 72 Gy) and subsequently demineralized under constant composition conditions showed a decrease in enamel acid solubility in vitro [Jansma et al., 1988]. Joyston-Bechal [1985] reported similar results after demineralization of enamel under less reproducible circumstances.

The reduced acid solubility of irradiated enamel could be ascribed to possible changes in the inorganic phase or to structural changes of the organic matrix, the latter influencing the permeability of enamel.

Earlier studies, using scanning electron microscopy and X-ray diffraction [Zach, 1976; Jansma et al., 1988], did not show any changes in the crystalline structure after irradiation. In view of the above reports, it was the purpose of this study to investigate possible changes in the permeability of irradiated enamel. Measuring techniques applied are complex impedance measurements and radioisotope diffusion experiments [Borggreven et al., 1977; Scholberg et al., 1984].

Materials and Methods

Preparation of Enamel Membranes

Enamel membranes (200 μm thick) were prepared from mature bovine incisors extracted just before eruption by sawing parallel to the labial surface of the tooth. The first slice (surface layer) was discarded. Only slices without microcracks and inhomogeneities were used for the experiments.

Measurement of Radioisotope Diffusion

Effective diffusion coefficients (D) of radioisotopes were measured according to the method described by Borggreven et al. [1977, 1983]. This method made use of a diffusion cell consisting of two compartments, between which an enamel membrane (membrane area 0.071 cm^2) was mounted (fig. 1). At the start of the experiment one compartment contained [^{3}H]-sorbitol, [^{14}C]-glycerol, $^{36}Cl^-$ and $^{86}Rb^+$ as radiotracers; to the other compartment equivalent amounts of nonradioactive components were added to maintain equal concentrations at both sides of the membrane. Radiotracers which did not show a strong interaction with enamel apatite were chosen, so as to obtain information about the transport of ionic as well as nonionic compounds of different molecular sizes. The concentrations of all compounds were chosen as described by Borggreven et al. [1977]. The solutions used were equilibrated for at least 10 days with powdered enamel at 4°C, before the radiotracers were added. Over a period of 2 weeks, samples were taken from the initially nonradioactive compartment and prepared for counting in a six-channel liquid scintillation spectrometer. The effective diffusion coefficients were calculated from the measured tracer concentrations [Borggreven et al., 1977]. Subsequently, the same enamel membranes were prepared for X-ray irradiation. For this purpose the chambers of the diffusion cell were emptied, and the enamel surface was carefully washed with water and wiped off with cleansing tissue. The diffusion cell was dismantled, with the exception of the core carrying the enamel membrane. After X-ray irradiation the diffusion cell was mounted again, and filled with labeled and nonlabeled solutions as described above, after which the diffusion was measured again over a period of 14 days to determine the effect of irradiation. Diffusion coefficients were also measured during two consecutive periods of 14 days, under similar conditions, but with-

out irradiation between both periods. All diffusion experiments were performed at 4 °C.

Electrical Impedance Measurements

A slice of enamel was mounted in the core of the same diffusion cell for the radioisotope measurements. This core was placed in a container (fig. 2) with approximately 30 ml of measuring solution, in such a way that only the lower surface of the slice was in contact with the measuring solution. This solution consisted of 2 mmol/l HEPES buffer (Merck, Darmstadt, FRG) of pH 7.4, 40 μmol/l Hibitane (ICI, Macclesfield, Cheshire, England) and 50 mmol/l rubidium chloride as an electrolyte. A calomel electrode was placed in this solution. Approximately 1 ml of measuring solution was subsequently put on the upper surface of the slice, and a second calomel electrode was placed in it. Thus the electrodes were connected via the enamel membrane. Impedance measurements were performed 48 and 24 h before X-ray irradiation and 6 h after X-ray irradiation of a specimen. Complex impedance measurements were performed with an Apple II+ microcomputer, which was used to operate a sine-wave function generator (range 0.1 Hz–1 MHz, Krohn & Hite 4141R, Avon, Mass.), an optimal amplifier to enable measurements of high impedances, a vector-impedance meter (range 1 Hz–1 MHz, Hewlett & Packard 3575A), a video-screen and a printer. The R_0 (the real impedance extrapolated to 0 Hz) of a membrane was determined as follows: the total impedance vector and the phase angle were measured at 49 frequencies (8 per decade) between 1 Hz and 1 MHz. These values were used to calculate the real and imaginary part of the impedance at each frequency. The values were plotted in a Cole-Cole plot [Cole and Cole, 1941] in which the real impedance is plotted against the imaginary impedance for each frequency. Using this plot, the R_0 was determined by extrapolation [Scholberg et al., 1987a, b].

X-Ray Irradiation

To simulate oral conditions during irradiation, the cores carrying the enamel membranes were placed in an open glass container, with the enamel membranes under 2 cm of water. The enamel membranes were irradiated in a single dose of 72 Gy (Linac, 6 MeV photon irradiation, source to specimen distance 100 cm, field size 15 × 15 cm). Irradiation was performed at room temperature. During transport to and from the irradiation unit the enamel membranes were kept in a humidified atmosphere.

Mathematical and Statistical Analysis

The mean diffusion coefficient for RbCl was calculated using the equation described by Borggreven et al. [1980b, 1983]:

$$D_{RbCl} = \frac{2\,D_{Rb} \times D_{Cl}}{D_{Rb} + D_{Cl}} \quad (1)$$

The normalized effective diffusion coefficient (D*) after irradiation was calculated as a percentage of the value before irradiation, as follows:

$$D^* = \frac{\text{D after irradiation}}{\text{D before irradiation}} \times 100\% \quad (2)$$

The normalized value of the impedance (R*) was similarly calculated from:

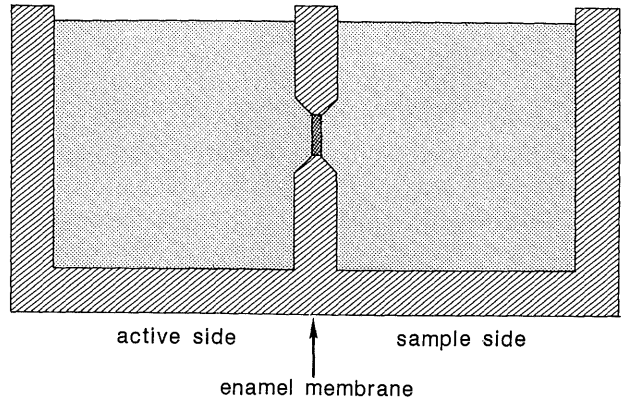

Fig. 1. Schematic drawing of the diffusion cell. Radiotracers were added to the active side at the start of the experiment. Effective diffusion coefficients were calculated from the tracer concentrations in the samples taken from the initially nonradioactive compartment over a period of 2 weeks. The core carrying the enamel membrane was taken out for irradiation.

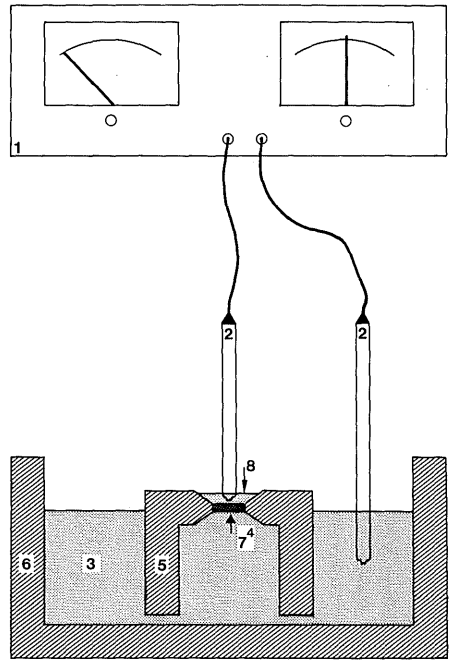

Fig. 2. Schematic representation of the electrical impedance measuring device. 1 = Vector impedance meter; 2 = calomel electrode; 3 = measuring solution; 4 = enamel membrane; 5 = supporting block; 6 = container; 7 = measuring solution in the supporting block which is in direct contact with the bulk measuring solution; 8 = measuring solution on the upper surface of the enamel membrane.

$$R^* = \frac{\text{R after irradiation}}{\text{R before irradiation}} \times 100\% \quad (3)$$

A matched two-tailed t test was used to analyze the changes in D and R.

Table 1. Effective diffusion coefficients (D) of [^{3}H]-sorbitol, [^{14}C]-glycerol, $^{36}Cl^-$, $^{86}Rb^+$ and RbCl for the enamel membranes before (I) and after (II) irradiation (n = 4)

Tracer	Enamel slice	I $cm^2 \cdot s^{-1} \cdot 10^8$	II $cm^2 \cdot s^{-1} \cdot 10^8$	D* (II/I) ×100%	D* %
Sorbitol	1	0.47	0.47	100	
	2	0.58	0.73	126	
	3	0.27	0.45	167	132 ± 28
	4	0.45	0.61	136	
Glycerol	1	0.61	0.63	104	
	2	0.76	0.98	129	
	3	0.38	0.66	174	135 ± 29
	4	0.66	0.89	135	
Cl	1	1.25	1.39	111	
	2	1.56	2.19	140	
	3	1.04	1.85	178	142 ± 27
	4	1.66	2.28	137	
Rb	1	1.79	1.73	97	
	2	2.28	2.58	113	
	3	1.11	1.84	166	127 ± 29
	4	1.84	2.41	131	
RbCl	1	1.47	1.54	105	
	2	1.85	2.37	128	
	3	1.07	1.84	172	135 ± 28
	4	1.74	2.34	134	

For D* mean values ± SD are given.

Table 2. Values of R_0 (kΩ) before (Ia, Ib) and after (II) irradiation of seven different slices of enamel

Enamel slice	Ia, kΩ	Ib, kΩ	II, kΩ	R*, %
1	35	33	38	115
2	95	95	99	104
3	339	328	305	93
4	182	174	183	105
5	136	131	143	109
6	293	284	278	98
7	33	32	33	102
Overall mean				104 ± 7.1

Ia = 48 h before irradiation; Ib = 24 h before irradiation; II = 6 h after irradiation.

Results

Table 1 shows the effective diffusion coefficients before and after irradiation of four different enamel membranes. The increase of the effective diffusion measured after irradiation was not significant ($p > 0.05$). In nonirradiated control membranes (n = 12) D* for sorbitol, glycerol and RbCl was 121 ± 24, 122 ± 18 and 127 ± 27%, respectively ($p > 0.05$).

The results of the impedance measurements on seven different enamel membranes are summarized in table 2. No significant changes in impedance values were observed ($p > 0.05$).

Discussion

The differences in the diffusion coefficients and R_0 of the various enamel slices used in the experiments (tables 1, 2) may be ascribed to biological variation or to heterogeneity of the enamel composition [Bakhos et al., 1976]. Because in our experiments the effects of X-ray irradiation on the enamel slices were compared to the preirradiation values of the same slices, these differences were of no importance.

In most enamel membranes the rate of transport increased after irradiation (table 1). This may be due to some solubilization of the enamel in the transport medium, as indicated by the results of the control experiments. The increase of the diffusion coefficients was about the same for the irradiated and the nonirradiated specimens. Solubilization of enamel may occur even in previously saturated media, because the surface composition and hence the solubility properties are different for each specimen of enamel [Patel and Brown, 1975]. The measured increases of transport are therefore not thought to be caused by irradiation, but by the relatively long stay (2 × 14 days) of the enamel in the transport medium. This is in accordance with the results of the much faster impedance measurements (table 2), in which solubilization effects are of less importance. To limit the possibility of solubilization during irradiation treatment all enamel membranes were irradiated in a single dose.

In the literature we could find no evidence of studies on the effect of X-ray irradiation on the organic matrix of enamel. Some authors suggested that X-ray irradiation may cause denaturation of the organic component of tooth substance which can be followed

by dissolution of the calcified component [Leist, 1925; Lüdin and Müller, 1936; Bianchi, 1943; Poyton, 1968].

Analogous with our findings [Jansma et al., 1988] and those of Joyston-Bechal [1985] after X-ray irradiation, it is known that laser-irradiated dental enamel also produces less subsurface demineralization than enamel not subjected to laser irradiation on exposure to acid [Fowler and Kuroda, 1986]. The laser-induced physical and/or chemical changes that cause this reduced subsurface demineralization are unknown. They are expected, however, to primarily arise from localized heating [Stern et al., 1972; Yamamoto and Sato, 1980; Borggreven et al., 1980a]. Because X-ray irradiation at a therapeutic level does not produce substantial heating of enamel it is difficult to draw any parallel with laser irradiation effects.

Since in the present study no decrease in permeability of enamel was found after X-ray irradiation in two independent experiments it might be that chemical modifications (solubility) rather than physical modifications (permeability) were responsible for the decreased subsurface demineralization rates observed for X-ray-irradiated enamel [Joyston-Bechal, 1985; Jansma et al., 1988]. As mentioned in the introduction neither SEM nor X-ray diffraction brought to light any changes in the crystalline structure of enamel after X-ray irradiation [Jansma et al., 1988]. Similar results were reported by Zach [1976] and Wiemann et al. [1972], who found no chemical or structural changes in enamel subjected to X-ray irradiation treatment using X-ray diffraction and dispersion staining procedures, respectively. Jervøe [1970], however, demonstrated changes in the crystalline structure of enamel with X-ray diffraction, but he irradiated at an extremely high single experimental dose of 10,000 Gy. He concluded that the effect of X-ray irradiation on enamel might not be exclusively a radiation-induced effect in the crystal structure but that it might also be possible that the effect in the crystal is the result of a chemical reaction caused by radiolysis.

Similar radiation-induced effects have been observed in the electron microscopy of octacalcium phosphate [Aoba et al., 1981]. The formation of voids, strain fields and even dislocations has been observed in that structure during radiation damage by the electron beam. That this is a case of radiation damage is clear from the fact that the octacalcium phosphate structure contains relatively loose water molecules and that irradiation took place in vacuum, which makes the structural changes irreversible in that case.

In our present study we irradiated with X-rays under wet conditions and we dealt with a calcium phosphate having an apatitic structure. The apatitic crystals of tooth enamel have incorporated some sodium, carbonate and magnesium by entrapment during their formation [Driessens and Verbeek, 1989]. X-ray irradiation at room temperature will probably mobilize the point defects in this apatite somewhat, whereby entrapped ions can be removed from the surface layer of the crystals (compare the data on irradiation effects for many other ionic compounds as given by Kröger [1964] and Hasiguti [1967]) through the aqueous solution in the pores of the enamel. Therefore, the expected result of irradiation under moist conditions on the inorganic phase of tooth enamel is that the surface layers of the apatite crystals are stabilized and, hence, will develop a decreased rate of dissolution into slightly acidic buffers.

This elucidates the decreased subsurface demineralization of dental enamel after X-ray irradiation somewhat but it seems that further research especially concerning the inorganic phase is needed in order to prove this hypothesis. Solubility experiments and tunneling microscopy studies could be of great value. In view of the high sensitivity reported for the techniques applied in the present study, it may be concluded that X-ray irradiation of dental enamel at a therapeutic level has no influence on its permeability and that it is probably not the organic phase which is responsible for the decreased subsurface demineralization after X-ray irradiation.

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