Maak uw keuze: artikel


Opmerkingen:

Naam: N.E. Geurts-Jaeger voor Jansma
E-mailadres: n.e.geurts-jaeger@umcg.nl
Kostenplaats: 7201
Afdeling: MKA-chirurgie
Telefoon / pieper: 12567
Bent u promovendus? ja
Aanvragen in het buitenland? ja
Akkoord met de voorwaarden? ja

1 6 0 2 2 3 5

6 oktober 2016
Aanvraagnummer:
Aantal pagina's:
Totaal bedrag: €
Leverdatum:
The Effect of X-Ray Irradiation on the Demineralization of Bovine Dental Enamel

A Constant Composition Study


*Department of Oral and Maxillofacial Surgery, and **Laboratory for Materia Technica, University of Groningen; 
*Department of Radiotherapy, University Hospital, Groningen, The Netherlands

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; Jervoe, 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 de­
minalization time for the irradiated and control specimens in the
presence of 6 µM 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.

Materials and Methods

Enamel Specimens Preparation
Labial enamel surfaces of freshly extracted mature bovine inci­
sors were partially ground flat (Siwat grid 600) and cut in rect­
tangles 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). Subse­
quently, they were ultrasonically cleaned in tap water for 10 min.
The embedded specimens (n = 56) were divided at random into
groups. Two groups were irradiated, two others served as non­
irradiated control groups.

X-Ray Irradiation
To approach oral circumstances during irradiation, the em­
bedded enamel specimens were immerged in an open glass con­
tainer under 2 cm of water and the irradiation was carried out frac­
tionally. All samples were irradiated twice daily with 2 Gy, during a
period of 18 days. The overall dose was 72 Gy (Linac, 8 MeV pho­
ton 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 in­
ternal control area.

Demineralization
The irradiated and control specimens were demineralized as de­
scribed by Buskes et al. [1985]. Artificial lesions were produced by
means of an acidic solution of 10 liters containing 3 mM
CaCl₂·2H₂O, 3 mM KH₂PO₄, and 50 mM CH₃COOH. The solution
was buffered by adding 48 ml 10 M KOH; the pH of the solution
to specimens. With a Jeol-35C Scanning Electron Microscope, a thin Au-layer (approximately 12.5 nm) was sputtered on the broken specimens were broken and cleaned with freon. Subsequently a thin Au-layer (approximately 12.5 nm) was sputtered on the broken specimens.

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.

Microhardness 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.

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

<table>
<thead>
<tr>
<th>Time, h</th>
<th>X-ray</th>
<th>ΔZ, kg·m⁻²</th>
<th>l_d, µm</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>+</td>
<td>0.029 ± 0.020</td>
<td>30 ± 15</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>0.047 ± 0.007</td>
<td>42 ± 3</td>
<td>15</td>
</tr>
<tr>
<td>96</td>
<td>+</td>
<td>0.050 ± 0.030</td>
<td>53 ± 21</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>−</td>
<td></td>
<td></td>
</tr>
<tr>
<td>140</td>
<td>+</td>
<td>0.044 ± 0.030</td>
<td>51 ± 27</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>0.093 ± 0.018</td>
<td>90 ± 15</td>
<td>25</td>
</tr>
</tbody>
</table>

ΔZ = Mineral loss; l_d = lesion depth; + = irradiated; − = non-irradiated. Demineralization was performed in a solution as described under Materials and Methods containing supplementary 6 µM MHDPh; 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 MHDPh showed no significant differences. The microhardness data for irradiated and control enamel specimens demineralized in an acid solution without MHDPh 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:

\[ ΔZ _{irradiated \ enamel} = \frac{1}{2} ΔZ _{non-irradiated \ enamel}. \]

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

Typical microradiograms of irradiated and control specimens after demineralization with or without MHDPh are shown in figure 2. After demineralization...
of irradiated and control enamel specimens in a de­
mineralization 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 be­
fore and immediately after irradiation were identical.
No differences for the characteristic reflections were
observed. SEM pictures of irradiated enamel com­
pared with the controls did not show structural differ­
ences.

Discussion

 Obviously there is much controversy in the litera­
ture 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] demineral­
ized in small volumes of liquid so the calcium and
phosphate concentrations increased with time. Joys­
ton-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 sur­
facial layer formation in nonirradiated enamel demin­
eralized under similar conditions has not been pub­
lished before. Because hardness is a qualitative pa­
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 differ­
ences in mineral loss and lesion depth might conse­
quently 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 ther­
apeutic level. However, when increasing the total
dose far beyond this level (10,000 Gy) Jervøe [1970]
found in an X-ray diffraction study irradiation-in­
duced changes in the crystalline structure of human
enamel. A better explanation for the alterations ob­
served at a therapeutic level might be that irradiation
of enamel causes changes in the organic matrix result­
ing in changed diffusion properties. A study concern­
ing the latter is in progress now.

Acknowledgements

 The authors would like to thank Mr. A.A. Canrinus for irradiat­
ing the enamel specimens, Dr. W.L. Jongebloed for performing the
scanning electron microscopy, Prof. Dr. W.G. Perdok for the X-ray
diffraction study and his valuable contribution to the discussion
and Mr. E.G.C. van Ommen for drawing the figures.

This study was supported by the Praeventiefonds (grant
28-1290).

References

xerostomia on human oral microflora. J Dent Res 1975;54:
740–750.
Brown LR, Dreizen S, Handler S: Effects of selected caries preven­
tive regimes on microbial changes following irradiation in­
duced xerostomia in cancer patients; in Stiles HM, Loesche WJ,
O’Brien TC (eds): Microbial Aspects of Dental Caries. Wash­
ington, Information Retrieval, 1976, vol I.
Buskes JAKM, Christoffersen J, Arends J: Lesion formation and
lesion remineralization in enamel under constant composition
composition of saliva from radiation-induced xerostomia pa­
tients and its effect on growth of oral streptococci. J Dent Res
Del Regato JA: Dental lesions observed after roentgen therapy in
ancer of the buccal cavity, pharynx and larynx. Am J Roentge­
Featherstone JDB, Duncan JF, Cutress TW: Surface layer pheno­
mena in in vitro early caries-like lesions of human tooth enamel.
Featherstone JDB, Duncan JF, Cutress TW: A mechanism for den­
tal caries based on enamel processes and diffusion phenomena
during in vitro caries simulation on human tooth enamel. Arch
Francis MD: The inhibition of calcium hydroxyapatite crystal
growth by polyphosphonates and polyphosphates. Calc Tiss


J. Jansma
Department of Oral and Maxillofacial Surgery
University of Groningen
Ant. Deusinglaan 1
NL-9713 AV Groningen (The Netherlands)