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Radiation caries—radiogenic destruction of dental collagen

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Summary Radiogenic dental damage is thought to be the result of reduced salivary flow as well as possible direct radiogenic damage. The exact nature of the latter is still to be elucidated. We set out to assess whether there was measurable direct and immediate radiogenic damage to the collagen component of dental hard and soft tissues.

A total dose of 31.5 Gy was applied to 40 human third molar teeth in vitro (cobalt 60, 6.3 Gy/day for 5 days) (group 1), 40 further third molar non-irradiated human teeth served as controls (group 2). Collagen fragments (split collagen) of mineralized tissue (a) and pulpal tissue (b) of groups 1 and 2 were isolated by ultrafiltration and pooled separately for each experimental group. Measurement of the mature collagen cross-links hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) by high performance liquid chromatography (HPLC) was used to determine the ratio of the amount of collagen fragments from irradiated as opposed to non-irradiated teeth and assessing mineralized from pulpal tissue separately.

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No significant difference was found between the concentration of collagen cross-links in probes of mineralized tissue between groups 1 and 2. The concentration of HP and LP in probes of irradiated dental pulp however was significantly increased (ratio: 3.4 and 3.4 times) as compared to pooled probes from non-irradiated pulp.

Irradiation does not measurably affect the collagen component in mineralized dental tissue, which may be due to the relatively low concentration of this protein in dentin and enamel. In contrast, direct and instant radiogenic damage of (extracellular matrix) pulpal tissue collagen could be demonstrated.

Introduction

Ablative surgery, radiotherapy, chemotherapy and reconstructive surgery are components of the curative or palliative treatment of oral cancer which are intended to improve survival time and quality of life.[1–6] In addition to the desirable anti-cancer effects, ionizing radiation causes damage to normal tissues located within the irradiation fields.[7] Signs and symptoms include mucositis, irreversible hyposalivation due to impaired salivary gland function, osteoradionecrosis and radiation caries.[8,9]

The dental crown is covered by enamel, which is the most mineralized of all mammalian tissue.[10,11] 95% of the total mass of human enamel is considered to be mineralized, organic compounds represent a share of 1%, the rest (4%) is water.[11] Our group has recently been able to show that there is a minor collagen content even in mature dental enamel.[12]

The principal component of the human tooth, however, is dentin. Human dentin is composed of mineral (70%), organic matrix (20%) and water (10%). 90% of the organic matrix component of dentin is believed to be collagen.[13–16]

Dental pulp is located within a central cavity and root canals and represents the vital part of the tooth. On its outer surface, the dental pulp has lining cells (odontoblasts) with processes extending into the dentinal tubules.[10,11] Necrosis and subsequent removal of dental pulp in the course of treatment of deep carious lesions results in a loss of elasticity and an increased risk of dentin fracture.[17,18] While bone is a highly adaptive tissue which constantly undergoes remodelling, dentin and enamel develop their definitive structure in the course of odontogenesis.[19,20]

Hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) are two non-reducible cross-links of mature collagen. HP is present in virtually all mature tissue while LP is found principally in dentin and bone.[13,21–24] HP and LP concentrations can be measured using highly sensitive biochemical methods. After separation of split collagen by ultrafiltration, measurement of HP and LP enables the assessment of differences in the amount of broken collagen between different experimental groups.[13,22,25–27]

On the one hand, irradiation is thought to have a direct destructive effect on dental hard tissue, especially at the dento-enamel junction. On the other hand, irradiation induced hypo-salivation is considered to cause an impairment of oral self-cleansing mechanisms and a decrease in local immune competence, facilitating the action of caries-producing micro-organisms.[28–31] When teeth are located in the irradiation field, hypo-vascularity results in a decrease in the circulation through pulpal tissue. The indirect effect of radiation on vascular flow to the dentition as a whole also plays a role in this multifaceted caries-promoting cycle.[32]

This study was designed to clarify whether there was a direct *and* instant radiogenic effect on the collagenous content/extracellular matrix of dental tissue in addition to the hypo-salivation component.

Materials and methods

Irradiation, preparation and hydrolysis of samples

Eighty extracted caries free permanent human teeth (third molars) from 36 patients (age: 16–63 years) were immediately stored at 4 °C in normal saline solution with 0.5% sodium azide. These teeth were stored in two different vials containing 40 teeth each. The period between storage and irradiation did not exceed six weeks.

We exposed the teeth in group 1 to a total dose of 31.5 Gy using a Cobalt 60 machine and two iso-

centric opposite fields with a single dose of 6.3 Gy/day, five fractions a week (see discussion).

Upon completion of the last dose, periodontal tissue including cementum was completely removed with currettes until exposed dentin was found. Teeth were horizontally separated at the cemento-enamel junction with fine high speed rotating diamond dental burrs (120,000 rpm, water spray coolant at 50 ml/min). Pulpal tissue was removed from the pulp cavity and the root canals in order to pool hard tissue and pulpal probes separately.

63.43 g of non-irradiated dental hard tissue, 54.23 g of irradiated dental hard tissue, 14.50 ml of non-irradiated dental pulp and 14.00 ml of irradiated dental pulp were obtained.

The samples were then taken out of the solution and dried at room temperature for 24 h. Dentin and enamel probes underwent dialysis (dialyzing tube: 12,000–14,000 Da) with a 0.5 M EDTA (pH = 7.6) solution for three weeks in order to remove the inorganic components (demineralization). EDTA was removed by exposing to 0.5% acetic acid for 2, 6 and 12 h. All probes were lyophilized.

Separation of the fraction of (radiogenic) broken collagen

After dialysis, aliquots of the sample volume were transferred to an ultra spin microfilter (Roth, 30,000 MW cut-off, Karlsruhe, Germany) and centrifuged at 4000*g* for 30 min at room temperature in order to obtain the collagen fragments in the ultrafiltrate. Following centrifugation, the ultrafiltrate was removed from the centrifuge and lyophilized.

Analysis of hydroxlysyl- (HP) and lysylpyridinoline (LP)

Samples were hydrolyzed by 1 ml of 6 M HCl at 110 °C for 24 h and centrifuged at 1000 rpm for 5 min. Ten microliter of each hydrolyzate (enamel and dentin) were taken for an analysis of hydroxyproline (see below). One milliliter of each hydrolysate was added to a mixture of 1 ml acetic acid, 2 ml *n*-butan-1-ol and 5 ml 10% CF-1-slurry (fibrous cellulose powder, Whatman, Maidstone, England). A column was prepared by adding the mixture of hydrolysate and CF-1-slurry described above to an econo-column polyprop (40 × 8 mm, Bio-Rad, München, Germany) and the resin was washed three times with 5 ml of the mobile phase. Subsequently, the pyridinium-containing eluate was eluted from the column with 3 × 2 ml distilled water into a 15 ml plastic tube and traces of *n*-butan-1-ol were removed from the surface of the eluate. Thereafter, the lyophylised eluate was again dissolved in 1 ml 0.22% (v/v) *n*-heptafluorobutyric acid (HFBA) and centrifuged at 1000 rpm for 5 min. Two hundred microliter of the sample was analyzed. The hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) content was quantified by reverse-phase high performance liquid chromatography using external standards as previously described.[13,22,27]

The variations within and between series were 2% and 4.8% respectively.

Ethical considerations

The study was conducted in accordance with the standards of the Ethics Committee of the University of Kiel (reference number: AZD 309/00, chairman: Jürgen Schaub, MD, PhD, Professor for Pediatrics, Head of the Department of Pediatrics, University of Kiel, Germany) and with the Helsinki Declaration of 1983. The patients were informed about the aim and design of the study.

Results

No significant difference was found between the concentrations of collagen cross-links HP and LP in probes of mineralized tissue in groups 1 and 2 (group 1: HP 11.70 pmol/ml, LP 2.28 pmol/ml; group 2: HP 11.58 pmol/ml, LP 2.1 pmol/ml; Table 1).

The concentrations of HP and LP in the pooled probe of irradiated pulp were significantly increased (ratio: 3.4 and 3.4 times respectively) when compared to pooled probe of non-irradiated pulp (Fig. 1a and b; Table 1; group 1: HP 63.04 pmol/ml, LP 18.37 pmol/ml; group 2: HP: 18.40 pmol/ml, LP: 5.42 pmol/ml).

Discussion

We set out to assess whether there was a direct and instant radiogenic effect on collagen of mature dental tissue following irradiation. This followed on previous papers in this field.[18,28–30]

Irradiation is thought to have a direct destructive effect on dental tissues and to cause hyposalivation, resulting in an impairment of oral self-cleansing mechanisms and a decrease in the local immune competence.[28–30] This facilitates the action of caries forming microorganisms in the oral cavity, e.g. *Streptococcus mutans*, *Lactobacillus*

Table 1 Concentrations of hydroxlysylpyridinoline (HP) and lysylpyridinoline (LP) indicating amount of radiogenic split collagen

	Irradiation	Unit	HP	LP
Mineralized tissue	Yes			
	Group 1	pmol/g	11.70	2.28
	No			
	Group 2	pmol/g	11.58	2.10
Pulpal tissue	Yes			
	Group 1	pmol/ml	63.04	18.37
	No			
	Group 2	pmol/ml	18.40	5.42

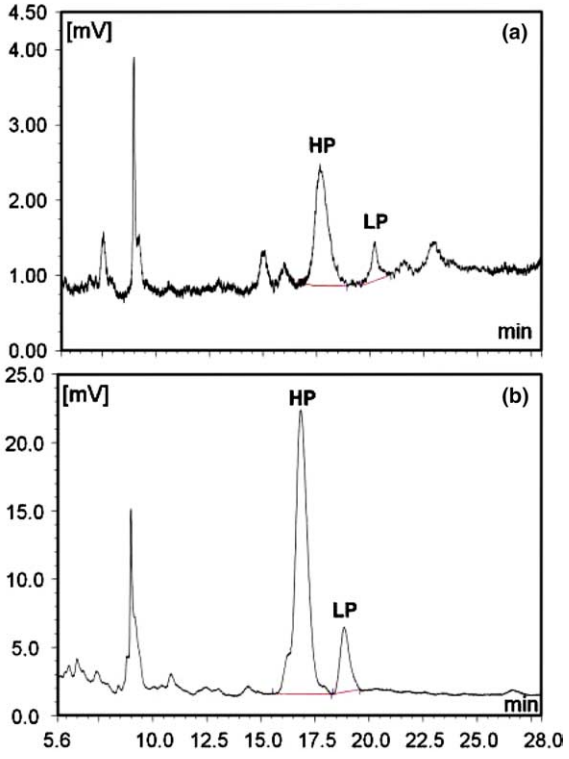

Figure 1 Chromatogram of the pooled non-irradiated dental pulp probe from group 2 (a) and irradiated dental pulp probe from group 1 (b). The fluorescence was monitored with excitation at 297 nm and emission at 397 nm. The HP peak arose at 17.5 min after injection, followed by the LP peak. The area under the peaks corresponds to the concentrations of HP and LP (Table 1). HP and LP in the fraction of broken collagen of the irradiated probe (b) were significantly higher as compared to the non irradiated probe (a). Please note that the peaks appear to be of the same size, but that the scale is different.

and their by-products. *Streptococcus mutans* is recognized as the principal etiological agent of dental caries. The ability to adhere to and form a biofilm on the tooth's surface, to metabolize carbohydrates, and to survive a low pH and other environmental insults is believed to be critical to the cariogenicity of this human pathogen.[31] When vital teeth are located in the field of irradiated tissue, hypovascularity results in a decreased circulation to pulp tissue.[32] Irradiation-induced caries therefore is the result of numerous host and non-host factors.

In the present study, 80 teeth were collected, 40 of which were irradiated in vitro. In vitro assays have previously been employed in order to evaluate the radiogenic effects on teeth and enable the establishment of standardized experimental conditions.[18] For the purposes of the present study, in vitro irradiation was felt to offer the advantage of studying the direct and instant radiogenic effects. By using the linear-quadratic model assuming an alpha/beta value of 3 Gy for the teeth, a total dose of 31.5 Gy and a single dose of 6.5 Gy was calculated and applied to the teeth within the period of 5 days.[33] This schedule is considered to be equivalent to a total dose of 60 Gy delivered in 2 Gy single doses per day, as used in clinical practice.[33]

The measurement of the concentration of the mature collagen cross-links HP and LP by an HPLC method in mineralized dental tissues and comparing this with an external standard is a reliable and sensitive method of obtaining reproducible results independent of the kind of tooth under study (incisor/molar etc.) and age.[12,13,22]

We were able to prove that there is a measurable content of hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) in both dentin *and* enamel, but that the collagenous content in dental enamel is extremely low.[12,13] Probes of isolated collagen fragments of dental hard tissue and dental pulp were pooled for the present study. Pooling was required to ensure a sufficient quantity for the analysis.[12,13]

Direct radiogenic effects on dental tissue have been described previously.[18,29,30] Besides

destruction at the dento-enamel junction, significant micro-morphometric differences in the demineralized nature of irradiated enamel have been shown, suggesting that enamel is less resistant to acid attack after irradiation.[29,30] When comparing the pooled probe of the fraction of collagen fragments of irradiated versus non-irradiated mineralized dental tissue, we detected no differences in the concentrations of HP and LP. This implies that the amount of damaged collagen is equivalent and that direct radiogenic damage of collagen within dentin and enamel does not play a crucial role in the course of the development of irradiation caries. Other authors have found that the mechanical properties of dentin irradiated in vitro were affected only after high experimental dosages of up to 500 Gy, suggesting that direct radiogenic damage without further cofactors such as hyposalivation does not significantly affect mineralized dental hard tissue.[18]

Based on an experimental study involving in vivo irradiation of maxillary arches in monkeys with therapeutic dosages, others have suggested that no microscopic alteration of the dental pulp can be induced by irradiation.[34] Other experimental studies involving in vivo irradiation of different tissues did show impaired vascularization and fibrosis in the long term.[35,36] Dose dependent radiogenic increases in the basement membrane components laminin and collagen IV have been shown to be late radiation effects in laryngo-tracheal specimens from Wistar rats.[37] These changes were proposed to play a role in structural and functional changes.[37]

We found a significant increase of the collagen cross-links HP and LP in dialyzed and ultrafiltrated probes of pulpal tissue of irradiated as compared to non-irradiated teeth, indicating significantly increased amounts of collagen fragments by direct radiogenic destruction. The dental pulp is proposed to play a crucial role in maintaining the odontoblastic metabolism and water balance of dentine.[17] Removal of the pulp and root canal filling is thought to cause an increased fragility of the tooth.[17] The decrease in elasticity of endondontically treated teeth seems to be comparable to that of irradiated teeth.[18] We suggest that radiogenic destruction of collagen within the dental pulp may contribute to secondary fibrosis and decreased vascularity, thereby impairing the odontoblastic metabolism. The obliteration of the dentine tubules, preceded by a degeneration of the odontoblast processes, was found to be the result of direct radiogenic cell damage with hampered vascularization and metabolism particularly in the area of the terminations of the odontoblast processes.[29] The authors used confocal laser scanning microscopy techniques in their study. We were able to show that cellular damage and damage to the extracellular matrix components follow irradiation in clinical doses. Grotz and coworkers suggested that a deficit in metabolism combined with a latent damage of the parenchyma ultimately resulted in functional symptoms such as subsurface caries.[29] Subsurface caries is a main factor contributing to the atypical and comparatively rapid progress of irradiation caries which may not be explained by hyposalivation alone.[38]

Irradiation did not measurably affect the extent of collagen destruction of mineralized dental tissue, which may be related to the relatively low concentration of this protein in dentin and enamel. However, radiogenic destruction of collagen in pulp tissue could be demonstrated. To the best of our knowledge, direct radiogenic alteration in pulpal collagen has not been demonstrated to date. The authors of the present study suggest that radiogenic destruction of collagen in the pulp represents an additional component in direct irradiation-induced damage, and that it contributes synergistically with hypo-salivation to the development of dental caries.

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