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Modulatory effect of fluoride and irradiation on rat molar rate of wear

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SUMMARY

The hypothesis was tested that fluoride (F^-) modulates molar wear rate in the irradiated rat and that enamel solubility and dentin hardness are involved in this process. Seventy five 21 day-old rats were divided into 5 groups. Groups received either F^-(25 ppm) in the drinking water or irradiation to the head (15 Gy in a single dose), or a combination of the two. The rate of occlusal wear was assessed by computerized planimetry. The amount of wear was significantly higher in the F^- and irradiation monotreated rats, while under combined treatment it did nor differ significantly from the control values. Fluoridation or irradiation suppressed enamel solubility, as measured by calcium release in the etchant. Dentin microhardness, expressed in Vickers hardness number, was enhanced after either treatment, but remained unaffected when F^- administration preceded irradiation. Enamel solubility and dentin microhardness did not correlate significantly with the rate of occlusal wear.

KEY WORDS:
Occlusal wear - Irradiation - Fluoride - Rat molars.

RESUME

L'hypothèse vérifiée dans cette recherche a été la modulation par le fluore (F^-) de l'usure des molaires du rat irradié et de l'éventuelle contribution de la solubilité de l'émail et de la dureté dentinaire dans ce processus. Soixante-dix rats agés de 21 jours ont été divisés en 5 groupes. Les groupes ont reçu soit 25ppm F^- dans l'eau de boisson, soit une dose unique d'irradiation (15 Gy) dans la sphère cranio-faciale, soit les deux traitements combinés. Le niveau d'usure occlusale des molaires a été determinée par planimétrie computerisée. La quantité d'usure a été plus prononcée chez les animaux recevant uniquement du F^- ou une irradiation tandis que les deux traitements combinés ont été suivis par des valeurs d'usure semblables a celles mesurées chez les témoins. La solubilité de l'émail, établie selon la quantité de calcium présente dans la solution corrosive et exprimée en mg/l Ca^{++}, a été réduite par chacun des deux traitements. La dureté de la dentine, exprimée en unités Vickers, a été amplifiée par chaque traitement, restant toutefois inchangée uniquement chez les animaux recevant du F^- avant l'irradiation. Les resultats de l'étude corrélative entre l'usure occlusale et la solubilité de l'émail ou la dureté dentinaire n'ont pas atteint des valeurs significatives.

MOTS CLES:
Usure occlusale - Irradiation - Fluor - Molaires de rat.

INTRODUCTION

Occlusal tooth wear, a physiological process that continues throughout life, ensues in irreversible loss of tooth substance. The process is the combined result of attrition, erosion and abrasion (Smith and Knight, 1984), its rate of progress depending on natural or acquired physical properties of the hard dental tissues, dietary constituents, and degree of lubrication of the surfaces in contact (Newman, 1974). Thus, therapies affecting penetration hardness and roughness of the sliding surfaces brought about by acid attack may alter the rate of wear (Rabinowicz, 1965; Smith, 1989). It has been shown in rats that posteruptive chronic ingestion of fluoride (F^-) enhances occlusal molar wear (Markitziu et al, 1985), whereas in another, similar, experimental study, reduced erosion of the lingual molar aspect was noted (Sorvari and Kiviranta, 1988). Chronic topical application of F^- in head and neck irradiated patients is the accepted therapy to prevent radiation caries. However, the effect of this life-long treatment on the phenomenon of tooth wear has never been investigated.

The aim of the present work was to study the occlusal surface wear in irradiated rat molars by planimetric methods. Special attention was paid to comparisons between nonfluoridated irradiated teeth and teeth treated with fluoride prior to and following irradiation. In an attempt to correlate molar rate of wear with eventual altered physico-chemical properties of the irradiated dental tissues, enamel solubility and dentin microhardness were determined.

MATERIALS AND METHODS

Seventy five male rats of the Sabra strain, aged 21 days and weighing about 80 g, were randomly divided into 5 groups and caged by 5. The animals were maintained on a standard animal house diet (Am/Rod 931 Chow, Anbar Ltd., Hadera, Israel, containing about 3 ppm F^-) and water (containing 0.3 ppm F^-) *ad libitum* and were weighed weekly. Treatments consisted of administration of F^-(25 ppm, added to the drinking water) and of a single dose of irradiation (15 Gy Co^{60}) delivered to the head under pentothal sodium (50 mg/kg) anesthesia. Only rats in which all 4 third molars had reached functional occlusion on the day of irradiation were included in the study. The assignments of the experimental groups to the various regimens is detailed in Fig. 1. The non-irradiated animals also underwent anesthesia to ensure comparable experimental conditions.

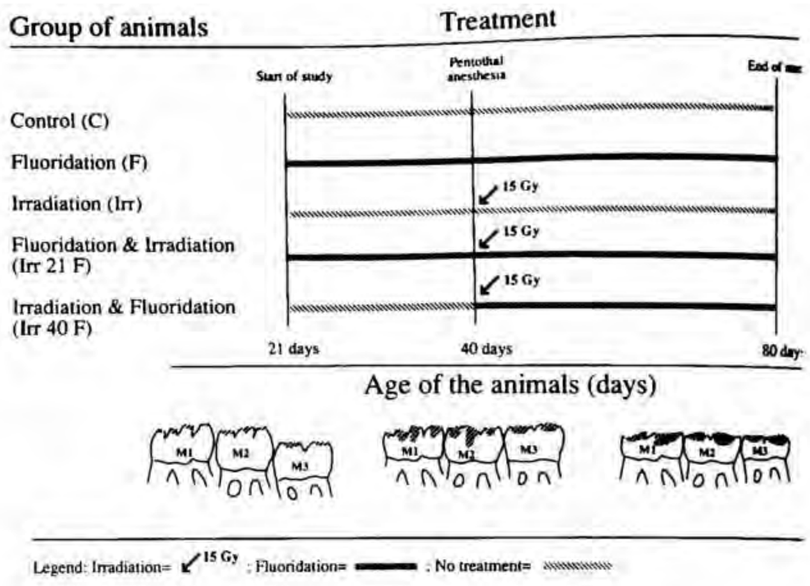

Fig. 1: Assignment of the rats to the five experimental groups and timing of the treatment regimens. Graphic representation of rat molars occlusal facets at the age of 21, 40 and 80 days of life.

Fig. 1 Tableau représentant la division des rats en cinq groupes expérimentaux et le temps d'application des différents régimes thérapeutiques. Schéma représentant les facettes occlusales des molaires du rat à l'âge de 21, 40 et 80 jours.

At 80 days of age all the 69 remaining animals were killed with CO_2. The jaws were dissected, defleshed and the soft tissue remnants were removed by several consecutive washes in 0.1 M $(NH_4)_2\ CO_3$ solution. The segments containing the molars (M1, M2 and M3 were trimmed mesially and distally.

Measurement of occlusal surface wear

The right maxillary and mandibular quadrants were ground apically and parallel to the occlusal surface until mid-root length, mounted on scanning electron microscope (SEM; Jeol 35C, Tokyo, Japan) stubs and gold sputtered to a thickness of 2,000 - 5,000 nm by evaporation in a high vacuum chamber. The specimens were positioned in the SEM at angle permitting maximal view of the occlusal surface, examined at 25 kV and the 3 molars in each specimen were microphotographed (x 4.08) separately. The micrographs were enlarged (x 10.29) and the occlusal surface of each molar was measured in mm2 with the aid of a computer-coupled graphic analyzer (Supergrid, Summagraphics Corp., Fairfield, CT, USA). The computerized integral of the surface was obtained by delineating the outermost limit of the enamel edge of the facet area by means of a manually operated digitizer. The measurements were repeated 3 times by the same operator at 2-week intervals with good reproducibility ($SE < 0.2$ mm²). The values were corrected for the magnifications and the mean value for each molar was utilized for statistical analysis. The sum of the worn areas in the 3 molars was designated total wear area.

Measurement of coronal dentin microhardness

The left mandibular quadrant was embedded in acrylic resin and ground mesiodistally until the mid-sagittal anteroposterior section of the 3 molars was equally exposed. Preparation of the tooth surface for hardness testing was described elsewhere (Markitziu et al., 1986). To avoid drying of the tooth material, the samples were stored on wet cotton in sealed polyethylene containers (100% relative humidity). Vickers hardness numbers were obtained with a Leitz microhardness tester (Wetzlar, Germany), using a 60 g load applied for 15 sec. The length of the square indentations was measured immediately and expressed in Vickers hardness number (VHN) according to the equation. Indentations bordering on the dentino-enamel junction or pulp cavity wall were excluded. The mean of 3 valid indentations per molar was calculated.

Determination of enamel solubility

The left maxillary jaw segment with its 3 molars was prepared under a dissection microscope. For accuracy of exposed enamel sampling, red nail varnish was carefully applied to the dentin on the tip of the molar cusps, to the enamel covered *in vivo* by the gingiva, and to the alveolar bone. The specimens were immersed for 5 sec into consecutive 3-ml aliquots of 0.25 N HCI buffer acetate solution, pH 5.5, being washed with deionized distilled water between immersions. The degree of enamel solubility was determined by analysis of the calcium content (Ca^{++}) in the 3 respective etchants, using an atomic absorption spectrophotometer (Perkin-Elmer Model 303, Perkin-Elmer Corp., Norwalk, CT, USA) at 422.7 nm with suppression by lanthanum chloride. In the present work, the mean of the 3 etchants was used for statistical analysis. The data for the 3 individual etchants are reported elsewhere (Markitziu, in press).

Statistical analysis

To assess the significance of differences between groups, the data were subjected to non-parametric statistics (Mann Whitney U test), a level of $p < 0.025$ being regarded as significant. The Pearson correlation coefficient and simple linear regression, relating wear with either enamel solubility or dentin microhardness, were computed to evaluate the strength of association between these variables.

RESULTS

The mean weight gain ($\Delta \pm SD$) of the control group at the end of the study amounted to 192 ± 17.24, which exceeded that of the F^- treated group (151.33 ± 20.47; $p < 0.001$), of the irradiated group (130 ± 35.89; $p < 0.0001$), of the irradiated group pretreated with F^- (156 ± 23.7; $p < 0.01$), and that of the group receiving F^- following irradiation (124.42 ± 20.23; $p < 0.0001$).

Total wear area (sum of 3 molars) in the F^-treated group and in the irradiated animals was greater than that of the controls. In the irradiated rats pretreated with F^- this value was similar to that of the control group (Table I). The mean values ($\pm SD$) of wear in each molar are presented in Table II.

Dentin microhardness values (mean of 3 molars) were higher in the F^- mono-treated and in the irradiated group than in the controls; the lowest hardness values were measured in the group receiving F^- pre-irradiation (difference not statistically significant compared with the control: Table I). The absolute VHN values in each molar are listed in Table II.

The mean Ca^{++} content in the 3 consecutive etchants was lower in the F^- monotreated and in the irradiated rats than in the controls. In the 2 groups in which F^- was administered, either prior to or following irradiation, mean enamel solubility was similar to that in the F^- mono-treated group, but lower than in the irradiated animals (Table I).

There were no significant correlations between either enamel solubility and occlusal rate of wear of the maxillary molars or between dentin microhardness and occlusal rate of wear of the mandibular molars.

DISCUSSION

The elevated rate of wear in the rat molar following fluoridation as established planimetrically in the present study is consistent with previous findings (Markitziu et al., 1985). The increased rate of occlusal wear measured after irradiation may,

TABLE I. Mean values (±SD) of enamel solubility*, dentine microhardness** and occlusal surface wear***.
TABLEAU I. Valeurs moyennes (±DS) de la solubilité de l'émail, microdureté de la dentine* et usure de la surface occlusale***.*

Animal group	No. of animals	Enamel solubility (mg/L Ca^{++})	Dentine microhardness (VHN•)	Occlusal surface wear mm^2
C	15	60.30 ± 16.8	67.95 ± 2.68	4.93 ± 0.31
F	14	46.91 ± 18.2^a *(U=30)*	78.39 ± 7.17^b *(U=38)*	5.59 ± 0.37^a *(U=33)*
Irr	13	56.47 ± 8.1^b *(U=40)*	70.82 ± 6.14^c *(U=46)*	5.41 ± 0.17^a *(U=31)*
Irr 21 F	14	45.53 ± 16.6^a *(U=35)*	65.35 ± 4.88	4.91 ± 0.46
Irr 40 F	13	46.80 ± 19.18^a *(U=31)*	71.91 ± 8.11	5.25 ± 0.44

Significance of differences between the experimental groups and the control group: a $p<0.001$; b $p<0.004$; c $p<0.01$.
* mean of three etchants; ** mean of 3 molars; *** sum of 3 molars.

• = Vicker's Hardness Number.
(U)=the observed value of *U* in the Mann-Whitney test.

Les différences significatives entre les groupes expérimentaux et le groupe témoin: a $p<0.001$; b $p<0.004$; c $p<0.01$.
** moyenne des trois solutions corrosives; ** moyenne des 3 molaires; *** somme des 3 molaires.*
•=unités de dureté Vicker.
(U)=la valeur obtenue pour "U" du test de Mann-Whitney.

TABLE II. Absolute values (mean±SD) of dentine microhardness (VHN) and occlusal surface wear (mm^2) in the three rat molars.
TABLEAU II. Valeurs absolues (moyenne±DS) de dureté dentinaire et d'usure de la surface occlusale (mm^2) dans chacune des trois molaires du rat.

Animal group	No. of animals	First molar (M_1)		Second molar (M_2)		Third molar (M_3)	
		VHN*	Wear	VHN	Wear	VHN	Wear
C	15	71.91 ± 5.11	2.31 ±0.23	72.37 ± 6.14	1.68 ±0.14	66.59 ± 8.42	0.91 ±0.08
F	14	81.90^c *(U=32)* ± 7.81	2.46 ±0.16	79.58^c *(U=47)* ± 7.76	1.93^b *(U=31)* ±0.19	71.88 ± 9.95	1.21^a *(U=29)* ±0.15
Irr	13	72.91 ± 7.86	2.44 ±0.16	75.53 ± 7.85	1.89^d *(U=35)* ±0.14	66.84 ± 8.05	1.07^d *(U=39)* ±0.10
Irr 21 F	14	62.28^c *(U=31)* ± 6.65	2.28 ±0.18	64.93 ± 6.19	1.61 ±0.18	68.85 ± 6.77	0.97 ±0.19
Irr 40 F	13	73.01 ± 7.36	2.40 ±0.22	75.57 ±10.16	1.79 ±0.16	67.31 ± 8.75	1.05 ±0.14

Significance of differences between the experimental groups and their respective controls: a $p<0.0001$; b $p<0.001$; c $p<0.001$-<0.009; d $p<0.01$; e $p<0.02$.
*=Vicker's Hardness Number.
(U)=the observed value of *U* in the Mann-Whitney test.

Les différences significatives entre les groupes expérimentaux et leurs témoins respectifs: a $p<0.0001$; b $p<0.001$; c $p<0.001$-<0.009; d $p<0.01$; e $p<0.02$.
**=unités de dureté Vicker.*
(U)=la valeur obtenue pour "U" du test de Mann-Whitney.

possibly reflect the decrease in wear resistance of irradiated dentin (Davis, 1975), since in rats the dentin on the tips of the cusps is naturally devoid of enamel (Schour and Massler, 1967).

As the pre-experimental dental age of the animals included in the study was the same, the intergroup comparisons of wear rate were based on the assumption that the occlusal surfaces of the homologous molars, too, were identical.
With respect to the tooth wear measurements, the degree of accuracy attainable by measuring this variable on microphotographs is limited, since tracing of the often ill defined boundaries of the worn area must be left to the subjective decision of the examiner. Nevertheless, the planimetric method provides quantitative measurements to a degree of precision that allows comparison between wear rate of teeth exposed to different environmental variables (Molnar et al., 1983).

Assuming that weight gain reflects masticatory activity, the increased tooth wear seen in all experimental groups can not be attributed to the effect of mastication, since the degree of tooth wear was lowest in the control animals which showed the highest weight gain.

It is tempting to relate the increased wear rate in the irradiated animals to cessation of compensatory eruption (Knychalska-Karawan et al., 1989; Markitziu et al., 1974). However, since irradiation was delivered at the age of 40 days, the finding that M_1 (the molar that reaches occlusal contact at day 25 of life (Kurahashi et al., 1968) was least worn, argues against the interference of therapy with tooth eruption (Molnar et al., 1983). On the other hand, the relative resistance to wear of this particular molar may imply altered dentin apposition (Molnar et al., 1983; Carpenter, 1978) in M_2 and M_3.

According to the chronology of rat molar odontogenesis, fluoridation and irradiation could have affected each of the 3 molars at different developmental stages (Schour and Massler, 1967; Kurahashi et al., 1968). In humans, abnormal abrasion and fracture of fluorotic teeth has been reported to be due to disturbances in amelogenesis (Newbrun, 1960). Since in rats, however, amelogenesis is completed on the 21st day of life in all 3 molars (Schour and Massler, 1967; Kurahashi et al., 1968), therapy-induced amelogenetic defects were precluded in the present study. On the other hand, fluorosis, which may also arise when high amounts of F^- are absorbed after completion of amelogenesis and prior to eruption (Shinoda, 1975; Fejerskov et al., 1974; Weatherell et al., 1977), could have occurred in the non-erupted M_3. This may explain the 33% higher rate of wear assessed in M_3 of the F^- mono-treated animals, compared with the control (Table II).

Since acid attacks causing erosion and reduced hardness of tooth material decrease wear resistance (Newman, 1974; Davis and Winter, 1980; Suckling et al., 1988) it was postulated that the alterations in enamel solubility and dentin microhardness would show a correlation with the rate of tooth wear. However, although irradiation and fluoridation, wether administered combined or separately, did alter enamel solubility and dentin hardness, the trend of these changes did not correlate with the amount of occlusal wear.

The depressed solubility seen after F^- treatments was, of course, to be expected; more surprizing, but still in keeping with previous reports (Joyston-Bechal, 1985; Jansma et al., 1988) was the decrease in enamel solubility measured subsequent to the irradiation.

The enhanced dentin microhardness following either treatment contradicts former assessments *in vitro* (Markitziu et al., 1986). The dichotomy between the *in vitro* and *in vivo* results may hint at a possible contribution of the long term exposure of tooth material to the altered oral microenvironment.

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