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Original Paper

Can α-Tocopherol and β-Carotene Supplementation Reduce Adverse Radiation Effects on Salivary Glands?

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In this study, we evaluated whether supplementation with antioxidant vitamins can reduce the adverse effects of irradiation on the salivary glands in the rat. Four groups of adult Sprague–Dawley rats were given a basic diet providing 0.6 mg α-tocopherol and no β-carotene per day. In two groups the basic diet was supplemented with 3.4 mg α-tocopherol and 6 mg β-carotene per day from 14 days before irradiation until 12 days after completed irradiation. One group of rats given basic diet and one group given supplemented diet were irradiated with 7 Gy daily for five consecutive days. Isoproterenol and pilocarpine-stimulated whole saliva was collected from all rats 2, 4 and 26 weeks after irradiation. Vitamin-supplemented irradiated rats had higher secretion rates on all three occasions compared with those of irradiated rats given basic diet. The changes in saliva composition seen in irradiated rats were less accentuated in vitamin-supplemented, irradiated rats. The proportions of acinar cells were significantly decreased both in parotid and submandibular glands 26 weeks after irradiation. Supplementation with α-tocopherol and β-carotene did not alter the morphology of the glands.

Key words: irradiation, antioxidants, saliva, salivary gland, xerostomia, rat
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INTRODUCTION

CONVENTIONAL RADIOTHERAPY, i.e. 2 Gy per day, used for treating tumours in the head and neck region usually embraces the major and minor salivary glands in the radiation field. It leads to a marked decrease in saliva flow rate and changes in saliva composition within the first week of radiation [1, 2]. Permanent xerostomia occurs in virtually all patients when the salivary glands are irradiated with maximal treatment dose, i.e. >60 Gy [1, 3]. However, at lower total doses, large inter individual variations in impairment in the salivary gland function are reported; the degree of impairment of the saliva flow varies and the reduction may be temporary or persist permanently [1, 2, 4]. This damage is believed to be caused mainly by free radicals formed during irradiation.

Free radicals are highly reactive metabolites formed in normal oxidative processes in a cell [5]. All organisms living in an oxygen environment have, therefore, developed systems protecting them against the consequences of free radical activity. Examples include superoxide dismutase, glutathione peroxidase, glutathione, catalase, uric acid, peroxidases and vitamins [6]. Some of these are predominantly active intracellularly whereas others mainly exist extracellularly. The standard diet may provide antioxidative vitamins and provitamins, such as tocopherols, ascorbic acid and β-carotene [7, 8]. Tocopherols are mainly ascribed a role as breakers of the lipid peroxidation chain [9] whereas β-carotene also quenches singlet oxygen [10].

Irradiation is known to induce increased levels of free radicals which cause damage to most cellular components, such as unsaturated bonds in membrane lipids, sulphur containing enzymes, nucleic acids, carbohydrates and DNA [5]. Since radiotherapy is always accompanied by tissue damage, it is obvious that the naturally occurring free radical scavenger system is not sufficiently active.

The aim of the present study was to investigate whether supplementation with antioxidants such as α-tocopherol in combination with β-carotene can reduce radiation induced effects in normal tissues, i.e. salivary glands.

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MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats, weighing 190–200 g (Möllegaard Breeding Centre Ltd, Ejby, Denmark) were randomised into four groups, each with 10 animals (Table 1). The rats were housed in plastic cages on bedding of wooden chips. The study was approved by the Ethics Committee for Animal Experiments at Umeå University.

Table 1. Details of experimental groups

Diet	Irradiation dose	No. of rats
Basic diet	No	10
Supplemented diet*	No	10
Basic diet	7 Gy × 5 days	10
Supplemented diet	7 Gy × 5 days	10

*Diet supplemented with 3.4 mg α-tocopherol and 6 mg β-carotene per day per rat.

Basic diet

A semisynthetic, powdered basic diet (a modified Diet MIT 200, Table 2) [11] and tap water were given *ad libitum*. The basic diet lacked β-carotene, but contained 1.5 mg retinol and 30 mg α-tocopherol per kg diet. Based on an estimated intake of 20 g per day, the average daily intake from the basic diet corresponded to 30 μg retinol and 600 μg α-tocopherol, an intake considered optimal for adult rats [12].

Supplemented diet

After a 2 week accommodation period, daily supplements of 3.4 mg α-tocopherol (Roche AB, Helsingborg, Sweden) mixed in 100 μl of corn oil and 6 mg starch-coated β-carotene (Dry β-Carotene 10% Water-soluble, gift from Roche AB, Helsingborg, Sweden) mixed in 300 μl of water were given to the rats in two groups (Table 1). The two supplements were administered separately within 5 min by pipetting the emulsions into the back of the mouth. The α-tocopherol and β-carotene supplements started 2 weeks before irradiation exposure begun and lasted until 12 days after the irradiation exposure was completed.

Radiation treatment

Irradiation was given in a field including all salivary glands, but limited to the head and neck region of the rat. Adjacent parts of the body, including the nose, were shielded by 10 cm lead. The irradiated rats were given daily fractions of 7 Gy for five consecutive days (6 MV and a dose rate of 2.19 Gy/min with a focus to skin distance of 100 cm), and the total dose was 35 Gy. The total radiation field, in which two rats were irradiated simultaneously, was 8 × 20 cm. The animals were immobilised during irradiation by intravenous injection of 0.1 ml Brietal (10 mg/ml of the active substance methohexital; Lilly, Indianapolis, Indiana, U.S.A.). The anaesthetised rats were placed in a plastic mould to fix the heads during exposure. The animals were observed via a television camera during radiation exposure. This experimental set-up has been used previously [13, 14], and has been shown to affect both the function and morphology of the salivary glands. To reduce the effect of dehydration, the irradiated rats were given 5 ml subcutaneous injections of Ringer solution with 2.5% glucose (Kabi Pharmacia, Uppsala, Sweden) starting at day 4 of the irradiation period and lasting for 2 weeks. The rats in the two non-irradiated groups were anaesthetised using the same procedure.

Saliva collection and analyses

Saliva was collected at 2, 4 and 26 weeks after completed irradiation. At 26 weeks, a reduced number of rats were used for saliva collection since a number of rats had been used for salivary gland extirpation after 5 weeks. Food, but not drinking water, was removed at 6.00 p.m. the night before saliva collection. Contemporaneously, a 5 ml subcutaneous injection of Ringer solutions with 2.5% glucose (Kabi Pharmacia, Uppsala, Sweden) was given to all rats. Saliva was collected at 9.00 a.m. the following morning. The rats were anaesthetised with 0.02 ml of Hypnorm® (Janssen Pharmaceutica, Beerse, Belgium) containing the active substances fluanisone (10 mg/ml) and fentanyl (0.2 mg/ml) and 0.02 ml of Stesolid® (Dumex A/S, Copenhagen, Denmark) containing 5 mg/ml of the active component diazepam. The Hypnorm® and Stesolid® preparations were given as two separate intramuscular injections in the thigh. During saliva collection, the rats were placed on a table that inclined 10° with the mouth placed over a plastic cup in a way that prevented contamination from nasal secretion and tears. Saliva secretion was stimulated by a subcutaneous injection of a combination of 2.5 mg/kg body weight of pilocarpine (Sigma Chemical Co., St Louis, Missouri, U.S.A.) and 2.5 mg/kg body wieght of isoproterenol (Sigma). Fresh solutions of the sialagogues were mixed every 30 min. Saliva was collected for 15 min. The saliva samples were kept chilled on ice. The volumes needed for the various analyses were pipetted into analytical test tubes and then immediately frozen and stored at −20°C until analysis. After saliva collection, 0.05 ml atropine (Kabi Pharmacia, Helsingborg, Sweden) and 0.5 ml of the Hypnorm® antidote Narcanti® (Du Pont, Stevenage, U.K.) [15], were given to stop the sialagogue activity and enhance recovery from anaesthesia.

Saliva flow rates were determined gravimetrically. In whole saliva, the concentrations of calcium, sodium, potassium and chloride precipitated as silver chloride were determined by atomic absorption (AAS, Varian Techtron AA6, Varian Associates, Instrument Group, Palo Alto, California, U.S.A.). The concentrations of total protein were determined by a Coomassie blue method with bovine serum albumin (Sigma) as a standard

*Table 2. Composition of the basic diet**

	g/100 g diet	Producer
Vitamin free casein	28	Sigma Chemical Co., St Louis, Missouri, U.S.A.
DL-Methionine	0.1	Sigma Chemical Co., St Louiss, Missouri, U.S.A.
Corn oil†	5	CPC Foods, Kristianstad, Sweden
Corn starch	39.3	CPC Foods, Kristianstad, Sweden
Sucrose	19.6	Swedish Sugar Co., Malmö, Sweden
α-Cellulose	4	Sigma Chemical Co., St Louis, Missouri, U.S.A.
Vitamin mix†	1	Ewos, Södertälje, Sweden
Salt mix	3	Ewos, Södertälje, Sweden

*A modified Diet 200 [11]; †Retinol, tocopherol and β-carotene content controlled by HPLC.

[16]. The concentrations of hexosamines were assessed after lyophilisation of the hydrolysate by an Elson–Morgan method modified after Blix [17]. The activities of amylase were measured as the degradation of an insoluble blue-coloured starch polymer (Phadebas® kit, Pharmacia AB, Uppsala, Sweden). Salivary bacteria agglutinating activities (defined by Ericson and associates [18] as BAGP, a glycoprotein with ability to aggregate *Streptococcus mutans*, serotype c (TH16)) were followed spectrophotometrically (Beckman DU50, Beckman Instruments Inc., Glenrothes, U.K.) for 60 min. The activity of salivary peroxidase (SPO) was measured at 400 nm by following the peroxidation of pyrogallol by 1 M H_2O_2 spectrophotometrically [19], and of myeloperoxidase (MPO) by following the oxidation of 5-thio-2-nitrobenzoic acid (Nbs) by OCl^- [20]. Further, the concentrations of albumin were measured by rocket electrophoresis as described by Laurell [21], using rabbit anti-rat albumin as antiserum (Nordic Immunologic Laboratory, Tilburg, The Netherlands) and rat serum albumin (Sigma) as a standard.

Salivary gland extirpation and morphometry

Morphometric determinations in the parotid and submandibular glands were made as described by Franzén and colleagues [13]. The parotid and submandibular glands were extirpated from three or four animals from each group at 5 and 26 weeks, respectively, after complete irradiation. The rats were anaesthetised with 0.5 ml of Hypnorm® (Janssen Pharmaceutica, Beerse, Belgium) and 0.5 ml of Stesolid® (Dumex A/S, Copenhagen, Denmark). Parotid and submandibular glands were removed and carefully freed from adjacent tissues, sliced, and fixed in glutaraldehyde. After rinsing in 0.2 ml/l phosphate buffer (pH 7.4), the salivary gland specimens were postfixed in 1% osmium tetroxide in the same buffer. After a cold buffer rinse, the specimens were dehydrated in graded ethanol solutions and embedded in Epon 812. Semithin (1 μm) sections were stained with toluidine blue, and used for morphometry. An ocular with 10 × 10 grid mesh was used and "hits" in the crossings were counted [22]. One to five hundred hits per section were counted.

Blood collection and tocopherols, carotenoids and retinol analyses

Blood was collected from a tail vein from all rats immediately before radiation exposure. The blood samples were protected from light and air exposure and left at room temperature for 1 h. Serum was prepared by centrifugation at room temperature for 15 min. Aliqouts of serum were then immediately frozen and kept under nitrogen gas at −80°C.

The concentrations of α- and γ-tocopherol, α- and β-carotene and retinol were measured simultaneously using reversed phase high performance liquid chromatography (HPLC) [23]. Throughout all procedures, the samples were protected from light and air. After extraction with 1 ml *n*-hexane (Merck, Darmstadt, Germany), the samples were centrifuged (Beckman J-6B Centrifuge, Galway, Ireland) for 10 min (at 1645 ***g***) and 800 μl of the hexane phase were evaporated to dryness under nitrogen. The samples were then dissolved in ethanol at room temperature for 30 min. A stainless steel column (200 mm × 4.6 mm) with Spherisorb ODS-2, C-18, 5 μm (HPLC Teknik AB, Umeå, Sweden) was used. The mobile phase was 95% methanol and 5% tetrahydrofluran (Merck, Darmstadt, Germany) with the addition of butylated hydrotoluene (Sigma), 0.15 g/l mobile phase. The separation was carried out at 30°C, with a flow rate of 1.5 ml per min (LKB 2150 HPLC Pump, Bromma, Sweden). A variable wavelength UV detector (LKB, Bromma, Sweden) was used to measure the absorbances at 325 nm (retinol), at 292 nm (tocopherols) and at 450 nm (carotenes). Tocol (gift from Roche AB, Helsingborg, Sweden) in ethanol (Merck) was used as an internal standard. Standards from Sigma Chemical Co., St Louis, Missouri, U.S.A. were used for α-tocopherol, γ-tocopherol, α-carotene, β-carotene and retinol. The concentrations of the standards were determined using molar extinction coefficients after the purity of the commercially available standards had been tested by HPLC separation.

Statistical analyses

The Statistical Analysis System (SAS release 6.03, SAS Institute Inc., Cary, North Carolina, U.S.A.) was used for statistical evaluation [24]. Differences between means were tested using unpaired Student's *t*-tests after the null hypothesis that all groups were samples from the same population was rejected by analysis of variance. All tests were two-sided and *P*-values below 0.05 were considered statistically significant.

RESULTS

No rats died during the irradiation treatment or as a result of anaesthesia. Nine died during the experiment; two died shortly after the sialogogues were administered at the first saliva collection (these rats were excluded from the analysis), three more rats died during recovery from anaesthesia at the same collection (these rats were included in the analysis). Three more non-irradiated rats died during recovery from anaesthesia after the second saliva collection and one irradiated rat receiving basic diet died after the third saliva collection.

The average body weights were similar in all four groups at the start of the study (Figure 1). Supplemented diet did not affect the growth of the rats. Irradiated rats began to lose weight at the end of the irradiation period but regained weight after another week. At the end of the experiment (26 weeks after irradiation), the irradiated rats given basic diet were slightly smaller than non-irradiated rats ($P < 0.05$) who received supplemented diet. Figure 1 shows the average body weights for rats participating at all three saliva colletions. A similar pattern was seen for rats participating until salivary gland extirpation 5 weeks after irradiation (data not shown).

In a serum sample collected 5 days before irradiation, rats given supplemented diet had significantly higher levels of α-tocopherol and β-carotene (Table 3), whereas the γ-tocopherol levels were lower and the retinol levels were similar in rats given

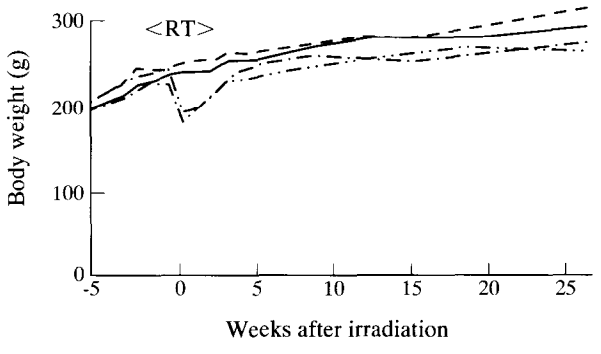

Figure 1. Mean body weights for rats participating at all three saliva collections. – – – non-irradiated, supplemented diet; ——— non-irradiated, basic diet; – · – · irradiated, supplemented diet; and – ·· – irradiated, basic diet.

Table 3. Serum concentrations (mean ± S.D.) of α- and γ-tocopherol, α- and β-carotene, and retinol at the beginning of the irradiation period

	Basic diet		Supplemented diet	
	— (n = 10)	7 Gy × 5 (n = 10)	— (n = 10)	7 Gy × 5 (n = 10)
α-Tocopherol (μmol/l)	23.9 ± 3.50*	23.0 ± 2.50†	41.4 ± 7.9*	42.6 ± 6.2†
γ-Tocopherol (μmol/l)	3.1 ± 0.8‡	3.7 ± 0.8§	0.5 ± 0.2‡	0.3 ± 0.2§
β-Carotene (μmol/l)	0 ± 0‖	0 ± 0¶	0.14 ± 0.17‖	0.14 ± 0.19¶
α-Carotene (μmol/l)	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Retinol (μmol/l)	0.98 ± 0.20	0.86 ± 0.14	0.99 ± 0.17	0.92 ± 0.13

*–¶Groups sharing a common superscript differ at the $P < 0.001$ when tested with an unpaired, two-sided t-test.

supplemented or basic diet. α-Carotene was not detected in any rat.

Salivary albumin concentrations were measured as an indicator of inflammation in the salivary glands, at all three saliva collections (Figure 2). Two weeks after irradiation, there was a substantial increase in albumin concentration in saliva from irradiated animals but in those given supplemented diet this increase was significantly less. Thus, a 30-fold increase in average albumin concentration was observed in the irradiated group given basic diet compared with a 15-fold increase in those irradiated given a supplemented diet. A slight increase in albumin concentration persisted in irradiated rats given basic diet after 4 weeks whereas it was nearly normalised in the irradiated rats on supplemented diet. At 26 weeks albumin concentrations were normalised in all groups.

Irradiation significantly reduced saliva secretion rate in both diet groups (Table 4, Figure 3). However, irradiated rats on supplemented diet had significantly higher flow rates after irradiation than those on basic diet (43.4 ± 17.4 μl/min versus 26.7 ± 7.5 μl/min, $P < 0.05$). At the collection 4 weeks after irradiation, flow rates were still higher in the supplemented diet group than in the basic diet group (41.5 ± 15.8 μl/min versus 29.2 ± 9.6 μl/min) but the group sizes were smaller and the difference was no more statistically significant (data not shown). At the collection 26 weeks after irradiation, saliva secretion rates had decreased further in all irradiated rats, but the decrease was less accentuated in those that received supplemented diet compared with those that received basic diet (analysis only of data from rats which were included in all three saliva collections). The groups in Figures 2 and 3 are, therefore, small. However, since no difference was seen between supplemented and basic diet, non-irradiated groups, these rats were merged to form one control group (n = 7) in Figures 2 and 3.

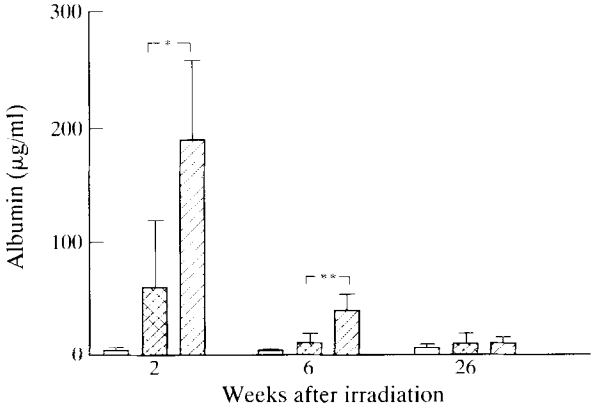

Figure 2. Albumin concentration (mean ± S.D.) in pilocarpine/isoproterenol stimulated saliva collected 2, 4 and 26 weeks after completed irradiation exposure. Only rats participating at all three saliva collections are included. The non-irradiated group includes both rats given basic and supplemented diet. Open box, non-irradiated, both diet groups; crosshatched, irradiated, supplemented diet; hatched, irradiated, basic diet. *$P < 0.05$; **$P < 0.01$, when means were tested with an unpaired, two sided t-test.

Two weeks after irradiation, the concentrations of calcium, sodium, potassium, total protein and hexosamines and the activities of amylase and salivary peroxidase were significantly increased in irradiated rats given basic diet. The effect of irradiation was somewhat less pronounced in those on supplemented diet (Table 4). Thus, in animals given supplemented diet only the sodium concentration and SPO activity were higher and BAGP activity lower in irradiated rats compared to non-irradiated. Apart from the higher flow rate, a lower total protein concentration was the only component differing significantly between the two diet groups which were irradiated.

The effects of irradiation and α-tocopherol and β-carotene supplementation, respectively and in combination, on saliva composition were similar 2 and 4 weeks after radiation exposure (data not shown). 26 weeks after irradiation, saliva composition was further affected in all irradiated rats, but the effects were less pronounced in those receiving supplemented diet (Figure 3).

The proportions of acinar and duct cells and stroma in parotid and submandibular glands were determined 5 and 26 weeks after irradiation. No obvious morphometric differences were seen between the non-irradiated basic diet group and any of the other three groups after 5 weeks. After 26 weeks, the proportions of acinar cells were significantly decreased both in irradiated parotid and submandibular glands, and the proportions of duct cells and stroma were increased in irradiated parotid glands. Supplementation with α-tocopherol and β-carotene did not alter the morphology of the glands. Data presented in Table 5 were, therefore, merged for supplemented and basic diet non-irradiated rats.

Table 4. Flow rate and composition of pilocarpine/isoproterenol stimulated saliva collected 2 weeks after irradiation

	Basic diet			Supplemented diet			
	— (n = 9)		7 Gy × 5 (n = 10)	— (n = 10)		7 Gy × 5 (n = 9)	ANOVA P-value
Flow rate (μl/min)	62.1 ± 27.7	**	26.7 ± 7.5[a]	67.6 ± 23.2	*	43.4 ± 17.4[a]	0.0004
Calcium (mmol/l)	4.4 ± 1.1	**	6.2 ± 1.3	4.7 ± 1.9		5.8 ± 1.6	0.0436
Sodium (mmol/l)	22.3 ± 11.2	**	44.3 ± 14.1	25.6 ± 11.4	*	38.0 ± 10.9	0.0008
Potassium (mmol/l)	64.4 ± 7.0	***	89.0 ± 11.8	73.0 ± 23.1		81.5 ± 16.3	0.0121
Chloride (mmol/l)	9.0 ± 5.8		9.8 ± 2.3	12.2 ± 5.5		12.7 ± 7.6	NS
Total protein (mg/ml)	5.33 ± 1.93	***	11.20 ± 2.23[b]	5.23 ± 2.93		7.82 ± 3.43[b]	0.0001
Hexosamines (μg/ml)	677 ± 169	***	1226 ± 186	704 ± 307		980 ± 325	0.0001
Amylase (U/ml)	2 299 ± 1 257	**	6 954 ± 3 490	2 435 ± 2 171		5 534 ± 4 530	0.0040
BAGP (m-value)	0.13 ± 0.04		0.12 ± 0.03	0.16 ± 0.03	**	0.10 ± 0.04	0.0290
SPO (ΔA_{400}/ml min)	23.4 ± 9.8	***	54.7 ± 12.0	16.7 ± 10.1	**	45.3 ± 22.8	0.0001
MPO (mU/ml)	0.03 ± 0.01		0.03 ± 0.02	0.02 ± 0.01		0.02 ± 0.01	NS

Data are presented as mean ± S.D.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ between non-irradiated and irradiated groups receiving basic or supplemented diet, respectively.
[a,b]Means sharing a common superscript differ at the $P < 0.05$ when tested with an unpaired, two-sided t-test.
SPO, salivary peroxidase; MPO, myeloperoxidase; BAGP, bacteria agglutinating activity; NS, not significant.

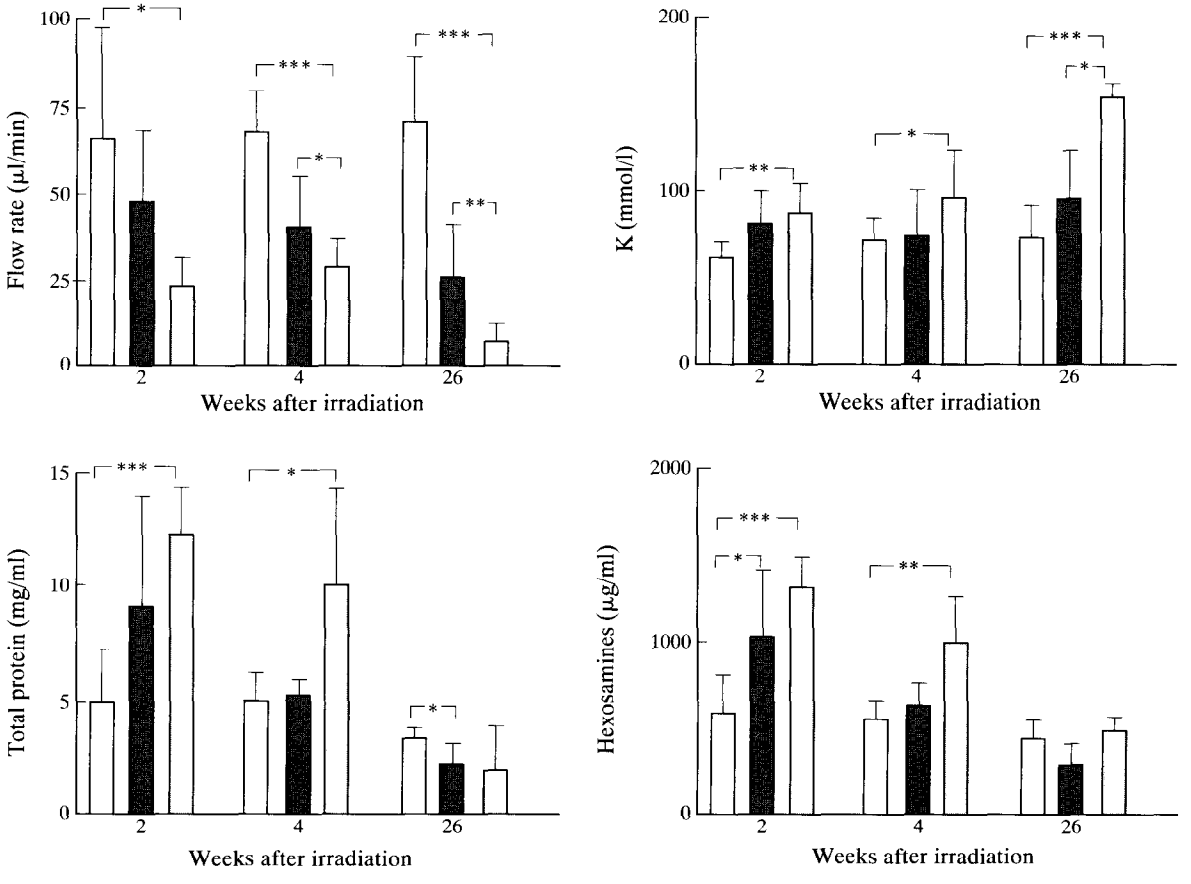

Figure 3. Flow rate and concentrations of potassium, total protein and hexosamines (mean + S.D.) in pilocarpine/isoproterenol stimulated saliva collected 2, 4 and 26 weeks after irradiation. Only rats participating at all three saliva collections are included. The non-irradiated group includes both rats given basic and supplemented diet. Open, non-irradiated; dark grey, irradiated, supplemented diet; light grey, irradiated, basic diet. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ when means were tested with an unpaired two sided t-test.

Table 5. Quantitative morphology in parotid and submandibular glands in irradiated and non-irradiated rats (both diet groups combined), 5 and 26 weeks after irradiation

	5 weeks		26 weeks		
	— ($n = 8$)	7 Gy × 5 ($n = 8$)	— ($n = 7$)		7 Gy × 5 ($n = 8$)
Parotid gland					
% acinar cells	77.0 ± 5.1	69.0 ± 4.5[a]	74.1 ± 2.3	***	42.3 ± 12.5[a]
% duct cells	17.3 ± 3.5	22.7 ± 4.4[b]	22.8 ± 8.0	***	38.1 ± 8.3[b]
% stroma	5.5 ± 5.9	8.0 ± 1.4	6.0 ± 2.6	**	19.5 ± 10.7
Submandibular gland					
% acinar cells	66.1 ± 7.0	60.9 ± 5.6[c]	61.5 ± 8.2	***	37.1 ± 13.3[c]
% duct cells	27.7 ± 10.0	34.6 ± 6.3	34.9 ± 7.6		49.2 ± 18.6
% stroma	6.2 ± 4.1	4.4 ± 1.1	3.5 ± 1.8		5.1 ± 1.9

Data are presented as mean ± S.D.*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ between non-irradiated and irradiated groups.

[a,b,c]Means sharing a common superscript differ at $P < 0.05$ when tested with an unpaired, two-sided t-test.

DISCUSSION

The present study demonstrated that the damage caused by irradiation in salivary glands, measured as saliva flow rate and composition, was less pronounced when daily supplements of α-tocopherol and β-carotene were given to rats shortly before and during irradiation. However, the vitamin supplementation as given in this study did not normalise the salivary function.

Radiotherapy of malignancies in the head and neck often involves the salivary glands in the treatment volume, even if they are not the primary target. The radiosensitivity of salivary glands is manifested by a decrease in saliva secretion, which in most cases is severe and leads to great discomfort for the patient, such as impaired eating, speaking, swallowing and impaired protection against infections [25]. It is, therefore, of great importance to find methods which reduce the adverse effects of irradiation on salivary gland function without hampering the treatment effect.

We wanted to estimate the value of antioxidant vitamins as radioprotectors of salivary glands considering both acute and delayed effects. Irradiation-induced cellular damage is caused mainly by the formation of free radicals. The radiation effects have been discussed both in relation to damage of cellular membranes and DNA. One of the primary targets for free radical reactions are the unsaturated bonds in membrane lipids. The lethal target is DNA which is mainly damaged by hydrogen and hydroxyl radicals formed by hydrolysis of water [6]. Antioxidants, given before and during irradiation, might, therefore, play a role as scavengers both during and after irradiation. The persistent higher saliva flow rates in the irradiated rats receiving supplemented diet compared with those in the irradiated rats given basic diet supports this hypothesis.

Vitamin E (α-tocopherol), the major lipid soluble antioxidant present in cellular membranes, and β-carotene protect against lipid peroxidation. The breaking of the lipid peroxidation chain by β-carotene complements that of vitamin E, since β-carotene is effective at low oxygen concentrations and vitamin E is effective at high oxygen concentrations. Further β-carotene is also a quencher of singlet oxygen [26] and vitamin E also has a sparing effect on β-carotene [27].

The rats given supplemented diet received a total of 4 mg α-tocopherol (3.4 mg as supplement and 0.6 mg from the basic diet) and 6 mg β-carotene per day, whereas the rats given basic diet received, on average, 0.6 mg α-tocopherol per day and no β-carotene. Rats in all groups received adequate intakes of retinol (30 μg/day). The intake by the rats in the basic diet groups corresponded to the daily recommended intake in adult rats for retinol and α-tocopherol [12]. Thus, the supplemented level of α-tocopherol was 6.7 times higher than the recommended intake. In humans, the recommended daily intake (U.S.A. RDA) of α-tocopherol is 10 mg per day and of retinol 1 mg per day [28]. There is no RDA recommendation for β-carotene intake, but the National Cancer Institute at NIH, Bethesda, U.S.A. has recommended a daily intake of 5–6 mg β-carotene in man [29]. Intakes of up to 180 mg β-carotene per day in humans have been taken for long periods without side-effects [30]. This would correspond to 30–36 times higher intake than is recommended by the National Cancer Institute, Bethesda, U.S.A. In humans, intakes of 3000 mg α-tocopherol/day, corresponding to 300 times the recommended daily intake, have been reported safe [31]. Thus, based on this current knowledge, the levels used in the present study would not be hazardous for use in man. However, extrapolation from animals must be done with care and human studies must verify the present findings.

No obvious effects of irradiation were seen on parotid or submandibular gland morphology 5 weeks after irradiation, but 26 weeks after irradiation the proportions of acinar cells were significantly diminished both in parotid and submandibular glands. The proportion of duct cells and stroma was significantly increased in irradiated parotid glands. These results are in accordance with previous observations that morphometric changes are not manifested in the early period after irradiation [13, 32], but rather is a delayed effect that may be associated with the turnover time for the acinar cell. The life-time for acinar and tubular cells is reported to be 2–4 months [33].

Diet supplementation did not lead to any detectable differences in the morphology of parotid or submandibular glands. However, the flow rates were markedly higher in irradiated rats given supplemented diet than in those given basic diet even 26 weeks after irradiation. It therefore appears that factors other than acinar cell destruction cause the reduction in flow rate in

the early phase, and that the salivary gland function in the late phase is more selective and complex than can be detected by morphological analysis. In comparison, it has been reported that a decreased flow rate in the elderly is not associated with visible degenerative morphological changes in the salivary glands [34, 35].

Thus, based on the present findings, we suggest that supplementation with α-tocopherol in combination with β-carotene, at least, to a certain degree protects the salivary glands of the rat from adverse effects following irradiation. Flow rates are partly preserved, the concentrations of proteins and electrolytes are partly normalised and signs of inflammatory reactions, evaluated by secretion of albumin, are less pronounced in rats given supplemented diet. The increased leakage of albumin and its use as an inflammatory parameter has also been discussed in lung tissue and bronchoalveolar lavage following irradiation in both humans [36] and rats [37]. In this paper, we did not evaluate the possibility that tumour cells may also be protected by supplementation with antioxidants. The potential effect of vitamins as radioprotectors in normal tissues must be confirmed and further investigated in relation to other radiation doses, other species and unintended effects on cancer management.

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