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Radioprotection of minipig salivary glands by orciprenaline-carbachol *

An ultrastructural and semiquantitative light microscopic study

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Summary. Parotid and submandibular glands of miniature pigs were exposed to 36 Gy X-irradiation given as 6×6 Gy in 3 weeks. Half of the animals received orciprenaline and carbachol before each dose. The effects were analysed 6 months later by light and electron microscopy. Ultrastructural examination showed less change in the pretreated glands. Semi-quantitative light microscopic data confirmed the significance of the differences. Acinar cells of both glands were significantly more numerous ($P < 0.01$) and the cells were better preserved ($P < 0.01$) in the pretreated group. The effect was more pronounced in the parotid gland, which appeared almost normal. Intercalated ducts of the parotid glands ($P < 0.01$) and striated ducts of the submandibular glands ($P < 0.05$) showed less change in pretreated animals. The findings confirm the radioprotective effect of pharmacologically induced degranulation of acinar cells. The biological effects of the radiation schedule (cumulative radiation effect value 18.76) as well as the dosage of orciprenaline and carbachol are within the normal range of medical treatment. Similar results may be expected from future studies in man.

Key words: Salivary glands – Radiation injury – Radioprotective substances – Miniature swine

Introduction

Bergonié and Spéder (1911) and later Ceresole (1912) were the first to notice xerostomia as a consequence of irraditation to the parotid region. Today, radiation therapy of head and neck tumours often includes salivary glands in the radiation field: 100% of the parotid gland is irradiated during irradiation of nasopharyngeal, advanced oropharyngeal and Waldeyer's ring lesions; for early oropharyngeal and hypopharyngeal lesions 30%–90% of the parotid gland is irradiated (Cheng et al. 1981). The consequence is a radiation-induced sialadenitis, leading to atrophy and sclerosis of the salivary glands (Hermann 1937; Busuttil 1977; Fajardo and Berthrong 1981; Fajardo 1982; Seifert and Geier 1971; Seifert et al. 1984; Dreyer et al. 1989). The patients suffer from severe xerostomia resulting in shifts in the oral microflora leading to extensive dental caries (Brown et al. 1975; Dreizen et al. 1977; Makkonen and Nordman 1987). The xerostomia is irreversible. Though the patient feels better subjectively in the months and years following radiation therapy, objective improvement cannot be substantiated (Dreizen et al. 1977; Wescott et al. 1978; Mira et al. 1981, 1982).

Previous studies have shown that radiation-induced changes in salivary glands can be reduced by pretreatment with isoproterenol (Schneyer et al. 1969; Hall 1974; Sodicoff et al. 1979; Sodicoff and Conger 1983) or amifostine (WR-2721; Sodicoff et al. 1978; Pratt et al. 1980; Menard et al. 1984; Takahashi et al. 1986). These studies have used high single-dose irradiation or have examined gland weights and saliva flow rates. To the best of our knowledge studies concerning the morphology of late radiation changes after fractionated radiation under radioprotective pretreatment with isoproterenol or its derivatives have not been reported.

Abok and co-workers (1984) reported that radiosensitivity of serous rat submandibular acinar cells increased with a higher content of secretory granules. Consequently we decreased the number of secretory granules present by administering orciprenaline and carbachol prior to irradiation.

The subject of this report is the light microscopic and ultrastructural changes in salivary glands of pretreated animals in comparison with non-treated animals 6 months after irradiation.

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Materials and methods

We used 20 male miniature pigs of the Göttingen strain, 4–8 months old, weighing 23–34 kg. Two animals served as controls, the other 18 were randomly divided into two groups and exposed to cobalt-60 irradiation in an opposed field technique: 2×6 Gy per week for 3 weeks, total dose 36 Gy; the CRE value (cumulative radiation effect, Kirk 1971) was 18.76 considering correction factors for late radiation effects. According to CT-assisted calculations, both the parotid and submandibular gland were completely within the 80% isodose. Animals of the first group were irradiated without premedication. The second group was given both 0.01 mg/kg body weight orciprenaline (Alupent; Boehringer, Mannheim, FRG) intramuscularly 30 min before radiation and 0.01 mg/kg body weight carbachol (Doryl; Merck, Darmstadt, FRG) intramuscularly 5 min before radiation. Three animals of each group died during the experiment; 4 died of pneumonia, 2 of narcotic accidents following anaesthesia with diethylether and thiopental for saliva collection by catheterization of the main duct.

Six months after radiation the animals were sacrificed and all parotid and submandibular glands were removed. Several specimens from the gland's central parts were fixed in 4% buffered formaldehyde or 3% glutaraldehyde. The formalin-fixed specimens for light microscopy were paraffin-embedded; slides were conventionally stained with haematoxylin and eosin (H&E), periodic acid-Schiff (PAS) and Astrablue (Romeis 1968). The glutaraldehyde-fixed specimens were washed in sodium cacodylate, postfixed in 1.5% osmium tetroxide and embedded in Epon 812 (Luft 1961). Ultrathin sections (60–90 nm according to Sitte 1985) were stained with uranyl acetate and Reynolds' lead citrate (Reynolds 1963) and examined in a Zeiss EM 9 electron microscope.

In addition to ultrastructural examination the radiation changes were examined using the light microscope semi-quantitatively by estimating the percentage of rest glandular tissue per slice area and acinar cells per gland area and by using a detailed evaluation-scheme with graduation of the changes from 0 to +++.

The right and left gland of the same animal were evaluated simultaneously. The percentages of all slices were listed to obtain the median value of the corresponding specimen. The mean value of all specimen values was regarded as representative for the animal. From the evaluation scheme we chose four main criteria, each with four subcriteria:

1. *Global gland changes:* loss of parenchyma, lobular atrophy, fibrosis, lymphocytic infiltration;
2. *Acinar cell changes:* vacuolization, loss of secretory granules, nuclear enlargement, clumping of chromatin;
3. *Intercalated duct changes:* nuclear enlargement, duct proliferation, dilatation, epithelial flattening;
4. *Striated duct changes:* nuclear enlargement, duct dilatation, epithelial flattening, goblet cell metaplasia.

For each of the four main criteria, the intensity values were added in each slice. The slice values were listed to obtain the median value of the corresponding specimen. The median value of these specimen values was regarded as representative for the animal. Thus it was possible to compare the radioprotective effect on different parts of the glands. The percentages and the intensity values were used to compare the two groups by van der Waerden's X-test (van der Waerden 1957).

Results

Controls

The parotid gland showed special serous[1] acinar cells with a vacuolated cytoplasm and dense nucleus. PAS and Astrablue reactions were negative (Fig. 1).

Ultrastructurally the cells were densely packed with translucent, mostly fused secretory granules. Small islands of cytoplasma contained rough endoplasmic reticulum (rER) and Golgi lamellae (Fig. 7).

[1] Nomenclature as suggested by Pinkstaff (1980) referring to the description by Ginsbach and Kühnel (1978)

Table 1. Estimation of percentage of rest-glandular tissue (mean-values ± standard deviation)

Animal no.	Number of slices	Parotid gland		Number of slices	Submandibular gland	
		% Gland tissue per slice area	% Acinar cells per gland area		% Gland tissue per slice area	% Acinar cells per gland area
Untreated controls						
3–3	40	89.17 ± 7.36	93.33 ± 2.58	25	97.0 ± 4.47	83.0 ± 4.47
3–4	35	88.33 ± 12,99	90.83 ± 1.77	30	97.5 ± 5.0	83.13 ± 5.54
Irradiated with pretreatment						
1–1	32	89.58 ± 7.49	92.92 ± 2.46	28	79.38 ± 10.48	66.25 ± 4.79
1–2	29	89.0 ± 16.35	90.5 ± 3.71	29	90.5 ± 4.47	79.5 ± 3.71
1–3	28	86.25 ± 11.09	82.5 ± 6.45	34	85.0 ± 12.91	71.88 ± 2.39
1–5	27	72.5 ± 8.29	68.0 ± 18.49	29	63.33 ± 29.3	46.67 ± 28.43
1–6	32	78.33 ± 25.18	76.67 ± 23.96	34	37.5 ± 28.72	40.0 ± 31.22
1–8	33	71.0 ± 25.35	76.5 ± 23.69	27	56.67 ± 20.82	50.0 ± 26.46
Irradiated without pretreatment						
2–1	59	15.0 ± 9.79	35.25 ± 11.81	44	16.67 ± 26.58	13.75 ± 5.30
2–3	50	6.44 ± 10.82	33.33 ± 5.77	24	0.0 ± 0.0	–
2–4	56	7.9 ± 9.88	15.83 ± 4.92	29	38.33 ± 18.93	5.0 ± 0
2–6	–[a]	–	–	24	27.5 ± 17.68	13.75 ± 5.30
2–7	44	36.0 ± 21.80	32.22 ± 11.69	39	92.0 ± 10.95	39.0 ± 7.42
2–9	53	26.61 ± 22.08	32.85 ± 9.51	35	76.0 ± 29.66	33.0 ± 6.71

[a] These "parotid gland"-slices contained submandibular tissue and therefore were not evaluated

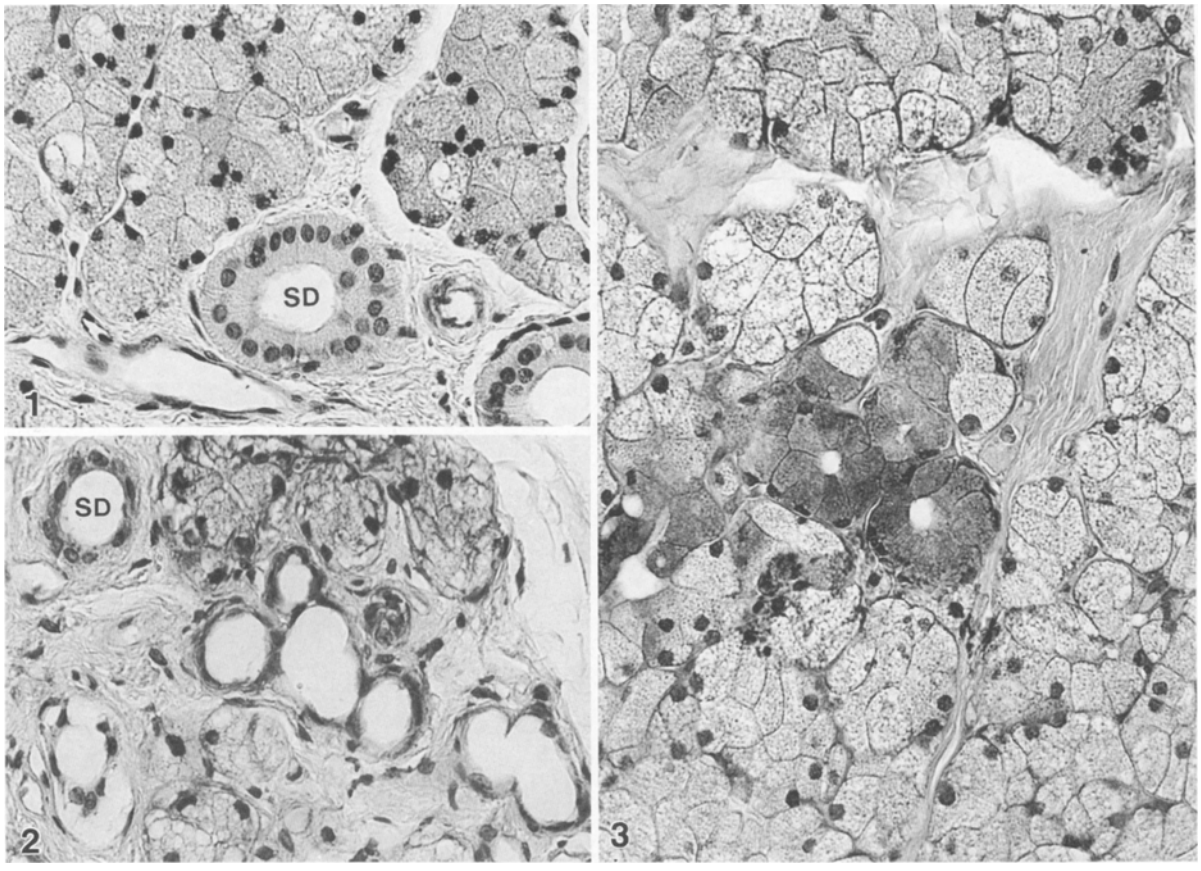


Fig. 1. Normal miniature pig parotid gland: acinar cells with slightly vacuolated cytoplasm, striated duct cells (*SD*) with distinct basal striation. Haematoxylin and eosin (H&E), ×300

Fig. 2. Parotid gland, irradiated without pretreatment: fibrosis, small groups of acini with vacuolated cells; dilated intercalated ducts with flattened epithelium. H&E, ×300

Fig. 3. Parotid gland, irradiated with pretreatment: mucous acinar cells staining Astrablue-positive within a well-preserved gland. Astrablue, ×300

The submandibular gland consisted of mucous tubules with serous demilunes, which stained weakly with PAS and Astrablue. The ratio of mucous to serous cells was approximately 1:1 (Fig. 4).

Ultrastructurally the mucous cells were filled with a translucent secretory pool with small islands of cytoplasm and a flat-shaped nucleus located at the base. Serous cells contained well-defined secretory granules of varying electron density; the round nucleus was located at the base (Fig. 10).

In both glands the secretory endpieces were followed by short intercalated ducts, then striated ducts with distinct basal striation and a few goblet cells in the interlobular ducts. We found a few periductular small lymphocytes and plasma cells.

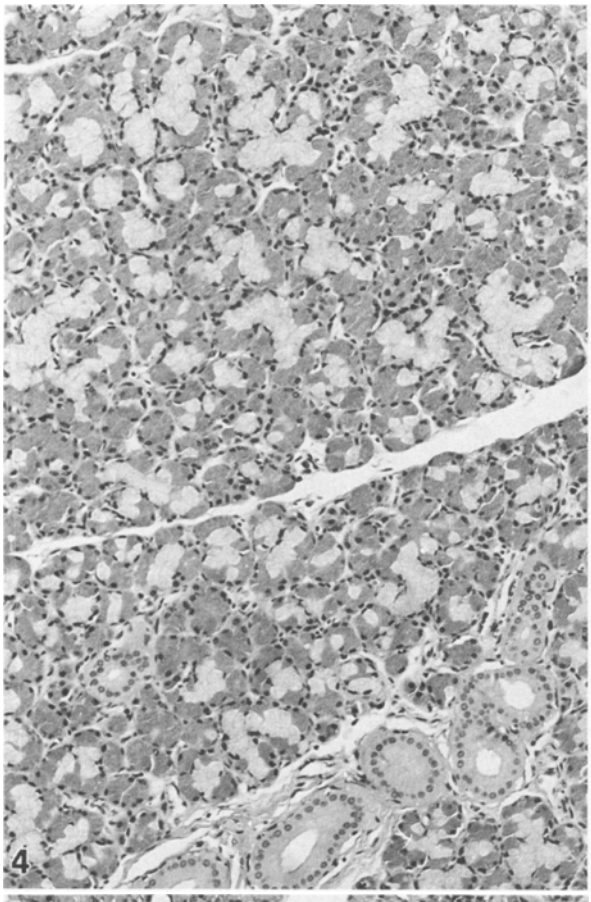
Irradiation without pretreatment

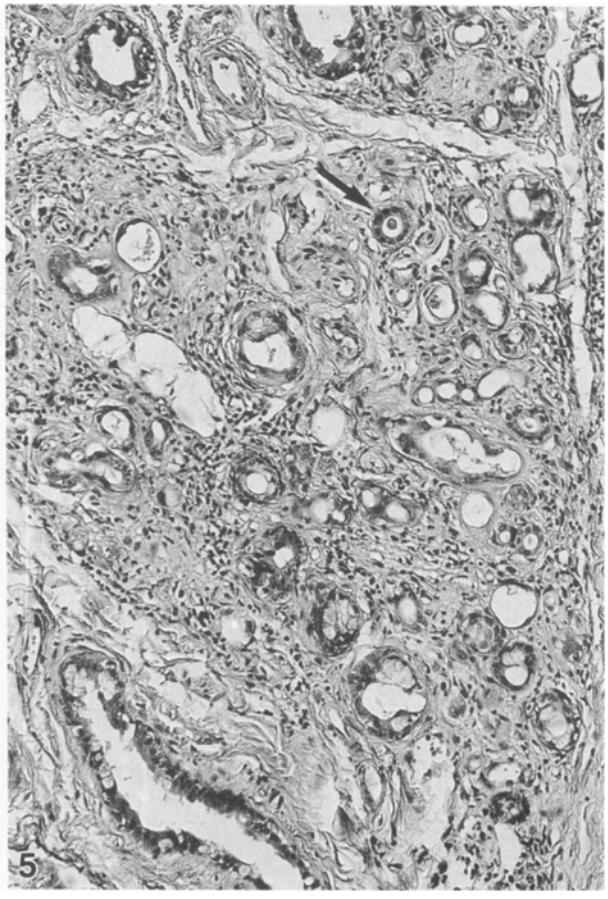
Parotid and submandibular glands showed extensive destruction (Figs. 2, 5) with moderate to complete loss of acinar cells (Table 1), proliferation of fatty tissue, interstitial fibrosis with destruction of lobular architecture, diffuse infiltration of lymphocytes, plasma cells and rare infiltrates of eosinophilic granulocytes. Small arterioles exhibited concentric intimal proliferation; some obliterated small vessels were found.

The remaining secretory endpieces of both glands consisted of diminished, degranulated and vacuolated cells (Fig. 2). Their nuclei were enlarged, with clumped chromatin and sometimes of bizarre shape. Karyolysis was rare.

The duct system showed proliferation of the intercalated ducts with nests of small ectatic ducts with endothelium-like flattened epithelium and enlarged nuclei. Striated ducts were dilated, the duct cells were flattened with almost complete loss of the basal striation and extensive goblet cell metaplasia, sometimes accompanied by epithelial dysplasia. One submandibular and one parotid gland contained small microliths within striated ducts.

Ultrastructural examination confirmed the light microscopic findings (Figs. 9, 12). Acinar cells showed ballooned Golgi and ER cisternae, the intercellular spaces were dilated. Some degenerating cells were also found with lysosomes and karyolytic nuclei. These changes





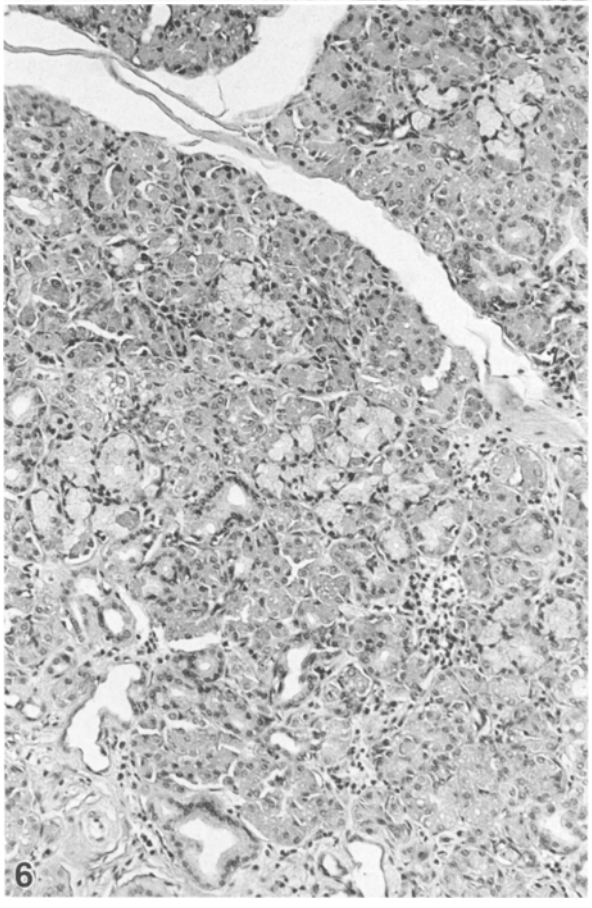
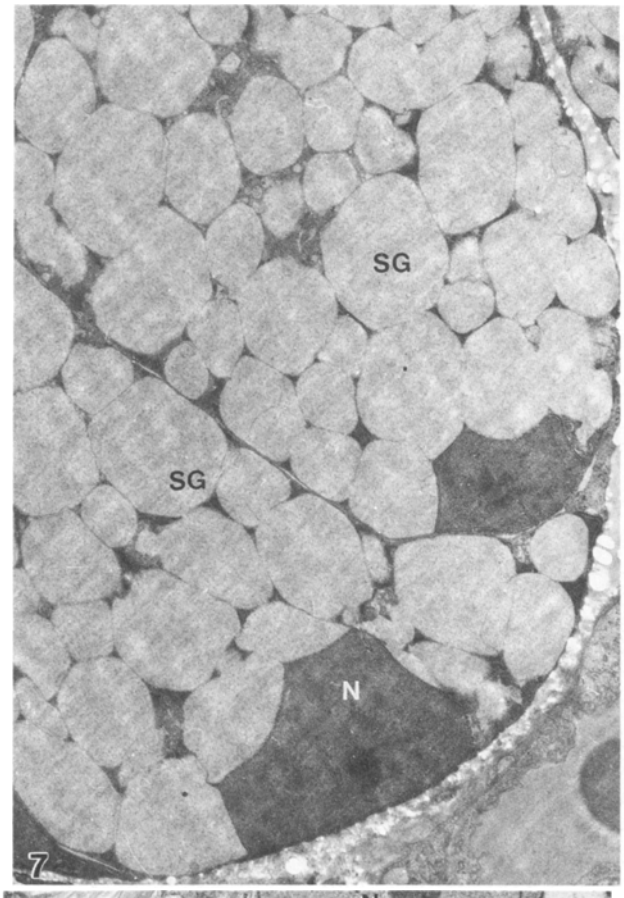
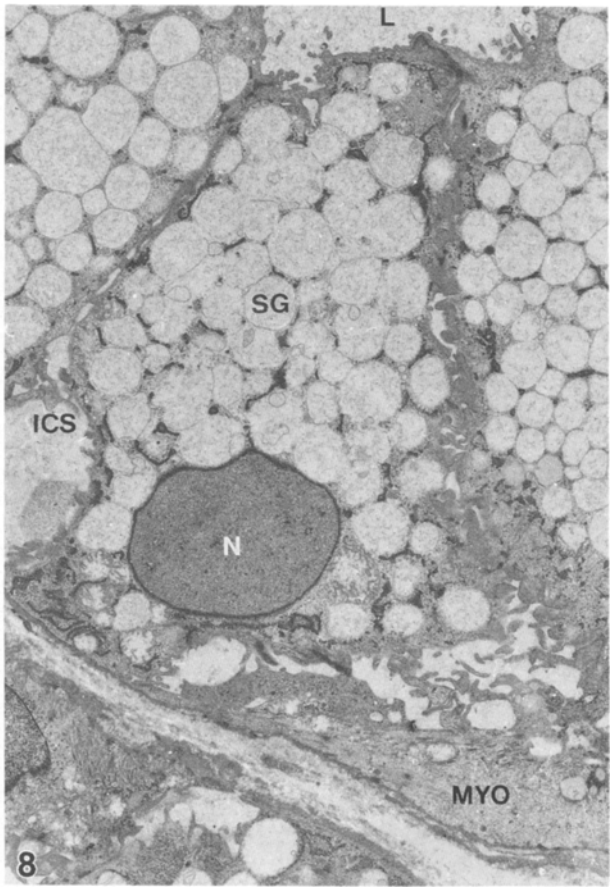


Fig. 4. Normal miniature pig submandibular gland: mucous tubules with serous demilunes, ratio of serous to mucous cells is approximately 1:1. H&E, ×120

Fig. 5. Submandibular gland, irradiated without pretreatment: destruction of glandular architecture, lymphocytic infiltration, almost complete loss of acinar cells, ectatic ducts, one containing a small microlith (*arrow*). H&E, ×120

Fig. 6. Submandibular gland, irradiated with pretreatment: well-preserved gland, serous and mucous cells are aranged focally. H&E, ×120





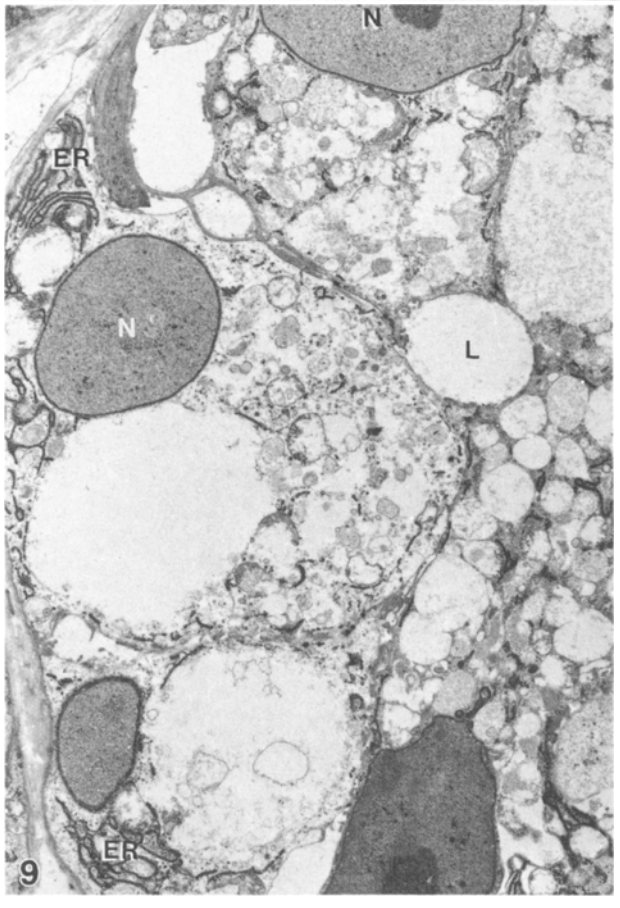


Fig. 7. Normal miniature pig parotid gland: acinar cells are densely packed with translucent, partly fused secretory granules (*SG*). ×4800

Fig. 8. Parotid gland, irradiated with pretreatment: well-preserved acinar cell, intercellular spaces (*ICS*) are dilated. Some mitochondria and endoplasmic reticulum at the cell basis. *L*, lumen; *MYO*, myoepithelial cell. ×4800

Fig. 9. Parotid gland, irradiated without pretreatment: degenerated acinar cells, degranulated with large vacuoles. ×4400

Table 2. Higher percentage of glandular tissue and acinar cells in the pretreated group in comparison to the non-treated group

	Parotid gland	Submandibular gland
% Gland tissue/slice area	$P<0.01$	not significant
% Acinar cells/gland area	$P<0.01$	$P<0.01$

Significance according to van der Waerden's X-test

Table 3. Reduction in extent of glandular changes in the pretreated group in comparison with the non-treated group (referring to the +++ graduation of the evaluation scheme)

	Parotid gland	Submandibular gland
Global gland changes	$P<0.01$	$P<0.05$
Acinar cell changes	$P<0.01$	$P<0.01$
Intercalated duct changes	$P<0.01$	Not significant
Striated duct changes	Not significant	$P<0.05$

Significance according to van der Waerden's X-test

were most pronounced in the serous submandibular acini, followed by the parotid special serous acini. The mucous submandibular cells were only slightly changed. Striated duct cells lost their basal membrane infoldings almost completely. Mitochondria were reduced in number and distributed basally in the cell. We rarely observed the onset of squamous cell metaplasia in submandibular striated ducts.

The ratio of serous to mucous cells within the submandibular gland showed great variation but no clear shift. Loss of parenchyma was about equal in the parotid and submandibular gland.

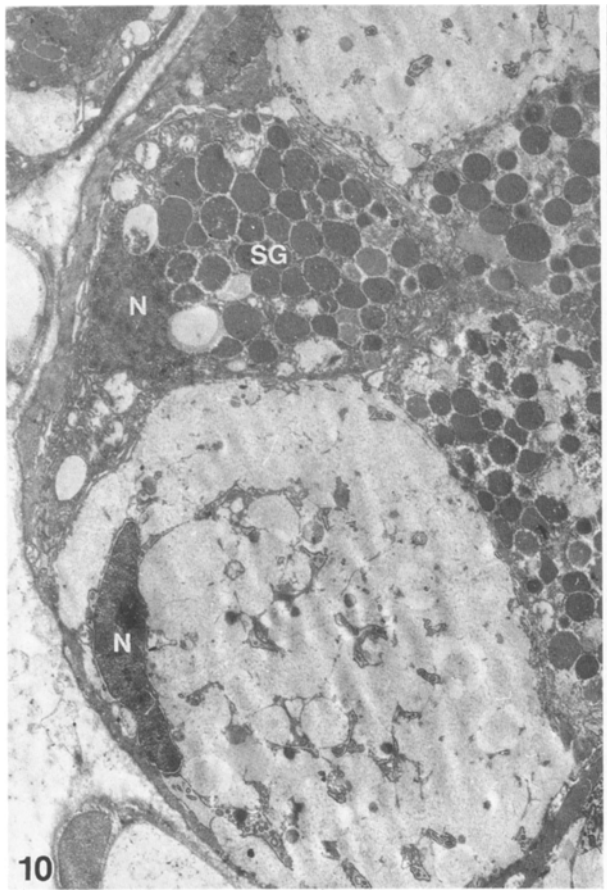
Irradiation with pretreatment

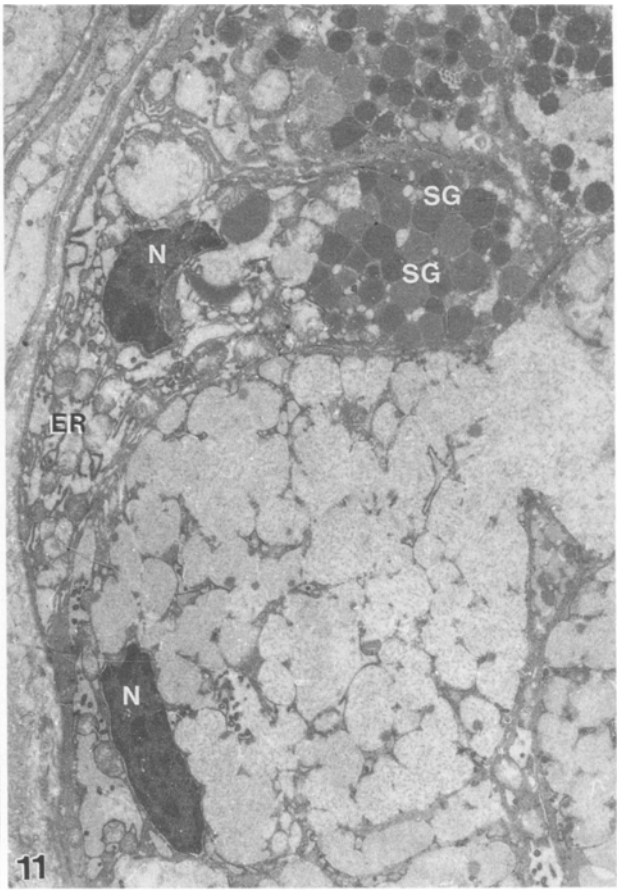
The changes were of the same kind as in the non-treated irradiated group, but were less pronounced when examined statistically in the pretreated glands (Tables 2, 3). The percentage of remaining glandular tissue was significantly higher in the pretreated parotid gland ($P<0.01$), but not in the submandibular gland. The global gland changes were significantly less pronounced in both the parotid ($P<0.01$) and submandibular glands ($P<0.05$). Wide areas of the parotid glands were almost indistinguishable from the controls. Occasionally we found mucous cells in the parotid glands (Fig. 3).

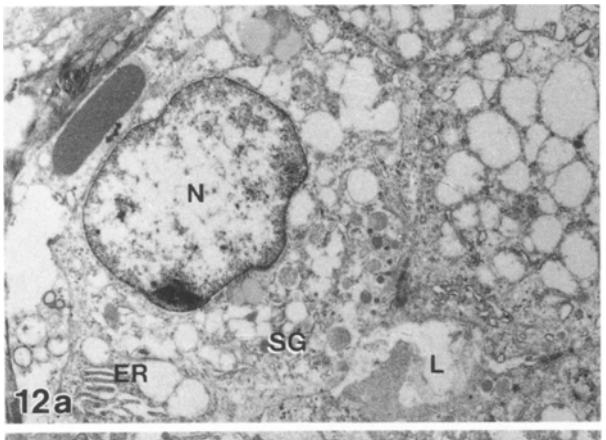
In both glands the percentage of remaining acinar cells was significantly higher ($P<0.01$). The cells themselves – serous as well as mucous cells – showed significantly fewer changes: loss of granules, cell vacuolization and nucleus changes were 1–2 grades less according to semi-quantitative analysis (Tables 4, 5). Submandibular acini were arranged focally in small, purely serous and almost purely mucous areas (Fig. 6). Intercalated ducts of the parotid gland showed significantly ($P<0.01$) less proliferation, epithelial flattening and nuclear enlargement. In striated ducts, the damage of the basal striation was less serious, the number of goblet cells was higher, duct dilatation and flattening of epithelium were about equal compared to the glands which were not pretreated. In the submandibular gland, the striated ducts were sig-

Table 4. Parotid gland: morphological evaluation (median values) of glandular changes according to a graduated scale (0 to +++)

	Controls		Irradiated and pretreated						Irradiated without pretreatment				
Animal number	3–3	3–4	1–1	1–2	1–3	1–5	1–6	1–8	2–1	2–3	2–4	2–7	2–9
Number of slices	40	35	32	29	28	27	32	33	54	33	37	44	46
Loss of parenchyma	0	0	0	0	+	++	0	0–+	+++	+++	+++	+++	+++
Lobular atrophy	0	0	0	0	0	+	0	0	+++	+++	+++	+++	+++
Fibrosis	0	0	0	0–+	+	++	0	0–+	+++	+++	+++	+++	+++
Lymphocytic infiltration	0	0	0	0–+	+	+	0	0–+	+	+	++	+++	++
Acinar cells													
loss of granules	+	+	+	+	+	+	++	++	+	+	+++	+++	++
vacuolization	+	+	++	++	++	++	++	++	++	++	+++	+++	+++
nuclear enlargement	0	0	++	+	+	+	+	+	++	+++	+++	++	++
clumping of chromatin	0	0	+	+	+	+	+	+	++	+++	+++	++	++
Intercalated ducts													
nuclear enlargement	0	0	0	+	+	++	0	0	++	++	++	++	++
duct proliferation	0	0	+	+	++	++	+	+	++	++	++	++	++
duct dilatation	0	0	+	++	+–++	++	++	+	++	++	++	++	++
epithelial flattening	0	0	+	+	+	+	+	++	++	++	++	++	++
Striated ducts													
nuclear enlargement	0	0	0	0	0	+	+	0	+	+	+	+	+
duct dilatation	0	0	+	+	+	++	+	+	+	+	+	+	+
epithelial flattening	0	0	++	++	++	++	++	++	+	++	+	++	+
goblet cell metaplasia	+	+	+	+	+	+++	++	+	0	0	++	++	+
loss of basal striation	0	0	+	+	++	++	++	++	++	+++	++	+++	++







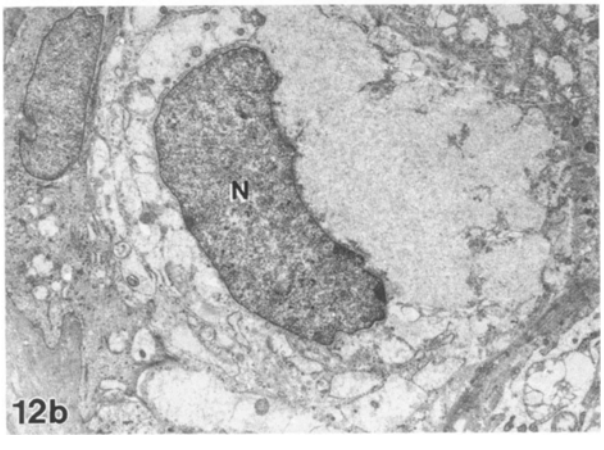


Fig. 10. Normal miniature pig submandibular gland: serous cell with clearly defined, electron-dense secretory granules (*SG*); mucous cell filled by a translucent "secretory pool". ×4300

Fig. 11. Submandibular gland, irradiated with pretreatment: serous cell with secretory granules (*SG*) restricted to the apical area, basally ballooned cisternes of the endoplasmic reticulum (*ER*). Mucous cell well preserved. ×4400

Fig. 12a, b. Submandibular gland, irradiated without pretreatment. **a** Serous cell degranulated with enlarged nucleus and ballooned endoplasmic reticulum. **b** Mucous cell with "secretory pool" and enlarged nucleus (*N*). ×4700

Table 5. Submandibular gland: morphological evaluation (median values) of glandular changes according to a graduated scale (0 to +++)

	Controls		Irradiated and pretreated						Irradiated without pretreatment					
Animal number	3–3	3–4	1–1	1–2	1–3	1–5	1–6	1–8	2–1	2–3	2–4	2–6	2–7	2–9
Number of slices	25	30	28	29	34	29	27	27	12		29	24	39	35
Loss of parenchyma	0	0	++	+	++	++	++–+++	++–+++	+++	–[a]	+++	+++	+++	++
Lobular atrophy	0	0	++	+	++	++	+–++	++–+++	+++		+++	+++	++	+++
Fibrosis	0	0	++	+	+	++	++–+++	+++	+++		+++	+++	+++	++
Lymphocytic infiltration	+	+	++	++	++	++	++	++	++		+++	+++	+++	+++
Serous acinar cells														
loss of granules	0	0	+–+ +	+	+	+	++	++	+++		+++	+++	+++	++
vacuolization	0	0	++	+	++	++	++	++	++		++	+++	+++	+++
nuclear enlargement	0	0	+	++	++	++	++	++–+++	+++		+++	+++	+++	+++
clumping of chromatin	0	0	0–+	+	++	+	++	++–+++	+++		+++	+++	+++	+++
Mucous acinar cells														
loss of granules	0	0	+	+	+	++	++	++	++		++	+++	++	++
vacuolization	0	0	+	+	+	+	+	+	++		++	+++	++	+
nuclear enlargement	0	0	+	+	+	+	++	++	+++		+++	+++	+++	+++
clumping of chromatin	0	0	+	+	+	+	+	++	+++		+++	+++	+++	+++
Intercalated ducts														
nuclear enlargement	0	0	+	+	+	+	++	++	++		++	+++	++	++
duct proliferation	0	0	++	+	++	++	++	++	0		++	++	+++	++
duct dilatation	0	0	++	++	++	++	++	++	++		++	++	++	++
epithelial flattening	0	0	++	+	++	++	++	++	++		++	++	++	+++
Striated ducts														
nuclear enlargement	0	0	0–+	+	+	+	+	+	++		+	++	++	++
duct dilatation	0	0	+	+	+	+	+	+	++		+	+	+	++
epithelial flattening	0	0	+	0	+	++	++	++	+++		++	+	++	++
goblet cell metaplasia	+–++	++	++	++	++	++	++–+++	++	++		++	+++	++	++
loss of basal striation	0	0	+	+	+	++	++	+	+++		+++	++	+++	+++

[a] These slices contained fatty and fibrous tissue only

nificantly ($P<0.05$) less altered – fewer nuclear enlargement, less dilatation and epithelial flattening. In two animals we found microliths in striated ducts.

Ultrastructurally the secretory granules of serous submandibular and parotid acinar cells (Fig. 8) were restricted to the apical cell area and extensive, sometimes dilated ER and Golgi lamellae were seen at the base of the cell. Most of the mucous submandibular cells appared normal, filled by a secretory pool. The serous cells were more intensively changed than the mucous cells.

In striated ducts of both glands the mitochondria were decreased in number. The basal membrane infoldings were reduced and had disappeared focally. The different extent of changes in submandibular and parotid gland as seen in light microscopy were not seen in the small specimens used for electron microscopy.

Discussion

Rat salivary glands pretreated with isoproterenol 20 min before irradiation show a reduction of weight loss that is equivalent to a 2.3–2.5 fold reduction of radiation dose. This effect is found for the acute as well as for the chronic period (Sodicoff et al. 1979). Our miniature pig study shows that glands pretreated with orciprenaline-carbachol are morphologically better preserved and that the protective effect can also be seen with fractionated irradiation comparable to radiation therapy in man.

The percentage of remaining acinar cells is significantly higher ($P<0.01$) for both the pretreated parotid and submandibular gland. Acinar cells of both glands show significant reduction ($P<0.01$) in loss of granules, vacuolization and nuclear changes. Morphologically the radioprotective effect is more pronounced in the parotid gland than in the submandibular gland; parotid gland specimens show wide areas that are almost indistinguishable from the controls. The percentage of rest parenchyma per slice is significantly higher ($P<0.01$) for the pretreated parotid gland only. The level of significance for global gland changes is higher in the parotid ($P<0.01$) than in the submandibular gland ($P<0.05$).

Abok and co-workers (1984) reported that serous acinar cells of rat submandibular gland show less radiation injury with a decreased content of secretory granules. Degranulated cells contain less proteolytic enzymes and less heavy metal ions. Radiation induced membrane damage, which is catalysed by heavy metal ions, leads to liberation of proteolytic enzymes and autolysis of the cells. To achieve a maximum of gland degranulation the miniature pigs were treated with both the isoproterenol derivate orciprenaline and the parasympathomimetic agent carbachol.

The reduction of changes in the pretreated glands confirm the protective effect of gland degranulation for late radiation changes and a fractionated therapy schedule.

With fractionated radiation and multiple orciprenaline applications there is, in addition, radiation-resistant hypertrophy and hyperplasia which isoproterenol treatment is known to produce (Selye 1961; Schneyer 1962; van den Brenk and Stone 1972; Ansel 1974; Baskerville et al. 1976; Wells and Humphreys-Beher 1985). That this hypertrophy is not prevented by subsequent irradiation has been reported previously by Sodicoff et al. (1979). Isoproterenol-induced mitosis in previously irradiated glands leads to proliferation-death of cells with latent damage (Sasaki 1976), which may accelerate their elimination. Healthy or non-irreversibly damaged gland cells will proliferate. This may explain the unusual focal arrangement of mucous and serous cells in the pretreated submandibular gland where the original arrangement is destroyed; surviving cells proliferate and form clusters of similar daughter cells. We believe that in addition to the radioprotective effect of gland degranulation, orciprenaline like isoproterenol assists the selection and proliferation of surviving cells. The regeneration of the gland may be improved.

The effect of pretreatment on the duct system is not uniform. In the pretreated parotid gland the intercalated ducts are significantly less altered, in the submandibular gland the striated ducts show significantly less damage – in particular less epithelial flattening and nuclear change. According to Boshell (1981) there is no effect of isoproterenol on the duct system in pigs. The protective effect on the duct system may be of secondary nature in that where acinar cell changes are less pronounced, there is less inflammatory reaction of the surrounding tissue as well as less change in secretory material. Consequently there are less duct alterations due to obstruction by viscous saliva.

The changes are less pronounced in the glands of the pretreated group. Qualitatively the radiation-induced changes are identical in pretreated and non-pretreated glands and are comparable to those described in man by other authors (Salis 1924; Hermann 1937; Busuttil 1977; Casarett 1980; Fajardo and Berthrong 1981; Seifert and Geier 1971; Seifert and Donath 1976; Seifert et al. 1984; Berthrong 1986; Dreyer et al. 1989) and in other animal studies (English et al. 1955; Cherry and Glücksmann 1959; Espinal et al. 1983; Stephens et al. 1986a, b). Loss of parenchyma extending to complete loss of acinar cells, fatty tissue proliferation, interstitial fibrosis, and lymphocytic infiltrates are seen. Surviving acinar cells are vacuolated, degranulated and their nuclei are enlarged. Ducts are ectatic with dysplastic epithelial regeneration, goblet cell metaplasia and proliferation of small ducts. Ultrastructural examination of acinar cells reveal dilatation and ballooning of the Golgi/ER system (Nicolatou 1981), widened intercellular spaces and a few degenerated cells with cytolysosomes and karyolytic nuclei. Striated duct cells lose their basal membrane infoldings (Chomette et al. 1981); mitochondria are reduced in number (Messelt and Dahl 1983) and no longer orientated along the basal infoldings (Espinal et al. 1983).

Atrophy and sclerosis of the glands following irradiation are caused by direct acinar cell death in interphase in the early period (Stephens et al. 1986a) and later by proliferation-death as well as ischaemic cell degeneration due to obliterative vasculitis (Seifert and Geier 1971; Fajardo and Berthrong 1981). Several authors describe duct obstruction by viscous secretory material (Seifert and Geier 1971; Busuttil 1977; Maillart 1981; Berthrong 1986). Damage to the saliva-producing cell organelles and replacement of normal duct epithelia by squamous cells and goblet cells cause dyschylia (Seifert 1964). Microliths may be correlated with chronic duct obstruction. Like Busuttil (1977), we think that the initial radiation sialadenitis is followed by an obstructive sialadenitis, causing additional acinar cell and duct degenerations.

Early radiation injury is reversible as long as it does not exceed a certain threshold (Dreyer et al. 1989). Several authors have observed complete gland regeneration after 3.5 Gy (Maillart 1981), 7.5 Gy (Stephens et al. 1986b) or 17.5 Gy (English et al. 1955). The "functional" threshold radiation dose leading to irreversible xerostomia in man is predictable and depends on the patient's pre-irradiation salivary flow rate (Mira et al. 1982; Eneroth et al. 1972). The aim of radioprotective pretreatment is to keep radiation damage at a reversible level, by increasing the threshold radiation dose. This aim was achieved for the parotid gland where extensive areas of the pretreated parotid gland are morphologically completely preserved.

The biological effect of our radiation schedule is within the range of common radiation schemes in man. The drugs Alupent and Doryl are well-known substances. On a mg/kg body weight basis the two drugs are used in comparable doses in the pig as in man (Forth et al. 1983). Therefore similar results should be expected in man.

This study underlines the importance of morphological analysis in functional disorders. Morphological studies are a necessary part of the process of understanding of diseases, and provide valuable information for their treatment and prevention.

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