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REGENERATION OF PAROTID ACINAR CELLS AFTER HIGH RADIATION DOSES

A morphological study in rat

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The acute and late effects of fractionated irradiation on rat parotid gland acinar cells were studied by light and electron microscopy. At 10 days after the last irradiation session (6 Gy or 9 Gy daily during five consecutive days) no effects were seen. At 180 days, minor loss of acini was detectable after a total dose of 30 Gy. After 45 Gy a massive acinar loss was seen at that time; the number of acini had diminished and minor duct-like structures and scattered amounts of fibrous stroma dominated the slides. The remaining acini were disorganized and usually larger compared with the control side and to non-irradiated animals. The acinar cells appeared larger than in the controls. The ducts were better preserved but the intercalated ducts often seemed to be larger than normal. We suggest that this phenomenon indicates a remaining capacity of the parotid gland to regenerate acinar cells even after high radiation doses.

A large amount of data provides the basis for several accepted concepts that describe the influence of total dose, overall treatment time and number of fractions on normal tissue damage when treating cancer with radiotherapy (1, 2). Radiotherapy of head and neck cancers usually embraces salivary glands in the treatment volume, with subsequent xerostomia and hampered quality of life. Effects of single doses of irradiation on salivary glands have been studied extensively in experimental animals (3–9). However, there are only a few studies, and as yet no conclusive results concerning the effects of fractionated irradiation on different morphological and physiological parameters. The few long-term studies have, however, unanimously described degenerative processes (10, 11). Although, in theory DNA is the essential sensitive target of irradiation, other structures, such as cell membranes (6, 12) and enzyme-containing secretory granules (7), have been suggested as primary targets for the noxious radiation. More recently, membrane coupled potassium efflux was shown to be affected early in a dose-dependent manner by fractionated irradiation (13, 14).

In the present study a system for unilateral fractionated irradiation of rat parotid glands was devised for evaluating different morphological aspects of salivary acinar cells. The long-term effects on the secretory capacity of proteins and electrolytes was correlated with gland morphology. The results suggested that 6 months after irradiation a regeneration of acinar cell structures had occurred. This may explain the recovery of the secretory capacity observed in the clinical situation.

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Material and Methods

Animals. White female albino rats of Sprague-Dawley strain (Alab, Södertälje, Sweden), 8 weeks old (approx. 200 g) were used. They were fed water and chow ad libitum and kept on a diurnal light scheme.

Irradiation procedure. Irradiation of the rats was carried out with 6 MV x-rays from a linear accelerator. The rats were anaesthetized with Brietal (methohexital) and fixed in

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a plastic mould holding then firmly in position during the whole irradiation. During this time the rats were checked through a TV camera and at movements larger than approximately 4 mm the treatment was temporarily interrupted. The total irradiation field was 8 × 20 cm when two rats were irradiated simultaneously. One side of the head—the reference side—was shielded with a thick lead block (80 mm). The geometrical margin between the irradiation field and the non-irradiated parotid gland was 10 mm in all cases and the distance between field edge and 95% dose level of this beam was 6–7 mm. With the arrangement used the parotid gland on the reference (control) side received an insignificant radiation dose. The dosimetry was checked with an ionization chamber in a rat-like phantom with all scattering material in the field kept constant. Total doses of 30 or 45 Gy were given, divided into five fractions delivered on consecutive days.

Tissue preparation. The rats were killed at either 10 or 180 days, after termination of the irradiation. At each time 5 rats were irradiated with 30 Gy (total dose) and 5 rats with 45 Gy. The parotid gland specimens were fixed immediately after removal in 3% glutaraldehyde in 0.1 M phosphate buffer for 3 h. After rinsing in buffer, the salivary gland specimens were post-fixed 1 h 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) as well as thin (70 nm) sections were cut on a LKB Ultrotome. The thin sections were collected on naked copper grids and stained with uranyl acetate and lead citrate. A JEOL 1200 EM electron microscope was also employed for studying the fine structure of the glands after 30 Gy and 45 Gy irradiation. Parotid gland specimens from irradiated glands and controls were fixed and embedded in parallel. Semithin sections stained with toluidine blue were used for light microscopy.

Results

Light microscopy. In the non-irradiated side the well-known lobular structure with densely packed acini and ducts was seen in all specimens. No infiltration of fat cells or inflammatory cells occurred (Fig. 1). On the irradiated side no changes were seen 10 days after termination of irradiation with 30 or 45 Gy. However, 180 days after the last irradiation session a loss of acini could be detected. Irradiation with 30 Gy caused a minor loss and the changes were unevenly distributed. The acini were replaced by scattered amounts of fibrous stroma. After irradiation with 45 Gy, a massive loss of acini was found. Apparently more than half of the acini had disappeared. In a few cases only one or two acini per mm^2 were identified. The remaining acini were disorganized, and usually larger than normal. The acinar cells also appeared larger than those of the control side (Figs. 2 and 3). The individual acinar cells

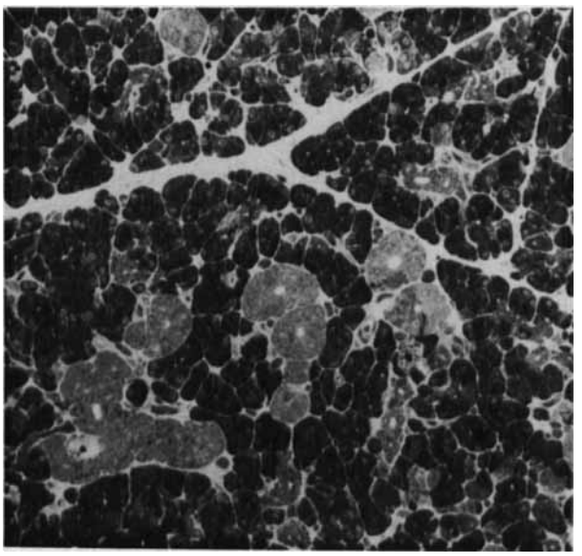

Fig. 1. Non-irradiated control side 6 months after 5 × 9 Gy irradiation to contralateral gland. Acinar and ductal configuration is normal. No fibrosis or inflammatory reactions are seen. × 200.

were otherwise of a normal form, often with larger amounts of secretory granules than in normal acinar cells. The ducts were generally less altered. In certain areas only ducts were seen surrounded by fibrous stroma. Striated ducts appeared generally unaltered, whereas intercalated ducts often seemed larger than normal with wider lumina.

Electron microscopy. After 45 Gy and 180 days following the last irradiation the cells of the remaining acini had sometimes a normal appearance and were packed with

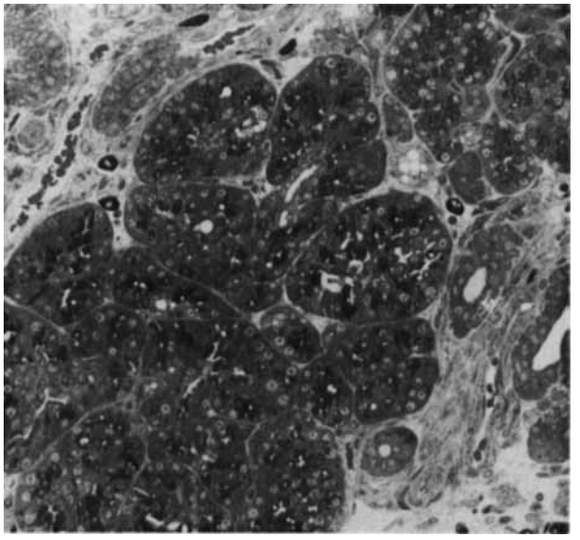

Fig. 2. Irradiated side, 6 months after 5 × 9 Gy. A nearly complete loss of acini. A single very large acinus is seen. Ductal structures look essentially normal, although the intercalated ducts seem to have larger lumina. × 200.

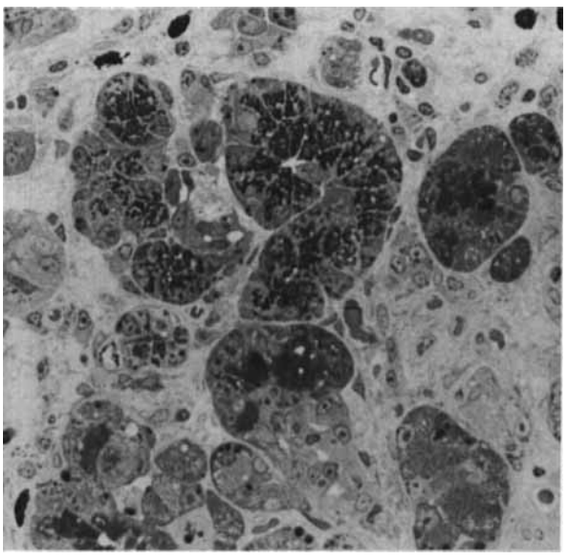

Fig. 3. Close-up view of an irradiated large acinus (5 × 9 Gy, 6 months). Acinar cells are irregularly distributed. They are larger than normal and often completely packed with secretory granules. × 400.

secretory granules. The overall organisation of the acini was, however, altered with up to 30 acinar cells in a single acinus (Fig. 4). The acinar cells were arranged in several layers and some cells seemed to be devoid of a secretory surface. This may, however, only be an illusion due to the plane of section as no 3D-reconstruction was attempted. Occasionally, the lumen seemed bordered by intercalated ducts in the centre of the acinus. In other areas acini were completely lost, and also cells in various stages of degeneration and necrosis were seen. Swollen mitochondria, dilated endoplasmic reticulum, and all degrees of nuclear deterioration could be seen in cells neighbouring well-preserved cells.

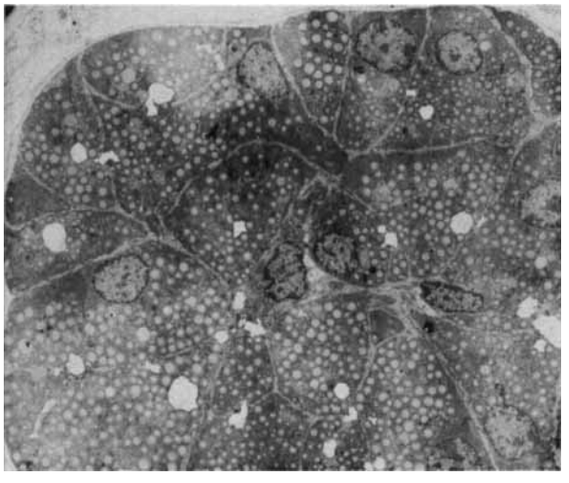

Fig. 4. Low power electron micrograph 6 month after 5 × 9 Gy irradiation showing approx. 20 acinar cells packed with secretory granules, and lying in a apparently disordered manner within the acinus. × 1000.

Discussion

An interesting implication from the present study is the signs of a capacity of the rat parotid gland to regenerate acinar cells following fractionated irradiation. Thus, the results may provide a morphological explanation for the recovery that can be seen in the clinical situation of salivation following irradiation of head and neck cancer (e.g., 15). The possibility of renewal of parotid acinar cells after fractionated irradiation has been suggested earlier by Glücksman & Cherry (16) in their work mainly dealing with the formation of adenomas. However, they only described persisting acini and enlarged acinar cells and the changes observed can be solely attributed to degenerative processes, which have been seen in other studies (10, 17). In none of these studies a regeneration of lost cells was documented, a discrepancy which may be due to the different period of observation and irradiation schedules. Similar observations have been reported not only in rodents but also in rhesus monkeys (18–20). Studies on other types of regeneration, i.e. after duct ligation, mainly have been done on the submaxillary gland for technical reasons. These studies have not shown cell death or mitotic activity indicating that the recovery was not dependent on cell division and differentiation (21, 22).

A pure degenerative process would most likely have resulted in small remaining acini with only few acinar secretory cells, if no new acinar cells developed. The presence of large irregular acini in the present study may thus indicate new formation of acinar cells. The structure of the basement membrane with a thickness comparable to the irradiated state in spite of the alterations in acinar diameter indicates an active adaption to a new state. This may further support the assumption of cell renewal. An increased amount of cytoplasmic filaments has been seen in acinar cells following radiation injury (Gustafsson et al., unpublished data). This might support the occurrence of regenerative capacity in analogy with the fact that epithelial cells contain enhanced actin filament amount during wound healing (23). The regenerated acinar cells also displayed normal structure. Consequently, cells responsible for the regeneration of acinar cells must persist in certain ductal-acinar units even after very high doses of irradiation. Whether this ability resides in the acinar cells themselves, or if it is a hypothetical intercalated duct reserve cell has been disputed (24, 25). Anyway, cells which can to some degree restore and regenerate secretory cells after irradiation injury may have interesting theoretical implications. A similar discussion of morphological analyses of renal tissue after irradiation has been presented by Withers et al. (26), who presented the mathematical calculations to determine whether a resulting tubular cell cluster stems

from one or more cells. There are problems of classical type for determining a regenerative capacity in the parotid glands after irradiation. A prerequisite for autoradiography of tissues after 3 H-labelled thymidine is a sufficient rate of the renewal processes. In the case of acinar cells, this rate can be extremely low (27). The parotid gland also shrinks markedly after irradiation and only small amounts of tissue remain for examination. A further complication is the appearance of ductal cells within the acinus (Fig. 4). Thus, a dividing cell within an acinus found by autoradiography can also be a duct cell.

An interesting point of view is the similarity between our finding of enlarged acini and some of the adenomas found after irradiation by Glücksman & Cherry (16). Ahlner et al. (28) also mentioned adenomatous ducts in the rabbit submandibular gland 6 months after irradiation but their illustrations are not convincing, as it only shows a slight enlargement of the duct and no form of disorganisation inside. The acini observed in the present study have greater similarities with the acinar microadenomas of the sublingual glands in female rats after 4–6 months than with the later appearing tubular adenomas of the male parotid glands. Probably there are only two ways of interpreting the same findings. In a well-differentiated acinar cell tumour (acinic cell carcinoma) the cells can be very similar to normal acinar cells. Even ultrastructural morphometry can sometimes be insufficient for discerning the two cell types (29). Furthermore the apparent disorganisation of acini found in the present study is also present in acinic cell carcinomas. Physiological studies may also be unable to discriminate between neoplastic and non-neoplastic acinar cells. Acinic cell cancer can contain amylase (29, 30), and tissue from this tumour type can also in vitro secrete amylase after adrenergic stimulation (29). In vitro studies recently performed, revealed that the remaining parotid tissue following irradiation with 30–45 Gy had a secretory capacity with regard to both electrolytes and exocytotic amylase release (13, 14, 31). However, the parotid glands notably diminish in size after irradiation. These acinar elements may thus fabricate only minimal amounts of saliva.

In conclusion, the present study gives some indications that the secretory units in salivary glands at least partly can be restored even after high doses of fractionated irradiation.

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