Expression of Neuron-specific Enolase in Irradiated Salivary Glands of the Rat: A Pilot Study

REINHARD E. FRIEDRICH1, MICHAEL S. DAVIDOFF2 and SYLVA BARTEL-FRIEDRICH3

1Department of Oral and Maxillofacial Surgery and
2Institute of Anatomy, Eppendorf University Hospital, University of Hamburg, Germany;
3Department of Otorhinolaryngology, Martin Luther University Halle-Wittenberg, Halle a.d.S., Germany

Abstract. External irradiation of advanced head and neck cancer often includes the major salivary glands, despite them not being affected by the disease. The aim of this study was to determine neuron-specific enolase (NSE) as a marker of inflammation in irradiated and non-irradiated salivary glands of the rat. This study shows that NSE expression in irradiated salivary glands is dependent on the topography of the organ related to the irradiation source. NSE immunostaining was increased in irradiated rats, both in glands directly exposed to the irradiation source and in shielded glands, and compared to glands of non-irradiated animals. Staining of NSE in salivary glands appears to indicate the inflammatory response of the organ to X-ray exposure. The increased immunoreactivity for NSE in shielded glands might be useful as a marker of irradiation in salivary glands, even for scattered radiation effects in major salivary glands.

The extent of radiogenic damage to the salivary glands depends on the radiation dosage, the fractionation and the volume effects of different radiation techniques (1). External irradiation of advanced head and neck cancer often includes the major salivary glands despite them not being affected by the disease. In these normal tissues, both single-cell damage (necrosis, apoptosis, functional cell death) and interstitial damage (oedema, fibrosis, vascular alterations, cellular infiltration) resulting in tissue remodelling can occur, depending on various parameters of the irradiation protocol (1-4). Neuron-specific enolase (NSE) is a catalyst relevant in glucose metabolism. NSE is predominantly synthesised in the brain, peripheral nerves and neuroendocrine cells (5).

Elevated NSE levels are found in different pathologies, such as in the course of cerebral trauma, brain tumours and small cell lung carcinoma (6, 7). However, acute inflammation can also induce increased serum levels of NSE (8).

In a recent study, evidence for dose-dependent morphological alterations of the facial nerve were noted following fractionated external irradiation (9). Therefore, NSE determination may be useful to investigate the effect of irradiation on salivary glands, focusing on both the epithelia and nerves inside the parenchyma. This pilot study was designed to identify NSE in irradiated and non-irradiated submandibular glands of the rat.

Materials and Methods

All experiments were conducted in accordance with the current version of the German law on animal protection. The experiments were approved by the Ethics Committee of the University of Hamburg. Three female Wistar rats weighing 200-250 g at the commencement of irradiation were used. Submandibular glands of a non-irradiated rat served as a control. The rats were 4-5 months of age at the beginning and 12-18 months old at the end of the study.

The irradiation field was the left side of the head and neck (Figure 1). To achieve irradiation restricted to such a small field, the animals were anaesthetised with isoflurane and positioned supine under a lead shield which covered the whole body but left the left neck unprotected from the clavicle to the external ear. The radiation field (i.e. the unprotected area) included the midline organs of the neck such as the pharynx, larynx, trachea and thyroid gland. The irradiated rats were subjected to X-rays under a fractionation scheme of 2 Gy/day, 5 days a week, up to total dosage of 20 Gy. At 6 months following completion of the irradiation, animals were sacrificed by fatal intraperitoneal injection of sodium pentobarbital followed by fixation of the tissues by intracardial perfusion with 4% buffered formalin. The technical details are described elsewhere in detail (10, 11).

Anti-NSE (Chemicon Ltd., Hofheim, Germany; 1:10) was applied according to the instructions of the supplier. For the visualization of the antigen, a combination of the peroxidase-anti-peroxidase (PAP) and the avidin-biotin-peroxidase complex (ABC) procedure was applied (12), including nickel-glucose oxidase amplification (13). The following controls were used: (a)
replacement by the primary, secondary and tertiary antibodies by PBS, and (b) preabsorption of primary antibody to the corresponding synthetic peptide at a final concentration of between 1 and 100 μg/ml.

Results

In the submandibular glands of the non-irradiated rat, NSE was detected in the excretory duct, intraglandular nerves (ganglion and fascicles) and in the walls of arterioles. Acini were barely stained. In the shielded submandibular gland of irradiated rats, immunoreactivity for NSE was shown in excretory ducts, in peripheral nerves, endothelia and smooth muscle cells of arterioles. Acini, intercalated ducts, granular convoluted tubules and striated ducts showed weak staining intensity. NSE staining was atypically found in nuclei of some epithelial cells. Submandibular glands of irradiated rats directly exposed to the radiation source showed intense NSE immunoreactivity of the acini but extenuated staining intensity and diffuse staining pattern in small excretory ducts and peripheral nerves (Figures 2-5).

Discussion

This study shows that NSE expression in irradiated salivary glands is dependent on the topography of the organ related to the irradiation source. At a dosage of 20 Gy, alteration of NSE expression in terms of increased immunostaining was predominantly confined to the acinar cells.

Saitoh et al. (14) studied NSE in the major salivary glands of normal and irradiated Sprague-Dawley rats by immunohistochemical methods. The salivary glands showed positive staining for NSE in striated ducts and granular convoluted tubule cells. The fractionation of the irradiation was avoided in favour of a single irradiation equivalent to 18.82 Gy and 27.97 Gy, respectively. Four groups of 5 rats each were used and the salivary glands were investigated 1, 2, 3 and 4 weeks after radiation. According to these authors, irradiated salivary glands indicated a remarkable reduction of NSE staining in granular convoluted tubule cells and a reduction, but to a lesser degree, in the striated duct of the submandibular gland. Interestingly, the immunohistochemical deposition of NSE was not changed in the sublingual glands of irradiated rats. They demonstrated that the reduction of NSE immunodeposition was dependent on the irradiation dose. However, the irradiation protocol of this study differs markedly from conditions in human radiotherapy for head and neck cancer.

Irradiation of 20 Gy allows restoration of the salivary glands (4). Peter et al. (15) studied the repopulation of salivary glands after irradiation. They aimed to measure the proliferative activity as a function of time in different epithelial cell compartments of rat salivary glands after irradiation with a single dose of 15 Gy restricted to the region of interest. They used bromodeoxyuridine-labeling index as a morphological marker of the recovery of radiation-induced tissue injury. In both parotid and submandibular glands, the irradiation caused cell death and a delay of the cell cycle within the first day. However, three days after irradiation, cell proliferation started in the intercalated duct and within six days was also observed in acinar and granular convoluted tubule cells. These results suggest that after 15 Gy of X-ray irradiation repopulation takes place in all cell compartments. In the present study, NSE immunostaining was increased in irradiated rats. Increased synthesis of NSE is a marker of nerve cell damage and recovery. These findings are in contrast to the report of Saitoh et al. (14) on reduced NSE immunoreactivity in salivary glands following X-ray exposure. However, this study used a fractionation scheme that allows recovery from irradiation damage, at least at a dosage of 20 Gy. Increased staining of NSE in salivary gland parenchyma appears to indicate the inflammatory response of the organ to X-ray exposure. This increased immunoreactivity for NSE in shielded glands might be useful as a marker of irradiation in salivary glands, even for scattered radiation effects in major salivary glands.

Acknowledgements

This study was generously supported by Hamburger Stiftung zur Förderung der Krebsbekämpfung (project No. 149) and in part by Deutsche Forschungsgemeinschaft (project FR 1035/1-2).
References


Received April 23, 2010
Revised May 3, 2010
Accepted May 3, 2010