A domestic porcine model for studying the effects of radiation on head and neck cancers

Christoph R. Arnold, MD, PhD,[a] Frank Kloss, MD, DMD, PhD,[b] Sarvpreet Singh, MD,[c] Danijela Vasiljevic, MD,[a] Robert Stigler, MD, DMD,[b] Thomas Auberger, MD,[d] Volker Wenzel, MD, PhD,[e] Günter Klima, MD, PhD,[f] Peter Lukas, MD, PhD,[a] Günter Lepperdinger, PhD,[c,g] and Robert Gassner, MD, DMD, PhD[b]

Background. Radiation therapy (RT) of the head and neck region is often accompanied by serious side effects. Research in this area is needed to improve treatment outcomes and ameliorate therapy tolerance. Laboratory rodents are barely matching today's clinical standards in RT research. Yet domestic swine (Sus scrofa domestica) have previously proved suitable for various advanced tests in clinical research and training. We therefore investigated whether S. scrofa domestica is also appropriate for irradiation of the mandible.

Study Design. A common scheme for irradiation treatment of S. scrofa domestica mandibles in a split-mouth design was acquired by applying computed tomography (CT) scanning under sedation. Basing on close anatomic resemblance, a standard treatment plan comprising 2 opposed irradiation fields could be accomplished.

Results. RT was carried out in a clinical environment with 2 × 9 Gy. The resulting operating procedure facilitated complication-free sedation, transport, positioning, CT scanning, and effective irradiation.

Conclusion. Based on common standards applied for RT in humans, domestic pigs can be employed to progress RT clinical research. Due to their human-like anatomy, physiology, size, and weight, the swine model is expedient for advancing experimental RT of the head and neck area. (Oral Surg Oral Med Oral Pathol Oral Radiol 2017;123:536-543)

Most tumors of the head and neck region develop from within the mucosa and are hence termed head and neck squamous cell carcinomas (HNSCCs).[1] HNSCC is the sixth leading cancer by incidence worldwide, accounting for approximately 3% of adult malignancies in North America and Europe.[2] The choice of therapeutic modality depends primarily on localization and disease progression. Early-stage HNSCC, to date, has most often been surgically treated,[3,4] but radiation therapy (RT) has become increasingly important. Currently, RT is the primary treatment for locally advanced HNSCC.[5-7]

In general, RT plays an important role in locoregional therapy for malignant diseases, not only of the head and neck region.[8-10] Being positioned between the surgical and chemotherapeutic approaches, RT effectively allows for addressing microscopic tumor extensions or draining lymphatic vessels. Commonly, RT is applied in an adjuvant or neoadjuvant setting, but is also often employed as a primary treatment with or without simultaneous chemotherapy. Around half of patients diagnosed with various types of cancer undergo RT, either with or without surgery or chemotherapy.[11,12] Recent technical innovations, such as 3-dimensional conformal RT (3-D−CRT) and intensity-modulated RT (IMRT), have resulted in improved clinical outcomes, in particular regarding locoregional disease control and survival rates. Owing to enhanced precision, these modern techniques yield reduced adverse effects, in particular by leaving surrounding healthy tissue untouched as much as possible.[13-18]

Although RT has been widely applied in treating malignant diseases for many years, its outcomes are considered far from satisfactory, often resulting in serious and impairing adverse effects. Especially complicating in the head and neck area are numbers, spatial location, radiosensitivity, and individual size of

Statement of Clinical Relevance

Domestic pigs can be easily employed as an experimental animal model to mimic therapeutic irradiation in the head and neck region to gain information about radiation-induced pathologies and improve existing or advance new radiotherapeutic strategies.

This work was presented as a poster at the DEGRO-ÖGRO annual conference in Mannheim, Germany, in June 2016. This work was supported by the Jubilee Fund of the Austrian National Bank, 2006 (project number 12246) and Synthes Austria.

[a]Department of Therapeutic Radiology and Oncology, Innsbruck Medical University, Innsbruck, Austria.
[b]Department of Maxillofacial Surgery, Innsbruck Medical University, Innsbruck, Austria.
[c]Institute for Biomedical Aging Research, Innsbruck, Austria.
[d]Department of Radiotherapy-Radiooncology, Hospital Traunstein, Traunstein, Germany.
[e]Department of Anaesthesiology and Critical Care Medicine, Innsbruck Medical University, Innsbruck, Austria.
[f]Division of Histology and Embryology, Innsbruck Medical University, Innsbruck, Austria.
[g]Department of Cell Biology, University Salzburg, Salzburg, Austria.

Received for publication Aug 1, 2016; returned for revision Oct 11, 2016; accepted for publication Nov 25, 2016.

2212-4403/$ - see front matter
http://dx.doi.org/10.1016/j.oooo.2016.11.017

tissues. Complications in the oral region after head and neck RT can be manifold and include mucositis, hyposalivation, xerostomia, excess mucous and secretions, radiation-induced caries and periodontitis, oral infections, trismus, taste and smell disorders, pathologic fractures, radiation-induced stenosis of the carotid arteries with severe neurologic consequences, and osteoradionecrosis (ORN).[19] ORN emerges as a delayed radiation injury emanating from defective bone healing for longer than 3 months. This occurs in approximately 2-8% of cases, most commonly affecting the body of the mandible, supposedly because of compromised vascularization.[19-23] Typically, ORN develops 2 to 4 years after RT completion. In severe cases it can lead to facial deformities, barely manageable pain, pathologic fractures, sequestration of devitalized bone, and oral-extraoral or oroantral fistulae. Suffice it to say, therapeutic strategies need to be improved considerably in order to reduce side effects and ameliorate clinical outcomes.

Research concerned with advancing RT is addressing cellular mechanisms, physical principles, technical innovations, and clinical routines. Basic research in medicine often relies on animal models. They provide insight into disease mechanisms, allow determination of efficacy and safety of therapies, and represent unprecedented options for testing novel therapies. For obvious reasons, small rodents are most commonly used. The advantages are availability, handling and effort in housing, maintenance costs, low ethical concerns, ease of genetic modifications, and reproductive traits.[24] On the other hand, limitations of these model organisms compared to humans are enhanced metabolic rates, disparate genetic backgrounds due to inbreeding, little immunologic challenge due to sterile housing, and relatively short lifespans. Therefore, studies involving large animals, which are more closely related to humans, are being pursued. Apparently, nonhuman primates would be an optimal match. However, these do not reach sexual maturity before the age of 3 years and give birth at best once a year, with only few offspring per litter. Furthermore, they require very sophisticated maintenance and handling, and, for political reasons, experimentation on primates is often banned. In contrast, domestic pigs (Sus scrofa domestica) can produce up to 30 offspring per year and are widely available, easily maintained, and relatively inexpensive. Moreover, many aspects of their physiology and anatomy greatly resemble the human situation.[25-29] Also, due to technical constraints, large animal models are found to be better suited for testing new RT approaches, in particular because rodents are too small for defined irradiation of specific anatomic regions, especially the head and neck region. Domestic swine have already been applied in RT research in many aspects. Distinguished protocols have been established for RT procedures on skin, lung, and kidneys.[30-33] The previously established experimental procedures, employing radiation technology, date back 2 to 3 decades and have been further developed by introducing more sophisticated techniques and powerful machines. We here provide an operation procedure for irradiation of the mandible employing domestic pigs, describing all procedures and technical refinements, which should ease future large animal studies for the advancement of RT, especially for the treatment of malignancies of the head and neck region.

MATERIAL AND METHODS

Permission to perform experimentation on S. scrofa domestica (domestic pigs, weight ~60 kg) was granted by the Austrian Government and National Ethics Committee (permission no. BMBWK-66.011/0143-BrGT/2006). All experimental and analytical work concerning animal experimentation was conducted in accordance to the European Union directive 86/609/EEC.

CT scanning for planning of the irradiation was performed on a Philips Brilliance Big Bore CT scanner (Philips, Hamburg, Germany). The irradiation plan was created using the PrecisePLAN treatment planning system, release 2.12-477.08 (Elekta, Stockholm, Sweden). Irradiations were exclusively performed with a 6 MV Elekta SLi Precise linear accelerator.

The biologically effective dose (BED) was determined using the modified linear quadratic model (LQ-L), which can be used to elicit biological responses to different irradiation fractionation regimes, also when employing high single doses.[34] The formula for the BED is as follows:

$BED_n = D_T + D_T^2/(\alpha/\beta) + [(\gamma/\alpha)(D - D_T)]$, where γ is the proportion of killed cells per Gy in the final linear portion of the survival curve, D_T is the dose at which the survival curve becomes linear, and D is the dose applied per fraction. γ/α was calculated according the formula $\gamma/\alpha = 1 + (2\ D_T/(\alpha/\beta))$. D_T was estimated to be 2 α/β. The α/β ratio was assumed to be 3.85, a value that is typically used for late-reacting tissues such as bone.[35-37]

Electronic portal images were taken via the Elekta portal imaging system.

For sedation, the following drugs were used: azaperone (Eli Lilly, Indianapolis, IN, USA), atropine (Braun, Melsungen, Germany), ketamine (Intervet, Vienna, Austria), and propofol (Fresenius, Bad Homburg, Germany).

For histologic analysis, biopsies from 16 irradiated and 8 nonirradiated animals were taken from equivalent regions of the mandible 8 weeks after irradiation. After embedding in poly-methacrylate, blocks were crude sawed, grounded, polished, and stained with toluidine blue.