Evaluation of Radiation-Induced Oral Mucositis by Optical Coherence Tomography

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Abstract

Purpose: Optical coherence tomography (OCT) imaging was evaluated to determine if radiation-induced mucosal damage could be noninvasively monitored in real time and correlated with histopathologic findings.

Experimental Design: Female C3H mice, ages 7 to 9 weeks, four per group, were immobilized in a custom-made Lucite jig and received 0, 15, 22.5, and 25 Gy in a single fraction to their oral cavity. OCT images were acquired of proximal, middle, and distal aspects of the dorsum of the tongue on days 0, 1, 3, 5, and 7 post-irradiation. Animals were sacrificed on day 7 and samples taken for histologic evaluation. OCT images were visually examined and also quantified by image analysis and compared with histologic findings.

Results: Tongues removed 7 days post-irradiation showed no visible damage; however, upon staining with toluidine blue, ulcers at the base of the tongue became visible (100% for 25 Gy, 75% after 22.5 Gy, and 0% after 15 Gy). Visual inspection of OCT images qualitatively compared with histologic findings and quantitative image analysis of the OCT images (effective light penetration depth) revealed significant changes 7 days post-irradiation compared with unirradiated controls for the base of the tongue.

Conclusions: OCT allows for direct noninvasive real-time acquisition of digitally archivable images of oral mucosa and can detect radiation-induced changes in the mucosa before visual manifestation. OCT may be a useful technique to quantify subclinical radiation-induced mucosal injury in experimental chemoradiation clinical trials.

Recent phase III randomized trials have shown that intensification of radiation therapy treatment by means of altered fractionation schedules and/or combination with chemotherapy have improved local tumor control, organ preservation rates, and survival of patients with head and neck cancer (1–3). This significant improvement in local control, survival, and organ preservation comes at the expense of a complex process, which results from direct and indirect effects of radiation or chemotherapy on the basal epithelial cells of the oral mucosa. Sonis proposed a four successive phase process for mucositis. The frequency was nearly as high for patients treated with conventional radiation therapy and combined chemoradiation, 97% and 90%, respectively. Chemotherapy alone had a frequency of 22%. Radiation-induced mucositis is not a transient and isolated event. It has a mean duration of nearly 40 days (range, 7–98 days) and is translated into 69% frequency of oral pain, 56% frequency of dysphagia, at least 53% frequency of opioid use, mean weight loss of 3 to 7 kg, 15% frequency of feeding tube insertion and hospitalization, and finally 11% of patients experienced a modification or interruption of treatment (4, 5).

Radiation-induced mucositis occurs as a function of cumulative tissue dose. It typically begins at doses around 15 to 20 Gy of standard fractionated (1.8-2 Gy per fraction) radiotherapy. Ulcerative mucositis mostly occurs at doses of 30 Gy. This corresponds to a period about 2 to 3 weeks after the onset of radiotherapy. During this period, mucositis evolves from asymptomatic focal hyperemia and edema to symptomatic patchy, then confluent desquamation (6). Mucositis is a complex process, which results from direct and indirect effects of radiation or chemotherapy on the basal epithelial cells of the oral mucosa. Sonis proposed a four successive phase process for mucosal barrier injury (7). The damage to the epithelial basal cells induces an inflammatory phase with activation of early response genes and proinflammatory cytokines. This is followed by an epithelial atrophy phase where multiple pathways mediated by nuclear factor-κB, p53, and ceramide lead to epithelial cell apoptosis. Subsequently, an ulcerative
and invasive phase follows and finally proliferation of basal cells results in a healing phase (7, 8).

Optical coherence tomography (OCT) is a new and evolving imaging technology, which can acquire high-resolution, cross-sectional imaging of the internal microstructure of biological tissues (9). It is analogous to ultrasound, except that it measures the intensity profile of back-reflected near-IR light rather than sound waves. Thus, imaging can be done directly without requiring a transducing medium and contrast enhancing agents. OCT performs two- and three-dimensional imaging of tissue microstructure in situ and in real time (10, 11). It can achieve spatial resolution approaching the cellular level over approximately the same depths as a conventional biopsy (15 μm axial resolution, 1-2 mm depth, 30 μm transverse resolution; refs. 12–14). OCT has been used ex vivo and in vivo to image retinal tissue (15), gastrointestinal tract (11), respiratory tract, and circulatory system to distinguish among normal, premalignant, and malignant lesions in these various systems (16, 17). OCT imaging and evaluation of oral cavity has been done (18–21); however, OCT has not been applied to acute mucosal damage during cancer therapy, other than to show structural changes in vocal folds after remote therapy (22).

In this feasibility study, the ability of OCT to detect oral acute mucosal changes in mice following single-dose radiation exposure was assessed.

Materials and Methods

Animals. Female C3H mice, produced by the National Cancer Institute Animal Production Area (Frederick, MD), were used for this study. The mice were 7 to 9 weeks of age at the time of experimentation and weighed between 20 and 30 g. All experiments were carried out under the aegis of a protocol approved by the National Cancer Institute Animal Care and Use Committee and were in compliance with the Guide for the Care and Use Of Laboratory Animal Resource, (1996) National Research Council. Mice, four per group, were immobilized without using anesthetics in a custom-made Lucite jig and received 0, 15, 22.5, or 25 Gy in a single fraction to their head using a Therapax DXT300 X-ray irradiator (Pantak, Inc., East Haven, CT) using 2.0-mm Al filtration (300 kVp) at a dose rate of 1.9 Gy/min. Custom-made lead shields limited the radiation to the head. Immediately after irradiation, animals were removed from the jig, housed in a climate and light/dark controlled environment, and allowed free access to food and water.

Optical coherence tomography imaging, sample collection, and histologic staining. Animals were examined daily for mucositis/ulcer formation and general appearance. OCT images were acquired using an Imalux OCT imaging system (Imalux Corp., Cleveland, OH) equipped with a flexible fiberoptic probe (≤2.2-mm free space depth scanning range, corresponding to 1.6-mm depth normalized to typical tissue refraction index of 1.4, 1.6-2.4 mm lateral scanning range). The OCT system operates at central wavelength 980 nm, with in-depth resolution of 14 μm (in free space units) and lateral resolution of 25 μm (beam waist diameter). The acquisition time for a 200 x 200 pixel image was 1.3 seconds. Images were acquired on days 0, 1, 3, 5, and 7 post-irradiation. For image acquisition, mice were anesthetized by administration of Ketaset (Ketamine; 100 mg/mL, Fort Dodge Animal Health, Fort Dodge, IA), Xyla-ject (Xylazine; 20 mg/mL) in sterile water by i.p. injection (10 μL/10 g, Phoenix, St. Joseph, MO). The tongues were gently extended using padded forceps and the fiber optic probe was gently pressed perpendicular to the surface of the tongue. This procedure did not cause any apparent damage to the tongue based on comparison of histologic sections of control animals over the course of the study. Images were made at the general area of the tip of tongue, middle of tongue, and base of tongue throughout the time course of the study. The time required for image collection at each site was ~30 seconds. Following image acquisition and recovery from anesthesia, mice were returned to their cages. On day 7 following the final image acquisition, animals were sacrificed by cervical dislocation. Tongues were stained in a solution of 1% toluidine blue in 10% acetic acid and analyzed macroscopically. Repeated wiping with gauze soaked in acetic acid was continued until there was no further recovery of dye. A negative result is indicated by no dye uptake or light, diffusely stippled uptake of dye. A positive result, identified as lack of epithelium and therefore an ulcer, is indicated by deep, royal blue staining in epithelium defects. Next, tongues were processed and embedded in paraffin, 3-μm sections were stained with H&E and microscopic analysis was conducted. Sections were made for each of the three general areas that OCT images were collected.

Optical coherence tomography image analysis. Temporal changes as a result of radiation treatment of tongue mucosa were made by visual comparison of OCT images and by using computer software to quantify changes in light penetration of the tissue. An NIH developed image analysis application was used to quantify data from the OCT image samples. The Medical Image Processing Analysis and Visualization (MIPAV) application enables quantitative analysis and visualization of biomedical imaging modalities, from micro to macro data sets (23). A new, specialized, and fully automatic plug-in was developed to quantify the effective light penetration depth of the tissue of interest from the OCT images. A 3 x 3 median filter was applied to reduce the noise followed by an automatic-thresholding technique to segment the inherently bimodal image data into two distinct regions, background and tissue of interest (24). The length of each pixel column (i.e., effective light penetration depth) was calculated and combined to form an average effective light penetration depth. Gaps in the binarized pixel column >5 pixels terminated the length calculation for the column. Because the algorithm is fully automated, the results are user invariant. The average effective light penetration depth was used for statistical comparison of the serially acquired OCT images. Values for light penetration depth for each day 0, 1, 3, 5, and 7 were compared and normalized to the untreated controls for each particular day. Three mice were used for all time and dose points. Means and SDs were calculated and used in a two-tailed Student’s t test with unequal variances. P values were then determined from the t values derived as shown in Fig. 4.

Results

Comparison of optical coherence tomography image with histologic section. Figure 1 shows a comparison of an OCT image taken at the base of tongue of a control animal and a corresponding histologic section. It was not possible in the present study to exactly align OCT images with histologic sections; however, in all cases, the histologic sections were made in the general vicinity of the OCT image. Histologically (right), the normal stratified squamous keratinized epithelium consists of several layers of cells. The cells of the basal layer are located just above the basement membrane. The succeeding prickle, granular, and squamous layers are superficial to the basal layer and consist of polyhedral cells that become flattened scales and keratin is formed and shed from the surface. The dorsal surface of the tongue shows hair-like filiform papillae formed by epithelium and keratin placement. The basement membrane is a thin mat of extracellular matrix that separates epithelial sheets from connective tissue. It is composed of mainly of type IV collagen, laminin, nidogen, and heparin sulfate proteoglycan. The basement membrane can be clearly visualized on the OCT images as a clear line projected between
the surface epithelium and the stroma (left). Supporting stroma is composed of connective tissue that contains muscle fibers, vascular, and neural elements and can include glandular structures. It should be noted that filiform papillae are not visible on the OCT image because the probe, when pressed against the surface of the tongue, flattens these structures.

**Visualization of radiation-induced ulcer formation.** From pilot studies, it was determined that radiation doses of 22.5 and 25 Gy produced ulcers at the base of tongue 7 days posttreatment, whereas a dose of 15 Gy did not (data not shown). The ulcers produced by the higher radiation doses were not clinically visible on day 7 posttreatment (see Fig. 2), unless the tissue was stained by toluidine blue, used in clinical practice for oral cancer screening (25) and evaluation of gastric mucosa (26, 27). Figure 2A-C shows control tongues with and without toluidine blue staining along with a
histologic section of normal mucosa. Figure 2D shows the tongue from an animal that received 25 Gy 7 days before tissue acquisition, which seems normal visibly. However, Fig. 2E shows that toluidine blue staining reveals an ulcer at the base of the tongue with marked tissue damage as shown in the histologic section (Fig. 2F). Figure 2F shows an ulcerated lesion on base of tongue covered by necrotic fibrinoid tissue, with complete depletion of the stratified squamous keratinized epithelium. The connective tissue shows an acute inflammatory infiltrate among the muscle fibers. None of the animals in the 0 and 15 Gy groups developed an ulcer during the course of the experiment (7 days). By day 7 post-irradiation, four of four (100%) mice from the 25 Gy dose group developed an ulcer on the dorsum of the tongue. Three of four mice (75%) in the 22.5 Gy dose group developed an ulcer at the same location on day 7.

**Optical coherence tomography and histologic comparison post-irradiation.** OCT images were collected on days 1, 3, 5, and 7 post-irradiation. Figure 3 shows a series of OCT images and accompanying histologic sections from the base of the tongue for animals exposed to three different doses of radiation 7 days posttreatment (columns A and B). Also shown in Fig. 3 are histologic sections taken from the middle and tip of tongue (columns C and D). Visual examination of OCT images revealed a thinning of the upper epithelial layer as a function of radiation dose by day 7 post-irradiation with a loss of stratification between layers; in particular, a loss of the basement membrane was clearly evident for 22.5 and 25 Gy treatments. Examination of the corresponding histologic sections revealed changes in the epithelium thickness and loss of filiform papillae for 15 Gy. More severe changes were noted for 22.5 and 25 Gy treatments, consisting of a complete loss of the keratinized filiform papillae, decrease in mucosal thickness, and overall breakdown of the epithelia creating an ulcer consisting of a thin layer of necrotic fibrinoid material at the base and margins of the ulcer and regions containing

![Fig. 3. OCT images taken at the base of tongue (A) and corresponding histologic samples taken from base of tongue (B), middle of tongue (C), and tip of tongue (D) after different radiation doses. OCT images and corresponding histologic sections were taken from the same mouse for each condition.](image-url)
inflammatory cells and active granulation tissue. OCT images are not shown for the other regions of the tongue, but there was a radiation dose-dependent increase in tissue damage for the middle and tip of tongue regions, as shown by histology in Fig. 3 (columns C and D).

**Time course changes in effective light penetration depth of optical coherence tomography images.** Whereas the OCT images shown in Fig. 3 clearly provide an indication of visual changes as a function of radiation dose at the base of tongue area, a more quantitative assessment of these changes was desired. Each OCT image was evaluated using the MIPAV application with a specialized plug-in to enable quantitative analysis with respect to light penetration depth. OCT images collected from the tip, middle, and base of tongue from control and irradiated animals over the 1- to 7-day time course were evaluated. Light penetration depth data derived from each OCT image of each radiation group was expressed as a percentage of control values as shown in Fig. 4. The gray area on each plot represents the range of untreated control values (mean ± SD) over the time course. Figure 4A shows that despite the radiation-induced changes observed at the tip of the tongue for all three radiation doses (Fig. 3, column D), OCT images were unable to detect this damage over the time course using MIPAV analysis. In contrast, Fig. 4B and C shows that OCT images could both before and by day 7 post-irradiation detect significant radiation-induced damage for the middle (P ≤ 0.046) and base of tongue (P < 0.005) as measured by light penetration depth. Detection of changes by OCT occurred before visual changes were observed. Whereas MIPAV analysis of OCT images did not show differences for the tip of tongue region over the time course (Fig. 4A), close visual inspection of the images clearly revealed that changes as a result of the radiation treatment could be discerned (see Fig. 5). Classic interpretation of OCT images is based on visualization of two-dimensional, cross-sectional tissue microstructure. Figure 5 provides a typical set of image examples, significant to this study, showing tip of tongue OCT images of a control mouse (A) and (B) mouse treated with 25 Gy. The progression from days 1 to 7 provides clear visual indication that mucosal tissue was significantly damaged. Note complete lack of visualization of the basal membrane by day 7 in the irradiated mouse.

**Discussion**

OCT images reveal internal microstructures in biological tissue and provide a noninvasive and rapid technique requiring <10 seconds per image acquisition (12). OCT offers the possibility of continuous noninvasive monitoring of mucosal changes without recourse to biopsy (12). This unique feature could be extremely useful in the field of chemoradiation therapy. Although chemoradiation has brought significant improvement in local tumor control, organ preservation, and survival in patients with cancer, it has come at a price of increased toxicity with normal tissues such as oral and gastrointestinal mucosa (1, 3, 28–32). To date, OCT has mainly been used in oncology to compare normal tissue with dysplasia, metaplasia, carcinoma in situ, and invasive lesions for diagnostic purposes (12, 14). OCT is also used in various organs to assess epithelial changes (33–35).

In the present feasibility study, we assessed the ability of OCT to detect changes in effective light penetration depth of the tongue in a rodent model of radiation-induced mucositis. Radiation-induced changes in the mucosa were detected both by visual inspection of the OCT images and quantitative image analysis with MIPAV of OCT images with respect to a decrease in the effective light penetration depth of the mucosa.
in effective light penetration depth. The use of the MIPAV analysis of the images is important in that changes can be quantified rather than relying on subjective visual image interpretation, with exception to measurements at the tip of the tongue. The unique finding of the study was that significant changes in the mucosa as registered by the OCT images could be discerned before visible macroscopic manifestations (ulcers) became apparent.

To our knowledge, this is the first use of OCT to evaluate radiation-induced acute mucosal damage and the use of MIPAV to quantify the changes in the mucosa. Whereas the depth of tissue that can be interrogated by OCT is limited (1-2 mm) and the resolution currently is not at the cellular level, OCT may have use in experimental chemoradiation trials where experimental drugs (radiation sensitizers or protectors) are evaluated in a dose escalation fashion. OCT could be used in such trials to noninvasively evaluate normal tissue toxicity and thus avoid reaching high-grade toxicity. Additionally, it is possible that OCT images of irradiated tissues may have some predictive value in determining whether late effects will be experienced and also may be of use in determining the efficacy of radiation protectors. These latter points will obviously require additional preclinical studies; however, the positive results of this preliminary study warrant a more extensive assessment of potential uses of OCT in experimental radiation oncology.

**Conclusions**

OCT can detect post-irradiation, premacrosopic mucosal changes both by visual inspection of the images and by quantitative image analysis in a murine radiation-induced mucositis model. OCT may be useful for real-time monitoring of tissue injury during experimental radiation and combined modality trials where a systematic study of novel radiation modifiers could be evaluated noninvasively without reaching high-grade toxicity.

**Fig. 5.** Examples of serial OCT images taken on the tip of tongue (TOT) area from days 1 to 7 for (A) control, untreated mouse and (B) mouse treated with 25 Gy on day 0.

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