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Original Research

Photodynamic diagnosis of oral carcinoma using talaporfin sodium and a hyperspectral imaging system: An animal study

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ABSTRACT

The purpose of this study was to examine the efficacy of detecting oral squamous cell carcinoma (SCC) using photodynamic diagnosis (PDD), a highly reliable method for tumor diagnosis, through the use of photosensitizers such as talaporfin sodium. Talaporfin sodium (5 mg/kg) was administered to 7 control rats and 43 rats that had ingested the carcinogen 4-nitroquinoline 1-oxide (4NQO). After 8 h, tongues were examined by spectral imaging and measurements were made using a hyperspectral imaging system. On each spectral image, macroscopically lesions and normal regions of the tongue were selected. Lesions comprised squamous cell carcinoma (SCC) in 22 cases, carcinoma in situ (CiS) in 9 cases, and dysplasia in 12 cases. The value $\Delta\alpha$ was determined as the absorbance spectrum of a lesion compared with normal tissue, or as the difference between $\Delta\kappa$ at the posterior of the lingual protrusion and $\Delta\kappa$ at the anterior of the lingual protrusion in controls. We compared $\Delta\alpha$ for SCC, CiS, and dysplasia with controls. The value $\Delta\alpha$ was $(2.4 \pm 1.78) \times 10^{-2}$ in SCC, $(1.2 \pm 1.34) \times 10^{-2}$ in CiS, $(-0.2 \pm 0.49) \times 10^{-2}$ in dysplasia, and $(0.21 \pm 0.63) \times 10^{-2}$ in controls. Both SCC and CiS showed significant differences from controls ($p = 0.037$ and $p = 0.021$, respectively). Histopathological analysis revealed that proliferating cell nuclear antigen (PCNA) expression increased gradually in the following order: controls, dysplasia, CiS, and SCC. Development of PDD using a hyperspectral imaging system may represent a useful technique for detecting oral SCC.

1. Introduction

In the diagnosis of oral squamous cell carcinoma (SCC), biological staining using solutions such as iodine or toluidine blue has been performed to ascertain the location of the SCC. In our department, iodine stain has also been used to determine the region for surgical resection. However, iodine binds with cytoplasmic glycogen granules, and since telomerase activities tend to be higher in SCC and atypical cells than in normal cells, the resulting glycogen deficiency prevents staining by iodine [1]. Conversely, toluidine blue stains atypical cells, but not normal tissues [2]. However, limitations exist when using these dyes for diagnostic purposes for example, such stains cannot be used in the detection of gingival SCC. In addition, since deeper regions of the tumor tissue are not penetrated, some tumor cells may remain after surgical resection, increasing the risk of recurrence. For these reasons, more sensitive methods have been sought for the diagnosis of oral SCC. Focus has recently been placed on the use of photosensitizers that accumulate in SCC as agents to facilitate photodynamic therapy (PDT) and photodynamic diagnosis (PDD) [3].

Photosensitizers exert cytotoxic activities upon laser irradiation and cause antitumor effects through tumor vessel occlusion [4]. Photosensitizers show high affinity towards molecules that are rich in lipoproteins, and thus have been shown to be incorporated more intensely in tumors over normal tissues [5]. Talaporfin sodium (Laserphyrin®; Meiji Seika, Tokyo, Japan) is a photosensitizer that accumulates quickly in SCC and is excreted more rapidly from the body than photosensitizers such as porfimer sodium, so side effects should be reduced [6]. The absorbance spectra of the tetrapyrrole ring of talaporfin sodium in phosphate-buffered saline (PBS) (pH 7.2) are in the Soret band (398 nm) and Q band (502, 530, 620 and 654 nm) regions. When absorbed by SCC, biomolecular binding and displacement shift the absorbance spectra to the higher wavelength side by 10 nm [7]. The absorbance spectra of SCC containing talaporfin sodium are thus examined at a wavelength of 664 nm. Since this wavelength is higher than the absorption bands

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of biological elements such as hemoglobin, effects on normal tissue are minimal [8].

PDD is a technique in which a low laser is irradiated to visually confirm fluorescence emitted by excitation of photosynthesizers such as talaporfin sodium that have accumulated in the SCC [9]. In lung cancers, PDD is used as an important tool to ascertain tumor lesions during surgical resection [10]. However, PDD is not always reproducible, as the images often lack high resolution. Since the laser used in PDD is a single wavelength and difficult with dispersal of light, PDD under monochromatic light from the irradiating laser does not clarify the edges of the lesion. To overcome these limitations, hyperspectral imaging systems have been incorporated into PDD [11]. A hyperspectral imaging system uses a halogen lamp to irradiate the target with a wide range of wavelengths, allowing dispersion of light and subsequent assembly of spectral images. The light source for the hyperspectral imaging system is a halogen lamp with line spectrum. The images collected by the hyperspectral imaging system can quantify total light reflected from samples with uptake of photosensitzers [12]. The purpose of this study was to examine the efficacy of PDD using talaporfin sodium and a hyperspectral imaging system in detecting oral SCC.

2. Materials and methods

This study was conducted in compliance with the Guidelines for the Treatment of Experimental Animals at Tokyo Dental College.

2.1. Experimental animals

A total of 69 male Sprague–Dawley rats (body weight, approximately 300 g) were used in this study. The carcinogen 4-nitroquinoline 1-oxide (4NQO; Tokyo Kasei, Tokyo, Japan) was reconstituted using the methods described by Katakura et al. [13]. Briefly, stock solution was prepared by dissolving 1.0 g of 4NQO in 50 ml of ethanol and 4,950 ml of distilled water, then stored at room temperature in the dark. The stock solution was diluted with tap water to a final concentration of 50 ppm, and this diluted solution was placed in light-resistant water bottles to be administered to 62 rats for 7 consecutive months [14]. The remaining 7 rats were used as controls by providing plain tap water instead of 4NQO-containing water. Both groups were provided with ad libitum access to food, as suggested in the experimental animal study guidelines.

2.2. Hyperspectral imaging system

The hyperspectral imaging system consists of an imaging spectrograph (V10-13-203; SPECIM, Oulu, Finland) attached to a charge-coupled device (CCD) camera (Texas Instruments, Tokyo, Japan), an objective lens and a motor-driven scanning stage [15]. A halogen lamp (BMAC 2344 and 2342; Japan PI, Tokyo, Japan) was used as a light source (Fig. 1). A line (width, 30 μm) on an object was focused onto the entrance slit of the imaging spectrograph. Light signals from all points along the line on the target object were dispersed by the imaging spectrograph along the direction perpendicular to the line (in the direction of the slit), and then output spectra were focused onto the CCD sensor and recorded in a single CCD frame (Fig. 2A). Both spatial and spectral resolutions were determined by the pixel size of the CCD sensor. Synchronizing the velocity of the scanning stage to the frame rate of the CCD sensor, spectra of all points on a two-dimensional (2D) image were acquired (Fig. 2B). In the present case, one dataset per single CCD frame contained spectral bands for ultraviolet spectral points and infrared rays. Spatial resolution was 50 μm in the longitudinal direction and 80 μm in the transverse direction on the 2D image (Fig. 2C). Spectral resolution was 0.93 nm. The frame rate of

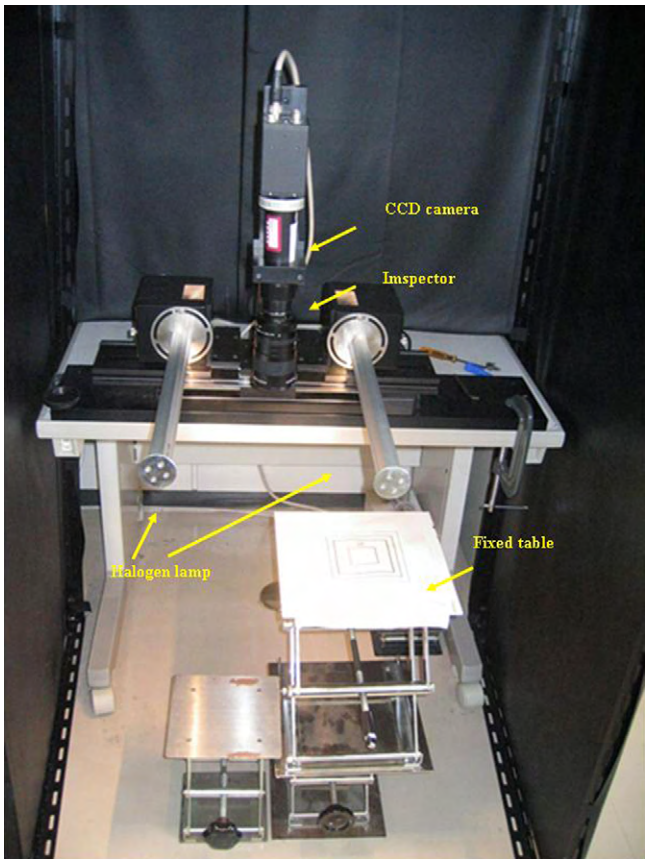

Fig. 1. Hyperspectral imaging system. The object was set on the fixed table and irradiated by the halogen lamp. The reflected lights were assembled to obtain spectral images, which were recorded by the CCD camera.

30 fps for the CCD sensor allowed the acquisition of all spectra of 480 × 321 pixels on the 2D image within 10 s (Fig. 2D). The spectral features of the present system restricted meaningful spectra to the wavelength region of 400–1,000 nm. A currency of measurements depends on the spectral characteristics of the system. A well-defined white plate reference (Spectralon, SRT-99-120; Labsphere) was used to calibrate spectra. The dark current correction was also made. The reflectance spectrum of the i-th pixel, $R(i,\lambda)$, was calculated as

$$R(i,\lambda) = \frac{I_{\text{object}}(i,\lambda) - I_{\text{dark}}}{I_{\text{ref}}(\lambda) - I_{\text{dark}}}$$

where I_{object} (i,λ) represents the light intensity measured at the i-th pixel, $I_{\text{ref}}(\lambda)$ is the light intensity measured using the white reference, and I_{dark} is the dark current intensity. The reflectance spectrum $R(i,\lambda)$ was converted to the absorbance spectrum $\alpha(i,\lambda)$ using Lanbert–Beer's law [16]:

$$\alpha(i,\lambda) = -\log_{10} R(i,\lambda)$$

2.3. Talaporfin sodium administration and absorbance spectra measurement

All rats, including controls, received 5 mg/kg of talaporfin sodium dissolved in 0.1 ml of physiological saline solution through the femoral vein under etherization and were kept in the dark with ad libitum access to food and water. At 8 h after administration of talaporfin sodium, 5 mg/kg of pentobarbital was administered intraperitoneally, and light absorbance by rats was measured and recorded by CCD camera on a fixed table.