Photofrin-mediated Photodynamic Therapy of Rat Palatal Mucosa: Normal Tissue Effects and Light Dosimetry

J.M. NAUTA a, H.L.L.M. VAN LEENGOED b, M.J.H. WITJES b, J.L.N. ROODENBURG a, P.G.J. NIKKELS c, S.L. THOMSEN d, J.P.A. MARIJNISSEN b, W.M. STAR b

aDepartment of Oral and Maxillofacial Surgery, University Hospital Groningen, The Netherlands
bDepartment of Clinical Physics, Dr Daniel den Hoed Cancer Centre, Rotterdam, The Netherlands
cDepartment of Pathology, University Hospital Groningen, The Netherlands
dLaser Biology Research Laboratory, Departments of General Surgery and Pathology, M.D. Anderson Cancer Center, Houston, Texas, USA

Correspondence to J. M. Nauta, Department of Oral and Maxillofacial Surgery, University Hospital Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands

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Abstract. Photodynamic therapy (PDT) is a treatment modality with potential application for premalignant lesions and squamous cell carcinoma of the oral mucosa. PDT in principle has dual selectivity. This may result from a 'preferential' retention of the photosensitizer in target tissue. In addition, the photodynamic activity will be limited to the irradiated area because PDT will not affect tissues in the absence of excitation light. The specificity of PDT is limited by the fact that normal tissues also retain the photosensitizer to some degree, which makes these tissues susceptible to PDT damage. To optimize PDT for oral malignancies, a study was undertaken on normal tissue to investigate the responses in rat palatal mucosa and surrounding anatomical structures. Eighty male Wistar rats were used in the study. Photofrin was administered i.v. at four doses (0, 2.5, 5 or 10 mg kg⁻¹ body weight). Irradiation for PDT was performed 24 h later. An argon pumped dye laser system was used to produce light of two different treatment wavelengths (514.5 and 625 nm), and various energy density levels (0, 25, 50, 100 or 200 J cm⁻²). Early effects of PDT were studied at 2 days and late effects at 2 months after treatment. Twenty-four hours after i.v. administration of Photofrin, it was found that PDT affects normal tissues of the oral cavity both macroscopically and microscopically. Combinations of photosensitizer doses ≥5 mg kg⁻¹ and light doses ≥100 J cm⁻² caused severe and permanent damage to the palatal mucosa and adjacent normal structures such as palatal bone and dentition.

Light scattering and internal reflection usually raise the fluence rate in tissue above the irradiance of the incident beam. In an additional study using six male Wistar rats, the energy fluence rate at two treatment wavelengths (514.5 and 625 nm) was measured ex vivo in the palatal mucosa and adjacent anatomical structures. As expected, the energy fluence rates were wavelength, tissue and depth dependent. At the air–mucosa boundary, light of 625 nm was found to have a three-times higher fluence rate than the primary incident beam. Under similar conditions, the fluence rate of 514.5 nm was found to be less, but still twice as high as the primary incident beam. At deeper levels of the rat maxilla, fluence rates were still elevated compared with the incident beam. For 625 nm light, this phenomenon was observed up to the level of the nasal cavity. These increased fluence rates could largely explain the pattern of damage to normal mucosa and surrounding anatomical structures.

INTRODUCTION

Photodynamic therapy (PDT) is a treatment modality with potential application for premalignant lesions and squamous cell carcinoma of the oral mucosa. PDT is based on the dye-sensitized photo-oxidation of biological matter in the target tissue (1). The photosensitizers Haematoporphyrin derivative (HpD), or more commonly Photofrin (the semi-purified version...
of HpD, enriched in the 'active fraction'), are frequently used for PDT. Currently, a number of photosensitizers are being clinically tested, but to date Photofrin is the only photosensitizer that has been approved for a limited number of indications in the USA, Canada, Japan and the Netherlands. The advantage of PDT over conventional surgical or radiotherapeutical treatment may be its potential dual selectivity. Selectivity may be obtained by a 'preferential' retention of the photosensitizer in target tissue. In addition, the photodynamic activity will be limited to the irradiated area because PDT will not affect tissues in the absence of excitation light. However, the selectivity of Photofrin is far from ideal because normal tissue also retains the photosensitizer to some extent, and is, therefore, susceptible to PDT damage (2-6). Knowledge of the morphological alterations induced by PDT in the area of the tumour and in the region of the adjacent normal tissues is therefore important with respect to the early and late complications of PDT (7). Furthermore, it is important to determine the optimal combination of photosensitizer and light dose that will result in minimal or at least reversible damage to the surrounding normal tissues. Proper knowledge of light dosimetry is, therefore, needed in order to compare, reproduce and predict the effects of PDT, and to establish the factors that determine success or failure. However, so far little attention has been paid to light dosimetry in clinical PDT (8-11). Frequently, the distribution of the photosensitizer in tissue remains unknown, and consequently, the actual light dose absorbed by the photosensitizer is unknown. In PDT of superficial tumours, the incident power per unit area (W m\(^{-2}\)) multiplied by the irradiation time is used to describe the light dose. In tissue, the energy fluence rate can vary, however, due to the phenomena of light scattering and internal reflection at tissue boundaries. In oral tissue there are potentially many boundaries which could influence the homogenous distribution of light, eg the air–mucosa and mucosa–bone boundaries. Better knowledge of the distribution of light in the treated tissues can be obtained by calculations using a mathematical description of light propagation in tissue, using estimates of the scattering properties and optical absorption of the treated tissue, and by in vivo measurement of the fluence rate of light (12-15). This may help to understand the treatment results.

Red light in the range of 625-630 nm is most commonly used in clinical PDT with porphyrin-based photosensitizers such as Photofrin. Although porphyrins have a low absorption at these wavelengths, this excitation wavelength is often chosen because of its increased optical penetration compared with light of shorter wavelengths. For PDT of small, superficially growing tumours, the use of 514.5 nm green light might be preferred. The porphyrin molecule can be excited at a wavelength where it has a higher absorption, and damage to anatomical structures beyond the target volume can be minimized (16-18).

The aim of this study was two-fold. First, to describe the effect of PDT on normal rat palatal mucosa and surrounding normal tissues; various combinations of photosensitizer and light doses at two different treatment wavelengths were compared. Second, to describe light fluence as a function of wavelength and site in the normal rat palate and adjacent anatomical structures using the two most common excitation wavelengths for Photofrin excitation, and to compare this with the treatment results.

**MATERIALS AND METHODS**

**Animals**

Eighty healthy, 6-8-week-old male Wistar rats (CDL-Groningen, The Netherlands) were used in this study. The rats were randomly divided into two groups of 40 rats. One group was treated with light of 625 nm, based on the study of Star et al (12), and the other group was treated with light of 514.5 nm. Each group of 40 rats was again randomly divided into 10 groups of four rats. Each group of four rats was treated with a different combination of photosensitizer dose and light dose (Table 1). In this way, it was possible to compare the effects of PDT at different excitation wavelengths using the same photosensitizer dose and light dose combinations.

**Excitation light**

A tuneable argon pumped dye laser system (Spectra-Physics, 171 and 375B) was used to produce 514.5 nm (green) light or 625 nm (red)