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

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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 radiotherapeutic 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 irradition 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). During administration of the photosensitizer and during photodynamic therapy, the animals were anaesthetized with a combination of N_2O/O_2/Halothane. Approval for this study was obtained from the University Animal Experiments Committee (FCC-0472).

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)

Table 1. Summary of the treatment plan of the effect of PDT on normal rat palatal mucosa and surrounding anatomical structures

Protocol number	Number of rats	Photofrin dose (mg kg^{-1} bw)	Incident light dose (J cm^{-2})
I	8	0	200
II	8	2.5	200
III	8	2.5	100
IV	8	5	100
V	8	2.5	50
VI	8	5	50
VII	8	10	50
VIII	8	5	25
IX	8	10	25
X	8	10	0

The irradiance of both treatment wavelengths (514.5 and 625 nm) was 60 mW cm^{-2} and the photosensitizer-light interval was 24 h. Half of the animals of each treatment protocol were killed after 2 days to examine the short-term effects. The remaining rats were kept alive up to 2 months after treatment to examine the long-term effects.

light. The fluence rate of the incident beam for both wavelengths was 60 mW cm^{-2}, and the light dose was 0–200 J cm^{-2} (Table 1). A light delivery system was developed which enabled the delivery of light to an area of about 10 mm in diameter, corresponding with the intermolar area of the rat palate (19).

Photodynamic therapy

Each rat was photosensitized with either 0, 2.5, 5 or 10 mg kg^{-1} bw Photofrin (Quadra Logic Technologies, Vancouver, BC, Canada), by intravenous injection in the tail vein. The animals were housed under reduced light conditions to avoid unwanted photodynamic action. Twenty-four hours after administration of the photosensitizer, the palates were irradiated. From every treatment group, two rats were killed 48 h after PDT and two after 2 months, to examine both short- and long-term effects. The rats were killed by an intracardial injection of sodium pentobarbital (Euthesate).

Histological preparation

The palate, including the surrounding hard and soft tissues, was removed in one piece and photographed. The specimens were fixed in 4% formalin, and subsequently decalcified for approximately 4 weeks in 25% formic acid with 0.34 M trisodium citrate dihydrate. The degree of decalcification was checked by X-ray analysis. The palates were dehydrated and embedded in paraffin. Histology slides of 7 μm were cut transversely through the region of the second molar. The slides were stained with Haematoxylin and Eosin (HE) for examination by light microscopy.

Macroscopic scoring system

Changes in the macroscopic appearance of the dissected palates 2 days after PDT treatment were given a numerical score: 0=no clinical effect, 1=slight erythema, 2=marked erythema, 3=ulceration or necrosis of the epithelium (focal), 4=ulceration or necrosis of the epithelium (entire area), 5=denuded bone.

Subsequently, the macroscopic appearance of the palate 2 months after PDT was given a score: 0=no clinical effect, 1=epithelium intact, scar tissue (locally), 2=epithelium intact, scar tissue (entire area), 3=ulceration or necrosis of the epithelium, 4=denuded bone (locally), 5=denuded bone (entire area).

Microscopic scoring system

The histological changes were described using two grading systems. One described the short-term histological effects (2 days after PDT), the other the long-term histological effects (2 months after PDT). In both grading systems, effects seen in the mucosa, the palatal bone and in structures beyond the palatal bone were evaluated separately. Each item of both systems was given a numerical score which varied from 0 to 2 (0=no effect, 1=slight effect, 2=marked effect). The overall PDT-induced damage score is summarized by adding up these individual scores. For the short-term effects (2 days after PDT), the maximum possible score of this grading system was 54; for the long-term effects (2 months after PDT), this was 40.

Microscopic changes 2 days after PDT were examined for the following items:

Mucosa: oedema, haemorrhage, parakeratosis, partial or total necrosis of the epithelial layer, vascular oedema, congestion or haemostasis of vessels, vascular thrombosis, vascular

Fig. 2. Diagram showing the results of the isotropic probe measurements of the energy fluence rates of 514.5 and 625 nm excitation light at various sites of the oral cavity and surrounding structures, with the palatal mucosa in situ. The fluence rate of the primary incident beam (calibrated in air) was 100 mW cm^{-2}. The beam diameter was 10 mm.

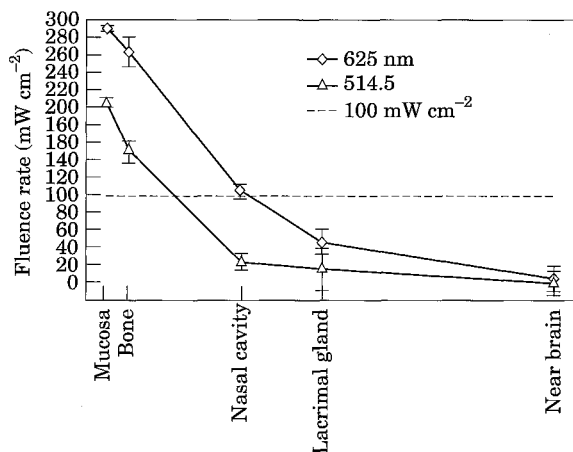mucosa and the light that is scattered by the mucosa only.

RESULTS

During follow-up, the rats were weighed twice a week. After an initial slight loss of weight during the first week after treatment, all rats showed an increase in weight, but no correlation was found between weight and treatment parameters.

Dosimetry

Figure 2 shows a line diagram of the fluence rates of the rat palatal mucosa and surrounding structures for both excitation wavelengths. The measurements plotted in this graph were taken with the mucosal layer in situ. The fluence rates at 625 nm were always higher than those of 514.5 nm, and found to be significantly different in a paired Student's t-test ($p < 0.05$). At the surface of the mucosa, the fluence rate at 625 nm was almost three times higher than that of the primary incident light beam. At the mucosa–bone boundary, the fluence rate was more than 2.5 times higher. The fluence rate of light at 514.5 nm was two times higher at the surface of the mucosa, and at the mucosa–bone boundary exceeded the irradiance of the incident beam.

In Table 2, the actual fluence rates and additional measurements taken after dissection of the palatal mucosa are listed. Overall results indicate that both mucosa and palatal bone contributed considerably to light scattering and internal reflection. The measurements taken after dissection of the palatal mucosa showed that the average fluence rate of 514.5 nm light at the mucosa–air boundary was about 0.8 times the value measured while the palatal mucosa was still attached to the palatal bone. Under similar conditions, the average fluence rate of 625 nm light was about 0.7 times the value measured while the palatal mucosa was still attached to the palatal bone. Measurements underneath the dissected palatal mucosa also revealed a decrease of the actual fluence rate of 0.8 and 0.7 times for 514.5 nm and 625 nm, respectively.

Macroscopic changes

Figure 3(a,b) depicts the mean overall macroscopic damage score 2 days after treatment of the normal palate with various combinations of photosensitizer and light doses for both treatment wavelengths. As expected, the effect on the normal palate and surrounding structures of the oral cavity was found to be both sensitizer- and light-dose dependent. Higher sensitizer doses and higher light doses resulted in more damage than combinations in which lower drug, or lower light doses, were used. Except for one rat in which locally denuded bone was evident, the most severe damage of the mucosa presented as ulceration or necrosis with haemorrhage and oedema of the entire treated area. When no sensitizer or no light was applied, no clinical damage of the mucosa of the intermolar area was observed. Two days after PDT, no real difference was observed in the amount of macroscopic damage between the two treatment wavelengths.

In Fig. 3(c,d) the mean overall macroscopic damage score at 2 months after treatment is shown. For most of the combinations of sensitizer–light doses, the epithelium of the intermolar area had macroscopically regenerated to an intact epithelial layer. In the majority of cases, however, scar tissue was evident. In these cases, the appearance of the normal anatomy of the intermolar area was disturbed. The rugal pattern had disappeared and the palatal mucosa gave a more whitish dense impression in which the underlying palatal

Table 2. Isotropic probe measurements of the fluence rates of light of 514.5 and 625 nm at various sites of the rat oral cavity and surrounding structures

| | 514.5 nm wavelength | | 625 nm wavelength | |
	Mean	s.e.m. (%)	Mean	s.e.m. (%)
Before dissection of the palatal mucosa				
Air–mucosa boundary (A*)	207	6	291	3
Mucosa–bone boundary (B*)	153	13	265	8
Nasal cavity (C*)	25	9	106	9
Lacrimal gland-near molars (D*)	17	24	48	14
Lacrimal gland-near brain (E*)	1	14	6	15
After dissection of the palatal mucosa				
At the mucosa (black background)	166	7	207	3
Under the mucosa (black background)	129	6	189	6
On denuded palatal bone	194	6	287	4
Nasal cavity	55	14	158	7

The fluence rate of the primary beam (calibrated in air) was 100 mW cm^{-2}. Measurements were taken both before and after dissection of the palatal mucosa to examine the specific influence of mucosa and bone on the fluence rates. A*–E* corresponds with the sites as depicted in Fig. 1.

Fig. 3. The overall macroscopic damage scores 2 days and 2 months after PDT. (a) Two days after PDT, at 514.5 nm. (b) Two days after PDT, at 625 nm. (c) Two months after PDT, at 514.5 nm. (d) 2 months after PDT, at 625 nm. The x-axis represents the light dose in Jcm^{-2}, the y-axis the damage score, and the z-axis the Photofrin dose in mg kg^{-1} bw.

blood vessels were no longer visible. In three rats, the epithelial integrity was disturbed with the denuded palatal bone clearly visible. Two of these rats were treated with 625 nm, the other with the 514.5 nm, all in Protocol IV. The absence of photosensitizer or light in the initial treatment caused no observable damage, resulting in normal macroscopic appearance of the palatal mucosa. Overall, no apparent differences were found between the two treatment wavelengths in the amount of macroscopic damage at 2 months.

Microscopic changes

Figure 4(a,b) shows the mean overall microscopic damage score of the effect of PDT on the normal palate and surrounding structures of the rat oral cavity, 2 days after treatment. The overall damage effect of light of 625 nm
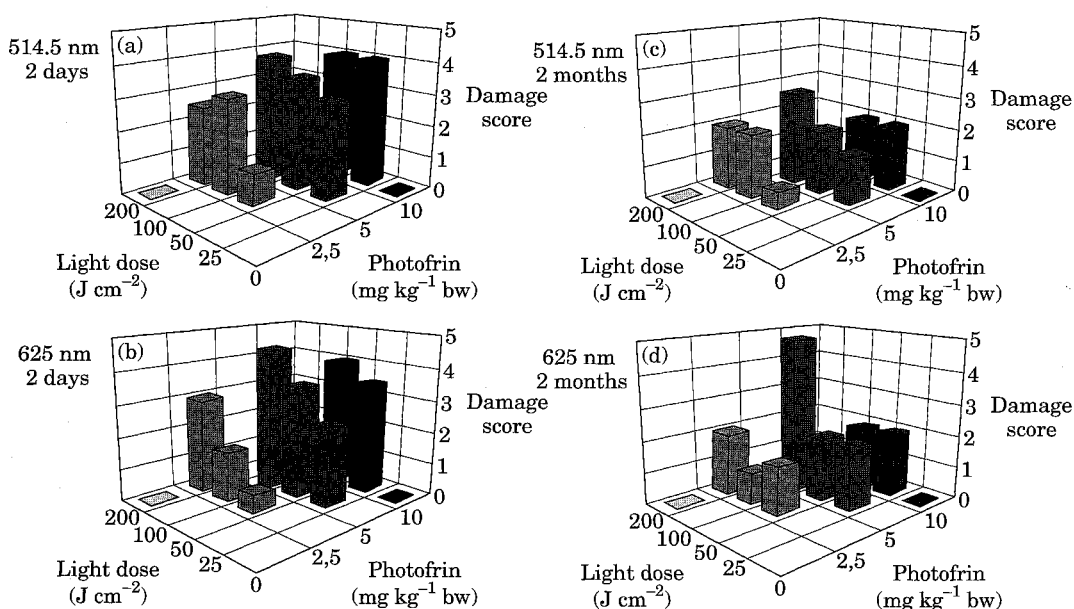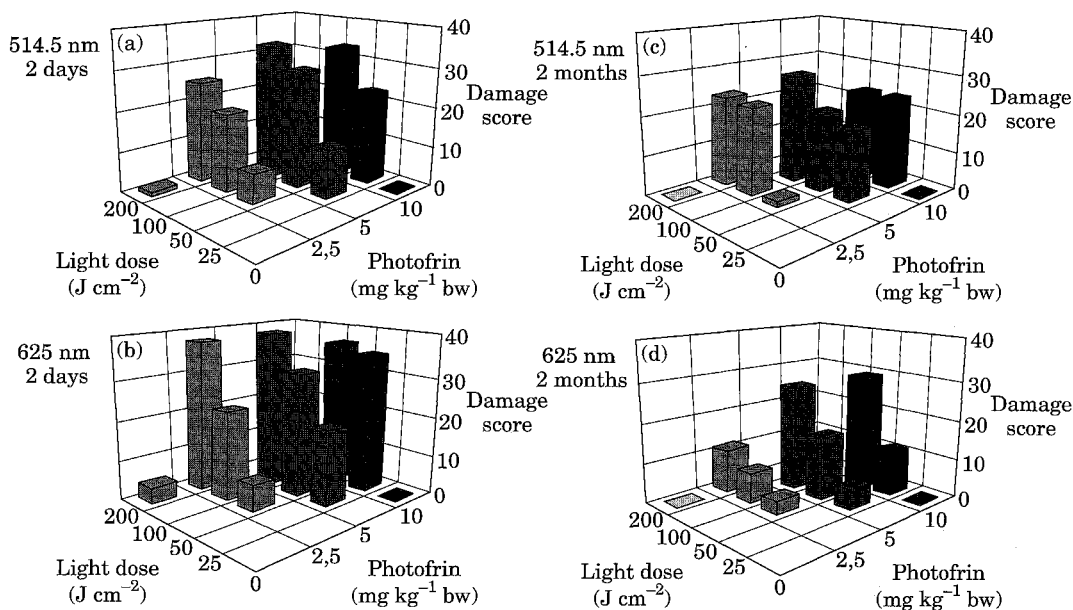
Fig. 4. The overall microscopic damage scores 2 days and 2 months after PDT. (a) Two days after PDT, at 514.5 nm.
(b) Two days after PDT, at 625 nm. (c) Two months after PDT, at 514.5 nm. (d) Two months after PDT, at 625 nm. The x-axis
represents the light dose in Jcm^{-2}, the y-axis the damage score, and the z-axis the Photofrin dose in mg kg^{-1} bw.

exceeded that of light of 514.5 nm. For both
excitation wavelengths, this overall effect
increases with increasing Photofrin dose, as
well as with increasing light dose. The damage
score of light at 625 nm mainly exceeded
that at 514.5 nm because of effects on struc-
tures beyond the palatal bone. As expected, the
highest Photofrin dose (10 mg kg^{-1}) used in
this study caused no measurable effect in the
absence of light (Protocol X). On the other
hand, a high dose of light (200 J cm^{-2}) in the
absence of the photosensitizer (Protocol I),
resulted in a small but distinctive amount of
damage. This effect was present for both wave-
lengths, and was noticed only in structures
beyond the palatal bone (ie epithelium of the
nasal sinus and lacrimal gland).

Figure 4(c, d) shows the mean overall micro-
scopic damage score 2 months after PDT. At
this time, in contrast to the effects seen at 2
days after PDT, the tissue damage of light of
514.5 nm exceeded that of 625 nm. When dam-
age to mucosa, palatal bone and structures
beyond the palatal bone were judged separ-
ately, the long-term damage to the mucosa was
found to contribute most to the overall conclu-
sion that light at 514.5 nm was more damaging
than that at 625 nm. Most striking differences
between the mucosa scores of both wave-
lengths were found in Protocols II and III. The
minor damage caused by light alone, as
observed at 2 days, was not present at 2
months. Increasing the drug dose or the light

dose resulted in more permanent damage at
2 months.

One of the presumptions of PDT is that
damage to normal tissues should be at least
reversible. However, it was found that several
combinations of photosensitizer and light
doses resulted in permanent tissue damage
(Fig. 5). This permanent damage was found
to be sensitizer, light-dose and wavelength
dependent. For both wavelengths, a marked
loss of the palatal bone was found in Proto-
cols IV and VI. In other combinations of
sensitizer and light doses (except Protocols I
and X), bone remodelling or partial loss of
the palatal bone was apparent. At 2 months,
the effect of light of 514.5 nm on bone slightly
exceeded that of 625 nm. Damage to molar
teeth was found for both wavelengths. Dam-
age was composed of resorption of dental
hard tissues, ankylosis of the root (ie a bony
attachment of the root with the surrounding
alveolar bone, indicating regeneration after
previous damage to the periodontal liga-
ment), and the formation of irregular dentin.
For both wavelengths (except in Protocols I,
V and X), damage to the dental hard tissue
was evident. No apparent differences existed
in the degree of damage to the dental hard
tissue induced by either wavelength. Further-
more, it was found that in all cases (except in
Protocols I, V and X), epithelial inclusion
cysts were found within the regenerated
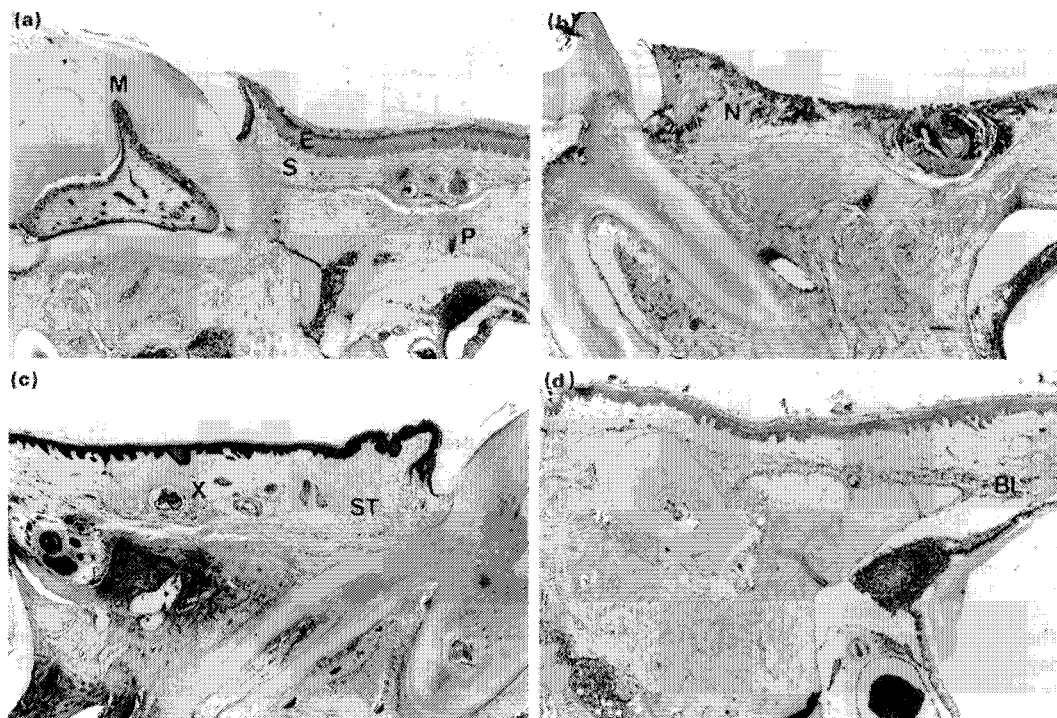epithelium (Fig. 6).

Fig. 5. Some typical examples of the histological appearance of the rat palatal mucosa after PDT. (a) Normal, untreated palatal mucosa. E=epithelial layer of the palatal mucosa, S=submucosa, P=palatal bone, M=molar (HE, ×40). (b) Two days after PDT. Treatment parameters: 625 nm, 5 mg kg^{-1}, 100 J cm^{-2}. Complete loss of epithelium, bacterial overgrowth in submucosal tissue and around vessels. Severe periosteal oedema. N=necrotic epithelial layer (HE, ×40). (c) Two months after PDT with more or less reversible damage. Treatment parameters: 514.5 nm, 5 mg kg^{-1}, 50 J cm^{-2}. The palate shows a normal intact epithelium with normal hyperkeratosis. The submucosa consists of a dense collagen-rich tissue (scar tissue) with epithelial islands within the submucosa. ST=scar tissue in the submucosa, X=epithelial islands and epithelial cyst in the submucosa (HE, ×40). (d) Two months after PDT with irreversible damage. Treatment parameters: 625 nm, 5 mg kg^{-1}, 100 J cm^{-2}. Note the marked loss (BL) of palatal bone (HE, ×40).

DISCUSSION

In order to treat premalignant epithelial lesions and squamous cell carcinoma of the oral mucosa with PDT, it is important to understand the effects on normal tissues. Sparing, or at least regeneration, of normal tissue is necessary in order to preserve function. Not only is it important to assess the 'treatment window' (the range of optimal combinations of sensitizer and light doses), but also to understand the light distribution in tissues to gain more knowledge of factors that lead to tissue effects. Among the large number of PDT studies, the effect of normal tissue and light distribution has received relatively little attention (12, 22–26).

Light dosimetry

Light scattering and internal reflection strongly influence the energy fluence rates in different tissues. The results of the present experimental study on the fluence rates in normal rat palates show that the distribution of light in oral tissue is far from homogenous. Palatal mucosa and palatal bone influence the fluence of 514.5 and 625 nm light. Energy fluence rates at the air–mucosa boundary were found to be twice as high for 514.5 nm light and three times as high for 625 nm light, relative to the primary incident beam with an irradiance of 100 mW cm^{-2}. The fluence rates of 625 nm light were always higher than those of the 514.5 nm. This was expected because the absorption and scattering properties of tissue for green light is different from those of red light, and longer wavelengths can penetrate deeper in tissues.

The conclusions of the present study are in accordance with those of Star and Marijnissen et al (8, 13, 27, 28). At several tissue surfaces (chicken muscle, rhabdomyosarcoma, dog bladder), they found actual fluence rates that were two to six times higher than the primary incident beam. Light of 630 nm gave higher actual fluence rates than 514.5 nm light. In
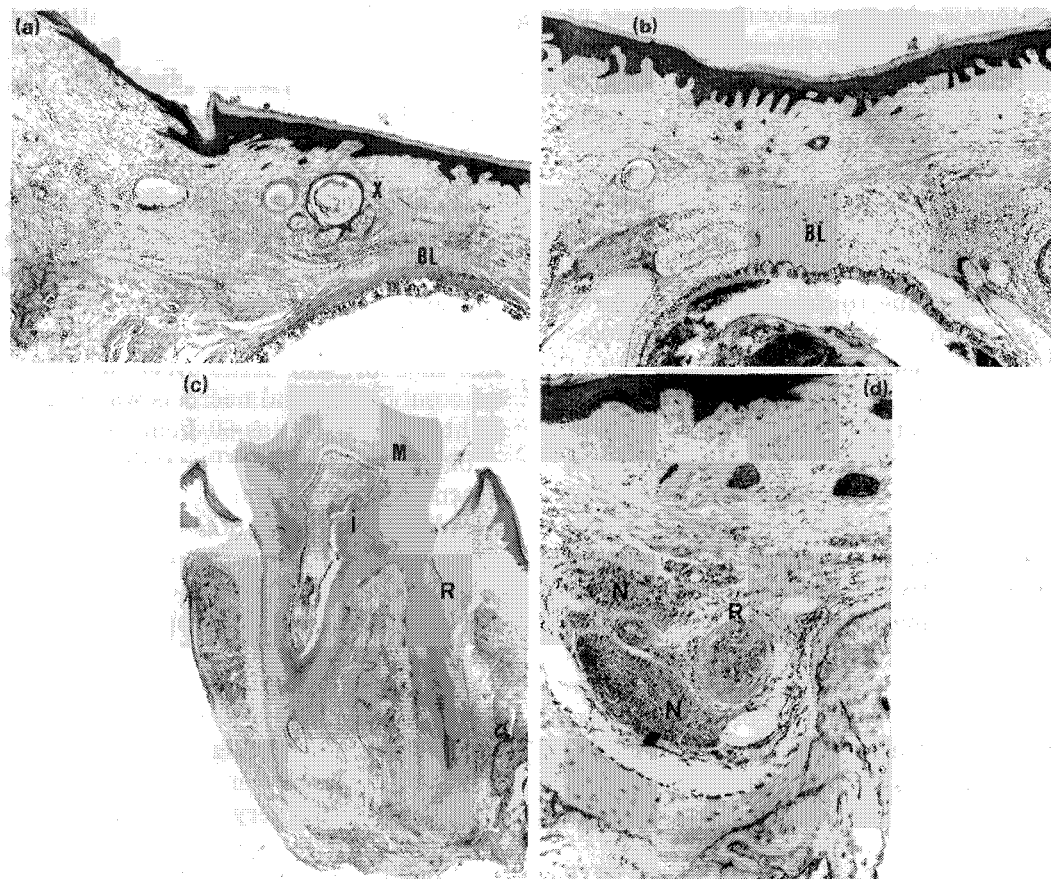
Fig. 6. Histological appearance of the long-term (2 months after treatment) specific damage of the rat palatal mucosa and surrounding tissues. (a) Under a normal intact epithelium with normal hyperkeratosis epithelial islands (inclusion cysts) with sebaceous differentiation, complete loss of the palatal vessel and nerve bundle can be seen. X=inclusion cyst, BL=palatal bone loss. Treatment parameters: 514.5 nm, 5 mg kg^{-1}, 100 J cm^{-2} (HE, ×64). (b) Marked loss of palatal bone. BL=palatal bone loss. Treatment parameters: 625 nm, 10 mg kg^{-1}, 50 J cm^{-2} (HE, ×64). (c) Damage to dental hard tissue and dental pulp. Root resportion (R) and formation of irregular dentin (I) can be seen. Treatment parameters: 625 nm, 5 mg kg^{-1}, 50 J cm^{-2} (HE, ×64). (d) Revascularization of the palatal artery (R) and proliferation of the palatal nerve (N) due to previous damage. Treatment parameters: 514.5 nm, 10 mg kg^{-1}, 25 J cm^{-2} (HE, ×160).

human whole bladder wall, PDT (630 nm) revealed a true light fluence (ie non-scattering incident light plus scattered light) almost five times larger than the non-scattered incident light fluence. In rat ears, Star et al (12) found the actual light fluence to be higher than the incident fluence. These actual fluence rates correlated with the relative biological effect, rather than with the incident fluence.

The results of the present dosimetry study clearly indicate that an incident light beam with an energy fluence of 100 mW cm^{-2} at the mucosa results in a true energy fluence rate which is twice as high for light of 514.5 nm and three times as high for light of 625 nm. Other structures in close proximity to the palatal mucosa, eg dentition, nasal cavity and lacrimal gland, therefore received higher doses of light. In this study, these increased fluence rates contribute considerably to PDT-induced

damage of normal anatomical structures. The fluence-rate data largely explain the damage pattern seen in this study.

Normal tissue effects

Knowledge of the morphological alterations induced by PDT, not only in the area of the tumour but also in the region of the adjacent normal tissue, is important with respect to the early and late complications of PDT (7). The mechanism of accumulation of photosensitizers in tumour tissue is still largely unknown. Autoradiographic studies using [^{14}C]- and [^{3}H]-labelled porphyrins have demonstrated photosensitizer uptake in normal tissue (2–4, 29). As normal tissue also retains the photosensitizer to some extent, it is also susceptible to PDT. The feasibility of clinical

PDT is determined, in part, by the response of normal tissue adjacent to tumour tissue. The ideal is to find an optimal therapeutic window, combining photosensitizer dose and light dose. Treatment under these parameters should lead to tumour destruction with only limited or at least reversible damage to the surrounding tissues. As seen in the present study, 2 days after PDT, the effect of 625 nm light exceeded that of 514.5 nm, due to damage of structures beyond the mucosa. At 2 months, these effects were reversed, caused by permanent damage of the mucosal layer. Dosimetry experiments in mucosa and structures beyond the mucosa revealed that actual fluence rates of 625 nm light were higher than those of 514.5 nm. Although light of 625 nm penetrates deeper into tissue, the increased absorption of 514.5 nm apparently results in more permanent damage (at a smaller tissue volume). The observation that light alone resulted in distinctive damage 2 days after PDT can be explained by the presence of endogenous porphyrins. For example, the Harderian gland, a lipid-secreting gland in the orbit in rodents, contains a large amount of endogenous porphyrin (30).

Only a few studies describe the effect of PDT with Photofrin on normal tissues of the oral mucosa. Treatment protocols used in these studies differ from the present study, so consequently it is difficult to find analogies in PDT-induced (normal) tissue damage. Biel et al (24) studied the effect of PDT (630 nm) with Photofrin ($2 \, mg \, kg^{-1}$) and various (incident) light doses (20, 50, 80, 100 and $125 \, J \, cm^{-2}$) in normal tissues of the oral cavity and larynx in dogs. Similar to the results of this study, the effects appeared to be tissue and light-dose dependent. The tongue was found to be more susceptible to PDT than cheek or larynx. The degree of oedema was found to be proportional to the degree of vascularization of the organ. Light doses of up to $50 \, J \, cm^{-2}$ gave reversible damage, which resulted in complete healing of the tongue by 7 weeks. A light dose greater than $80 \, J \, cm^{-2}$ resulted in permanent ulceration with muscular fibrosis. The cheek mucosa was more resistant and tolerated treatments of up to $125 \, J \, cm^{-2}$ with minimal permanent tissue damage. These results are in accordance with the present results, where a combination of photosensitizer dose and light dose $\geq 5 \, mg \, kg^{-1}$ and $\geq 100 \, J \, cm^{-2}$ resulted in distinct permanent damage. Due to the smaller dimension of the rat oral cavity, damage to several

anatomical structures beyond the mucosal layer were observed in the present study.

Monnier et al (25) studied PDT 72 h after injection of Photofrin ($2-3 \, mg \, kg^{-1}$ bw) in normal buccal mucosa in humans. Light doses varied from $60-240 \, J \, cm^{-2}$ ($50-200 \, mW \, cm^{-2}$) and thermal effects were ruled out. A control group irradiated with a light dose of $240 \, J \, cm^{-2}$ but received no Photofrin, showed no necrosis of the buccal mucosa. Four days after PDT, all Photofrin-treated patients showed necrosis of the irradiated, normal buccal mucosa. Superficial necrosis was induced with light doses as low as $60 \, J \, cm^{-2}$.

In a clinical PDT study, Biel (31, 32) described the treatment of 65 patients with neoplastic disease of the head and neck. Among these were seven patients with a T1 squamous cell carcinoma of the tongue or the floor of the mouth. Patients were treated 48 h after injection of Photofrin ($2.0 \, mg \, kg^{-1}$) with 630 nm light at $50-75 \, J \, cm^{-2}$ and $150 \, mW \, cm^{-2}$. For tumours greater than 3 mm depth, cylindrical diffuser implants were used. No adverse reactions were described. The results of this study are very promising because PDT was found to be effective, especially in the treatment of T1 tumours and carcinoma in situ (CIS). However, much less impressive results were achieved in treating deeply-invasive carcinomas. The present authors fully agree with the conclusions of the study that adequate delivery of the laser light to the tumour bed plays an important role, and that it is difficult to compare or evaluate the effectiveness of PDT because of the lack of well-defined standards for PDT treatments with regard to drug dosage, light delivery systems and optical dosimetry.

CONCLUSIONS

Internal reflection and light scattering have a strong influence on the true-light fluence in tissue. Normal tissues do retain the photosensitizer and are susceptible to PDT. The amount of PDT-induced damage was found to be photosensitizer and light-dose dependent.

An additional aim of this study was to define a treatment window for PDT of chemically induced dysplastic lesions and squamous cell carcinoma of the rat palatal mucosa. In this study, combinations of photosensitizer $\geq 5 \, mg \, kg^{-1}$ and light $\geq 100 \, J \, cm^{-2}$ resulted in distinct permanent damage. As damage to

normal tissues should be at least reversible, these values define the upper limits of the treatment window. Furthermore, the use of light of 514.5 nm seems to be more appropriate for PDT of superficial mucosal lesions than 625 nm because it is capable of adequately exciting porphyrin without damaging structures at deeper levels. In humans, the dimensions in the oral cavity are much larger than in rats, so the effects of depth of penetration to surrounding anatomical structures, will be far less devastating than those described in this study. Nevertheless, this study shows that anatomical structures beyond the target volume are prone to permanent PDT damage. Phenomena, such as the occurrence of epithelial inclusion cysts, ankylosis of teeth, pulp necrosis and necrosis of bone, seriously complicate clinical application of PDT in the oral region. Additional in vivo PDT studies are, therefore, needed to further establish optimal treatment protocols and to define the true merit of PDT in the treatment of premalignant and malignant lesions of the oral mucosa.

ACKNOWLEDGEMENTS

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