Photodynamic therapy (PDT) using topically applied δ-aminolevulinic acid (ALA) for the treatment of oral leukoplakia

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Abstract
Background: Photodynamic therapy (PDT) is a non-invasive method for topical and selective treatment of pre-malignant lesions of oral cavity. The aim of our study was to determine therapeutic response to PDT in patients with oral leukoplakia.
Methods: Twelve patients participated in our study. Lesions affected a variety of intraoral sites. The most common location was buccal, gingival and mandibular mucosa. Patients were treated with topically applied 10% δ-aminolevulinic acid (ALA) and light from an argon-pumped dye laser. Irradiation was performed in several (6–8) sessions using light at 635 nm wavelength, delivering a total dose of 100 J/cm^2 per session.
Results: A complete response was obtained in 10 out of 12 treated patients. One recurrence was observed during 6 months.
Conclusion: Photodynamic therapy appears to be a feasible alternative to conventional therapy of pre-malignant lesions of oral cavity.

Key words: δ-aminolevulinic acid (ALA); oral leukoplakia; photodynamic therapy

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Leukoplakia is defined as a white patch or flat thickening of oral mucosa, which neither can be removed by scrapping nor can be symptomatic for other illnesses except for the results of tobacco smoking. It should be clearly stressed that the term 'leukoplakia' is only a clinical notion with no specific recognizable pathological pictures (1). In microscopic inspection, distinct levels of keratosis are observed to coexist with different grade of dysplasia.

Leukoplakia, being a pre-cancerous alteration, plays a crucial role in pathogenesis of squamous cell carcinoma in oral cavity. The risk of neoplasmatic transformation of leukoplakia varies from 0.3 to 25%. On the other hand, presence of dysplasia in leukoplakia lesion increases malignancy incidence over 30% (2). Therefore, the reduction of oral cavity cancers may be achieved by both implementation of early diagnostic tools and successful eradication of pre-cancerous lesions (3, 4).

The idea of photodynamic diagnosis (PDD) is based on detection of the light emitted by activated chemical agents, which

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were previously absorbed by a tissue. Photosensitizer is a chemical compound which after having absorbed radiation is not undergoing photochemical transformation. Absorbed quantum of radiation becomes 'stored' in the excited electronic energy levels and may efficiently be transferred to oxygen or biological molecules.

It was Sutro in 1933 who had initiated fluorescence diagnosis (5). He had made an observation that surgically removed breast tissue irradiated with Wood lamp light, emitted either red light (breast cancer) or green light (normal breast tissue). Afterwards, the above-mentioned observations were confirmed with regard to cancer of skin and oral cavity (6). Red fluorescence tended to be associated only with clinically advanced stages of cancer (6). Neoplastic or dysplastic tissue shows reduced intensity of fluorescence, especially in the green range of light spectrum, whereas fluorescence in the red range is negligibly lowered (7–9). Reasons for the phenomenon to occur have only partly been defined and hitherto existing explanations refer to changes in structure and stratification of dysplastic and neoplastic tissue and to their distinct biochemical composition (6, 10).

Cytotoxic effect of photodynamic therapy (PDT) is associated with reactive oxygen species (ROS) production by excited photosensitizer, which are capable of damaging crucial cell components, such as structural proteins, enzymes, DNA and phospholipids (11).

A variety of treatments are known to be effective in leukoplakia. Acceptance of PDT as an alternative and complementary modality requires to demonstrate PDT to be at least as effective as currently used therapies. We have documented clearance, recurrence rates and adverse reactions in patients with oral leukoplakia after PDT.

Patients and methods

The study was performed at the Department of Internal Diseases and Physical Medicine, Center for Laser Diagnostics and Therapy, Silesian Medical University and in the Department of Conservative Dentistry and Periodontology, Silesian Medical University. Clinical enrolment examination consisted of determining the localization and extension of oral leukoplakia. Each patient had the lesions accurately measured and photographed for further evaluation. Fragments of the diseased mucosa were biopsied for pathological analyses. Further, the cure of concomitant oral pathology had been carried out. In case of existing fungal infection, mycogramme-based fungicidal treatment was administered to the patient. The average duration time of persistent leukoplakia prior to PDT initiation was 19.5 months (period range, 12–27 months). Seven individuals had undergone unsuccessful surgical treatment or cryotherapy before PDT was applied. In five cases, PDT was not preceded by other treatment modalities.

Patient population

Twelve patients participated in our study (range: 32–70 years old). Patients were informed of the investigational character of our study and signed a consent form. Ethical committee approval was obtained for their treatment. Photodynamic therapy was a primary treatment or a recurrent one after previous therapy, i.e., cryosurgery, surgical excision, electroexcision. A total of 12 patients with 24 sites were treated. Nine of them reported smoking cigarettes.

Lesions affected a variety of intraoral sites. The most common locations were buccal, gingival and mandibular mucosa. There were homogenous and non-homogenous types of foci (Fig. 1a,b).

Usually lesions were small, plaque-like, but in some cases they were very extensive, reaching even 4 cm in diameter. The detailed, individualized description of each treated patient is presented in Table 1, while localization of lesions is summarized in Table 2.

Drug application

In our study, δ-aminolevulinic acid (ALA; Medac GmbH, Germany) was used as a precursor in the biosynthesis of haem. A thin layer of an oil-in-water emulsion containing 10% ALA was applied topically to the lesions with a margin of 4 mm from the surrounding normal tissue.

PDT light source

An argon-pumped dye laser (Coherent Inc., USA) tuned to emit radiation at 635 nm was used for the light treatment. The fibre optic was terminated in a microlens that defocused the laser radiation homogeneously into a circular field. Laser radiation emitted from the fibre was monitored with a power meter (Coherent Inc., USA). The power density of the laser radiation was 150 mW/cm^2.

Treatment procedure

An oil-in-water emulsion containing 10% ALA was applied topically to the lesion sites. The sites were then occluded with a

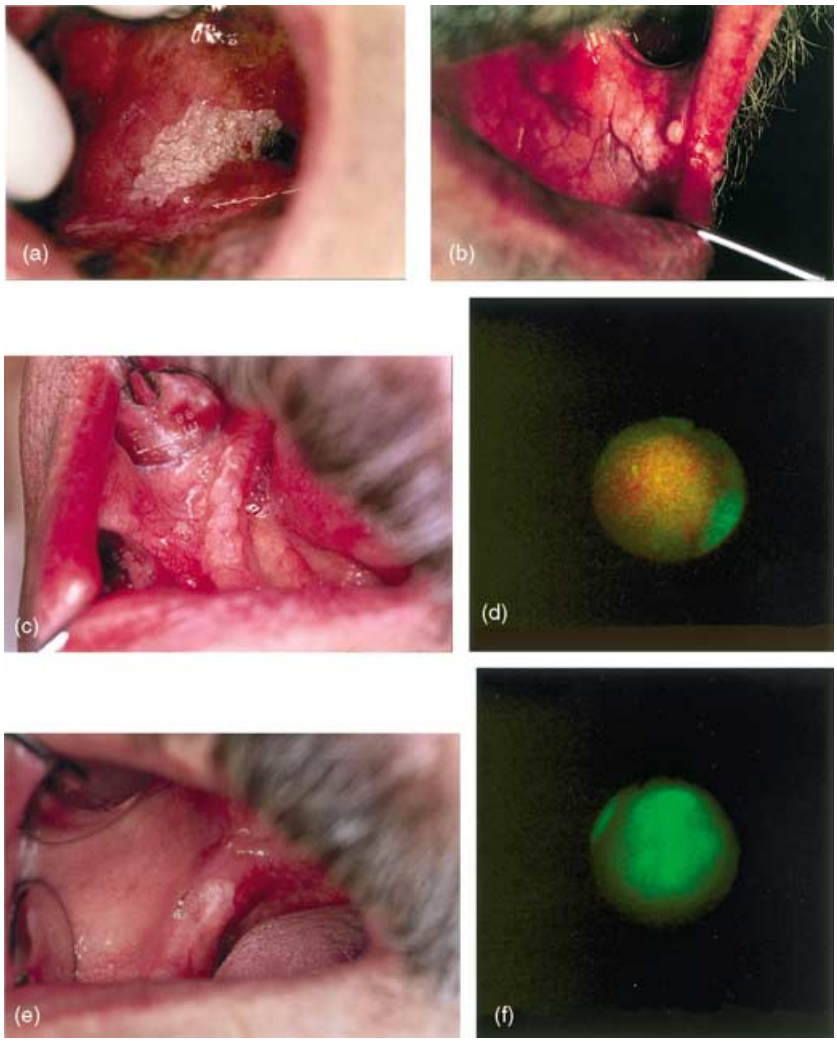

Fig. 1. Homogenous (a) and non-homogenous (b) type of oral mucosal leukoplakia. White light (c,e) and corresponding fluorescent (d,f) images of gingival leukoplakia prior to (two middle pictures) and after (two lower pictures) photodynamic therapy (PDT); note the red fluorescence as a result of protoporphyrin IX (PpIX) synthesis within a lesion (d) and the subsequent photobleaching yielding the green fluorescence (f).

self-adhesive foil for 4 h. Before light treatment, leukoplakia patch fluorescence had been assessed visually and recorded onto the computer hard disk for further analysis. Tissue accumulation of protoporphyrin IX (PpIX) was seen as a red fluorescence under excitation at 442 nm (Xillix Technologies Corp., Canada) (Fig. 1d). Irradiation was performed in several (6–8) sessions using light from an argon-pumped dye laser at 635 nm wavelength, delivering a total dose of 100 J/cm^2 per session. We included at least 10–20% margin around lesions in the field of irradiation. The treatment time was about 15 min at low fluence rate and was interrupted periodically to prevent excessive photosensitizer bleaching. Consequently, after the session was terminated the irradiated site were reinspected with Xillix machine to monitor mucosa for PpIX (Fig. 1f). All the patients had received local anaesthesia, and 30% of patients analgesic drugs. Afterwards, they were reviewed at 2-week intervals and treatment was repeated if required.

Follow-up study

Patients were seen 4 weeks after the treatment for the first time. The post-treatment observation period ranged from 4 to 34 months. One recurrence was observed during 6 months. Complete response was judged as absence of leukoplakia lesion on clinical inspection, white light and fluorescent imaging (Figs. 1e,f, 2b,d and 3b,d) confirmed by specimen biopsy.

Results

Evaluation

A complete response (total disappearance of leukoplakia patches on visual inspection) was obtained in 10 out of 12 treated patients.

Table 1. Individualized description of each treated patient

Patient	Treatment site	Lesion size (cm)	H-P	Number of treatments	Last response	Follow-up time (month)	Full or partial response	Recurrence	Habits
1	Buccal R	4 × 0.5	No dysplasia	8	24-06-1999	34	Full		Non-smoker
	Sublingual	1 × 0.7		6			Full		
2	Buccal R	0.9 × 0.7	Low-grade dysplasia	6	11-01-2001	15	Full		Smoker
3	Buccal L	1 × 0.5	No dysplasia	8	11-04-2000	24	No response		Non-smoker
	Lips	0.4 × 0.5		7			Partial		
4	Mandibular L	2 × 0.7	No dysplasia	10	4-02-2000	26	No response		Smoker
	Gingival	0.4 × 0.3		10			No response		
5	Buccal L	3 × 2	No dysplasia	7	4-02-2000	26	Full	Yes	Smoker
	B-G sulcus	0.5 × 0.5		6			Full		
6	Buccal L	0.7 × 1	No dysplasia	4	1-08-2000	20	Full		Smoker
	Mandibular L	0.5 × 1.2		7			Full		
7	Gingival	0.7 × 0.5	No dysplasia	6	16-10-2000	18	Full		Smoker
	B-G sulcus	1 × 0.5		7			Full		
8	Buccal L	1 × 1.5	Low-grade dysplasia	8	30-01-2002	3	Full		Smoker
	Gingival	0.4 × 0.5		7			Full		
9	Buccal L	1.5 × 1.5	No dysplasia	10	9-01-2002	3	Full		Non-smoker
	Gingival	0.5 × 0.7		8			Full		
10	Gingival	0.7 × 0.7	No dysplasia	9	20-12-2001	4	Full		Smoker
	Mandibular R	0.9 × 0.5		9			Full		
11	Buccal R	0.5 × 1	No dysplasia	7	16-05-2001	11	Full		Smoker
	Buccal L	1.5 × 1		8			Full		
	Lips	0.5 × 0.5		8			Full		
12	Buccal R	1 × 1	No dysplasia	8	17-10-2001	6	Full		Smoker
	Buccal L	0.5 × 1		8			Full		

Table 2. Localization of lesions of the treated patients

Localization	Buccal mucosa	Mandibular mucosa	Gingival mucosa	Lips	Sublingual mucosa	Bucco-gingival sulcus
Lesions	11	3	5	2	1	2

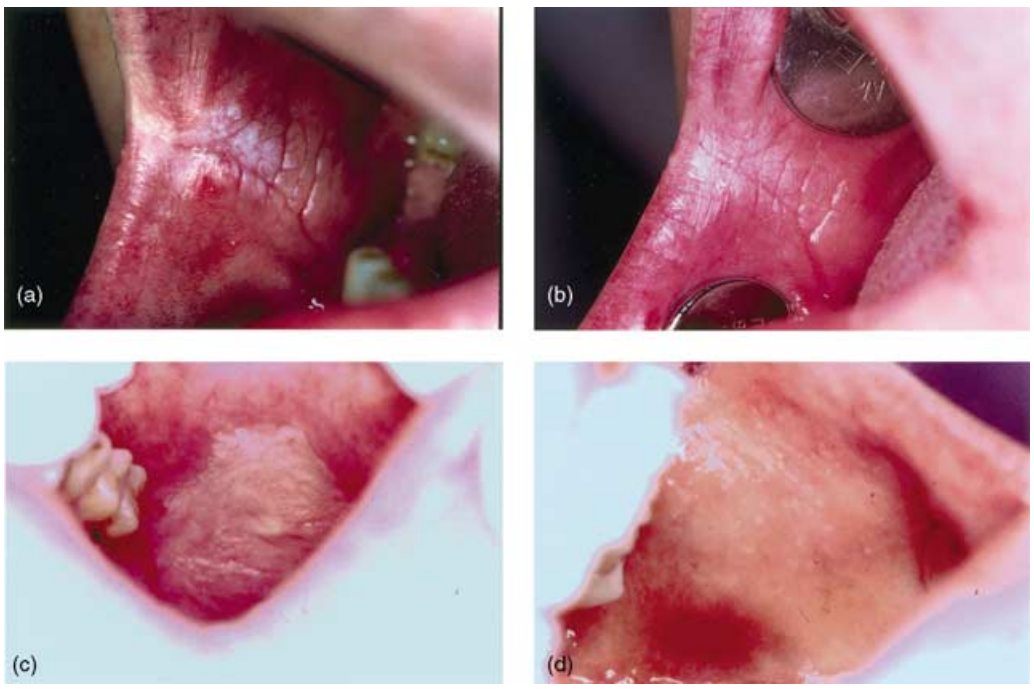

Fig. 2. White light images of angular (a,b) and palatal (c,d) leukoplakia in two individuals prior to (two left-hand side pictures) and after (two right-hand side pictures) the full course of PDT treatment which has resulted in complete response. The white spots visible on post-PDT pictures are caused either by flash lamp light reflection from mucosa or tissue scarring process.

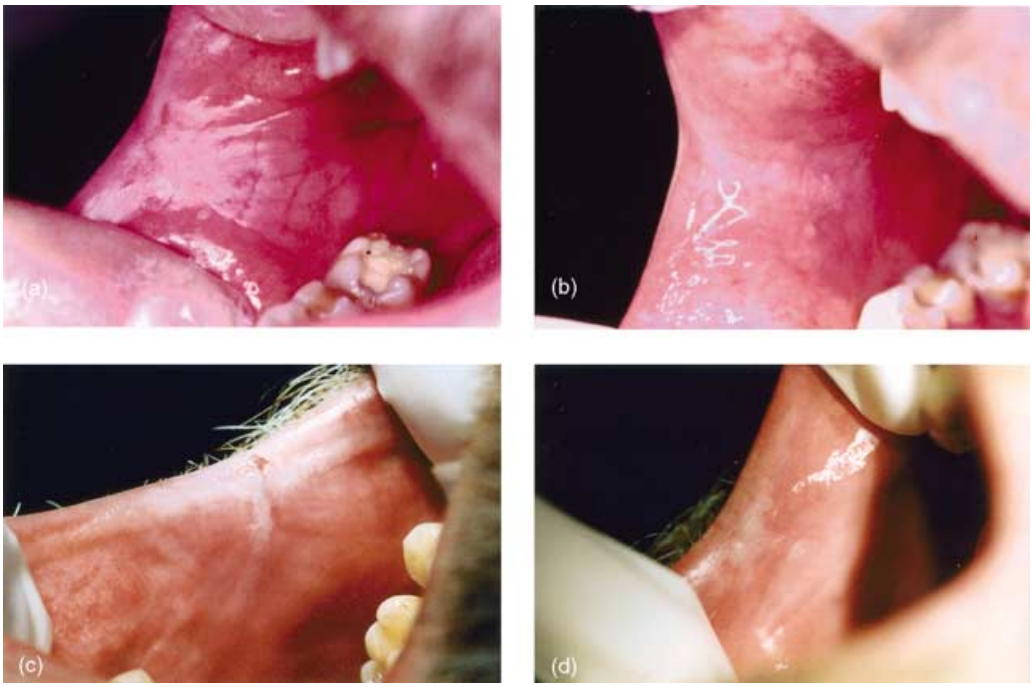

Fig. 3. Examples of angular leukoplakia complete cure in two individuals after the full course of PDT treatment. Left-hand side pictures (a,c) show mucosa before PDT and right-hand side pictures (b,d) illustrate the same regions post-PDT course. The white spots visible on post-PDT pictures are caused either by flash lamp light reflection from mucosa or tissue-scarring process.

Table 3. Lesion response to photodynamic therapy (PDT)

Lesion type	Patients	Complete response	No response	Recurrence
Oral leukoplakia	12	10	2	1

The results are summarized in Table 3 and illustrated in Figs. 2 and 3.

Adverse reaction

The treatment was well tolerated by the majority of patients. Moderate pain during treatment, localized erythema and oedema were observed immediately after light exposure and for the consecutive 1–2 days. Thirty per cent of patients had an acute pain. Sometimes, PDT treatment was associated with secondary infection (three patients).

Discussion

Clinical studies confirm high effectiveness of PDT in the treatment of early neoplastic and pre-neoplastic pathologies within oral mucosa. Although not a huge number of patients reported in the literature have undergone PDT, the results are promising.

The main goal of photodynamic diagnostics in the oral cavity is to unequivocally assess the range of the neoplastic tissue as well as post-operative evaluation of the results of treatment. Local application of photosensitizer (0.4% solution of 5-ALA) was used in the clinical trial carried out by Leuning and coworkers (12–14). The fluorescence of the examined area was recorded after 2 h by the use of digital camera and spectroscope at the range 375–440 nm. Even a relatively low concentration of photosensitizer let the authors obtain the sufficient contrast between neoplastic tissue and healthy one (10:1) in 16 patients. According to Leuning, higher concentration of photosensitizer applied locally is necessary only in case of PDT. However, in case of photodynamic diagnostics within oral mucosa, the use of photosensitizer with higher concentration (20–30%) leads to fluorescence of healthy tissue as well as the diseased one. The authors emphasize the advantages of local application of photosensitizer and the lack of side-effects related to general application of 5-ALA.

The results of PDT treatment of leukoplakia at our centre are consistent and comparable to those presented by other authors. Kubler et al. (15) performed a series of PDT procedures in 12 patients with leukoplakia of oral mucosa. They applied 20% preparation of 5-ALA locally in the form of cream 2 h before

procedure. Therapy was performed using laser light at 630 nm wavelength and energy dose of 100 J/cm^2. Complete cure was observed in five patients, partial in four patients. Three patients showed lack of effects of treatment. In one individual partial with relapsed leukoplakia, the series of repeated PDT procedures were completely successful. Zakrzewska et al. (16) carried out PDT in five cases in a group of 10 with proliferative verrucous leukoplakia. Other patients underwent conventional therapy (surgical treatment, cryotherapy, chemotherapy). PDT resulted in three total cures. Although the examined group was small, the author confirms that PDT is superior to other forms of treatment. Additionally, the usefulness of PDT seems to be extremely high in case of multiple foci of disease in the oral cavity. Grant et al. (17–19) applied photodynamic therapy in 11 patients with early stages of spinocellular carcinoma coexisting with the leukoplakia of oral cavity mucosa. Photofrin in the dose of 2 mg/kg body weight was administered intravenously. After 24 h, the focus of disease was irradiated by a laser emitting light at 620 nm. Eight weeks later, a total recovery was noted in 10 patients. Within 12 months, relapse of leukoplakia and erythroplakia was observed in two cases, however, without neoplastic foci. Fan et al. (20) used PDT both in patients with the cancer of the oral mucosa and those with pre-neoplastic pathologies. 5-ALA in the dose 60 mg/kg of body weight was administered orally and irradiated at 628 nm with energy density of 100–200 J/cm^2. The authors obtained excellent results in the treatment of pre-neoplastic conditions by evaluating the effectiveness of therapy. In another study, mTHPC (Foscan) was applied intravenously followed by a laser irradiation at 652 nm after 72–96 h. Nineteen patients with various stages of oral cavity cancer with coexisting fields of hyperkeratosis underwent treatment. Positive effects of therapy in 9 out of 14 cases of T1 and T2 foci were obtained. Recovery was obtained in one case out of six T3 foci. In T4 group, positive effects were obtained in three out of six cases. Biel (21) analysed the results of PDT of neoplasms localized in the oral cavity, larynx, head and neck in the years 1990–97. Within the group of 29 patients with pre-invasive squamous cell carcinoma of the oral cavity and tongue in which PDT was the only feasible method of treatment, the percentage of complete recovery after 70 months reached 80%. The relapse was observed only in five patients after this period.

Literature presents high effectiveness of PDT in the treatment of leukoplakia, erythroplakia and early stages of cancer of the oral cavity, especially in case of multiple localization of pathologies. Therapy and PDD becomes one of the basic methods in the treatment of neoplasms and pre-neoplastic conditions in the oral cavity. In addition, PDT possesses some features which surpass classic methods of treatment like cryotherapy, surgery and radiotherapy (22, 23). These features are non-invasiveness, good tolerance, excellent cosmetic effect, possibility of treatment multifocal changes, as well as repeated use without a threat of toxicity accumulation. Further clinical studies are needed to evaluate the effectiveness of PDT in the treatment of oral leukoplakia as related to surgical methods.

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