The effect of photodynamic therapy with pheophorbide-a on chemically induced papillary skin lesions in hairless mice

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The effect of photodynamic therapy with pheophorbide-a on chemically induced papillary skin lesions in hairless mice

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June 2013

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치과대학에 계신 모든 교수님께 감사의 인사를 드리며, 최근의 저에게 많은 영감을 주셨던 이승일 교수님께 특히 감사의 말씀을 전합니다. 제가 개원가에서 일하고 있을 때, 구강악안면외과의로서의 길을 계속 갈 수 있도록 용기를 주셨던 이연종 선생님과 이재휘 선생님, 그리고 구강악안면외과 이외의 다른 영역에 대한 지식을 전수해 주신 전영식 선생님께 이 지면을 빌려 감사드립니다.

마지막으로, 저의 어린 시절을 함께 해주신 아버지, 어머니, 그리고 세 언니들을 포함한 우리 가족에게 감사와 사랑을 담아 이 책을 바칩니다.

2013년 6월 27일

최은주 올림

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ABSTRACT

The effect of photodynamic therapy with pheophorbide-a on chemically induced papillary skin lesions in hairless mice

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Photodynamic therapy (PDT) is an alternative treatment option for early stage oral cancer and precancerous lesions due to the limitations of conventional treatment modalities. Pheophorbide a (Pa) is a chlorine-based photosensitizer extracted from chlorophyll-a, which is known to be activated by light at a wavelength of 670nm, is rapidly metabolized, and is selectively accumulated in tumor cells over a longer duration. The aim of this study is to validate the antitumor efficacy of PDT after the injection of Pa in murine papillary skin lesions. Hairless mice were randomly divided into two groups, namely, group I (control group, 5 mice) and group II (experimental group, 33 mice). Group I animals received topical application of acetone (100$\mu\ell$ per mouse) throughout the study. Group II animals received a topical application of 7,12-Dimethylbenz(a)anthracene (DMBA) 100μg in 100$\mu\ell$ of acetone for 2 weeks followed by a topical application of 12-O-tetradecanoylphorbol-13-acetate (TPA) 2.5μg in 100$\mu\ell$ of acetone for 5 weeks every second day. Animals in group II were further divided into 2 subgroups, namely group IIA (21 mice) and group IIB (6 mice). Group IIA received Pa-based laser treatment, and group

IIB received Pa treatment alone. 200㎍ of Pa was directly injected into the 3 largest lesions over the back. After 24 hours, the animals were exposed to a light dose of 100J/㎠ using a laser diode at a wavelength of 664nm. One week after the first cycle of treatment, the second cycle of treatment was performed using the same method in each group. One week after completion of the respective treatments, mice were euthanized in a CO2 chamber. In Group II, 6 mice that were found to have non-papillary skin lesions were excluded from this experiment. After treatment, one papillary lesions was sloughed away in 8 mice, 2 papillary lesions were sloughed away in 5 mice, and 3 papillary lesions were sloughed away in 2 mice of group IIA; whereas, one papillary lesion was sloughed away in 1 mouse out of 6 mice in group IIB. As a final outcome, Pa-based PDT was effective in 54% of 63 papillary lesions, and Pa treatment alone was effective in 11.1% of 18 papillary lesions.

Based on these results, the authors concluded the following:

Results of this experiment showed that Pa does not have any remarkable effect on papillary skin lesion in the absence of illumination (11.1%) though the previous reports had demonstrated that pheophorbide a (Pa) has antitumor activity and a potential immunomodulatory effect on macrophages.

PDT with Pa was observed to have an effect on early stage of tumor lesions although the success rate was relatively low (54%). More than 3 cycles of Pa-based PDT treatment can be predicted to cause more favorable treatment outcomes, especially in papillary skin lesions.

Development of a dysplasia model is necessary to validate the efficacy of Pa-based PDT in premalignant lesions.

Key words : photodynamic therapy, pheophorbide a, papillary skin lesion

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I. INTRODUCTION

Oral cancer is the most common malignancy in the head and neck area, and the eighth most common cancer worldwide. (Petersen 2009, 454-460) Surgery and postoperative radiotherapy have been used as conventional treatment modalities for oral cancer. However, surgical treatment frequently results in functional and esthetic defects, and radiotherapy is accompanied by complications such as endarteritis, xerostomia, mucositis, and osteoradionecrosis. (Grant et al. 1993, 1140-1145) Additionally, it has been reported that one-third of patients who were treated with surgery and adjuvant therapy had local recurrence, regional, and/or distant metastasis. (Greenberg et al. 2003, 508-15) Early diagnosis and treatment is critical for oral premalignant lesions in order to reduce the complications and to improve the treatment success in oral cancer.

However, the management of patients with premalignant lesions of the oral mucosa presents a considerable problem for the surgeon because of “field cancerization” with

multicentric foci of invasion. (Jerjes et al. 2011, 192-9) Dysplastic oral mucosal lesions have multicentric foci and undefined margins, and the risk of progression of these lesions to cancer varies considerably from 6% to 36% in the reports; (Reibel 2003, 47-62) consequently, these lesions are difficult to manage. Moreover, when leukoplakia occurs in conjunction with one or more primary tumors, indicating the likelihood of a wide field change, these difficulties are greatly compounded. (Grant et al. 1993, 1140-1145, Jerjes et al. 2011, 192-9) Even after surgical treatment, the incidence of second primary tumor has been reported in 25~30% of patients, (De Vries, and Gluckman 1990, 4-5, Ho et al. 2013) and 76% of patients who have squamous cell carcinoma (SCC) arising from oral epithelial dysplasia are known to develop local recurrence and a second primary tumor in 5 years. (Ho et al. 2013)

Photodynamic therapy (PDT) is an alternative treatment option for early stage oral cancer and precancerous lesions due to the limitations of conventional treatment modalities. (Grant et al. 1993, 1140-1145, Jerjes et al. 2011, 192-9) PDT is a treatment, in which there is a nonthermal photochemical reaction between a photosensitizing agent and light of a wavelength specific to that photosensitizer in the presence of oxygen, thus liberating reactive oxygen species and resulting in tissue necrosis. PDT consists of a 2-stage process: first, the photosensitizer is administered, and then the illumination is carried out when a differential photosensitizer concentration develops between the tumor tissue and adjacent normal tissue, resulting in optimal efficacy and tolerability. (Hopper et al. 2004, 138-46, Lofgren et al. 1994, 1355-1362) PDT is known to have an antitumor effect due to 3 inter-related mechanisms of action: direct cytotoxic effects on tumor cells, damage to the tumor vasculature, and induction of a robust inflammatory reaction that

can lead to the development of systemic immunity. (Agostini et al. 2011, 998-1000; author reply 1000-1)

The type of the photosensitizer is one of the important components to determine optical photodynamic efficacy. (Ahn et al. 2012, 1772-8) There are several photosensitizers such as porphyrin derivatives; however, they have limitations in selective localization and tumor absorption. The use of treatment with second generation photosensitizers (5-aminolaevulanic acid and porfimer sodium) is limited to tumors having a depth of 0.2 cm to 0.5 cm. (Hopper 2000, 212-219) Pheophorbide a (Pa) is a chlorine-based photosensitizer extracted from chlorophyll-a, is an active component of *Scutellaria barbata*, a type of a ethnopharmacological herb, which is frequently used for treatment of tumors in Asia. (Tang et al. 2009) Pa can be synthesized by eliminating a magnesium ion and a phytyl group from chlorophyll-a. (You et al. 2011, 5383-91) Pa is known to be activated by light with a wavelength of 670nm, which is greater than a wavelength of 630nm, is rapidly metabolized, and is selectively accumulated in tumor cells over a longer duration.

Pa has been studied in the fields of hepatocellular carcinoma and uterine sarcoma, and the possibility of application of Pa in the field of oral cancer has been suggested. However, there is no report about the efficacy of Pa-based PDT in a chemically-induced head and neck carcinogenesis model. The aim of this study is to validate the antitumor efficacy of PDT after the injection of Pa in murine papillary skin lesions.

II. MATERIALS AND METHODS

All experiments were performed under protocols approved by the Institutional Animal Care and Use Committee at the Yonsei University Medical Center (2012-0064).

1. Carcinogenesis

Six-week-old female SKH-1 hairless mice were used. 7,12-Dimethylbenz(a)anthracene (DMBA), 12-O-tetradecanoylphorbol-13-acetate (TPA), and acetone were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Thirty-eight mice were housed 5 per cage, and provided fresh tap water ad libitum and a fresh diet that was changed twice a week. The animal rooms were maintained at 24℃, and 50–60% humidity. Mice were randomly divided into two groups, namely, control group (group I, 5 mice) and experimental group, DMBA/TPA (group II, 33 mice). Group I animals received a topical application of acetone alone (100$\mu\ell$ per mouse) throughout the study. Group II animals received a topical application of DMBA (100μg per 100$\mu\ell$ of acetone) for 2 weeks (every second day) followed by a topical application of TPA (2.5μg per 100$\mu\ell$ of acetone) every second day for 5 weeks.

2. Preparation of Pa

Pa was synthesized according to the previously described procedure (You H, 2011). Briefly, treatment of the ethanolic solution of chlorophyll-a in an acidic condition (1 N

HCl, pH 2.5) enabled to remove the magnesium ion easily to afford a crude pheophytin in the form of precipitates. The pheophytin was subsequently hydrolyzed by reacting with 80% TFA in water to obtain Pa as a fine powder (Ahn et al. 2012, 1772-8)

3. Photodynamic therapy

Animals in Group I were further divided into 2 subgroups, namely group IA (3 mice) and group IB (2 mice). Group IA received Pa-based laser treatment, and group IB received Pa treatment alone. In group II, 6 mice which were found to have non-papillary lesions were excluded from this experiment (Fig. 1). The remaining 27 animals in group II were further divided into 2 subgroups, namely group IIA (21 mice) and group IIB (6 mice). Group IIA received Pa-based laser treatment, and group IIB received Pa treatment alone (Fig. 2).

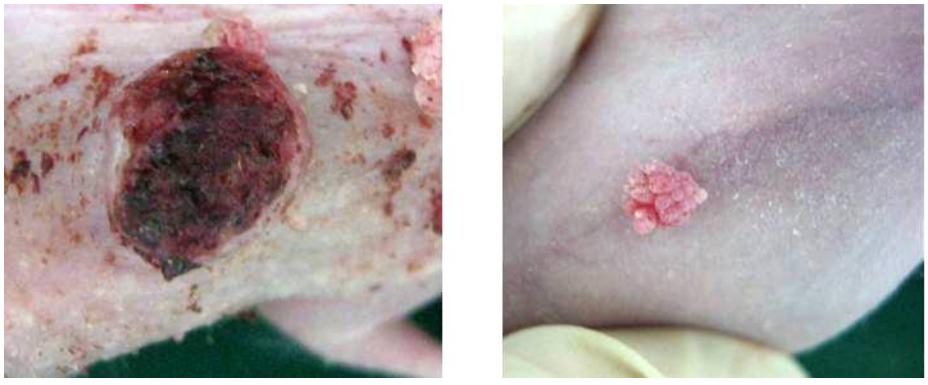

Figure 1. Clinical features of non-papillary ulcerative sessile lesion (A) and papillary lesion (B).

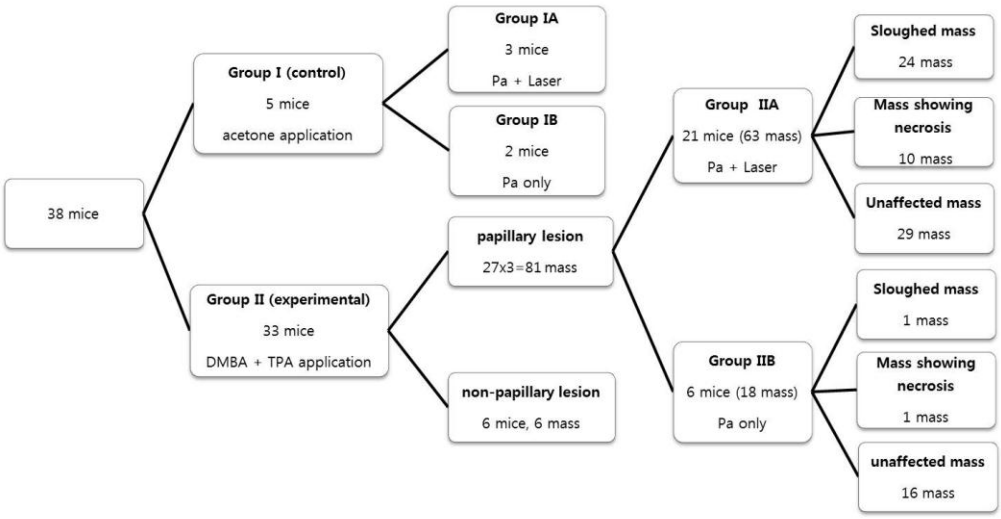

Figure 2. Flow diagram of the experiment.

Pa was dissolved in dimethylsulfoxide (DMSO) solution. 200㎍ (0.1 ml) of Pa was directly injected into the 3 largest lesions over the back. After 24 hours, the animals were exposed to a light dose of 100 J/㎠ using a laser diode (Geumgwang Co., Ltd., Daejeon, Korea) at a wavelength of 664nm. One week after the first cycle of treatment, the second cycle of treatment was performed using the same method in each group. Animals were housed under direct illumination after the Pa injection. The size of the tumors was measured before and after treatment, and the number of treated tumors was counted after treatment. For comparing the difference between groups, Mann-Whitney test was used in this study. P value less than 0.05 was considered statistically significant. SPSS 13.0 was used in this study. One week after completion of the respective treatments, mice were euthanized in a CO2 chamber.

4. Histology

Tumors were sampled carefully; and formalin-fixed, paraffin-embedded tissue samples were cut into 4㎛ sections, deparaffinized with xylene and hydrated with graded ethanol. After incubation in hematoxylin solution (Sigma) at RT for 5 min, the sections were treated with eosin (Sigma) at RT for 30 seconds. Subsequently, the sections were dehydrated and then mounted using a mounting medium (DAKO).

III. RESULTS

1. Carcinogenesis

Any abnormal lesion such as a papillary or a hyperplastic lesion was not observed on clinical and histological examination in mice of group I, in which acetone alone was applied (Fig. 3). DMBA application for 2 weeks did not cause any clinical changes in mice of group II. Multiple, pedunculated papillary tumors of 1~4mm in size were observed at five weeks after the application of TPA. However, 6 animals showed an induration of the skin or a sessile mass with superficial ulceration. TPA was discontinued after the 18^{th} application; however, even after discontinuation of TPA, the lesions did not stop growing.

2. Photodynamic therapy

After Pa-based PDT or Pa treatment alone, the size of papillary lesion was not significantly reduced, whereas the number of papillary lesion was decreased (Figs. 4, 5). The overall number of papillary lesions was reduced in 15 out of 21 mice in group IIA. One papillary lesion was sloughed away in 8 mice, 2 papillary lesions were sloughed away in 5 mice, and 3 papillary lesions were sloughed away in 2 mice of group IIA; whereas, only one papillary lesion was sloughed away in 1 mouse out of 6 mice in group IIB (Table 1). The proportion of persistent papillary lesion was 61.9% in group IIA and 94.4% in group IIB, and this difference was statistically significant using the 95% confidence interval (P=0.026, Fig. 6).

3. Histology

Histologic specimens of some of the persistent papillary lesions showed necrotic changes (Fig. 5C). Ten of 39 persistent papillary lesions in Group IIA, and one of 17 persistent papillary lesions in group IIB showed necrosis. As a final outcome, Pa-based PDT was judged to be effective in 54% of 63 papillary lesions, and Pa was judged to be effective in 11.1% of 18 papillary lesions.

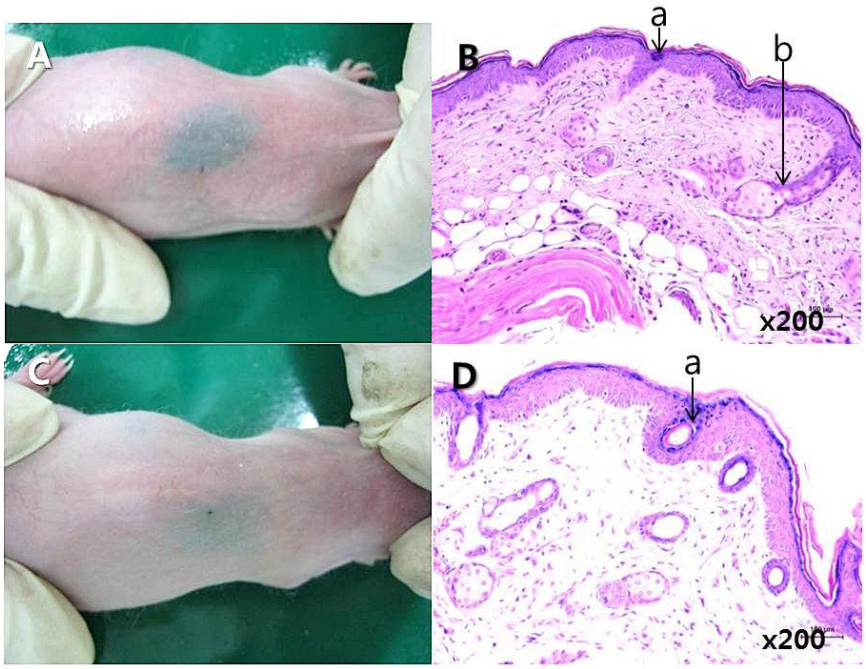

Figure 3. Clinical and histologic findings of group I.

A, B : Group IA (acetone application and Pa-based laser treatment)

C. D : Group IB (acetone application and Pa treatment alone)

Any abnormal lesion such as a papilloma or dysplasia was not observed on clinical and histological examination in mice of Group I, in which acetone was applied (A, C). Animals in Group I were further divided into 2 subgroups, namely Group IA and Group

IB. Group IA received Pa-based laser treatment (A, B), and Group IB received Pa treatment only (C, D). H-E staining showed superficial Pa accumulation, (a) and there was no remarkable inflammation or necrosis.

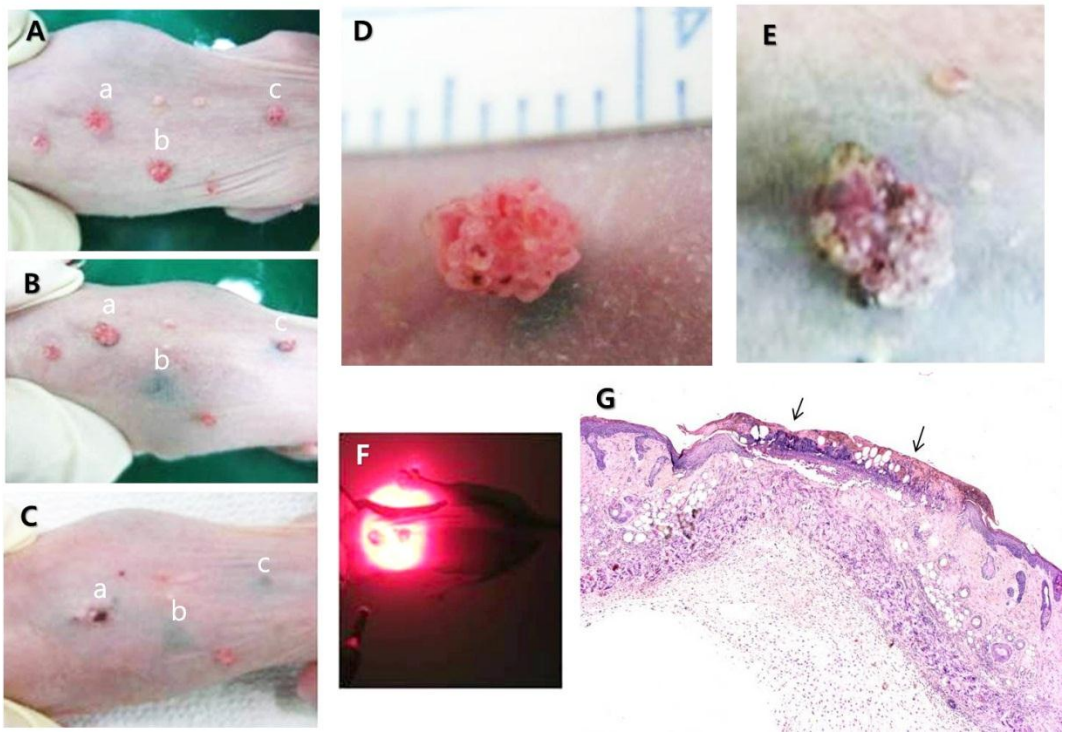

Figure 4. Treatment sequences in mice of group IIA.

The largest 3 lesions per mouse were selected as a target for Pa-based PDT (A: a, b, c). One week after the first cycle of treatment, lesion b was sloughed away (B). One week after the second cycle of treatment, lesions a and c were sloughed away (C). Papilloma was 4 mm in size before treatment (D). Pa was intensely distributed in the tumor when it was injected into the microvasculature around the neck of the pedunculated tumor. (E). 24 hours after Pa injection, the animals were exposed to a light dose of 100J/㎠using a laser

diode at a wavelength of 664nm (F). H-E staining showed that inflammatory cells and eschar persisted in the sloughed site (G).

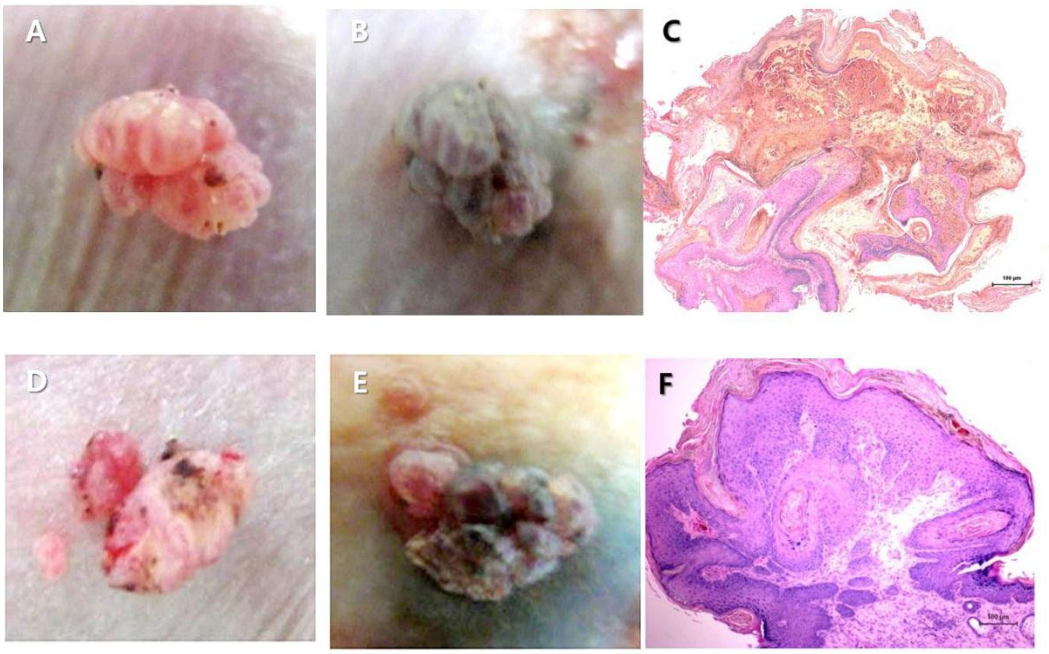

Figure 5. Clinical and histologic findings of persistent papillary lesion after Pa/Pa-based laser treatment. Before (A, D) and after (B, E) Pa-based laser treatment. H-E staining showed necrotizing papillomas (C) with no remarkable necrosis (F).

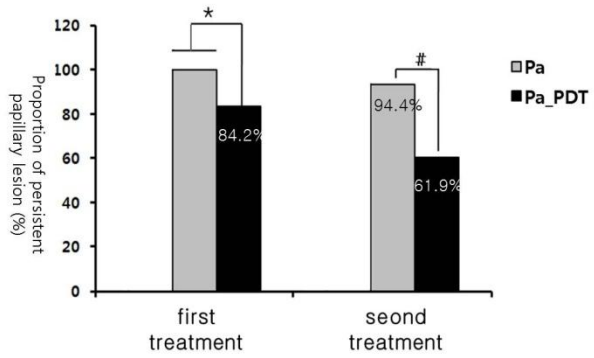

Figure 6. Proportion of persistent papillary lesion.

Proportion of persistent papillary lesion after two cycles of PDT was 61.9% in group IIA and 94.4% in group IIB, and this difference was statistically significant using Mann-Whitney analysis with 95% confidence level. (* P=0.069; # P=0.026)

Group	No.	The number of pretreatment papillary lesions	Sloughed papillary lesions after 1st round	Sloughed papillary lesions after 2nd round	Number of papilloma showing necrosis	Unaffected papillary lesions
Group IIA Pa+laser	**1**	3	0	1	1	1
	2	3	1	1	1	0
	3	3	0	0	2	1
	4	3	0	0	2	1
	5	3	0	1	0	2
	6	3	0	1	2	0
	7	3	1	1	1	0
	8	3	0	0	0	3
	9	3	0	1	0	2
	10	3	0	1	0	2
	11	3	0	0	0	3
	12	3	0	0	0	3
	13	3	0	1	0	2
	14	3	1	1	0	1
	15	3	1	0	0	2
	16	3	1	0	1	1
	17	3	1	1	0	1
	18	3	1	1	0	1
	19	3	1	2	0	0
	20	3	2	1	0	0
	21	3	0	0	0	3
	Total	**63**	**10**	**14**	**10**	**29**
Group IIB Pa alone	**1**	3	0	0	0	3
	2	3	0	0	0	3
	3	3	0	0	0	3
	4	3	0	1	0	2
	5	3	0	0	1	2
	6	3	0	0	0	3
	Total	**18**	**0**	**1**	**1**	**16**

Table 1. Changes in the number of papillary lesions.

The number of papillary lesions was decreased in 15 out of 21 mice of group IIA. Tumor regression rate was 38.1% in group IIA and 5.6% in group IIB. Necrosis was observed in 10 mice of group IIA and in 1 mouse of group IIB. Proportion of papillary lesions that showed the effect of the treatment was 54.0% in group IIA and 11.1% in group IIB.

IV. DISCUSSION

Many authors have reported the use of animal experimental models of oral carcinogenesis. Subcutaneous tumor cell inoculation, the most widely used method, has limitations since the tumor grows within areas encapsulated in a fibrous tissue, without invading the surrounding tissue. Furthermore, the inoculated tumor cells tend to grow without causing precancerous lesions. Another problem is that oral carcinogenesis causes restriction on the dietary intake of animals, and consequently the animals do not survive until the treatment is completed. (Chung 2008, Kim 2008) Because of the easy accessibility and similarities between mouse head and neck epithelia and human head and neck epithelia, the mouse skin model of carcinogenesis has frequently been used for assessing tobacco-associated SCC. (Bassi, and Klein-Szanto 2007, Unit 14 2, Mitsunaga et al. 1997, 81-9, Ruggeri et al. 1993, 1013-7, Yuspa 1994, 1178-89) Therefore, this experimental model was selected for evaluating the effect of Pa-based PDT. Bassi et al. reviewed various carcinogenesis models that could be used in experiments of head and neck SCC.

In this experiment, SKH-1 hairless mice were used; and these mice were reported to be useful for assessing the tumorigenic activity of the carcinogen that was painted on the back skin. (Roemer et al. 2010, 155-61) However, development of lesions such as papilloma or dysplasia takes more than 10 weeks with conventional methods. The authors applied the drugs every second day; consequently, papilloma and even SCC were induced within 7 weeks with DMBA and TPA as suggested by Bassi et al. The application of

carcinogens induced papillary lesions in almost all the animals, but it also induced a sessile mass with superficial ulceration suggestive of carcinoma in several animals. The initial aim of this study was to investigate the treatment efficiency of PDT on precancerous lesions such as dysplasia; however, in practice a papillary lesion develops during the induction of carcinogenesis, and not a dysplastic lesion. Indeed, many authors induced carcinogenesis in mice, but most of them observed the development of papillomatous lesions. (Miyamoto et al. 2008, 418-26, St Clair et al. 2005, 209-14)

PDT has little adverse effects on the underlying functional structure and it rarely causes a cosmetic defect. Moreover, previous reports support the role of PDT as a treatment modality for oral cancer due to its effect on field cancerization. (Biel 1998, 1259-68, Gluckman 1991, 1559-67) The clinical efficacy of PDT has been validated in the fields of urothelial bladder cancer, bronchogenic carcinoma, and dermatological carcinoma. PDT has been approved for treatment of oral cancer in United Kingdom and Japan.

PDT is composed of 3 essential factors; photosensitizer, laser light, and oxygen. Photofrin is the first generation photosensitizer, which has adverse effects such as photosensitivity due to its low selectivity. Second generation photosensitizer, 5-aminolevulinic acid (ALA) is widely used for treatment of non-malignant disorders such as actinic keratosis because of its several advantages such as the possibility of various types of administration including topical, oral, intravenous administration, and activation by various light sources. However, the drawback of ALA is that it is only effective in tumors having a depth of less than 0.2 cm, and hence it is not possible to use it in deep tumors. (Fan et al. 1996, 1374-1383, Grant et al. 1993, 1140-1145)

Therefore, photosensitizers have been continuously studied to improve their tumor selectivity, improve the effective treatment depth, and reduce the treatment time. Pa is a natural photosensitizer, and Pa-based photodynamic therapy is known to inhibit tumor growth and reduce the dose of chemotherapy. (Hajri et al. 2002, 140-8, Lee et al. 2004, 119-26, Lim, Ko, and Lee 2004, 1-6, Rapozzi, Miculan, and Xodo 2009, 1318-27) The interest in this compound has increased recently, since new studies have revealed that Pa satisfies most of the criteria for a good photosensitizer. Pa was proved to have antitumor activity and a potential immunomodulatory effect on macrophages. (Bui-Xuan et al. 2011, 60-7)

The selectivity of photosensitizer is important because it is inversely related to the photosensitivity of normal tissue. In this regard, Pa is known to have a high tumor sensitivity and thus it lowers skin sensitization (Fujishima et al. 1991, 257-63) Also, it is activated by the light with a wavelength of 670nm compared to conventional photosensitizers that are activated by the light with a wavelength of 630nm. The use of a photosensitizer that is activated by a light with a longer wavelength results in better penetration of light than that in those activated by a light with a shorter wavelength, and hence Pa can be used for the treatment of deep tumors. Pa has been reported to be effective in deep tumors with more than 10 mm depth, while second generation photosensitizers showed limitations in their efficacy in tumors having a depth of more than 2 mm. (Stiel, Marlow, and Roeder 1993, 181-6)

Pa is soluble in aqueous solution, and can be injected intravenously. Pa can be dissolved in a water-soluble or an oily medium according to the target organ; however, water soluble form is recommended for the distribution of Pa in the skin or mucosa.

(Nishiwaki, Nakamura, and Sakaguchi 1989, 254-63) DMSO does not have remarkable toxicity when injected intravenously. (Wood, Weber, and Palmquist 1971, 520-527) In this experiment, Pa was dissolved in DMSO, and then injected subcutaneously. After several injections, the authors found that Pa was more intensely distributed in the tumor when it was injected into a feeding vessel located on the neck of the pedunculated papilloma than when it was injected subcutaneously.

The interval between the sensitizer administration and light exposure was one of the key factors that influence PDT efficacy. In 15 minutes after drug administration, the photosensitizer was accumulated predominantly in the vascular compartment. This resulted in vascular stasis and thrombus formation, followed by indirect tumor cell killing. (Dolmans, Fukumura, and Jain 2003, 380-387) In the treatment of most cancers, the interval between drug and light administration is typically as long as 24 to 72 hours. (Dolmans et al. 2002, 2151-2156, Dougherty 2002, 3-7) Although the localized injection of a sensitizer may exhibit dose accumulation potential in a short time, the optimal time for illumination after injection needs to be investigated further.

Assessment of results after PDT is difficult in this model because tumor size and characteristics were variable. There are many reports about carcinogenesis of the mouse back skin; however, most of them have studied prevention of carcinogenesis, and not the effect of photodynamic therapy after induction of carcinogenesis. One of the reports demonstrated that photodynamic therapy was effective in DMBA and TPA induced two-stage carcinogenesis. (Takahashi et al. 2012, 221-4) In the above report, the efficacy of photodynamic therapy was assessed by tumor regression; therefore, the authors assessed the efficacy of PDT using the same method in this study. In this experiment, 24 papillary

lesions were sloughed away and 10 showed necrosis out of 63 papillary lesions after Pa-based PDT. Thus, Pa-based PDT was effective in 34 papillary lesions (54%). Yet it is not certain whether the injection of Pa into the feeding vessel and application of PDT caused necrosis and resultant sloughing in these 24 papillary lesions. Only one papillary lesion was sloughed away in group IIB, and because this difference was statistically significant, it could be interpreted as the effect of PDT. There have been a number of reports of PDT causing microvascular collapse. (Dolmans, Fukumura, and Jain 2003, 380-387, Fingar, Wieman, and Haydon 1997, 513-517) Star et al. observed vascular changes after intraperitoneal injection of hematoporphyrin derivative and photoradiation. After an initial blanching of the tumor accompanied by apparent vasoconstriction, reperfusion was observed with a slowing down of the tumor circulation, vasodilatation, and eventually a complete stasis, together with diffuse hemorrhages and subsequent necrosis. (Star et al. 1986, 2532-2540) There is another report demonstrating that pyropheophorbide derivative PDT caused a dose-dependent biphasic blood flow stasis and vascular hyperpermeability. (Dolmans et al. 2002, 2151-2156) It has been suggested that vasoconstriction or blood flow stasis in a narrow attachment might cause sloughing of the papillary lesion.

Pa has been reported to generate ROS and singlet oxygen after illumination, and change the permeability of the mitochondrial membrane, thereby inducing apoptosis. (Hoi et al. 2011, Xodo et al. 2012, 799-807) Moreover, Bui-Xuan et al. reported that Pa induces the release of interleukin-6 and tumor necrosis factor-alpha, and enhances the phagocytic activity even in the absence of illumination, and hence it has an immunostimulating activity. In this experiment, necrosis of persistent papillary lesion was

observed selectively in group IIA, this is supposed to be the effect of Pa-based PDT, and not that of Pa treatment alone.

A report by Takahashi et al. did not show tumor regression after PDT using ALA and TONS501 (13,17- bis(1- carboxypropionyl) carbamoylethyl- 3- ethenyl- 2- hydroxy- 3-hydroxyiminoethlidene- 2,7,12,18- tetramethylporphyrin sodium salt), but in the graph, the number of persistent papillary lesion was shown to be approximately 10%. In this experiment, the effect of PDT was seen in 54% of papillary lesions, which is lower than that reported in the previous experiment by Takahashi et al. (Takahashi et al. 2012, 221-4). However, in the previous report, tumor induction was performed by a fewer applications of the drugs, (single DMBA application and 16 TPA applications) and the size of the papillary lesion was smaller based on the supplementary photographs, which were considered to result in a more favorable outcome. Another study also demonstrated a better result of ALA-PDT and meta-tetra (hydroxyphenyl) chlorin (mTHPC)- based PDT in 147 patients, in which only 13 patients showed no improvement or showed aggravation of oral dysplasia. (Jerjes et al. 2011, 192-9) Better results were expected from this study because further cycles of treatment i.e., up to five cycles of PDT were given per patient.

The results of this experiment show that two cycles of Pa-based PDT was an effective treatment modality in 54% of papillary skin lesions. However, this result can be expected in papillary lesions, but not in dysplasia lesions because the authors could not induce dysplasia. Development of a dysplasia model is necessary to validate the efficacy of Pa-based PDT for treatment of premalignant lesions.

V. CONCLUSION

Results of this experiment showed that Pa does not have any remarkable effect on papillary skin lesion in the absence of illumination (11.1%) though the previous reports had demonstrated that pheophorbide a (Pa) has antitumor activity and a potential immunomodulatory effect on macrophages.

Photodynamic therapy (PDT) with Pa was observed to have an effect on early tumor lesions although the success rate was relatively low (54%). More than 3 cycles of Pa-based PDT treatment can be predicted to cause more favorable treatment outcomes, especially in papillary skin lesions.

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ABSTRACT (IN KOREAN)

화학적으로 유도된 쥐의 피부 유두상 병소에서 pheophorbide-a를 이용한 광역학치료 효과

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구강암과 구강전암병소에 대한 기존 치료 방법의 한계로 인해 광역학 치료가 대안으로 생각되고 있다. Pheophorbide a (Pa)는 chlorophyll-a에서 추출된 염소기반의 광감작제로서, 630nm보다 큰 670nm 파장의 빛에서 활성화되고, 빠르게 대사되며, 더 오랜 기간 동안 종양에 선택적으로 축적되는 것으로 알려져 있다. 이 연구는 헤어리스 마우스에서 7,12- Dimethylbenz(a)anthracene (DMBA)와 12- O-tetradecanoyl-phorbol- 13- acetate (TPA) 를 이용하여 유도된 암 모델에서 Pa를 이용한 광역학치료의 효과를 분석하고자 하였다. 실험동물은 두 군으로 분류하였고 I군 (5마리)은 대조군으로 배부에 아세톤 100$\mu\ell$를 적용하였다. II군 (33마리)은 실험군으로 아세톤에 용해된 DMBA (아세톤 100$\mu\ell$ 당 100μg)을 2주간 격일로 적용한 후, TPA (아세톤 100$\mu\ell$ 당 2.5μg를 5주간 격일로 적용하였다. 종양이 형성된 것으로 확인한 후, 임상적으로 유두상을 보이지 않는 진행된 암성 병소가 관찰된 동물은 이후 실험에서 제외하였다. II 군의 동물들

은 다시 2군으로 분류하여 IIA 군(21마리)은 Pa와 레이저를 이용한 광역학치료를, IIB군(6마리)은 Pa 주입만을 시행하였다. 각 동물당 세 개의 가장 큰 병소를 선택하여 200㎍의 of Pa를 주입하였고, 24시간 뒤 동물들을 664nm의 파장을 가지는 laser diode를 이용한 100J/㎠의 빛에 노출시켰다. 첫번째 처치 후 1주일 후에 같은 처치를 반복하였다. 1주일 후 모든 동물들을 희생하여 조직학적 검사를 시행하였다. 치료 결과 Pa 주입 후 광역학치료를 시행한 경우는 54%에서 치료효과를 보였고, Pa 주입만 한 경우는 11.1%에서 치료효과를 보였다. 이 실험의 결과로, 저자 등은 다음과 같은 결론을 내릴 수 있었다.

이전에 Pa 자체는 레이저 조사 없이도 마크로파지에 대한 면역조절 능력을 잠재적으로 가지고 있고 종양 억제 효과가 있다는 보고가 있었으나, 이 연구에서 Pa는 레이저 조사를 하지 않는 경우 피부의 유두상 병소에 대한 치료 효과가 미약하였다 (11.1%).

Pa를 이용한 광역학치료는 초기 종양병소에 적용하였을 때 상대적으로 치료효과는 낮았다 (54%). 그러나 향후 실험에서는 치료회수를 증가함으로써 피부의 유두상 병소에서 치료효과가 개선될 것이라고 예상된다.

이러한 광역학치료의 효과를 구강전암병소에서 확인하기 위하여 상피이형성을 형성할 수 있는 동물실험 모델의 개발이 필요하다.

핵심되는 말: 광역학치료, pheophorbide a, 피부 유두상 병소