GL-1

Symposium-1: In hope of getting over the present stage of clinical PDT

○Norio Miyoshi[1], Katsushi Inoue[2,] and Toru Tanaka[2]
[1]Division of Tumor Pathology, National University of Fukui, [2]SBI ALA-Promo, Ltd. Co.

Argon-dye laser, excimer-dye, gold-vapor and Nd-YAG pumped optical parametric oscillator (OPO) larger lasers had been used in Japan as the light source for photodynamic (fluorescent) diagnosis (PDD) and photodynamic therapy (PDT). Recently, compact and cheaper LD lasers have been used as the light sources of PDD and PDT, respectively. We found that the photoproduct of Pp-IX is produced by the irradiation for Pp-IX to create a new photosensitizer. In the results, we could produce the double wavelength light source (LD laser) against Pp-IX and the photoproduct. In future, we will develop the 3 wavelength LD laser adding the excitation wavelength to the double wavelength. In hope for the new type LD laser development, compact, low cost and convenience to use and move.

On the other hand, it will be hoped to develop these photosensitizers (photofroduct and Pp-IX) to create the other derivative photosensitizer (photoproduct, Chlorine-E6 derivatives) during the irradiation. 5-Aminolevulinic acid (5-ALA) is produced in our body to take metabolized of porphyrine synthesized cycle to produce hem from .Pp-IX. It is true to accumulate in a tumor tissues 4 hr after the administration of 5-ALA. It will need to know the metabolism system of 5-ALA in the normal organs. It will be very important to use the efficiently the 5-ALA combined with the LD lasers for the PDD and PDT against tumor treatments to know the metabolite providences in the body.

IS-2

Photodynamic Therapy and Photodynamic Diagnosis in Korea

Jong Ki Kim, PhD[1], Young Key Shim, PhD[2],
Chan Sup Shim, MD[3], Kyu Wan Lee, MD [4], Doo Yeun Lee, MD [5],
Sei Jun Han MD[6], Woong Shick Ahn, MD, PhD[7],

[1]*Department of Biomedical Engineering, Catholic University of Daegu, Daegu, Korea,*
[2]*Department of Nano System Engineering, Inje University, Gimhae, Korea,*
[3]*Department of Internal Medicine, Kunkook University Medical Center, Seoul, Korea,*
[4]*Department of Obstetrics and Gynecology, Korea University Anam Hospital, Seoul, Korea,*
[5]*Department of Thoracic and Cardiovascular surgery, Kangnam Severance Hospital, Seoul, Korea,* [6]*Department of Obstetrics and Gynecology, Chosun University Hospital, Gwangju, Korea,*[7]*Department of Obstetrics and Gynecology, The Catholic University of Korea College of Medicine, Seoul, Korea*

The Korean Photodynamic Association (KPA) was founded in 2002 in Seoul under the instigation of Professor Chung-Ku Lee (Department of Otorhinolaryngology, Medical Laser & Device Regional Innovation Center, Dankook University) who became the first president of KPA. Its aim was the development of photodynamic therapy (PDT) and photodynamic diagnosis (PDD) in clinical practice. The KPA gathers together many expert PDT practitioners worldwide every year including Japanese. It provides a unique occasion of exchange and interaction between basic researchers and clinicians.

The first meeting of KPA was held in Seoul in 2002 under the presidency of Professor Lee. The following year (2003) the second congress took place in the Catholic University of Daegu, Daegu. Prof. Woong-Shick Ahn M.D., Ph.D. was elected 2nd President of KPA on Aug 21, 2004. In 2004 and 2005 the 3rd national meeting by new cabinet and 4th congresses were held in Samil-Pharm, Co. Ltd., Seoul and in The Catholic University of Korea, Seoul respectively. The numbers of KPA members are 300 now and they are actively attended national and international meetings also the members experienced PDT, PDD using 6-10 photosensitizer and 2-3 different laser in there hospital.

The meeting expanded year by year. We had a 1st Japan-Korea Joint & Asian Photodynamic Therapy meeting in 2009. PDT related Universities, Hospitals and Companies in Korea increased more than 30 this year. We published the journal of KPA and PDT edition.

Based on KPA we have implemented PDT in Korea with great success. The initial resistance of physicians over the use of PDT has been overcome by good results and the country is experiencing a spread of this treatment with many new clinics and hospitals involved in PDT. The current results are satisfactory but we feel there is room for improvement. In particular, further expansion of the indications of our protocols and better understanding of the concept of PDT dosimetry are critical. We have observed the collaborative ventures between researchers in different fields as well as the extrapolation of experimental models to the clinical situation produce considerable improvement in our results. We strongly believe that the next generation of treatments will show enhancement over current results and are optimistic about PDT becoming a mainstream treatment option in Korea.

IS-3

The effect of PDT on *H. influenza* biofilm in vitro and vivo

Chung-Ku Rhee, MD, PhD; So-Young Chang, MS; Phil-Sang Chung, MD, PhD; Jin-Chul Ahn, PhD, Medical Laser Research Center & Department of Otolaryngology-HNS, Dankook University, Cheonan,-city, Korea

Biofilm formation has been demonstrated for many mucosal pathogens such as *Haemophilus influenzae*. The presence of mucosal biofilms with chronic otitis media with effusion (COME) suggests that bacteria do not clear by antibiotics. **Aim:** To test the effect of photodynamic therapy (PDT) on *H. influenzae* induced biofilm in vitro and vivo. **Methods:** In vitro: Sixteen biofilms of *H. influenzae* were maintained on flow cell system culture plates. The biofilms were divided into control, laser, photofrin, and PDT groups. For laser group, 7.2 J/cm^2 (4 mw x 30 min) of 632 nm LED was irradiated to the biofilms. For photofrin group, photofrins 5 and 25ug/ml were added to the media. For PDT group, photofrins 5 and 25 ug/ml were added to the media and LED 7.2 J/cm^2 was irradiated to the biofilms. Then the biofilms were cultured for 3 hours. Live/Dead (DAPI/PI) stain was performed and biofilms were examined under confocal laser microscope for thickness and density of biofilms. In vivo: Sixteen bullae of 8 gerbils were injected with 200μl (10^7CFU/ml) of *H. influenza* and formation of biofilms in the bullae was obtained by 5 days. The bullae were divided into control, laser, photofrin, and PDT groups. The control group received no treatment. For laser group, 120 J/cm^2 (100 mw x 20 min) of 632 nm LD laser was irradiated into the bullae by a fiber inserted directly into the bullae. For photofrin group, photofrin 40μl (1mg/ml) were injected into the bullae. For PDT group, photofrin same as in photofrin group was injected into the bullae and LD laser was irradiated into the bullae same way as in laser group. The mucosal tissues in bullae were examined by H/E staining, and SEM. **Results:** In vitro: By PI staining 40 - 52 % reduction of biofilms in PDT group was noted. The thickness of the biofilms were as followings; control group 124.3±11.7um, laser group 118.5±18.6um, photofrin 5 ug group 110 um, photofrin 25 ug group 71 um, PDT group with 5 ug photofrin 110 ug, PDT group with 25 ug photofrin showed no growth of H. influenzae. In vivo: The control, laser, and photofrin groups have shown well formed biofilm. Two third of the PDT group bullae have shown well resolved biofilm while 1/3 of the bullae have shown incompletely resolved biofilms. **Conclusion:** The results of this study demonstrated that PDT appears to be effective to treat experimental *H. influenzae* induced biofilms in vitro and vivo. Further trial in different dose combinations of photosensitizer and laser needs to be tried for better results in PDT group. Clinical implication: PDT may be an alternative to antiobiotic treatment on otitis media with biofilm formation.

IS-4

Organic Photodynamic Modulated Cell Therapy in Plastic ,Aesthetic Surgery & Dermatology

BestWell　Institute , Seoul, Korea. Dept. of Plastic surgery*　and　Dept. of Dermatology

Prof .Jin Wang Kim MD.PhD*　, Prof. Joung Ok Lee MD.PhD.

Introduction :

Recently developed technology applied light modulated cell therapy enables destroy tumor cell as well as photorejuveantion, fat cell reduction, promote wound healing, scar reduction,hair restoration　etc.

*

Purpose.

Clinical and meta analysis of the result and compare to traditional method.

Material & Method

1000 patients underwent PDT modulated Cell therapy with LED & Laser treatment since 1992 to 2010 with or without photosensitizer.

Result

Patient's response was as belows

Excellent 81 % ,Good 18 % Moderate 1% Poor 0%

Discussion

1. Detailed Mechanism of　PDT Modulated Fat Cell Reduction.
2. Efficacy of PDT modulated Cell Therapy and Diagnosis and its patent in Aesthetic Surgery & Dermatology
3. Efficacy of Photorejuvenation by PDT modulated complication management.
4. Efficacy of hair restoration and hair removal.
5. Efficacy of PDT combine surgery.
6. Efficacy of PDT modulated removal of wart, nevus,aging spot,hemangioma
7. PDT lasers & LED for next 10 years.

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Kang Nam Gu Sin Sa Dong 638-5 BestWell Institute , Seoul, Korea Zip 135-110

www.lasercongresskorea.org

E-mail : khg000@unitel.co.kr

Tel : 82-2-511-3713 ,Fax: 82-2-517-3713

IS-5

Nanotechnology for photodynamic detection and therapy of cancers

Ji-Yao Chen

State Key Laboratory of Surface Physics and Department of Physics, Fudan University, Shanghai 200433, China. Email: jychen@fudan.edu.cn

Although the photosensitizer (PS) based photodynamic detection and therapy (PDT) has achieved a great success, some PS disadvantages including the weak light absorption, non-specific binding for cancers and skin phototoxicity limit PDT further applications. The semiconductor nanocrystals with the size of a few nanometers, also known as quantum dots (QDs), have unique photoluminescence (PL) properties such as the tunable PL band with their size dependent, broad absorption region with the high extinction coefficients, the large two-photon absorption cross section and higher photostability. The surfaces of QDs can be linked with different ligands to become functional QDs for different purposes. The QD based nanotechnology may provide a chance for PDT improvements. In this talk, the works of our group focused on nanotechnology PDT exploring will be introduced from four aspects.

1. The conjugates of QDs with folic acids. Folic acid (FA) has been recognized as a marker for a variety of tumors such as ovarian, prostate, and breast cancers, especially for epithelial cancer cells. The FA-QDs were found to specifically associate to human nasopharyngeal carcinoma cells (KB cells) but not the normal human embryonic kidney cells (293T). Thus the FA-QDs could be promising agents for detection of those cancers having the positive FA receptor.
2. The conjugates of QDs with AlPcS. The photosensitizer AlPcS has the poor light absorption in visible region, while the absorption coefficients of QDs are much higher. In AlPcS-QDs conjugates, QDs absorb the light and work as the donor transferring the energy to the AlPcS (acceptor) via a way of FRET (fluorescence resonance energy transfer). QD-AlPcS can fully utilize the visible region lights as the excitation to effectively produce $^1O_2$ for PDT.
3. (PNIPAM)-coated Fe_3O_4@SiO_2@CdTe multifunctional nanoparticles were used as carriers to deliver AlPcS. Due to the thermal-sensitive property of polymer (PNIPAM), AlPcS can be carried and then released by increasing the temperature to fulfill a specific tumor release.
4. Modifying QDs as the PSs for PDT. The surface traps of QDs can hold photo-induced electrons as long as in milliseconds which may function as the triplet of the PS transferring the electron to nearby oxygen molecules to produce reactive oxygen species ($O_2^{\cdot-}$). When the naked QDs were photooxidized, the surface traps were produced on purpose. These oxidized QDs were found to effectively photoinactivate cancer cells with the efficiency similar to commonly used PSs, and thus show potentials to be explored as a new kind of PS.

IS-6

Sonodynamic therapy with 5-aminolevulinic acid on a rat intracranial glioma

Eun-Ju Chung,[1] Seung-Jun Seo,[1] Ju-Hang Lee,[1] Young-Jun An,[1]Ki-Hong Kim,[2] Hong-Tae Kim,[3] Gi-Hwan Choi,[4] Sanghoon Jheon,[5] Jong-Ki Kim[1*]

Departments of Biomedical Engineering,[1] Anatomy,[3] Neurosuregry,[4] School of Medicine, and Department of Optometry,[2] Catholic University of Taegu, Taegu, Korea, Department of Thoracic surgery,[5] College of Medicine, Bungdang Hospital of Seoul National University, Korea

*: All correspondence should be addressed to Professor Jong-Ki Kim, jkkim@cu.ac.kr

Sonodynamic therapy is the use of low-level ultrasound and this produces tumor destruction from the non-thermal effects of ultrasound, especially cavitations in malignant cells. The photodynamic agents are often sensitive also to ultrasound frequencies. In order to develop new SDT methodology for the treatment of malignant gliomas, we attempt to study sonodynamic therapy with 5-aminolevulinic acid on the orthotopic rat C6 glioma model.

Intracranial gliomas were prepared by inoculating $5x10^6$ C6 glioma cells stereotactically 5 mm deep into the frontal lobe of the left hemisphere after craniotomy in Wistar rats. Animals were divided over 4 experimental groups (1 Radachlorin+SDT, 3 ALA+SDTs each of 3, 14, 30 days post-SDT follow up) and 5 control groups (3 Sham-operated non treatment groups each of 10, 13, 24 days post implantation of tumor cells, 2 SDT only groups each of 3, 14 days post-SDT follow up)

Experimental intracranial glioma, one week after implantation, were sonicated from a sonotransducer(1 Mhz, 152 mw/cm^2, 20 min) through 2 mm-diameter craniometer with or without intravenous injection of the photosenstizer either 5-ALA (60 mg/kg body weight) or Radachlorin (40 mg/kg body weight) four hours prior to irradiation. Antitumor effect was estimated by measuring tumor size after sonodynamic therapy. Tumor areas were measured using imaging software from the hsitopathologic microscopic images of the tissue sections of fixed brain that were removed from rat after heart perfusion with 4% formalin fixing agent.

At seventh day post-implantation of tumor cell, the tumor areas of control group (n=5) were 5.52 ±0.66 mm^2. At 3rd day post–SDT, tumor areas in sham-operated rats and in rats that received sonodynamic therapy without and with either ALA or Radachlorin were 9.95 ± 2.75, 13.37 ± 2.37, 2.72 ± 0.64 and 14.41 ± 1.26, respectively. At 14^{th} day post-SDT, tumor areas in sham-operated rats and in rats that received sonodynamic therapy without and with ALA were 31.18 ± 4.20, 22.11 ± 2.75 and 3.49 ± 0.80, respectively.

Tumor areas were significantly smaller in the SDT+ALA group than in the sham control and SDT only group. ($p < 0.001$) Sonodynamic therapy only group showed 29 % tumor volume reduction ($p < 0.05$) compared with sham-operated control group, and sonodynamic therapy with ALA group demonstrated 88 % tumor volume reduction ($p < 0.001$) compared with sham-operated control group. In conclusion, these data suggested that antitumor effects of ultrasound could be enhanced in the presence of photosensitizer (PpIX, derived from 5-ALA) which might be involved in a sonochemical mechanism.

IS-7

Real-time Clinical Singlet Oxygen Dosimetry for Photodynamic Therapy

Seonkyung Lee[1] and Steven J. Davis[1]

(1) Physical Sciences Inc., 20 New England Business Center, Andover, MA 01810, USA

Photodynamic Therapy (PDT) is a promising modality for cancer treatment. However, the outcomes of PDT treatments are highly variable depending on the individual patient. We have developed two, lab-prototype, *in vivo* capable, real-time dosimeters for PDT: a non-imaging ultra-sensitive point sensor and a 2D imaging sensor. These systems are optical detection probes based on a pulsed diode laser excitation of singlet oxygen emission and photosensitizer (PS) fluorescence.

PDT Dosimetry Development at PSI

PDT is a light activated chemotherapy that is dependent on three parameters: PS concentration; oxygen concentration; and light dosage. PDT uses certain compounds known as photosensitizers that are preferentially retained in malignant tumors. When combined with visible light, the photosensitizers initiate a reaction that selectively kills the malignant cells to which they are attached.

Due to highly variable treatment response, the development of accurate dosimetry to optimize PDT treatment outcome is an important requirement for practical application. During PDT treatment, oxygen molecules in the metastable singlet delta state, $O_2(^1\Delta)$, are generated and are believed to be the active agents responsible for the destruction of cancer cells. The singlet O_2 monitoring is a direct measure of available molecular oxygen multiplied by the PS concentration induced by light dosages ([singlet O_2] $\propto \int [PS(t)]\,[O_2]\,\Phi(t)\,dt$). Therefore, measurement of the singlet O_2 emission is an effective measurement of the PDT treatment efficacy. We have developed two ***singlet oxygen monitor systems as in vivo, real time dosimeters***. These sensors thereby enable direct temporally- and spatially-resolved measurements of the active agent concentration of PDT treatment process. We have completed both *in vitro* and *in vivo* measurements, and these results are promising for the development of the real-time dosimeter for PDT. In animal study, the higher singlet O_2 produced during PDT, the larger tumor reduction volume was observed. .

The point sensor is the most sensitive in singlet O_2 detection and an important step in the development of a useful dosimeter with potential clinical applications. However, only monitoring singlet O_2 production does not distinguish whether the PDT efficacy is limited by available oxygen or available PS. Our 2D imaging system produces 2D spatial maps of both the production of singlet O_2 and the location of the excited PS in a tumor during PDT. Comparison of the images prior to PS injection, the images after PS injection (Cl-e6, ALA, and BPD) shows much higher intensities of singlet O_2 from the tumor area due to the preferential accumulation of PS in the tumor. This would have several important advantages over a single point sensor approach. With such a sensor, we could visualize the particular locations of singlet O_2 emission within tissue. We could also correlate the singlet O_2 production with the PS fluorescence. The 2D imaging sensor would be an important tool for better understanding the kinetics of PDT, the relationship between singlet O_2 production and photobleaching of the PS, and for developing more effective treatment modalities.

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AS-8

Singlet Oxygen Detection in PDT

Toru Hirano,[1] Eiji Kohno,[1] Kazutaka Hirakawa,[2] Junkoh Yamamoto,[3] and Shigetoshi Okazaki[1]

1) Photon Medical Research Center, Hamamatsu University School of Medicine, Hamamatsu, Japan
2) Faculty of Engineering, Shizuoka University, Hamamatsu, Japan
3) Department of Neurosurgery, University of Occupational and Environmental Health, Kitakyusyu, Japan

Photodynamic therapy (PDT) is a cancer treatment modality which utilizes the cytotoxicity of active singlet oxygen ($^1O_2$) that is derived from the irradiation of a tumor-accumulated photosensitizer. As the $^1O_2$ radiates an emission of 1270 nm wavelength when it decays to the triplet state, detection of the emission helps us understand the mechanism of PDT. We describe the $^1O_2$ detection system and some its applications below.

(1) Singlet Oxygen Detection System

We detected the 1270 nm emission due to $^1O_2$ from photosensitizers used in PDT *in vitro* and *in vivo* by means of highly sensitive NIR detectors (R5509-42 or NIR-II; Hamamatsu Photonics, Hamamatsu, Japan). Photosensitizer samples in liquid contained in a quartz cuvette or in animal tumors were exposed to a tunable pulse laser (YAG/SHG-Dye, 355 nm /600~820 nm, ~5 nsec width), and the scattered light from them was guided into the NIR detector via a silicon filter and a 1270 nm interference filter. The detection was conducted with a time gate of a 5 μ sec delay from the laser pulse and a 50 μ sec width to eliminate the photosensitizer fluorescence.

(2) Singlet Oxygen in ALA-PDT

We monitored $^1O_2$ generation during PDT with 635 nm irradiation using ALA in 9L glioma cells *in vitro* and in a subcutaneous rat tumor model inoculated with 9L cells. At a low fluence rate of irradiation, the $^1O_2$ signal at the low level gradually decreased, while, at a high fluence rate, it initially showed a high level and immediately decreased. Consequently, the cumulative $^1O_2$ value at a low fluence rate tended to be higher than that at a higher fluence rate, and the higher cumulative $^1O_2$ value was coincident with the higher effect of PDT *in vitro* and *in vivo*.

(3) Singlet Oxygen from Irradiation of ICG

Indocyanine green (ICG) has a wide optical absorption band extending from 600 nm to above 800 nm, and the maximum absorption exists at around 800 nm. We observed $^1O_2$ generation when a solution of ICG was exposed to a light that has a wavelength in the above absorption band. To investigate the irradiation effect, ICG was injected in a HeLa tumor grown in a nude mouse. Just before laser irradiation, ICG was injected at a dose of 40 mg/kg. Irradiations of 100 mW/cm^2 power and 100 J/cm^2 energy were performed with lasers of 635 nm, 670 nm, and 820 nm. We observed tumor necrosis, as seen in ordinary PDT, with 635 nm and 670 nm. With 820 nm irradiation, the necrosis was particularly severe, and this was suspected to be the result of the combination of PDT and the thermal effect due to the strong optical absorption.

(4) Singlet Oxygen from Irradiation of the TiO_2 particle

Titanium dioxide (TiO_2) is a semiconducting photocatalyst that shows well-known bactericidal activity when exposed to UV light. TiO_2 is also studied as a candidate photosensitizer of PDT. We observed $^1O_2$-mediated 1270 nm emission during irradiation of TiO_2 powder dispersed in liquid when exposed to a 355 nm laser. As this emission diminished with SOD addition, we understood that $^1O_2$ was produced via the oxidation of superoxide (O_2^-). Irradiation of TiO_2 generated $^1O_2$, and this was supposed to be a main cause of its phototoxicity to biomolecules.

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AS-9

PDD-PDT spectrophotometoric fluorescence monitoring systems using HeLa-tumors in nude mice and author's seborrheic keratosis

○Takato O. Yoshida[1], Eiji Kohno[1], Katsushi Inoue[2], Takashi Sakurai[1], Seiji Yamamoto[1] and Susumu Terakawa[1]

[1] Photon Medical Research Center, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, 431-3192, Japan

[2] R&D Dept., SBI ALApromo CO., LTD., 8-15-1 Todoroki Setagaya-ku, Tokyo, 158-0082, Japan

On December 1997, we found that the mitochondrial area changed its fluorescence color from red to green, and the cells were simultaneously swelling in the cultured HeLa cells, during 488 nm irradiation after Photofrin®-staining *in vitro* (Porphyrins 7, 327-377, 1998). Since 1998, we have tried to search the mechanisms of the mitochondrial area changed its fluorescence from red to green color under a Nipkow disk-scanning confocal microscope in the HeLa cultured cells after labeling them with DsRed1-Mito and staining the cell with Photofrin. The change in fluorescence color from red to green in the area of mitochondria was tightly associated with their swelling during irradiation. It was confirmed that the green fluorescence, which appeared and increased during the Photofrin-irradiation and, was really a cell-death signal as it was compared with those of cell-death induced by FCCP (carbonyl cyanide-*p*-trifluoromethoxyphenyl-hydrazone) and ionomycin. And we established a PDD-PDT spectrophotometric real-time fluorescence monitoring system in HeLa-tumors in nude mice during Photofrin-PDT and ALA-PDT using a YAG dye pulse laser (630 nm or 635 nm) combined with a spectral multichannel analyzer (PMA-11, 405 nm). Because, in the PDT practice for tumor patients, the dose and irradiation time for the treatment are chosen by experience of doctors and not by the real need. (Patent: Japanese 2004- 361814/ open 2006-167046). We have reported at the annual meeting of JPA and the world congress of IPA as "New models of PDD-PDT spectrophotometric real-time fluorescence monitoring system *in vivo*" using Photofrin and 5-ALA in HeLA tumor bearing BALB/c nude mice and author's seborrheic keratosis. The results were published as a title "Novel PDD-PDT system based on spectrophotometric real-time fluorescence monitoring and MALDI- TOF-MS analysis of tumors" (Proc. SPIE vol. 7380 1l-1~6, 2009).

At this 2nd Asia joint symposium of PDT, the PDD-PDT data of 5-ALA in author's seborrheic keratosis used with LED* irradiation (635 nm, 200 mW/cm^2) combined with VLD** intermittent spectrophotometric monitoring (405 nm, 90 mW/cm^2), and VLD irradiation and intermittent spectrophotometric monitoring (405 nm, 90 mW/cm^2) systems will be presented and discussed as compare with the previous 5-ALA results of "Novel PDD-PDT spectrophotometric real-time fluorescence monitoring system".

*LED: light-emitting diode　**VLD: violet laser diode

AS-10

Terahertz Spectroscopy and Imaging of Biological Samples

Hiromichi Hoshina[1], Aya Hayashi[1], Norio Miyoshi[2], Yuichi Ogawa[3], Shigeaki Ueno[3], Yukihiro Fukunaga[2], Fumiaki Miyamaru[4], and Chiko Otani[1]

[1] RIKEN, Sendai, 980-0845, Japan
[2] University of Fukui, Fukui, 910-1193, Japan
[3] Tohoku University, Sendai 981-8555, Japan
[4] Shinshu University, Matsumoto, 390-8621, Japan

By virtue of the penetrative property and the fingerprint spectrum, THz wave have been spotlighted in the various application fields. In the medical field, the difference of absorbance between cancer and normal tissue was found, and the potential of the THz imaging for the pathologic diagnosis has been expected. However, the absorption spectra of the cancer tissue show no remarkable structure, and show different patterns with the individual disease types. Thus the THz imaging can not be applied for the cancer diagnosis straightforwardly. We have demonstrated the Chemometrics technique (partial component analysis and cluster analysis) introduced for the systematic analysis of the THz spectroscopic images of the plural tumor samples.[1]

Another problem for terahertz imaging is strong absorption of water contained in the tissues. Most of the research has been done by the transmission measurement of dried, thin samples or by the reflection measurement. One of the possible methods to reduce water absorption is by freezing, because the absorption of ice is one order weaker than that of liquid water in our frequency range. We demonstrate terahertz spectroscopic imaging of frozen biological samples. The samples were frozen to -40^{o}C and the spectra were measured using a THz time domain spectrometer. The transmission image shows clear contrast originated in the water content in the tissues.[2] We also found a difference of THz transmission spectra between rapidly frozen tissues and slowly frozen ones, which is mainly due to the difference of ice crystal size. In order to clarify this effect, we also measured the THz transmission spectra of frozen gelatin solution, which was colored by Rodamin B and the particle shape of the ice was observed using an optical microscope.

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AS-11

Enhancement of 5-Aminolevulinic acid-induced oxidative stress by gold nanoparticles

Shinji Ito[1], Norio Miyoshi[1], William G. DeGraff[2], Kunio Nagashima[3], Louis J. Kirschenbaum[4], and Peter Riesz[2]

[1]*Faculty of Medical Sciences, University of Fukui,* [2]*Radiation Biology Branch, National Cancer Institute, National Institutes of Health,* [3]*Electron Microscope Laboratory, National Cancer Institute, SAIC Frederick, National Institutes of Health,* [4]*Department of Chemistry, University of Rhode Island*

5-Aminolevulinic acid (5-ALA) and its methyl ester (5-ALA-Me) at mM concentration levels induce oxidative stress via the production of reactive oxygen species (ROS). Human cancer cell lines (MCF-7 mammary adenocarcinoma, HepG2 hepatocellular liver carcinoma, and A549 lung carcinoma cells) incubated in the dark in the simultaneous presence of 5.0 mM or more 5-ALA or 5-ALA-Me and 7 μg/mL of 15 nm citrate capped gold nanoparticles (AuNPs) were damaged more seriously compared to those in the presence of the levulinic acid alone. Damage (for MCF-7) is visible in electron micrographs which reveal similar morphology both in the presence or absence of AuNPs. Cytotoxicity was observed irrespective of the presence of serum and medium. Production of ROS in cell free samples containing 5-ALA-Me was monitored by EPR as the DMPO-OH spin adduct, and also showed a catalytic effect of AuNPs. Both SOD and CAT inhibited the production of ROS and also reduced cytotoxicity in the cell samples. These observations can be explained by initial attack on the cell membrane by ROS produced in the medium outside the cell and provide insight into possible uses of 5-ALA in cancer chemotherapy.

AS-12

Development of production method and usage of 5-aminolevurinic acid (5-ALA)

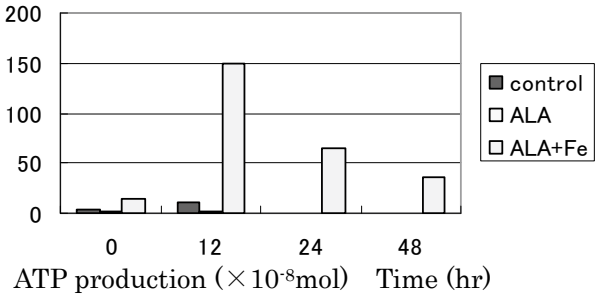
Dr. Tohru Tanaka, SBI ALApromo CO., LTD

5-ALA is a precursor of heme and chlorophyll. We have established production methods of 5-ALA by fermentation using the mutant strain of photosynthetic bacteria *Rhodobactor sphaeroides* and now we are developing usage of 5-ALA. Functional fertilizer contain 5-ALA named PENTAKEEP can improve photosynthesis and it become the center of public attention in world agricultural field. Additionally, 5-ALA improves salt tolerance and water utilization ratio of plants. It's already use for greening of desert land and seems effective for biomass energy production too.

In the medical field, 5-ALA-PDT, which selectively kills cancer cells by lightening accumulated PPIX, has been practically used. It is getting popular, for it can get rid of cancer without scar. In 1993, Prof. Bown and his team at Laser Medical Center published on the lancet the report that fluorescence of Porphyrin is found in larynx cancer thorough oral application of 5-ALA. It inspired R&D of 5-ALA-PDT and PDD. A lot of researches to be conducted since then show ALA administration near perfectly accumulate Porphyrin, which could be checked by its fluorescence. SBI ALApromo CO., LTD has been established as a bio-venture for developing 5-ALA in medical field by Cosmo oil and SBI holdings. SBI ALApromo and Tokyo Institute of Technology conducted a preliminary joint study to measure Porphyrin in blood and urine of cancer patients after 5-ALA administration, for we assumed the accumulated Porphyrin should be flown into blood and urine, and its analysis enables cancer screening. Pre-clinical and small scale clinical studies so far clearly support our assumption.

Today we would like to introduce PDD on cancer using 5-ALA at first. And we would like to introduce administration effect of 5-ALA with iron. It promotes electron transfer system of mitochondria and recover basic metabolism of human.

Promotion effects of 5-ALA on plants



Effects of ALA on ATP production

AS-13

Possibility of carotenoids as a photosensitizer in photodynamic therapy

H. Yoshii,[a] Y. Yoshii,[b] K. Hobo,[c] A. Fujii,[c] Y. Fujii,[c] T. Asai,[c] T. Furukawa,[d] S. Takaichi,[e] and Y. Fujibayashi[d]

[a] Faculty of Medical Sciences, University of Fukui, Eiheiji, Fukui 910-1193, JAPAN
[b] Biomedical Imaging Research Center, University of Fukui, Eiheiji, Fukui 910-1193, JAPAN
[c] Faculty of Engineering, University of Fukui, Fukui, Fukui 910-8507, JAPAN
[d] National Institute of Radiological Sciences, Chiba, Chiba 263-8555, JAPAN
[e] Department of Biology, Nippon Medical School, Kawasaki, Kanagawa 211-0063, JAPAN

In the present study, a possibility of carotenoids as a photosensitizer in photodynamic therapy (PDT) in dermatology was explored.

Singlet oxygen ($^1O_2$), one of reactive oxygen species, is known to have toxicity to cells. Green plants use several carotenoids, which have $n > 10$ length of conjugated double bonds, to relax $^1O_2$ involuntarily generated by photosynthetic process. Since these carotenoids have the T_1 level (first triplet excited level) just below the energy level of $^1O_2$, they have $^1O_2$ quenching ability. Conversely, we hypothesized that carotenoids with $n < 10$ length of conjugated double bonds, of which the T_1 level is above the energy level of $^1O_2$, might be able to generate $^1O_2$ via photo-excitation and damage tumor cells.

A375 human melanoma cells were incubated in medium containing carotenoids, which have conjugated double bond length of n = 9-11, under blue light irradiation. After 5-hour incubation, number of living cells was counted. As results, we found that cell death rate was increased with shortening conjugated double bond length, and cell death rate was particularly high with n = 9 carotenoids. This result could indicate the positive correlation between cell death ability and T_1 state energy of carotenoid. In order to examine whether photo-excited n = 9 carotenoids generate $^1O_2$, electron spin resonance (ESR) spectra of photo-excited fucoxanthin, which has n = 9 conjugated length, was measured with 2,2,6,6-Tetramethyl-4-piperidone hydrochloride (TMPD) as a $^1O_2$ trapping agent. Samples containing fucoxanthin and TMPD were irradiated with blue light for 0, 20, 40, 60, 80 and 100 min with presence or absence of oxygen, and ESR spectra were measured just after irradiation. The yields of ESR peaks observed under presence of oxygen were proportional to irradiation time below 60 min. This result could indicate $^1O_2$ production via photo-excitation of fucoxanthin. The yields of ESR peaks observed under presence of oxygen stagnated after 60 min and ESR spectra under absence of oxygen have no peaks. This oxygen concentration dependence indicates that energy transfer from photo-excited fucoxanthin to oxygen could occur.

These findings indicated that photo-excited n = 9 carotenoids can attack to tumor cells and photo-excited fucoxanthin can generate $^1O_2$. Production of $^1O_2$ via photo-excitation of carotenoids and their toxicity to tumor cells were observed for the first time. The photosensitizing ability of carotenoids might be utilized in therapy for skin disease.

AS-14

Determination of the optical properties of the tissues treated by photodynamic therapy using Inverse Monte Carlo method between 350 nm and 1000 nm

Norihiro Honda[1], Takaya Terada[1, 2], Takuya Nanjo[1], Katsunori Ishii[1], Kunio Awazu[1, 2, 3]

1. Medical Beam Physics Laboratory, Graduate School of Engineering, Osaka University, Japan
2. Development of System and Technology for Advanced Measurement and Analysis, Japan Science and Technology Agency, Japan
3. Research Institute of Nuclear Engineering, University of Fukui, Japan

In photodynamic therapy (PDT), the accurate knowledge about the optical properties (absorption coefficient μ_a [mm^{-1}], reduced scattering coefficient μ_s' [mm^{-1}], etc) of the target tissue is important for the understanding and prediction of propagation and distribution of light in tissues, since the PDT efficacy depends on the photon propagation and fluence rate distribution within irradiated tissues. Recently, optical properties of various normal and pathologic tissues have been determined at single wavelength or over broad wavelength range. However, there are a few reports on the alteration of the tissue optical properties during and after PDT. The objective of this study is determination of the optical properties of the tissues treated by PDT in the wavelength range from 350 nm to 1000 nm using double integrating sphere measurement system combined with an Inverse Monte Carlo (IMC) method.

In this study, mouse tumor tissues obtained from subcutaneously implanted Lewis lung carcinoma cell line were treated by a normal PDT procedure used a Talaporfin Sodium (Laserphyrin®, MEIJI SEIKA KAISHA, LTD.) as a photosensitizer at a concentration of 5 mg/kg body weight and a laser diode (UM1000 Dental_665, JENOPTIK AG) with a wavelength of 664 nm. For determination of the tissue optical properties during PDT, laser irradiation was performed for 1, 5 and 10 min at the power density of 100 mW/cm^2. For determination of the tissue optical properties 1, 2, 7 days after PDT, light exposure was performed with a total energy density of 100 J/cm^2 at the power density of 100 mW/cm^2. The diffuse reflectance R_d and the transmittance T_t of the PDT-treated tissues were measured by the double integrating sphere system. After the measurements, the μ_a and μ_s' of tissues were calculated in the wavelength range from 350 nm to 1000 nm.

During PDT, the μ_a spectra and the μ_s' spectra were not changed significantly. 7 days after PDT, the μ_s' at the wavelength of 664 nm was increased from 0.64 mm^{-1} to 1.24 mm^{-1}. Consequently, the optical penetration depth in the treated tissues was about 44 % lower than that in the tissues before PDT at the wavelength of 664 nm. To ensure the effective procedure, an adjustment of the laser parameter for the decrease of penetration depth is recommended for a second PDT.

AS-15

Photodynamic effect of pheophorbide *a* on MRSA, *Pseudomonas aeruginosa* and viruses

Masami Kobayashi[1]*, Akiyoshi Saito[2], Toru Yamamoto[2], Katsuaki Taira[2], Takanori Iriuchishima[2], Junpei Saito[3], Yu Nagai[2], Masaaki Okuda[1], Yasuhiro Abe[1], Masataka Nakazato[4], Hiroshi Sentsui[5]

[1] Institute of Materials Science, University of Tsukuba
[2] School of　Medicine, Nihon University
[3] Department of Matsudo Dentistry, Nihon University
[4] Chlorophyll Research Institute
[5] Department of Veterinary Medicine, Nihon University, JAPAN

Plasmid DNA was efficiently photocleaved by water-soluble sodium pheophorbide (Na-Phde) *a*, prepared from chlorophyll *a*, in the absence of oxygen as well as in the presence of oxygen. No singlet oxygen production by Na-Phde *a* with visible light was observed in water, but with remarkable efficiency in organic solvents. Singlet oxygen pathways for DNA photocleavage by Na-Phde *a* in water is unlikely, even though it is widely thought that singlet oxygen seems to be involved in the mechanism of photocleavage of DNA by various photosensitizers. Evidence for the electron capture from nucleic acid bases, guanine and adenine, by porphyrin in the singlet excited state was obtained; guanine- and adenine-attached porphyrins showed the strongest and the second strongest fluorescence reduction, respectively, while no decrease was observed in cytosine- or thymine-attached porphyrins. Oxidation potential of guanine is lower than that of adenine, but both potentials are low enough to reduce porphyrin in the singlet-excited state, supporting the idea that oxidation of guanine and/or adenine by the singlet-excited Na-Phde *a* is the first step in the oxygen-independent photocleavage of DNA .

Fluorescence microscopic observation showed a rapid incorporation of Na-Phde *a* into nuclei, mitochondria and lysosome in the cells. Bright and strong red fluorescence rapidly decayed during the microscopic observation, due to the photoreaction of Na-Phde *a* causing crucial damage to these organelles.*

Methicillin-resistant *Staphylococcus aureus* (MRSA) was completely sterilized by Na-Phde *a* with 30 min laser irradiation (670 nm, 300 mW) *in vitro*. The bactericidal photoeffect *in vivo* was also observed in MRSA arthritis of DBA/1J mice.

Gram-positive bacteria (MRSA*, β-Streptococcus, Enterococcus faecalis*), were sterilized by Na-Phde *a* with 75 W halogen lamp, but Gram-negative bacteria (*E. coli and Pseudomonas aeruginosa*) could not be killed *in vitro*. However, *P. aeruginosa* was sterilized in the presence of 15% ethanol, indicating that singlet oxygen production by Na-Phde *a* with visible light is indispensable to the *P. aeruginosa* sterilization.

Antiviral effects of Na-Phde *a* was investigated using several viruses: bovine herpesvirus 1(dsDNA), bovine viral diarrhea virus, Getah virus (+ssRNA), bovine parainfluenza virus 3 and vesicular stomatitis virus (-ssRNA). Na-Phde *a* suppressed the replication of all viruses with the irradiation of 20 W fluorescent lamp. Though the genotypes of these viruses are different, all viruses form the double strand nucleic acids in the process of viral replications. This step would be affected by Na-Phde *a,* and photodynamic antiviral actions were induced.

AS-16

筑波大学
University of TSUKUBA

Hirofumi MATSUI, M.D., Ph.D.
Division of Gastroenterology,
Graduate School for Comprehensive Human Sciences
University of TSUKUBA.
Email: hmatsui@md.tsukuba.ac.jp
1-1-1 Ten-nohdai, TSUKUBA, IBARAKI 305-8575, Japan
Tel: +81-29-853-3466, Fax: +81-29-853-3218

Gastric Cancer Specific Porphyrin Accumulation

Hirofumi MATSUI and Tsukyoshi KANEKO

[Introduction]　Policard reported the tumor specific porphyrin accumulation in 1924. Utilizing this phenomenon, Dougherty proposed a photodynamic therapy (PDT) with hematoporphyrin (HP) in 1979. Moreover, Kennedy proposed a new PDT method with aminolevulinic acid (ALA), a precursor of heme biosynthesis. However, mechanisms of these cancer specific porphyrin accumulations still remain unknown. We have been continuously investigating these mechanisms in vitro system with a rat gastric epithelial cell, RGM1 and its tumorigenic mutant RGK-1. Recently, we have gotten several evidences to prove Nitric Oxide (NO) induced these phenomena. I would like to propose a possible mechanism of cancer specific porphyrin accumulation after both the ALA and the HP treatment in this lecture.

[Study 1: porphyrin accumulation after the ALA]

Gastric cancer cells emitted porohyrin specific fluorescence 3hours after 1 mM ALA treatment. HPLC analysis revealed this fluorescence was indicative of protoporphyrin, the last precursor in heme biosynthesis. We investigated to find that a ferrocheletase activity particularly decreased in cancer cells. Since the active-site of the enzyme, an iron-sulfur-cluster (ISC), should irreversibly react with NO to be inactivated it, we hypothesized that cancer specific high concentration of NO plays an important role to induce this phenomenon. To elucidate this hypothesis, we examined intracellular NO concentrations in each cell, treated cells with a chemical NO donor, and inserted NOS2 (iNOS) cDNA in a normal cell to induce high NO conditions. In cancer cells, the NO concentration was higher than that of the normal cell. The NO donor treatment induced a cancer cell specific increase of fluorescence. The NOS2 DNA insert resulted that the cell emitted porphyry fluorescence. Moreover, we investigated whether dinitrosyl dithiolate iron complex (DNIC), a molecules induced by the reaction between NO and ISC, was formed in each cells or not, and found that it particularly formed in cancer cells. We thus concluded that NO induced cancer specific fluorescence.

[Study 2: porphyrin accumulation after the HP]

As described above, cancer specific porphyrin accumulation was well known since 1924, the mechanisms of this phenomenon still remain unknown; on the contrary, the cellular mechanisms of heme/ porphyrin transport are not revealed. In 2005, a new heme transporter, Heme-Carrier-Protein-1 (HCP-1) was reported. This protein is located in intestinal epithelial cell surface. We investigated the expression of HCP-1 in gastric cells, and found that this protein was particularly existed in cancer cells. Moreover, we elucidated the effect of erythropoietin (EPO) on the expression of this protein, because EPO regulates heme biosynthesis in bone marrow system. EPO treatment induced a HCP-1 increase, and an inhibitor of GATA, an element for EPO, clearly decreased the expression of both HCP-1 and EPOR. EPO treatment also induced the increase of cancer specific porphyrin fluorescence. We thus concluded that HCP-1 was one of heme/ porohyrin carrier, and its cancer specific expression induced cancer specific fluorescence after the HP treatment. We also proposed that EPO/ EPOR regulate the expression of this protein.

AS-17

The application of infrared microscope to medical field

Kenichi Akao, Norio Miyoshi

IR & Raman Application laboratory JASCO Corporation.
Fukui University

Infrared(IR) microscope can be used not only to simply measure the micro region to obtain IR spectrum, but also to perform IR imaging, which enables to visualize the information on distribution of molecules in specified measurement area. Since IR imaging of pathological sample can be obtained without dyeing, it is expected to be applied to simple and easy pathological diagnosis. However, using previous type of IR microscope, there needs enormous amount of time to obtain the data of IR imaging. On the other hand, the current multi-channel IR microscope with built in multi-channel detector, rapid scan and high performance stage, can reduce the measurement time to be less than 1/100. In addition, it is possible not only to analyze molecular structure but also to estimate the secondary structure (SSE) of proton using IR spectra. Here, we will discuss the possibility of applying the results obtained by using multi-channel IR microscope and protein SSE imaging to the medical field.

AS-18

Facile Synthesis and Characterization of Pyropheophorbide-a -Taxol Conjugate

Pankaj Kumar Chaturvedi[1], Hyo Jun Kim[1], Gantumur Battogtokh [1], Sook Hee Kim[1], Lan Ying Wen[1], Sumi Bae[1], Woong Shick Ahn[2].
[1]Cancer Research Institute, The Catholic University of Korea, Seoul, Korea; [2]Dept. of Ob/Gyn, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea,

Chlorin-based photosensitizers are considered the ideal photosensitizer (second generation) in photodynamic therapy (PDT). They have improved efficacy and have lesser side effects compared to porphyrin-based photosensitizers (first generation). Paclitaxel (PTX) is one of the most effective and widely used anticancer a drug. Although chemotherapy is a powerful and important modality in cancer treatment, the effectiveness of the chemotherapy is limited due to systemic toxicity and side effects.
For the improvement of photosensitizers (chlorin; apoptosis, necrosis, local immune response) and increased anticancer drug (PTX; apoptosis), we combined photosensitizer and PTX.
For the synthesis, naturally occurring chlorin derivative was used as a starting material. It was prepared with the acidic treatment of methyl pyropheophorbide-a from *Spirulina maxima* algae according to the previously reported methods [1-2]. PTX should contain three hydroxyl groups at 2^1-position and 1-position and 7-position, respectively. A hydroxyl group at 2^1-position has more reactivity than others. To the synthesize target compound, we carried out an esterification reaction between the hydroxyl group at 2^1-position for the PTX moiety and carboxyl group at 17^3-position for the pyropheophorbide-a. The conjugate was performed as reaction the pyropheophorbide-a and the commercial PTX by using DMAP/DCC coupling agent. The structure of chlorin- based PTX conjugate was fully characterized by 1D and 2D NMR techniques and also by MALDI-MS and UV-Vis. As well as, the conjugate was determined the purity of 99.45% by analytical reversed phase HPLC at 660 nm. This conjugate can be applied as anticancer agent and photosensitizer for PDT. This compound is promising to be able to afford some synergistic effects in tumor cell.

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AS-19

Synthesis of chlorin-based fatty acid conjugate as photosensitizer for photodynamic therapy

Gantumur Battogtokh [1], Sook Hee Kim[1], Lan Ying Wen[1], Sumi Bae[1], Woong Shick Ahn[2].
[1]Cancer Research Institute, The Catholic University of Korea, Seoul, Korea; [2]Dept. Ob/Gyn, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea

Photodynamic therapy (PDT) is promising an approach to cancer treatment that requires the combination of photosensitizer (PS), tissue oxygen, and light. Many PSs have been developed for the PDT; however, very few have made in clinical trials because improved drugs that are more selective and that can be used conveniently and without sustained skin photosensitivity are needed. Tumor targeting drug delivery is another attractive strategy in cancer treatment.

In this study, our purpose is to improve their selectivity on tumor cell as conjugating of chlorin-based PSs with unsaturated fatty acids (UFAs) that are taken up rapidly by tumors from the arterial blood, presumably for use as biochemical precursors and energy sources.

We have selected methyl pyropheophorbide-d-OH (MPPd) and pyropheophorbide-a-17^3-N-hexanol (PPa-N-He) that possess reactive hydroxyl groups for the molecule as developing photosensitizers, which were conjugated with docosahaxaenoic acid (DHA) and oleic acid (OA) by esterification reaction DCC-mediated, respectively, and gave in enough yield. Our selected PSs were obtained according to Tamiaki et al.[1] and Smith et al.[2] reported. Novel MPPd-DHA, MPPd-OA, PPa-N-He-DHA, and PPa-N-He-OA were prepared and structure of them was confirmed by spectroscopy's methods (1D and 2D NMR, UV-vis, and MS). The spectrum data of the compounds was clear and easy to characterize.

Our synthesized chlorin-based fatty acid conjugates that could be present a synergistic effect for PDT through a more accumulation of the conjugates on tumor cells due to both of those produce a reactive oxygen substrate (ROS). The conjugates have a high lipophilicity because the UFAs were linked to the PSs and the lipophilicity of PS has proven to be an important factor because it influences the biodistribution and clearance and thus the bioactivity of drugs.

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AS-20

Enhance efficacy of photodynamic therapy in combination with selenium in TC-1 animal model

Lan Ying Wen[1], Sumi Bae[1], Ki Ryung Choi[1], Myung Ha Song[1], Jin Hwan Do[1], Tae Kyu Ahn[3], Sung Hwan Lee[3], Sei Jun Han[3], Jeon Woo Kyu[4], Woong Shick Ahn[1,2].

[2]The Catholic University of Korea, Seoul, Korea; [2]Dept. of Ob/Gyn, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea; [3]Dept. of Ob/Gyn, Chosun University Hospital, Kwangju, Korea; [4]Dept. of Internalmedicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea

Photodynamic therapy (PDT) is a promising cancer treatment modality that involves the interaction of the photosensitizer, molecular oxygen and light of specific wavelength to destroy tumor cells. To improve efficacy of PDT, the efforts are underway to develop combination protocols. Selenium is an essential trace element and has anticarcinogenic properties on a various human cancer model. The aim of this study was to investigate the synergetic anti-tumor effects of PDT plus selenium in vitro and in vivo.

To compare the anti-proliferative effects by PDT, Selenium, or PDT plus selenium, MTT assay, microscopic observation and FACS analysis were performed. Apoptosis signaling pathways were analyzed using mouse signal transduction pathway^{RT} profiler PCR array (Superarray). For the vivo study, when tumor size was approximately 8 to 10 mm, Radachlorin (10mg/kg b.w.) was injected in to the tail vein at 3 hrs before irradiation with 100 J/cm2 of light and then selenium (1ug/kg b.w) was administrated daily during 20 days.

Our results demonstrate that combination of selenium with PDT strongly inhibits growth of TC-1 cell and tumor in TC-1 cell implanted mice when compared to the other groups. Moreover, PDT and selenium treatment in TC-1 cells had apoptosis features in microscope and FACS analysis. Increased apoptosis was associated with tumor inhibition in the combination therapy group. In signal transduction pathway Superarray, genes closely relate to NFkB, p53, and phopholipase C pathway such as vcam1, mdm2, and fos were very significantly down-regulated at least 10 fold in TC-1 cell following co-treatment of PDT and selenium. Furthermore, anti-apoptosis related genes and tumor-promoting genes, birc2, birc3, hk2, ccl2, ptgs2, wisp1, mmp10 and wisp1 were also significantly down-regulated. Up-regulated genes by co-treatment of PDT and selenium had relatively low ranges of fold change than fold range of down-regulated genes and were related with cell survival and proliferation mechanism.

These data indicate that combination therapy of selenium and PDT could more effectively induce tumor suppression response compared to PDT or Selenium alone. We suggest that combination therapy using PDT and selenium can be a useful to for inducing synergistic anticancer effect.

AS-21

Synthesis and characterization of chlorin based anticancer drug conjugate

Sohail Ahmed Ansari[1], Gantumur Battogtokh[1], Lan Ying Wen[1], Su-Mi Bae[1], Woong Shick Ahn [3]
[1]Cancer Research Institute, The Catholic University of Korea, Seoul, Korea; [2]Dept. of Ob/Gyn, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea,

Photodynamic therapy (PDT) is a promising approach to cancer treatment. PDT is based on the combination of a photosensitizing agent and light. Chlorin-based photosensitizers are considered to meet requirements for the ideal photosensitizer in PDT. They have improved efficacy and have lesser known side effects compared to porphyrin-based photosensitizers. Cis platinum is the most effective and widely used drug for chemotherapy. Although chemotherapy is a powerful and an important modality in cancer treatment, the effectiveness of the chemotherapy is limited by systemic toxicity and side effects of agents used.

For the syntheses, naturally occurring chlorin derivatives were used as starting material. It was prepared with the acidic treatment of methyl pyropheophorbide-a (MPPa) from *Spirulina maxima* algae according to the previously reported methods [1-2]. Then, pyropheophorbide-a (PPa) was prepared from MPPa. To synthesize the chlorin-based platinum conjugate, PPa bearing free amine was primarily prepared, which was reacted by treating with potassium tetrachloroplatinate in the mixture of tetrahydrofuran and water. The structures of chlorin-based cis platinum conjugate was fully characterized by ^{1}H NMR (1D and 2D), MALDI-MS, UV-vis, ICP mass, IR and elemental analysis techniques.

The conjugates can be applied as anticancer agents for PDT of tumor cell and is promising to be able to afford some synergistic effects.

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AS-22

9-hydroxypheophorbide α–mediated Photodynamic Therapy derived Oxidative Stress initiates Elevation of intracellular Calcium Level and Apoptosis in AMC-HN-3 cells

Jin-Chul Ahn [1,2], Peijie He [1,2,3], Jang-In Shin [1] and Phil-Sang Chung [1,2]

[1] Medical Laser Research Center, [2] Dept .of Otolaryngology-Head & Neck Surgery, Dankook University, Cheonan, Korea, [3] Dept .of Otolaryngology-Head & Neck Surgery, affiliated Eye, Ear, Nose & Throat Hospital, Fudan University, Shanghai, China

The role of 9-hydroxypheophorbide α (9-HPbD)-PDT induced oxidative stress on intracellular calcium level and apoptosis in AMC-HN-3 head and neck cancer cells was investigated. Apoptosis and alteration of intracellular calcium level were monitored by confocal microscopy and flow cytometry. A significant calcium elevation in AMC-HN-3 cells 2 h post irradiation, being observed in dose-dependent manner, was significantly inhibited by pretreatment with glutathione (GSH) or ascorbic acid. After 9-HPbD-PDT, apoptosis with dose-dependent manner was also significantly inhibited by GSH or ascorbic acid pretreatment. The above observations demonstrate that oxidative stress induced by 9-HPbD-PDT play a causative role on the elevation of intracellular calcium and apoptosis in AMC-HN-3 cancer cells.

Presentation type: Poster

Corresponding Author

Phil Sang Chung, MD

Department of Otolaryngology-Head and Neck Surgery, College of Medicine, Dankook University Hospital, Cheonan, Korea, 330-714

Tel : 82-41-550-3975 Fax : 82-41-556-1090

Email : pschung@dankook.ac.kr

AS-23

Photochemical approaches for drug delivery and combination therapy

Woo-Dong Jang

Department of Chemistry, College of Science, Yonsei University
262 Seongsanno, Seodaemun-gu, Seoul 120-749, Korea

Recently, less-invasive technologies for cancer therapy are attracting increasing attention for the improvement of quality of life. For example, photochemically-driven approches are being assumed as an emerging technology because the delivery of light can be successfully controlled to the target organ. Accroding to the recent advances of laser technology, light delivery and dose control are becoming easier, and wide range of light sources are becoming useful. Within the various photochemically-driven therapeutic approaches, photochemical delivery of several therapeutic agent will be introduced in this symposium. Recently, we have developed dendritic photosensitizers to minimize collisional quanching of photosensitizing unit. And also, we have successfully designed dendrimer-incorporated polymeric micelle using the charged nature of dendrimer surface. Currently, we are developing the ternary complex micelle, which is composed of cisplatin, dendritic photosensitizers, and diblock copolymers, for the combination of photodynamic therapy and cisplatin delivery. The ternary complex have shown sustained release of cisplatin and generation of singlet oxygen under light irradiation. As another approach, we have designed plasmid DNA-incorporated ternary complex system for the selective gene experssion on the specific site. By the laser irradition, we could control the gene expression in defined area. More detailed research results will be reported in the symposium.

AS-24

Evaluation of Fluorescence Image and Excitation Light Source for PDD

Hyun Soo Lim, Ph.D

Department of Biomedical Engineering, College of Medicine

Chungnam National University

Daejeon, Korea

Abstract

Photodynamic diagnosis (PDD) is a method to diagnose the possibility of cancer, both by the principle that if a photosensitizer is injected into an organic tissue, it is accumulated in the tissue of a malignant tumor selectively after a specific period, and by a comparison of the intensity of the fluorescence of normal tissue with abnormal tissue after investigating the excitation light of a tissue with accumulated photosensitizer.

Currently, there are two methods of PDD: The first is a way to acquire incitement fluorescence by using a photosensitizer, and the second is a way to use auto-fluorescence by green fluorescence protein (GFP) and red fluorescence protein (RFP) such as NADH+ active factors within the organic body. Since the selection of the wavelength band of excitation light has an interrelation with fluorescence generation according to the selection of a photosensitizer, it plays an important role in PDD. This study aims at designing and evaluating light source that can stably generate light with various kinds of wavelengths in order to make possible PDD using a photosensitizer and fluorescence image using the developed light source and mouse model. The light source was a Xenon lamp and filter wheel, composed of an optical output control through Iris and filters with several wavelength bands. It also makes the inducement of auto-fluorescence possible because it is designed to generate a wavelength band of 380-420nm, 430-480nm, 480-560nm. The transmission part of the light source was developed to enhance the efficiency of light transmission. To evaluate this light source device, the characteristics of light output and wavelength band were verified. To validate the capability of this device as PDD, the detection of fluorescence using mouse models was performed.

Key words: PDD, light source, wavelength bands, photosensitizer, fluorescence Image

AS-25

A Case of Inoperable Biliary Papillomatosis Treated by Photodynamic Thearpy

Chan-Sup Shim, Bynug Kook Kim, Tae Yoon Lee, Yeon Soo Kim, Jung Hyun Lee
Digestive Disease Center, Konkuk University Medical Center, Seoul, Korea

Objectives: Because biliary papillomatosis is a high-risk premalignant lesion of bile duct cancer, surgery such as a resection of the involved liver segments or liver transplantation has been treatment of choice for the cure in the patients with biliary papillomatosis. However, if the extent of the lesion is inoperable, how can we manage it? We recently got the good outcome by applying a photodynamic therapy to such lesion and report the case.

Aims and methods: A 68-year-old male presented with intermittent abdominal pain and recurrent episodic jaundice. He was referred to our hospital under the impression of cholangiocarcinoma. Abdominal CT scan and MR images revealed perihilar mass suggesting Klatskin tumor Bismuth type 4 and mildly enlarged lymph node in aortocaval space. However, cholangioscopy through percutaneous transhepatic route showed the typical findings of a non-mucin producing biliary papillomatosis. The biopsies were taken under the direct visualization of cholangioscopy. Finally, papillary neoplasm with low grade dysplasia was diagnosed by pathologic study. Although liver transplantation was only option for cure, the donor was not available. Therefore, we considered photodynamic therapy (PDT) as a second-line therapy. We applied laser irradiation (630 nm) to the bile duct lesion with papillomatosis via videocholangioscopy (Olympus) with 180J/cm, forty-eight hours after injection of 2 mg/kg of photosensitizer Photofrin II®. All two sessions of biliary PDT have been performed.

Results: Follow up PTCS was taken therapy on 2 days after PDT. It showed necrosis and inflamed mucosa from right proximal IHD to mid CBD. On the day 17 after the first photodynamic therapy, laboratory data had been normalized. Also, no discrete papillomatosis lesion was found except skipped inflamed and necrotized bile duct mucosa, where previous PDT was performed (which is from proximal Rt. IHD to mid CBD). But, mild narrowing of IHD near the bifurcation was observed. An 18 Fr-Foley catheter had been placed for drainage of the biliary tract during observation period. The patient remained asymptomatic for several months. Follow-up cholangioscopy was performed, three months after initial treatment. In the study, bile duct looked nearly normal except that several remnant small adenomatous lesions remained which need for further intervention.

Conclusions: PDT can be considered as an alternative local treatment modality for unresectable biliary papillomatosis.

Key words: photodynamic therapy, biliary papillomatosis

AS-26

Photodynamic Therapy in dermatology: beyond for non-melanoma skin cancers

○Sachiko Kosaka, Seiji Kawana

Department of Dermatology, Nippon Medical School

Interest in the use of photodynamic therapy (PDT) in dermatology has rapidly increased in recent years for many different spectrums of disease. PDT using δ-aminolevulinic acid (ALA) -induced protoporphyrin IX (PpIX), termed ALA-PpIX, is the most popular application, mainly due to the availability of this topical formulation out of the vast selection of photosensitizers. Besides neoplastic and preneoplastic dermatoses such as Bowen's disease, actinic keratosis, and basal cell carcinoma, management of other skin disease could benefit from PDT. Recently, several authors have reported on the efficacy of this procedure for acne, psoriasis, superficial infections, and for photorejuvenation.

One of the most promising applications of PDT is acne vulgaris, in which high efficacy of the procedure has been shown in previous clinical studies of ALA-PDT. However, the protocols have been different in each study, and the relatively severe side effects have hindered the uptake of ALA-PDT as a treatment for acne. We have performed a series of experiments to establish an optimal incubation time and ALA concentration to increase the selectivity of ALA-PpIX accumulation in sebaceous glands, thereby contributing to the development of a feasible ALA-PDT clinical protocol with minimal side effects for acne.

IPL is a common treatment modality for rejuvenation and concurrent use of ALA with IPL has recently been proposed as an effective treatment option. The previous trials were performed mainly in light skin types, and in our clinical study in Japanese, photoaging scores decreased significantly over the course of treatment on both sides of the face in all subjects, but there was no significant difference in the change in scores between the IPL-ALA-PDT- and IPL-treated sides.

Updates of ALA-PDT for cutaneous superficial infections, proliferative dermatosus will also be discussed in this session.

AS-27

Topical ALA-PDT for the treatment of Japanese Bowen's disease

Yoichi Akita, Yoshinari Matsumoto, Aki Nakano, Yasuhiko Tamada, Daisuke Watanabe
Department of Dermatology, Aichi Medical University School of Medicine, Aichi, Japan

Bowen's disease (BD) is considered as *in situ* squamous cell carcinoma. BD can be treated with cryotherapy, many topical agents and topical ALA (5-aminolevulinic acid)-PDT (photodynamic therapy), as alternatives to traditional surgical techniques.

Though we had performed ALA-PDT for BD, we obtained the lower cure rate (41.6%) than that reported for BD in Caucasian patients. We investigated an optimal approach for BD referring to the treatment results until 2004. As a protocol of ALA-PDT for BD, after application of 20% ALA for 4 h, exposure to an excimer-dye laser or a light at 630 nm was performed at a dose of 100 J/cm^2 (100 mW/cm^2) three times at an interval of a week. The cure rate of BD was up to 74.4% in totality, especially the cure rate on the trunk was 100%. The present study demonstrated that the protocol of ALA-PDT might be useful for the treatment of Japanese BD.

AS-28

Quantitative measurement of fluorescence and statistical analysis for malignant glioma using 5-ALA and talaporfin

Takashi Maruyama, Yosuke Hirakawa, Yoshihiro Muragaki, Osami Kubo,
Hiroshi Iseki, Ichiro Sakuma, Tomokatsu Hori
Department of Neurosurgery, Tokyo Women's Medical University
Faculty of Advanced Techno-Surgery(FATS), Institute of Advanced
Biomedical Engineering & Science ,Graduate School of Medicine, Tokyo
Women's Medical University
Bio-Medical Precision Engineering Laboratory, The University of Tokyo

Photodynamic diagnosis (PDD) is a technique using fluorescence in the surgical field as a substitute for histological assessment. Diagnostic accuracy should be evaluated comparing quantitative measurement of fluorescence and histopathology. We created a quantitative measurement system of extracted tissue samples and measured effective parameters to enhance diagnostic accuracy from the measurement results of tumor samples.

fluorescent peak intensity, fluorescence wavelength peak, tissue reflectometry and tissue auto-fluorescence were the candidate for quantitate measurement of 5-ALA. Peak of the fluorescence wavelength was useful to distinguish between the tumor group and nontumor group by both 5-ALA and talaporfin. However, tissue specificity was not accurate for talaporfin. 5-ALA was more sensitive for malignant glioma as PDD sensitizer. Quantitative observation of fluorescence enabled more accurate PDD using several parameters.

Key words　5-ALA, PDD, malignant glioma

AS-29

Outcome of photodynamic therapy using NPe6 for bronchogenic carcinomas in central airways more than 1.0 cm in diameter

Jitsuo Usuda, Shuji Ichinose, Taichirou Ishizumi, Keishi Ohtani, Tatsuya Inoue, Sachio Maehara, Hidetoshi Honda, Tatsuo Ohira, Harubumi Kato, Norihiko Ikeda

Department of Thoracic Surgery, Tokyo Medical University
6-7-1, Nishishinjuku, Shinjuku-ku, Tokyo-160-0023, Japan
Tel: 81-3-3342-6111
Fax: 81-3-3349-0326
Email: jusuda@tokyo-med.ac.jp

Abstract

Backgroud: Photodynamic therapy (PDT) is recommended as a treatment option for centrally located early lung cancers (CLELCs). Although PDT using Photofrin has not been recommended for large tumors or deeply invasive tumors , in the past, if their mass is reduced by electrocautery, PDT with the NPe6 second-generation photosensitizer has been found to be capable of destroying the residual cancer lesion. NPe6 is a second-generation photosensitizer, and since it has a longer absorption band (664 nm) than Photofrin (630 nm), we hypothesized that NPe6-PDT would exert a strong antitumor effect against cancer lesions greater than > 1.0 cm in diameter.

Methods: Between June 2004 and December 2008, 75 patients (91 lesions) with CLELC underwent NPe6-PDT after the extent of their tumors had been assessed by fluorescence bronchoscopy for photodynamic diagnosis (PDD) and tumor depth had been assessed by optical coherence tomography (OCT).

Results: Seventy-four cancer lesions ≦1.0 cm in diameter and 21 lesions >1.0 cm in diameter were identified, and the CR rate was 94.0% (66/70) and 90.4% (19/21), respectively. After the mass of large tumors and deeply invasive tumors, had been reduced by electrocautery, NPe6-PDT was capable of destroying the residual cancer lesions.

Conclusion: NPe6-PDT has a strong antitumor effect against CLELCs >1.0 cm in diameter, thereby enabling the destruction of residual cancer lesions after mass reduction of large nodular or polypoid type-lung cancers by electrocautery. The PDT guidelines for lung cancers should therefore be revised, because use of NPe6-PDT will enable expansion of the clinical indications for PDT.

AS-30

Photodynamic Medicine in Malignant Gliomas

-focus on PpIX accumulation in malignant glioma tissues-

Sadao Kaneko[1], Kohichi Tokuda, Tetsuyuki Yoshimoto, Tohru Yamauchi, Shin Fujimoto, Takeshi Yoshizumi, Hideo Yamaguchi, Masao Nishimura, Takeshi Kashiwaba, Masao Kondou[2]

[1]Dept. of Neurosurgery, Kashiwaba Neurosurgical Hospital, Sapporo, Japan

[2]Tokyo City University, Tokyo, Japan

The survival time of glioblastoma patient is slightly 16 months. However, with development of recent Photodynamic Medicine, the treatment results of the malignant glioma are improving. Photodynamic medicine is a therapeutic method that used photodynamic diagnosis and photodynamic therapy.
In photodynamic medicine using ALA as a photosensitivity material, it is possible basically because that ALA induced PpX is accumulated in malignant glioma tissues more than normal brain tissues.
It is not still clarified why ALA induced PpIX is accumulated to the malignant glioma cell. We paid our attention to porphyrin metabolic system to clarify the mechanism that PpIX was accumulated to the malignant glioma tissues. We measured various porphyrins, each enzyme and trace elements in the human malignant glioma tissues and normal brain tissues. We got interested knowledge.
In this symposium, I will report clinical and laboratory data of malignant glioma patients.

PS-1

Amplified Apoptotic and Anti-proliferation Efficacy of Photodynamic Therapy with Genistein

Yoon-Joo Lee[1], Jang-In Shin[1,2],Peijie He[1,2],Jin-Chul Ahn[1,2*]

[1] Medical Laser Research Center, and [2]Department of Otolaryngology-Head and Neck Surgery, College of Medicine, Dankook University, Cheonan, 330-715, Korea

Genistein, a natural isoflavone compound found in soy products, has been shown to exhibit numerous effects on various cell functions. Photodynamic therapy (PDT) is based on a photosensitizer (PS) activated by light of appropriate wavelength. Activation of a PS leads to generation of singlet oxygen and free radicals responsible for the cytotoxic effect.

In this study, we focused on the combination effect of genistein and photofrin-mediated PDT in HeLa cells.

Cell viability was measured by MTT assay. We observed morphological changes by Hoechst 33342 and propidium iodide (PI) staining. Apoptotic cell nuclei were visualized and photographed using a fluorescence microscope.

We observed that the proliferation of HeLa cells was inhibited by genistein. Moreover, the cytotoxicity was shown a synergic effect in the cells treated 25 μM of genistein with 3.2 μg/ml of photofrin-PDT compared with each single treatment. The apoptotic rate was increased after combination therapy in photofrin dose-dependent manner at 6 h. The cells exhibited typical apoptotic features including cell shrinkage, chromatin condensation and fragmented nuclei.

The results of this study indicate that genistein significantly attenuated the apoptosis and anti-proliferation efficacy of PDT, compared to that of PDT or genistein alone.

Key word: genistein, photofrin, photodynamic therapy, HeLa cells, apoptosis

Presentation type : Poster

Corresponding Author

Jin Chul Ahn, PhD

Dankook University Medical Laser and Device Research Center, Cheonan, Korea 330-714

Tel : 82-41-550-1786Fax : 82-41-550-1788

Email : jcahn@dankook.ac.kr

PS-2

Enhanced Anticancer effect of the Radachlorine-mediated Photodynamic Therapy when combined with Propolis on AMC-HN-4 cell lines

Yoon-Joo Lee, Jang-In Shin, Phil-Sang Chung, Chung-Ku Rhee, Jin-Chul Ahn
Medical Laser Research Center, Dankook University, Cheonan, Korea

Key words **: propolis, photodynamic therapy, apoptosis, cell death, human head and neck cancer cell**

Propolis, a resinous hive product collected from various plant materials by honeybees, is reported to exhibit several biological activities including antibiotic, antiinflamatory, antioxidant, antiviral and tumor cell arrest.

Photodynamic therapy (PDT) is method of treating malignant tumors based on the principle of photodynamic damage to tumor cells through a photochemical reaction. Chlorins have represented the second generation of photosensitizers with promising physicochemical properties and high photodynamic efficiency. Radachlorin is especially known to be effective when excitedby optical radiation at a wavelength of 662 ± 5 nm.

In this study, we focused on the effect of propolis and radachlorin-mediated PDT on AMC-HN-4 cancer cells.

Using MTT assay, we demonstrated the role of propolis and radachlorin in the suppression of the proliferation of AMC-HN-4 cells based on a concentration-dependent manner.

We observed morphological changes induced by propolis and radachlorin in AMC-HN-4 cells by Hoechst 33342 and propidium iodide (PI) staining. Apoptotic cell nuclei were visualized and photographed using a fluorescence microscope.

Cell viability was measured by MTT assay. Results showed that treated propolis with 0.63 ug/ml radachlorin-induced PDT has synergic effect. Combined PDT with propolis increased apoptotic cell death in AMC-HN-4 cells.

We observed that the proliferation of AMC-HN-4 cells was inhibited by propolis. The apoptotic rate was increased after combination therapy in radachlorin dose-dependent manner for 6h. The cells exhibited typical apoptotic features including cell shrinkage, chromatin condensation and fragmented nuclei.

The results of study indicate that radachlorin-induced PDT attenuated the apoptosis and anti-proliferation efficacy of PDT plus propolis was significantly enhanced, compared to that of PDT or propolis alone.

Presentation type : Poster

Corresponding Author
Chung Ku Rhee, MD
Dankook University Medical Laser and Device Research Center, Cheonan, Korea 330-714
Tel : 82-41-550-1780Fax : 82-41-550-1788
Email : rheeck@dankook.ac.kr

PS-3

Anticancer Effect of Emodin and Photodynamic Therapy combined in HN3 Cancer Cell Line

So-Young Chang[1], Yu-Ri Ahn[1], Jang-In Shin[1, 2],Peijie He[1, 2],Jin-Chul Ahn[1, 2 *]

[1] Medical Laser Research Center, and [2]Department of Otolaryngology-Head and Neck Surgery, College of Medicine, Dankook University, Cheonan, 330-715, Korea

Emodin(1,3,8-trihydroxy-6-methylanthraquinone) was isolated from Rheum palmatum L. , is reported to suppress the growth of tumor in many clinical situations and effectively inhibit tumor metastasis in *vitro*.
Photodynamic therapy (PDT) is a treatment modality that has been used in the successful treatment of a number of diseases and disorders. PDT uses a combination of a selectively localized light-sensitive drug (known as a photosensitizer) and light of an appropriate wavelength.
This study evaluated the efficacy of a combination treatment of two modalities (emodin and PDT) in head and neck cancer cell lines. HN3 Cells were treated with different concentration of emodin (10, 20, and 30 μM) or combined with PDT, the cell viability was measured by MTT assay. We observed morphological changes induced by emodin and PDT in HN3 cells by Hoechst 33342 and propidium iodide (PI) staining. Apoptotic cell nuclei were visualized and photographed using a confocal microscope. Through western blotting analysis, we found that the apoptosis-related protein Bcl-2 was decreased and the Bax was increased after emodin and PDT treatment.
In conclusion, this study demonstrates that the combined modality resulted in additive apoptotic cell death as well as cytotoxic effect on AMC-HN-3 cell in *vitro.*
Key words: Emodin, photodynamic therapy, apoptosis, AMC-HN3 cells

Presentation type: Poster

Corresponding Author
Phil Sang Chung, MD
Department of Otolaryngology-Head and Neck Surgery, College of Medicine, Dankook University Hospital, Cheonan, Korea 330-714
Tel : 82-41-550-3975Fax : 82-41-556-1090
Email : pschung@dankook.ac.kr