Review

Sonodynamic Cancer Therapy: A Non-invasive and Repeatable Approach Using Low-intensity Ultrasound with a Sonosensitizer

HIROTOMO SHIBAGUCHI[1], HIROFUMI TSURU[1], MOTOMU KUROKI[2] and MASAHIDE KUROKI[1]

[1]*Department of Biochemistry and* [2]*School of Nursing, Faculty of Medicine, Fukuoka University, Jonan-ku, Fukuoka, Japan*

Abstract. *The low-intensity ultrasound that is used in clinical diagnoses, such as abdomen echo inspection, is a non-invasive treatment, and penetrates deeper into the body than light. Recently, sonodynamic therapy (SDT), which uses low-intensity ultrasound together with a sonosensitizer, has been developed for cancer therapy in applying such properties of ultrasound. So far, most sonosensitizers that have been developed are sensitive to light as well as ultrasound, implying that the shortcomings of photosensitizers used during photodynamic therapy, such as skin sensitivity, still need to be overcome in SDT. Some exceptions were, however, reported in recent studies in which sensitizers were activated mainly by ultrasound but not by light. Furthermore, recent in vivo studies have demonstrated that SDT with a sonosensitizer has a great potential as a non-invasive and repeatable treatment for cancer therapy.*

Major treatments for malignant tumors are surgery, radiotherapy, chemotherapy and a combination of these. Although combination therapy is considered to be an option with potential additive benefits, the increasing side-effects often cause patients to elect to discontinue treatment, such as radical radiotherapy or chemotherapy, which are limited to a single course even if patients have recurrent disease or a second primary manifestation in the irradiated field. Novel therapeutic strategies, preferably consisting of non-invasive treatments, are therefore required.

Correspondence to: Hirotomo Shibaguchi, Department of Biochemistry, Faculty of Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka, 814-0180 Japan. Tel: +81 928011011, Fax: +81 928013600, e-mail: shiba-h@fukuoka-u.ac.jp

Key Words: Low-intensity ultrasound, sonodynamic therapy, sonosensitizers, review.

The use of light is one of the options that could be considered for non-invasive treatments. Light treatment for therapeutic purposes has been performed for thousands of years but more recently, the use of light with certain chemicals in photodynamic therapy (PDT) has been developed to treat diseases, especially in oncology (1-4). It is known that PDT requires a sensitizing agent, light energy and oxygen to generate reactive oxygen species (ROS), such as singlet oxygen and free radicals, which mediate cellular toxicity (5-7). Thus far, many chemical products can act as photosensitizers and new agents are regularly discovered. Very few, however, are carried through to clinical trials, and even fewer become clinical photosensitizers. Ideal photosensitizers should not be toxic chemicals and not create new toxic byproducts. Photofrin, which has the longest clinical history and patient track record, is a hematoporphyrin derivative. In clinical PDT, red light (50-500 J/cm^2) is needed to activate photofrin (8). Once a sensitizer is activated by specific wavelengths of light from its ground state into an excited state, there are two types of reactions that occur during PDT: (i) the activated sensitizer can react directly with substrates or molecules, transforming a hydrogen atom to form radicals, and then the radicals need with oxygen produce oxygenated products, or (ii) the activated sensitizer can transfer the energy to oxygen resulting in singlet oxygen formation, and then this highly reactive oxygen species oxidizes surrounding substrates. In PDT, cancer cell death occurs directly by the efficient induction of apoptosis, as well as through a non-apoptotic pathway. Recent evidence indicates that autophagy, in addition to necrosis as a mode of non-apoptotic cell death, is induced by PDT in order to allow repair and survival of key photodamaged organelles and can be turned into a death signal when the initial recovery response fails (9, 10). These signaling cascades are triggered in cancer cells exposed to photodynamic stress and, depending on the subcellular localization of the damaging ROS or sensitizers, transduce

these signals into a cell death response (11, 12). As mentioned above, PDT is a useful non-invasive treatment for cancer therapy; however, there are at least two notable shortcomings that need to be overcome: limited penetration of light into deep tumor tissue, which is required to activate the photosensitizer, and certain potentially serious side-effects, such as long-lasting skin sensitivity due to the retention of the photosensitizer in cutaneous tissues (13, 14).

When considering the other non-invasive therapy that overcomes the problems of PDT, low-intensity ultrasound together with a sonosensitizer, termed sonodynamic therapy (SDT), is a promising candidate because of the non-invasive and deeper penetrating properties of ultrasound. Recent *in vivo* studies have demonstrated that SDT with a sonosensitizer has great potential as a non-invasive and repeatable treatment for cancer patients, even when tumors are located too deep to be treated using regular PDT.

Ultrasound

Ultrasound is a type of mechanical sound wave with periodic vibrations in a continuous medium at frequencies greater than 20 kHz over the range of human hearing (16-20 kHz). Changes in ultrasound, such as scattering, reflection and absorption, among others, which allow ultrasound to reach the object, are useful to explore the object's inside or interface. The actions changing an object by ultrasound are classified as either actions by acoustic cavitation or others. Acoustic cavitation involves the formation, growth and near-adiabatic collapse of gas bubbles in liquids by irradiating ultrasound (15). When gas bubbles in liquid violently collapse temperature and pressure reach values in excess of 10,000 K and 10,000 atm with shockwaves and microjets. Acoustic cavitation also generates light, an emission known as sonoluminescence.

Ultrasound mediates both thermal and non-thermal effects in biological tissues. Ultrasound can penetrate into tissue better than light, and generally its bioeffects are intensity- and frequency-dependent. A higher intensity results in efficient heat production, and a lower frequency facilitates acoustic cavitation (15, 16). Because ultrasonic waves can be focused like optical and audio waves using an acoustic lens, a bowl-shaped transducer or electronic phased array, high-intensity focused ultrasound (HIFU) has been developed to mediate thermal effects (17, 18). Once the energy density at the focus point is high enough, tissue is damaged. During HIFU treatment, the temperatures become much greater than 80˚C. In such a case, thermal toxicity or irreversible cell death from coagulative necrosis occurs immediately within one second. Recently, magnetic resonance imaging (MRI)- or ultrasound-guided HIFU has been developed not only for prostate cancer but also for liver cancer (19, 20). Non-thermal bioeffects, on the other hand, are generally associated with oscillating or cavitating bubbles, but also include non-cavitating effects, such as radiation pressure, radiation torque, and acoustic streaming.

Drug Delivery System and Gene Therapy with Ultrasound

Due to the highly disorganized nature of tumor vasculature, high blood pressure in the tumor tissue and high blood viscosity, the administration of drugs alone does not work at the site of tumor mass. In order to improve anticancer therapy, various strategies have been attempted in the last two decades to deliver anticancer drugs to the site of interest and minimize the dose, such as liposomes, micelles, micro/nanoparticles, polymer-drug conjugates and implants (21-23). Recently, new strategies using low-intensity ultrasound have been established in order to introduce new methods of drug delivery and to develop useful carrier systems for anticancer agents (24-26). Ultrasound increases membrane permeability and intracellular drug uptake by cavitation on the cell membrane, called sonoporation, although the mechanism of sonoporation is still unclear. Furthermore, cavitating and/or non-cavitating effects help to release a drug from micelles and the increase in concentration at tumor site enhances intracellular uptake (27, 28). In the first studies investigating the efficacy of ultrasound for gene therapy, typical ultrasound frequencies employed in drug delivery studies are in the range of 20-90 kHz and the optimal power density (intensity) of ultrasound ranges from 1 to 5 W/cm^2, depending on the irradiation time, which is usually 30-60 s at continuous ultrasound irradiation. However, therapeutic ultrasound that uses frequencies of 1-3 MHz and intensities of 0.5-3 W/cm^2 with pulse-mode has been employed because of tissue damage with higher frequencies along with cavitation. Although experimental conditions with high power ultrasound, including pulsed HIFU exposure, led to higher efficacy in enhancing drug delivery, there is still considerable debate regarding the development of standard protocols for successful anticancer therapy. More recently, researchers developed ultrasound-mediated gene delivery by injecting gene and nano/microbubbles (bubble liposomes) into blood flow (29, 30). According to these reports, bubble liposomes quickly transduced plasmid DNA into the tissue of interest by cavitation, even with the existence of a blood stream. After significant successes of *in vitro* studies of ultrasound-mediated drug and gene delivery, a recent *in vivo* animal study indicated that low-frequency ultrasound significantly reduced tumor size in xenograft models (31-33). Alternatively, a combination of tumor-associated antigens and bubble liposomes was developed in dendritic cell (DC)-based cancer immunotherapy with low-intensity ultrasound (34). The exogenous antigens, interestingly, were recognized as endogenous antigens when delivered to the cytosol using

Table I. *Sonosensitizers used in SDT in an in vivo animal model and SDT conditions.*

Sensitzer	Cell	Intensity (W/cm^2)	Frequency (MHz)	Time (min)	Animal	Ref.
5-ALA	C6 glioma	10.0	1.0	5.0	rat	(43)
ATX-70	DMBA-induced	1.0-5.0	1.92	15.0	rat	(40)
DCPH-P-Na(I)	MKN-45	1.0	1.0	10.0	mouse	(38)
Photofrin	DMBA-induced	1.0-5.0	1.92	15.0	rat	(41)
PPIX	Hepatoma-22	3.0	1.43	3.0	mouse	(42)
TiO2	C32 melanoma	1.0	1.0	2.0	mouse	(45)

bubble liposomes in combination with ultrasound, resulting in presenting antigens to MHC class I, which is essential for activating tumor-specific cytotoxic T lymphocytes.

Sonodynamic Therapy

Exposure to ultrasound and subsequent cavitation collapse can have similar effects in producing free radicals by facilitating porphyrin derivatives, such as the effect of light on PDT (35, 36). Thereafter, SDT together with a sonosensitizer was developed for cancer therapy (37). Although the mechanisms for activating sensitizers by ultrasound irradiation from a steady state to an exited state are still unclear, it is thought that the process is likely to be identical to that when light is cast on PDT, as mentioned above. This hypothesis is doubtful for two sonosensitizers: 13,17-bis(1-carboxyethyl)-8-[2-(2,4-dichlorophenyl-hydrazono)ethylidene]-3-ethenyl-7-hydroxy-2,7,12,18-tetramethylchlorin, disodium salt [DCPH-P-Na(I)], a novel porphyrin derivative, and titanium deoxide (TiO_2), a photocatalyst. Both sensitizers showed different reactivity from those so far reported to light and ultrasound irradiation. The former had quite weak reactivity to visible light of approximately 6,000 lux for 10 min compared to ATX-70, a strong photosensitizer, which showed potent cytotoxicity (38). The latter required 60-fold longer periods of UV irradiation (5 mW/cm^2) to obtain similar cytotoxicity on ultrasound exposure (1.0 W/cm^2) (39). Furthermore, the cytotoxicity of TiO2 induced by UV exposure was completely inhibited by a radical scavenger while that by ultrasound irradiation was only partly inhibited. Taking into consideration the fact that ATX-70 demonstrated less sonotoxicity than DCPH-P-Na(I) on a human gastric cancer cell line, MKN-45, in addition to the results mentioned above, a promising sonosensitizer candidate in SDT might be facilitated by different mechanisms observed on PDT, namely cavitation and collapsing energy, but not sonoluminescence. Skin hypersensitivity or 30 days of sunlight photosensitivity might be a small price to pay for non-invasive or painless treatment for patients who have been through surgery, radiotherapy and/or multiple chemotherapy agents, whereas SDT with a sonosensitizer without photosensitivity may be an ideal treatment in non-invasive and repeatable cancer therapy.

Sonosensitizer on SDT in an *In Vivo* Animal Model

Except in cell cultures, there are very few recent studies of ultrasound-mediated antitumor effects in combination with a sonosensitizer performed on animal models, as summarized in Table I. Knowledge of the mechanism of sonoporation in SDT is still very limited, and constitutes a major obstacle in determining the factors affecting acoustically triggered activation of sensitizers and in the development of standard protocols for successful anticancer therapy.

Porphyrin derivatives thus far used most often as a sonosensitizer in *in vivo* animal models are photofrin, protoporphyrin IX (PPIX), ATX-70 and DCPH-P-Na(I) (38, 40-42). Although the mechanisms by which porphyrin derivatives selectively accumulate in tumors are complex and not fully understood, it is presumably because of the high vascular permeability of the agents, as well as their affinity for proliferating endothelium and the lack of lymphatic drainage in tumors. Pharmacokinetic parameters of these derivatives were investigated except for DCPH-P-Na(I), and showed similar patterns in tumor, skin and muscle, supporting this hypothesis. Furthermore, the antitumor effect of DCPH-P-Na(I) in SDT indicated a higher efficacy for preventing tumor growth from 6 up to 24 h after intravenous administration (unpublished data).

SDT using 5-aminolevulinic acid (5-ALA) was also reported in this issue with low-intensity but focused ultrasound in deep-seated glioma model (43). 5-ALA-induced fluorescence has been used in malignant glioma in order to render more complete resection in surgical operations (44). Neoplastic cells synthesize abundant intracellular PPIX after administration of 5-ALA. Therefore, the administration of 5-ALA may work with mechanisms similar to that of PPIX in SDT. The other advantage of using 5-ALA in malignant glioma in SDT is that it can be orally administered to patients.

The potential of TiO_2 nanoparticle as a novel sonosensitizer was reported recently using C32 melanoma

tumor cells *in vivo* (45). In the chemical industry and environmental treatment, TiO2 is well known as a photocatalyst that has a strong oxidizing activity and produces oxidative radicals with irradiating UV light or ultrasound (46, 47). These properties are useful not only for photosensitizers in PDT, but also for sonosensitizers in SDT. The lack of selective accumulation of particles in a tumor mass resulting in insufficient selectivity and low efficiency is one of the shortcomings in a clinical setting.

Conclusion

Theoretically, SDT using low-intensity ultrasound in combination with a sonosensitizer might be effective in all types of cancer without a need for choosing the target molecules, proteins and/or genes. Recent reports performed in both *in vivo* and *in vitro* studies support this hypothesis, and thus, SDT is a promising candidate for non-invasive and repeatable cancer therapy. Most sonosensitizers reported thus far, including a very recent one, mono-l-aspartyl chlorin e6 (NPe6), a chlorophyll-like substrate, are also known as photosensitizers, implying that skin sensitivity, a serious adverse effect of such sonosensitizers in PDT, still remains a problem that needs to be to overcome in SDT (48). It is expected, however, that a growing amount of experimental data and number of sensitizers would lead to the extensive application of SDT in various cancer models *in vivo* in the near future.

References

1 Ackroyd R, Kelty C, Brown N and Reed M: The history of photodetection and photodynamic therapy. Photochem Photobiol *74*: 656-669, 2001.

2 Roelandts R: The history of phototherapy: something new under the sun? J Am Acad Dermatol *46*: 926-930, 2002.

3 Wilson BC and Patterson MS: The physics, biophysics and technology of photodynamic therapy. Phys Med Biol *53*: R61-109, 2008.

4 Morton CA, McKenna KE and Rhodes LE: Guidelines for topical photodynamic therapy: update. Br J Dermatol *159*: 1245-1266, 2008.

5 O'Connor AE, Gallagher WM and Byrne AT: Porphyrin and nonporphyrin photosensitizers in oncology: preclinical and clinical advances in photodynamic therapy. Photochem Photobiol *85*: 1053-1074, 2009.

6 Buettner GR: Thiyl free radical production with hematoporphyrin derivative, cysteine and light: a spin-trapping study. FEBS Lett *177*: 295-299, 1984.

7 Robertson CA, Evans DH and Abrahamse H: Photodynamic therapy (PDT): a short review on cellular mechanisms and cancer research applications for PDT. J Photochem Photobiol B *96*: 1-8, 2009.

8 Triesscheijn M, Baas P, Schellens JH and Stewart FA: Photodynamic therapy in oncology. Oncologist *11*: 1034-1044, 2006.

9 Reiners JJ, Jr., Agostinis P, Berg K, Oleinick NL and Kessel D: Assessing autophagy in the context of photodynamic therapy. Autophagy *6*: 7-18, 2010.

10 Buytaert E, Callewaert G, Hendrickx N, Scorrano L, Hartmann D, Missiaen L, Vandenheede JR, Heirman I, Grooten J and Agostinis P: Role of endoplasmic reticulum depletion and multidomain proapoptotic BAX and BAK proteins in shaping cell death after hypericin-mediated photodynamic therapy. FASEB J *20*: 756-758, 2006.

11 Buytaert E, Dewaele M and Agostinis P: Molecular effectors of multiple cell death pathways initiated by photodynamic therapy. Biochim Biophys Acta *1776*: 86-107, 2007.

12 Morgan J and Oseroff AR: Mitochondria-based photodynamic anticancer therapy. Adv Drug Deliv Rev *49*: 71-86, 2001.

13 Nielsen KP, Juzeniene A, Juzenas P, Stamnes K, Stamnes JJ and Moan J: Choice of optimal wavelength for PDT: the significance of oxygen depletion. Photochem Photobiol *81*: 1190-1194, 2005.

14 Moriwaki SI, Misawa J, Yoshinari Y, Yamada I, Takigawa M and Tokura Y: Analysis of photosensitivity in Japanese cancer-bearing patients receiving photodynamic therapy with porfimer sodium (Photofrin). Photodermatol Photoimmunol Photomed *17*: 241-243, 2001.

15 Suslick KS: Sonochemistry. Science *247*: 1439-1445, 1990.

16 Didenko YT, McNamara WB, 3rd and Suslick KS: Molecular emission from single-bubble sonoluminescence. Nature *407*: 877-879, 2000.

17 Lindner U, Trachtenberg J and Lawrentschuk N: Focal therapy in prostate cancer: modalities, findings and future considerations. Nat Rev Urol *7*: 562-571, 2010.

18 Ahmed HU, Moore C and Emberton M: Minimally-invasive technologies in uro-oncology: the role of cryotherapy, HIFU and photodynamic therapy in whole gland and focal therapy of localised prostate cancer. Surg Oncol *18*: 219-232, 2009.

19 Chopra R, Burtnyk M, N'Djin WA and Bronskill M: MRI-controlled transurethral ultrasound therapy for localised prostate cancer. Int J Hyperthermia *26*: 804-821, 2010.

20 Padma S, Martinie JB and Iannitti DA: Liver tumor ablation: percutaneous and open approaches. J Surg Oncol *100*: 619-634, 2009.

21 Lammers T, Kiessling F, Hennink WE and Storm G: Nanotheranostics and image-guided drug delivery: current concepts and future directions. Mol Pharm *7*: 1899-1912, 2010.

22 Shim MS and Kwon YJ: Efficient and targeted delivery of siRNA *in vivo*. FEBS J *277*: 4814-4827, 2010.

23 Weinberg BD, Blanco E and Gao J: Polymer implants for intratumoral drug delivery and cancer therapy. J Pharm Sci *97*: 1681-1702, 2008.

24 Tezel A, Sens A, Tuchscherer J and Mitragotri S: Frequency dependence of sonophoresis. Pharm Res *18*: 1694-1700, 2001.

25 Kushner JT, Blankschtein D and Langer R: Evaluation of hydrophilic permeant transport parameters in the localized and non-localized transport regions of skin treated simultaneously with low-frequency ultrasound and sodium lauryl sulfate. J Pharm Sci *97*: 906-918, 2008.

26 Wolloch L and Kost J: The importance of microjet *vs*. shock wave formation in sonophoresis. J Control Release *148*: 204-211, 2010.

27 Husseini GA and Pitt WG: Micelles and nanoparticles for ultrasonic drug and gene delivery. Adv Drug Deliv Rev *60*: 1137-1152, 2008.

28 Schroeder A, Kost J and Barenholz Y: Ultrasound, liposomes, and drug delivery: principles for using ultrasound to control the release of drugs from liposomes. Chem Phys Lipids *162*: 1-16, 2009.
29 Suzuki R, Oda Y, Utoguchi N and Maruyama K: Progress in the development of ultrasound-mediated gene delivery systems utilizing nano- and microbubbles. J Control Release *149*: 36-41, 2011.
30 Hernot S and Klibanov AL: Microbubbles in ultrasound-triggered drug and gene delivery. Adv Drug Deliv Rev *60*: 1153-1166, 2008.
31 Nelson JL, Roeder BL, Carmen JC, Roloff F and Pitt WG: Ultrasonically activated chemotherapeutic drug delivery in a rat model. Cancer Res *62*: 7280-7283, 2002.
32 Myhr G and Moan J: Synergistic and tumour selective effects of chemotherapy and ultrasound treatment. Cancer Lett *232*: 206-213, 2006.
33 Suzuki R, Namai E, Oda Y, Nishiie N, Otake S, Koshima R, Hirata K, Taira Y, Utoguchi N, Negishi Y, Nakagawa S and Maruyama K: Cancer gene therapy by IL-12 gene delivery using liposomal bubbles and tumoral ultrasound exposure. J Control Release *142*: 245-250, 2010.
34 Suzuki R, Oda Y, Utoguchi N, Namai E, Taira Y, Okada N, Kadowaki N, Kodama T, Tachibana K and Maruyama K: A novel strategy utilizing ultrasound for antigen delivery in dendritic cell-based cancer immunotherapy. J Control Release *133*: 198-205, 2009.
35 Yumita N, Nishigaki R, Umemura K and Umemura S: Hematoporphyrin as a sensitizer of cell-damaging effect of ultrasound. Jpn J Cancer Res *80*: 219-222, 1989.
36 Miyoshi N, Misik V, Fukuda M and Riesz P: Effect of gallium-porphyrin analogue ATX-70 on nitroxide formation from a cyclic secondary amine by ultrasound: on the mechanism of sonodynamic activation. Radiat Res *143*: 194-202, 1995.
37 Rosenthal I, Sostaric JZ and Riesz P: Sonodynamic therapy–a review of the synergistic effects of drugs and ultrasound. Ultrason Sonochem *11*: 349-363, 2004.
38 Hachimine K, Shibaguchi H, Kuroki M, Yamada H, Kinugasa T, Nakae Y, Asano R, Sakata I, Yamashita Y and Shirakusa T: Sonodynamic therapy of cancer using a novel porphyrin derivative, DCPH-P-Na(I), which is devoid of photosensitivity. Cancer Sci *98*: 916-920, 2007.
39 Yamaguchi S, Kobayashi H, Narita T, Kanehira K, Sonezaki S, Kudo N, Kubota Y, Terasaka S and Houkin K: Sonodynamic therapy using water-dispersed TiO(2)-polyethylene glycol compound on glioma cells: Comparison of cytotoxic mechanism with photodynamic therapy. Ultrason Sonochem *18*: 1197-1204, 2010.
40 Yumita N, Okuyama N, Sasaki K and Umemura S: Sonodynamic therapy on chemically induced mammary tumor: pharmacokinetics, tissue distribution and sonodynamically induced antitumor effect of gallium–porphyrin complex ATX-70. Cancer Chemother Pharmacol *60*: 891-897, 2007.
41 Yumita N, Okuyama N, Sasaki K and Umemura S: Sonodynamic therapy on chemically induced mammary tumor: pharmacokinetics, tissue distribution and sonodynamically induced antitumor effect of porfimer sodium. Cancer Sci *95*: 765-769, 2004.
42 Wang X, Wang Y, Wang P, Cheng X and Liu Q: Sonodynamically induced antitumor effect with protoporphyrin IX on hepatoma-22 solid tumor. Ultrasonics *51*: 539-546, 2011.
43 Ohmura T, Fukushima T, Shibaguchi H, Yoshizawa S, Inoue T, Kuroki M, Sasaki K and Umemura S: Sonodynamic therapy with 5-aminolevulinic acid and focused ultrasound for deep seated intracranial glioma in rat. Anticancer Res in press, 2011.
44 Hefti M, von Campe G, Moschopulos M, Siegner A, Looser H and Landolt H: 5-Aminolevulinic acid induced protoporphyrin IX fluorescence in high-grade glioma surgery: a one-year experience at a single institutuion. Swiss Med Wkly *138*: 180-185, 2008.
45 Harada Y, Ogawa K, Irie Y, Endo H, Feril LB Jr., Uemura T and Tachibana K: Ultrasound activation of TiO_2 in melanoma tumors. J Control Release *149*: 190-195, 2011.
46 Ashikaga T, Wada M, Kobayashi H, Mori M, Katsumura Y, Fukui H, Kato S, Yamaguchi M and Takamatsu T: Effect of the photocatalytic activity of TiO(2) on plasmid DNA. Mutat Res *466*: 1-7, 2000.
47 Wang J, Wu J, Zhang Z, Zhang X, Pan Z, Wang L and Xu L: Sonocatalytic damage of bovine serum albumin (BSA) in the presence of nanometer anatase titanium dioxide (TiO_2). Ultrasound Med Biol *32*: 147-152, 2006.
48 Yumita N, Iwase Y, Nishi K, Ikeda T, Komatsu H, Fukai T, Onodera K, Nishi H, Takeda K, Umemura S, Okudaira K and Momose Y: Sonodynamically-induced antitumor effect of mono-l-aspartyl chlorin e6 (NPe6). Anticancer Res *31*: 501-506, 2011.

Received April 7, 2011
Revised June 1, 2011
Accepted June 2, 2011