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Elimination of adult T cell leukemia cells by ultrasound in the presence of porfimer sodium

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Sonodynamic effects using porfimer sodium (Photofrin®; Pf) on leukemic and normal cells were evaluated. The purpose of this experiment was to compare cell survival among MT-2 cells, normal peripheral mononuclear cells (PMNCs) and adult T cell leukemia (ATL) patients' PMNCs after sonodynamic treatment. Cells were exposed to 450 kHz ultrasound at an intensity of 500 mW/cm^2. The survival rate of MT-2 cells exposed to ultrasound alone for 80 s was 20.1 ± 4.8%, whereas survival rates exposed to ultrasound in combination with 25, 50 and 100 μg/ml of Pf resulted in 11.5 ± 2.9, 3.2 ± 1.6 and 1.6 ± 1.4%, respectively. There was a significant difference of cell survival between the group exposed to ultrasound alone and the Pf-combined groups ($n = 6$, $p < 0.05$). On the other hand, in the normal human PMNCs, no significant differences of cell survival rates were found between ultrasound-treated groups with and without Pf. We similarly examined the survival rate of PMNCs in the peripheral blood of five acute-type ATL patients ($n = 5$) after ultrasound (60 s, 300 mW/cm^2) exposure with or without 100 μg/ml of Pf. Comparison of cell survival rate between ultrasound alone and ultrasound plus Pf showed significant differences (69.4 ± 22.5 and 30.0 ± 23.0%, respectively). There were no significant cytotoxicities in all Pf alone treated groups of the MT-2 cells, the normal PMNCs and the ATL patients' PMNCs ($p < 0.05$). It was suggested from this study that there was a specific selectivity of sonodynamic effects to MT-2 cell lines and ATL patients' PMNCs. It is anticipated that this new method of treatment, i.e. sonodynamic therapy, could be used for extracorporeal blood treatment of acute-type ATL patients.

Key words: Adult T cell leukemia, Photofrin®, porfimer sodium, sonodynamic therapy, therapeutic ultrasound.

Introduction

Adult T cell leukemia (ATL) is a malignant blood disease which involves the T cell system and is caused by the lymphocytes of the peripheral blood associated with human T cell lymphotrophic type I (HTVL-I) virus.[1] The incidence rate is high, especially in the southwestern part of Japan and southeastern part of the US.[1,2] Prognosis of ATL patients has been reported to be generally poor and resistant to chemotherapy.[2–5] Recently, selective elimination of leukemic cells by photodynamic therapy using light-activated photosensitizing compounds has been reported.[6,7] Such compounds as hematoporphyrin derivatives or merocyanine-540 (MC540) have shown selective uptake by tumor cells and are activated by light, resulting in a necrotic effects.[6,8] In the clinical situation, porfimer sodium was recently approved in Japan and the US for photodynamic treatment of esophageal, gastric and lung tumors.[9,10] However, application of this treatment to other non-solid cancers has been limited due to poor penetration of light energy through human tissues at the most photosensitive (600–700 nm) laser wavelength range.[8–16]

An alternative energy, ultrasound (US) activation of photosensitizing drugs, frequently referred to as sonodynamic therapy, is a promising new approach, mainly for its higher transmission rate through human tissues and cost efficiency compared with lasers. Sonodynamic therapy has shown significant killing effects to solid tumors with hematoporphyrin, porfimer sodium and ATX-70.[17–24] However, as of now, no data has been reported on the use of sonodynamic therapy to selectively eliminate leukemic cells or its toxicity to actual cells derived from leukemic patients. In this paper, the sonodynamic cell killing effects were evaluated on an ATL cell line, MT-2 cells, in conjunction with the photosensitizer porfimer sodium. Sonodynamic cell killing of peripheral mononuclear cells (PMNCs) from normal subjects and acute-type ATL patients was also examined.

Materials and methods

Agents

Porfimer sodium (Photofrin®) was a gift from QLT PhotoTherapeutics (Vancouver, Canada). Com-

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pounds were diluted to 1 mg/ml with 5% glucose. All stock solutions of the compound were stored in light protected containers at −50°C until use.

Cell line

The MT-2 cell line[25] was a kindly provided by Dr Tomita (First Department of Surgery, School of Medicine, Fukuoka University, Japan). Cell lines were cultured in RPMI 1640 medium (Gibco/BRL Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (Gibco/BRL Life Technologies) and antibiotics (100 U/ml of penicillin G and 50 mg/ml of streptomycin and glutamine) in a humidified atmosphere of 5% CO_2 in air at 37°C. Cells were routinely tested for Mycoplasma contamination. All studies were performed during the exponential growth phase.

Leukemic samples

PMNCs were collected from peripheral blood of five acute-type ATL patients (four female and one male) with a median age of 53.2 years (range 42–88 years). The diagnostic criteria and valid cases of HTVL-I-associated ATL for this study were defined as follows: (i) histologically and cytologically proven lymphoid malignancy with surface antigens of T cells ($CD4^+$, $CD25^+$, $CD8^-$), (ii) positive antibody to HTLV-I and clonal integration of HTLV-I proviral DNA in the lymphocytes of patients at diagnosis. All blood samples were obtained during crisis, in which two cases had not been treated previously. The other three cases that had been treated with chemotherapy (CHOP) 3 weeks earlier were considered to have no response to anticancer agents. The white blood cell count was $26104 \pm 15482/mm^3$, and average proportions of ATL cells and lymphocytes were 59.8 ± 33.9 and $5.1 \pm 3.0\%$, respectively.

Normal PMNCs were collected from 200 ml of peripheral blood from a healthy male volunteer the age of 35. Prior blood examination for ATL diagnosis revealed negative findings.

Informed consent had been obtained under a protocol approved by the Fukuoka University Committee on Human and Animal Investigation from all participants.

Experiment protocol

The PMNCs were isolated from heparinized peripheral blood from ATL patients and the healthy volunteer by Ficoll-Conray density centrifugation (Lymphocyte Separation Medium, Organon Teknika, Durham, NC) at 300 *g* for 30 min under room temperature. The cells at the interface were carefully harvested with a Pasteur pipette, subsequently washed three times with phosphate buffered saline (PBS, pH 7.4, osmotic pressure 280 mOs/kg; Gibco/BRL Life Technologies) by centrifugation and resuspended in the required volume of PBS. The cells were resuspended in PBS at a concentration of 3×10^6 cells/ml. The cell suspensions were stored on ice until used in the experiments. The cell viability was checked before every treatment and only cell suspensions whose viability was above 99% were used in all series of treatments. All cell separations and purification were performed within 2 h after extraction from subjects. The cell were suspensions at a density of 3×10^6 cells/ml. Final drug concentrations of cell suspensions were 0, 25, 50 and 100 μg/ml, and were incubated for 30 min before treatment. All experiments were separately repeated six times for each condition.

Figure 1 shows a schematic diagram of the set-up for exposing suspended MT-2 cells, and PMNCs of ATL patients and healthy volunteers to ultrasound. For each condition, 1.5 ml of the cell suspensions was placed in a Pyrex test tube (12 mm in diameter, 10 cm in length). A miniature ceramic piezo ultrasound-emitting element (diameter 1.3 mm, thickness 0.62 mm) was directly inserted into the cell suspension. The cell suspensions were gently swirled continuously to prevent settling during treatment. Ultrasound frequency was 450 kHz, and intensities was varied at 0, 300, 400 and 500 mW/cm^2 in continuous wave mode. Ultrasound was produced

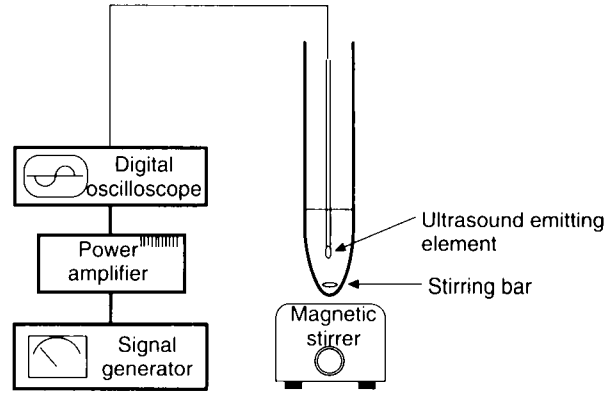

Figure 1. Schematic diagram of the method of ultrasound exposure.

by a signal generator (1940 Multifunction Synthesizer, NF Electric Instruments, Yokohama, Japan) driven by a high-speed power amplifier (4025 High Speed Power Amplifier/Bipolar Power Supply; NF Electric Instruments). Driving signals were monitored by an oscilloscope (Digital Storage Oscilloscope DCS-704; Kenwood, Tokyo, Japan). Temperature changes of cell suspension immediately before and after treatment by ultrasound were measured by a needle thermometer (Tele-Thermometer; Yellow Springs Instrument Co., Yellow Spring, OH).

Assessment of cell viability and survival

Trypan blue exclusion was performed by mixing 20 μl of cell suspensions with 380 μl of a 0.4% Trypan blue solution (Sigma, St Louis, MO) in PBS. After 4 min of incubation, the number of cells excluding Trypan blue was counted using a improved Neubauer-type hemocytometer. The number of surviving cells before exposure to ultrasound or drugs was calculated as 100%.

Statistics

The data from each treatment groups was compared using a two-sided Student's *t*-test to determine statistical variation. Significance was set at $p < 0.05$.

Results

In order to accurately measure the acute cytotoxicity of cells, the survival rates were obtained immediately after ultrasound irradiation. Although viability was evaluated by Trypan blue staining, exposure to ultrasound alone or in combination with porfimer sodium appeared to destroy the cell structure completely, leaving only the unstained cells visible for counting. Porfimer sodium alone at all concentrations (25, 50 and 100 μg/ml) to MT-2 cells and normal PMNCs did not show any cytotoxicity ($p < 0.05$). The effects of the survival rate of MT-2 cells subjected to ultrasound alone or in combination with various porfimer sodium concentrations are shown in Figure 2. Ultrasound irradiation for more than 20 s in the presence of 50 and 100 μg/ml porfimer sodium showed significant differences ($p < 0.05$) compared to ultrasound alone, with the exception of irradiation of ultrasound at an intensity of 300 mW/cm^2 for 40 s. The cell survival rate

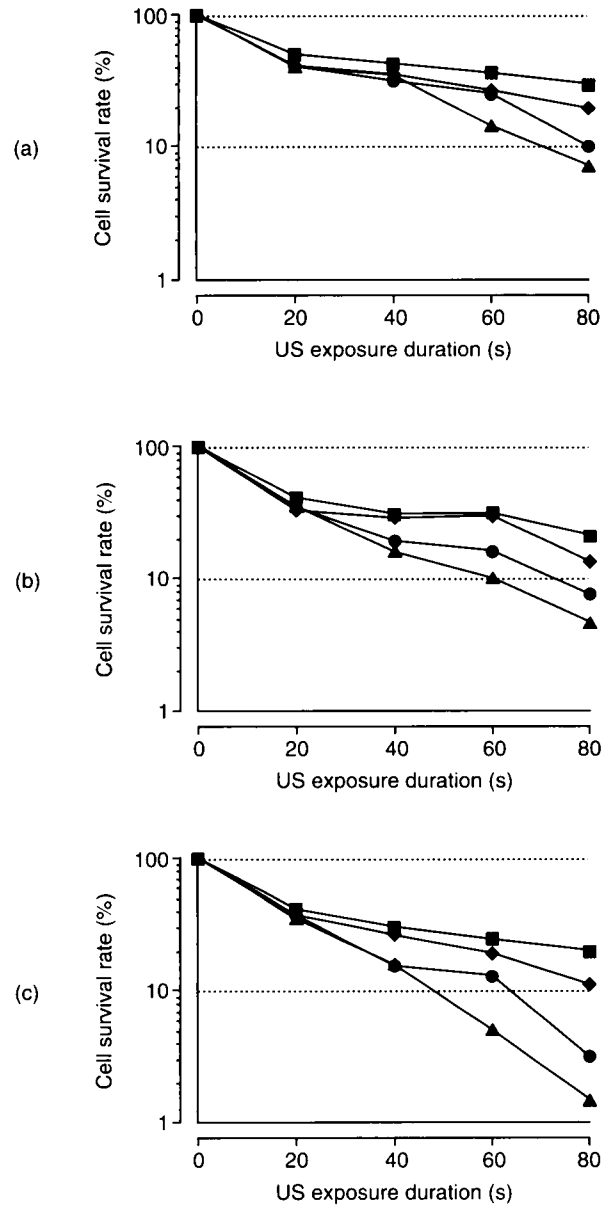

Figure 2. Cell survival rate of MT-3 cells ($n = 6$) after treatment. Ultrasound alone; (■). Ultrasound + 25 μg/ml porfimer sodium (◆). Ultrasound + 50 μg/ml porfimer sodium (●). Ultrasound + 100 μg/ml porfimer sodium (▲). Ultrasound intensity: (a) 300, (b) 400 and (c) 500 mW/cm^2.

tended to decrease with greater drug concentration and exposure duration. The survival rates of cells exposed to 500 mW/cm^2 ultrasound for 80 s were 20.1 ± 4.8%, whereas ultrasound in combination with 25, 50 and 100 μg/ml of porfimer sodium resulted in 11.5 ± 2.9, 3.2 ± 1.6 and 1.6 ± 1.4%, respectively. There was a significant difference of cell survival rate between the group exposed to

ultrasound alone and the porfimer sodium-combined group at all drug concentrations examined ($p < 0.05$).

The difference of cell killing by ultrasound in combination with 100 μg/ml of porfimer sodium and ultrasound alone, which could be interpreted as pure sonochemically induced effects, showed a peak cytotoxicity of $22.6 \pm 6.1\%$ when exposed at 400 mW/cm^2 for 60 s, and when irradiated at 300 mW/cm^2 for 60 and 80 s, 22.3 ± 8.2 and $23.0 \pm 11.8\%$, respectively (Figure 3). No significant sonodynamic effects were obtained with 20 s irradiation at all ultrasound intensities. It is of interest that an increase of ultrasound intensity or irradiation duration did not necessarily result in greater sonodynamic effects. Cell survival rates of normal PMNCs exposed at identical ultrasound intensities, exposure durations and drug concentration are shown in Figure 4. No significant difference ($p < 0.05$) between cells exposed to ultrasound alone and in combination with porfimer sodium were observed. There was also no clear dependency on drug concentration. As shown in Figure 5, there was a distinct difference of the cell survival rate between MT-2 cells and normal PMNCs that were treated at the same conditions. Ultrasound treatment to ATL patient's PMNCs resulted in a significant difference in cell survival in groups with or without porfimer sodium (Figure 6). Mean survival rates with porfimer sodium alone, ultrasound alone and ultrasound with porfimer sodium were as follows: 98.5 ± 2.7, 69.4 ± 22.5 and $30.0 \pm 23.0\%$.

Temperature measurement of the cell suspension immediately before and after all treatments by ultrasound gave 26 ± 1°C.

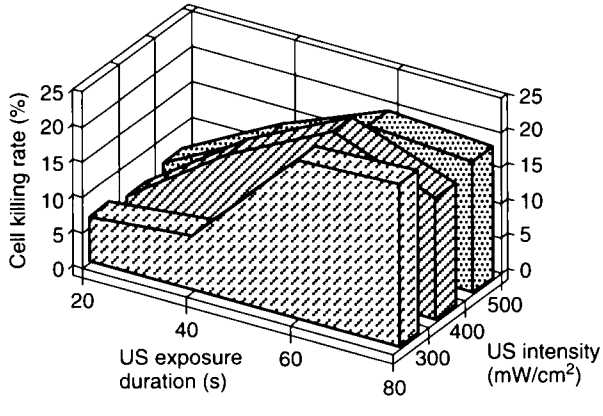

Figure 3. Cell killing rate of MT-2 cells ($n = 6$) after treatment by ultrasound for 20–80 s and 100 μg/ml porfimer sodium.

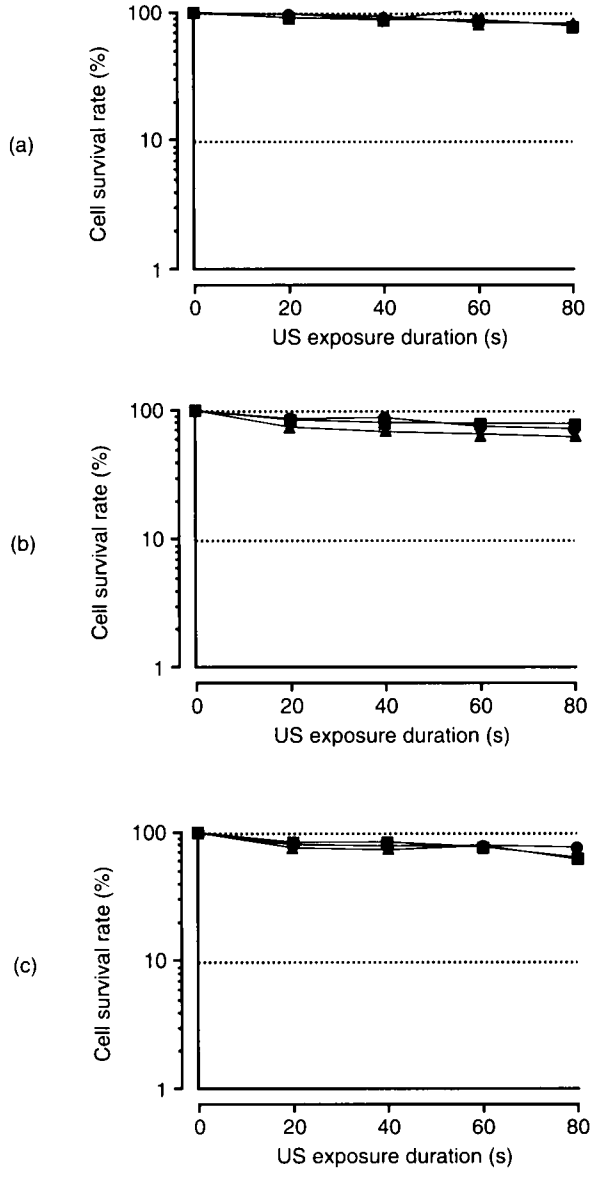

Figure 4. Cell survival rate of normal PMNCs after treatment. Ultrasound alone (■). Ultrasound + 50 μg/ml porfimer sodium (●). Ultrasound + 100 μg/ml porfimer sodium (▲). Ultrasound intensity: (a) 300, (b) 400 and (c) 500 mW/cm^2.

Discussion

Porfimer sodium is currently used for cancer photodynamic therapy *in vivo*.[8–10] Although the mechanism of action is not clearly understood, it appears that the cellular damage is mediated by the generation of reactive oxygen and hydroxyl radicals only when stimulated with light.[10,26–29] In addition, greater drug uptake or binding of porfimer sodium to malignant leukemic cell as compared to normal cells

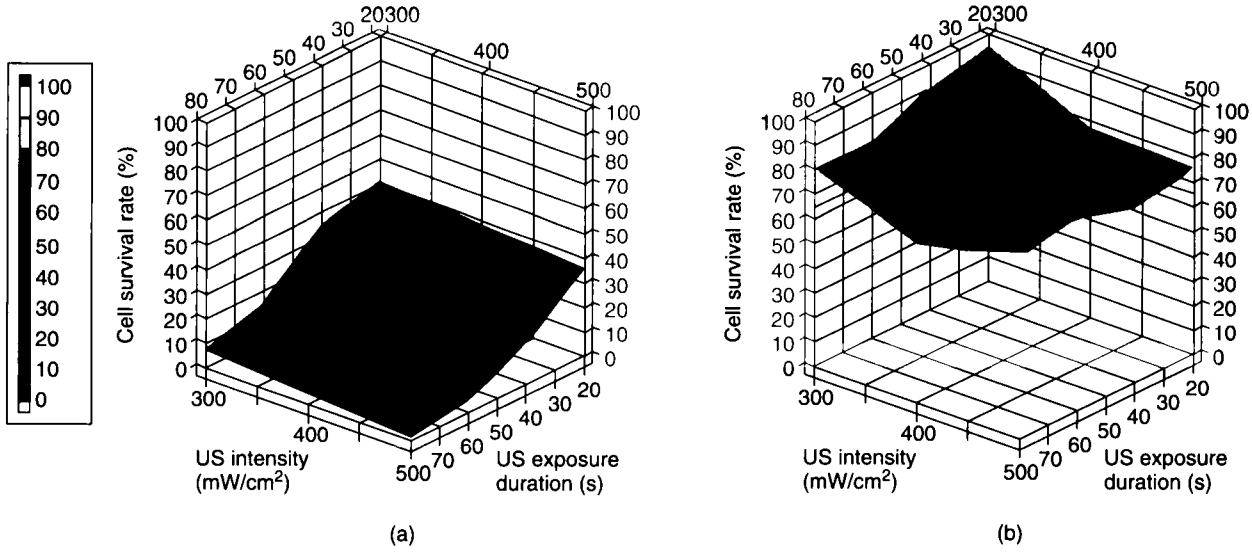

Figure 5. Comparison of cell survival rate between MT-2 cells (a) and normal PMNCs (b) with ultrasound in the presence of 100 μg/ml porfimer sodium.

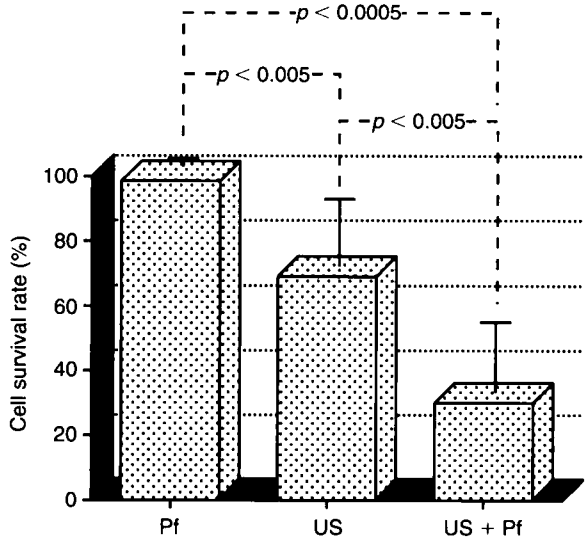

Figure 6. Survival rate of PMNCs from ATL patients ($n = 5$) after treatment by US for 60 s and/or porfimer sodium (100 μg/ml).

permits an ideal means to selectively kill malignant cells.[8] The use of photodynamic therapy has been proposed as a purging agent to selectively eliminate malignant cells in bone marrow or peripheral blood stem cells in autologous stem cell transplantations.[6–8,30–33] Such drugs as MC-540, an alipophilic polymethine dye, are currently undergoing phase II clinical trials for purging bone marrow grafts from patients with leukemia, lymphoma or metastatic neuroblastoma.[6–8,30–34] Numerous reports have also documented photodynamic therapy with hematoporphyrin and porfimer sodium for local treatment in a variety of solid tumors.[8–16] On the other hand, sonodynamic therapy is a new approach in which the photosensitive agents are activated by ultrasound energy.[17–24] The literature is limited in this new modality; however, it seems to be a promising form of energy to replace lasers due to the fact that ultrasound can penetrate much deeper into human tissues than the visible spectrum lasers used for photodynamic therapy, in addition to its cost efficiency.

In the present study, we have evaluated the potential role of porfimer sodium coupled with ultrasound exposure for the *ex vivo* treatment of peripheral lymphocytes of adult T cell leukemia cells. Sonodynamically treated MT-2 cell lines showed greater cytotoxicity compared to ultrasound alone. We also showed that increases in drug concentration, exposure duration and ultrasound intensity tended to result in more cell killing. These results are in agreement with earlier reports indicating cytotoxic effects of photosensitive agents stimulated by ultrasound energy. In contrast, sonochemically induced cell killing was not observed with PMNCs of normal subjects. Previous reports with photodynamic therapy have demonstrated selective killing of murine leukemia cells with porfimer sodium in comparison with normal murine bone marrow progenitor cells.[8] Other investigators have showed that selective elimination of clonogenic cells from patients with acute myelogenous leukemia

(AML) could be achieved with porfimer sodium and light irradiation.[8] It is suggested from the present study that porfimer sodium has a higher affinity to MT-2 cell lines compared to normal PMNCs, which results in a significant difference of cell survival after ultrasound treatment. Additionally, the present study was designed to evaluate the capability of sonodynamic therapy to purge malignant cells in clinically active ATL patients. The result showed that there was significant sonochemically induced cell killing of PMNCs in all ATL patients observed. There have been several previous attempts to apply photodynamic therapy to ATL patients by circulating blood extracorporeally for light irradiation.[35] However, the outcome of this treatment has not resulted in a satisfactory clinical response. This in part is due to light absorption and attenuation by red cell contamination at the wavelength used. Furthermore, the duration of light irradiation for photodynamic therapy is far greater, of the order of minutes versus seconds in the case of sonodynamic therapy. Although further investigation is needed to obtain the optimal ultrasound irradiation condition and drug dosage, a more efficient cell killing may be achieved by ultrasound energy with minimal damage to normal cells than photodynamic therapy.

Use of ultrasound irradiation for tumor treatment has been relatively well investigated with respect to thermal effects due to ultrasound absorption; however, few studies have reported on the non-thermal effects demonstrated in the present study.[17–24] Recent reports by Umemura[19] have associated the mechanism of toxicity to hydroxyl radicals. Experiments with and without active oxygen scavengers showed that free radical formation clearly influenced the cell killing effect of ultrasound with hematoporphyrin.[24] A similar mechanism may also have contributed to damage MT-2 cells and ATL patients' PMNCs. Furthermore, cell destruction observed immediately following ultrasound irradiation in the present study suggests a very rapid form of chemical reaction at the cell membrane. Kessel[23] have also described a similar phenomenon in experiments where sonodynamic effects were obtained only when porphyrins were present in the incubation medium, whereas cell viability was not altered when cells containing intracellular porphyrins were exposed to ultrasound.

The direct mechanism of activation of photosensitizing agents by ultrasound has not been fully explained. Jeffers[36] evaluated the ultrasound cell lysis of HL-60 cells in combination with dimethylformamide. These experiments in the presence of albumin microbubbles during ultrasound exposure suggested mechanisms related to acoustic cavitation. Others have suggested sonoluminescence-related activation of hematoporphyrin by ultrasound.[19] Although all these results lead to acoustic cavitation involvement, more investigation is anticipated.

It is concluded that there was specific selectivity of sonodynamic cell killing with porfimer sodium to MT-2 cells and acute-type ATL patients' PMNCs compared to PMNCs from healthy subjects. Although further evaluation is needed to optimize ultrasound conditions in the clinical situation, it may become feasible to apply sonodynamic therapy during extracorporeal blood treatment of ATL patients.

References

1. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood* 1977; **50**: 481–92.
2. Blattner WA, Clark JW, Gibbs WN, *et al.* Human T-cell leukemia/lymphoma virus: Epidemiology and relationship to human malignancy. In: Gallo RC, Esses ME, Gross L, eds. *Human T-cell leukemia/lymphoma virus. The family of human T-lymphotropic retroviruses: their role in malignancies and association with AIDS.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press 1984: 267–74.
3. Tamura K. Treatment for adult T-cell leukemia. *Jpn J Clin Hematol* 1986; **27**: 701–7.
4. Suzuki M, Uno H, Kawano K, *et al.* Prognostic factor analysis for adult T-cell leukemia/lymphoma. First report. *J Miyazaki Med Ass* 1989; **13**: 29–33.
5. Lymphoma Study Group. Major prognostic factors of patients with adult T-cell leukemia-lymphoma: a cooperative study. *Leukemia Res* 1991; **15**: 81–90.
6. Sieber F, Spivak JL, Sutcliffe AM. Selective killing of leukemic cells by merocyanine 540-mediated photosensitization. *Proc Natl Acad Sci USA* 1984; **81**: 7584–7.
7. Atzpodien J, Gulati SC, Clarkson BD. Comparison of the cytotoxic effects of merocyanine-540 on leukemic cells and normal human bone marrow. *Cancer Res* 1986; **46**: 4892–5.
8. Levy JG, Dowding C, Mitchell D, Sorrenti R, Yip S, Jamieson C. Selective elimination of malignant stem cells using photosensitizers followed by light treatment. *Stem Cells* 1995; **13**: 336–43.
9. Dougherty T. Photosensitizers: therapy and detection of malignant tumors. *Photochem Photobiol* 1987; **45**: 879–89.
10. Kato H. History of photodynamic therapy; past, present and future. *Gan-To-Kagaku-Ryoho* 1996; **23**: 8–15.
11. Dougherty TJ, Kaufman JE, Goldfarb A, Weishaupt KR, Boyle D, Mittleman A. Photoradiation therapy for the treatment of malignant tumors. *Cancer Res* 1978; **38**: 2628–35.
12. Dougherty TJ, Lawrence G, Kaufman JH, Boyle D, Weishaupt KR, Goldfarb A. Photoradiation in the

treatment of recurrent breast carcinoma. *J Natl Cancer Inst* 1979; **62**: 231–7.
13. Hayata Y, Kato H, Konaka C, Ono J, Takizawa N. Hematoporphyrin derivative and laser photoradiation in the treatment of lung cancer. *Chest* 1982; **81**: 269–77.
14. Soma H, Akiya S, Nutahara S, Kato H, Hayata Y. Treament of vaginal carcinoma with laser photoirradiation following administration of haematoporphyrin derivative. *Ann Chir Gynaecol* 1982; **71**: 133–6.
15. Ward BG, Forbes IJ, Cowled PA, *et al.* The treatment of vaginal recurrences of gynecologic malignancy with phototherapy following hematoporphyrin derivative pretreatment. *Am J Obstet Gynecol* 1982; **142**: 356–7.
16. Tsuchiya A, Obara N, Miwa M, Ohi T, Kato H, Hayata Y. Hematoporphyrin derivative and laser photoradiation in the diagnosis and treatment of bladder cancer. *J Urol* 1983; **130**: 79–82.
17. Yumita N, Nishigaki R, Umemura K, Umemura S. Hematoporphyrin as a sensitizer of cell-damaging effect of ultrasound. *Jpn J Cancer Res* 1989; **80**: 219–22.
18. Yumita N, Nishigaki R, Umemura K, Umemura S. Synergistic effect of ultrasound and hematoporphyrin on Sarcoma 180. *Jpn J Cancer Res* 1990; **81**: 304–8.
19. Umemura S, Yumita N, Nishigaki R, Umemura K. Mechanism of cell damage by ultrasound in combination with hematoporphyrin. *Jpn J Cancer Res* 1990; **81**: 962–6.
20. Tachibana K, Sugata K, Meng J, Okumura M, Tachibana S. Liver tissue damage by ultrasound in combination with photosensitizing drug, Photofrin II. *Cancer Lett* 1994; **78**: 177–81.
21. Tachibana K, Kimura N, Okumura M, Eguchi H, Tachibana S. Enhancement of cell killing of HL-60 cells by ultrasound in the presence of photosensitizing drug Photofrin II. *Cancer Lett* 1993; **72**: 195–9.
22. Umemura S, Yumita N, Nishigaki R. Enhancement of ultrasonically induced cell damage by a gallium–porphyrin complex, ATX-70. *Jpn J Cancer Res* 1993; **84**: 582–8.
23. Kessel D, Jeffers R, Fowlkes JB, Cain C. Porphyrin-induced enhancement of ultrasound cytotoxicty. *Int J Radiat Biol* 1994; **66**: 221–8.
24. Miyoshi N, Misik V, Fukuda M, Riesz P. Effects of gallium–porphyrin analogue ATX-70 on nitroxide formation from a cyclic secondary amine by ultrasound: On the mechanism of sonodynamic activation. *Radiat Res* 1995; **143**: 194–202.
25. Miyoshi I, Kubonishi I, Yoshimoto S, *et al.* Type C virus particles in a cord T-cell line derived by co-cultivating normal human cord leukocytes and human leukemic T cells. *Nature* 1981; **294**: 770–1.
26. Weishaupt KR, Gomer CJ, Dougherty TJ. Identification of singlet oxygen as the cytotoxic agent in photoinactivation of a murine tumor. *Cancer Res* 1976; **36**: 2326–9.
27. Cannistaro S, Vorst AV. Photosensitization by hematoporphyrin: ESR evidence for free radical induction in unsaturated fatty acids and for singlet oxygen production. *Biochem Biophys Res Commun* 1977; **74**: 1177–85.
28. Das M, Dixit R, Mukhtar H, Bickers DR. Role of active oxygen species in the photodestruction of microsomal cytochrome P-450 and associated monooxygenases by hematoporphyrin derivative in rats. *Cancer Res* 1985; **45**: 608–15.
29. Takemura T, Nakagima S, Sakata I. Mechanism of photodynamic therapy. *Gan-To-Kagaku-Ryoho* 1996; **23**: 16–21.
30. Sieber F, Rao S, Rowley SD, Sieber-Blum M. Dye-mediated photolysis of human neuroblastoma cells: implications for autologous bone marrow transplantation. *Blood* 1986; **68**: 32–6.
31. Sieber F. Elimination of residual tumor cells from autologous bone marrow grafts by dye-mediated photolysis: preclinical data. *Photochem Photobiol* 1987; **46**; 71–6.
32. Itoh T, Messner HA, Jamal N, Tweeddale M, Sieber F. Merocyanine 540-sensitized photoinactivation of high-grade non-Hodgkin's lymphoma cells: potential application in autologous BMT. *Bone Marrow Transplant* 1993; **12**: 191–6.
33. Köhler A, Müller A, Zintl F. Investigation of the usefulness of the photodynamic reaction for the purging of bone marrow from metastatic neuroblastoma cells. *Photochem Photobiol B: Biol* 1993; **19**: 227–9.
34. Valinsky JE, Easton TG, Reich E. Merocyanine 540 as a fluorescent probe of membranes: staining of electrically excitable cells. *Cell* 1978; **13**: 475–86.
35. Yamaguchi K, Futami G, Ishii T, Takatsuki K. Extracorporeal photochemotherapy. *Jpn J Transfusion Med* 1991; **37**: 70–2.
36. Jeffers RJ, Feng RQ, Fowlkes JB, Hunt JW, Kessel D, Cain CA. Dimethylformamide as an enhancer of cavitation-induced cell lysis *in vitro*. *J Acoust Soc Am* 1995; **97**: 669–76.

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