in vivo *21*: 205-214 (2007)

Review

Acridine Orange could be an Innovative Anticancer Agent under Photon Energy

KATSUYUKI KUSUZAKI[1], HIROAKI MURATA[3], TAKAO MATSUBARA[2], HARUHIKO SATONAKA[2], TORU WAKABAYASHI[2], AKIHIKO MATSUMINE[2] and ATSUMASA UCHIDA[2]

Departments of [1]Cancer Center and [2]Orthopaedic Surgery, Mie University Faculty of Medicine, Tsu; [3]Department of Orthopedic Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan

Abstract. *Acridine orange (AO) was extracted as a dye from coal tar over a hundred years ago. It has various unique biological activities and has been shown to be a useful fluorescent dye specific for DNA and RNA, a pH indicator, photosensitizer, antitumor and antimalarial drug, and detector of bacteria and parasites. It has recently been found that AO accumulates in musculoskeletal sarcomas and that after illumination of the tumors with visible light or irradiation with low-dose X-rays, the dye rapidly exerts selective cytocidal effect against the sarcoma cells. Therefore, surgery combined with photo- (PDT) or radiodynamic therapy (RDT) with AO (AO-PDT and -RDT) has been applied to human musculoskeletal sarcomas. The results of a clinical study on the outcome of this therapeutic strategy revealed that it yielded better local control and remarkably better limb function than wide resectional surgery. Based on our experimental studies, it was clarified that AO accumulates in acidic organelles or structures, especially lysosomes, depending on the acidity. An enormous number of protons are produced in cancer from lactate or CO_2 under hypoxic conditions, which are moved into the extracellular fluid or lysosomes to maintain the intracellular fluid pH. Therefore, AO shows marked accumulation in the acidic lysosomes of cancer cells. Photon energy from visible light or X-rays excites the AO accumulated in lysosomes; the excited AO emits fluorescence and forms activated oxygen from intra-cytoplasmic oxygen. The activated oxygen destroys lysosomes, with the released lysosomal enzymes causing rapid death of the cancer cells. On the other hand, normal cells can exclude AO quickly because they are not acidic. Thus, AO-PDT and AO-RDT exhibit strong and selective cytocidal effect against malignant tumors. In conclusion, we believe that AO-PDT and AO-RDT exhibit selective anticancer cell activity and that AO excited by photon energy has excellent potential as an anticancer agent.*

Correspondence to: Katsuyuki Kusuzaki, MD, Department of Cancer Center, Mie University Faculty of Medicine, Edobashi 2-174, Tsu city, Mie Prefecture 514-8507, Japan. Tel: +81 59 231 5296, Fax: +81 59 231 5211, e-mail: kusu@clin.medic.mie-u.ac.jp

Key Words: Acridine orange, photodynamic therapy, photon energy, radiation, musculoskeletal sarcoma, review.

While selective elimination of every single cancer cell from the human body would represent the ideal therapy for cancer, no anticancer agents with such highly selective activity have yet been found. Most anticancer drugs currently available are cytotoxic not only to cancer cells but also to normal cells, and are, therefore, associated with a high risk of dangerous adverse effects. Molecule-targeted therapy has been found for some cancers, but not all, as no universal cancer-specific target has yet been identified.

It has recently been found that photodynamic or radiodynamic therapy with acridine orange (AO) (referred to as AO-PDT and AO-RDT, respectively) exerts a strong cytocidal effect against mouse osteosarcoma cells both *in vitro* and *in vivo*. We have, therefore, attempted to apply intralesional or marginal excision combined with AO-PDT or AO-RDT, to avoid any damage to intact tissues, to patients with musculoskeletal sarcoma in order to obtain better limb function with a lower or at least equal risk of local recurrence, compared to conventional limb salvage surgeries (1-6).

In this paper, we present our latest data from clinical studies and on the mechanism of the cytocidal effect of AO-PDT and AO-RDT investigated by quantum mechanical analysis.

Acridine Orange

Acridine orange (AO; chemical structure: $C_{17}H_{20}ClN_3$-$1/2ZnC_{l2}$, CAS No.10127-02-3) was first extracted from coal tar as a weak basic dye over a 100 years ago (Figure 1). It has various unique biological activities,and has been shown to be a useful fluorescent dye specific for DNA and RNA, a pH indicator, photosensitizer, antitumor and antimalarial drug, and a detector of bacteria and parasites, apoptosis and sperm mobility (7-17).

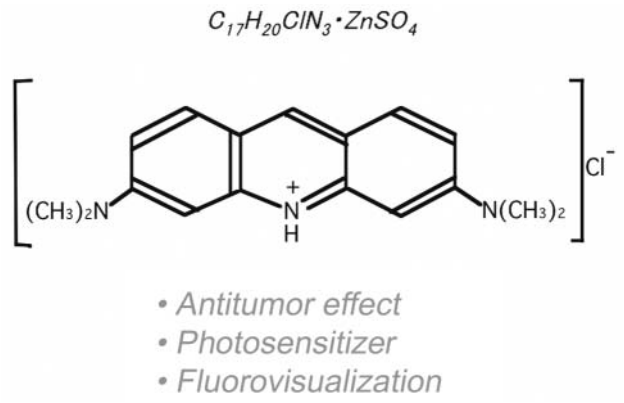

Figure 1. *Chemical structure of acridine orange (AO).*

It has a low molecular weight of 369.94 and can therefore rapidly diffuse into the cytoplasm of cells within a few seconds to bind to the DNA, RNA, lysosomes or other acidic vesicles in the cells. Figure 2 shows a fluorescence micrograph of cultured living mouse osteosarcoma cells exposed to 1.0 μg/ml of AO under blue light excitation. The green fluorescence is speculated to be from AO mainly binding to RNA and the orange fluorescence from aggregated AO binding to lysosomes or acidic cellular vesicles (18).

The International Agency for Research on Cancer (IARC) of the WHO reported that this agent was considered to be non-carcinogenic for humans (class 3) (19).

Fluorovisualization Effect of AO

Since AO has the ability to emit fluorescence after excitation with blue light (466.5 nm) (Figure 2), it is possible to macroscopically observe green fluorescence from tumor tissues with AO accumulation. Figure 3 shows a photograph of a mouse osteosarcoma transplanted into the subcutis on the back of a mouse after intravenous injection of 1 mg/ml of AO. The photograph on the left shows a macroscopic view of the tumor under normal light and that on the right shows a macroscopic view of the tumor under blue light excitation using a resorption filter (>520 nm), where the tumor can easily be distinguished from the surrounding muscle by the strong green fluorescence emitted from the tumor alone. With the use of a stereomicroscope, even lesions smaller than 1 mm in diameter can be detected (3).

Cytocidal Effect of AO-PDT

AO-PDT has a strong cytocidal effect against mouse osteosarcoma cells, including multi-drug resistant osteosarcoma cells (1). Mouse osteosarcoma cells show swelling of the cytoplasm and nucleus within 24 hours, and die within 72 hours of exposure to AO-PDT. AO-PDT has also been shown to be effective in inhibiting the tumor growth of mouse osteosarcoma *in vivo* (2).

Cytocidal Effect of AO-RDT

We also found that AO-RDT, which refers to low-dose irradiation by X-rays, at less than 5 Gy, of tumors treated with AO, exhibited a similar cytocidal effect to AO-PDT *in vitro*; furthermore, AO-RDT was also found to exert growth-inhibitory effect against mouse osteosarcoma *in vivo*. These findings indicate that X-rays can also excite AO, like photon energy. X-rays have an advantage over photon energy in that they can penetrate deeper into areas of the human body than a light beam can. Since AO quickly penetrates into muscle and bone tissues, satellite lesions of osteosarcoma could also be destroyed by AO-RDT procedure (Figure 4).

Mechanism of AO Accumulation in Malignant Tumors

To reveal the mechanism of AO accumulation in malignant tumors, we investigated the sequential changes in the fluorescence intensity emitted from mouse osteosarcoma and muscles after AO injection. AO rapidly diffused into both the tumor and muscles, but while it was rapidly eliminated from the muscles within 2 hours, it was eliminated from the tumor much more slowly that is, AO was retained for longer periods of time in the tumor than in the muscles (3). AO is well known to bind to acidic structures, such as DNA, RNA and lysosomes, depending on the acidity (9, 10, 16, 17). Most sarcoma cells were found to be more acidic than cells constituting benign tumors or normal soft tissues like muscle and adipose tissue, perhaps due to the more hypoxic environment of these cells. Thus, AO binds strongly to the acidic sarcomas (20). High-grade malignant sarcomas produce enormous amounts of protons due to the active cell metabolism under hypoxic conditions. These protons are secreted into lysosomes or the extracellular fluid by the cellular proton pump. The binding of AO to lysosomes, acidic vesicles, and intracellular protons, prevents its rapid elimination from malignant tumor cells (Figure 5).

Quantum Mechanics in AO-PDT and AO-RDT

The photoreaction involved in the cytocidal effect of AO-PDT and AO-RDT is illustrated in Figure 6. AO excited by *hv* energy from a light beam or X-rays produces activated oxygen which induces cell death (21). Figure 7 summarizes the mechanism of the cytocidal effect of AO-PDT and AO-RDT. Quantum mechanics of the electron energy transfer from photons to oxygen through AO is central to the efficacy of this therapy. The activated oxygen

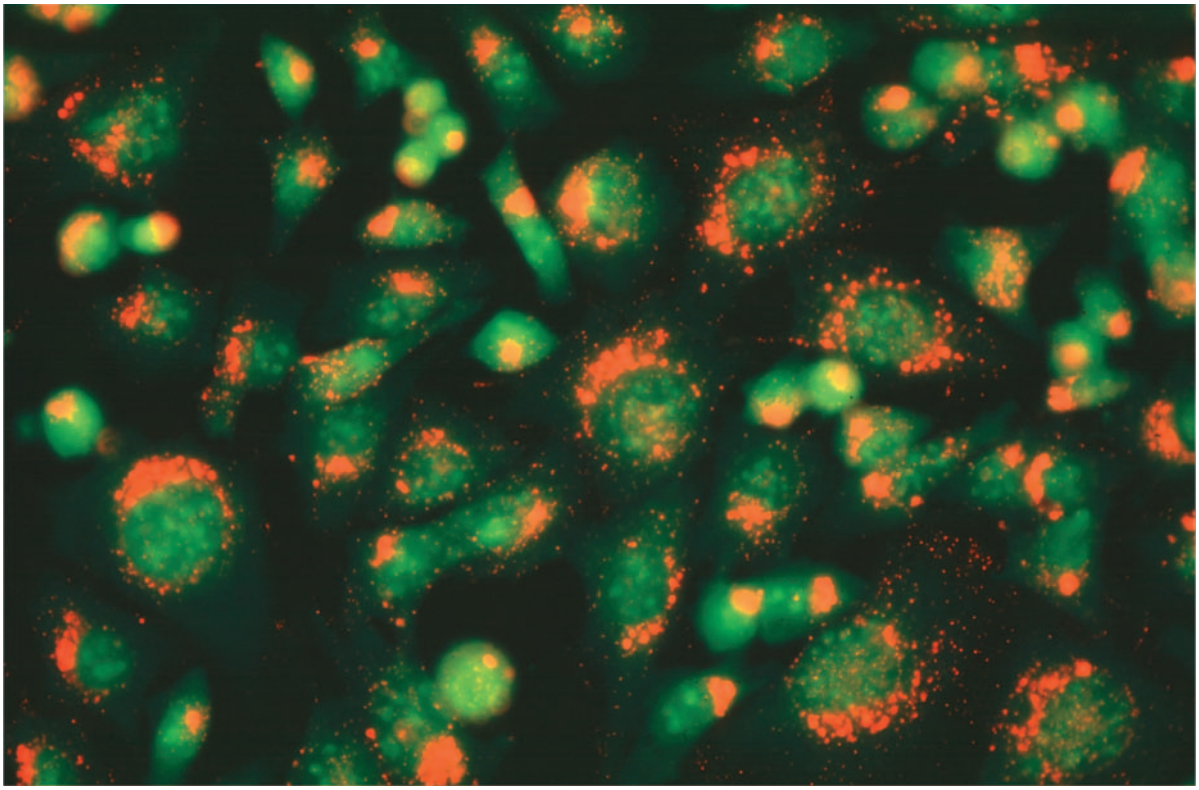

Figure 2. *Fluorescence image of living mouse osteosarcoma cells exposed to AO under blue light excitation.*

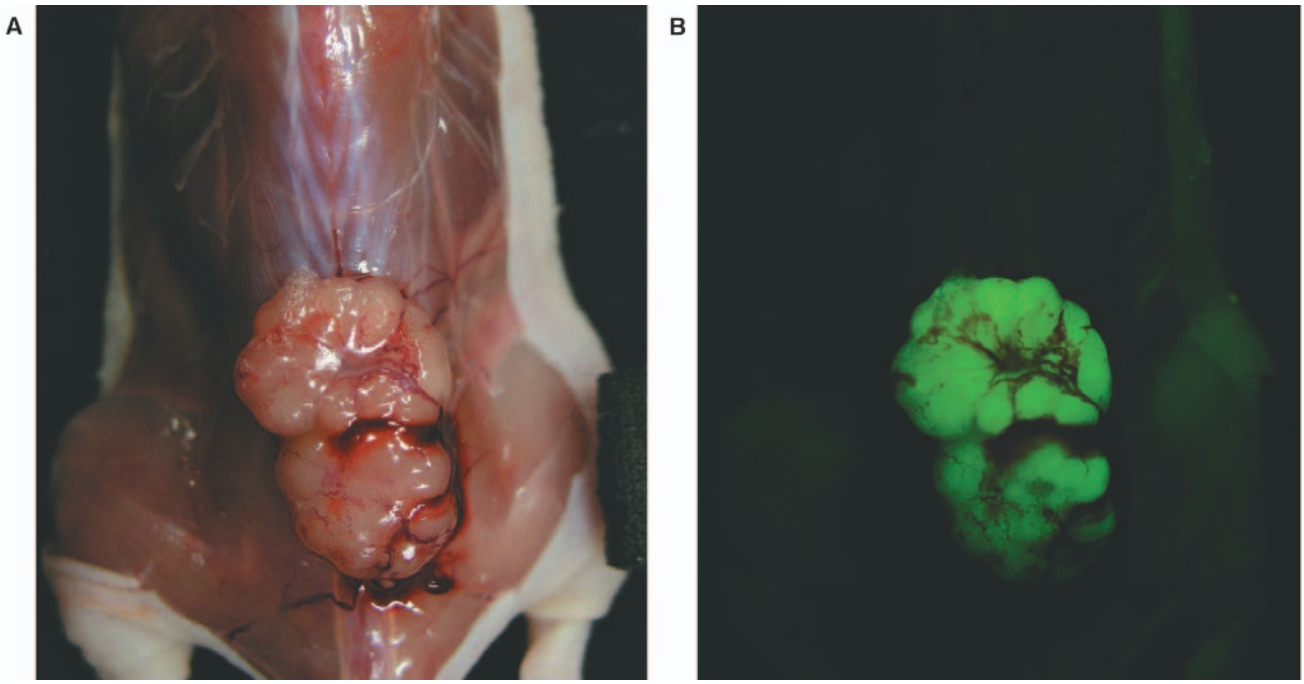

Figure 3. *Fluorovisualization effect in a mouse osteosarcoma inoculated subcutaneously into the skin on the back of a mouse (A: under ordinary light, B: under blue light).*

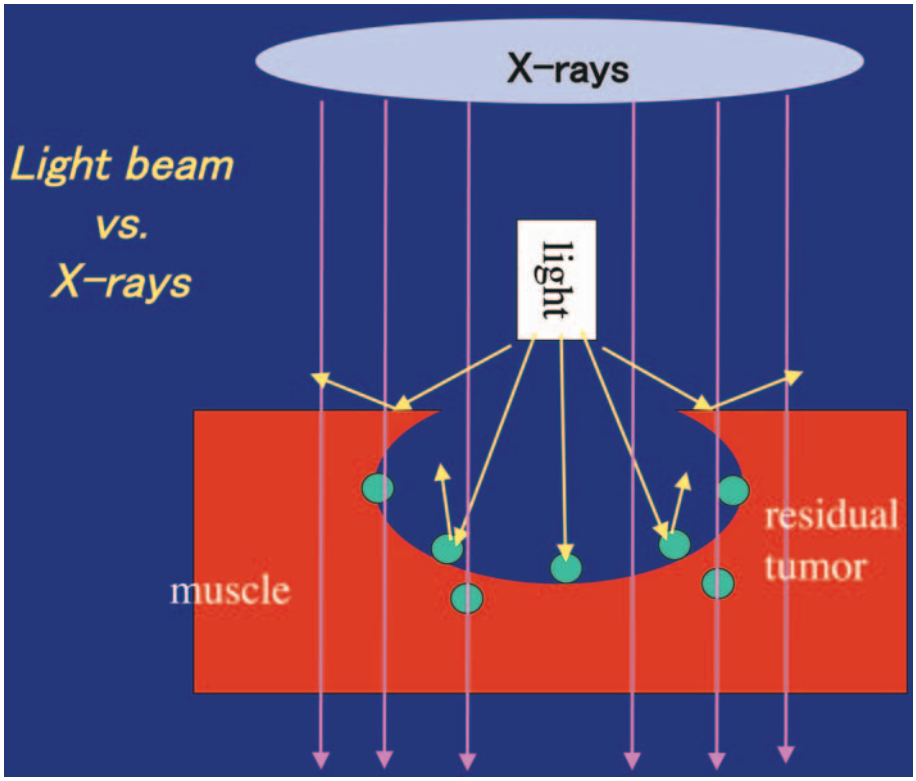

Figure 4. *Cytocidal effect of AO-PDT vs. AO-RDT against a residual tumor mass in the muscle*

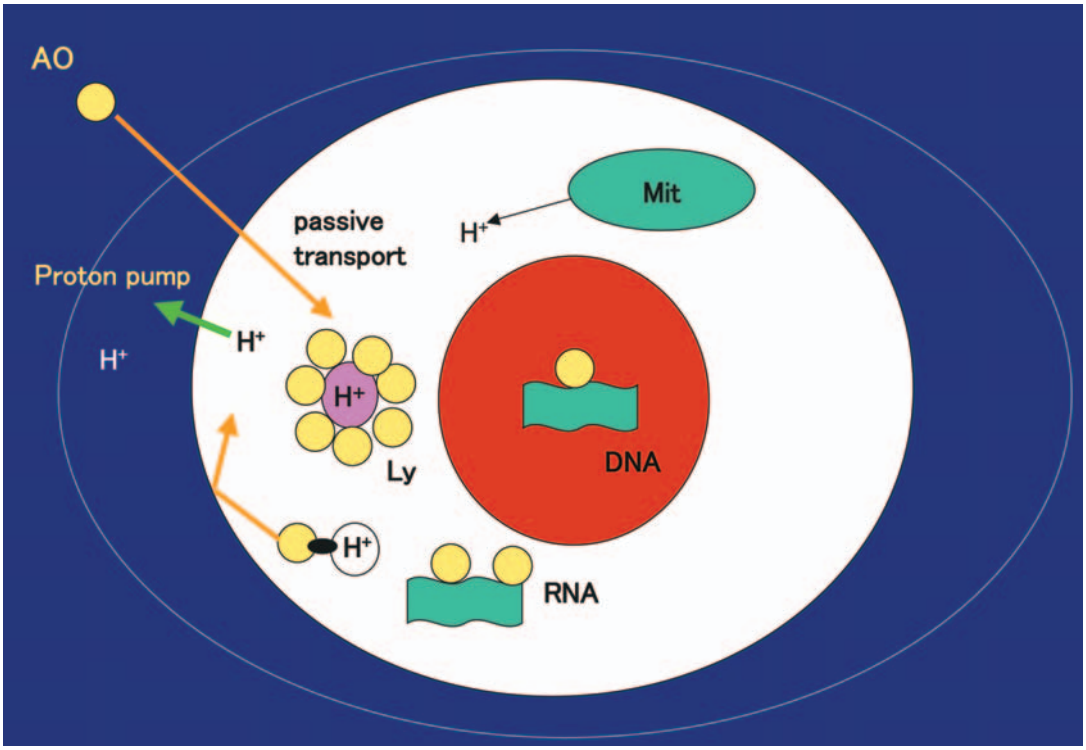

Figure 5. *Mechanism of AO accumulation in malignant tumors.*

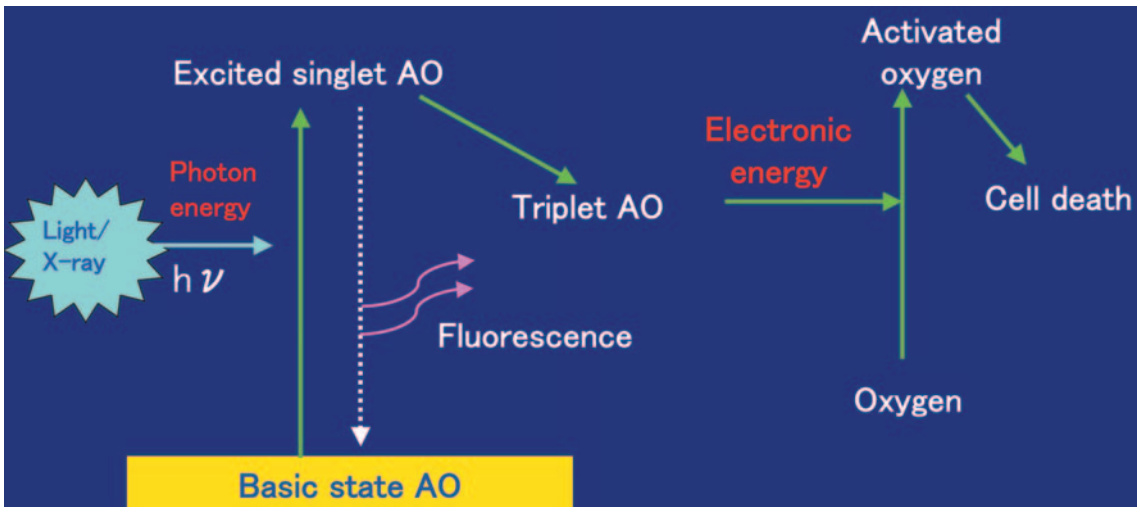

Figure 6. *Cytocidal mechanism of AO-PDT and AO-RDT.*

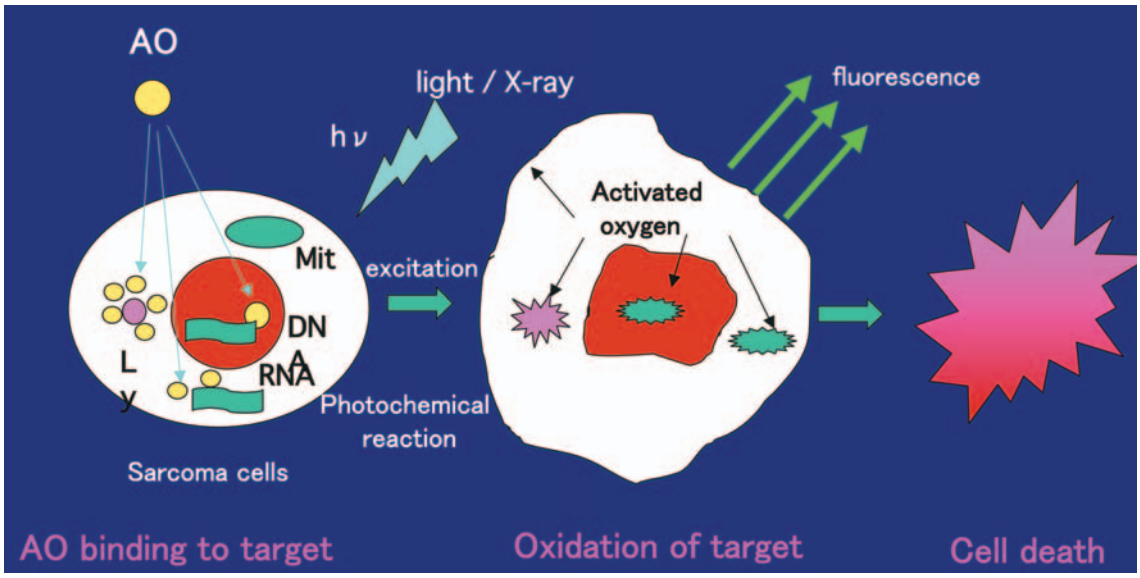

Figure 7. *Quantum mechanics underlying the cytocidal effect of AO-PDT and AO-RDT.*

produced by the electron transfer oxidizes the lipid constituting the lysosomal or cellular membranes, thereby causing rapid cell death.

AO Accumulation in Human Musculoskeletal sarcomas

The fluorovisualization effect with AO in surgically resected human musculosketetal sarcomas was examined before clinical application of AO-PDT and AO-RDT. The cut-surfaces of the resected tumors were exposed to 1 μg/ml of AO solution, washed with saline and then illuminated with blue light. Most of the sarcomas examined emitted green fluorescence from light-excited AO binding selectively to the tumor tissue (3). Figure 8 shows an osteosarcoma invading the muscle; it can be seen that only the tumor tissue emits green fluorescence.

Clinical Application of AO-PDT and AO-RDT

These results of our basic studies suggested that AO-PDT and AO-RDT might be applicable to limb salvage operation for malignant bone and soft tissue tumors. If effective, patients can obtain almost normal limb function

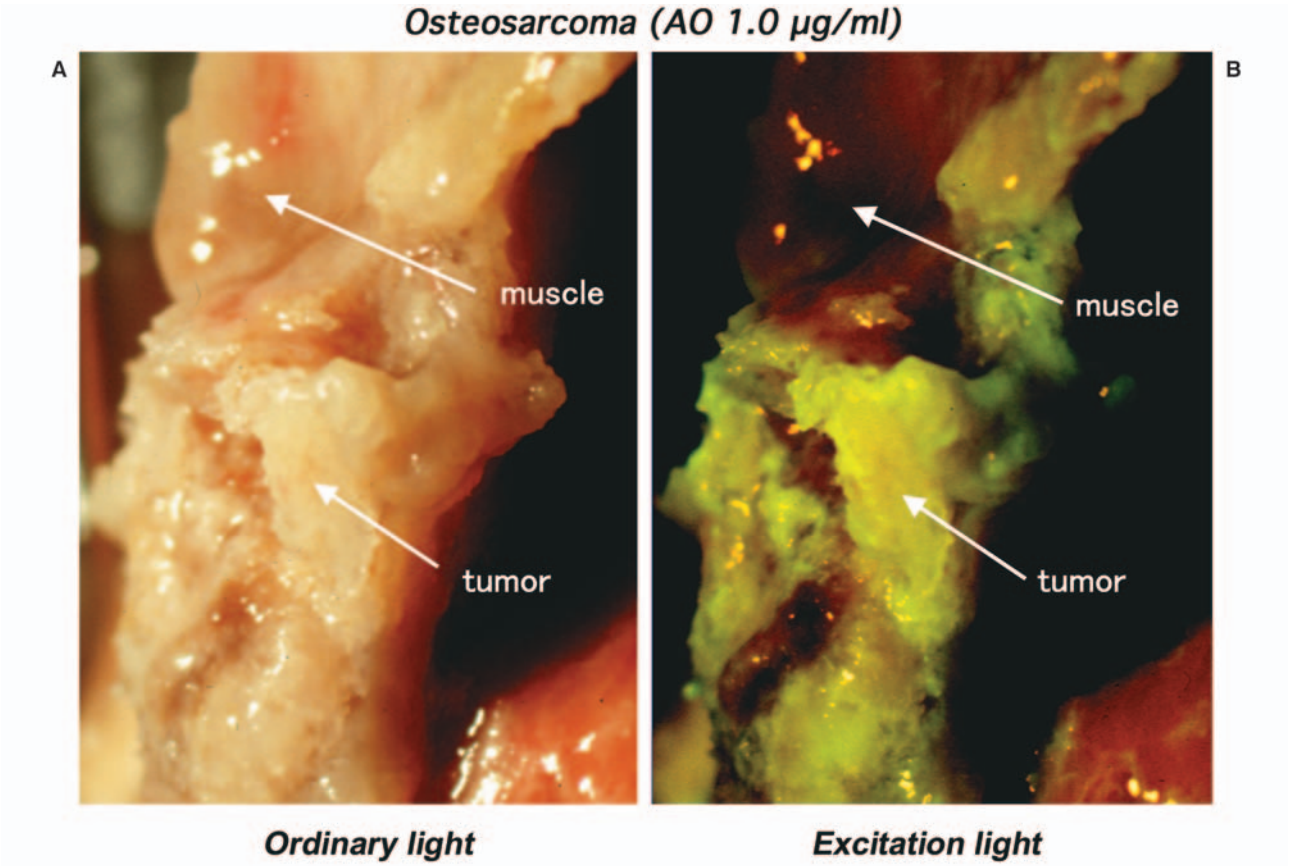

Figure 8. *AO accumulation and the fluorovisualization effect in a surgically resected specimen of human osteosarcoma (A: under ordinary light, B: under blue light).*

with a low risk of local recurrence because normal tissues such as muscles, bones and major nerves and vessels adjacent to the tumor can be preserved after intralesional or marginal tumor excision combined with AO-PDT and AO-RDT. Our strategy for clinical application of AO-PDT and AO-RDT is as follows; Step 1: macroscopic curettage of the tumor; Step 2: additional microscopic curettage with an ultrasonic surgical knife using a fluorescence surgical microscope under tumor fluorovisualization after local treatment with 1 μg/ml of AO solution for 5 minutes and excitation with blue light (we call this procedure photodymanic surgery: AO-PDS); Step 3: AO-PDT with unfiltered full light from a xenon lamp is applied to the tumor curettage area for 10 minutes using a surgical microscope; Step 4: after closure of the surgical wound, without washing-out of AO solution, the resected area is irradiated with X-rays at a dose of 5 Gy for AO-RDT (5, 6). Our fluorescence surgical microscope (Carl-Zaiss, Germany) is equipped with a xenon lamp (500 W) and special filters for excitation and fluorovisualization of AO (5).

Clinical Outcome in our Human Sarcoma Study

Thirty-two patients with 33 lesions of musculosketetal sarcomas were treated by intralesional or marginal excision combined with AO-PDT and AO-RDT during the period from July 1999 to February 2005. All the patients were followed up for longer than 1 year after treatment. Of the 33 lesions, 21 were primary lesions, 10 were recurrent lesions and 2 were metastatic lesions. The average age of the patients, consisting of 19 males and 13 females, was 29 years (range 1 to 82 years). The follow-up period ranged from 12 to 74 months. Histologically, all were high-grade malignant sarcomas.

This clinical trial was officially certified by the IRB committee of our university. Informed consent was obtained from each patient and his/her family after a full explanation of the purpose and method of the study. Out of the 21 patients receiving primary treatment with AO-PDT and AO-RDT, oncologically 19 were continuously disease-free (CDF) and 2 of the patients with lung metastasis (M1) were died of disease (DOD). Local tumor recurrence occurred in only one

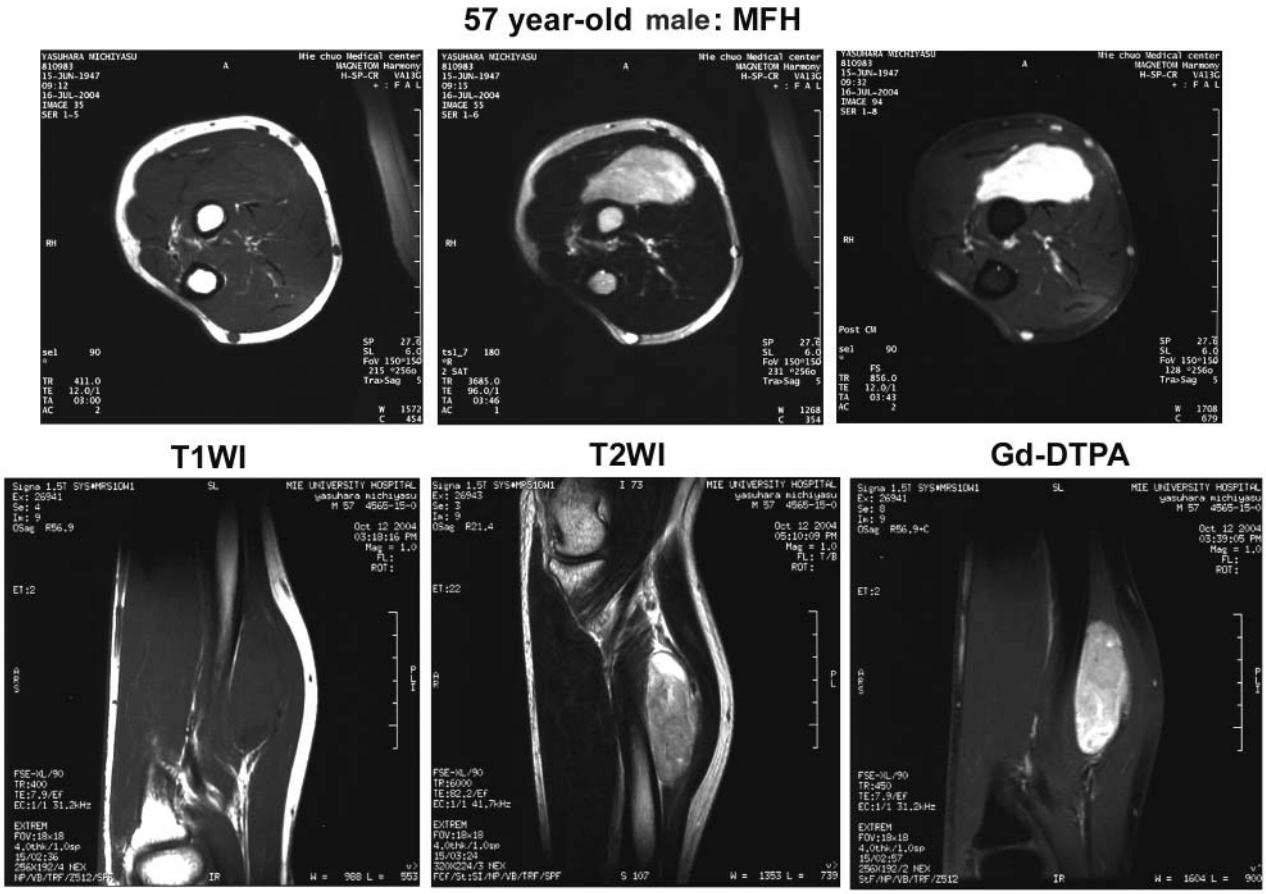

Figure 9. *MRI findings of malignant fibrous histiocytoma (MFH) (57 year-old, male).*

of these patients, with a local recurrence rate of 4.8%, which is an acceptable rate for musculoskeltal sarcomas (22, 23). All of the patients recovered excellent limb function, almost normal. None of the patients developed systemic or local complications of AO administration or AO-PDT/AO-RDT. Three of the 12 cases with recurrent lesions after primary conventional surgery showed recurrence again even after AO-PDT and AO-RDT. Although this represents a recurrence rate of 25%, this is also an acceptable figure for recurrent lesions, as compared with that following conventional surgeries (22, 23).

Case Presentation

This patient was a 57 year-old man with malignant fibrous histiocytoma (MFH) in the forearm (Figure 9) who presented with an increase of the tumor size despite intensive preoperative chemotherapy. To preserve the hand function, we performed AO-PDS and AO-PDT followed by AO-RDT. The surgical margin of the resected tumor was histologically positive (Figure 10). Following the treatment, the hand and finger function were restored to near normal in this patient (Figure 11). The patient is now working as a machine mechanic without any obvious handicap. There is no evidence of local tumor recurrence even after 20 months.

Discussion

Recently, various photosensitizers have been applied experimentally for photodynamic therapy *in vivo* using animal tumor models, however, only hematoporphyrin and its derivatives or precursors are available for clinical use. Clinical results with these agents showed that photodynamic therapy using these photosensitizers was effective and clinically useful only for early-stage superficial cancers such as these of the lung, esophagus, skin and bladder. These agents accumulated not only in cancer cells but also in normal cells and laser beam irradiation of normal cells would result in their death (21).

Thus, an ideal photosensitizer should accumulate only in cancer cells. We found that as compared to these hematoporphyrin agents, AO has the ability to more selectively accumulate in sarcoma cells relative to normal

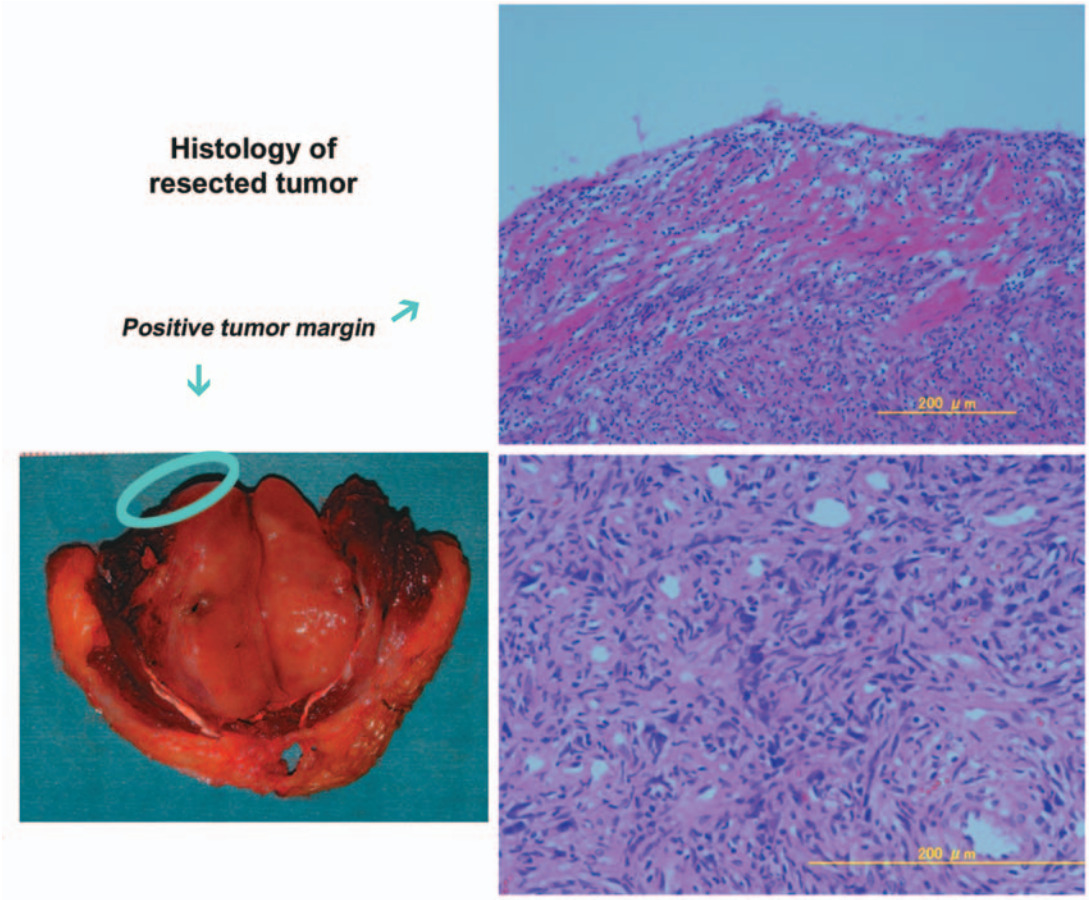

Figure 10. *Macroscopic and histological findings of the resected tumor in the case of MFH shown in Figure 9.*

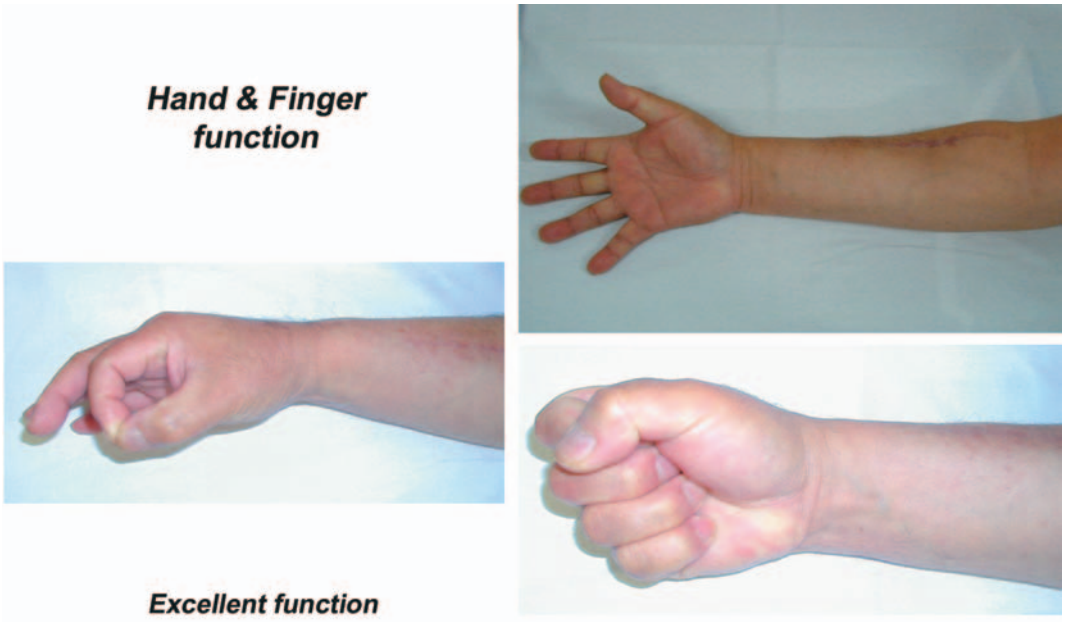

Figure 11. *Postoperative function of the hand and fingers of the patient with MFH shown in Figures 9 and 10.*

cells. It is well known that AO is an excellent pH indicator in living tissues or cells, because it binds to acidic cellular vesicles or vacuoles, of which lysosomes are representative (3, 9, 17, 24-26). Since malaria and other parasites also have such acidic vesicles, AO is used for vital staining of these parasites (14). Cancer cells are also well known to be acidic because they produce large numbers of protons (H^+) from lactate or CO_2 to generate ATP energy in hypoxic environments (27). However, since cancer cells also need to maintain a neutral intracytoplasmic fluid pH, they pump out protons into the extracellular fluid and lysosomes or acidic vesicles in the cytoplasm *via* the proton pump. Cancer cells therefore have numerous lysosomes and acidic vesicles in the cytoplasm. AO may also bind to acidic structures such as nucleic acids, DNA and RNA or newly produced protons in the intracytoplasmic fluid; however, as reported by us previously, AO does not bind to mitochondria (3), while the target of hematoporphyrin is mitochondria (21). It was recently shown that high-grade malignant sarcomas are more acidic than benign tumors like lipomas or schwannomas and normal muscle or adipose tissue (20). Bafilomycin, which inhibits the proton pump of cytoplasmic and lysosomal membranes and converts intracytoplasmic pH from acidic to neutral, disturbs the binding of AO to cancer cells and thereby also the cytocidal effect of AO-PDT (20, 25). Normal cells or benign tumor cells can eliminate AO quickly, because they are not acidic, whereas cancer cells accumulate AO.

Although AO does not specifically accumulate in cancer cells, it is retained for significantly longer periods of time in cancer than in normal tissues (3, 20); this is the reason for the apparently selective accumulation of this compound in tumors. AO also has the ability to emit fluorescence when excited by blue light. These two features allow direct visualization of AO accumulation in tumors; we call this phenomenon the fluorovisualization effect (3), which is extremely useful for detecting tumor tissue during surgery. Under a fluorescence surgical microscope, only tumor tissue exhibits green fluorescence emitted by the accumulated AO excited by blue light. After tumor excision with an intralesional or marginal margin, residual tumor tissues, even if less than 1 mm in diameter, can be detected and excise. Such additional excision utilizing the AO-fluorovisualization effect is probably better called "photodynamic surgery". After photodynamic surgery with AO (AO-PDS), tumor cells at the microscopic level can be attacked using AO-PDT (1, 2). Recently it was reported that AO-PDT using strong unfiltered full xenon light exhibited a stronger cytocidal effect than that using blue light for the AO excitation because the luminance (lux) of a light beam appears to be more important than the wavelength of the light for AO-PDT (28), although the wavelength (blue) is a critical factor for fluorovisualization.

After closure of the wound without washing out the AO, the patients are brought to the X-rays room for irradiation of the tumor resection area at a dose of 5 Gy. The difference between a light beam and X-rays is in their wavelength. X-rays have much shorter wavelength and stronger energy than a visible light beam, therefore. Logically, X-rays must also excite photosensitizers. After our report that AO could be excited by low- dose X-rays to exert cytotoxicity against osteosarcoma cells (4), it was found that porphyrin, popularly used for PDT for cancer, could also be excited by X-rays (29). An American group also reported that AO enhanced the sensitivity to radiation of radio-resistant chondrosarcoma cells (30).

The outcome of our clinical trial of AO-PDS, AO-PDT and AO-RDT for high-grade malignant musculoskeletal sarcomas revealed that as compared with conventional surgery, namely tumor resection with a wide excisional margin, this modality was sufficient to inhibit local tumor recurrence and to restore excellent limb function in patients, most of whom from our series have been living as high a quality of life as healthy people. Therefore, we believe that AO-PDS, AO-PDT and AO-RDT are innovative surgical modalities for limb salvage in patients with musculoskeretal sarcomas, although the results must be confirmed by a longer follow-up and a larger number of patients. We also believe that this modality may be applicable to all cancers that are sensitive to AO; high grade malignant cancers are highly likely to be sensitive to AO because they are very acidic.

We hope that in the near future, AO-RDT, without any surgery after intravenous administration of AO, might be curative in patients with cancer, although it still remains to be confirmed whether even low-dose AO is entirely safe for the human body.

Conclusion

AO has potential as an excellent anticancer agent when excited by photon energy, and AO-PDS followed by AO-PDT and AO-RDT could represent a successful innovative surgical modality in cancer therapy.

Acknowledgements

This work was supported in part by a Grant-in-Aid (14207058) for scientific research from the Ministry of Education, Science, Sports and Culture of Japan

References

1 Kusuzaki K, Minami G, Takeshita H, Murata H, Hashiguchi S, Nozaki T, Ashihara T and Hirasawa Y: Photodynamic inactivation with acridine orange on a multidrug-resistant mouse osteosarcoma cell line. Jpn J Cancer Res *91*: 439-445, 2000.

2 Kusuzaki K, Aomori, K, Suginoshita, Minami G, Takeshita H, Murata H, Hashiguchi S, Ashihara T and Hirasawa Y: Total tumor cell elimination with minimum damage to normal tissues in musculoskeletal sarcomas by photodynamic reaction with acridine orange. Oncology-Bazel *59*: 174-180, 2000.

3 Kusuzaki K, Suginoshita T, Minami G, Aomori K, Takeshita H, Murata H, Hashiguchi S, Ashihara T and Hirasawa Y: Fluorovisualization effect of acridine orange on mouse osteosarcoma. Anticancer Res *20*: 3019-3024, 2000.

4 Hashiguchi S, Kusuzaki K, Murata H, Takeshita H, Hashiba M, Nishimura T, Ashihara T and Hirasawa Y: Acridine orange excited by low-dose radiation has a strong cytocidal effect on mouse osteosarcoma. Oncology-Bazel *62*: 85-93, 2002.

5 Kusuzaki K, Murata H, Matsubara T, Miyazaki S, Okamura A, Seto M, Matsumine A, Hosoi H, Sugimoto T and Uchida A: Clinical outcome of a new photodynamic therapy with acridine orange for synovial sarcomas. Photochem Photobiol *81*: 705-709, 2005.

6 Kusuzaki K, Murata H, Matsubara T, Miyazaki S, Okamura A, Seto M, Matsumine A, Hosoi H, Sugimoto T and Uchida A: Clinical trial of photodynamic therapy using acridine orange with/without low dose radiation as new limb salvage modality in musculoskeletal sarcomas. Anticancer Res *25*: 1225-1236, 2005.

7 Lewis RM and Goland PP: *In vivo* staining and retardation of tumors in mice by acridine compounds. Am J Med Sci *215*: 282-289, 1948.

8 Korgaonkar K and Sukhatankara J: Anti-tumor activity of the fluorescent dye, acridine orange, on Yoshida sarcoma (ascites). Br J Cancer *17*: 471-473, 1963.

9 Zdolsek JM, Olsson GM and Brunk UT: Photooxidative damage to lysosomes of cultured macrophages by acridine orange. Photochem Photobiol *51*: 67-76, 1990.

10 Sastry KS and Gordon MP: The photodynamic inactivation of tobacco mosaic virus and its ribonucleic acid by acridine orange. Biochim Biophys Acta *129*: 32-41, 1966.

11 Zampieri A and Greenberg J: Mutagenesis by acridine orange and proflavin in *Escherichia coli* strain S. Mutat Res *2*: 552-556, 1965.

12 McCann J, Choi E, Yamasaki E and Ames BN: Detection of carcinogenesis as mutagens in the Salmonella/microsome test: assay of 300 chemicals. Proc Natl Acad Sci USA *72*: 5135-5139, 1975.

13 Giorgio A, Rambaldi M, Maccario P, Ambrosone L and Moles DA: Detection of microorganisms in clinical specimens using slides prestained with acridine orange (AOS). Microbiologica *12*: 97-100, 1989.

14 Rickman L, Long G, Oberst R, Cabanban A, Sangalang R, Smith J, Chulay J and Hoffman S: Rapid diagnosis of malaria by acridine orange staining of centrifuged parasites. Lancet *14*: 68-71, 1989.

15 Kapuscinski J, Darzynkiewicz Z and Melamed MR: Interactions of acridine orange with nucleic acids. Properties of complexes of acridine orange with single stranded ribonucleic acid. Biochem Pharmacol *32*: 3679-3694, 1983.

16 Amagasa J: Mechanisms of photodynamic inactivation of acridine orange-sensitized transfer RNA: participation of singlet oxygen and base damage leading to inactivation. J Radiat Res (Tokyo) *27*: 339-351, 1986.

17 Zelenin AV: Fluorescence microscopy of lysosomes and related structures in living cells. Nature *212*: 425-426, 1966.

18 Kusuzaki K, Murata H, Takeshita H, Hashiguchi S, Nozaki T, Emoto K, Ashihara T and Hirasawa Y: Intracellular binding sites of acridine orange in living osteosarcoma cells. Anticancer Res *20*: 971-976, 2000.

19 International Agency for Research on Cancer. Acridine Orange. *In*: IARC Monographs Program on the Evaluation of Carcinogenic Risks to Human. IARC Press, Lyon *16*: 145, 1978.

20 Matsubara T, Kusuzaki K, Matsumine A, Shintani K, Satonaka H and Uchida A: Acridine orange used for photodynamic therapy accumulates in malignant musculoskeletal tumors depending on pH gradient. Anticancer Res *26*: 187-194, 2006.

21 Vrouenreats MB, Visser GWM, Snow GB and van Dongen GAMS: Basic principles, applications in oncology and improved selectivity of photodynamic therapy. Anticancer Res *23*: 505-522, 2003.

22 Zagar GK, Ballo MT, Pisters PW, Pollock RE, Patel SR, Benjamin RS and Evans HL: Prognostic factors for patients with localized soft-tissue sarcoma treated with conservation surgery and radiation therapy: an analysis of 225 patients. Cancer *97*: 2530-2543, 2003.

23 Duffaud F, Digue L, Mercier C, Dales JP, Baciuchka-Palmaro M, Volot F, Thomas P and Favre R: Recurrences following primary osteosarcoma in adolescents and adults previously treated with chemotherapy. Eur J Cancer *39*: 2050-2057, 2003.

24 Krolenko SA, Adamyan SY, Belyaeva TN and Mozhenok TP: Acridine orange accumulation in acidic organelles of normal and vacuolated frog skeletal muscles fibers. Cell Biol Int *20*: 1-7, 2006

25 Millot C, Millot JM, Morjani H, Desplaces A and Manfait M: Characterization of acidic vesicles in multidrug-resistant and sensitive cancer cells by acridine orange staining and confocal microspectrofluorometry. J Histochem Cytochem *45*: 1255-1264, 1997.

26 Hiruma H, Katakura T, Takenami T, Igawa S, Kanoh M, Fujimura T and Kawakami T: Vesicle disruption, plasma membrane bleb formation, and acute cell death by illumination with blue light in acridine orange-loaded malignant melanoma cells. J Photochem Photobiol B: Biology *86*: 1-8, 2007.

27 Griffiths JR, McIntyre JO, Howe FA and Stubbs M: Why are cancers acidic? A carrier-mediated diffusion model for H^+ transport in the interstitial fluid. *In*: The Tumour Microenvironment: Causes and Consequences of Hypoxia and Acidity. Wiley, Chichester (Novartis Foundation Symposium 240), pp. 46-67, 2001.

28 Ueda H, Murata H, Takeshita H, Minami G, Hashiguchi S and Kubo T: Unifiltered xenon light is useful for photodynamic thrapy with acridine orange. Anticancer Res *25*: 3979-3984, 2005.

29 Pogue BW, O'Hara JA, Demindenko E, Wilmot CM, Goodwin IA, Chen B, Swartz HM and Hasan T: Photodyamic therapy with vertephorin in the radiation-induced fibrosarcoma-1 tumor causes enhanced radiation sensitivity. Cancer Res *63*: 1025-1033, 2003.

30 Moussavi-Harami F, Mollano A, Martin JA, Ayoob A, Domann FE, Gitelis S and Buckwalter JA: Intrinsic radiation resistance in human chondrosarcoma. BBRC *346*: 379-385, 2006.

Received November 22, 2006
Revised January 10, 2007
Accepted January 15, 2007