Photodynamic Therapy with ATX-S10·Na(II) Inhibits Synovial Sarcoma Cell Growth

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Abstract Photodynamic therapy (PDT) is an effective cancer treatment modality that allows selective destruction of malignant tumor cells. We asked whether PDT could inhibit *in vivo* and *in vitro* growth of synovial sarcoma cells. We analyzed PDT using ATX-S10·Na(II) and a diode laser for a synovial sarcoma cell line (SYO-1). Photodynamic therapy with ATX-S10·Na(II) showed an *in vitro* cytotoxic effect on the cultured SYO-1 cells. The *in vitro* effect of PDT depended on the treatment concentration of ATX-S10·Na(II) and the laser dose of irradiation. ATX-S10·Na(II) was detected in the tumor tissue specimens that

were excised from nude mice bearing SYO-1 within 6 hours after intravenous injection, but it was eliminated from the tumor 12 hours after injection. Photodynamic therapy suppressed the tumor growth of nude mice bearing SYO-1, and high-dose irradiation induced no viable tumor cells in histologic specimens. Photodynamic therapy performed after marginal resection of the tumor of nude mice bearing SYO-1 reduced the rate of local recurrence of the tumor. Our results suggest PDT using ATX-S10·Na(II) and laser irradiation may be a potentially useful treatment for synovial sarcoma, especially to reduce the surgical margin and preserve critical anatomic structures adjacent to the tumor.

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Introduction

Photodynamic therapy is a unique cancer treatment modality based on the dye-sensitized photooxidation of biologic matter in target tissue [25, 26]. An intravenous injection of a light-sensitive agent (the photosensitizer) is retained selectively by tumor cells. The photosensitizer can be focally excited by laser light in the presence of oxygen using light of a wavelength matched to an absorption peak of the photosensitizer [3], and it transfers energy from photons to oxygen molecules. Direct killing of tumor cells, vascular damage, and inflammatory responses contribute to tumor destruction [2, 7]. Photodynamic therapy, with systemic administration of photosensitizer and laser irradiation, has been used clinically in recent years. It has advantages such as anatomic and functional preservation of adjacent normal tissues, enabling minimally invasive procedures and adjuvant therapy for unresectable cancers [16, 29, 31]. Various authors report PDT has an antitumor effect

in lung [10, 18], esophageal [8], bladder [1, 12], and dermatologic cancers [24, 33]. Photodynamic therapy also has been used to treat noncancerous diseases such as choroidal neovascularization [22], atherosclerosis [30], and benign hyperproliferative skin [28].

Malignant musculoskeletal tumors typically require wide surgical resection with normal surrounding tissue. However, a wide surgical resection often results in poor physical function postoperatively according to the amount of excised normal tissue, including muscle, vessels, and nerves. Preservation of such normal surrounding tissue can lead to better postoperative function for the patient, although there is a higher risk of local recurrence. Adjuvant treatment may reduce the risk of local recurrence when decreasing the surgical margin. The effect of chemotherapy and radiotherapy remains controversial regarding musculoskeletal tumors [4, 6, 32]. Photodynamic therapy could be a novel adjuvant treatment for musculoskeletal tumors. Several papers report the treatment of osteosarcoma with acridine orange PDT and chondrosarcoma with BPD PDT [5, 13, 14].

ATX-S10-Na(II) is one of the hydrophilic chlorine photosensitizers and has some advantages compared with other photosensitizers. ATX-S10-Na(II) can be eliminated rapidly from normal tissue, usually within 48 hours after injection, thus resulting in reduced skin photosensitization [27]. Its absorption maximum lies at 670 nm, which allows deeper penetration of laser beams into tissues than a 630-nm laser [19]. Photodynamic therapy with ATX-S10-Na(II)

can be more effective for treatment of deeply located or large tumors, but has not been explored for use in soft tissue sarcomas.

Exploring the possibility that PDT using ATX-S10-Na(II) could be a new therapy for synovial sarcoma we asked four questions: (1) Does PDT have a cytotoxic effect on human synovial sarcoma cells in vitro?; (2) Can ATX-S10-Na(II) accumulate specifically in the tumor after intravenous injection in vivo, and be eliminated from the tumor quickly?; (3) Can PDT using ATX-S10-Na(II) and laser irradiation cause tumor necrosis and inhibit tumor progression?; and (4) Can PDT using ATX-S10-Na(II) and laser irradiation reduce the rate of local recurrence after marginal resection of synovial sarcoma? In this study, we conducted in vivo and in vitro experiments using a human synovial sarcoma cell line to assess these questions.

Materials and Methods

To answer these four questions we conducted four series of experiments (Fig. 1). To assess an in vitro cytotoxic effect of PDT, we measured the cell viability of synovial sarcoma cells after PDT using laser irradiation (10–50 J/cm²) following incubation with ATX-S10-Na(II) (3.25–50 µg/mL) for 24 hours. To assess the in vivo accumulation of ATX-S10-Na(II) in the tumor, we performed fluorescence microscopic examination of the tumor xenograft of synovial sarcoma cells on nude mice after an intravenous

Fig. 1 The diagram shows the experiments we performed for observation of in vitro and in vivo antitumor effects on synovial sarcoma.

1. In vitro

Cell viability after PDT

SYO-1 cells (2 × 10³ per well)
ATX-S10-Na(II) for 24 hours incubation
(0, 3.13, 6.25, 12.5, 25, and 50 µg/mL)
10, 20, and 50 J/cm² irradiation
MTT assay

2. In vivo

Tumor (SYO-1) xenograft

SYO-1 cells (10⁵ cells/per mouse)
BALB/c nude mice

Accumulation of ATX-S10-Na(II)

Fluorescence microscopic examination at
0, 3, 6, 12, and 24 hours after injection

Antitumor effect of PDT alone

ATX-S10-Na(II) (5 and 10 mg/kg)
100 J/cm² and 200 J/cm² irradiation
Tumor size at Days 0, 4, 7, 11, 15
Histologic examination at Day 15

PDT as adjuvant therapy

Laser irradiation (100 J/cm²) 3 hours
after intravenous injection of ATX-S10-Na(II) (10 mg/kg) following
marginal resection of the tumor

injection of ATX-S10·Na(II) (10 mg/kg) during a course of 0 to 24 hours. To assess the efficacy of PDT on the tumor in vivo, we measured the size of the tumor xenograft on nude mice for 15 days after PDT treatment with laser irradiation (100, 200 J/cm²) following intravenous injection of ATX-S10·Na(II) (5, 10 mg/kg). Because the maximal laser penetration depth was reported as less than 8 mm [17], PDT was performed when the diameter of the tumor xenograft on the nude mice was 5.5 mm. The size of each treated tumor (five mice for each group) at Day 15 after PDT was compared with the size of the untreated control tumors (five mice). Histologic examination of the tumor xenograft was performed on Day 15 after PDT. To answer the question of PDT reducing local recurrence of the tumor, we compared the rates of local recurrence after marginal resection followed by PDT with a 10 mg/kg injection of ATX-S10·Na(II) and 100 J/cm² laser irradiation, or after marginal resection alone (five mice in each group). In all experiments, the concentration of ATX-S10·Na(II) and the dose of laser irradiation were not randomized.

ATX-S10·Na(II), 13,17-bis(1,2-dicarboxyethyl)carbamoyl ethyl-8-ethenyl-2-hydroxy-3-hydroxyiminoethylidene-2,7,12,18-tetramethylporphyrin sodium salt was synthesized by a commercial laboratory (Photochemical Co, Ltd, Okayama, Japan). We dissolved ATX-S10·Na(II) in phosphate-buffered saline, and diluted in a culture medium at appropriate concentrations. A diode laser, ALD-1 (Hamamatsu Photonics KK, Hamamatsu, Japan), was used as the light source to excite ATX-S10·Na(II). The diode laser is a continuous-wave laser and its wavelength is 670 nm.

A human synovial sarcoma cell line, SYO-1 [11], was incubated in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and antibiotics (penicillin and streptomycin) in a humidified atmosphere with 5% CO₂ at 37°C.

We incubated SYO-1 cells (2 × 10³ per well) in a 96-well microplate with 0, 3.13, 6.25, 12.5, 25, and 50 µg/mL ATX-S10·Na(II) for 24 hours. The cells were washed with medium twice and then irradiated with 10, 20, and 50 J/cm² using the 670-nm diode laser. The laser power was fixed at 150 mW/cm². After additional 24-hour incubation of the cells at 37°C, cell viability was assessed by an MTT assay (Chemicon International, Inc, Temecula, CA). Quantitation then was measured using a Model 550 microplate reader (BioRad, Hercules, CA). We prepared eight wells for each condition and the cytotoxicity rate in treated cells was represented as a percentage of MTT value, and compared with that of untreated control cells.

Fifty 4-week-old male BALB/c nude mice (BALB/c nu/nu) were used for implantation of SYO-1 cells. We injected single cell suspensions of SYO-1 cells (10⁵ cells per

mouse) subcutaneously into the back of the mice. Growth of the tumors was measured using a slide caliper. When the diameter of the tumor reached 10 mm (1.5–2 weeks), ATX-S10·Na(II) (10 mg/kg) was injected intravenously into five SYO-1-bearing mice. The tumors were removed at 0, 3, 6, 12, and 24 hours after injection, respectively. Four frozen sections of the tumor were embedded in OCT compound and prepared for fluorescence microscopy coupled with a cooled CCD camera (Axioplan 2 Imaging; Carl Zeiss, Inc, Thornwood, NJ). A 5-µm unstained frozen section was excited by 520 nm light, and the fluorescence of ATX-S10·Na(II) was examined under a 585-nm band pass filter by the CCD camera.

When the diameter of the tumor on the back of the nude mice reached 5.5 mm, ATX-S10·Na(II) (5 and 10 mg/kg) was injected intravenously. We irradiated the tumor site with 670 nm laser light (100 J/cm², 200 J/cm²) 3 hours after injection (five mice for each group). The duration of PDT was related to the laser dose (11 minutes for 100 J/cm² and 22 minutes for 200 J/cm²). After PDT, the animals were maintained in a dark room to avoid skin irritation. The length and width of the tumors were measured at Days 0, 4, 7, 11, 15 after laser irradiation and then tumor volume was calculated by using the equation: tumor volume (mm³) = [maximum diameter (mm)][minimum diameter (mm)]²/2. The mice were euthanized by an intraperitoneal injection of a barbiturate (120 mg/kg) on Day 15 after PDT. Tumor specimens excised from mice were fixed with 4% PFA and embedded in paraffin before undergoing microscopic examinations. Slides of the 4-µm-thick specimens were stained with hematoxylin and eosin. All sections were viewed by two (TK, SM) individuals who were blind to results. Results did not differ between observers.

We examined the efficacy of PDT as an adjuvant treatment to reduce surgical margins, such as for marginal resections of a tumor, without excising surrounding normal tissue. It might be difficult to treat tumors that are large or deeply localized with PDT alone owing to limitations in laser penetration. After the diameter of the tumor on the back of the mice was 12 mm, a marginal resection of the tumor on mice bearing SYO-1 was performed 3 hours after intravenous injection of ATX-S10·Na(II). We divided the mice into two groups: (1) marginal resection without PDT (control), which means resection of the tumor without excision of the surrounding normal tissue; and (2) marginal resection followed by PDT with 100 J/cm² laser irradiation 3 hours after intravenous injection of 10 mg/kg ATX-S10·Na(II) (n = 10 for each group). The mice were observed until 8 weeks after marginal resection of the tumors.

Each continuous variable of the tumor volume resulting from PDT to the tumor xenograft was expressed as

mean + SD. A nonparametric Mann-Whitney U test was used to compare the tumor volume on Day 15 after PDT with each condition (5 and 10 mg/kg ATX-S10·Na(II) and 100 J/cm² and 200 J/cm² laser irradiation) with tumor volume of the untreated control group. We compared the rate of tumor recurrence after marginal resection of the xenograft with or without PDT using Fisher's exact probability test. Taking into account our power analysis, we considered a probability value less than 5% as significant throughout the study. All statistical analyses were performed using Stat View 5.0 software (SAS Institute Inc, Cary, NC).

Results

MTT assay revealed PDT using ATX-S10·Na(II) had a cytotoxic effect on and inhibited the growth of SYO-1 cells in vitro. The cytotoxic effect of PDT on SYO-1 cells was dependent on the ATX-S10·Na(II) concentration and the level of laser irradiation (Fig. 2). A higher dose of laser irradiation induced a strong antitumor effect under each concentration of ATX-S10·Na(II). No antitumor effect was observed in the SYO-1 cells treated with laser irradiation without ATX-S10·Na(II) and also with the exposure to ATX-S10·Na(II) without laser irradiation. Photodynamic

Fig. 2 An MTT assay showed an in vitro antitumor effect of PDT using ATX-S10 Na(II) with a diode laser on SYO-1 cells. The cells were incubated with 0 to 50 µg/mL ATX-S10 Na(II) at 37°C for 24 hours before 670-nm diode laser radiation with 0 to 50 J/cm². The cytotoxicity rate in the treated cells is represented as a percentage of MTT value and in comparison to that of the untreated control cells. Higher dye concentrations and laser irradiation levels induced an antitumor effect in a dose-dependent manner.

therapy inhibited tumor growth of SYO-1-bearing mice by Day 15 compared with the untreated control group (ATX-S10·Na(II) 5 mg/kg and laser 100 J/cm², $p = 0.047$; 10 mg/kg and 100 J/cm², $p = 0.021$; 5 mg/kg and 200 J/cm², $p = 0.007$; and 10 mg/kg and 200 J/cm², $p = 0.007$) (Fig. 3). The tumors had disappeared completely 4 days after injections of 5 and 10 mg/kg ATX-S10·Na(II) and laser irradiation with 200 J/cm². Regrowth of the tumor was not observed until 15 days after PDT under such conditions. However, the tumor was growing gradually after PDT with injections of 5 and 10 mg/kg ATX-S10·Na(II) and laser irradiation with 100 J/cm². Injection of ATX-S10·Na(II) without irradiation or laser irradiation without injection of ATX-S10·Na(II) did not inhibit tumor growth.

ATX-S10·Na(II) accumulated into the tumor xenograft of SYO-1 cell on nude mice after the intravenous injection. A marked fluorescence of ATX-S10·Na(II) was observed in the tumor excised from SYO-1-bearing mice 3 and 6 hours after intravenous injection of ATX-S10·Na(II) (Fig. 4). The fluorescence of ATX-S10·Na(II) in the tumor was minimally evident 12 and 24 hours after injection of ATX-S10·Na(II). ATX-S10·Na(II) injected intravenously was incorporated into the SYO-1 forming tumor and thereafter remained inside the tumor for at least 6 hours. ATX-S10·Na(II) was almost totally eliminated from the tumor within the next 6 hours.

Fig. 3 The tumor growth of SYO-1-bearing mice after ATX-S10·Na(II) PDT is shown. Tumor growth was inhibited (* $p < 0.01$, ** $p < 0.05$) within 15 days after PDT with 5 and 10 mg/kg ATX-S10·Na(II) and 100 and 200 J/cm² laser irradiation. No tumor regrowth was seen within 15 days after PDT with 5 and 10 mg/kg ATX-S10·Na(II) and 200 J/cm² laser irradiation. The data are represented as mean + standard deviation.

Fig. 4A–C The illustrations show microscopic distribution of ATX-S10-Na(II) fluorescence in SYO-1-forming tumors on nude mice at (A) 3 hours, (B) 6 hours, and (C) 12 hours after intravenous injection of ATX-S10-Na(II). A marked fluorescence of ATX-S10-Na(II) was

observed in the tumor resected from SYO-1-bearing mice at (A) 3 and (B) 6 hours after the intravenous injection of ATX-S10-Na(II), but was not observed at (C) 12 hours after injection.

The tumor was growing on Days 11 and 15 after PDT with injection of 5 mg/kg ATX-S10-Na(II) and laser irradiation of 100 J/cm² (Fig. 5A), but was not observed macroscopically on Day 15 after PDT with 10 mg/kg ATX-S10-Na(II) and 100 J/cm² laser irradiation (Fig. 5B) and with 10 mg/kg ATX-S10-Na(II) and 100 J/cm² laser irradiation (Fig. 5C). No necrotic areas were observed inside the tumors on Day 15 after PDT with injection of 5 mg/kg ATX-S10-Na(II) and irradiation with 100 J/cm² (Fig. 5D, E). Photodynamic therapy with injection of 10 mg/kg ATX-S10-Na(II) and irradiation with 100 J/cm² caused partial necrosis of the tumor in the mice. However, an area of viable tumor cells remained under the necrotic area (Fig. 5F, G). With PDT with injections with 5 and 10 mg/kg ATX-S10-Na(II) and irradiation with 200 J/cm², necrotic tissue without viable tumor cells was seen in the entire tumor area (Fig. 5H, I). Histologic analysis of the skin overlying a tumor showed a normal structure in all of the PDT-treated mice 15 days after treatment.

Photodynamic therapy substantially suppressed local recurrence after marginal resection of the tumor. Nine of 10 mice had local recurrence within 8 weeks after marginal resection of the tumor without PDT. Two of 10 mice had local recurrence after marginal resection of the tumor followed by PDT (Table 1). No side effects, such as delayed wound healing or skin defects, were evident with PDT with marginal resection (Fig. 6). None of the mice experienced walking disability until 8 weeks after PDT. The rate of local recurrence after marginal resection followed by PDT was lower ($p = 0.006$) than that after marginal resection only. Local tumor recurrence occurred in the peripheral area of PDT.

Discussion

Limb salvage surgery is considered the standard procedure for soft tissue sarcoma such as synovial sarcoma. Wide resection of the tumor with the surrounding normal tissue is necessary to prevent local recurrence for limb salvage surgery. A curative surgical margin sometimes requires

excision of the surrounding normal tissue, such as the nerves, vessels, and muscles with tumor, that sometimes leads to poor postoperative limb function. Therefore, reduction of the surgical margin such as preservation of major vessels and nerves could result in better postoperative function. However, this might increase the risk of local tumor recurrence. In searching for better adjuvant therapy, we explored the possibility that photodynamic therapy using ATX-S10-Na(II) could be a novel therapy for synovial sarcoma. We asked whether PDT with ATX-S10-Na(II) could have an *in vitro* cytotoxic effect on synovial sarcoma cells, whether ATX-S10-Na(II) accumulated in the tumor *in vivo*, whether PDT could inhibit tumor progression in nude mice, and whether PDT could suppress local tumor recurrence after marginal resection.

Our study has several limitations. The major limitations of our investigation are limited penetration depth of laser light and skin photosensitization after PDT. Because soft tissue sarcomas, such as a synovial sarcoma, often occur in deep layers of soft tissue, the tumor should not be treated only with PDT. We believe PDT can be applied to the surrounding tissue after tumor resection as an adjuvant therapy to reduce the surgical margin. Photodynamic therapy after tumor resection also may result in less skin complications. Various studies on PDT using ATX-S10-Na(II) have been reported, but ATX-S10-Na(II) has not been studied for clinical use in humans. No data are available regarding clinical complications or toxicities caused by PDT using ATX-S10-Na(II). According to some studies, major complications or toxicities were not observed by the injection of ATX-S10-Na(II) into experimental animals [20, 27, 28]. The lethal dose of ATX-S10-Na(II) for a rat seems to be approximately 1000 mg/kg (personal communication, Dr. Isao Sakata, December 26, 2007).

Patients treated with PDT using Photofrin stayed in a dark room for approximately 1 week after treatment because of hyperphotosensitivity of the skin induced by the photosensitizer [20]. ATX-S10-Na(II) was developed as a novel hydrophilic chlorine photosensitizer that was rapidly eliminated from normal tissues after injection, thus

Fig. 5A–I The illustrations show the change of macroscopic findings of the tumor after (A) PDT with 5 mg/kg ATX-S10-Na(II) and 100 J/cm² laser irradiation, (B) 10 mg/kg ATX-S10-Na(II) and 100 J/cm² laser irradiation, and (C) 5 mg/kg ATX-S10-Na(II) and 200 J/cm² laser irradiation. Histologic samples with hematoxylin and eosin staining, (D) shown at $\times 4$ and (E) $\times 200$ magnification, were made on Day 15 after PDT with 5 mg/kg ATX-S10-Na(II) and 100 J/cm² laser irradiation, at (F) $\times 4$ and (G) $\times 200$ magnification for 10 mg/kg ATX-S10-Na(II) and 100 J/cm² laser irradiation, and at (H) $\times 4$ and (I) $\times 200$ magnification for 5 mg/kg ATX-S10-Na(II) and 200 J/cm² laser irradiation. (A–C) A dusky skin was observed at the treatment location,

which thereafter gradually decreased in size and then normal skin developed. (A) The tumor grew gradually 11 days after PDT with 100 J/cm² laser irradiation. No necrotic area was seen inside the tumor on Day 15 after PDT using (D) 5 mg/kg ATX-S10-Na(II) injection and 100 J/cm² irradiation and (E) SYO-1 cells were all viable. Photodynamic therapy (F, G) using an injection of 10 mg/kg ATX-S10-Na(II) and irradiation with 100 J/cm² caused necrosis of the tumors, although an area of viable tumor cells remained under the necrotic area. (H, I) An injection of 5 mg/kg ATX-S10-Na(II) and irradiation with 200 J/cm² resulted in a completely necrotic area of the tumor and no viable tumor cells were observed.

indicating ATX-S10-Na(II) could cause less skin photosensitization [19]. Our data suggest ATX-S10-Na(II) was incorporated in implanted synovial sarcoma on mice until 6 hours after intravenous injection, and it was eliminated from tumor tissues less than 12 hours after injection. ATX-S10-Na(II) has been reported to be excreted rapidly, within 3 hours after administration, from normal surrounding

organs such as muscles, lungs, and brain [17, 19, 21]. Prolonged light protection might not be required after administration of ATX-S10-Na(II) PDT because of its rapid clearance compared with other photosensitizers [23].

Photodynamic therapy on SYO-1-bearing mice can inhibit tumor growth in 15 days, thus indicating PDT may be an effective therapy for synovial sarcoma. Moreover, PDT with 200 J/cm² irradiation showed no local recurrence after 15 days. However, SYO-1-bearing mice showed regrowth of the tumor after PDT with 100 J/cm² irradiation. Photodynamic therapy with lower laser irradiation therefore might not sufficiently abolish tumor cells in deep layers. Preventing local recurrence after PDT also may require higher laser irradiation. The *in vivo* mechanism of PDT remains controversial. ATX-S10-Na(II) was reported to produce singlet oxygen, which thus induces cell death directly [21]. Furthermore, PDT using ATX-S10-Na(II)

Table 1. Local control after marginal resection of the tumor with or without PDT

Treatment	Recurrence		Total
	Yes	No	
Marginal resection + PDT	2	8	10
Control (marginal resection alone)	9	1	10

PDT = photodynamic therapy.

Fig. 6A–D Marginal resection followed by PDT 3 hours after an intravenous injection of 10 mg/kg ATX-S10 Na(II) was performed on the SYO-1-forming tumors. The illustrations shows the macroscopic findings of mice received marginal resection followed by PDT at (A) Day 1, (B) Day 14, (C) Day 28, and (D) Day 56 after

treatment. The skin (A) in the treatment area lost its normal pink color on Day 1 after treatment. (B) A dark scab was observed approximately 2 weeks later, (C) which peeled off and finally healed with a normal appearance. (D) No tumor recurrence was observed by 56 days after this procedure.

and a diode laser was reported to have an indirect antitumor effect induced by vascular shutdown and inhibition of nutritional supply to tumor cells [20].

Musculoskeletal tumors often are located in deep layers and usually grow to greater than 5 cm in size. Photodynamic therapy alone therefore is insufficient for clinical treatment of musculoskeletal sarcomas because of the limited penetration of laser light. The maximal depth of necrosis induced by ATX-S10-Na(II) PDT is reportedly 7.9 mm in the treated tumor [17]. We developed PDT as adjuvant treatment after a marginal resection, which can preserve such adjacent critical tissues as nerves and vessels. Adjuvant PDT could eliminate residual tumor cells after a marginal resection. In our study, a marginal resection followed by adjuvant ATX-S10-Na(II) PDT in mice reduced the local recurrence rate of SYO-1-forming tumors. This suggests adjuvant ATX-S10-Na(II) PDT can be effective for the remaining residual cells after marginal resection. However, a 20% recurrence rate is not clinically acceptable. Because these local recurrences were in the peripheral area of PDT, it is possible wider irradiation or higher laser dose might have prevented local recurrence of the residual tumor cells in those areas. Kusuzaki et al. reported acridine orange PDT followed by radiotherapy suppressed tumor growth of osteosarcoma-bearing mice [15]. Jin et al. also suggested the use of a combination of PDT and sonodynamic therapy improved the tumoricidal effects in squamous cell carcinoma in mice [9]. Okunaka et al. reported the pulsed wave laser showed a higher effect of PDT than the continuous-wave laser [23]. Additional therapy such as radiotherapy and sonodynamic therapy and the use of a pulsed wave laser therefore also might reduce the risk of local tumor recurrence after ATX-S10-Na(II) PDT.

We observed the *in vitro* and *in vivo* antitumor effects of ATX-S10-Na(II) PDT on synovial sarcoma. Although

additional clinical trials are necessary, our results indicated ATX-S10-Na(II) PDT may be a potentially useful treatment modality, especially as adjuvant therapy to reduce the size of the surgical margin for synovial sarcoma. Preservation of critical muscles, nerves, and vessels adjacent to tumors treated with ATX-S10-Na(II) PDT would result in a better postoperative functional outcome and improved quality of life.

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