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Enhancement of the Antitumor Effect by Combined Use of High-energy Shock Waves and ATX-70

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The antitumor effects of high-energy shock waves (HESW) in combination with ATX-70 [a gallium-porphyrin complex, 2,4-bis(1-decyloxyethyl)-Ga(III)-1,3,5,8-tetramethylporphyrin-6,7-dipropionyl diaspartic acid] were investigated. *In vitro*, the cell damage to mouse MH134 hepatoma after HESW treatment was enhanced by adding ATX-70. *In vivo*, HESW and ATX-70 combination therapy inhibited cell growth. However, neither HESW treatment alone nor ATX-70 treatment alone inhibited cell growth. These results imply that the antitumor effects of HESW and ATX-70 combined therapy are caused by activation of ATX-70 by HESW.

Key words: High-energy shock wave — Hematoporphyrin — ATX-70 — Antitumor effect

High-energy shock wave (HESW) therapy is widely utilized for treatment of cholecystolithiasis or urolithiasis. Recently, HESW was reported to have antitumor effects *in vitro* and *in vivo*.[1-3] HESW can be concentrated on a small region, and is considered to be applicable to local cancer therapy.

Hematoporphyrin (Hp) is known to be effective for cancer therapy in combination with laser beam exposure, and this therapy is called photoradiation therapy or photodynamic therapy.[4] However, the indication for this treatment is restricted to early cancers such as lung cancer, gastric cancer or cervical cancer because of the shallow penetration of the laser beam.[5]

ATX-70, a gallium-porphyrin complex, is reported to have a much longer phosphorescence lifetime than Hp or Hp derivatives and to accumulate in tumors at high concentration.[6] A combination of ATX-70 and pulsed laser beam exposure showed significant tumor destruction.[7] Moreover, ultrasonically induced cell damage was enhanced by ATX-70.[6] In this study we examined the combined effects of HESW and ATX-70 and investigated whether the photosensitizer could be applied in the treatment of deeply located cancers.

HESW exposure: A shock wave lithotripter (LT-01, EDAP Co., Marne, France) generated HESW with a peak pressure of about 90 MPa. Samples were placed at the focal point in water of room temperature.

ATX-70 was provided by Toyohakka Kogyo Ltd. (Okayama). Cells of MH134, a transplantable ascitic tumor from a spontaneous hepatoma in a C3H/HeN mouse, were maintained through intraperitoneal implantation in C3H/HeN mice.

For the *in vitro* studies, MH134 cells suspended in RPMI solution (1×10^7 cells/ml) were placed in polypropylene tubes. The test tubes were exposed to either 250, 500 or 1000 shots of HESW (50 MPa, 20 shots/s). Cell viability, assessed in terms of trypan blue exclusion, after HESW was dose-dependent; increasing HESW number was associated with decreased cell viability. Addition of ATX-70 (1000 μg/ml) caused enhancement of the antitumor effects of HESW (Fig. 1).

In vivo, tumor cells (1.0×10^6 cells) were injected subcutaneously into the thighs of 6-week-old C3H/HeN mice (Nihon SLC Co., Shizuoka), which were divided into the following four groups: a) control group, b) HESW-alone group [2000 shots of HESW (10 shots/s, 90 MPa) were administered to the tumor 6 days after tumor inoculation], c) ATX-70-alone group [40 mg/kg ATX-70 was injected intravenously after 4 days], d) HESW+ATX-70 group [2000 shots of HESW were administered after 6 days, 48 h after injection of 40 mg/kg ATX-70]. From 6 days after tumor cell inoculation, the tumor size was measured with calipers. The tumor volume was calculated using the formula, volume (V) = $\pi/6\times$ length (L) $\times$ width (W) $\times$ height (H). Either 40 mg/kg ATX-70 injection alone or 2000 shots of HESW exposure alone failed to inhibit tumor growth. But the combination of 40 mg/kg ATX-70 and 2000 shots of HESW caused a significant decrease of tumor growth on the 12th day after tumor inoculation in comparison with the control group ($P<0.05$) (Fig. 2).

These results show that HESW activated ATX-70. It was reported that Hp was activated by the cavitation caused by ultrasound.[8] HESW was also reported to

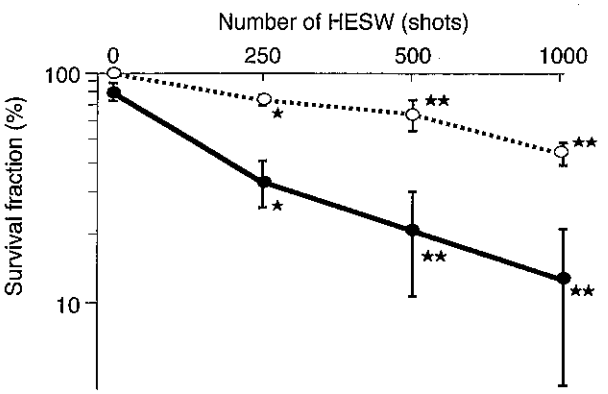

Fig. 1. Survival fraction after HESW exposure. ○, HESW alone; ●, HESW+1 mg/ml ATX-70. Points and vertical bars show the mean±SD (n=3–6). ★ and ★★ indicate statistical significance: ★, $P<0.0001$; ★★, $P<0.005$.

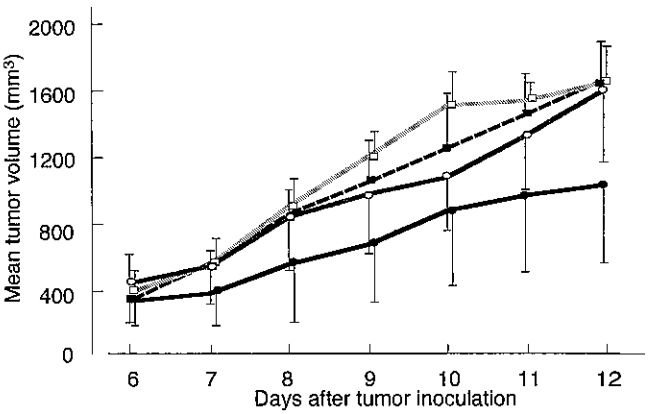

Fig. 2. Mean tumor volume after treatment. ■, Control (n=4); □, ATX-70 (40 mg/kg) alone (n=4); ○, HESW alone (n=3); ●, ATX-70+HESW (n=5). Vertical bars show the SD.

induce cavitation,[9] and this may be the mechanism by which HESW activated ATX-70.

At present, photodynamic therapy is restricted to endoscopic therapy because of the shallow penetration of the laser beam. However, HESW can be focused on deep structures. Using HESW, photosensitizer therapy may be applicable to deep lesions, such as liver tumors, pancreatic cancers, breast cancers and others.

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REFERENCES

1) Rosso, P., Stephenson, R. A., Miles, C., Huryk, R., Hesston, W. D. W., Melamed, M. R. and Fair, W. R. High energy shock waves suppress tumor growth in vitro and in vivo. *J. Urol.*, **135**, 626–628 (1986).
2) Randazzo, R. F., Chaussy, C. G., Fuchs, G. J., Bhuta, S. M., Lovrekovich, H. and deKernion, J. B. The in vitro and in vivo effects of extracorporeal shock waves on malignant cells. *Urol. Res.*, **16**, 419–426 (1988).
3) Hoshi, S., Orikasa, S., Kuwahara, M., Suzuki, K., Shirai, S., Yoshikawa, K. and Nose, M. Shock wave and THP-adriamycin for treatment of rabbit's bladder cancer. *Jpn. J. Cancer Res.*, **83**, 248–250 (1992).
4) Dougherty, T. J., Kaufman, J. E., Goldfarb, A., Weishaupt, K. R., Boyle, D. and Mittleman, A. Photoradiation therapy for the treatment of malignant tumor. *Cancer Res.*, **38**, 2628–2635 (1978).
5) Kato, H., Konaka, C., Suzuki, S., Yoshida, K., Hiki, Y., Shimao, H., Ito, Y., Goto, K., Noguchi, Y., Tenjin, Y., Sugishita, T., Ota, M., Mizuno, K., Hisazumi, H., Uchibayashi, T. and Hayata, Y. Clinical phase III trial of photodynamic therapy for superficial early stage cancers using excimer dye laser and photosensitizer polyhematoporphyrin ester/ether (PHE). *J. Jpn. Soc. Cancer Ther.*, **29**, 1757–1766 (1994) (in Japanese).
6) Uemura, S., Yumita, N. and Nishigaki, R. Enhancement of ultrasonically induced cell damage by a gallium-porphyrin complex, ATX-70. *Jpn. J. Cancer Res.*, **84**, 582–588 (1993).
7) Nakajima, S., Maeda, T., Omote, Y., Hayashi, H., Yamazaki, K., Kobo, Y., Takemura, S., Shindo, Y. and Sakata, I. Tumor localizing Ga-porphyrin complex (ATX-70) as a new photosensitizer excited with YAG-laser. *J. Jpn. Soc. Laser Med.*, **10**, 225–228 (1989) (in Japanese).
8) 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).
9) Coleman, A. J., Saunders, J. E., Crum, L. A. and Dyson, M. Acoustic cavitation generated by an extracorporeal shockwave lithotripter. *Ultrasound Med. Biol.*, **13**, 69–76 (1987).