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Shock Wave and THP-Adriamycin for Treatment of Rabbit's Bladder Cancer

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Focused high-energy shock waves (6,000 to 10,000 shots) were targeted under ultrasound guidance onto implanted urinary bladder cancer in rabbits to elucidate its effect. Although only focal necrosis of the tumor was seen following 6,000 to 10,000 shots daily for 3 days or following chemotherapy (THP-adriamycin) alone, almost total tumor necrosis was observed following a combined shock-wave therapy for one day and THP-adriamycin administration, demonstrating an additive and/or synergetic effect on rabbit urinary bladder cancer.

Key words: Underwater shock wave — Shock wave and anti-cancer drug — Bladder cancer — Animal tumor

High-energy shock waves (SW) have been reported to kill tumor cells in suspension and to delay the growth of solid tumors exposed *in vivo*.[1-5] We previously reported that SW treatment of VX2 cancer implanted in the rabbit bladder induced tumor necrosis and decreased the tumor growth.[6,7] SW induce vascular damage in the tumor, which may be the primary cause of the tumor necrosis.[7]

In the present experiments we have examined the effects of a combination therapy of SW and THP-adriamycin. THP-adriamycin was selected because of its effectiveness on VX2 cancer in rabbits.[8] The VX2 cancer is a highly malignant transplantable tumor originating from Shope virus-induced papilloma of a domestic rabbit, and a VX2 bladder cancer model exhibits metastasis to the perivesical and mesenteric lymph nodes as well as to the lung.[7]

Male rabbits (Japanese white) of mixed breed weighing from 2.5 to 2.7 kg were used. To establish our research model, the urinary bladder was exposed through a lower abdominal incision and 0.2 ml of a cell suspension containing 5×10^6 VX2 cancer cells was injected into the bladder submucosa from the opening in the bladder mucosa. Five days after the introduction of the VX2 cancer cells, the animals except for the control group were treated by chemotherapy alone, SW alone or a combination of SW and chemotherapy.

SW exposure: The rabbits were anesthetized by pentobarbital 25 mg/kg (i.v.), then the rabbit bladder was emptied by introducing a 4 Fr. feeding tube from the urethra, and infused with 30 ml of saline. Subsequently the bladder tumor, visualized by transabdominal ultrasound, was exposed to focused SW, generated by a 24-piece piezo ceramic of 300 mm aperture with a $2 \times 2 \times 19$ mm focus zone and about 1 MPa peak pressure, at a shot-rate of 5 shots per second, using a specially designed treatment apparatus constructed by Toshiba Corporation (Japan).[9]

Experimental animals were classified according to the treatment they received into the following 4 groups ($n=12$): 1) Group A, the SW+chemotherapy group; the tumor was exposed to 10,000 shots of SW just after intravenous injection of 2 mg/kg of THP-adriamycin ($n=3$). 2) Group B, the SW alone group; the tumor was subjected to 6,000 to 10,000 shots of SW daily for three days ($n=3$). 3) Group C, the chemotherapy alone group; 2 mg/kg of THP-adriamycin was injected intravenously ($n=3$). 4) Group D, the control without any treatment ($n=3$).

Before the start of treatment, the tumor was measured by ultrasound from various angles and the largest tumor areas were scored. The mean areas just before the treatment were: Group A, 45.3 mm^2; Group B, 43.8 mm^2; Group C, 42.5 mm^2 and Group D, 46.1 mm^2, respectively. In all groups the bladder tumor was removed on day 10, fixed in 10% formalin and prepared for light microscopic examinations.

Only focal tumor necrosis was observed following chemotherapy alone or SW alone, whereas almost complete tumor destruction was induced by the combined treatment using SW and chemotherapy (Fig. 1). Histologically, calcified lesions were observed in the necrotic foci of the tumor cells in the group treated with SW and chemotherapy in combination (Fig. 2). Thus, the SW and anti-cancer drug combination therapy has an additive and/or synergetic effect on rabbit urinary bladder cancer.

Underwater shock wave has been clinically used for the treatment of urinary stones.[10] The fragmentation of urinary stones by underwater shock wave is presumed to be caused by tensile stress at the solid-water acoustic

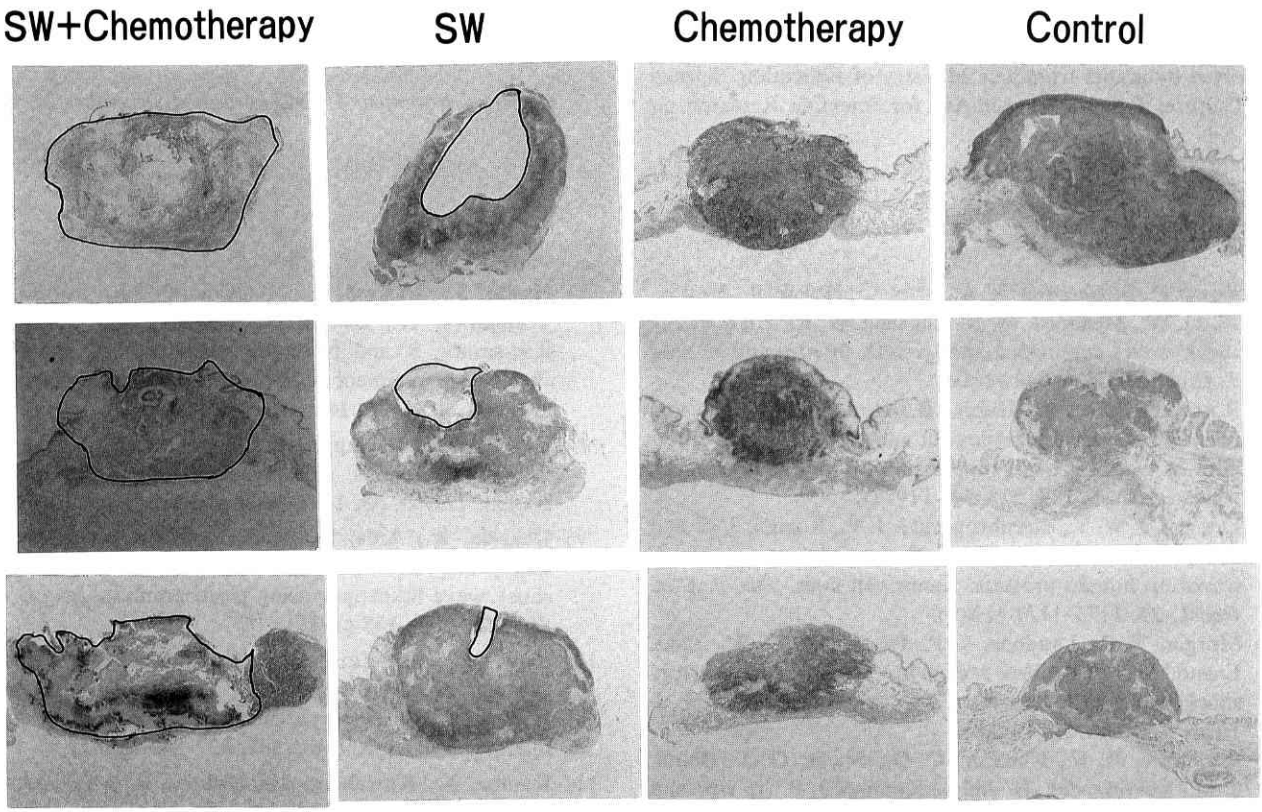

Fig. 1. Cut surface of bladder tumor (the solid line outlines the necrotic area). Hematoxylin-eosin staining. Reduced from ×3.2.

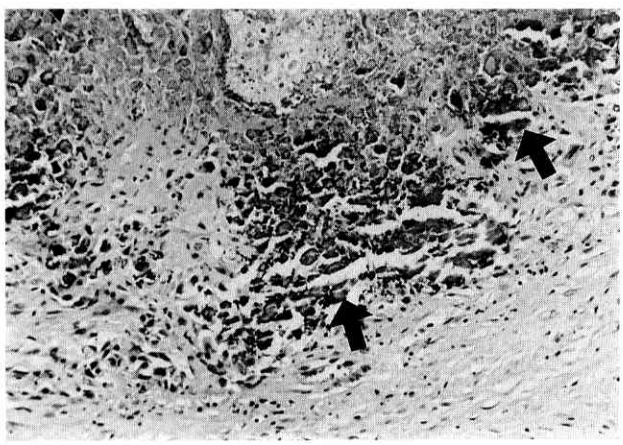

Fig. 2. Representative photomicrograph of tissue damage in one case of Group A. Calcified lesions (⬆) were observed in the necrotic foci of the tumor cells. Reduced from ×100.

interface[11] or by the cavitation phenomenon.[12] SW have been reported to suppress tumor growth *in vitro* and *in vivo*.[1-5] We previously investigated the effects of SW on implanted urinary bladder cancer in rabbits[6,7] and found that SW induced destruction of the vascular wall and caused ischemic changes in the tumor tissue through vascular changes, which may be the main cause of the tumor necrosis. However, serial SW exposures alone can not induce complete tumor necrosis.[7] Accordingly we experimented with a combined therapy using SW and an anti-cancer drug (THP-adriamycin), which produced almost complete necrosis of the bladder tumor.

Compared to our previous reports, serial SW exposure did not induce wider tumor necrosis. In the submucosal injection model, severe bladder mucosal edema occurred around the tumor after the first day of exposure and made it difficult to focus the SW onto the tumor on the second and third days. This may be the reason why serial SW was less effective than in the muscle injection model.[7]

Ultrasound can visualize deeply situated solid tumors and shock waves can be focused onto the tumor extracorporeally. Application of these techniques in combination with anti-cancer drugs may improve the response rate in cancer treatment.

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