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The Effect of High-energy Underwater Shock Waves on Implanted Urinary Bladder Cancer in Rabbits

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We have examined the effects of high-energy shock waves (HESW) on implanted urinary bladder cancer in rabbits. The bladder cancer was exposed to 2000 to 6000 shots of focused HESW under ultrasound guidance. Although only focal necrosis of the tumor was seen in the one-day HESW exposure (2000 shots), wider and deeper necrosis was observed in the tumors following serial HESW (4000 or 6000 shots; 2 or 3 days). These results indicate that serial HESW exposure has destructive effects on implanted bladder cancer in rabbits.

Key words: Underwater shock wave — Bladder cancer — Implanted tumor — Animal examination

An underwater shock wave can be used to focus extracorporeal energy onto a target organ. If this energy has a cytotoxic effect on cancer cells, the cancer tissue may be selectively destroyed by focused shock waves. High-energy shock waves (HESW) have been reported to suppress tumor growth *in vitro* and *in vivo*.[1-5] In the present experiments we have examined the effect of HESW on implanted urinary bladder cancer in rabbits.

The VX2 cancer is a highly malignant transplantable tumor which originated in Shope virus-induced papilloma of a domestic rabbit. Male rabbits (Japanese white) of mixed breed weighing from 2.5 to 2.7 kg were used. The experimental animals were grouped as follows. Group 1; After the exposure of the urinary bladder through a lower abdominal incision, 1 ml of cell suspension containing 1×10^7 VX2 cancer cells were injected into two separate parts of the posterior bladder wall[6] to induce bladder tumors in each rabbit (n=5). One bladder tumor was exposed to 2000 shots of HESW on day 14 after tumor cell injection, and the rabbits were killed on day 21. Group 2; Cell suspension (1 ml) containing 1×10^7 VX2 cancer cells was injected into a part of the posterior bladder wall. From the 14th day after the tumor cell injection, the tumor was subjected to serial HESW consisting of 4000 or 6000 shots (2 or 3 days). The rabbits were killed on day 21 (n=4). Four other rabbits similarly treated, but not exposed to HESW, served as the control. Group 3; Three normal rabbits received 1000 shots of HESW to the posterior bladder wall and were killed 5 days later.

HESW exposure: The rabbit was anesthetized with pentobarbital, 25 mg/kg (iv). The rabbit bladder was emptied by introducing a 5 Fr. feeding tube from the urethra, then infused with 30 ml of saline. The bladder tumor was visualized by transabdominal ultrasound (Fig. 1). The tumor was exposed to focused HESW, generated by a 24-piece piezo ceramic of 300 mm aperture, 35×4 mm focus zone, 1.3 kilobar peak pressure, at a shot-rate of 5 shots per second (a specially designed treatment apparatus constructed by Toshiba Medical Instruments, Tokyo). Hyperechoic regions were detected by ultrasound in the focused area when the bladder tumor was exposed to shock waves.

The bladder was fixed in 10% formalin and prepared for light microscopic examinations. Comparison was based on the extent of necrosis in the cut surface of bladder tumors.

The results in each examination group were as follows.

Group 1 (one exposure): Focal necrosis was seen in the exposed tumor (Fig. 2), however, no differences were observed in the tumor volume.

Group 2 (two or three serial exposures): Wider and deeper necrosis of tumors was found in those with exposure to HESW than in those without (Fig. 3). Histologically, deeper and wider tumor necrosis and severe hemorrhage were observed in HESW-exposed tumor, associated with vascular damage. Neutrophil infiltration was found in the border between the necrotic tumor cells and the viable tumor cells.

Group 3 (one exposure of normal bladder): Severe edema and a central dimple were observed. Microscopically we noted disconnection of the muscle layer, muscles were changed to fibrous tissues, and subcutaneous edema and bleeding were found. Significant hemorrhagic foci were not observed.

Underwater shock waves have been clinically used for treatment of urinary stones.[7] The fragmentation of urinary stones by underwater shock waves is presumed to be

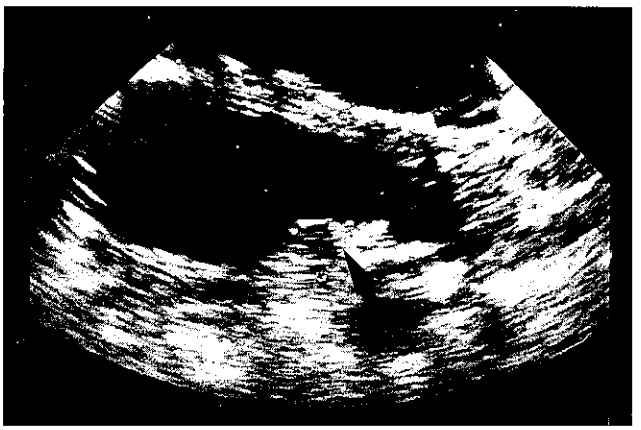

Fig. 1. The bladder tumor (↗) was exposed to focused HESW under transabdominal ultrasound guidance.

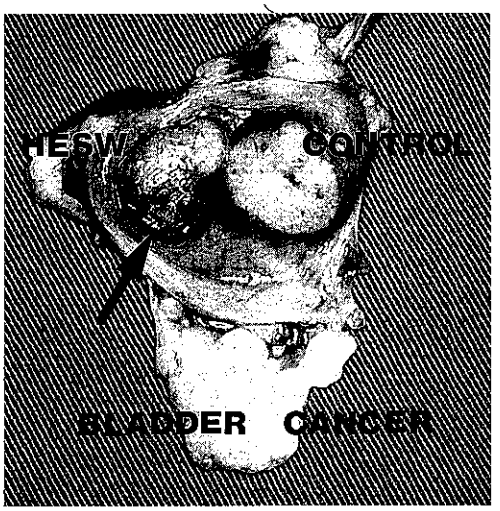

Fig. 2. Only one of two bladder tumors was exposed to HESW. One-day exposure induced only focal tumor necrosis (↗) in the exposed tumor.

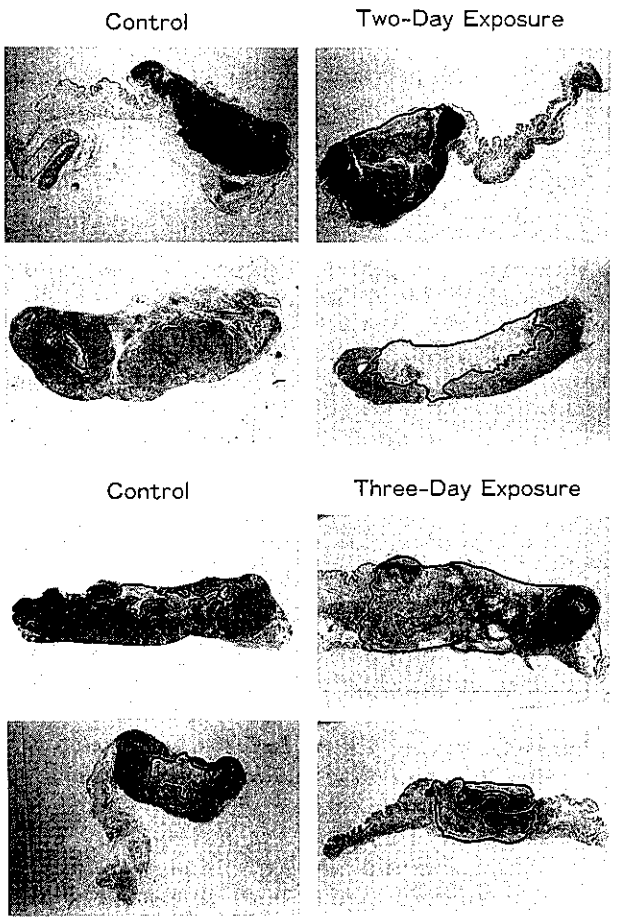

Fig. 3. Cut surface of bladder tumor after two or three days of serial exposure. Two or three days of serial exposure induced wider and deeper tumor necrosis than in the unexposed tumor (the traced lines indicate necrotic areas).

caused by tensile stress at the solid-water acoustic interface,[8] or by the cavitation phenomenon.[9] Ellwart *et al.*[10] reported that microcavitation might cause damage to cell membranes. A hyperechoic region in living tissue was observed by ultrasound in the focused area of shock waves and it is probably due to cavitation bubbles.[9] Such a hyperechoic region was also detected in our present examination.

In regard to renal tissue injury induced by the focused shock wave, Ioritani *et al.*[11] stated that the primary lesion was in the vessels, mainly in arterial walls. They stated that the injury to the arterial wall induces successive pathological changes in the renal tissue, i.e. arterio-venous fistula, interstitial bleeding, regional infarction, and subcapsular hematoma. The exposed area finally becomes a fibrotic lesion in the late stage. Their findings suggest that living cells will be injured not only by the direct effect of the shock waves, but also by an indirect effect arising from the arterial injury in the focused area.

HESW have been reported to suppress tumor growth *in vitro* and *in vivo*. However, the mechanism of the destructive effect of HESW on the living cell is not known in detail. Rosso *et al.*[2] reported that, in an *in vivo* examination, no remarkable tumor necrosis was apparent in rat prostatic cancer tissues. However, they used only one exposure. In our rabbit experiments, a single exposure did not cause any remarkable tumor necrosis, but 2–3 days of serial exposures induced marked tumor necrosis.

The VX2 bladder cancer is a rapidly proliferative cancer which undergoes spontaneous focal necrosis. Thus, it is difficult to differentiate the significance of cell death caused by HESW from that of spontaneous destruction. In fact, tumor necrosis had already occurred 3 weeks after the tumor cell implantation in our experiments even without HESW. However, our results in group 2 showed that the implanted tumor tissue is definitely more injured when it had been exposed to HESW as compared to control implanted tumor tissue. Tumor necrosis in HESW-treated groups was associated with severe hemorrhage, suggesting that HESW may induce ischemic changes in the tumor tissue through vascular changes rather than by direct cell injury. To evaluate in detail the effect of HESW on the tumor tissue, investigation of serial HESW-induced cell damage and growth retardation in earlier phases is required.

Ultrasound can visualize a deeply situated solid tumor and shock waves can be focused onto the tumor extracorporeally. The combination of these techniques has the potential of becoming an effective treatment for cancer.

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