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Effect of high energy shock waves on tumor cells

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Summary. Exposure of bladder tumor cell strain HT-1197, chronic bonemarrow leukemic cell strain K-562, and African green-turtle normal kidney cell strain Vero to high energy shock waves resulted in ultrastructural changes and a reduction in cell viability as determined by ^{3}H-thymidine incorporation assay and flowcytometer. K-562 was the most sensitive while Vero was the most resistant to the high energy shock wave. By flowcytometry using anti BrdU antibody, described K-562 in the S phase was found to be inhibited by the exposure. Electron microscopy revealed destruction of microvilli over the cell surface and swollen mitochondria in K-562 and HT-1197. These effects were related to the number of high energy shock wave exposures. Our study demonstrates that a high energy shock wave has an anti-tumor effect in vitro.

Key words: High energy shock wave – Tumor cell

Extracorporeal shock wave lithotripsy (ESWL) is a therapeutic method that has changed conventional therapeutic methods for urolithiasis. ESWL is based on the theory that calculi can be broken into pieces by the power generated when the high energy shock wave passes through substances with different acoustical impedances [1]. Tumor cells are considered to have a greater variability of acoustical impedance than normal cells, although not as great as that of calculi. High energy shock waves have been reported to be cytotoxic to tumor cells [3–5] but little is known about their anti-tumor effect. To determine whether high energy shock wave could be applied to the treatment of tumors, we investigated its effect on several cancer cell strains.

Materials and methods

Culture cell strains used were urinary bladder tumor cell strain HT-1197, chronic bone-marrow leukemic cell strain K-562 and African green-turtle normal kidney cell strain Vero (Flow Laboratories Inc).

The cells (5×10^5) were suspended in appropriate culture solutions (RPMI 1640 medium containing 10% bovine calf serum for K562 cells and Vero cells and EAGLE-MEM for HT 1197 cells) and 2 ml of each cell-suspension was placed into a polypropylene tube (NUNC Co.) to simulate the passage of high energy shock wave through soft tissues.

Irradiation of high energy shock wave: The high energy shock waves were generated by a shock wave lithotripter (Dornier Co. HM-3). Samples were placed at the focus of the high energy shock wave under roentgenic fluoroscopy and submerged in water at 37°C. High energy shock waves were generated at a voltage of 18 KV and emitted at a rate of 100 times per min. A control tube containing the same concentration of cells was placed in the water bath.

^{3}H-Thymidine incorporation assay: Cell-suspensions (200 μl) were put into 96-well micro-plates. Then 1 μl of ^{3}H-thymidine was added to each well and incubated for 24 hours in a 5% CO_2 humidified incubator at 37°C. Then cells were harvested into glass wool filters with the semiautomatic cell harvester, and after they were allowed to dry for 24 hours, the radioactivity was measured for 1 min by a liquid scintillation counter and expressed as cpm.

Measurement by the two-color flow cytometer using the anti-BrdU antibody [2]: To cell-suspensions, bromodeoxyuridine (BrdU) was added so as to achieve a concentration of 10 mole, and then they were placed in the CO_2 incubator at 37°C for 30 min. After washing with PBS twice, the cells were fixed with 5–10 ml of 70% cold ethanol for 30 min while stirring with a vortex mixer. After the addition of 2 ml of 4H HCl, they were kept for 30 min at room temperature and then neutralized with 1 ml of 0.1 M sodium tetraborate ($Na_2B_4O_7$), pH 8.5. Cells were centrifuged and then suspended in PBS. After the addition of 5 ml of fluorescein isothiocyanate (FITC)-labeled anti-BrdU antibody (Becton Dickinson Co.), they were kept for 30 min at room temperature. Finally cells were washed three times and suspended in 1 ml of PBS containing propidium iodide (PI) (20 μg/ml), and 30 min later they were measured by flow cytometer (FACStar Becton Dickinson Co.)

Electron microscopy: Following shock wave exposure, the cell suspensions were centrifuged at 1,500 rpm for 7 min to form pellets, then fixed for two hour in 2.0% glutaraldehyde at 4°C, and then fixed with 1.5% osmium tetroxide. Graded alcohol dehydration and overnight embedding in 100% propylene oxide and Epon was performed. Sections were examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Results

^{3}H-Thymidine incorporation assay: For HT-1197, the control value was 25,869.5 ± 4,157.6 cpm ($n=6$); up to 200 exposures of the high energy shock wave did not cause any change in the uptake of ^{3}H-thymidine, but 500 exposures reduced the uptake of ^{3}H-thymidine into cells to about half (13,949.2 ± 2,916.5 cpm), and 1,000 exposures significantly inhibited the uptake to 5,916.0 ± 419.0 cpm (Fig. 1).

For K-562, the control value was 12,791.9 ± 135.9 cpm ($n=6$), which was inhibited to 4,610.0 ± 466.0 cpm ($n=6$) by 100 exposures; the value remained unchanged later (Fig. 2).

For Vero, the control value was 10,653.8 ± 587.5 cpm ($n=6$), and was unchanged by up to 500 exposures. One thousand aexposures caused a 50% reduction of ^{3}H-thymidine uptake (Fig. 3).

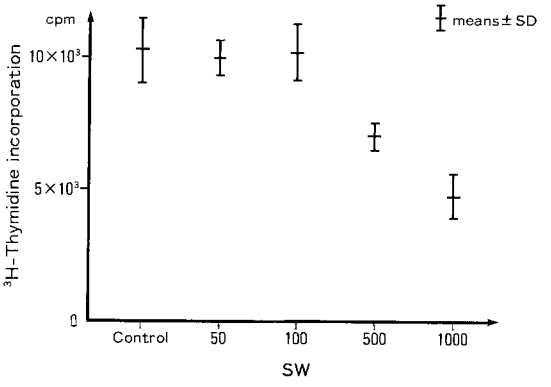

Fig. 3. ^{3}H-thymidine incorporation assay of Vero exposed to high energy shock wave

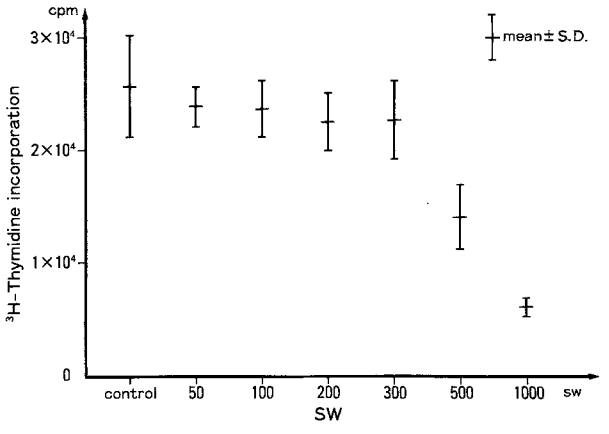

Fig. 1. ^{3}H-thymidine incorporation assay of HT-1197 exposed to high energy shock wave

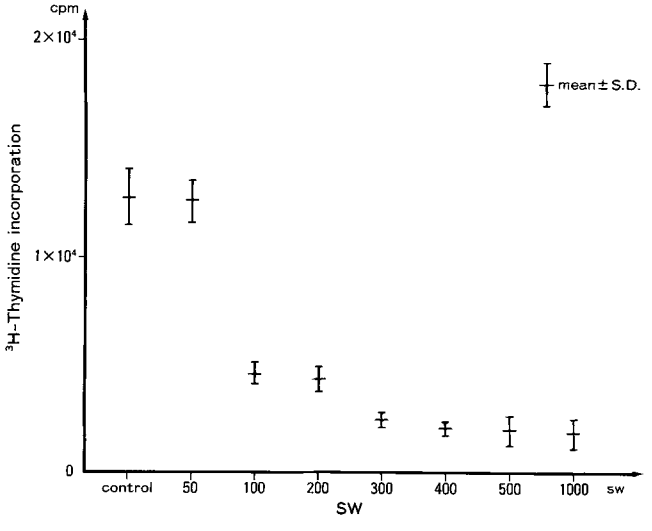

Fig. 2. ^{3}H-thymidine incorporation assay after exposure of K-562 to high energy shock wave

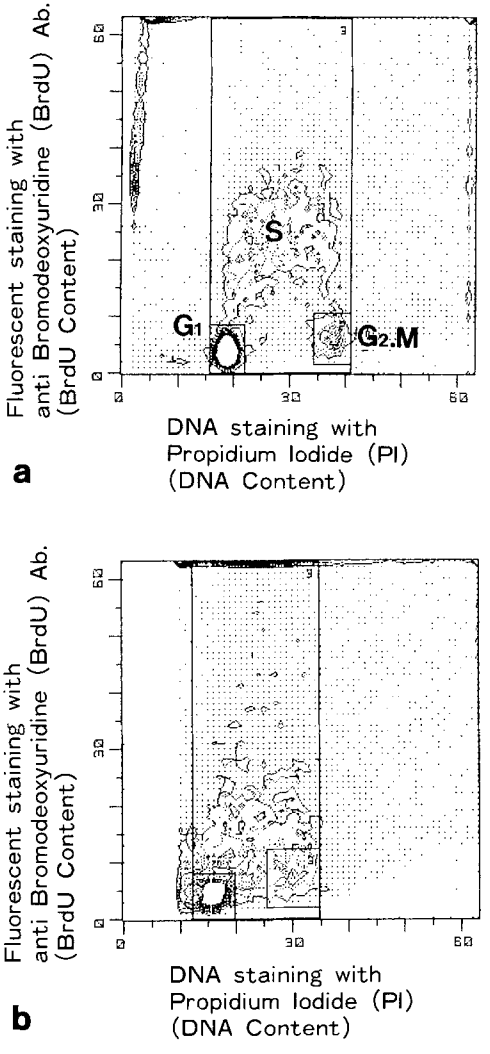

Fig.4 a and b. Two-color flowcytometry using anti BrdU antibody in K-562 cells. **a** control, **b** 100 exposures. The abscissa shows the DNA contenf after staining with propidium iodide, and the ordinate shows the content of the bromodeoxy uridine (BrdU), a derivative of thymidine. The high energy shock wave decreased the content of BrdU in S phase cells.

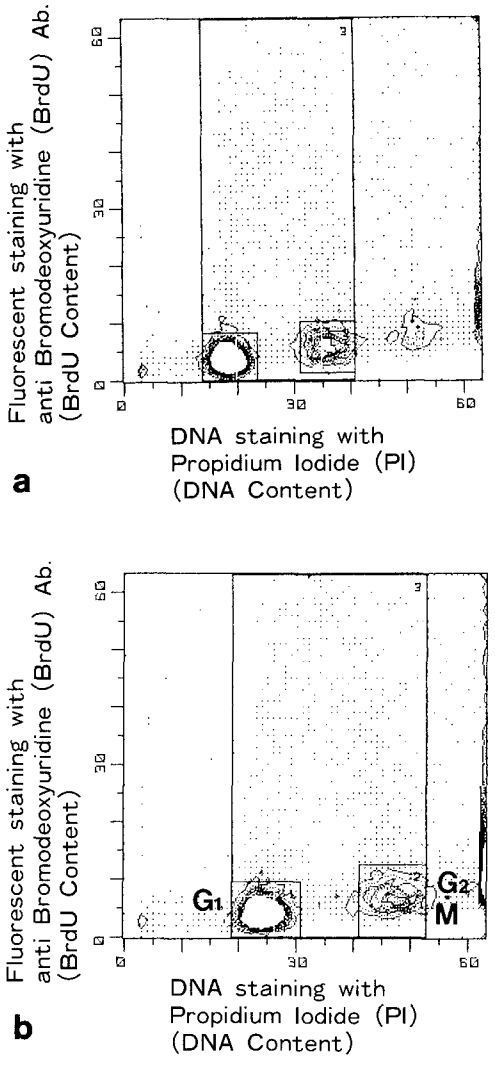

Fig. 5 a and b. Two-color flowcytometry using the anti BrdU in vero cells. a control, b 100 exposures

Measurement by the two-color flow cytometer using the anti-BrdU antibody: In the case of K-562, cells in the S phase of the cell cycle which were seen in the control condition were found to be significantly inhibited by exposure to the high energy shock wave (Fig. 4). In the case of Vero cells, on the other hand, no difference was observed between the control and the shock-wave-exposed groups (Fig. 5).

Observation of cells by electron microscopy: Observation of HT-1197 by electron microscopy demonstrated that numerous microvilli that were observed over the cell surface in the control were significantly reduced and flattened after 500 exposures (Fig. 6). Observation of HT-1197 at a high magnification demonstrated mitochondria clearly observable as a small figure was swollen with its margin indistinct after 500 exposures (Fig. 7). In the case of K-562 as in the case of HT-1197, microvilli were reduced and flattened, and mitochondria were markedly swollen after 150 exposures (Fig. 8). Numerous degenerating cells and an increase in cellular debris and fragments were also observed.

Discussion

In this study, we found that the treatment by means of the high energy shock wave could destroy tumor cells. The anti-tumor effect of the high energy shock wave was found to be more potent for the liquid type (K-562) malignant tumors than for the solid type (HT-1197) malignant tumors from both cell viability and cell structure. So far the anti-tumor effect of high energy shock waves has been found to be proportional to the number of high energy shock wave exposures used [2] as in this study. About 100 exposures were required for the anti-tumor effect to appear for the chronic bone-marrow leukemiccell strain (K-562) and about 500 exposures for the urinary bladder

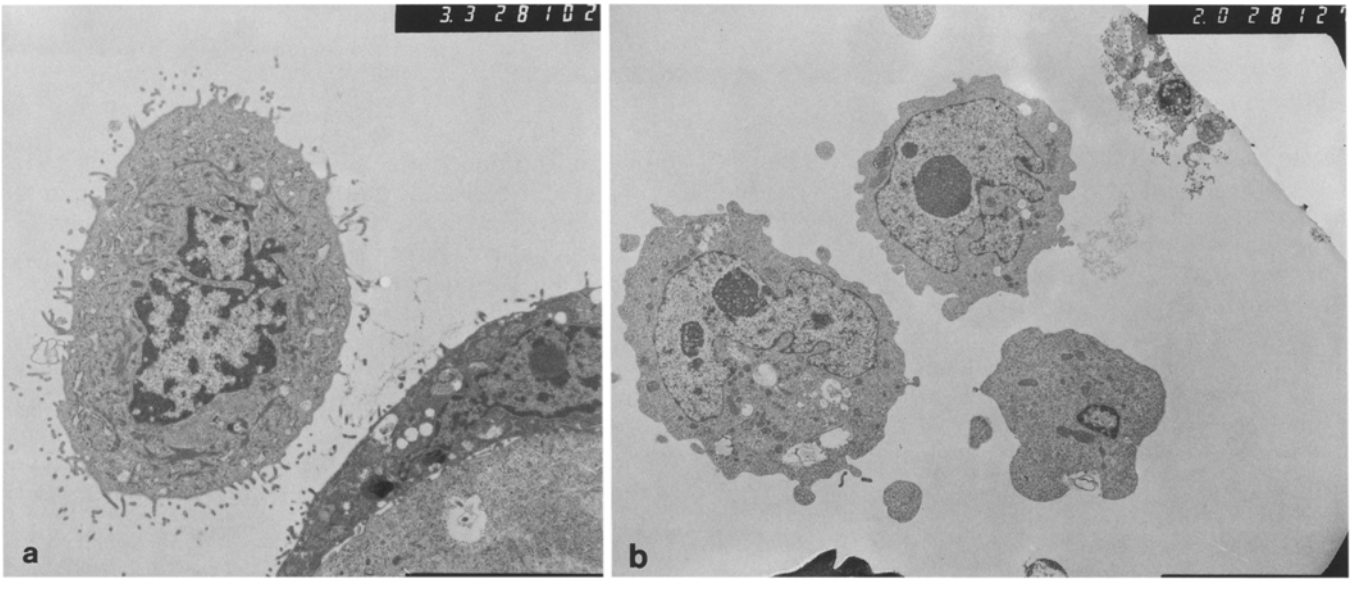

Fig. 6 a and b. Effects of high energy shock wave exposure. Microvilli on the cell surface of HT-1197 were decreased and flattened. a control (×3300), b 500 exposures (×2000)

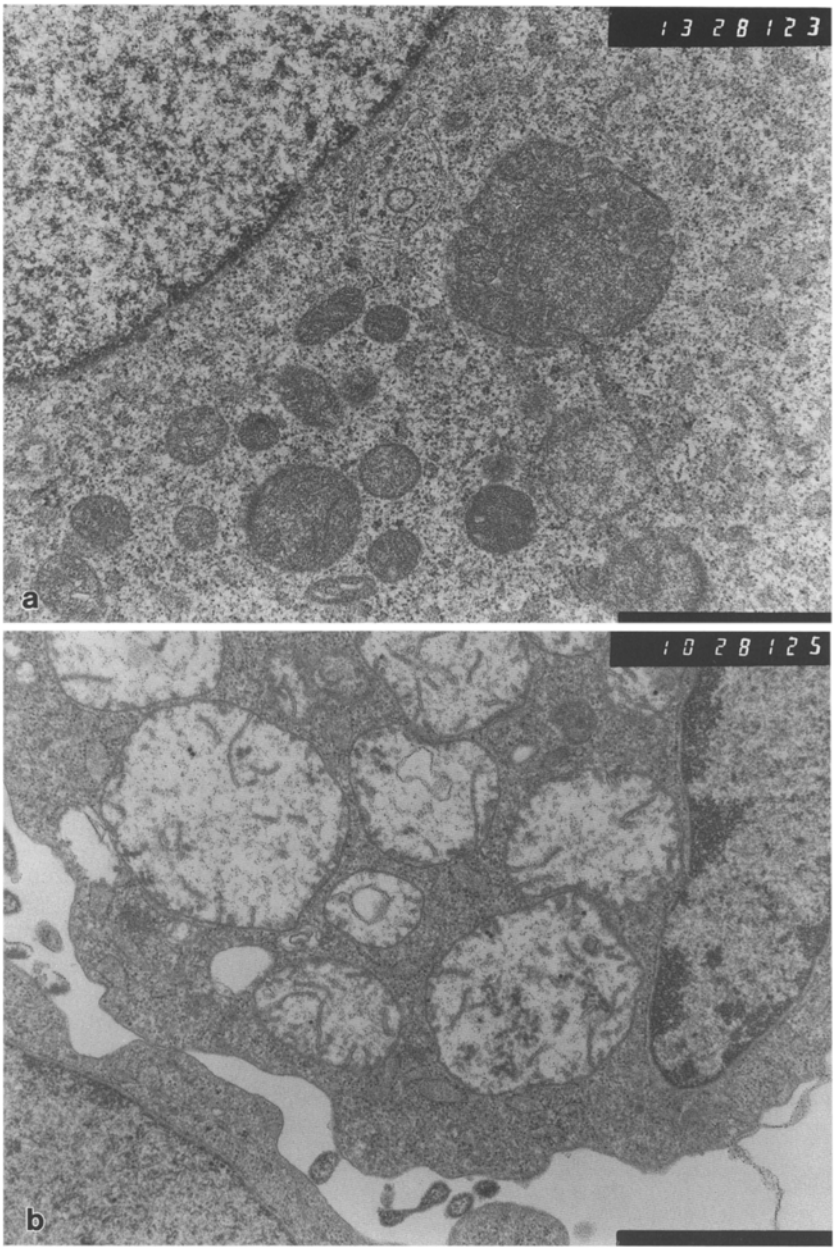

Fig. 7 a and b. Mitochondria in HT-1197 were swollen after high energy shock wave exposure. **a** control (×13,000), **b** 300 exposures (×10,000)

tumor cell strain (HT-1197); these figures were almost consistent with those reported by Russo et al. According to the suggestion made of Russo et al., the high energy shock wave exerts its anti-tumor effect by blocking G_2 and M phases of the cell cycle. In this study using the two-color flowcytometer, the high power shock waves seemed to inhibit mainly S phase of the cell cycle.

Besides, scanning electron microscopy demonstrated that microvilli existing over the cell surface were destroyed by high energy shock wave. Russo et al. demonstrated that pellets formed from centrifuged high energy shock wave exposed cells were distinctly layered with a superficial band of cell fragments and debris overlying a middle zone of viable appearing cells admixed with fragmented cells microscopically. Ultrastructural examination showed high energy shock wave exposed cells contained swollen mitochondria.

The anti-tumor effect of the high energy shock waves observed in this study may have been greater than in the organism, since in the experimental method employed in the study, cells suspended in a culture solution were exposed to the high energy shock waves. It is well known that in the treatment of urolithiasis by the high energy shock waves, calculi are more easily destroyed if they are in contact with water. Tumors in contact with urine as observed in the tumors of the urinary bladder may respond to the high energy shock waves, but some modifications may be necessary in the actual treatment of tumors having a poor contact with water, for instance, tumors of the kidney and the prostate.

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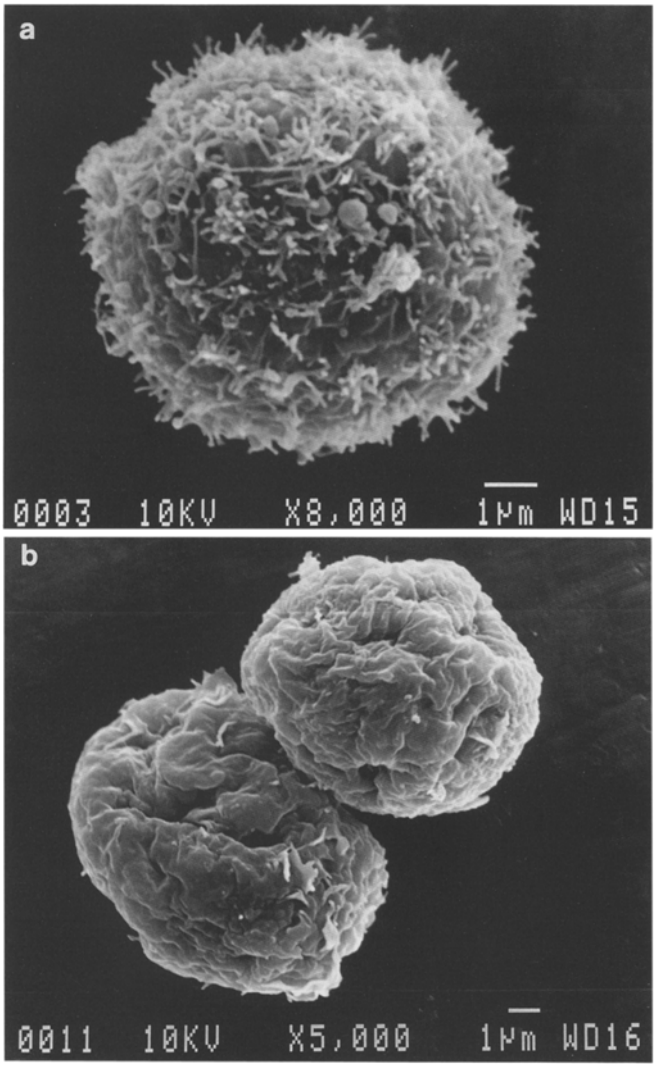

Fig. 8 a and b. Scanning electron microscopic photo. Microvilli of K-562 were destroyed by high energy shock wave exposure. **a** control (×8,000), **b** 200 exposures (×5,000)

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