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The Effect of High Energy Shock Waves (HESW) on Human Bone Marrow

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Summary. The effect of High energy shock waves (HESW) on the viability and proliferation of normal human bone marrow cells was evaluated. A dose dependent increase in cytotoxicity with an increase in the number of HESW was demonstrated. In general 700 HESW immediately reduced the cell viability of bone marrow cells by 50%. The CFU-GM assay provides a method to evaluate the effect of HESW on the proliferative capacity of bone marrow. Following HESW treatment the colony forming ability of trypan blue excluding cells also felt in a dose dependent fashion, but some variation in sensitivity was noted. By comparing the sensitivity of various cells, the cells of normal human bone marrow were felt to be less sensitive to HESW effects than those of other tissue cultured cells or malignant cell lines.

Key words: HESW – High energy shock waves – CHO cells – Chinese hamster ovary cells – CFU-GM – Colony forming units – Granulocyte and macrophage

Introduction

High energy shock waves (HESW) have been utilized to treat patients with renal calculi in many medical centers. The effect of HESW on various normal human cells has still not been evaluated. The purpose of this study was to investigate the cytotoxic effect of HESW on the proliferative capacity of human bone marrow progenitor cells.

Material and Methods

Bone Marrow Aspiration

Five ml of normal human bone marrow was obtained from healthy donors after informed consent and was placed in 20 ml of McCoy's 5A solution and centrifuged at 1,800 rpm for 12 min. Interphase cells which usually contain the hematopoietic precursors were collected between plasma and red blood cells, and then diluted to 5 ml with McCoy's 5A solution. The cell count was determined by a hemocytometer following a 50 fold dilution with 0.2% acetic acid which dissolved the red blood cells. Viability was determined by trypan blue staining. Normally the cell count would range from 25 to 40 x 10^6 cells/ml with viability greater than 90%. The concentration of cells was then adjusted to approximately 10 x 10^6/ml with McCoy's 5A solution. Aliquots (1.5 ml) of the cell suspension were transferred to a 5 ml polypropylene test tube (Falcon) and kept in ice during transportation.

HESW Treatment

A specifically designed test tube holder (constructed by the Medical Physics Department, Memorial Sloan-kettering Cancer Center, New York, N.Y.) fitted directly on the brass ellipse of the lithotripter, supported the test tube in the second focus of the Dornier Lithotripter (HM-3). The tub was then filled with water, and the test tube with cell suspension was submerged in the water bath at 37 °C. The cell suspension was placed in the second focus of the lithotripter as determined by fluoroscopy. The operating voltage was set at 18 kilovolts and 100 HESW/min were delivered to the cell suspension. Different aliquots of the cell suspension received 400, 800 and 1,500 HESW respectively. The control was submerged in a water bath on the edge of the tub for the time equivalent to 1,500 HESW.

In Vitro Assay for Hematopoietic Stem Cells (CFU-GM Assay)

After HESW treatment, the repeat cell count was obtained by diluting each aliquot of cells as above. Granulocyte-macrophage progenitor cells were grown from these cells by a modification of

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Table 1. The effect of HESW on human bone marrow cells: Following the HESW treatment, the number of viable cells were determined by trypan blue stain after dilution with 0.2% acetic acid. Then 2×10^5 viable cells were plated in guadruplicate. Colony formation (i.e. > 50 cells) was counted 10 days after incubation

		Cell count x 10^6 (viability)	%	No. of colonies	%
Case 1 Control		13.3 (92%)	100	33.3 ± 7.4	100
	400 s	12.7 (83%)	86	22.0 ± 5.5	66
	800 s	9.6 (76%)	60	14.8 ± 1.5	44
	1,500 s	4.4 (76%)	27	13.8 ± 3.4	41
Case 2 Control		11.5 (97%)	100	45.3 ± 5.5	100
	400 s	7.9 (90%)	63	40.5 ± 5.4	89
	800 s	5.0 (79%)	34	41.0 ± 3.4	90
	1,500 s	2.6 (78%)	18	32.0 ± 5.2	70
Case 3 Control		11.3 (90%)	100	43.0 ± 6.7	100
	400 s	10.1 (86%)	86	29.8 ± 5.6	69
	800 s	4.9 (88%)	42	27.8 ± 8.9	65
	1,500 s	3.5 (81%)	28	11.5 ± 4.0	27

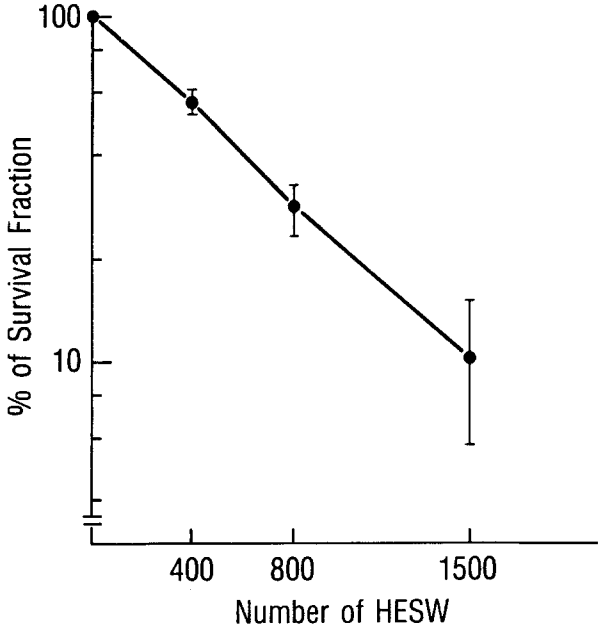

Fig. 1. Survival fraction of normal human bone marrow following HESW treatment expressed as the percentage of control. Survival Fraction = Number of Viable Cells x Number of Colonies

the technique of Pike and Robinson [1, 2]. Following HESW treatment, 2×10^5 viable marrow cells (trypan blue excluded) were plated on medium with a final concentration of 40% McCoy's methylcellulose (MC), 30% McCoy's 5A tissue culture medium (TCM), 10% GCT-condition medium (Gibco, medium obtained from the growth of giant cell tumor), 1% penicillin and 20% of cells in Fetal calf serum. Quadruplicate cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air for ten days. The colonies and clusters were counted under an inverted microscope. All aggregates consisting of more than 50 cells were called colonies, and those of 20–49 cells were scored as clusters.

Results and Discussion

Following HESW treatment the number of viable cells dropped in a dose dependent fashion. The CFU-GM growth of the cells also decreased significantly following 400 HESW treatment in case 1 and case 3, and following 1,500 HESW treatment in case 2 (Table 1). Compared to the untreated control, the calculated survival fraction of 57.7 + 3.1% was obtained for cells exposed to 400 HESW, 27.7 + 4.2% to 800 HESW and only 10.7 + 5.0% noted to 1,500 HESW (Fig. 1).

The cytotoxicity of HESW on various tumor cell lines has been previously evaluated by Russo and associates [3, 4]. But the sensitivity of the HESW on normal human cells has not been previously investigated. The human bone marrow stem cell assay used in this study provided a method to evaluate the effect of HESW on human bone marrow. Our results obtained with bone marrow stem cell culture are similar to the results obtained with tumor cells or Chinese hamster ovary (CHO) cells [3–5], and exhibit a dose dependent cytotoxicity to HESW exposure with both immediate cell killing (as evaluated by trypan blue exclusion) and the ability of the treated cells to form colonies. By comparing the ability of HESW exposed cells to exclude trypan blue and to form colonies in a clonogenic assay, the human bone marrow cells were felt to be less sensitive than those of CHO (Chinese Hamster Ovary), PC-3 (Prostatic Cancer), MCF-7 (Breast Cancer), DU-145 (Prostatic Cancer) and Sk-Mel-28 (Malignant Melanoma) cell lines [3–5]. The reason for the different sensitivity of various cells to HESW is still unknown. While HESW exhibited an effect on human bone marrow cells in an in-vitro assay system and the extrapolation of this results to may potential toxicity to human bone marrow cells in patients receiving extracorporeal shock wave lithotripy is not yet warranted.

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