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High energy shock waves do not affect either primary tumor growth or metastasis of prostate carcinoma, R3327-MatLyLu

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Summary. We investigated the possibility that extracorporeal high energy shock wave (HESW) treatment of solid tumors increased metastatic tumor cell spread using a rapidly metastasising rat prostate tumor variant (R3327-MatLyLu). Volume of HESW treated primary tumor, the volume of metastatic lymph nodes and the number of lung tumor nodules formed were compared with values in untreated control rats. HESW treatment resulted in marked hemorrhage and readily visible hematoma at the focal point of treatment. Tumor histology directly after treatment showed extravascular blood cells due to breakage of blood vessels. Contrary to previous reports we could not observe a delay in tumor growth rate in either small or medium sized tumors. More importantly the extent of metastatic spread was not influenced by HESW treatment.

Key words: High energy shock waves – Prostate tumor – Metastasis – R3327-MatLyLu

Introduction

High energy shock wave (HESW) therapy has become a standard treatment for upper tract renal calculi [1]. In vitro and in vivo studies suggest that it is a safe treatment modality [2, 9]. Renal function appears not to be affected [6]. In addition to physical and possibly thermal effects contributing to tissue damage, the local production of free radicals during treatment has been suggested [8].

Because HESW provide a noninvasive means of delivering focussed energy and causing local tissue destructive effects, a role in the local control of malignancy has been proposed for HESW treatment by Russo et al. [10]. Using Dunning R3327-AT prostatic tumor cells treated both in vitro and in vivo, a delay in tumor cell proliferation and tumor growth was reported. It was reported that it was not possible to find clear histopathologic correlates of this growth delay within tumor tissue, except for hemorrhage and necrosis in surrounding normal tissue [11].

It is known that physical manipulation can accelerate metastatic spread of tumor cells [3]. We have observed that surgical manipulation of the primary tumor increases metastatic growth of prostate tumor R3327-MatLyLu [5]. It is conceivable that the substantial physical force exerted by HESW treatment of tumor could cause a hematogenous liberation of tumor cells and an increase in metastatic spread. The observed hemorrhage following HESW treatment would support this concept [11]. These considerations warrant a critical evaluation of the effects of HESW treatment on metastasis from either a treated solid tumor or from malignant lesions in the immediate vicinity of renal calculi. In the present study we address the question whether HESW affect the rate of metastatic spread using a highly metastatic rat prostate tumor variant (R3327-MatLyLu).

Materials and methods

Animals and tumors

Copenhagen rats, originally obtained from the Mammalian Genetics and Animal Production Section, National Cancer Institute, Bethesda, MD, were bred (brother × sister) and housed in our animal facilities according to institutional animal welfare regulations. In all experiments only male rats older than 90 days were used. The R3327-MatLyLu tumor variant was originally obtained from Dr. J. T. Isaacs (Johns Hopkins School of Medicine, Baltimore, Md) and has been maintained in castrated male Copenhagen rats by trocar transplantation performed under Hypnorm[1] anaesthesia (1 ml/kg, i.m.).

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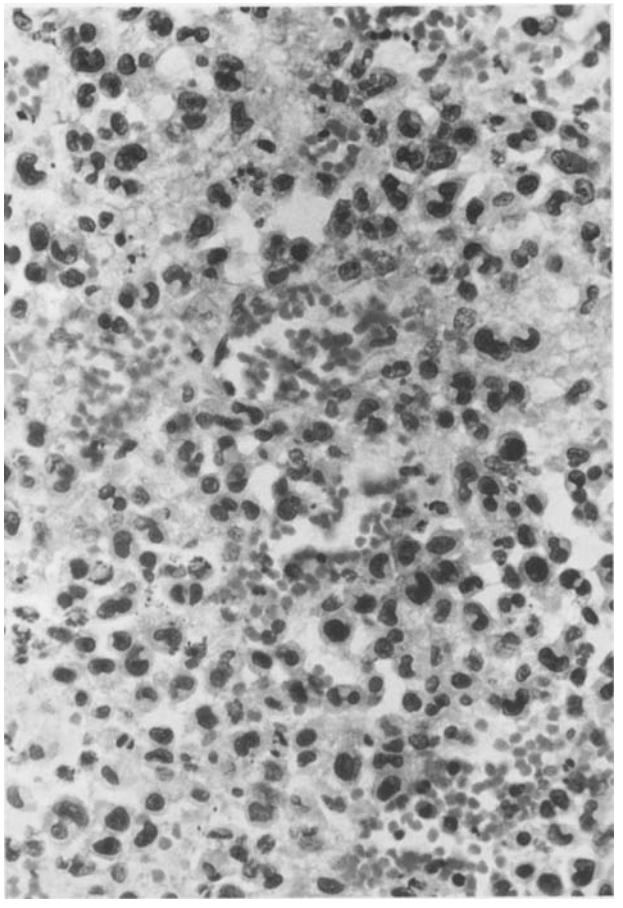

Fig. 1. Photomicrograph of R3327-MatLyLu tumor area exposed to HESW. Note presence of blood cells in and outside blood vessels. Haematoxylin and Eosin staining. Magnification ×430

Experimental setup and analysis

Extracorporeal high energy shock wave (HESW) treatment was performed using a Piezolith 2300 lithotripter (Wolf, Germany), delivering 1,500 pulses at a rate of 100 HESW/min/animal. Maximal pulse pressure delivered at the focal point (effective dimensions of 8×3×3 mm) was 800 bar. The HESW generated in this lithotripter at low intensity was estimated to yield a pulse pressure of about 250 bar at the focal point. Animals were exposed to HESW treatment at high and low rates after local shaving of hair and a further removal of hair remnants using a "hair remover" paste. This procedure facilitated in minimizing air bubble formation near the tumor. Treatment was followed by echoscopy while the animal was under Hypnorm anaesthesia (1 ml/kg; i.m.).

In the initial experiment 12 male animals bearing large tumors (7.48 ± 3.17 cc) were randomly distributed over 3 groups and treated. Apart from a control group (receiving no HESW), a second group received 1,500 pulses at maximal intensity and a third group 1,500 pulses at low intensity. Tumor volume measurement was performed by measuring a tumor with calipers in three mutually orthogonal dimensions while the rat was under light ether anaesthesia. A double skin flap dimension of 1.3 mm was subtracted from each caliper reading. Tumor volume was calculated using the formula lxwxhxΠ/6 [7]. Six days after treatment (day 20 after tumor transplantation) animals were killed, tumor volume measured, extent and volume of lymph node metastases determined and the number of pleural lung metastases counted after immersion of the isolated lungs in Bouin fixative [4]. In a second experiment 8 male rats bearing small tumors (0.8 ± 0.2 cc) were randomly distributed over 2 groups and treated. One control group received no HESW and one treatment group 1,500 pulses at maximal intensity. Eight days after treatment (day 13 after tumor transplantation) animals were killed, changes in body weight recorded, tumor volume measured, extent and volume of lymph node metastases determined and the number of lung metastases counted after the immersion of isolated lungs in Bouin fixative.

Statistical evaluation of numerical data was performed using Student's *t*-test.

Samples of tumor tissue in separately treated animals were taken at 3 hours and on day 6 and 8 after HESW treatment, fixed in phosphate buffered formalin (4%) and processed for histology. Paraffin sections (4 μm) were stained with Haematoxylin and Eosin.

Results

HESW treatment

General effects. Tolerance of tumor HESW treatment in tumor bearing rats was good as observed in different experiments. Body weight 5 days before treatment was 218 ± 17 g (mean ± SD). In HESW treated rats body weight increased on an average by 8.0 ± 5.0 g (determined on the 8 th day after treatment), while in untreated control rats body weight was increased on an average by 9.6 ± 3.6 g in this same period.

There was a clearly visible hemorrhage immediately under the skin overlying the tumor locally on the spot where the pulse front was focussed. This hemorrhage was more pronounced after treatment with high intensity HESW. The hematoma cleared within one or two days. Histology of this tumor area 3 h after treatment showed erythrocytes outside disrupted blood vessels (Fig. 1). Histology of treated tumors at day 6 or 8 after treatment failed to reveal clear signs of treatment induced tissue damage or the presence of erythrocytes due to breakage of capillaries. Necrotic areas present were indistinguishable from necrosis normally present in control tumors, both in extent and in morphologic appearance.

Effects on primary and metastatic tumor growth. In the first experiment 12 male animals bearing large tumors were used either as control or treated with low or high intensity HESW. Six days later primary tumor and axillary lymph node volumes were determined (Table 1). It appeared that in these large tumor bearing rats there were no treatment induced alterations in size either in the primary tumor volume or in the (considerable) metastatic lymph node volume. It was not feasible to determine the exact number of lung metastases in these animals since metastatic tumor nodules had become almost confluent within the lungs. This was

Table 1. HESW treatment (low and high intensity) of large R3327-MatLyLu tumors (tumor size at treatment: 7.48 ± 3.17 cc). Effects on primary and metastatic tumor growth measured 6 days after treatment. Means ± SD are given (n=4)

	Control	Low int. HESW	High int. HESW
Primary tumor volume (cc)	22.9 ± 8.5	22.3 ± 6.0	23.8 ± 5.6
Axillary lymph node volume (cc)	0.61 ± 0.50	0.60 ± 0.41	0.67 ± 0.54

Table 2. HESW treatment (high intensity) of small sized R3327-MatLyLu tumors (tumor size at treatment: 0.8 ± 0.2 cc). Effects on primary and metastatic tumor growth measured 8 days after treatment. Means ± SD are given (n=4).

	Control treated	HESW treated
Tumor volume (cc)	12.6 ± 2.4	14.1 ± 3.3
Axil. lymph node vol. (cc)	0.008 ± 0.011	0.028 ± 0.026
Number of lung nodules	52.7 ± 49.0	28.0 ± 20.7

consistently observed in animals from all three treatment groups.

In a second experiment, 8 male animals bearing small palpable tumors were treated with high intensity HESW or used as controls. Eight days after treatment, primary tumor volume, axillary lymph node volume and number of lung tumor nodules were determined (Table 2). There were no statistically significant differences between these two treatment groups in either primary tumor volume or axillary lymph node volume or in the number of lung tumor nodules (P=0.05).

Discussion

In the present study HESW treatment was applied to a rapidly metastasising rat prostate tumor variant (R3327-MatLyLu). Primary and metastatic tumor growth was found not to be altered by HESW treatment compared to control. HESW has been said to suppress tumor growth in vitro and in vivo [9]. After in vivo treatment with 1,500 HESW a tumor growth delay of approximately 2 days can be expected from these results. However an extremely small tumor burden (diameter 2.5 mm) at the time of treatment was used by these authors, which may make it technically difficult to aim the focussed pulse precisely on the tumor. This is partly reflected by necrosis observed in the normal surrounding tissue by those investigators. Moreover we feel that the use of a minimally established tumor at the time of treatment does not fully reflect an eventual clinical presentation of malignant disease. Furthermore using somewhat larger sized tumors we were able to measure tumor volume changes with more precision. In the present study using both small and larger sized tumors we could not observe any direct effect on the basis of either an enhancement or retardation of tumor growth rate, although a marked hemorrhage in the tumor confirmed the effective delivery of the pulse energy dose in the tumor. Therefore, we conclude that effective in vivo activity of HESW against solid tumors was not conclusively demonstrated. A temporary cessation of nutritional support to the tumors due to blood vessel damage following HESW treatment similar to that observed by us in tumor histology, may also be a cause for the tumor growth delay observed by Russo et al. [9].

Since physical manipulation can increase the rate of metastatic spread [3, 5] and HESW was shown to disrupt the integrity of the walls of small local blood vessels (Fig. 1), an increase in the rate of metastatic spread after HESW was conceivable. However, under the conditions used, no evidence for increased metastasis of either lymphogeneous or of hematogeneous type was obtained in this study using a rapidly metastasising tumor variant. We therefore feel that in HESW application there is no rationale to restrain from treating renal calculi in the immediate vicinity of lesions of possible malignancy. Our present observations indicate that HESW treatment of solid tumors is unlikely to increase the rate of tumor cell spread. It is also clear that HESW will be of little use as a monotherapy in the management of cancer.

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