High-Energy Shock Waves Induce Blood Flow Reduction in Tumors¹

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ABSTRACT

We have studied the effect of extracorporeally applied high-energy shock waves (HESW) on blood flow in amelanotic melanomas (A-Mel-3). Two tumors were implanted in the dorsal skin of 21 Syrian golden hamsters. One of the tumors was treated with 200 HESW, and the other served as an intralndividual control. Mean blood flow in the whole tumor, or a tumor excluding necrotic areas, was quantitatively measured using autoradiography with iodine¹⁴C)antipyrine at 30 min (n = 5), 1 h (n = 5), 3 h (n = 5), and 12 h (n = 6) after HESW treatment. As measured for the whole tumor, blood flow in the controls was 23.4 ± 7.9 ml/100 gm/min (median ± SE) and thus within the range reported in the literature. Thirty min or 1 h after the application of HESW, tumor perfusion was reduced to 6 ± 4% or 5 ± 4% (median ± SE) of the corresponding controls, respectively. Three h after treatment, perfusion increased slightly to 7 ± 5% and after 12 h increased significantly to 55 ± 25% of the corresponding controls. Values measured excluding the necrotic areas were higher in all groups. Temporary reduction of tumor perfusion after treatment with HESW was interpreted as a consequence of HESW-induced damage to tumor microcirculation. These effects should be taken into account for maximizing the therapeutic efficiency of HESW on tumors and for combining HESW treatment with other therapeutical modalities.

INTRODUCTION

Extracorporeally generated HESW³ have become the standard treatment for fragmentation of kidney stones (1) and have been applied for lithotripsy of gallstones (2). This was made possible because shock waves can be focused on targets within the body with a minimized effect on the tissue surrounding those targets.

The use of HESW as a means for nonsurgical, local tumor treatment has also been suggested (3). First investigations have been carried out demonstrating the cytotoxic effects of HESW on tumor cells in vitro (3, 4). Furthermore, treatment of experimental tumors in vivo with HESW has been shown to induce the delay of tumor growth (3, 5, 6). HESW are known to have damaging effects on the vasculature of normal tissues; side effects of shock wave lithotripsy of renal stones or gallstones include edema formation, hemorrhage, and reduction of tissue perfusion (7, 8). Damage to microcirculation has been confirmed by histology, electron microscopy, and intravital microscopy (9, 10).

Similar effects of HESW have been observed on tumor vasculature (11, 12). This is of particular interest if HESW are to be used for tumor therapy. HESW-induced damage of tumor vasculature with subsequent impairment of perfusion would contribute to tumor cell death, in addition to the direct cytotoxic effects of the shock wave itself. Cell death secondary to perfusion defects would depend on their extent and duration. Such mechanisms of action have been elucidated for modalities of tumor therapy like hyperthermia (13) or photodynamic therapy (14, 15). Moreover, perfusion changes after HESW have to be taken into account for therapeutic approaches combining HESW and chemotherapy or other treatment modalities.

The aim of this study was therefore to quantify the extent and duration of perfusion changes in tumors after the application of HESW.

MATERIALS AND METHODS

Animals and Tumors. The experiments were performed in 21 male Syrian golden hamsters (70-80 g) bearing two amelanotic hamster melanomas (A-Mel-3) (16) in the dorsal skin. During tumor implantation, HESW application, and blood flow measurements the animals were anesthetized with pentobarbital (Nembutal; Sanofi-Ceva, Hannover, Germany; 60 mg/kg i.p.). For tumor implantation the dorsal skin was shaved and chemically depilated (Pilcamed; Schwarzkopf GmbH, Lübeck, Germany). About 5 × 10⁷ A-Mel-3 cells were inoculated s.c. at two paravertebral sites (thoracic and lumbar regions) in the dorsal skin. Seven days after implantation tumors had grown to diameters of 7-10 mm, corresponding to volumes of 160-250 mm³ [volumes were calculated as described by Weiss et al. (5)].

HESW Application. HESW were electrohydraulically generated with the Dormier lithotripter model XL1 (Dormier Medizintechnik, Germersing, Germany), as described previously (1). Briefly, an underwater spark discharge produces a radially expanding shock wave which is reflected on a metal semielipsoid (Fig. 1). Thus the shock wave is concentrated at a focal site where maximum pressures of 800 bar are reached within 1 ms. The 50% isoare of the pressure field is currently defined as the shock wave focus; it has a diameter of 5 mm and measures 22 mm in the longitudinal axis (17).

At day 7 of tumor growth the animals were placed into plexiglas tubes which were covered with 2 mm of styrofoam on the inner side to protect the body from the high-pressure field of the shock waves. The tumor-bearing dorsal skin was elevated through a slit in the tube, fixed with three sutures (Opraflex; Lohmann GmbH, Neuwied, Germany) shielding the space between the skin and the tube. This arrangement made it possible to submerge the tumors under water (water bath temperature, ≤36°F) while the rest of the animals remained dry inside the tube.

One of the tumors was randomly chosen for HESW treatment. It was positioned at the shock wave focus, which was localized by two low-energy laser beams intersecting there. Two hundred HESW were applied on the tumor at a fixed discharge voltage of 15 kV, a condenser capacity of 80 nF, and a HESW application frequency of 2.3 Hz. Thus the overall treatment time was 87 s. The second tumor, located at a distance of about 30 mm from the first one, was not exposed to HESW (Fig. 1).

Measurement of Tumor Blood Flow. Tumor blood flow was measured with the autoradiographic tissue equilibration technique developed by Kety (19) and Sakurada (18). Polyethylene catheters (Portex, Ltd., Hythe, Kent, England) were implanted into the right carotid artery (outer diameter, 0.96 mm; inner diameter, 0.58 mm), femoral artery, and superior vena cava (outer diameter, 0.61 mm; inner diameter, 0.28 mm). Forty μCi of the inert, readily diffusible compound IAP (NEN Research Products, Du Pont de Nemours, Dreieich, Germany) were evaporated to dryness and redissolved in 0.5 ml 0.9% NaCl solution. The carotid catheter was cut at a length of 35 mm, and two arterial blood samples of approximately 20 μl were drawn into heparinized glass capillaries. The IAP solution was then injected through the superior vena cava catheter by means of an infusion pump (Harvard Apparatus, Ltd., Kent, England) with a constant flow over 30 s. During the infusion period, further arterial blood samples (approximately 20 μl each) were withdrawn from the freely flowing carotid catheter every 2-3 s. MAP in the femoral artery was registered continuously during the experiment. Exactly 30 s after the start of the IAP infusion both tumors were rapidly resected, immediately frozen in liquid nitrogen, and stored at −70°C.
infusion of IAP, $\lambda$ is the blood tissue partition coefficient of IAP in A-Mel-3 tumors, and $K$ is a parameter which is related to blood flow $F$ as follows:

$$F = K \times \lambda/m$$

where $m$ is a value between 0 and 1 defining the extent to which IAP diffusional equilibrium is established between tissue and blood. We assumed no diffusion barriers for IAP between tumor vessels and interstitial space and therefore chose $m = 1$.

Twenty autoradiograms corresponding to 20 levels of each single tumor were evaluated, and the mean blood flow value of each tumor was calculated. The blood tissue partition coefficient $\lambda$ was determined in separate experiments. Two hamsters, each bearing three tumors in the dorsal skin, were tracheotomized and artificially ventilated with a 70% N2O/30% O2 mixture. Anesthesia was maintained by 1.5% enflurane. Catheters were placed in the right carotic and femoral arteries and superior vena cava. To avoid metabolic degradation of IAP during the time needed for equilibration between blood and tissue, laparotomy was performed and both renal arteries and veins, the hepatic portal vein, and hepatic artery were ligated (18, 20). Spleen, stomach, and the small and large intestine were carefully removed, and the abdominal wall was closed. After 30 min, 40 $\mu$Ci IAP were injected i.v. Twenty-μl arterial blood samples were drawn before and every 15 min following IAP. Ninety min after injection, the last blood samples were drawn, and the tumors were resected and deep frozen. 14C concentrations in blood and tumor tissue were determined as described above. The tissue-blood partition coefficient of IAP was calculated as:

$$\lambda = C_i(T)/C_i(T)$$

**Experimental Protocol.** In hamsters bearing two A-Mel-3 tumors, one of the tumors was randomly chosen for treatment with HESW; the other served as an intraindividual, untreated control. After exposure to HESW the animals were randomly assigned to four groups. Tumor blood flow was measured 30 min after treatment with HESW in the first group ($n = 5$), 1 h after HESW in the second ($n = 5$), after 3 h in the third ($n = 5$), and after 12 h in the fourth group ($n = 6$).

**Statistics.** For each investigated group the median blood flow ± SE was calculated using the mean values of each single tumor. Blood flow values in the control tumors of the different groups or in the different groups of HESW-treated tumors were analyzed for statistical significance using the Kruskal-Wallis test for nonparametric one-way analysis of variance and multiple comparisons on ranks for independent samples (21). Tumors treated with HESW and their corresponding controls were statistically compared with the Wilcoxon matched pairs signed rank test. This test was also used to compare values measured in ROI 1 and 2 of the same tumors (22). The relationship between blood flow in the control tumors and MAP was examined by linear regression and correlation analysis (22). $P < 0.05$ was regarded to be significant.

**RESULTS**

**Tissue-Blood Partition Coefficient of Iodot14Cantipyrine.** Between 60 and 90 min after injection of IAP, its concentration in blood did not change further. It was assumed therefore that an equilibrium had been reached in the IAP distribution between blood and tissue. As assessed by autoradiography, the distribution of IAP within the tumors was homogeneous.

The blood-tissue partition coefficient ($\lambda$) of IAP in the tumors was $0.86 \pm 0.06$ (mean ± SD). $\lambda = 0.86$ was later used for the determination of tumor blood flow in control and HESW-treated tumors.

**MAP during Blood Flow Measurements.** MAP values in the different groups during the injection of IAP are shown in Table I in detail. At the beginning of IAP injection the MAP was 92.5 ± 4.9 mm Hg (median ± SE of all animals). MAP and blood flow of the control tumors correlated significantly. Measurements reflected no significant differences in MAP between the experimental groups. MAP remained unchanged during the injection of IAP and withdrawal of blood samples.
Table 1 Middle arterial blood pressure during injection of IAP (mm Hg)

MAP as measured through a catheter in the femoral artery at the beginning (t = 0 s), during (t = 15 s), and the end (t = 30 s) of IAP injection and release of arterial blood samples. The values are given as medians ± SE in mm Hg. No significant differences between the experimental groups or between the values for different times during IAP injection were measured.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Time during IAP injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t = 0 s</td>
</tr>
<tr>
<td>30 min after HESW (n = 5)</td>
<td>80 ± 7.8</td>
</tr>
<tr>
<td>1 h after HESW (n = 5)</td>
<td>97 ± 17.3</td>
</tr>
<tr>
<td>3 h after HESW (n = 5)</td>
<td>94 ± 11.5</td>
</tr>
<tr>
<td>12 h after HESW (n = 5)</td>
<td>95 ± 12.7</td>
</tr>
<tr>
<td>All together (n = 20)</td>
<td>92.5 ± 4.9</td>
</tr>
</tbody>
</table>

Table 2 Blood flow in control tumors

Blood flow (ml/100 g/min) in control tumors as measured in ROI 1 (whole tumor) and ROI 2 (tumor without necrotic areas). Values are medians ± SE.

<table>
<thead>
<tr>
<th>Time after application of HESW</th>
<th>Blood flow (ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROI 1</td>
<td>ROI 2</td>
</tr>
<tr>
<td>30 min (n = 5)</td>
<td>19.0 ± 15.5</td>
</tr>
<tr>
<td>1 h (n = 5)</td>
<td>23.4 ± 8.9</td>
</tr>
<tr>
<td>3 h (n = 5)</td>
<td>60.0 ± 18.7</td>
</tr>
<tr>
<td>12 h (n = 6)</td>
<td>24.5 ± 15.2</td>
</tr>
<tr>
<td>All control tumors (n = 21)</td>
<td>23.4 ± 7.9</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The objective of this study was to quantify changes of tumor perfusion during the first hours after a single treatment with HESW. We chose the amelanotic hamster melanoma A-Mel-3 (16) for our experiments because previous studies performed on this tumor model in our laboratory had addressed the effects of HESW on tumor microcirculation (12) and tumor growth (5). A-Mel-3 is a rapidly growing and well-vascularized tumor (16, 23). Vascularization occurs between 4 and 10 days after implantation, with necrotic areas appearing on the fourth day.

The experimental Dornier lithotripter XL1 used here is similar to other commercially available Dornier models (like the MPL 9000 or HM3) for disintegration of kidney stones or gallstones in patients. Maximal shock wave pressures of the XL1 are higher than those of the MPL 9000 or HM3 (factor 1.25 or 2.6) (17), but for each stone disintegration up to 10 times more HESW than was used in our experiments are currently applied (24).

The major advantage of the autoradiographic tissue equilibration technique to measure blood flow is its high spatial resolution, which exposed to HESW was significantly reduced at 30 min, 1 h, and 3 h after treatment as well (P < 0.05), whereas in the group 12 h after HESW no significant differences of perfusion were measured between untreated and treated tumors.

**Blood Flow in the Control Tumors.** Blood flow of the untreated control tumors is summarized in Table 2. No statistically significant differences were found between the groups of control tumors. Taking all control tumors together (Fig. 2), 23.4 ± 7.9 ml/100 g/min (median ± SE) were measured in ROI 1, corresponding to the whole tumor, and 32.5 ± 5.6 ml/100 g/min in ROI 2, corresponding to the tumor without necrotic areas. Blood flow in the control tumors and MAP correlated significantly (P < 0.05; Spearman’s correlation coefficient = 0.57). Values measured in ROI 1 were significantly less than those measured in ROI 2 (P < 0.001). Blood flow as measured in ROI 1 ranged between 2 and 80 ml/100 g/min in the different tumors. Within one tumor, maximum and minimum values ranged between 0 and 110 ml/100 g/min if measured in small ROIs including about 1 mm² of a tumor cross-section.

**Blood Flow in the HESW-treated Tumors.** We observed that the tumors became hemorrhagic and edematous even during the application of HESW. Tumor and tumor overlaying skin maintained their macroscopic structure and were not ulcerated after the application of HESW. The following results are given as median ± SE (Table 3).

Treatment with HESW induced a breakdown of tumor perfusion. As measured in ROI 1 (Fig. 3a), tumor blood flow was reduced to 1.7 ± 0.7 ml/100 g/min 30 min after application of HESW and to 1.1 ± 0.9 ml/100 g/min 1 h after HESW. In ROI 1 tumor blood flow 3 h after HESW was slightly increased to 4.1 ± 1.4 ml/100 g/min. Twelve h after exposure to HESW tumor perfusion was 11.5 ± 6.9 ml/100 g/min and thus significantly higher as compared to 30 min, 1 h, and 3 h after treatment (P < 0.01). The following perfusion values were measured in ROI 2 (Fig. 3b). Thirty min and 1 h after exposure to HESW tumor blood flow was 2.7 ± 1.2 and 2.0 ± 1.5 ml/100 g/min, respectively. A significant increase of tumor perfusion (P < 0.05) was assessed 3 h after HESW: 4.0 ± 1.6 ml/100 g/min. Twelve h after treatment tumor perfusion further increased to 24.9 ± 12.8 ml/100 g/min (P < 0.01 versus 30 min and 1 h after HESW). Some tumors had blood flow values exceeding those measured in the corresponding control tumors.

Measurements of tumor perfusion in ROI 1 after treatment with HESW were always significantly lower than perfusion in the corresponding controls (P < 0.05). In ROI 2 blood flow in the tumors...
allows blood flow determination in demarcated tissue volumes (25). Horton et al. (26) compared this method with the microspheres technique and found that both provide comparable perfusion values in the brain. This autoradiographic technique has been applied to measuring blood flow in some brain tumors (27-29) and RT-9 tumors implanted s.c. (30). Recently, Tozer and Morris (31) measured blood flow in LBDS fibrosarcomas implanted s.c. and different tissues of rats with IAP autoradiography and stated that the technique provides "reasonable" values for tumor and normal tissues.

For assessing blood flow in A-mel-3 tumors we took into account the recommendations of Patlak (32) and Williams et al. (33) for accurate measurements: use of a short, freely flowing catheter for the withdrawal of arterial blood, an experimental time of \( T = 30 \) s, and fast removal and freezing of the tissue samples. Theoretically, errors in the estimation of blood flow with IAP have to be expected in tissues under ischemic conditions. Potential error sources are changes in the factors \( \lambda \) (i.e., the blood-tissue partition coefficient of IAP) and \( m \) (i.e., the extent to which IAP diffusional equilibrium is established between tissue and blood) because of modifications in tissue composition or changes in vascular permeability, respectively (34). Marked differences in \( \lambda \) would also lead to a heterogeneous distribution of IAP between perfused and ischemic regions in untreated tumors after 90 min of equilibration time. Since we did not detect any regional heterogeneities in IAP concentration between necrotic and vital regions of untreated tumors in the experiments for determination of \( \lambda \), we exclude major changes of \( \lambda \) in ischemic tissues. Inaccuracy in the determination of \( \lambda \) (10-15\%) would result in small, tolerable errors in the calculation of blood flow (27, 32, 35). Changes in \( m \) during ischemia are more difficult to assess. An increase in the permeability of vessels would not affect the results since this would just shift \( m \) toward unity, whereas \( m = 1 \) had been already assumed. If there is an incomplete mixing of the tracer along vessels with low flow conditions, reduction of the true \( m \) would lead to underestimation of blood flow (34). To date we are not aware of any changes of \( m \) during ischemia.

The blood flow values we measured in control tumors were comparable to those reported for other experimental tumors implanted s.c. (30, 36, 37). Mean blood flow values of the control tumors were found to vary within a wide range. Perfusion was also regionally heterogeneous within each tumor. Both findings are characteristic for tumor blood flow (36, 38). By intravital microscopy, Endrich et al. (23) determined the following total perfusion values for A-Mel-3 tumors: 40.4 and 21.1 ml/100 g/min on the 4th and 12th days after tumor implantation, respectively. These data correspond to our measurements. In control tumors as well as in tumors exposed to HESW the blood flow values assessed in ROI 1 (whole tumor) were significantly different from those of ROI 2 (tumor without necrotic regions) since necrotic areas were in general characterized by low perfusion values. Such relations between histology and blood flow have been described by Tozer and Morris (31), Kuhle et al. (25), and Walenta et al. (39). Measurements in ROI 2 reflect perfusion of the vital tumor regions.

In this study, HESW had been focused on one tumor at a distance of 3 cm from the intraindividual control tumor in the same animal. The possibility cannot be completely excluded, however, that the control tumor and/or the tissue surrounding the control tumor were affected by HESW. The assumption that HESW had no relevant effect on the perfusion of the control tumors is supported by the facts that their blood flow values were in the range as expected for tumors implanted s.c. and that no significant differences in perfusion rates were measured that depended on the time after application of HESW.

Thirty min and 1 h after application of HESW tumor blood flow was significantly reduced to values which were not clearly discernible from the background level. Ischemia was induced in the whole tumor, and maximum blood flow values within one tumor did not exceed 7 ml/100 g/min as measured in small ROIs (1 mm²; data not shown in detail). Reduction of tissue perfusion is a consequence of HESW-induced damage of tumor microcirculation. Some of the effects of HESW on renal and other tissues are hemorrhage, edema, venous thrombosis, and focal necrosis (7, 40). Histological and electron microscopic studies have revealed defects and loss of endothelial cells, rips in capillaries and venular walls with extravasation of red blood cells and leukocytes, and formation of platelet plugs (10). These morphological changes of renal vasculature cause the reduction of renal plasma flow (8), which may become permanent (24, 41). By intravital microscopy of the microcirculation of the dorsal skin of hamsters, arteriolar vasoconstriction, venular hemorrhages, and thrombus formation have been documented following exposure to HESW (9). Similar damaging effects of HESW on tumor microcirculation have to be expected. Indeed, interstitial hemorrhage and vessel damage in tumors after the application of HESW have been described (5, 11, 12, 42).
Three h after treatment a significant increase of blood flow was measured in ROI 2 as compared to the values 30 min or 1 h after HESW. This finding might be interpreted as a beginning of tumor reperfusion. It should be noted, however, that measurements in ROI 2 might overestimate blood flow after HESW. This is due to the fact that we were not able to determine whether the necrotic areas, which are excluded in ROI 2, had increased in size as a consequence of HESW or not. Thus ROI 1 might be more reliable for the analysis of tumor perfusion in the treated tumors. As measured in both ROI 1 and 2, tumor blood flow 12 h after the application of HESW was significantly higher as compared to earlier measurements. Values obtained in ROI 2 indicate no differences in perfusion rates of treated and control tumors beyond 12 h after exposure to HESW, leading to the conclusion that tumor reperfusion had started between 3 and 12 h after treatment. Possible explanations for the reperfusion of the A-Mel-3 tumors are the relaxation of long-lasting vasoconstriction in the supplying arterioles or recanalization of thrombosed vessels. Changes in tumor blood flow after the application of HESW were not dependent upon macrohemodynamic parameters as suggested by MAP, which was the same in all groups. To exclude the influence of systemic effects on blood flow measurements, tumors after the application of HESW were compared to intraindividual control tumors; the breakdown of tumor perfusion and early reperfusion between 3 and 12 h after HESW were confirmed.

Blood flow reduction after HESW is probably one of the main mechanisms leading to the delay of tumor growth. The findings of Oosterhof et al. (6) that HESW are more effective on well vascularized tumors support this statement. Tumor cell death secondary to ischemia plays an important role in therapeutic modalities like hyperthermia (13, 15, 36) or photodynamic therapy (14, 15) and could be essential for tumor therapy with HESW. In addition to focusing the shock waves on the tumor, an increased sensitivity of tumor vasculature to the treatment could constitute a factor enhancing its selective action.

We postulate that repeated applications of HESW in short intervals, i.e., before tumor reperfusion after each exposure occurs, would prolong tumor ischemia and have a more pronounced therapeutic effect. Indeed, the same number of HESW is more effective if applied in many fractionated doses, as shown by Oosterhof et al. (6) and Hoshi et al. (42) or Weiss et al. (5) for A-Mel-3 tumors. However, complete tumor remission after repeated applications of HESW has not been achieved yet. Since the extent of perfusion defects and the time needed for reperfusion had not been considered in those studies we suppose that the intervals chosen between the exposures to HESW (12, 24, or 48 h) had been too long.

The effects of HESW on tumor blood flow must also be taken into account for combined treatment with other agents like chemotherapeutics. Several studies have demonstrated additive and/or synergistic effects of HESW and chemotherapeutic agents or biological response modifiers (43–45). According to our results, the chemotherapeutic agent must be given prior to the application of HESW to make possible its intravascular transport into the tumor. On the other hand, if HESW are applied after the chemotherapeutic agent has accumulated in the tumor the blood flow reduction induced would contribute to a slower washout of the agent. Based on our knowledge, only agents that are active under ischemic conditions should be considered.

We conclude that HESW have significant effects on tumor perfusion which most probably determine their therapeutic efficiency. Perfusion changes should be taken into account to optimize tumor therapy with HESW and/or the combined treatment with HESW and other therapeutic strategies.
TUMOR PERFUSION AFTER SHOCK WAVES


High-Energy Shock Waves Induce Blood Flow Reduction in Tumors


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