Effects of high-energy shock waves combined with biological response modifiers or Adriamycin on a human kidney cancer xenograft

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Summary. We have studied the effect of high-energy shock waves (HESW) alone or in combination with biological response modifiers (BRMs) or Adriamycin on the growth of the NU-1 human kidney cancer xenograft. When HESW are administered repeatedly (four sessions of 800 shock waves on days 0, 2, 4 and 6) a prolonged delay in tumor growth was found compared with that following a single administration. This effect was temporary, and several days after stopping the HESW administration the tumor regained its original growth potential (same doubling time). Tumor growth was suppressed for a longer period by the combination of 4 sessions of HESW and a single administration of Adriamycin, 5 mg/kg. Combination of HESW treatment with interferon alpha (5.0 ng/g body weight, three times/week) and tumor necrosis factor alpha (500 ng/g body weight, 5 days/week) s.c. around the tumor resulted in a complete cessation of tumor growth. While Adriamycin had an additive effect on HESW treatment, the combination with BRMs was highly synergistic.

Key words: High-energy shock waves – In vivo cytotoxicity – Biological response modifiers – Adriamycin

Electromagnetically generated high-energy shock waves (HESW) can alter the growth characteristics of tumor cells in vitro [8]. Furthermore, HESW can provoke suppression of tumor growth in vivo [10-13]. The tumor growth suppression observed in vivo is temporary and results rather in an elongated lag-phase than in a permanent effect. This clearly indicates that HESW is not likely to be useful as monotherapy. The number of shock wave sessions, the number of shock waves per session and the role of tumor volume at start of treatment appeared to be relevant in determining the antiproliferative effect of HESW in vivo [9]. Also, the effects depend on the tumor line used, which may be related to doubling time and vascularization of the tumor [9].

Alternatively, HESW exposure can potentiate the effect of cytotoxic drugs [3, 6, 9, 10]. Our studies clearly revealed the enhancement of cytotoxicity by vinblastine or Adriamycin after shock wave exposure [8, 9].

Biological response modifiers (BRMs) are known to have an antiproliferative effect on human renal cancer xenografts [2]. We now studied the combination of HESW and Adriamycin or BRMs (Interferon alpha + tumor necrosis factor alpha) on the NU-1 human kidney cancer xenograft to establish possible additive or synergistic effects on tumor growth of this combination therapy.

For the in vivo studies the (commercially available) Siemens lithotriptor (Lithostar) was used. The pressure profile of the electromagnetically generated shock waves differs from that of electrohydraulically generated HESW and has been described elsewhere [8].

Materials and methods

Animals

Xenografts were transplanted in 6-week-old male Balb C nu/nu (Bornholt Gárt, Rye, Denmark). The mice were kept in groups of five in PAG type 2 cages (IFFA Credo) covered with an iso-cap for sterile conditions. The mice were fed ad libitum with irradiated SRM food (Hope Farms, Woerden, The Netherlands), and drinking water was acidified with 0.7 ml concentrated HCl/ml.

Tumors

The human renal cell carcinoma NU-1 xenograft was used. This tumor was established in our laboratory by serial s.c. transplantation of tumor pieces after original s.c. transplantation of small primary tumor pieces directly after nephrectomy. The tumors were transplanted s.c. as trocar pieces in the hind limb of each animal under ether anesthesia. This human renal adenocarcinoma is well vascularized and grows with a doubling time of 3–4 days. Histologically the tumor is characterized as sarcomatous and shows spontaneous hemorrhagic necrosis (Fig. 1A).
Fig. 1. A NU-1 human kidney adenocarcinoma. H&E, 40X. B NU-1 kidney tumor after exposure to four sessions of 800 HESW: vasodilatation, hemorrhage, some neutrophilic migration and pyknotic tumor cells. H&E, 40X. C, D NU-1 kidney tumor after exposure to four sessions of 800 HESW combined with BRM treatment. Pronounced necrosis, neutrophilic migration and infiltration, fibrin clots within the dilated capillaries. H&E, 40X

**HESW treatment**

The shock waves were generated electromagnetically by the Lithostar (Siemens). The experimental set-up and way of administration of the shock waves have been described in detail elsewhere [8]. For HESW exposure the animals were placed in a water-filled container and kept fixed in position by means of a plastic cocoon.

With in vitro pressure measurements, using a piezo-electric crystal transducer (Imotec) connected with an oscilloscope (Gould, DSO, 4072), the focal area was determined (area limited by pressures which are half the maximum pressure (P_max 50)). It appeared that there is a marked pressure fall in the lateral plane, indicating the importance of immobilizing the animal and positioning the tumor precisely [8, 14]. The diameter of the focal area in the lateral plane is only 6 mm, e.g. the pressure in the center of the focal area is 3.75 MPa, at the edge 24 MPa and 2 mm outside the focal area 7.5 MPa (18.4 kV).

After the desired tumor volume (60–70 mm³) was reached, the tumor-bearing animals were randomly divided into a sham-treated control group, a group receiving shock waves, a group receiving BRMs or Adriamycin, and a group receiving both treatment modalities. Each group consisted of 6 or more animals.

The nude mice were anesthetized with ketamine hydrochloride (Ketalar, Parke-Davis) 150 mg/kg and received two, three or four sessions of 800 shock waves (18.4 kV) on days 0, 2, 4 and 6. As BRM we used interferon alpha (IFN alpha) and tumor necrosis factor alpha (TNF alpha). IFN alpha was given three times a week at a dose of 5.0 ng/g body weight and TNF-alpha, five times a week at 500 ng/g body weight, s.c. around the tumor during the study period. Adriamycin (Adriablastine, Pharmitalia) 5 mg/kg was administered once on day 0, i.p., just before the first HESW exposure.

**Biological response modifiers**

Human IFN alpha and TNF alpha, kindly supplied by Boehringer Ingelheim, Alkmaar, The Netherlands, were produced in *Escherichia coli* by recombinant DNA technology. The specific activity of IFN alpha was 3.2 × 10^8 units/mg protein. It was measured by inhibition of encephalomyocarditis (EMC) virus replication in A549 cells with reference to the National Institute of Health (NIH) leukocyte IFN alpha standard Go 23-901-527. The purity of IFN was >98% as determined by SDS polyacrylamide gel electrophoresis and the amount of endotoxin was less than 1.0 ng/mg protein for IFN alpha based on the limulus amebocyte lysate assay. The specific activity of TNF alpha determined in the presence of actinomycin-D was 6 × 10^7 units/mg protein (L-929 cytotoxicity assay). The purity was >99% as determined by SDS-polyacrylamide gel electrophoresis, and it contained 1.0 ng or less endotoxin/mg protein based on the limulus amebocyte lysate assay. The drugs were dissolved in the accessory dissolvent and diluted with unsupplemented RPMI medium (Gibco, Paisley, UK). After dilution, the drugs were stored in small aliquots at −80°C until use.

**Evaluation of tumor growth**

Tumor volume was determined in vivo every other day by using a precision sliding caliper to measure the three dimensions, i.e. maximum diameter (L) and the perpendicular diameters (W, H); the volume was expressed as the tumor size index (TSI) calculated from the equation L × W × H/0.52. In this way, tumor growth patterns were evaluated by calculating the mean volume of the tumors in each group.

**Statistical analysis**

Per animal, loglinear regression over the first 2 weeks was used to estimate the growth rate (α), i.e. the number of times the tumor volume doubles per day (the doubling time equals 1/α). Treatment versus control differences in growth rate were then analyzed by two-sided t-tests.

For combined treatments, two treatments were considered partly additive if the decrease in growth rate resulting from the combined treatment was larger than the decreases resulting from either of the treatments alone. They were considered synergistic if the decrease...