Original Article

Effects of Tandem Shock Waves Combined with Photosan and Cytostatics on the Growth of Tumours

(electrical discharges in water / focused shock waves / cavitations / tandem shock waves / tumour growth)

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Abstract. Shock waves, pressure waves manifested as a sharp increase in positive pressure followed by a decrease and the negative part of the wave, are not only used to treat concrements in medicine. Recently, research has been focused on the possibility of their use for damaging the tumour tissue. In contrast to concrements, which are different from the surrounding tissue by their acoustic impedance, the tumour tissue has the same acoustic impedance as the surrounding soft tissue. Therefore, we have developed a new source of shock waves, which is based on the principle of multichannel discharge. This new source generates two successive shock waves (tandem shock waves). The first shock creates acoustic non-homogeneity and cavitations in the tissue, and the second shock is damped in it. In this work we demonstrated the effect of tandem shock waves on the muscle tissue in depth. The damage is shown on the images from the magnetic resonance imaging and histological sections. In the further part of the experiment, we investigated the *in vivo* effects of tandem shock waves in combination with Photosan and cisplatin on the tumour tissue. The application of tandem shock waves resulted in the inhibition of tumour growth, compared with controls, in both parts of the experiment. The largest inhibition effect was observed in the groups of tandem shock waves combined with Photosan and in the second part with cisplatin.

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Introduction

Shock waves have been used in medicine for as long as 20 years (Chaussy et al., 1982). They represent microsecond pressure surges. There is first a sudden pressure jump (up to values about 100 MPa), which is followed by a smaller negative wave (about 10 MPa) (Delius, 2002; Shrivastava and Kailash, 2005). The shock wave is today frequently being used for the disintegration of renal or hepatic concrements with the help of a lithotripsy procedure, where the shock wave is generated beyond the patient's body and concentrated with the help of a reflector into the focus in which the concrement is situated (Beneš et al., 1987, 1989; Beneš, 2000).

Excellent results in the field of the concrement disintegration initiated discussion about further use of shock waves in medicine. For example, they have rather long been used in orthopaedics for the treatment of syndromes involving insertions. However, the greatest interest is being paid to a possible use for damaging tumour tissues. In contrast to concrements, which are different from the surrounding tissue by their acoustic impedance, the tumour tissue has the same acoustic impedance as the surrounding soft tissue. Thus, another approach should be selected, since common generators employed for the disintegration of concrements are designed in such a way that they do not cause clinically important damage to cells (Coleman and Saunders, 1993; Wilbert 2002). Due to that, in our experiments we use a new generator of shock waves developed at the Institute of Plasma Physics of the Czech Academy of Sciences. It is based on the principle of a multichannel discharge, which occurs at a composite electrode. The electrode is situated in strongly conductive water and after a pulsed voltage application, numerous discharge channels are produced, which are distributed throughout the electrode surface (Šunka and Babicky, 1997, 2002; Šunka et al., 2004; Stelmashuk and Šunka, 2006). If we want to achieve the effect in an acoustically homogene-

ous medium, it is necessary to use either cavitation effects or two shock waves generated one after another in a short time interval, where the first wave forms an acoustic non-homogeneity and the second one is damped in it (Beneš et al., 1997). In our work, we employ two subsequently generated shock waves, called tandem shock waves (Loske et al., 2002). The new generator was formerly shown to cause haemolysis of erythrocytes and also necrosis of tumour cell lines (Beneš et al., 2001, 2007). *In vivo* effects were demonstrated in rats whose liver tissue was exposed to shock waves. In the work presented here we intended to demonstrate damage to deeper structures without involving surface tissue and structures through which the shock wave passes. The shock wave was focused on a region in the rabbit femoral muscle. Magnetic resonance was used to image the effects. In a further part of the experiment, we investigated *in vivo* effects of the shock wave on B-lymphoma and syngeneic sarcoma tissue. The tumour growth over time was also followed and compared with the effects of Photosan and cytostatics.

Material and Methods

The shock waves were generated by a new source, which was based on the multichannel discharge principle. For a scheme of the generator see Fig. 1. The cylindrical composite anode consists of two parts (A1 - ∅ 60 × 70 mm, A2 - ∅ 77 × 25 mm). Each part of the anode is separately supplied, which makes it possible to switch the two parts with a certain time delay. If they are switched on simultaneously, the shock wave from the part with the larger focus diameter is obtained 5 ms before that from the part having the smaller diameter. The electrode itself is made of stainless steel and coated with a thin layer (d = 0.2–0.3 mm) of porous ceramics produced by plasma spraying. The whole equipment for the shock wave application is divided into two parts, which are separated one from another by an acoustically transparent membrane. The first part is filled with a very conductive salt solution (with specific conductivity of 5–20 mS/cm), in which the composite anode is situated coaxially with the parabolic reflector forming the cathode. The focal point at which the shock waves are aimed is present in the second part of the equipment. The second part is filled with degassed water. The design of the composite anode makes it possible to simultaneously generate many discharge channels, which are homogeneously distributed on the anode. Every discharge channel forms a quasi-spherical pressure wave. A cylindrical pressure wave is obtained by superimposition of these particular waves. The pressure wave is reflected from the reflector to the focal point and is transformed to the shock wave close to it.

Laboratory rabbit, body weight of 3,100 g, was selected as the experimental model. The rabbit was totally

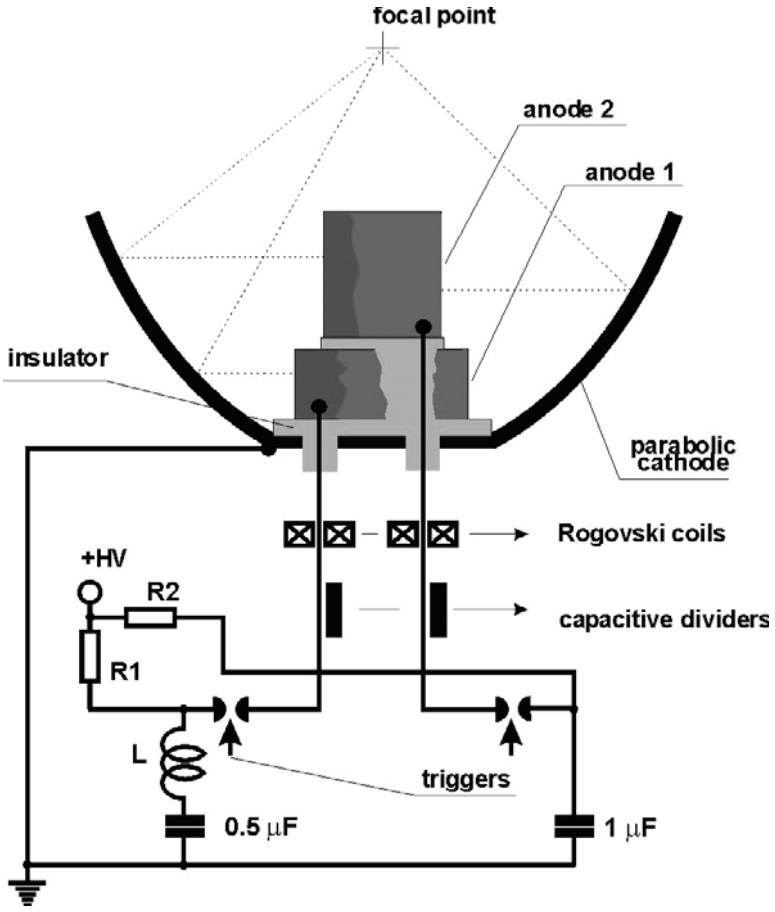

Fig. 1. Shock wave source

anaesthesized and depilated on the hind limb. The animal was then situated into the experimental equipment to have its femoral muscle in the shock wave focus. In total 1,800 tandem shock waves were applied.

MR scanning was provided in the MR tomograph Siemens Magnetom Trio 3 T (Siemens AG, Erlangen, Germany) at the Institute of Clinical and Experimental Medicine. TE and TR times were set as follows: TE = 20 ms and TR = 642 ms. The layer thickness was set to 2 mm. The imaging procedure, in the course of which the rabbit was under anaesthesia, was carried out on the first, third and seventh days after the exposure to shock waves. On the seventh day after the exposure, the animal was dissected and samples for histology were taken.

In the second part of the experiment, we used experimental outbred SD/Cub strain rats. Their body weight ranged between 200 and 220 g. They were administered with T-lymphoma tumour cells in a number of $1*10^6$ per rat in the abdominal region. Tumours started growing in all the animals. The rats were divided into the following groups; each group included six animals.

1) The first group of rats were exposed to shock waves only.
2) The second group of rats were administered intravenously with Photosan (391C 358, Seehof Laboratorium GmbH, Wesselburen, Germany) in the vena caudalis at a dose of 25 mg/kg. After 48 h, the animals were exposed to shock waves.
3) The third group of rats included controls.

The shock wave application was carried out in such a way that the rats were first anaesthetized. The tumour tissue was then situated in the focus of shock waves and each animal was exposed to 1,200 tandem shock waves. The tumour volume was measured on the 3rd, 7th, 10th, 14th, 17th and 21st days.

In the third part of the experiment, Lewis strain experimental rats were used. Their body weight ranged between 200 and 250 g. Tumour cells of the syngeneic sarcoma were intradermally administered caudally on the right and left sides. The rats were subsequently divided into groups. Each group included 10 animals.

1) The first group of rats were exposed to the effects of shock waves.
2) The second group of the animals were administered with cisplatin (50 mg MO1030AC, Medac, Hamburg, Germany) at a dose of 5 mg/kg before the shock wave application. After that they were exposed to the shock waves.
3) The third group of the animals were administered with cisplatin at a dose of 5 mg/kg only.

The contralateral tumour served as a control. The method of the shock wave application was the same as in the preceding part with the difference that 120 shocks were applied at a certain angle and 120 shocks were applied at a different angle.

Results

Fig. 2 and Fig. 3 show an MR image of the rabbit femoral muscle in two different sections, transversal and frontal, through the same site. The femoral muscle was selected to make possible a comparison with the femoral muscle on the second limb, which served as a control. The femoral muscle exposed to the shock waves is shown on the right side of the figures. We can see damage to the muscle (the arrows). In the focus of the shock waves a haematoma was developed surrounded by perceptible oedema. The control limb was not damaged. On the third and seventh days, we repeated the imaging procedure and the damage to the muscle tissue

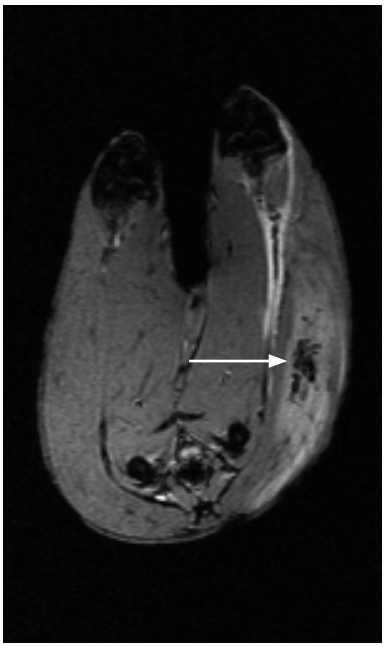

Fig. 2. MR image one day after shock wave exposure, transversal section

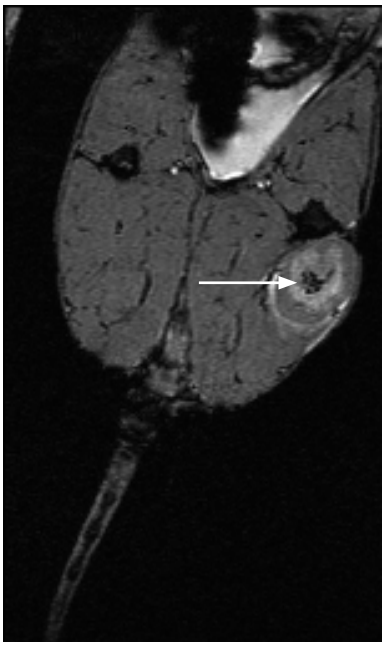

Fig. 3. MR image one day after shock wave exposure, frontal section

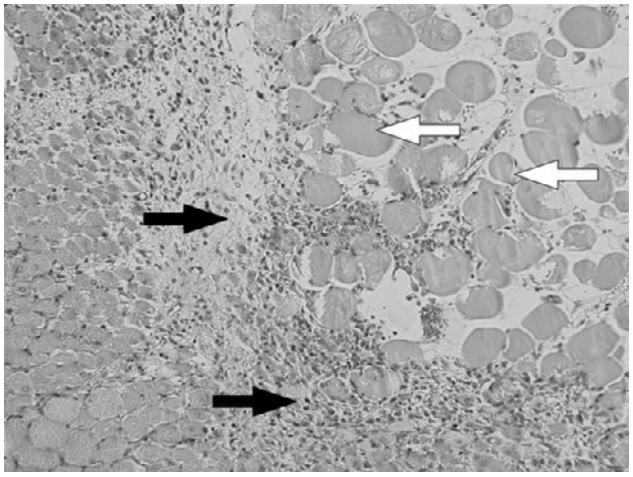

Fig. 4. Histology of damaged femoral muscle. Magnified 200×, stained with haematoxylin-eosin

was still visible. The damage was also obvious in a subsequently performed dissection.

Fig. 4 shows a histological section of the damaged femoral muscle with an extensive focus of granulation tissue (black) in the field of subacute dystrophic changes in muscle fibres (white).

In the second part of the experiment, the tumour volume was followed after 1,200 shock waves applied at the intervals described. The volume was already measured before the exposure and on the 3rd, 7th, 10th, 14th, 17th and 21st days after the exposure. The results measured were recorded. Fig. 5 shows average values of the tumour volume on particular days of the measurement. Colour differentiation was used for the three experimental groups.

In rats of this group, the exposure to shock waves delayed the tumour growth compared to controls. In the experimental groups of rats first administered with Photosan and exposed to shock waves 48 h after that, there was a higher inhibiting effect compared to the group exposed to shock waves only.

The highest number of animals were used in the third part of the experiment. Tumour volumes were followed and measured on the 1st, 4th, 7th and 11th days after the shock wave exposure. Average values and standard errors were calculated. Fig. 6 shows particular groups with the use of colour differentiation and the table summarizes the average values of tumour volume in percent of the baseline volume including the standard error.

In all the groups, the tumour growth was delayed compared to controls. The largest effect was observed in the group of cisplatin combined with tandem shock waves. The cisplatin administration alone had a rather lower inhibiting effect compared to the action of the tandem shock wave alone.

Discussion

The results of the first experiment indicate that the tandem shock wave is able to cause local damage inside the tissue even in an acoustically homogeneous muscle medium. This is in agreement with the damage that was described previously (Beneš et al., 2007). The damage is produced at the site of the shock wave focus, and the superficial structures through which the shock wave passes remain intact. The damage is formed in such a way that the first wave produces non-homogeneity in the acoustically homogeneous muscle tissue medium and the second one is subsequently absorbed in it.

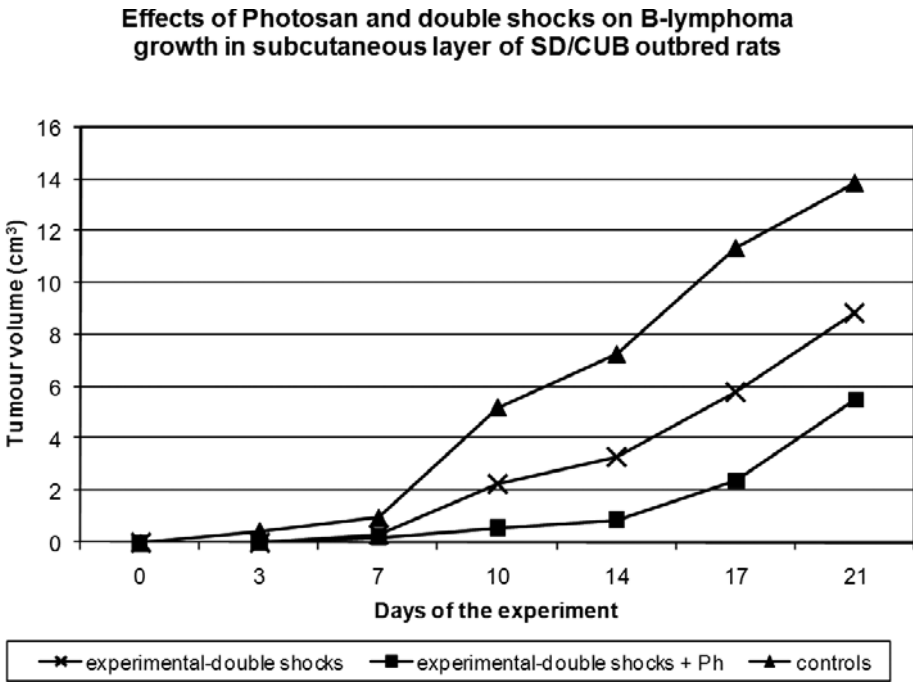

Fig. 5. Tumour volumes on particular days

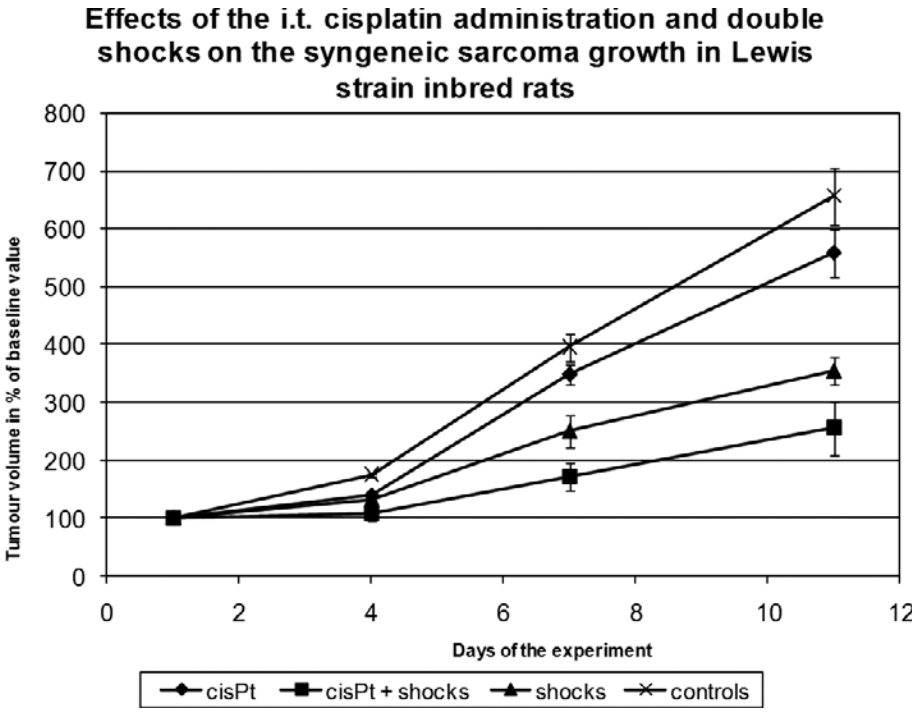

Fig. 6. Tumour volumes on particular days

The results of the second part of the experiment suggest that exposure of growing tumour (T-lymphoma) to shock waves delays tumour growth compared to controls. The delay of the tumour growth is significantly increased by administration of Photosan before the shock wave application. Photosan is a substance which is effective in photodynamic therapy, and it should be elucidated whether substances exerting effects in photodynamic therapy may also be sensitive to cavitations.

The third part is of importance not only because their result demonstrates an effect at least comparable with cisplatin, but particularly by the number of tumour types investigated.

The results of the third part indicate that the tandem shock wave is able to damage the tumour tissue and thus delay the tumour growth. An even higher effect is achieved in combination with cisplatin. The approach based on the application of these shock waves is still at the experimental stage only, but this is the only currently known type of energy that can be concentrated in a small volume without damaging the surrounding tissues, and the question remains whether the toxic effects of tandem shock waves occur in the focus, or molecules are activated by cavitations, or micelles containing the cytostatic are involved.

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