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In vivo effects of focused shock waves on tumor tissue visualized by fluorescence staining techniques

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ABSTRACT

Shock waves can cause significant cytotoxic effects in tumor cells and tissues both in vitro and in vivo. However, understanding the mechanisms of shock wave interaction with tissues is limited. We have studied in vivo effects of focused shock waves induced in the syngeneic sarcoma tumor model using the TUNEL assay, immunohistochemical detection of caspase-3 and hematoxylin–eosin staining. Shock waves were produced by a multichannel pulsed-electrohydraulic discharge generator with a cylindrical ceramic-coated electrode. In tumors treated with shock waves, a large area of damaged tissue was detected which was clearly differentiated from intact tissue. Localization and a cone-shaped region of tissue damage visualized by TUNEL reaction apparently correlated with the conical shape and direction of shock wave propagation determined by high-speed shadowgraphy. A strong TUNEL reaction of nuclei and nucleus fragments in tissue exposed to shock waves suggested apoptosis in this destroyed tumor area. However, specificity of the TUNEL technique to apoptotic cells is ambiguous and other apoptotic markers (caspase-3) that we used in our study did not confirmed this observation. Thus, the generated fragments of nuclei gave rise to a false TUNEL reaction not associated with apoptosis. Mechanical stress from high overpressure shock wave was likely the dominant pathway of tumor damage.

1. Introduction

Shock waves have been used in medicine for many years, particularly in extracorporeal lithotripsy (ESWL), which uses focused shock waves to non-invasively treat patients with stone diseases (mostly, urinary stones) [1–3]. The treatment involves focusing shock waves generated by the ESWL device (lithotripter) outside of the patient's body to disintegrate the stone at a depth in tissue. In electrohydraulic lithotripters – the most common clinical type – an underwater high-current spark discharge between a pair of electrodes is generated at the focus of the ellipsoidal reflector, and the emerging spherical shock wave produced by the plasma at the spark gap is concentrated on the kidney stone located at the second focus of the ellipsoid. After this urinary stone treatment, the stone debris passes through the urinary tract. The success of the ESWL stimulated research on the applications of focused shock waves (FSWs) in other branches of medicine. Lithotripter-generated shock waves have been applied to treatment of cells and soft tissues of various cancers both in vitro and in vivo; however, only with a limited degree of success [3–5]. In vitro studies have demonstrated that ESWL can cause various types of cell damage including fragmentation of cells [6–9]. However, in vivo treatment of tumors by lithotripter-induced shock waves has been shown to be ineffective in inhibiting tumor growth [9–12]. It has been found that shock wave-induced cavitation plays an important role in the cell damage [13–16]. The collapse time of ESWL-induced cavitation bubbles was found to be significantly reduced going from in vitro to in vivo conditions, suggesting that in vivo bubble expansion may be severely constrained by the surrounding tissue and may explain why ESWL effects are significantly lower in vivo than in vitro [17,18]. Therefore, controlling the formation and subsequent oscillations of cavitation bubbles seems to be a crucial factor in producing optimal shock wave-induced bioeffects in vivo.

Shock waves are characterized by a violent change in pressure which induces subsequent changes in characteristics of the medium through which they are propagated. A typical pressure waveform at the lithotripter focus in water consists of a leading shock wave front (compressive wave) with a peak positive pressure in the range of 30–150 MPa and a phase duration of 0.5–3 μs, followed by a tensile wave with a peak negative pressure down to −20 MPa and a duration of 2–20 μs. The negative pressure part of the shock wave produces cavitation [1]. To accelerate stone comminution and/or reduce/enhance tissue

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damage, special generators of shock waves are being designed to modify the cavitation field and to control cavitation. The corresponding methods manipulate the timing between pulses or modify the lithotripter waveform [19–22]. A promising approach to control bubble growth and collapse is the use of two focused successive (tandem) shock waves, which intensify the collapse of cavitation bubbles by sending a second shock wave before the bubbles produced by the first shock wave begin to collapse [23–28]. Shock waves produced by these modified generators have been shown to cause significant cytotoxic effects in tumor cells both in vitro and in vivo. These effects were considerably higher than those reported using traditional shock wave lithotripters. Furthermore, tandem shocks were shown to delay tumor growth and this effect was significantly potentiated with cytostatic drugs [27–35].

However, despite the demonstrated effectiveness in experimental tumors, understanding the mechanism of interaction between the shock waves and tumors is limited. Both positive and negative pressures and inertial cavitation especially are thought to play roles in the interaction of lithotripter-induced shock waves with biological tissue [2]. Little is also known about how cavitation bubbles develop within tissue. One reason is that the acoustic impedance of fluid media is similar to that of soft tissues in the body, and shock waves can propagate through tissues without significant energy loss. Small-scale inhomogeneities in different layers of tissue (skin, fat and muscle) may scatter the wave, thereby distorting the wavefronts and slightly attenuating the shock wave. There is no acoustical difference between cancerous and healthy tissues in contrast to solid objects such as kidney stones in urine; thus, the localized action of shock waves is attributed to the cavitations produced in tissue in vivo. Collapsing cavitations create strong secondary shock waves of nanosecond duration (tens of micrometers in scale) that can interact with cell scale-structures. Local thermal effects (on the order of μm dimensions) accompanying cavitations collapse (sonoluminescence) and the production of chemical radicals may also play a role in cell damage [36–39]. Also, it might not be necessary to produce cavitation in vivo. Previously, we have found that when using tandem shock waves with short delays (between approximately 10 and 15 μs), the main role of the first shock wave is to produce an inhomogeneous medium at the focal zone, i.e., a low-density region [29–34]. In a low-density inhomogeneous medium, the velocity of propagation of the second shock front is slower than in a homogeneous medium. This inhomogeneity is the reason why the second shock interacts with an otherwise acoustically transparent/homogeneous liquid medium. The second shock thus propagates with growing strength through a medium with a negative density gradient and interacts with targeted tissue. However, further research is required on the optimal shock wave profile and on understanding mechanisms of interaction between the shock waves with tissue in vivo treatment.

Huber and Debus [35] reported histopathological changes in Dunning prostate tumors transplanted into the thighs of Copenhagen rats upon in vivo treatment by double shock waves. They observed a large number of pyknotic nuclei, severe intracellular and pericellular vacuoles, and patchy necrosis in the treated tumors. These tissue changes were more extensive after treatment by a higher number of double shocks which produced more severe effects on the tumor histopathology such as hemorrhaging, tissue disruption, and necrosis. Apart from mechanical effects, the shock waves may cause cavitation-induced sonochemical effects in exposed tumor tissue which could lead to other changes than necrosis, e.g., to apoptosis. Apoptosis or programmed cell death is an important physiological process whose goal is to eliminate damaged cells or redundant cells during normal development [40]. In oncology, apoptosis plays an important role in both carcinogenesis and cancer treatment. Apoptotic cells are characterized by specific morphological and biochemical changes. Participation of numerous proteins in apoptosis, several apoptotic signaling pathways, and their regulation have been well described [40,41]. The apoptotic mode has been shown to be triggered by a variety of antitumor drugs, radiation, or pulsed electric fields with pulse duration of nanosecond range [42–50]. Focused shock waves represent to be another potential local cancer treatment strategy. However, only few studies on apoptotic pathways in tissues exposed to shock waves have been reported with no conclusive results whether shock wave may induce apoptotic changes in tissue [51–55].

In this work, we used histological and immunohistochemical techniques to investigate the effects induced by focused shock waves in tumor tissue in vivo. The Lewis rats with syngeneic sarcoma (developed after subcutaneous inoculation of tumor cells into thighs) were used as experimental animal models. A multichannel pulsed-electrohydraulic discharge generator with cylindrical ceramic-coated electrode was used as source of focused shock waves [56–59]. In previous work, we have demonstrated that focused shock waves produced by such a type of generator can cause localized lesions at a predictable location deep within soft tissue. The biological effects caused by these shock waves were demonstrated both in vitro and in vivo [56,57]. Present research was undertaken to contribute to better understanding of the mechanisms of interaction between the shock waves with in vivo tissue treatment. We evaluated serial cryosections of tumor tissue exposed to focused shock waves using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) technique and immunohistochemical detection of caspase-3. In addition, tissue morphology was evaluated using hematoxylin–eosin staining. The observed changes in tumor tissue upon exposure to focused shock waves were correlated with the direction and the shape of shock wave propagation in water determined using high speed shadowgraphy.

2. Experimental

2.1. Shockwave generator

Fig. 1 shows a scheme of the experimental setup. The shock wave generator consisted of a cylindrical high-voltage composite electrode (anode) placed along the axis of the outer metallic parabolic reflector (cathode) [57]. The dimension of the cylindrical composite electrode was 60 mm diameter × 100 mm long. The generator was divided into two sections by an acoustically transparent membrane (Mylar foil). The inner part was filled with a highly conductive saline solution (18 mS/cm) and a contained electrode system. The focal point of the

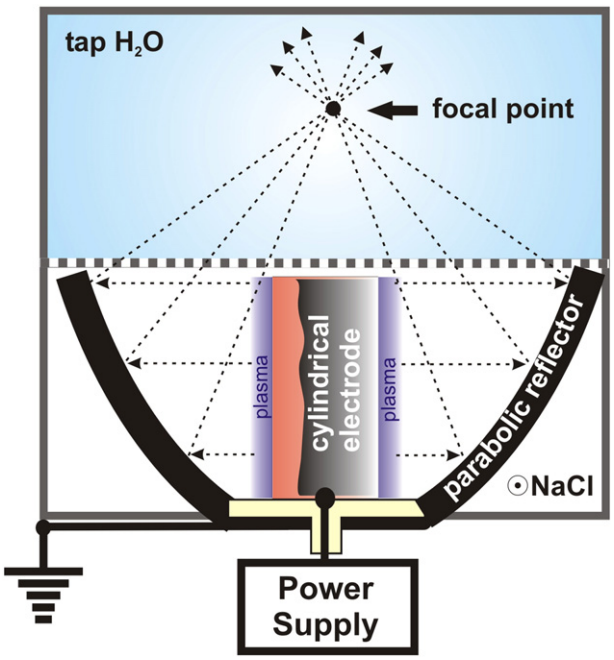

Fig. 1. Scheme of shock wave generator.