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● *Original Contribution*

SYNERGISTIC EFFECTS OF SONOPORATION AND TAUROLIDIN/TRAIL ON APOPTOSIS IN HUMAN FIBROSARCOMA

Adrien Daigeler,* Ansgar M. Chromik,† Kathrin Haendschke,‡ Sabine Emmelmann,‡ Monica Siepmann,§ Karin Hensel,§ Georg Schmitz,§ Ludger Klein-Hitpass,‖ Hans U. Steinau,‡ Marcus Lehnhardt,* and Joerg Hauser‡

*Department of Hand, Plastic and Reconstructive Surgery, Burn Center, BG-Unfallkrankenhaus, Ludwigshafen, Germany; †Department of General and Visceral Surgery, St. Josef Hospital, Ruhr-University, Bochum, Germany; ‡Department of Plastic Surgery, Burn Center, Sarcoma Reference Center, BG-University Hospital Bergmannsheil, Bochum, Germany; §Institute for Medical Engineering, Ruhr-University Bochum, Bochum, Germany; and ‖Institute for Cell Biology (Tumor Research), University of Duisburg-Essen, Essen, Germany

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Abstract—Sonodynamic therapy, in combination with ultrasound contrast agents, proved to enhance the uptake of chemotherapeutics in malignant cells. HT1080 fibrosarcoma cells were treated *in vitro* with a combination of ultrasound SonoVue™-microbubbles and taurolidine (TRD) plus tumor necrosis factor related apoptosis inducing ligand (TRAIL). Apoptosis was measured by TdT-mediated dUTP-biotin nick end labelling (TUNEL) assay and fluorescence activated cell sorting (FACS) analysis. Gene expression was analysed by RNA-microarray. The apoptotic effects of TRD and TRAIL on human fibrosarcoma are enhanced by sonodynamic therapy and additional application of contrast agents, such as SonoVue™ by 25%. A broad change in the expression of genes related to apoptotic pathways is observed when ultrasound and microbubbles act synchronously in combination with the chemotherapeutics (*e.g.* BIRC3, NFKBIA and TNFAIP3). Some of these genes have already been proven to play a role in programmed cell death in human fibrosarcoma (HSPA1A/HSPA1B, APAF1, PAWR, SOCS2) or were associated with sonication induced apoptosis (CD44). Further studies are needed to explore the options of sonodynamic therapy on soft tissue sarcoma and its molecular mechanisms. (E-mail: adaigeler@bgu-ludwigshafen.de) © **2010 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasound, Taurolidine, TRAIL, Soft tissue sarcoma, Fibrosarcoma, Sonoporation, Sonodynamic therapy, Sonication, Apoptosis.**

INTRODUCTION AND LITERATURE

Within the group of soft tissue sarcomas with an incidence of about 2-4/100,000 (Singer et al. 1995), fibrosarcoma represents a rare entity. It accounts for approximately 2.6% of soft tissue sarcomas. Response rates to established chemotherapeutic agents like doxorubicin and ifosfamide are still disappointing (Donato Di Paola and Nielsen 2002). Therefore, new chemotherapeutic agents like tumor necrosis factor related apoptosis inducing ligand (TRAIL) and taurolidine (TRD) that have already been proven effective in a variety of malignant cells like esophageal cancer, colon, pancreas, and liver carcinoma *in vitro* and *in vivo* (Braumann et al. 2004, 2006; Calabresi et al. 2001; Chromik et al. 2007, 2010; Daigeler et al. 2008b; McCourt et al. 2000; Stendel et al. 2007) have previously been tested and turned out to be potent inductors of apoptotic cell death in human fibrosarcoma (Daigeler et al. 2008a). To date, surgical resection with clear margins is still the therapy of choice for fibrosarcoma. Most of the soft tissue sarcomas are located in the extremities, sometimes making it difficult to preserve limbs or limb function, especially in large tumors or those adjacent to important vessels or nerves (Daigeler et al. 2009; Steinau et al. 1995). However, this predominant localization at the extremities makes the tumors accessible by other therapeutic strategies. Isolated limb perfusion with chemotherapeutic agents like melphalan and TNF alpha was effective in a number of cases but side effects, including a possible loss of the treated limb, are still considerable (Cherix et al. 2008; Grunhagen et al. 2006). Another available option is the

Address correspondence to: Adrien Daigeler, M.D., Department of Hand, Plastic and Reconstructive Surgery, Burn Centre, BG-Unfallkrankenhaus, Ludwig-Guttmann-Str. 13, 67071 Ludwigshafen, Germany. E-mail: adaigeler@bgu-ludwigshafen.de

use of sonodynamic therapy—ultrasound dependant enhancement of cytotoxic activities of certain compounds. The interaction of ultrasound with a bulk fluid causes pre-existing gas bubbles to oscillate, grow and collapse, and thereby send little shock waves to the surrounding tissue, an effect called "cavitation". This cavitation can create transient defects of the cell membrane ("sonoporation"). These pores persist for several minutes and facilitate the uptake of simultaneously applicated cytotoxic drugs (Rosenthal et al. 2004; Schlicher et al. 2006; Tachibana et al. 1999; Wu et al. 2002). Many studies confirmed these synergistic effects of ultrasound and chemotherapeutics, then referred to as "sonosensitizers", *in vivo* and *in vitro* in various cells (Harrison et al. 1991; Lentacker et al. 2010; Loverock et al. 1990; Mizuno et al. 2005; Mohamed et al. 2003; Sur et al. 1999; Yu et al. 2001) including sarcoma (Saad and Hahn 1992; Yumita et al. 2002). The effect can be enhanced by the addition of ultrasound contrast-agent gas bodies (so-called "microbubbles"). Oscillating microbubbles can cause transient membrane pores of a size approximately 100 nm (Schlicher et al. 2006; Tachibana et al. 1999; Wu and Nyborg 2008). This effect exceeds the impact of sonodynamic therapy and sonoporation without contrast agents, leading to an enhanced uptake of molecules for several minutes after sonoporation (Karshafian et al. 2009; Khanna et al. 2006; Kodama et al. 2002).

Ultrasound can penetrate deeply into tissues and can be focused on regions of tumor growth to effectively activate sonosensitizers while peripheral healthy tissue is preserved. Sonoporation by the use of microbubbles can be employed to enhance drug delivery in tumor sites, potentially making it a valuable clinical application.

The aim of this study was to elaborate if the proapoptotic effects of TRD and TRAIL on fibrosarcoma can be enhanced by ultrasound and/or additional contrast agent SonoVue™ and, if so, to gain insight into possible mechanisms on a molecular basis.

MATERIAL AND METHODS

Cells and cell culture

Human fibrosarcoma cells, HT1080, were purchased from ATCC (Cell line CCI 121; Wesel, Germany) and maintained with Dulbecco's modified Eagle's medium (DMEM) + 10% FBS supplemented with 1% penicillin (100 U/mL) and streptomycin (100 μg/mL), 1% sodium pyruvate and 1% L-glutamine. Cells were cultured in a humidified atmosphere with 5% CO_2 at 37°C in 25 cm^2 flasks. For the sonication experiments, cells were seeded in dishes to a subconfluent monolayer. Approval from the institutional review board for the experiments was obtained.

Reagents

Taurolidine (TRD) (Taurolin® 2%; Geistlich Pharma, Suisse/Boehringer Ingelheim, Germany) containing 5% povidon was used as supplied by the manufacturer at a final concentration of 250 μmol/L. Recombinant human TRAIL/Apo2L (Bender MedSystems, Vienna, Austria) was dissolved in distilled water according to the manufacturer's instructions and used in a final concentration of 500 ng/mL. Culture medium in equal volume served as control.

SonoVue™ (Bracco S.p.A., Milano, Italy) contains hexafluoride gas bubbles surrounded by a phospholipid monolayer shell with a mean diameter of about 2.5 μm (Schneider et al. 1995). Microbubbles were used in a dispersion according to the manufacturer's instructions. A quantity of 100 μL (approximately 16×10^6 microbubbles) were added to the dishes already filled with 8.9 mL culture medium resulting in a final concentration of about 32 microbubbles per sarcoma cell, observed microscopically (Fig. 1)(similar to Fig.1). Reagents were added immediately before sonication.

Ultrasonic exposure system

The set-up used for the evaluation of sonoporation enhanced chemotherapy consisted of a therapy transducer (A392S; Olympus NDT, Waltham, MA, USA) with a center frequency of 1 MHz, an element diameter of 38 mm and a focus depth of 75 mm. The –6 dB lateral focal width was measured by a calibrated hydrophone (Force MHA; 239,5 S/N311, Copenhagen, Denmark) to be 4.6 mm. Ultrasound therapy inducing oscillation of the microbubbles was conducted with a signal consisting of five cycle sine bursts (center frequency 1 MHz) repeated 150 times at a burst frequency of 10 Hz with peak negative pressures (PNP) ranging from 0.72 to 1.09 MPa. The effective exposure time of each sample

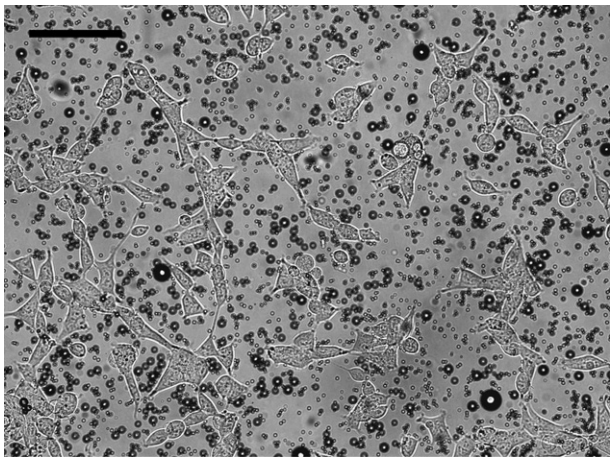

Fig. 1. SonoVue™-microbubbles next to vital cells. The bar measures 100 μm.