Sensitivity of Normal and Malignant Cells to Shock Waves

FRANZ BRÜMMER, PH.D., DIERK SUHR, DIPL.-BIOL., and
DIETER F. HÜLSER, PH.D.

From the Universität Stuttgart, Biologisches Institut, Abteilung Biophysik, Stuttgart, Germany

BRÜMMER, F., ET AL.: Sensitivity of Normal and Malignant Cells to Shock Waves. We examined the cytotoxic effect of shock waves for primary (embryonic chick kidney and thigh muscle) and permanently growing normal and malignant cells (human, rat, and mouse) in suspension. To avoid the influence of different media, the cells were suspended in phosphate buffered saline and shock wave treated. In all cases the acute cytotoxic effect (measured by flow cytometry) was a function of the applied shock waves. The investigated cells differed in their LD_{50} values which, however, do not reveal a general difference in sensitivity to shock waves for normal and malignant cells. (J Stone Dis, Vol. 4, No. 3, July 1992)

Introduction

In the last decade, a noninvasive technique with extracorporeal induced shock waves has revolutionized the clinical treatment of kidney stones.[1] Although in clinical use for only a short period, extracorporeal shock wave lithotripsy (ESWL) is the standard therapy today for urinary stone disease and, in addition, a promising tool in the noninvasive treatment of gallstones.[2] The treatment of salivary gland stones has even been reported.[3]

The treatment with shock waves, however, produces tissue damages consisting primarily of intraparenchymal and perirenal hemorrhages.[4] Guided by the observed damages, attempts have been made to apply shock waves on tumor cells and tumors to investigate their influence on cell viability and growth. Effects of shock waves on tumor cells were first reported by Russo and coworkers.[5,6] Meanwhile, other groups have investigated the cytotoxic effect of extracorporeal shock waves,[7–11] including our own studies.[12,13]

In this in vitro study, we compared the acute effect of shock waves on different permanently growing normal and malignant cell lines, as well as on primary embryonic chick cells. For this measurement, a rapid and reliable detection of cell damage is necessary. We, therefore, determined the concentration of intact cells before and after shock wave treatment with an electronic cell counter. Together with a double staining technique using flow cytometry, the proportion of intact cells was screened for physiologically active (viable) cells and seriously damaged (dead) cells.[13] In addition to the rapidity and reliability of this assay, the number of cells investigated for each experiment was much higher (up to 10,000) compared to other tests like trypan blue dye exclusion test where the totally disintegrated cells are not considered. Since experimental conditions like temperature and oxygen content of the lithotripter water bath influence the cytotoxic effect to a considerable extent,[14,15] great caution was taken to perform each experiment under identical conditions.

Materials and Methods

Cell Culture

Permanently Growing Cells. The suspension culture L1210[16] and four monolayer lines (FL,[17]

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Address for reprints: Dr. F. Brümmer, Universität Stuttgart, Biologisches Institut, Abt. Biophysik, Pfaffenwaldring 57, D-7000 Stuttgart 80, Germany.

Table 1. Characterization of Permanently Growing Cell Lines Used in This Study

Cell Line	Cell Diameter/μm*	Origin	Growth Medium**
L1210	11.0 ± 0.5	Mouse leukemia	RPMI 1640 + 15% FCS
FL	15.7 ± 0.3	Normal human amnion	DMEM + 10% NCS
BICR/M1R$_k$	18.3 ± 0.7	Rat mammary tumor	DMEM + 10% NCS
MGH-U1	16.3 ± 0.3	Human bladder carcinoma	RPMI 1640 + 10% FCS
F9	12.9 ± 0.3	Mouse teratocarcinoma	DMEM + 10% NCS

* Cell diameters are presented as mean ± standard deviation; ** FCS = fetal calf serum, NCS = newborn calf serum.

BICR/M1R$_k$,[18] MGH-U1,[19] and F9[20]) have been investigated. For characterization and further details see Table 1.

Primary Embryonic Chick Cells. Single cell suspensions of 12-day-old embryonic chick kidneys and thigh muscles were prepared as described by Freshney.[21] Briefly, organs were explanted from 12-day-old embryos and placed overnight in 1 mL ice-cold trypsin (0.25% trypsin in phosphate buffered saline [PBS] without calcium and magnesium). After trypsin removal, the tissue was incubated in the residual trypsin at 37°C for 15 minutes, dispersed by gentle pipetting in 2 mL Minimum Essential Medium (Eagle), Dulbecco's Modification (DMEM, Biochrom KG, Berlin, Germany), and seeded in three to six Petri dishes of 60-mm diameter (Falcon, 3002 F, Becton Dickinson, Mountainview, CA, USA). After this procedure, the cell diameter was 12.9 ± 1.2 μm for kidney and 14.7 ± 0.8 μm for thigh muscle cells as measured with a flow cytometer.[22]

Culture Conditions. All cell cultures were cultivated at pH 7.4 and 37°C in a humidified incubator with an atmosphere of 8% CO_2 in air. Monolayer cells were grown in tissue culture flasks up to subconfluence (Nunc, Roskilde, Denmark) and passaged with 0.25% trypsin in PBS without calcium and magnesium, L1210 cells were maintained as suspension under the same conditions.

Shock Wave Generation

Shock Wave Source. Shock waves were generated by underwater spark discharge at an operating voltage of 18 kV and at a frequency of 1 Hz with an experimental lithotripter XL-1 (Dornier Medizintechnik, Germering, Germany). Electrodes were replaced after 1,500 discharges. A laser system was used to position the test tube with the cell suspension into the target focus.

Water Processing. Water in the lithotripter was partially degassed (2.4–2.7 mg O_2/L) by a vacuum pump (Maprotec, Idstein, Germany), and the temperature of the water bath was regulated thermostatically. Oxygen concentration was determined by an oxygen probe (oxygen electrode EO 196–1.5 and oximeter OXI 196, WTW GmbH, Weilheim, Germany), which simultaneously measured the temperature of the water bath (37°C).

Determination of Surviving Cells

For shock wave treatment, adherent cells were trypsinized, concentrated (2–5 × 10^5 cells/mL), and transferred into polyethylene pipettes that were positioned 10-cm under the water surface in the focal point. Cells were either suspended in growth medium or in PBS (8.0 g/L NaCl, 0.2 g/L KCl, 1.44 g/L $Na_2HPO_4 \cdot 2H_2O$, 0.2 g/L KH_2PO_4, 0.1 g/L $MgCl_2 \cdot 6H_2O$, 0.1326 g/L $CaCl_2 \cdot 2H_2O$; pH 7.4), untreated controls were kept under the same conditions. Phosphate buffered saline was only used after storage at 4°C for at least 14 days.

After shock wave treatment, fractions of intact and destroyed cells were determined as described earlier.[13] Briefly, the cell damage was quantified by counting the geometrically intact cells in a Coulter Counter (Model Industrial D, Coulter Electronics, Hialeah, FL, USA) and by determining the propor-

tion of viable cells within the geometrically intact population by flow cytometry. For discrimination between viable and dead cells, we applied a double staining technique using propidium iodide for characterization of damaged cells and hydrolyzed fluorescein diacetate for identification of viable cells. Every experiment was repeated at least three times.

Statistics

The data are presented as means ($n \geq 3$) with standard deviation. Regression analyses and graphics were calculated by least square fittings to single exponential decay functions[23] using Multigraf (midas micro-Datensysteme, Frankfurt/M., Germany). The LD_{50} was calculated using the function obtained by the weighted regression analyses.

Results

Since the cell lines used for these studies are growing in different media, we first investigated the influence of different suspension media on shock wave efficacy. With L1210 cells, we found an insignificant difference ($\alpha > 0.05$) in cell damage after shock wave treatment in their recommended growth medium RPMI 1640 (lethal dosage 50% [LD_{50}] = 249 shock waves) or in DMEM (LD_{50} = 243 shock waves). In PBS (LD_{50} = 291 shock waves), their sensitivity was significantly reduced ($\alpha < 0.01$) when compared to both media (Fig. 1). This result indicates that the use of different growth media has no effect on the shock wave efficacy for L1210 cells. For BICR/M1R_k cells, however, treatment in their recommended growth medium DMEM (LD_{50} = 407 shock waves) resulted in a higher sensitivity ($\alpha < 0.01$) than in RPMI 1640 medium (LD_{50} = 517 shock waves) and, in contrast to L1210 cells, in PBS (LD_{50} = 249 shock waves), their sensitivity was considerably increased with $\alpha < 0.01$ when compared to both media (Fig. 2). Further experiments were carried out with cells suspended in PBS without serum. In any case, this procedure has the advantage of reducing the influence of unknown parameters that may stem from the required medium components, e.g., serum. The controls of all cell cultures showed 99%–100% viable cells after the time required for the experiments.

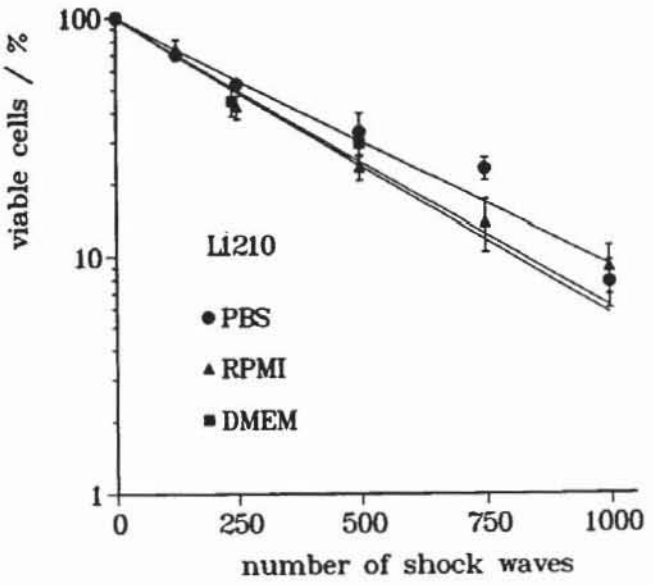

Figure 1. *Viability of L1210 cells after shock wave exposure in different suspension media. Every point represents the mean ± standard deviation of at least three experiments.*

The resulting dose–effect curves are shown in Figure 3. For these data, exponential regression curves were fitted for the L1210 cells grown as suspension and the cell lines growing permanently as monolayers (FL, BICR/M1R_k, MGH-U1, and F9), as well as the primary embryonic chick cells. The LD_{50} values for each cell line were calculated from the

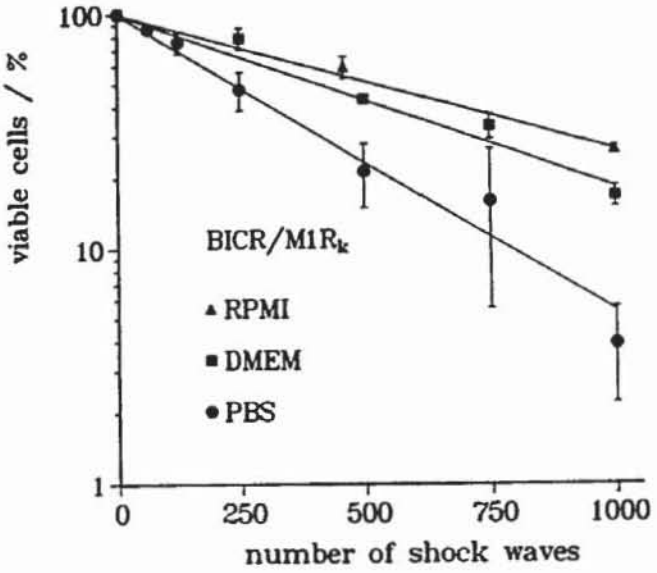

Figure 2. *Viability of BICR/M1R_k cells after shock wave exposure in different suspension media. Every point represents the mean ± standard deviation of at least three experiments.*

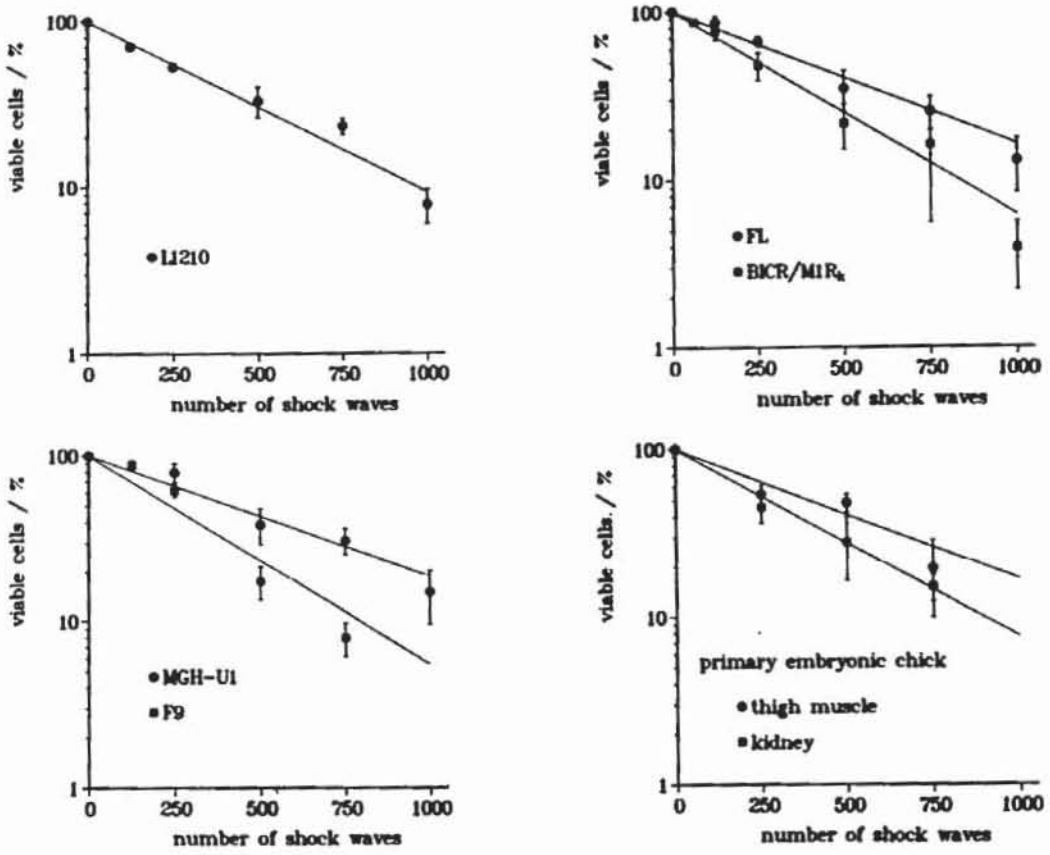

Figure 3. *Viability of normal and malignant cells after shock wave treatment in phosphate buffered saline. Every point represents the mean ± standard deviation of at least three experiments.*

resulting regression analyses and are summarized in Figure 4. LD_{50} values ranged from 412–238 shock waves. Only thigh muscle and FL as well as BICR/$M1R_k$ and F9 differed insignficantly ($\alpha > 0.05$); MGH-U1 and thigh muscle differed with $\alpha < 0.05$, and all other LD_{50} values differed with $\alpha < 0.01$. For our investigated cell lines, the cell size seems not to be correlated with cell sensitivity to shock waves (compare Fig. 4 with cell diameter results given in Materials and Methods and Table 1).

Discussion

Since Russo and co-workers[5] described in vitro and in vivo cytotoxic effect of shock waves on tumor cells, a dose-related reduction in cell viability after shock wave treatment has been demonstrated for both spark gap[5–8,24] and electromagnetically[9,10,15] generated shock waves. Our results presented in this study also show a dose-related effect and are thus in accordance with our earlier findings,[13] as well as with investigations from the groups mentioned above.

Since temperature and oxygen content of the lithotripter water bath influence the applied shock wave energy and thus the amount of surviving cells,[14] and since viscosity of suspension media effects the survival rate,[13,15] we carefully controlled these parameters and kept them constant for comparison of the shock wave efficacy.

The mechanism of shock wave induced cell death and possible different sensitivities of cell to shock wave application is still unknown. Cavitation, micro jets, acceleration, shearing forces, and the formation of free radicals are mechanisms that may cause the injury of suspended cells.[12,13,24–28] On the other hand, parameters like cell size and pretreatment of the cells prior to shock wave application may influence the survival rate.

In vitro experiments have been performed with cells suspended in their appropriate growth media. From ultrasonic research, however, it is known that parameters like gas content, surface tension, and

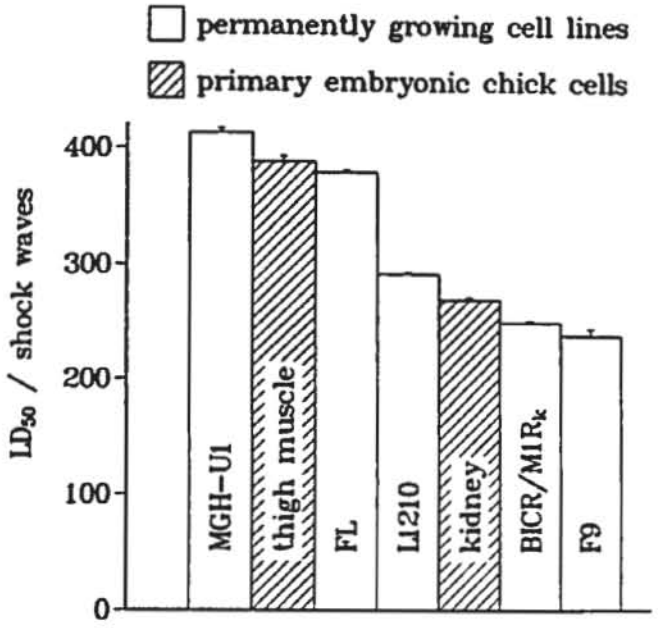

Figure 4. *LD_{50} values of shock wave treated normal and malignant cells; LD_{50} values ranged from 412–238 shock waves. Only thigh muscle and FL as well as BICR/M1R$_k$ and F9 differed insignificantly ($\alpha > 0.05$); MGH-U1 and thigh muscle differed with $\alpha < 0.05$, and all other LD_{50} values differed with $\alpha < 0.01$.*

temperature of the suspension media can influence the threshold for acoustic cavitation.[29–32] Hemolysis after ultrasonic treatment does not only depend on variations in temperature, osmolarity, viscosity, and density of suspension medium,[28] but also on cell concentration[33] and age of the suspension medium.[34] Furthermore, we cannot exclude a biological influence (i.e., change in cellular sensitivity) of different suspension media in addition to the well-documented alterations of physical parameters. To reduce the effect of different contents of cavitation nucleation sites or variations in the viscosity and/or the mass density of the media, we used only PBS (> 14 days old) without serum to suspend the cells for the shock wave treatment. In all cases the acute cell-damaging effect was a function of the applied shock waves; but with regard to their dose response, the investigated cell lines differ in their calculated LD_{50}. However, we could not detect a specific difference in the sensitivity to shock waves between normal and malignant cells as can be seen from Figure 4, where the cell lines have been arranged by ranking their LD_{50}. This ranking, however, will certainly be changed when the cells were treated in another suspension medium, as can be judged from Figures 1 and 2. Nevertheless, this procedure should not result in a separation of normal and malignant cells with regard to their shock wave sensitivity.

It may well be that embryonic cells cannot be considered as normal cells. Whether they are more or less sensitive to shock waves than normal adult tissue cannot be answered from our presented data. Whereas permanently growing cells of normal origin (like FL cells, in culture since 1956) may rather be malignant cells, the investigated embryonic cells of 12-day-old chicken embryos were already differentiated and may well represent normal tissue.

Our results presented here indicate that under carefully controlled and constant experimental conditions, cells have different sensitivities to shock waves, but no general difference between normal and malignant cells can be seen.

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