BIOLOGICAL EFFECTS AND PHYSICAL CHARACTERIZATION OF SHOCK WAVES GENERATED BY AN XL-1 EXPERIMENTAL LITHOTRIPTER

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Suspended L1210 cells have been exposed to shock waves under different experimental conditions (temperature, oxygen content). Pressure waveforms have been measured with two different types of PVDF-needle-hydrophones. The efficacy of the shock wave treatment is compared with physical characteristics of the shock waves.

INTRODUCTION

Extracorporeal shock wave lithotripsy (ESWL) has become the clinical standard method for non-invasive disintegration not only of concrements in kidney and urinary tract but also of gallstones. Despite the widespread clinical use of ESWL, the mechanism of stone destruction is not yet really understood, but several possibilities (cavitation, shock wave reflection) are discussed [1,2]. The final cause of various side effects is still under discussion [reviewed in: 3,4,5]. Nevertheless, during the last few years much effort was put into possible extensions of ESWL applications [tumor treatment: 6, revision total arthroplasty: 7]. On the other hand, physical characterizations of shock waves are rarely reported [8,9] and combined measurements of biological effects in vitro and physical characterization of the applied shock waves are not available. We, therefore, examined the influence of water temperature and gas content on the shock wave efficacy in biological systems and determined several physical characteristics (pressure amplitudes, rise time etc.) of the shock waves under the same experimental conditions.

MATERIAL AND METHODS

Shock wave generation

Shock waves were generated by an experimental lithotripter XL-1 (Dornier Medical Systems GmbH, Germering, FRG) by underwater spark discharge. The XL-1 has the same generator and brass semiellipsoid as the clinical system HM3. Electrodes were not used prior the first 50 ignitions which were done at 18 kV with a frequency of 1 Hz, they were replaced after 1500 discharges. Water in the lithotripter was partially degassed by a vacuum pump (Maprotec GmbH, Idstein, FRG). Oxygen concentration was determined by an oxygen probe (oxygen electrode EO 196 - 1,5 and oximeter oxi 196, WTW GmbH, Weilheim, FRG) which simultaneously...
measured the water bath temperature.

Pressure measurements

The pressure measurements in the XL-1 were carried out at a generator charging voltage of 14 kV, using two different kinds of PVDF-needle-hydrophones. Both the commercial available (Imotec GmbH, Würselen, FRG) [10] as well as the hydrophones which were constructed in our laboratory (glued, spotpoled PVDF-foil, bandwidth 10 MHz) have sensing elements with a diameter of about 0.5 mm. Calibration is performed by means of the acoustic impulse response of a piezoelectric disk [11].

By now there is no commonly accepted method for a standard pressure measurement of focused shock waves. Different hydrophones show at least different ratios of the peak-positive- and peak-negative-pressure as well as different risetimes according to their bandwidth. Additionally, decreasing sensitivities after shock wave exposure are reported [12]. We, therefore, took care that neither hydrophone damage nor changes in the shock wave source characteristics (due to electrode deterioration) occurred during our experiments: we finished each series of experiments with the same water conditions from the beginning of the experiment (see table 1, 2). Additionally, by calibrations before and after shock wave measurements it was ensured that hydrophone sensitivity and frequency response were unchanged.

All our measurements started with a definite water temperature and definite gas content with a new electrode in the shock wave generator. At the point of maximal pressure we recorded waveforms at a repetition-rate of about 1 discharge per minute with a fast digital storage oscilloscope (Philips, FRG) which allowed cursor measurements on the screen. The shock waves were described by the rise time (10% - 90%) of the shockfront, \( t_r \), the peak of positive (\( p^+ \)) and of negative pressure (\( p^- \)), and the positive (\( t_{p^+} \)) and negative pressure halfwidth (\( t_{p^-} \)).

Two different experiments were repeated several times to test, if there is a significant change in one of the pulse parameters due to water temperature (21°C, 37°C) at a fixed oxygen content of 3 mg/l or due to oxygen content (3 mg/l; 5.7 mg/l; 7.2 mg/l) at a fixed temperature of 37°C.

Because of the large amplitude scattering of the XL-1 (± 30%), we evaluated 20 single-shots at each temperature and oxygen content. The experiments were done in the order listed in table 1 and 2.

Cell culture

All experiments were performed with L1210 cells, a lymphocytic mouse leukemia cell line, which were cultured as described before [13]. For shock wave treatment, cells were concentrated to 4-5 x 10^6 cells/ml and transferred to polyethylene pipettes (4.5 ml, Multimed, Kirchheim/Teck, FRG). Samples were fixed under water in the second focal point of the semiellipsoid, which was indicated by two crossing laser beams. Treatments were carried out with two different numbers of shock waves (250 and 750) at 18 kV with a frequency of 1 Hz at different water bath temperatures (21°C, 37°C and 41°C) and oxygen contents (2.4 ± 0.3 and 7.0 ± 0.3 mg/l). The application of shock waves at 1 Hz follows clinical standards.

Untreated controls were kept under the same conditions. After shock wave treatment fractions of intact and destroyed cells were determined by a Coulter Counter, viable and dead cells were separately counted by
a double-staining method using flow cytometry [13].

Statistics

All data are presented as means with standard deviations. Significance was tested using the Mann-Whitney U-test and error probability p is indicated.

RESULTS

Under all experimental conditions, we found a dose-dependent cell damage after shock wave treatment (fig. 1 and 2). The influence of temperature on cell viability is shown in fig. 1. The fraction of viable cells decreased with rising temperature: e.g. after 250 shock waves 84.68 ± 5.08 % viable cells were measured at 21°C whereas at 41°C only 31.06 ± 3.05 % survived this shock wave treatment. Higher oxygen content leads to less cell damage as is shown in fig. 2. After treatment of the cells with 750 shock waves almost 2.5 fold more cells survived in water containing 7 mg O₂/l than in water with 3 mg O₂/l.

The hydrophone was fixed at the maximal p+ for every given temperature. At the beginning of the experiments (21°C) this point was 4 mm behind the geometrical focus in source axis (position a), and at 37°C it was at 6 mm (position b). However, because of large shot to shot scattering, experimental focus determination was only accurate to about ± 1.5 mm in direction of the source axis direction and to about ± 1 mm in the plane perpendicular. Within this accuracy the focus did not move due to temperature, but there was always an significant increase in p+ (p ≤0.01) from 21°C to 37°C accompanied by a significant reduction of t+½ (p ≤0.01), whereas for the negative pressure data no correlation to temperature was found (table 1).

In another set of experiments (see table 2) we observed no significant changes (p >0.1) for all pulse parameters due to different oxygen contents at a fixed temperature of 37°C and a repetition rate of 1 discharge per minute, except for t-½ which is significantly higher at 3 mg O₂/l (p ≤0.01) than for the other oxygen values. The values of table 2 were obtained by positioning the hydrophone in the plane perpendicular to the source axis for maximum p+ at a distance given by the geometrical focus. A measurement in this position results in a typical waveform with positive and negative pressure components (see fig. 3). Comparing the data at 37°C and 3 mg oxygen of table 1 with corresponding data of table 2 a 35 % increase in p+ and a 40 % decrease in t+½ towards position b is ascertained.

DISCUSSION

Linear acoustic theory predicts equivalent positive and negative pressure time integrals. According to fig. 3, this does not hold in the nonlinear case. A negative pressure breakdown is mainly caused by cavitation during propagation. Furthermore, a small waveform distortion due to a slightly increased high frequency hydrophone sensitivity (about 20% for the shockfront) must be taken into account.

In order to facilitate numerous measurements with the same hydrophone, the generator charging voltage was kept at 14 kV. This enabled us to prove that the hydrophone characteristics remained unchanged by repeating the starting conditions at the end of an experimental series (see
table 1 and 2) as well as by a recalibration of the hydrophone. After correct alignment of the hydrophone to the XL-1 source axis about 80\% of the recorded waveforms looked like fig. 3 and similar waveforms were obtained with both types of hydrophones. This form is quite different to those reported before [14].

Suspension cultures exposed to shock waves in the focal area are damaged in a dose dependent manner [6, 13, 15]. Our presented data demonstrate, that rising the temperature or lowering the oxygen content of the lithotripter waterbath results in a further decrease of viable cells. Our experiments followed the protocol given for clinical treatments of patients (18 kV, 1Hz, degassed water). Confirming [16], we observed rising gas bubbles which we attribute to cavitation induced by previous shock waves. To avoid uncontrolled interactions, we, therefore, reduced the repetition rate to 1/min and performed pressure measurements with differently constructed needle-hydrophones. Special care was taken to remove all gas bubbles from the ellipsoid, the electrode and the hydrophone. The measured increase in $p+$ with higher temperatures correlates with the increase in damaged cells. This might be attributed to higher shear forces caused by enhanced agitation of the suspended cells. We cannot exclude, however, additional effects such as higher enzyme activities.

With an oxygen content above saturation no reduction of pressure amplitudes was recorded, $t-\frac{1}{2}$, however, was significantly lower at higher oxygen contents. This might be explained by an increased number of cavitation in the waterbath between ellipsoid and target focus. Thus, a greater amount of energy may be transferred to cavitation bubbles. The same mechanism explains the reduced number of destroyed cells, an effect which is enhanced by the treatment of cell suspension at 1 Hz. Simultaneously, a reduction of $p+$ is expected due to shockfront interaction with rising gas bubbles. Reduced $p+$ and $t-\frac{1}{2}$ lead to diminished shear forces and less cavitation in the test tube, explained the decreased cell damage under higher oxygen content.

REFERENCES

11. Eisenmenger, W.; Acustica (1962) 12 165

Fig. 1: Temperature dependent cell damage at constant oxygen content (2.4 ± 0.3 mg O₂/l) demonstrated for 250 and 750 shock waves.

Fig. 2: Influence of oxygen content on cell viability (250 and 750 shock waves at 37°C).

Fig. 3: Typical recording of a shock wave generated at 14 kV in an experimental lithotripter XL-1 measured in the geometrical focus with a glued, spotpoled PVDF-needle-hydrophone. Time base: 2 μs (For pressure specifications see table 2; calibration accuracy ± 15 %)
Table 1: Influence of waterbath temperature on "focal" pressure.
(generator voltage 14 kV, oxygen content 2.8 - 3.3 mg/l)

<table>
<thead>
<tr>
<th>T [°C]</th>
<th>hydrophone position</th>
<th>n</th>
<th>p+ [MPa]</th>
<th>p- [MPa]</th>
<th>t_r [ns]</th>
<th>t+½ [ns]</th>
<th>t-½ [ns]</th>
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<tr>
<td>21</td>
<td>a</td>
<td>21</td>
<td>65 ± 12</td>
<td>10 ± 1</td>
<td>84 ± 14</td>
<td>376 ± 90</td>
<td>474 ± 72</td>
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<tr>
<td>37</td>
<td>a</td>
<td>20</td>
<td>75 ± 16</td>
<td>10 ± 1</td>
<td>73 ± 7</td>
<td>350 ± 121</td>
<td>469 ± 95</td>
</tr>
<tr>
<td>37</td>
<td>b</td>
<td>20</td>
<td>82 ± 18</td>
<td>9 ± 1</td>
<td>71 ± 11</td>
<td>314 ± 122</td>
<td>487 ± 75</td>
</tr>
<tr>
<td>21</td>
<td>b</td>
<td>20</td>
<td>68 ± 16</td>
<td>9 ± 1</td>
<td>73 ± 9</td>
<td>350 ± 113</td>
<td>387 ± 86</td>
</tr>
<tr>
<td>21</td>
<td>a</td>
<td>25</td>
<td>61 ± 14</td>
<td>10 ± 1</td>
<td>81 ± 13</td>
<td>410 ± 114</td>
<td>404 ± 82</td>
</tr>
</tbody>
</table>

* position a and b were adjusted to maximal p+ and are separated by 2 mm.

means ± standard deviation

Table 2: Influence of oxygen content on "focal" pressure.
(generator voltage 14 kV, waterbath temperature 37°C)

<table>
<thead>
<tr>
<th>oxygen content</th>
<th>n</th>
<th>p+ [MPa]</th>
<th>p- [MPa]</th>
<th>t_r [ns]</th>
<th>t+½ [ns]</th>
<th>t-½ [ns]</th>
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<tr>
<td>waterbath [mg/l]</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>2.9 - 3.1</td>
<td>20</td>
<td>60 ± 8</td>
<td>10 ± 1</td>
<td>111 ± 20</td>
<td>543 ± 72</td>
<td>433 ± 57</td>
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<tr>
<td>7.2 - 7.5</td>
<td>20</td>
<td>59 ± 8</td>
<td>10 ± 1</td>
<td>113 ± 27</td>
<td>564 ± 101</td>
<td>398 ± 28</td>
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<tr>
<td>5.5 - 5.7</td>
<td>20</td>
<td>58 ± 8</td>
<td>9 ± 2</td>
<td>107 ± 17</td>
<td>535 ± 72</td>
<td>385 ± 36</td>
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<tr>
<td>3.0 - 3.1</td>
<td>20</td>
<td>63 ± 8</td>
<td>10 ± 1</td>
<td>106 ± 23</td>
<td>545 ± 101</td>
<td>426 ± 39</td>
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</table>

* oxygen saturation: 6.52 mg O₂/l corrected for the local atmospheric pressure and water temperature

means ± standard deviation