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Damaging effects of high energy shock waves on cultured Madin Darby Canine Kidney (MDCK) cells

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Summary. Shock wave lithotripsy (ESWL) has become an almost non-invasive standard treatment modality for urolithiasis. Several investigations, however, demonstrated that ESWL is not completely free of side effects. Among others alteration of renal tubular function has been reported. To study the effect of shock waves on tubular cells directly an in-vitro model with cultured Madin Darby Canine Kidney (MDCK) cells was established. Suspensions of MDCK cells (7 groups of 6 containers each) were exposed to 0, 16, 32, 64, 128, 256 shock waves (Dornier HM4, 18 kV). Before and 0, 1, 3, 6, 9, 12, 24 h after ESWL the following parameters were measured in the nutrient medium: lactate dehydroxygenase (LDH), glutamate oxalacetate transaminase (GOT), electrolytes. LDH and GOT increased depending on the number of shock waves indicating a membrane damage of MDCK cells. The MDCK model seems suitable for further studies on the effect of shock waves on renal tubular cells.

Key words: ESWL – Shock waves – MDCK cells – LDH – GOT

For several years extracorporeal shock wave lithotripsy (ESWL) is a standard treatment modality for urolithiasis. About 90% of all urinary stones can be treated by this method. With second generation lithotriptors ESWL has become an almost painfree and non-invasive treatment. Despite of these improvements ESWL is not completely free of side effects. Apart from intra- and perirenal hematomas and edemas [1] arterial hypertension has been reported in some percent of patients treated by ESWL [5]. Furthermore, alteration of the glomerular and tubular function has to be considered. As described by Wilbert et al. [8] the excretion of β-2-microglobulin increases after ESWL caused by impaired proximal tubular reabsorption. Tamm-Horsfall excretion is decreased as a consequence of impaired synthesis of this glycoprotein in the distal tubules.

Proceeding from these findings we were looking for an in-vitro model to investigate the effects of high energy shock waves on renal tubular cells directly. Because of that cultured Madin Darby Canine Kidney Cells (MDCK-cells) were used. MDCK cells are an established cell line originally derived from dog kidney cortex [3].

Material and methods

MDCK cells were grown in culture flasks containing Dulbecco's modified Eagles medium and fetal calf serum. About 10 days after seeding when forming domes (indication of a certain differrentiation of MDCK cells [7]) cells were trypsinated. By this procedure cell suspensions were obtained which were solved in a medium containing Dulbecco's modified Eagles medium and fetal calf serum. The cell concentration was adjusted to 70 million cells/ml. For shock wave exposure especially designed small containers (kindly supplied by Siemens AG, Erlangen/FRG) (Fig. 1) were used which can be filled with 1.1 ml of the cell suspension thus containing about 77 million cells. It is essential to note that the containers were completely filled to avoid acoustic air-fluid interfaces within the containers.

A total of seven collectives of six containers each has been examined. The first collective served as control and was not exposed to shock waves, the other collectives underwent shock wave exposure with impulse rates of 16, 32, 64, 128 and 256 shock waves respectively. The shock waves were generated by a Dornier HM4 lithotripter. The generator voltage was 18 kV. The containers with the MDCK-cells were adjusted to the second focus of the ellipsoid by a specially constructed holder (Fig. 2).

After shock wave exposure the containers were emptied and the MDCK cell suspension was mixed with ll ml of the nutrient medium

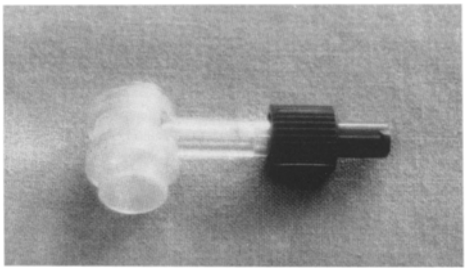

Fig. 1. MDCK cell container for shock wave exposure (Volume 1.1 ml) with silicone bottom

Table 1. LDH concentration (means and standard deviations) in the nutrient medium of controls and exposed MDCK cells. Significant difference: $*p < 0.05$; $**p < 0.01$

Time \ Impulses	0 Control	16	32	64	128	256
Immed.	265 ± 19.2	382 ± 25**	450 ± 86**	611 ± 108**	1,237 ± 275**	1,479 ± 463**
1 h	321 ± 21.2	763 ± 61**	519 ± 70**	561 ± 109**	1,057 ± 231**	1,508 ± 491**
3 h	429 ± 39.3	554 ± 58**	597 ± 112**	674 ± 58.8**	1,134 ± 246**	1,430 ± 468
6 h	846 ± 76.2	945 ± 61	860 ± 218	1,025 ± 104	1,419 ± 223**	1,882 ± 420**
9 h	891 ± 74.3	815 ± 156	957 ± 157	1,027 ± 142	1,535 ± 214*	2,055 ± 465**
12 h	953 ± 46.1	981 ± 102	1,029 ± 120	1,230 ± 88**	1,677 ± 197**	2,150 ± 411**
24 h	1,561 ± 101.9	1,618 ± 154	1,710 ± 84.5	1,973 ± 134**	2,204 ± 129**	2,590 ± 289**

LDH (X ± S)

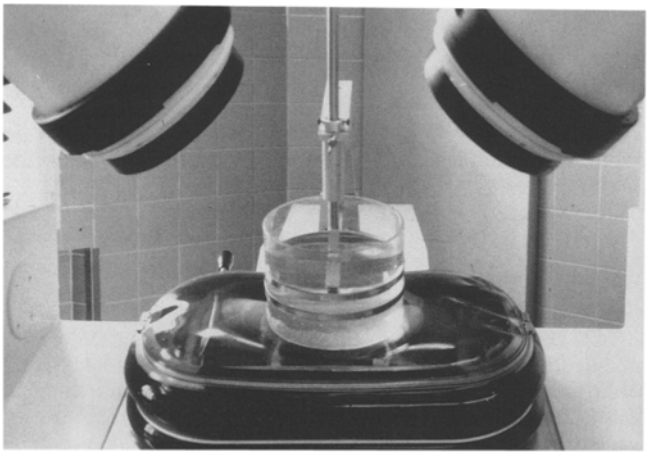

Fig. 2. Specially constructed holder for shock wave exposure of MDCK cell containers on a Dornier HM4 lithotriptor

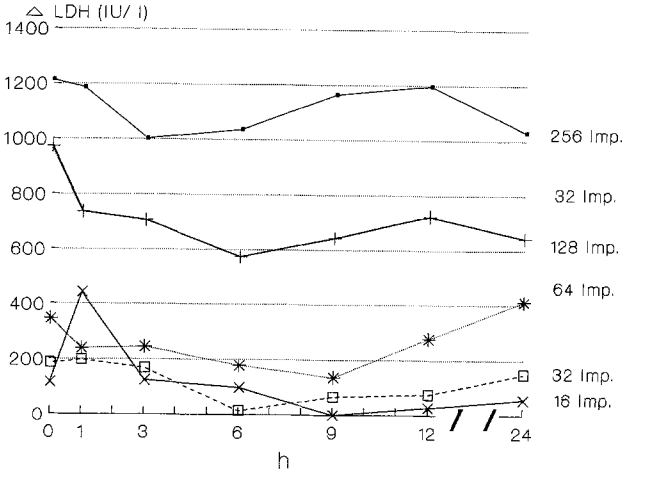

Fig. 3. LDH increase in the nutrient medium (delta LDH exposed minus control cells) immediately, 1, 3, 6, 9, 12 and 24 h after shock wave exposure

described above. The same was done with the control containers. The diluted suspension was divided into 7 equal portions of 1.7 ml each. One of these portions respectively was centrifuged immediately, 1, 3, 6, 9, 12 and 24 h later.

The supernatant was examined for lactate dehydoxygenase (LDH), glutamate oxalacetate transaminase (GOT), sodium, potassium, calcium and magnesium.

For statistical analysis the Mann-Whitney U-Test was used.

Results

The LDH concentration in the centrifuged nutrient medium increased already immediately after shock wave exposure. The increase was dependant on the number of shock waves applied. For high impulse rates of 64, 128 and 256 differences were highly significant compared to the controls (Table 1, Fig. 3).

At the various times of examination after shock wave exposure the mean differences of LDH concentration between exposed and control groups showed an s-shaped relation to the impulse rates with a linear dependence in the middle range. As an example Fig. 4 demonstrates this dependence for the time immediately after shock wave exposure.

Similar results were obtained for GOT. The mean concentration increased already immediately after shock wave exposure (Table 2, Fig. 5). At the various times of examination after shock wave exposure the mean differences of GOT concentrations showed an s-shaped relation to the impulse rates as well (Fig. 6).

The other parameters examined (sodium, potassium, calcium and maganesium) did not show any significant changes after shock wave exposure.

Discussion

Our experiments reveal a significant increase of LDH and GOT concentration in the nutrient medium after shock wave exposure. The amount of the increase is dependant on the number of shock waves applied. As LDH is a brush border enzyme and GOT occurs in the cytosol and mitochondria an extracellular increase of these enzymes marks a damage of the cell membrane. Thus high energy shock waves exerted a damaging effect on the membranes of the MDCK cells. These findings are consistent with electron microscopic changes as loss of microvilli and cell

Table 2. GOT concentration (means and standard deviations) in the nutrient medium of controls and exposed MDCK cells. Significant difference: * $p < 0.05$, ** $p < 0.01$

Time \ Impulses	0 Control	16	32	64	128	256
Immed	11.3 ± 0.47	17 ± 2.2**	13.5 ± 3.6*	14.8 ± 2.7*	22.6 ± 2.7**	25.16 ± 4.3**
1 h	11 ± 0.5	20.1 ± 2.1**	15.3 ± 3.7*	13.3 ± 2.6	22 ± 3.6**	24.5 ± 5.3**
3 h	10.6 ± 0.8	18.6 ± 0.47**	15.3 ± 3.1**	15 ± 2.6**	21 ± 3.7**	23.6 ± 3.1**
6 h	12.6 ± 2.05	20 ± 1**	16 ± 3.1*	16.3 ± 2.4*	22.6 ± 1.8**	23.8 ± 4.6**
9 h	15.6 ± 2.35	16.3 ± 1.25	17.5 ± 2.6	18.16 ± 1.2*	23.2 ± 5.2**	23.5 ± 3.1**
12 h	17.3 ± 1.37	17.3 ± 0.94	19.5 ± 1.3*	19.33 ± 2.13	25.3 ± 0.5**	25.8 ± 5.4**
24 h	17.2 ± 1.2	21.8 ± 2.5**	20.8 ± 1.2*	23 ± 1.7**	26.3 ± 1.25**	29.5 ± 3.5**

GOT (X ± S)

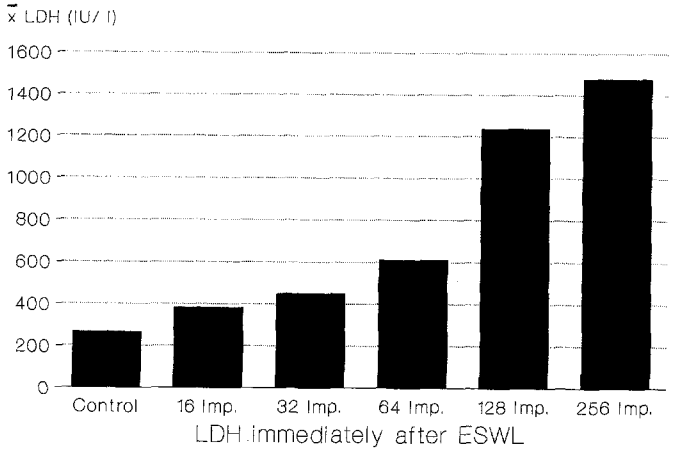

Fig. 4. LDH concentrations in the nutrient medium of MDCK cells immediately after shock wave exposure

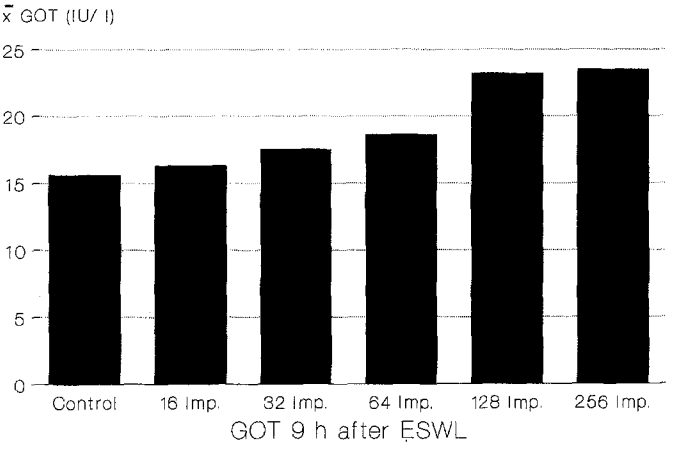

Fig. 6. GOT concentrations in the nutrient medium of MDCK cells 9 h after shock wave exposure

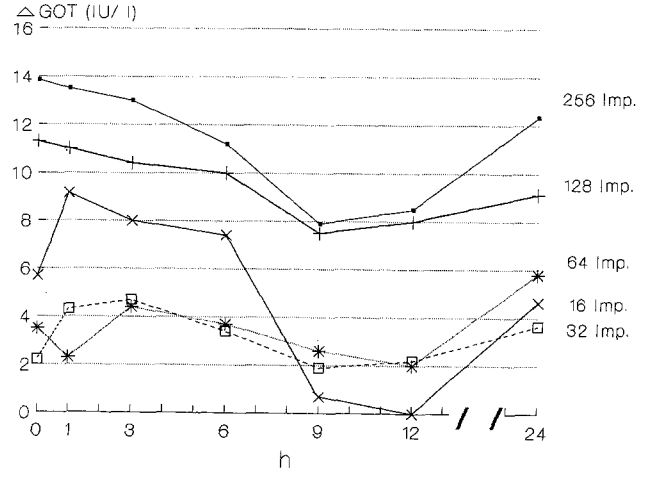

Fig. 5. GOT increase in the nutrient medium (delta GOT exposed minus control cells) immediately, 1, 3, 6, 9, 12 and 24 h after shock wave exposure

vacuolization [6]. The exact mechanism is not known yet. One possible mechanism is the formation of cavitation bubles resulting in the emission of a tissue destructing liquid jet [2].

As our in vitro results demonstrate the proteinuria and enzymuria observed after shock wave lithotripsy of renal stones [8] may be explained by a direct impairment of renal tubular cells by shock waves. The grade of damage may be different to some extent when using immobilized cells on agar gel as this has been reported for cultured tumor cells [4]. As differentiated MDCK cells form domes similar to renal tubules they are lifted from the culture dish thus normally not being completely immobilized. So cell suspensions were used in this model. As could be shown by Laudone et al. [4] for red blood cells the damaging effect of high energy shock waves on immobilized (clotted) erythrocytes was only slightly different from suspended erythrocytes in completely filled containers when compared to erythrocytes in partially filled tubes. Obviously the air-fluid interface enhances disruption of the cell membrane by cavitation effects. As we have used cell suspension in completely filled containers the damage may be only gradually different from immobilized cells. Thus, this model of MDCK cells seems to be suitable for further investigation on the mechanisms of shock wave induced impairment of renal tubular function especially for comparison between the different types of lithotripters and their effects on tubular cells. It is essential, however, always to examine a negative control group not under-

going shock wave exposure for correct interpretation of the results obtained.

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