Shock Waves and Free Radicals: Cell Protection by Vitamin E *in vitro* and *ex vivo*

Dierk SUHR, Franz BRÜMMER, Uwe IRMER, Manfred SCHLACHTER*, and Dieter F. HÜLSER

University of Stuttgart, Biology Institute, Biophysics Department,
Pfaffenwaldring 57, D-70550 Stuttgart, Germany, and
*F. Hoffmann-La Roche Ltd., CH-4002 Basle, Switzerland

The application of extracorporeal generated shock waves in medicine for the fragmentation of human kidney and gall stones proved to be a very successful technique. Shock wave lithotripsy, however, is not free of tissue damaging side effects. One major mechanism for the fragmentation of stones as well as for the side effects is cavitation, *i.e.* the formation and movement of bubbles in liquids exposed to tensile forces. Collapse of cavitation bubbles is accompanied by local "hot spots" of several 1,000 K, thus generating free radicals. We investigated the contribution of these free radicals to cellular injury by varying the cellular amount of a well known scavenger of free radicals, α-tocopherol.

Keywords: lithotripsy, cell damage, α-tocopherol

INTRODUCTION

Since 1980 a new technology for the treatment of urinary and biliary calculi revolutionized the treatment of stone disease. Shock waves, generated extracorporeally by underwater spark discharge, electromagnetic or piezoceramic reflection destroy human stones inside the body when focussed on them. The so-called extracorporeal shock wave lithotripsy was such a successful technique that it nowadays became the method of choice. The shock wave treatment, however, is not free of side effects, like haematuria, haematoma, and temporary reduction of organ function (Reviews: [1,2]). These damaging effects have also been demonstrated using cell cultures (*e.g.*, [3-7]). One major mechanism discussed for these side effects is cavitation, *i.e.*, the generation and movement of bubbles in liquids exposed to tensile forces. During the collapse of these cavitation bubbles, gas or vapour inside the bubble is nearly adiabatically compressed without heat exchange with the surrounding medium, generating "hot spots", where the temperature rises up to several 1,000 K. This high temperature can lead to a homolytic cleavage of molecules, causing the generation of free radicals, short living, highly reactive atoms or molecules. These cavitation-generated free radicals cannot only be detected in water [8,9] but even in shock wave treated cells [10]. Their involvement in cell killing, however, is discussed controversially [6,10]. We, therefore, varied the status of antioxidative vitamins of rat erythrocytes *in vivo* and of L1210 mouse leukemia cells *in vitro* using all-rac-α-tocopherol, a well known scavenger of free radicals, and investigated the resulting cell damage after shock wave treatment.

MATERIAL & METHODS

L1210 Cells *in vitro*

The permanently growing suspension cell line L1210 was cultured for 14 days in the absence or presence of different concentrations of α-tocopherol. Vitamin E was applied as an emulsion of 5% α-tocopherol and 15% modified fish gelatine, or as pure α-tocopherol, partially emulsified in the culture medium by sonication. Cells were washed twice before treatment, so that only α-tocopherol incorporated in the cell membrane could be effective. Shock waves were applied in an experimental Dornier spark gap lithotripter XL-1 (generator voltage 18 kV, capacitor 80 nF). Water in the lithotripter bath (37°C) was partially degassed (~2 mg O_2/l). After administration of 250 shock waves at a repetition frequency of 1 or 8 Hz, cell viability was determined using a cell counter and a double staining technique: cells that were able to enzymatically hydrolyze the membrane-permeable dye fluorescein-diacetate and exhibit a green fluorescence of accumulated fluorescein, were considered as viable, whereas cells which cannot exclude the membran-impermeable dye propidium iodide and exhibit a red fluorescence are physiolgically damaged and must be regarded as dead. The amount of viable and dead cells was determined using flow cytometry [4]. Additionally, the growth rate of cells after shock wave treatment and untreated controls were compared for at least 9 days by seeding equal concentrations of cells and counting them daily.

Rat Erythrocytes *ex vivo*

Rats were kept for 6 weeks on a adequate or tocopherol-free diet [11]. After tocopherol depletion of the rats, ascertained by an increased serum pyruvate kinase activity [11], blood was obtained by heart puncture and stabilized by citrate and dextrose. Blood samples of each animal were split for three treatments, and the cells were washed with phosphate buffered saline for shock wave treatment. After treatment with 250 shock waves at a repetition frequency of 1 Hz, cells were centrifuged, the number of intact cells was determined in an electronic cell counter, and the amount of free haemoglobin in the supernatant was determined photometrically.

RESULTS

In all three experiments - the viability of L1210 cells, the growth rate of L1210 cells, and the haemolysis of rat erythrocytes - a difference between low and high content of α-tocopherol cells could be demonstrated after shock wave treatment.

L1210 cells supplemented with α-tocopherol were less damaged in both acute and long term investigations (table 1 & fig. 1). The acute viability of L1210 cells exposed to 250 shock waves after culture in the presence of α-tocopherol revealed a protective effect of the highest concentration (table 1). The protection of 10^{-2} M α-tocopherol was more pronounced for a shock wave repetition frequency of 8 Hz, where more free radicals are produced than at 1 Hz [8,10].

A more sensitive method than the determination of acute cell death after shock-wave treatment may be the investigation of long-term effects, since cells injured by shock waves and free radicals will be altered physiologically. As could be demonstrated with α-tocopherol pretreated L1210 cells exposed to 250 shock waves, a concentration

dependent protective effect is visible after a lag-phase of 72 h (fig. 1).

Erythrocytes from tocopherol depleted rats showed an enhanced haemolysis compared to adequate fed animals (table 2). The percentage of geometrically destroyed erythrocytes after 250 shock waves, as determined with a cell counter, was 17.5 ± 1.4% for the control animals (mean ± s.d of n = 6 animals) versus 30.6 ± 5.3% for the tocopherol-depleted rats (n = 5).

CONCLUSIONS

From our results, we conclude an involvement of cavitation-generated free radicals in shock wave induced cell damage *in vitro*. Scavengers of free radicals may be also helpful for clinical use *in vivo*.

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Table 1 Vital L1210 cells after 250 shock waves

pulse repetition frequency	1 Hz	8 Hz
without tocopherol	45.9 ± 4.4%	51.7 ± 3.9%
supplemented with 10^{-2} M toc.	54.2 ± 1.8%	66.4 ± 1.4%

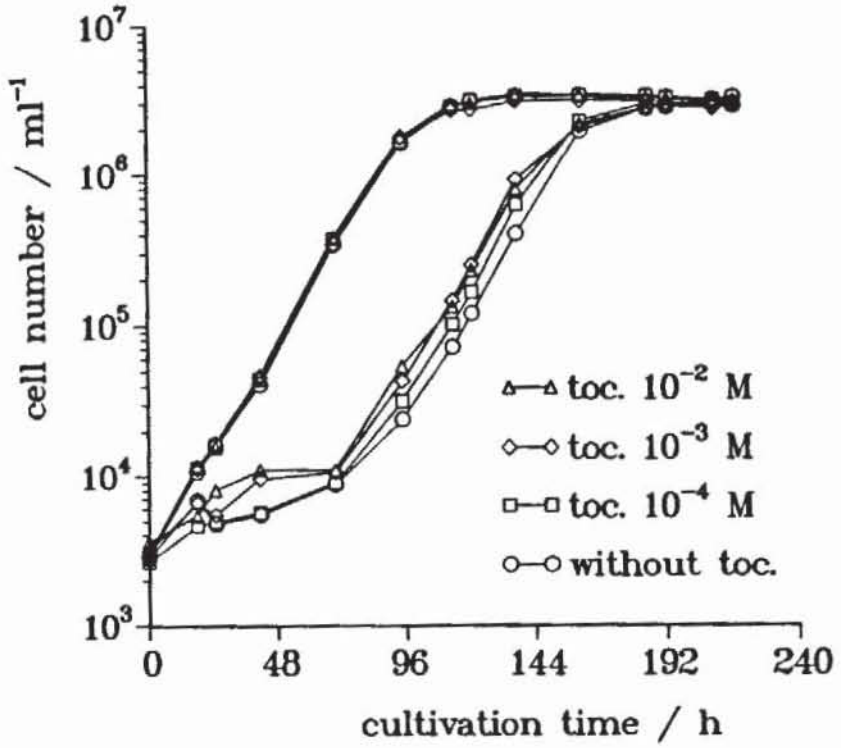

Fig. 1 Growth of L1210 cells after a 14-day incubation with different concentrations of α-tocopherol. Upper curves: controls, lower curves: after 250 shock waves. For further information see text.

Table 2 Destroyed erythrocytes (%) after 250 shock waves

	animal #	mean	s.d.
control group	1	17.2	3.0
	2	19.1	3.8
	3	18.0	1.4
	4	18.2	1.0
	5	14.9	3.6
	6	17.5	2.5
tocopherol-depleted group	7	32.2	2.7
	8	34.6	1.1
	9	32.9	1.6
	10	32.1	2.1
	11	21.3	2.5

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