Toxin and Gene Transfer into Cells by Extracorporeal Shock Waves: in Vitro and in Vivo Effects

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Abstract: Shock wave application to cells in vitro causes a transient increase of the permeability of the cell membrane which does not lead to cell death. It was hypothesized that shock waves might be a new method of transferring therapeutic agents directly into cells. To test this, biological effects resulting from the acoustical transfer of proteins and nucleic acids into cells were examined. Protein transfer was examined with the ribosome inactivating proteins gelonin and saporin. Dose response curves were established with tumor cell lines in the presence and absence of shock waves. Compared to the controls, shock waves enhanced the action of gelonin and saporin from 300 to 40,000 fold. In vivo experiments with an animal tumor model established that acoustic transfer of these agents into cells occurred also in vivo. Gene transfer by shock waves was examined in vitro in a number of cell lines with plasmid vectors carrying standard reporter genes. Transfer succeeded yet the transduction efficiency was lower than with other established methods of gene transfer. In vivo experiments revealed a similar result.

INTRODUCTION

Extracorporeal shock waves are clinically used for lithotripsy (1,2). A few years ago it was shown that shock waves cause a transient increase in the permeability of the cell membrane which does not lead to cell death when the waves are applied to cells in vitro. Molecules with sizes > 2 MDa can be transferred directly into the cellular cytoplasm. The effect is mediated by cavitation and is suppressed by minimal static excess pressure in the exposure vial (3).

In the following it was examined whether shock waves can be used to transfer two sorts of molecules into the cytoplasm of cells and exhibit a defined action. In vitro and in vivo experiments were performed with

1. ribosome inactivating proteins (RIP) with a molecular weight of 30 kDa. They act by a strong inhibition of protein synthesis. The toxic effect of RIPs leads to cell death.
2. nucleic acids. They were considered to assess the possibility of gene transfer. Using standard plasmids the de novo synthesis of marker proteins was assessed

METHODS AND RESULTS

RIP transfer in vitro
Cells of different tumor cell lines were incubated for 40 minutes with the RIPs gelonin or saporin (or others) and exposed to shock waves (250 discharges at 25 kV in a Dornier XL1 experimental lithotripter). Controls were not exposed to shock waves. The MTT test was used to assess cell proliferation and toxicity. Dose-response curves were obtained. Enhancement factors were calculated from the ribosome inactivating protein concentrations which lowered the cell proliferation to 50% (IC50) (Fig. 1). Shock waves enhanced the effect of shock waves in all cases and shifted the IC50 from the mM to the nM range. The enhancement factors varied from 300 to 40,000. A typical example is shown in the figure on the left.

Fig. 1: Effect of shock waves with saporin (+SW) or saporin without shock waves (-SW) on SSK2 fibrosarcoma cells. The enhancement factor was 7,000 (range 6,000-8,000) as calculated by nonlinear regression analysis.

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RIP transfer in vivo

SSK2 fibrosarcoma tumors (5-8 mm diameter) were grown under the dorsal skin of C3H mice and divided into four treatment groups (Fig. 2). Shock wave exposure was with 500 discharges of the XL1 lithotripter focused to the tumor under water immersion (experiment 1) or with 3 times 500 discharges 2 hours apart (experiment 2). Tumor growth curves were determined and Kaplan Meier curves were generated. The event was reaching a tumor volume of 2.5 cm³.

A single shock wave exposure in experiment 1 did not reveal a difference in tumor growth in all four treatment groups.

An example of the effect of three consecutive shock wave treatments in experiment 2 is shown on the left.

Fig. 2: Kaplan Meier curve after three shock wave treatments. The first group was exposed three times to shock waves after injecting saline intraperitoneally, the second three times to shock waves after injecting 1 mg gelonin intraperitoneally, the third after injecting saline without shock waves and the fourth after injecting 1 mg gelonin without shock waves. In 40% of the tumors treated with shock waves and gelonin no tumor regrowth was detected over a period >180 days.

The result demonstrates for the first time that membrane permeabilization of a tumor by shock waves can transfer cytotoxic substances into it and induce long term tumor remissions.

Gene transfer in vitro

A number of cell lines were exposed to shock waves in suspension (250 discharges at 25 kV in a Dornier XL1 experimental lithotripter) in the presence of a reporter plasmid (4). The transfection efficiency was assessed by determining the number of transfected cells or the amount of protein secreted into culture. The transfection by shock waves was compared to the transfection by electroporation, another established method of gene transfer. The transfection efficiency from shock waves was found to depend on the number of discharges and the amount of plasmid added. It was in a similar range as the efficiency from electroporation. Compared to other established methods of gene transfer the efficiency of shock waves was low (<1%).

Gene transfer in vivo

The first approach to the gene transfer situation was removal of a tumor under anesthesia and its transfer into a test tube. Subsequently, plasmid DNA was injected directly and shock waves were applied. The next step was shock wave exposure of a tumor in vivo with tumor removal directly afterwards. The third experiment was exposure to shock waves in vivo and tumor removal after 24 hours. Single cell suspensions were made and screened for transfected cells.

In all protocols, the number of transfected cells was 3-5 fold increased by the shock wave application. The number of transfected cells 24 hours after shock wave exposure was very low (0.002% positive cells).

CONCLUSIONS

1. Extracorporeal shock waves can be used to transfer substances into cells and document the resulting changes both in vitro and in vivo.
2. Shock waves enhance the in vitro action of ribosome inactivating proteins by four orders of magnitude.
3. Treatment with shock waves and RPs can induce lasting long term remissions in vivo.
4. Shock waves are a novel method of gene transfer in vitro and in vivo. The transfection efficiency was found to be quite low. To enhance efficiency further modifications are required (e.g. autogene expression systems).

References