194g Experimental and Theoretical Modeling of Intracellular Drug Delivery Following Acoustic Cavitation

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Acoustic cavitation can irreversibly lyse cells, shatter kidney stones, and drive chemical reactions. Recently, cavitation has also been shown to reversibly increase plasma membrane permeability to drugs, proteins and DNA in living cells and animals independently of cell or drug type, suggesting a ubiquitous mechanism; however, this mechanism has not been previously defined. It was recently shown that cavitation generated by ultrasound facilitates cellular incorporation of macromolecules up to 28 nm in radius through repairable disruptions in the plasma membrane for as long as 4 min after exposure. Evidence of micron-scale membrane disruptions in cavitation-exposed cells was also found using electron and fluorescence microscopy. The aim of this study was modeling of drug transport through cellular membranes reversibly disrupted by acoustic cavitation. Fluorescent molecules with different sizes (gyration radii - 0.6 nm calcein, 3.5 nm bovine serum albumin and FITC-dextrans from 1.4 to 28 nm) were added to cell suspensions prior to or at defined time intervals after ultrasound treatment. Cells were allowed to recover for 15 min at room or body temperature, washed to remove extracellular fluorescent solution and analyzed by flow cytometry for viability and intracellular uptake. In this study we used two different types of pulsed ultrasound treatment – by 24 kHz (0.7 MPa, 2 s on-time) and 1.1 MHz (0.5-2 MPa, 0.01-1 s on time) ultrasound in the presence of stabilized perfluorocarbon bubbles (Optison) to serve as cavitation nucleation sites. Characteristics of intracellular transport and cellular recovery were similar for both types of ultrasound treatment thus consistent with a common mechanism of bioeffects caused by sonication. Analysis of experimental results revealed two phases of drug uptake after sonication. First, fast uptake occurred in the first seconds after sonication. This stage accounted for up to 60% of overall drug uptake depending on the molecule studied. In this phase transport was likely driven by highly localized convective jets caused by cavitation and/or osmotic fluxes caused by differences in ion concentration across the disrupted membrane. Our estimation showed that on the second phase (1 s – 15 min) transport was primarily driven by passive diffusion through long-lived holes in the membrane. A combination of analytical and numerical methods was used to model drug transport through the cell membrane. We analyzed passive diffusion transport through randomly damaged membrane and showed that the radius of the damaged area is a better indicator of its permeability than total area. Analysis of intracellular uptake for molecules of different sizes revealed sizes of membrane disruptions at different stages of membrane healing. The effective sizes of membrane disruptionss depended on molecular size (due to diffusional hindrance) and time after sonication (due to wound healing). Our estimation showed that the initial size of the disruption measured by calcein transport immediately after sonication was of the order of a micron. A few seconds after disruption, the microstructure of the membrane wound resealed sufficiently to effectively hinder passive diffusion of macromolecules on the order of tens of nanometers in size. The effective mesh size of the healing membrane as well as the disruption size progressively became smaller over time ($t_{1/2} \sim 20$-40 s) until even the smallest used molecule, calcein, could not penetrate through it. The proposed model allows characterizing the mechanism of drug transport and identifying characteristics of reversibly damaged membranes. This information can be used to better design ultrasound-based methods to target drug and gene delivery to required sites, minimize side effects, lower drug dosages, and improve efficacy.