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Ultrasound-induced bbb opening: a morphological study in an in vitro cellular model

Jacopo Junio Valerio Branca[1], Matteo Boninsegna[2], Gabriele Morucci[1], Donatello Carrino[1], Massimo Gulisano[1], Leonardo Bocchi[3], Alessandra Pacini[1]

[1] University of Florence, Department of Experimental and Clinical Medicine, Florence
[2] University of Florence, Department of Information Engineering, Florence
[3] University of Firenze, Department of Information Engineering, Florence

Degenerative diseases of the central nervous system are significant causes of mortality among aging population in industrialized countries, as well as expensive for the national health systems and caregivers [1]. Nowadays, pharmacological therapies are still not decisive because they are hindered by the blood brain barrier (BBB) [2]. Although in the last decades many researchers have conducted in vivo experiments to better identify a therapeutic approach of ultrasound (US) in neurodegenerative diseases, little is known about their role in increasing the BBB permeability [3,4]. The present study aims to identify, the effect of focused US on a rat brain endothelial cell line (RBE4). After ultrasound stimulation (10-20-30 min.), MTT and western blotting assay were conducted to demonstrate the non-toxic effects of US. Furthermore, immunostaining of stimulated cells was performed to detect changes in cytoskeletal F-actin fibers and Zonula occludens-1 (ZO1) tight juntion. BBB opening was evaluated by measuring the extent of the intercellular space, in Papanicolaou stained cells. The results evidenced an ultrasonic-dependent mechanical action on F-actin fibers that altered their distribution within the cells showing the formation of numerous stress fibers. F-actin alterations were accompanied by an alteration of the ZO-1 distribution, exhibiting a "zipper-like" staining pattern and holes that became visible between cells. Papanicolaou staining confirm the opening of the BBB evidencing many wider areas of intercellular space. All these structural changes on RBE4 cell line occurred without significant alterations in metabolic activity as well as in absence of apoptotic or endoplasmic reticulum stress markers. In conclusion, these results confirm and highlight the potential role of ultrasound in the permeabilization of the BBB, thus suggesting new ways for drugs administration.

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Key words

BBB in vitro model, RBE4 cell line, permeabilization, ultrasound.