AIRWAY CELL EXOSOME UPTAKE IN MICROGLIA IS RELATED TO MITOCHONDRIAL SUPPRESSION AND ROS PRODUCTION

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Upper respiratory tract infections are the most common [1], and approximately 80% of them are caused by viruses [2]. The viruses do not spread far from the infection site, but an inflammatory signal can be transmitted through liquid and exosomal communication [3]. Previous studies have shown that the exosomes from virus-primed cells may have viral genetic material or other inflammatory factors [4]. Exosomes can pass membrane barriers, including the brain blood barrier, and transmit the inflammatory signal away from periphery to the brain [5]. It is known that immune cells including microglia respond to viral infection via mitochondrial antiviral signaling protein including changes in mitochondrial function and increased production of reactive oxygen species (ROS) [6]. However, it is not clear whether exosomes from virus-affected airway cells might induce similar immuno-metabolic changes. The aim of our study was to analyse the effect of exosomes from virus infection-mimicking polyinosinic polycytidylic acid (poly (I:C)) sequence-primed respiratory tract cells (RTC) on mitochondrial functions and on the formation of ROS in primary rat microglia.

Primary microglial cells were isolated from mixed glial culture that was prepared from the brain hemispheres of 5-7 days old Wistar rats. Exosomes were isolated from the culture medium of heterogeneous rat RTC culture by "Total Exosome Isolation Reagent" (Thermo Fisher Scientific) according to the manufacturer protocol. For simulation of viral infection, the RTC were stimulated with 1 μg/ml poly (I:C). The amount of exosomal protein was determined by spectrophotometric Bradford assay. Microglial cells were identified using fluorescence microscope after staining them with Isolectin GS-IB4-Alexa Fluor 488 conjugate, and RNA of exosomes was labeled by a specific die conjugated with Alexa Fluor 555 (BLOCK-iT Alexa Fluor Red Fluorescent Control, Thermo Fisher Scientific). Respiration of microglial mitochondria was evaluated by Oroboros Oxygraph-2k. ROS formation in microglial cells was determined by DCFDA fluorescence intensity calculated from fluorescent images by ImageJ software. Statistical data analysis was performed by SigmaPlot 12.5 software.

The study revealed that exosomes from both poly I:C-primed and not primed RTC enter microglia within 30 min by direct pathway (endocytosis, phagocytosis or micropinocytosis). After 16-18 hours of incubation both primed and unprimed exosomes significantly reduced the activity of oxidative phosphorylation of microglial mitochondria by suppressing the respiratory chain and increased ROS production. The level of ROS induced by exosomes was similar to that caused by 3 hour direct treatment of microglia with 1 μg/ml poly (I:C). The obtained data show that exosomes from both poly I:C-primed and not primed RTC similarly affect mitochondrial respiration and ROS production in microglia.

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