Characterization of plasma extracellular vesicles of sleep-restricted C57/BL6 mice

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INTRODUCTION: Extracellular vesicles (EVs) can be classified as: exosomes, microvesicles and apoptotic bodies. EVs are involved in many biological processes, can be found in a lot of fluid bodies, and they are an important factor in cellular intercommunication, where they can share proteins, genetic material and other structures. But up to now the origin, direction, secretion and destination of these EVs are still little known. So our research group decided to analyze the content of the EVs protein profile presented in plasma of submitted sleep-restricted mice.

OBJECTIVE: Collect and characterize plasma EVs of submitted 21 days protocol sleep-restricted mice, and then analyze these proteins content in liquid nano chromatography and mass spectrometry.

METHODS: We utilize two groups of C57/BL6 mice, experimental and control. Sleep-restriction begins in day one, and the experimental group was submitted to restriction for 21 consecutive days, when they stay 18 hours awake and 6 hours asleep. After the sleep-restricted protocol, we took animals blood, isolated plasma and freeze it in -80ºC for storage. Extracellular vesicles were isolated by the Total Exosome Isolation Kit, it was performed Dot Blot to check biomarkers CD9, CD81 and CD69, and for concentration and size we used Nanoparticle Tracking Analysis (NTA) in Nanosight NS300, and the protein profile was processed in mass spectrometry by Orbitrap and will be analyzed in Perseus.

RESULTS: The analysis by NTA presented that the most EVs of the experimental group had an average diameter of 165 nm, and the control group 197 nm (there was no significant difference), In proteomic analysis based on data from both groups and realized by Orbitrap we found 76 proteins.

CONCLUSION: Our NTA results show us that the EVs from restricted mice group are smaller than the control group, suggesting a possible influence of the sleep deprivation on EVs morphology. In the next step analyzing these 76 proteins on Perseus software, our expectation is to find some significant protein expressions differences between the groups, which will give us good insights to understand better the different characterization between them.

Agradecimentos: Agradecemos às agencias de fomento, instituições e colaboradores envolvidos no desenvolvimento do projeto.