INHIBITION OF AUTOPHAGY BY CHLOROQUINE STIMULATES NITRIC OXIDE PRODUCTION AND PROTECTS ENDOTHELIAL FUNCTION DURING SERUM DEPRIVATION

Pestana, C.R.¹; Oishi, J.C.²; Salistre-Araújo, H.S.²; Rodrigues, G.J.²

¹Instituto Latino-Americano de Ciências da Vida e da Natureza, Universidade Federal da Integração Latino-Americana, Foz do Iguaçu, Paraná, Brazil; ²Departamento de Ciências Fisiológicas, Universidade Federal de São Carlos, São Carlos, São Paulo, Brazil

Introduction and objectives: Autophagy plays a fundamental role in cell survival under stress conditions such as nutrient deprivation. Decreased nitric oxide (NO) production, which may contribute to vascular dysfunction, is one of the consequences of autophagy in endothelial cells. The antimalarial drug chloroquine (CLQ) has been proposed as an adjuvant chemotherapy in other diseases. CLQ inhibits autophagy by blocking autophagosome formation. This work investigates the stimulation of NO production by autophagy inhibition chloroquine in endothelial cells under deprivation.

Materials and methods: Autophagy was induced by serum deprivation in Human Umbilical Vascular Endothelial Cells (HUVEC) as demonstrated by formation of Acidic Vesicular Organelles (AVOs), conversion of Microtubule-associated protein 1 light chain (LC3) and Sequestosome-1 (SQSTM1/p62) degradation. NO production was determined by diaminofluorescein-2 (DAF-2) fluorescent dye and cell proliferation using MTT assay. NO/endothelium-dependent vasorrelaxation were conducted in an ex vivo rat aortic ring model pre-constricted with phenylephrine.

Results and conclusions: The inhibition of autophagy by CLQ restored NO levels, preserved morphology as well as proliferation of HUVEC under serum deprivation. Interestingly, the incubation of ex vivo rat aortic rings with CLQ resulted in endothelium-dependent relaxation mediated by the increase of NO. These findings emphasize the importance of autophagy in endothelial function and demonstrate the potential use of autophagy inhibitors to protect vascular function during nutrient deprivation.

Keywords: autophagy; chloroquine; vascular reactivity

Acknowledgements: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) process number 475715/2013-6 and Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp) process number 12/24477-8.