The Resveratrol Caspase-mediated Apoptosis Effect is Accompanied by Mitochondrial Membrane Potential Impairment and ROS Production

Martins, L.A.M.\textsuperscript{1}; Vieira, M.Q..\textsuperscript{1-2}, Vasconcelos, M.\textsuperscript{1}, Ilha, M., Guma, F.C.R.\textsuperscript{1}

\textsuperscript{1}Departament of Biochemistry, ICBS – UFRGS, Porto Alegre/RS, Brazil, \textsuperscript{2}Electron Microscopy Center, UFRGS, Porto Alegre/RS, Brazil

INTRODUCTION Mitochondria play a central role in many cell death pathways. Apoptosis and autophagy are two dependent events in which signalling can be mediated by impaired mitochondria. Indeed, mitophagy may remove damaged organelles contributing to the cell surviving. We previously found that treatment with Resveratrol, a phytoalexin related to the beneficial effects of red wine, increased autophagosomes and promoted mitophagy in GRX, a hepatic stellate cell line. Here, we analysed the Resveratrol-mediated cell death in GRX. We also evaluated the mitochondrial membrane potential, this organelle superoxide production, and intracellular reactive oxygen species (ROS).

MATERIALS AND METHODS GRX were treated for 24h with Resveratrol diluted in medium (1, 10 and 50µM). The most assays were performed by flow cytometry. Mitochondrial membrane potential was accessed by JC-1. Cell death was evaluated, using annexin V and Propidium Iodide as apoptosis and necrosis indicator. The caspase activation was probed by FITC-VAD-FMK. The mitochondrial superoxide was quantified using MitoSox. The intracellular reactive species (RS), among which H$_2$O$_2$, were measured by DCFH-DA. Optic microscopy images were also acquired to access cell morphology and population density.

RESULTS AND DISCUSSION Resveratrol doses at 10 and 50 µM decreased mitochondrial membrane potential. However, while all treatments induced cell death by caspases-mediated apoptosis, 50 µM treatment also promoted cell death by necrosis, justifying the visible decrease of cell density showed by optic microscopy images. While 1µM of Resveratrol increased mitochondrial superoxide, 10 and 50µM decreased this molecule production. Paradoxily, there were an increase of intracellular RS in cells treated with 10 and 50µM of Resveratrol. The mitochondrial superoxide decrease could be associated to the lower mitochondrial activity. Also, the increased intracellular H$_2$O$_2$ may be related to the superoxide dismutation.

CONCLUSION More studies are underway to better understand these findings.

Keywords: Caspases-mediated apoptosis, hepatic stellate cells, mitochondrial ROS, resveratrol.

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