Title

Effects of TSPO and STAR inhibitors on cell death in a cardiomyocyte model of hypoxia-reoxygenation

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Key words

Hypoxia-reoxygenation TSPO-STAR

Introduction We previously demonstrated that reperfusion of an ischemic myocardium induces an increase in mitochondrial cholesterol (CL) content accompanied by a generation of oxysterols. The translocator protein (TSPO) and the steroid acute regulatory protein (STAR) are involved in CL transport at the mitochondrial membrane in steroidogenic tissues but in the heart their role remain uncertain. The TSPO ligand 4'-chlorodiazepam (4'CDZ) has been demonstrated to inhibit STAR and sterols mitochondrial accumulations and to reduce infarct size. These data suggest that targeting mitochondrial sterol accumulation could participate to the protective effects of TSPO ligands. **Objective** To analyze the mechanisms and the role of the mitochondrial CL transport in cell death with a cardiomyocyte model of hypoxia-reoxygenation (HR). **Method** AC16 human cardiomyocytes were submitted to different durations of hypoxia (1% O_2) followed by reoxygenation (21% O_2) to achieve 50% mortality. Cells were then treated at reoxygenation with 4'CDZ and novel TSPO and STAR inhibitors, known to delay steroidogenesis *in vitro* by targeting CL specific binding sites of these proteins (CRAC and START, respectively). Cell mortality was assessed with MTT and crystal violet assay and CL was identified by means of fluorescent probes. **Results** HR induced 41±3% mortality and modified membrane CL pattern in the cells. In this model, 4'-CDZ (10 µM) did not display cardioprotective effect (44±3% mortality). Similar results were observed with the TSPO inhibitors (CRAC benzamide 100 µM, CRAC triol 100 µM) and the STAR inhibitor (START triol 100 µM) (46±1% 43±3% and 54±3% mortality, respectively). **Conclusion** This preliminary study suggests that TSPO ligands do not exert cardioprotection through a direct action on cardiomyocytes. This conclusion needs to be confirmed with the use of adult primary cardiomyocytes.