Simulated ischemia/reperfusion injury in an in vitro hypercholesterolemic comorbidity model in cardiac myocytes

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Introduction:
Metabolic diseases including hypercholesterolemia are risk factors for ischemic heart disease. We have previously shown that endogenous cardioprotective mechanisms are largely impaired in in vivo and ex vivo hypercholesterolemic models. In vitro hypercholesterolemic cell culture models would be suitable for exploration of intracellular signaling pathways or cardioprotective drug testing in hypercholesterolemia and ischemia/reperfusion comorbidity conditions.

Aim:
Our aim was to set up an in vitro comorbidity model of ischemia/reperfusion and hypercholesterolemia in cardiac myocytes.

Methods:
Cardiomyocytes were isolated from neonatal rats and cultured for 3 days in control or cholesterol-containing supplement (SP) with increasing concentrations of components (group SP1, SP2, SP3). Filippin staining was performed to quantify the membrane cholesterol content changes. We applied a 4-hour simulated ischemia followed by 2-hour of simulated reperfusion (SI/R). Normoxic groups served as controls. After the SI/R the cell viability was measured. For the assessment of oxidative stress, 2',7'-dichlorodihydrofluorescein-diacetate staining (for reactive oxygen species - ROS) and dihydroethidium (for superoxide level) were applied.

Results:
The membrane cholesterol concentration was increased in the treated groups (SP1: 135.8±13%, SP2: 170±20%, SP3: 190±26%). The viability of neonatal cardiomyocytes decreased significantly at the highest cholesterol concentration after SI/R (SP1: 135±35%, SP2: 132±40%, SP3: 58±28%). In normoxic condition, superoxide level elevated significantly (SP1: 137±11%, SP2: 134±19%, SP3: 183±14%) and ROS level in neonatal cardiomyocytes elevated only after SI/R (SP3: 252±157% - all data are expressed in percentage compared to the vehicle control groups).

Conclusion:
We have found that SI/R injury in the presence of hypercholesterolemia aggravated cell death and increased oxidative stress in vitro, which mimics the effect of hypercholesterolemia in vivo. Therefore, the present in vitro comorbidity model may be suitable for testing SI/R injury in the presence of hypercholesterolemia.