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P341

Hydrogen sulphide treatment of human adipose derived stem cells increases their proliferation, decreases their mitochondrial activity and enhances their antioxidant defenses

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Purpose: The potential of cell-based therapies in diseases involving ischemia-reperfusion is greatly hampered by the excessive loss of the administered cells in the harsh, oxidative environment. Our earlier studies indicated that preconditioning of human adipose derived stem cells (ASC) with the hydrogen sulphide donor sodium sulphide (NaHS) increased their survival and efficacy in an in vitro model of cell-based therapy for myocardial infarct. Our current aim was to better understand the mechanism of action of sodium sulphide on ASCs.

Methods: ASCs were treated with 0.3 μ M (ASC-0.3), 3 μ M (ASC-3) or 30 μ M (ASC-30) NaHS 4 times with 3 days between treatments whereas a control group (ASC) received vehicle. The effect of endogenous hydrogen sulphide was investigated using the cystathionine- γ -lyase inhibitor propargylglycine

(PAG, 10 mM). Proliferation was followed by microscopical evaluation. Mitochondrial activity was measured by resazurin test at the beginning of the treatments and on the 9th day. The changes are expressed as percentages of the initial value. The antioxidant effect of NaHS pretreatment (3 and 30 μ M) was examined after H₂O₂ treatments (2 hours, 2 mM) by lactate dehydrogenase (LDH) release.

Results: By the 9th day NaHS dose-dependently increased the proliferation of cells (ASC: $234 \pm 25\%$; ASC-0.3: $331 \pm 67\%$; ASC-3: $405 \pm 61\%$ ****; ASC-30: $471 \pm 33\%$ ***, vs control, * $p < 0.05$, **** $p < 0.0001$). There was no change in the overall mitochondrial activity as measured with resazurin test. PAG treatment decreased the proliferation from the 3rd day (control: $156.2 \pm 10.3\%$; 10 mM PAG: $81.7 \pm 4.7\%$ ****). LDH-release was reduced when cells were pretreated with 3 μ M NaHS (control: $90.3 \pm 7.9\%$; 3 μ M NaHS: $67.0 \pm 5.7\%$ **; 30 μ M NaHS: $89.5 \pm 10.2\%$, $p < 0.01$).

Conclusions: Hydrogen sulfide can dose-dependently increase the proliferation of human adipose derived stem cells. The unchanged overall mitochondrial activity of increased amount of cells indicates a decreased cellular metabolism that may lead to reduced release of reactive oxygen species from the mitochondria. Antioxidant defenses are more efficient after treatment with 3 μ M NaHS. These combined effects potentially explain the beneficial outcome of NaHS pretreatments of ASCs found in our in vitro model of cell-based therapy for myocardial infarct.