

Hypothermia reduces inflammation in a co-culture model of neuronal and microglial cells

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Background:

Deep Hypothermia is a standard method for neuroprotection during cardiac surgery in children. However, the cellular mechanisms which are induced by deep hypothermia have not been clearly understood in vitro. Therefore we investigated the effects of deep hypothermia and rewarming on the co-culture model of a murine BV-2 microglial cell line and a murine HT-22 neuronal cell line.

Methods:

HT-22 neuronal cells and BV-2 microglial cells were co-cultured 24 hours before the experiment at 37°C. The co-culture cells were exposed to 17°C for 2 hours and then slowly rewarmed to 37°C within 2 hours. Non-specific microglial cell activation was performed by incubation of cells for 4 hours with 1µg/ml Lipopolysaccharid (LPS). In addition, the cells were damaged with 100 mM Glutamate to imitate neuronal damage for 4 hours. Morphology was documented using phase- contrast microscopy. The viability of the co-culture model was quantified by MTT assay. Flow cytometric analysis was performed with CD11b to differentiate both cell types. IL-6, MCP-1 and TNFα levels were measured as markers inflammation.

Results:

There was no significant difference in cell viability under hypothermic conditions in comparison to the control cells kept at 37°C for 24h.

Furthermore deep hypothermia led to morphological changes. from a ramified and resting status under 37°C to amoeboid shaped cells under 17°C even without LPS stimulation. Neuronal cells change their network structure. Under 37°C the long neurites are connected, while under 17 °C neuritis become short and cell bodies touch directly.

The secretion of the pro-inflammatory cytokine IL-6 was significantly decreased after the induction of deep hypothermia and rewarming in comparison to normothermic controls. After 2 and 4 h the IL-6 concentration assimilated for normothermic and hypothermic cells. Moreover, MCP-1 release was significantly decreased after 2 and 4h in the normothermia and hypothermia groupe.

Conclusion:

Deep hypothermia has no influence on the cell viability but reduces the release of the proinflammatory cytokines IL-6, MCP-1 and TNFα in our co-culture model of neurons and microglial cells