Reactive oxygen species production by cell-free oxidized hemoglobin drives the damaging pathway following exposure to a mixed glia cell culture: Possible implications for preterm intraventricular hemorrhage?

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Introduction

Severe cerebral intraventricular hemorrhage (IVH) in preterm infants continues to be a major clinical problem, occurring in about 15-20% of very preterm infants. In contrast to other brain lesions the incidence of IVH has not reduced over the last decade, but actually slightly increased. Cerebro-cerebellar deposition of the redox active cell-free hemoglobin (Hb) from hemorrhagic cerebrospinal fluid (CSF) in the intraventricular space has been shown to be central in the pathophysiology of brain injury following preterm intraventricular hemorrhage (IVH). Using a preterm rabbit pup model of IVH, intraventricularly administered haptoglobin (Hp), a cell-free Hb scavenger, blocked the damaging effects following IVH. An increased understanding and appreciation of the causal pathway and metabolites involved is essential for the continued development and implementation of the cell-free Hb scavenger,haptoglobin as a neuroprotective agent in the preterm infant with IVH**.**

AIM

To deduce the important Hb metabolite/metabolites causally involved in the damaging cellular response in preterm IVH

Methodology

Exposure of primary rat mixed glial cell cultures to hemorrhagic CSF obtained from preterm IVH infants (containing a mixture of the Hb metabolites) and to pure Hb metabolites (Met/oxidized Hb, OxyHb or Heme). Evaluation of cellular response, reactive oxygen species (ROS) generation, production of proinflammatory cytokines and oxidative markers. Blockade of these responses by Hp was also studied.

Conclusion

Met/oxidized Hb(Fe^{3+}) and not oxyHb(Fe^{2+}) drives the damaging pathway following IVH. Early administration of Hp to minimize the spontaneous autoxidation of cell-free oxyHb to Met/oxidized Hb may provide a therapeutic window for preterm **IVH**

Results

Exposure to Met/oxidized Hb, but not to oxyHb, resulted in a similar significant ($p < 0.05$) damaging response as exposure to hemorrhagic CSF with rate of ROS production positively correlated with the rate of production of proinflammatory and oxidative markers. Congruently, Met/oxidized Hb caused a disintegration of the polygonal cytoskeletal structure of the glial cells in addition to upregulation of F-actin proteins in microglial cells, a marker of microglia metamorphosis, motility, activation and cytokinesis. Administered Hp partially reversed the damaging response of CSF and not of pure Met/oxidized Hb