Erythropoietin attenuates hyperoxia-induced cell death and proteome changes in the developing brain

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Objective: Oxygen toxicity appears to contribute to the pathogenesis of adverse neurological outcome in survivors of preterm birth. We demonstrated previously that hyperoxia-induced apoptosis during infancy is associated with oxidative stress, decreased expression of neurotrophins, increase of pro-inflammatory cytokines and changes in brain proteins associated with neuronal circuit formation and cell growth. Since a tissue-protective effect has been observed for recombinant erythropoietin (rEpo), a factor used in neonatal medicine for its hematopoietic effect, we examined the effect of rEpo on hyperoxia-induced damage of the developing brain.
Methods: Six-day old *C57Bl/6* mice or Wistar rats were exposed to 80% oxygen for 24 hours and received 20,000 IE rEpo i.p., while sex-matched littermates kept at room air and injected with normal saline served as controls. We performed TUNEL staining on brain sections from treated rats and controls. Total protein extracts prepared from single murine brain-halves were separated by 2-DE, and proteins found to be reproducibly altered by hyperoxia+Epo were identified by mass spectrometry and partially further analyzed with RT-PCR and Western blots.
Results: rEpo-treatment reduced hyperoxia-induced apoptosis in infant rodent brains and inhibited most proteome changes observed when hyperoxia was applied exclusively. Proteome analysis allowed for an identification of protein changes that offer a link to the observed protective effect of erythropoietin, e.g., through reduction of oxidative stress, increased expression of neurotrophins, increased activation of neurotrophin-activated pathways and decreased activation of pro-inflammatory cytokines.
Conclusion: Our results demonstrate a protective effect of rEpo on hyperoxia-induced brain damage and suggest mechanisms involved in this process. Our findings are highly relevant from a clinical perspective since oxygen administration to neonates is often inevitable and rEpo has been identified as a candidate for adjunctive neuroprotective therapy.