

(2)

HYPOXIA-ISCHEMIA INTENSIFIES BILIRUBIN-INDUCED CELL DEATH AND TNF- α RELEASE IN CULTURED ASTROCYTES

AS Falcão, A Fernandes, MA Brito, RFM Silva, D Brites

Centro de Patogénese Molecular (UBMBE), Faculdade de Farmácia, University of Lisbon, Lisbon, Portugal

Background: Deposition of unconjugated bilirubin (UCB) in the central nervous system is the major factor causing encephalopathy during severe neonatal hyperbilirubinemia¹. Hypoxia-ischemia is another cause of brain injury that has been associated with neonatal morbidity and mortality². Interestingly, some studies report the onset of cerebral hypoxia as a risk factor for UCB encephalopathy^{3,4}. In fact, this condition often precedes neonatal hyperbilirubinemia. However, the cellular mechanisms triggered by hypoxia that may enforce UCB deleterious effects are not well elucidated.

Aims: In this study we investigate whether hypoxia (HP) or hypoxia-ischemia (IS), followed by reoxygenation, intensifies UCB-induced release of cytokines and glutamate, as well as apoptosis and necrosis.

Methods: Primary cultures of rat astrocytes were incubated for 4 h, at 37°C, with 50 or 100 μ M purified UCB and 100 μ M human serum albumin in conditions lacking or including a prior 4 h exposure to HP (95% N₂, 5%CO₂) or to IS (HP without glucose) plus 12 h recovery in normoxic conditions. Non-treated cells were used as controls in all conditions. Apoptosis was estimated by evaluation of nuclear morphology (staining with Hoechst dye 33258) and necrosis by the release of LDH using a commercial kit (Roche). Secretion of TNF- α and IL-6 was measured with specific DuoSet® ELISA Development kits (R&D Systems, MN), while the release of glutamate was determined by an adaptation of the L-Glutamic acid kit (Roche).

Results: UCB-induced apoptosis (7-8%) was aggravated by both HP and IS (~9 and 10%, respectively, $p < 0.01$). Necrosis due to interaction of UCB with astrocytes (7-8%) was similarly enhanced by HP and IS conditions (9-10%, $p < 0.05$). Interestingly, although glutamate efflux was stimulated by UCB in normoxia and HP, it was greatly reduced by glucose deprivation (~40%, $p < 0.01$). As expected, HP led to an increase in the TNF- α secretion both in controls and UCB exposed cells, (~20%, $p < 0.01$). This effect was markedly enhanced in IS conditions (~70%, $p < 0.01$). Surprisingly, both HP and IS conditions suppressed the ~50% inhibition in the IL-6 secretion due to UCB in the normoxic condition. Moreover, glucose deprivation in IS showed to induce an elevation in the release of IL-6 ($p < 0.01$ vs. normoxia).

Conclusions: Our results suggest that some of the deleterious effects of UCB are enhanced by HP and IS, namely the production of the pro-inflammatory cytokine TNF- α and the cell death by apoptosis or necrosis. Conversely, the UCB-induced release of glutamate from astrocytes did not increase in our HP model. It should also be noted that oxygen-glucose deprivation (IS) led to a decrease of extracellular glutamate pointing to an energy-dependent process. In sum, these findings provide a basis for the increased susceptibility of the jaundiced newborns to UCB deleterious effects in the presence of hypoxia or hypoxia-ischemia.

References

1. Hansen TWR. Mechanisms of bilirubin toxicity: clinical implications. *Clin Perinatol* 2002;29:765-78.
2. Patel J, Edwards AD. Prediction of outcome after perinatal asphyxia. *Curr Opin Pediatr* 1997;9:128-32.
3. Mayor F Jr, Pages M, Diez-Guerra J, Valdivieso F, Mayor F. Effect of postnatal anoxia on bilirubin levels in rat brain. *Pediatr Res* 1985;19:231-6.
4. Kim MH, Yoon JJ, Sher J, Brown AK. Lack of predictive indices in kernicterus: a comparison of clinical and pathologic factors in infants with or without kernicterus. *Pediatrics* 1980;66:852-8.