

(4)

UNCONJUGATED BILIRUBIN ENHANCES THE RELEASE OF PRO-INFLAMMATORY CYTOKINES AND GLUTAMATE BY RAT MICROGLIA**C Gordo, A Fernandes, S Falcão, A Brito, RFM Silva, D Brites**

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

Background: Recently, we have shown that unconjugated bilirubin (UCB) has immunostimulant effects leading to astrocyte production of pro-inflammatory cytokines such as TNF- α and IL-1 β . Microglia plays central roles in responding to brain injury and infection². In fact, when the brain is injured, the resting ramified microglia transform into “activated microglia” that produce several potentially cytotoxic molecules, including pro-inflammatory cytokines that in turn can be harmful to neurons^{3,4}.

Aims: To determine if microglia is activated by UCB and produces pro-inflammatory cytokines and cytotoxic factors such as glutamate, and the impact of these factors to induce cell death both by apoptosis and necrosis supporting participation of microglia in UCB neuropathology.

Methods: Primary cultures of microglia, prepared according to the method of Saura et al. (2003)⁵, were incubated with 50 μ M or 100 μ M purified UCB and 100 μ M human serum albumin (HSA), for 4 h at 37°C. Controls of non-treated cells were included. 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-1 β 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 at 50 μ M (UCB/HSA of 0.5) increased the production of both TNF- α and IL-1 β by ~1.9-folds ($p < 0.01$). Moreover, UCB also induced glutamate efflux from 14.7 \pm 5.4 μ g/ml to 68.7 \pm 1.7 μ g/ml ($p < 0.01$) and cell death, either by necrosis or apoptosis (1.7- or 1.9-fold, respectively, $p < 0.01$). Enhanced effects were obtained with UCB at 100 μ M (UCB/HSA of 1.0) for glutamate release (5-fold increase, $p < 0.01$), apoptosis (3-fold increase, $p < 0.01$), necrosis and IL-1 β secretion (2-fold increase, n.s.), and TNF- α (1.3-fold increase, n.s.).

Conclusions: By virtue of the potent effects produced by UCB in microglia leading to a significant increase in the release of cytokines and glutamate together with a harmful action on microglia survival we speculate that neurons would be greatly affected by these detrimental consequences. Understanding the mechanism of UCB action on glial activation may help gain further insight into the UCB-induced neuropathology.

Supported by FCT-POCTI/39906/FCB/2001

References

1. Fernandes A, Falcão AS, Silva R, Brito MA, Brites D. Cytokine production, glutamate release and cell death in rat cultured astrocytes treated with unconjugated bilirubin and LPS J Neuroimmunol. 2004;153:64-75.
2. Grossmann R, Stence N, Carr J, Fuller L, Waite M, Dailey ME. Justavascular microglia migrate along brain microvessels following activation during early postnatal development. Glia 2002;37:229-40.

3. Nakajima K, Kohsaka S. Microglia: neuroprotective and neurotrophic cells in the central nervous system. *Current Drug Targets-Card & Haemat Dis* 2004;4:65-84.
4. Uwe-Karsten H. Microglia as a source and target of cytokines. *Glia* 2002;40:140-55.
5. Saura J, Tusell JM, Serratosa J. High-yield isolation of murine microglia by mild trypsinization. *Glia* 2003;44:183-9.