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**RELEVANCE OF OXIDATIVE STRESS IN THE PATHWAYS OF NEURONAL DAMAGE BY UNCONJUGATED BILIRUBIN****MA Brito<sup>a</sup>, A Fernandes<sup>a</sup>, AS Falcão<sup>a</sup>, RFM Silva<sup>a</sup>, DA Butterfield<sup>b</sup>, D Brites<sup>a</sup>**

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**Background:** Cytotoxicity by unconjugated bilirubin (UCB) involves profound disturbances of membrane structure<sup>1-3</sup> and accumulation of extracellular glutamate<sup>4</sup>. More recently, a typical inflammatory response was further linked to cell death<sup>5</sup>. These types of events were reported to trigger elevated free radicals production, as well as impairment of calcium homeostasis, and to result in loss of cell membrane integrity<sup>6</sup>.

**Aims:** This study was designed to investigate whether interaction of clinically relevant concentrations of free UCB with synaptosomal membrane vesicles could be linked to oxidative stress, cytosolic calcium accumulation and perturbation of membrane function.

**Methods:** Synaptosomal vesicles were prepared from gerbil cortical brain tissue and incubated with purified UCB (0.1  $\mu$ M), for 4 h at 37°C. Intracellular concentrations of reactive oxygen species (ROS) and calcium were determined by dichlorofluorescein and BAPTA fluorescent probes, respectively. Membrane protein and lipid oxidation were evaluated by slot-blot, and phosphatidylserine exposure by annexin V binding. Levels of reduced and oxidized glutathione (GSH and GSSG, respectively), as well as activities of the Mg<sup>2+</sup>-ATPase aminophospholipid translocase (flippase) and Na<sup>+</sup>,K<sup>+</sup>-ATPase were also measured.

**Results:** Our studies showed that 0.1  $\mu$ M UCB induced a rise in ROS content (~17%,  $P<0.01$ ), together with a decrease in GSH/GSSG ratio (~30%,  $P<0.01$ ), and oxidation of protein (~20%,  $P<0.01$ ) and lipid (~10%,  $P<0.05$ ) components. In addition, synaptosomes exposed to UCB exhibited increased externalization of phosphatidylserine (~10%,  $P<0.05$ ), together with decreased flippase and Na<sup>+</sup>,K<sup>+</sup>-ATPase (~15%,  $P<0.05$ ) activities, events that were accompanied by enhanced intracellular calcium levels (~20%,  $P<0.01$ ).

**Conclusions:** The results of this study show that hyperbilirubinemia promotes oxidative stress in synaptosomal membrane systems. The oxidative damage of cellular components, together with the accompanying calcium intrusion, leads to the loss of membrane assembly and functionality. Damaged neurons have exposed phosphatidylserine on the outer cell surface, as a signal for phagocytic engulfment by microglial cells. Taken collectively, our data establish a link between hyperbilirubinemia and injury to neocortical synaptosomes, where oxidative lesion appears as a relevant component of the pathways of neuronal damage by UCB.

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