NEUROPROTECTIVE EFFECTS OF CELECOXIB AGAINST β-AMYLOID-INDUCED DAMAGE THROUGH THE REGULATION OF HEME OXYGENASE-1IN SH-SY5Y CELLS

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Introduction: Neuroinflammation and free radical production, as consequences of both amyloid-β-peptide (Aβ) and tau protein deposition, are responsible for the neuronal death occurring in Alzheimer's disease (AD) patients. A well-known approach to counteract neural damage in AD is the enhancement of cell stress response. In this regard, several studies have demonstrated an up-regulation of heme oxygenase-1(HO-1) in both brain tissue and lymphocytes from AD patients. The rationale underlining HO-1overexpression is the attempt of brain tissue to react against reactive oxygen species (ROS)-induced damage due to the pleiotropic neuroprotective effects of HO-1by-products, mainly carbon monoxide (CO). The present study was aimed to evaluate the differential modulation by oligomeric versus fibrillar Aβ (oAβ and fAβ, respectively) on HO-1expression. Furthermore, the antioxidant and neuroprotective outcomes of the antinflammatory drug celecoxib (CXB), whose involvement in the prophylaxis of AD is still questioned, secondary to HO-1regulation, has been investigated.

Materials and methods: To study the effects of celecoxib on oAβ- and fAβ- mediated regulation of HO-1, the human neuroblastoma cell line SH-SY5Y has been used. Both oAβ and fAβ structural conformation has been evaluated by light scattering and atomic force microscopy. HO-1protein has been assayed by Western-Blot, whereas intracellular ROS have been measured by the fluorescent probe dichlorofluorescein. The nuclear factor (erythroid-derived 2)-like 2(Nrf2) has been detected by immunofluorescence and confocal imaging.

Results: Treatment with oAβ (6.25-50 nM; for 24h) or fAβ (1.56-12.5nM; for 24h) dose-dependently increased HO-1expression in SH-SY5Y neurons. CXB (0.5-10 μM) for 1h, further up-regulated oAβ (25nM)- and fAβ (6.25nM)- induced HO-1levels. Furthermore, CXB (10 μM) counteracted the oAβ (25nM)- and fAβ (6.25nM)- related increase of ROS levels. In order to link CXB-mediated ROS reduction with HO-1over-expression, the HO inhibitor Zn-protoporphyrin-IX (Zn-PP-IX) was used. Indeed, Zn-PP-IX (2.5μM) for 24h reverted the antioxidant effects of CXB (10μM), suggesting that one of HO-1by-products is responsible for the CXB-mediated ROS decrease. The CO donor CORM-2(50 nM) reverted both oAβ- and fAβ- mediated ROS production, whereas the antioxidant effect of bilirubin (50 nM) was limited to fAβ. With regard to the mechanism(s) through which CXB over-expresses HO-1induction, the drug has been shown to favor the nuclear translocation of the transcriptional inducer Nrf2, and this effect was greater in SH-SY5Y cells exposed to oAβ than fAβ. Finally, both CO and BR have been demonstrated to impair Aβ oligomerization and fibril formation, thus widening the mechanism(s) involved in their neuroprotective outcomes.

Discussion and conclusions: These results unraveled a novel mechanism of action for CXB, independent of cyclooxygenase-2(COX-2) blockade and mediated by HO-1induction. In addition, the greater effects of CXB against oAβ-mediated Nrf2translocation and ROS generation, lend support to the hypothesis of a more efficient neuroprotective role of this drug during the early stage of Aβ oligomerization. However, since Aβ transition from soluble oligomers to insoluble fibrils is a continuum and considering the effects of CXB on both oAβ- and fAβ- mediated HO-1over-expression, it is possible to argue a potential therapeutic synergy between COX-2inhibition and HO-1enhancement over the whole stages of AD development. In conclusion, this study may help in refining our knowledge into the pathophysiological underpinnings of a multifactorial disease such as AD. A multi-target drug design accompanied by an early fast-acting treatment stratagem will offer novel clues for the development of innovative and efficient therapeutic strategies.