EFFECT OF DONEPEZIL ON THE EXPRESSION AND RESPONSIVENESS TO LPS OF CHRNA7 AND CHRFAM7A IN MACROPHAGES: A POSSIBLE LINK TO THE CHOLINERGIC ANTI-INFLAMMATORY PATHWAY

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Introduction: The Central Nervous System (CNS) exerts a control on innate immunity through the “Cholinergic Anti-Inflammatory Pathway” (CAIP), in which the Vagus nerve induces an anti-inflammatory response by stimulating the release of acetylcholine (ACh) that in turns activates the α7nicotine receptor (CHRNA7) expressed by the macrophages, attenuating the release of pro-inflammatory cytokines. This makes α7nAChR a suitable target for the pharmacological treatment of disorders characterised by systemic inflammation supported by alterations in its expression and function. Recent studies indicated that acetylcholine esterase (AChE) inhibitors, widely used for the symptomatic treatment of Alzheimer’s disease and other dementias, have neuroprotective properties mediated via α7nAChRs and also significantly modulate innate immunity possibly as a result of the increased availability of acetylcholine activating the CAIP. The human CHRNA7gene underwent a recombination event that gave rise to the human-restricted CHRFAM7A gene, the product of the partial duplication and fusion of exons 5-10 of CHRNA7gene with the novel FAM7A gene. The CHRFAM7A gene is particularly expressed in the CNS and immune system, and it differs from the α7conventional subunit for the N-terminus domain. The CHRFAM7A subunit is capable of assembling with CHRNA7subunits and exerting a dominant negative effect on α7nAChR function, suggesting a role in the pathogenesis of neurodegenerative and inflammatory diseases. Little is known about the expression and functional mechanisms of CHRFAM7A in inflammatory diseases, although alteration in its expression has been reported in inflammatory bowel disease and colon cancer. Interestingly, we have shown that acute stimulation of human primary monocytes and macrophages with LPS down-regulates CHRFAM7A expression at mRNA and protein levels by a mechanism driven by NF-κB in a way that is paralleled by the up-regulation of CHRNA7mRNA, which suggests a regulatory role in activating the CAIP. The regulation of CHRFAM7A expression may be a key step in the modulation of inflammation, but the mechanisms driving its transcription in human immune tissues are largely unknown. The identification and detailed analysis of the CHRFAM7A gene’s regulatory region, shown here, reveals some of the complex mechanisms driving its expression and responsiveness to pro-inflammatory stimuli in a human immune cell model. Given the anti-inflammatory potential of the AChE inhibitor donepezil, the little current knowledge about its mechanism of action, and the role of CHRFAM7A in the regulation of CHRNA7function in humans, we explored the link between the CAIP and the AChE inhibitor donepezil, by focusing on the regulation of CHRFAM7A and CHRNA7expression in human immune cell models to better understand their role in peripheral and central inflammation, and define a human restricted mechanism modulating the inflammatory response.

Materials and methods: Human primary macrophages and the THP-1 monocytic-like cell line have been treated with donepezil in the presence or absence of LPS and CHRNA7and CHRFAM7A expression level detected by means of qPCR and western blotting experiments.

Results: The analysis of the CHRFAM7A gene’s regulatory region reveals some of the mechanisms driving its expression and responsiveness to LPS in human immune cell models. Moreover, we show that donepezil modulates CHRFAM7A and CHRNA7 responsiveness to LPS, thus contributing to its therapeutic potential.

Discussion and conclusions: The unexpected up-regulation of both the CHRFAM7A and CHRNA7 genes by donepezil suggests that the immunomodulatory potential of the drug may be exerted by regulating the activation of CAIP through the modulation of the expression of α7nAChR and CHRFAM7A at transcriptional level. Given the great therapeutic potential of donepezil, we consider that the results provided could contribute to a better characterization of its pharmacological activity.