EFFECT OF ROFECOXIB TREATMENT ON BRAIN INFLAMMATION AND CHOLINERGIC HYPOFUNCTION INDUCED BY Ab-(1-42) INJECTION INTO THE NUCLEUS BASALIS OF RATS

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Alzheimer's disease (AD) is one of the most common form of dementia and is characterized by progressive cognitive impairment. The accumulation of neuritic plaques, and of neurofibrillary tangles, accompanied by the loss of cholinergic neurons in basal forebrain nuclei are the neuropathological hallmarcks of this disease. Recent studies suggest that brain inflammatory processes associated to the neuritic plaques contribute to the neurodegeneration through the release of proinflammatory cytokines, reactive oxygen species, nitric oxide and excitatory amino acids (Gonzalez-Scarano & Baltuch, Annu. Rev. Neurosci. 22: 219-240, 1999). Hensley et al. (J. Neurochem. 72: 2053-2058, 1999) demonstated p38 mitogen-activated protein kinase (p38MAPK) activation in glial and neuronal cells in neuropathological studies of AD.
In many experimental models of AD non-steroidal anti-inflammatory drugs (NSAIDs) seem to delay or reduce the incidence of the disease. The present study investigated the effects of the treatment with rofecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor, on the inflammatory reaction and cholinergic impairment induced by the injection of the β-amyloid peptide (1-42) (Aβ-(1-42)) into the nucleus basalis (NB) of rats. Pre-aggregated Aβ-(1-42) (5 μg/μl) was stereotaxically injected into the right NB of adult rats. Rofecoxib (3 mg/kg/day) was administered orally for 7 days. A group of rats was deeply anaesthetized and perfused transcardially with 4% paraformaldehyde and used for histochemistry and immunohistochemistry. Congo Red staining was used to visualize the aggregation of Aβ-(1-42) peptide in the site of the injection. The activation of microglial and astrocytic cells was detected by means of two monoclonal antibodies directed against the MHC-II (OX-6) and glial fibrillary acid protein (GFAP), respectively. The expression of COX-2 and inducible nitric oxide sinthase (iNOS) was revealed with specific antibodies. For detection of cholinergic neurons, the polyclonal antibody raised against the enzyme choline-acetyltransferase (ChAT) was used. Activation of p38 MAPK was detected with a polyclonal primary antibody raised against phospho-(Thr180-Tyr182)p38MAPK. Colocalization of phospho-p38 MAPK with microglial or astocytic cells was detected by the use of double labeling confocal microscopy using an anti-phospho-p38 MAPK antibody coupled with OX-6 or GFAP antibodies respectively. In another group of rats, the extracellular levels of acetylcholine (ACh) was measured in the parietal cortex ipsilateral to the injection site by transversal microdialysis and the ACh collected in the dialysate was assayed by HPLC.
The Aβ-(1-42) peptide was Congo Red-positive and induced a rapid activation of glial cells and a significant reduction in the number of ChAT-positive neurons. Microdialysis results revealed a significant decrease in K^+-stimulated ACh release in the cortex ipsilateral to the injection site. Many iNOS-positive cells were observed in the injected site, at 24 hours and 7 days post-injection. COX-2-immunoreactivity in microglial cells was observed 7 days after injection. Many phospho-p38 MAPK positive cells were present around the deposit 7 days after the injection. Double labeling confocal laser microscopy showed that phospho-p38 MAPK colocalizes with microglial cells but not with astrocytes. Seven days of rofecoxib treatment produced a strong attenuation of both microglial and astrocytic cells, a significant reduction in the number of iNOS and phospho-p38 MAPK positive cells, and attenuated the reduction in the number of ChAT-positive neurons. The treatment with rofecoxib significantly attenuated the decrease in K^+-stimulated ACh release induced by the deposit.
These results reveal that the Aβ-(1-42) deposit induces a strong glial activation, COX-2 and iNOS immunoreactivity, activation of p38MAPK signal trasduction pathway, loss of cholinergic neurons, and demonstrate that the treatment with Rofecoxib attenuates these processes. In conclusion this study charachterized the molecular mechanisms by which Aβ deposition lead to brain inflammation and neuronal degeneration, and support the therapeutic use of NSAIDs in AD.