In silico identification and pharmacological evaluation of 5-Methoxyflavone as a neuroprotective agent against β-amyloid-induced toxicity

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According to the 'cell cycle hypothesis of Alzheimer's disease (AD)', apoptotic death is the result of a failed attempt of neurons to divide. Consistent with this assumption, DNA replication has emerged as an obligatory step in the apoptotic pathway triggered by the β-amyloid protein (Aβ) in neurons. Neuronal DNA replication is unusual, since it is carried-out by DNA polymerase-β (DNA pol-β) and likely lasts months before the occurrence of neuronal death (Copani et al., 2008). DNA pol-β might therefore represent a relevant target for neuroprotection in AD. Known DNA pol-β inhibitors are not selective for the enzyme. Dideoxycytidine, which we have shown to prevent Aβ-induced DNA replication and apoptosis (Copani et al., 2002), is a preferential inhibitor of DNA pol-β over other polymerases.
In the present work, we searched for selective DNA pol-β inhibitors by virtual screening of a database containing more than 4,000 natural and over 20,000 drug-like compounds. Nine compounds were selected for their best scores, and 5-methoxyflavone (5-MF) was a top-scored compound when docked into the 8-kDa lyase domain of DNA pol-β.
All selected compounds were tested on both wild type and DNA pol-β-null mouse fibroblasts, which are hypersensitive to the DNA-methylating agent methylmethanesulfonate (MMS). Among the tested compounds, only 5-MF was able to enhance cellular sensitivity to MMS in wild type but not DNA pol-β null cultures. MMS sensitivity resulting from 5-MF exposure in wild type cells mimicked that observed in cells devoid of pol-β, showing that 5-MF was able to inhibit the base-excision repair activity of DNA pol-β required for MMS resistance. Similarly, 5-MF directly inhibited human DNA pol-β activity on a gapped DNA substrate in a cell-free assay. In pure cultures of rat cortical neurons, 5-MF was devoid of intrinsic toxicity when applied for 48 h and up to a concentration of 10 μM. These cultures are a useful model to investigate potential inhibitors of Aβ-induced DNA replication and apoptosis (Copani et al., 2002). Consistent with an inhibition of DNA pol-β, 5-MF (5-10 μM) was able to reduce both the number of S-phase neurons and apoptosis triggered by Aβ**.**To our knowledge, this is the first demonstration that a flavonoid compound is able to halt the apoptotic pathway triggered by Aß via a definite mechanism, thus achieving the status of promising drug candidate.

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