Fingolimod induces neuroprotective factors in human astrocytes.

Abstract:
Fingolimod (FTY720) is the first sphingosine-1-phosphate (S1P) receptor modulator approved for the treatment of multiple sclerosis. The phosphorylated active metabolite FTY720-phosphate (FTY-P) interferes with lymphocyte trafficking. In addition, it accumulates in the CNS and reduces brain atrophy in multiple sclerosis (MS), and neuroprotective effects are hypothesized. Human primary astrocytes as well as human astrocytoma cells were stimulated with FTY-P or S1P. We analyzed gene expression by a genome-wide microarray and validated induced candidate genes by quantitative PCR (qPCR) and ELISA. To identify the S1P-receptor subtypes involved, we applied a membrane-impermeable S1P analog (dihydro-S1P), receptor subtype specific agonists and antagonists, as well as RNAi silencing. FTY-P induced leukemia inhibitory factor (LIF), interleukin 11 (IL11), and heparin-binding EGF-like growth factor (HBEGF) mRNA, as well as secretion of LIF and IL11 protein. In order to mimic an inflammatory milieu as observed in active MS lesions, we combined FTY-P application with tumor necrosis factor (TNF). In the presence of this key inflammatory cytokine, FTY-P synergistically induced LIF, HBEGF, and IL11 mRNA, as well as secretion of LIF and IL11 protein. TNF itself induced
inflammatory, B-cell promoting, and antiviral factors (CXCL10, BAFF, MX1, and OAS2). Their induction was blocked by FTY-P. After continuous exposure of cells to FTY-P or S1P for up to 7 days, the extent of induction of neurotrophic factors and the suppression of TNF-induced inflammatory genes declined but was still detectable. The induction of neurotrophic factors was mediated via surface S1P receptors 1 (S1PR1) and 3 (S1PR3). We identified effects of FTY-P on astrocytes, namely induction of neurotrophic mediators (LIF, HBEGF, and IL11) and inhibition of TNF-induced inflammatory genes (CXCL10, BAFF, MX1, and OAS2). This supports the view that a part of the effects of fingolimod may be mediated via astrocytes.