Adenosine A_{2A}Rs as a target for the treatment of Niemann Pick C disease

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Niemann Pick C (NPC) disease is a fatal neurovisceral syndrome caused, in the 95% of cases, by the loss of function of the NPC1 protein and, as a consequence, accumulation of unesterified cholesterol and other lipids in the lysosomal and/or late endosomal compartment of cells. The disease is characterized by progressive hepato-splenomegaly, neurodegeneration and premature death. There are no disease modifying treatments currently approved for NPC.
In a previous paper, our group demonstrated that the stimulation of the adenosine A2A receptor (A2AR) in fibroblasts from NPC patients was able to restore many compromised cellular functions: calcium homeostasis; decreased mitochondrial membrane potential and cholesterol accumulation (Visentin et al., 2012). These results are important, since a reduced cholesterol accumulation in fibroblasts is the main criterion currently used for identification of a compound or pathway that would be beneficial for NPC disease.
Since, however, NPC disease is also strongly characterized by the development of progressive neurological disease, in the present study we extended our analysis to the CNS. Considering that both neurons and oligodendrocytes are particularly affected in the disease, we obtained human genetic models of NPC neurons and oligodendrocytes by transfecting neuroblastoma SH-SY5Y and oligodendroglial MO3.13 cell lines with siRNA for NPC1. siNPC1cell lines were treated with different concentrations of CGS21680 and, after 24h, cholesterol distribution was examined by Filipin III staining. In SH-SY5Y, siNPC1 transfection induced a significant increase in mean fluorescence intensity (MFI) of Filipin III with respect to CTR, indicating intracellular cholesterol accumulation; CGS21680 dose-dependently reduced MFI in siNPC1-treated cells (CGS 100 nM: 96.68±4.5 vs. 104,1±1.61, $p>0.05$, n=3-5; 500 nM: 83,7±2,79 vs. 104,1±1.6, $p<0.001$, n=3-5). The effect of CGS was completely abolished by the A_{2A}R antagonist ZM241385 (500nM, 83,7±2,79 vs. 98,13±2,55, n=3-5). Analogously, in the oligodendroglial cell line Mo3.13, while 100nM CGS did not reduce MFI of Filipin III in siNPC1 cells vs. vehicle, at 500nM it significantly decreased the accumulation of cholesterol (110,1±1,66 vs. 83,65±3,85, $p<0.001$, n=3-6). CGS-induced effect was completely abolished by ZM241385 (500nM, 83,65±3,85 vs. 99,63±3,33, n=3-5). Both concentrations of CGS were ineffective in CTR SH-SY5Y and MO3.13 cells.
In order to evaluate the role played by calcium in CGS-induced effects, the calcium chelator BATPA-AM was applied on siNPC1 cells before and along with CGS. BAPTA application completely abolished the effect of CGS on cholesterol accumulation both in siSH-SY5Y (100.3±0.81 vs. 83.7±2,79,$p<0.05$, n=3-6) and in siMO3.13 (101.6±5.08 vs.81.49±3.23, $p<0.05$, n=3-6). These results demonstrate that A2AR effects are mediated by their ability to increase intracellular calcium levels.
Our data demonstrate that the stimulation of A2AR can ameliorate NPC phenotype in both peripheral and CNS genetic models, thus indicating A2AR agonists as a new, promising therapeuthic approach to NPC.

Visentin S, De Nuccio C, Bernardo A, Pepponi R, Ferrante A, Minghetti L,
Popoli P. The stimulation of adenosine A2A receptors ameliorates the pathological phenotype of fibroblasts from Niemann-Pick type C patients. J Neurosci. 2013, 33(39):15388-93.