

## The stimulation of adenosine A<sub>2A</sub> receptors ameliorates the pathological phenotype of fibroblasts from Niemann Pick type C patients.

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Niemann–Pick type C1 (NPC1) disease is a rare neurovisceral disorder characterized by intracellular accumulation of unesterified cholesterol, sphingolipids and other lipids in the lysosomal compartment. Although how lipid storage causes cell death in NPC1 is not yet well understood, a deregulation of lysosomal calcium ions (Ca<sup>2+</sup>) has been identified as one of the earliest pathogenetic steps. Adenosine A<sub>2A</sub> receptors (A<sub>2A</sub>Rs) are G–protein coupled receptors exerting a variety of physiological effects. In the brain, A<sub>2A</sub>Rs are effective modulators of neuronal damage and have attracted much interest as potential targets for neurodegenerative diseases. Since A<sub>2A</sub>Rs control lysosome trafficking and restore altered lysosomal pH, which closely regulates lysosomal calcium, we have hypothesized a role for these receptors in NPC1. A valuable and widely used model for studying NPC1 is represented by human fibroblasts from NPC1 patients. The aim of this study was thus to evaluate the effects of the A<sub>2A</sub>R agonist CGS21680 on fibroblasts from control (healthy) and NPC1 individuals. By using the Ca<sup>2+</sup>-sensitive dye Fura-2-AM, we found that CGS21680 significantly increased lysosomal calcium levels in NPC1 fibroblasts, an effect prevented by the A<sub>2A</sub>R antagonist ZM241384 (500 nM). Furthermore, CGS21680 (100nM for 24h) rescues mitochondrial functionality (mitochondrial inner membrane potential and expression of the complex IV of the mitochondrial respiratory chain), which is compromised in NPC1 cells. Such an effect was abolished by ZM241384, supporting a specific involvement of A<sub>2A</sub>Rs in restoring mitochondrial functionality. The effects of A<sub>2A</sub>R activation on lysosomal calcium are not mediated by the cAMP/PKA pathway but they appear to involve the phosphorylation of ERK1/2. Finally, CGS21680 reduces cholesterol accumulation (Filipin III staining) in NPC1 fibroblasts.

In conclusion, our results show that the stimulation of adenosine A<sub>2A</sub>Rs normalizes intralysosomal calcium levels, rescues mitochondrial functionality and reduces cholesterol accumulation in human NPC1 fibroblasts.

Since the main criterion currently used to identify a compound or pathway that would be beneficial for NPC disease is the ability to reduce Filipin III staining in NPC1 fibroblasts (Karten et al., 2009), our findings strongly support the hypothesis (to be confirmed by means of extensive *in vivo* studies) that A<sub>2A</sub>R agonists may represent a therapeutic option for this disease.

### References:

Karten B, Peake KB, Vance JE (2009) Mechanisms and consequences of impaired lipid trafficking in Niemann-Pick type C1-deficient mammalian cells. *Biochim Biophys Acta* 1791: 659-670.