Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol12Issue3abst267P.pdf

Development of an antisense oligonucleotide-based method to manipulate RNA editing of AMPAR subunits

H Chaytow, L Popplewell, G Dickson, P Chen. Royal Holloway University, Egham, UK

Background: AMPA receptors (AMPARs) are a subset of glutamate receptors composed of one or more of four different subunits (GluA1-4). AMPARs are generally permeable to calcium, unless the GluA2 subunit is present. AMPAR subunits undergo RNA editing at specific sites in their sequence, a post-transcriptional modification caused by deamination of an adenosine subunit. This forms inosine, which is read by the cell's translation machinery as guanosine therefore changing the codon, critical for the GluA2 subunit's impermeability to calcium. Studies have shown that reduced editing is found in spinal cord motor neurons of MND patients, and this may lead to their cell death via increased calcium influx (Kawahara et al., 2004). Deamination is carried out by a family of enzymes called Adenosine Deaminases Acting on RNAs (ADARs). ADAR2 has multiple alternatively-spliced variants within mammalian cells: some have been shown to reduce the efficiency of ADAR2's deamination. One such alternatively-spliced transcript contains the addition of an exon containing an AluJ sequence. We can interfere with endogenous exon splicing using Antisense oligonucleotides (ASOs); short complementary nucleotide sequences which can be used to prevent AluJ exon inclusion.

Objectives: We designed ASOs targeting the splice sites surrounding the AluJ-containing exon in ADAR2 and examined their effects on AluJ inclusion and the RNA editing process in mammalian cell lines.

Methods: HeLa, NSC34 and SHSY5Y cells were grown in culture and treated with ASOs (n=3 per treatment group). HeLa cells do not endogenously express AMPARs, and so a plasmid containing a section of the GluA2 subunit was transfected into cells in order to measure RNA editing. Editing efficiencies were measured using a RT-PCR based assay on RNA extracts.

Results: Endogenous ADAR2 within HeLa cells exhibited an inclusion rate of the AluJ exon of 59.23% (±0.62) within ADAR2 transcripts (i.e. there is endogenous exon skipping of around 40%). When exposed to 2 µM of ASO, exon skipping was enhanced to 99.37% (±0.41, $p<0.05$). Exclusion of the AluJ exon in ADAR2 significantly increases editing of a minigene containing a section of GluA2 from 23.1 ± 1.07 % to 29.5 ± 0.98 % ($p<0.05$). We are now examining the effects of our ASOs in neuronal-like cell lines (NSC34s and SHSY5Ys) known to endogenously express the GluA2 subunit.

Discussion and conclusions: This shows promising preliminary data for the use of ASOs to alter RNA editing of AMPARs. Ultimately this ASO will be tested in primary neuronal cultures, where this increased editing efficiency can be enhanced and may prove to be beneficial to neuronal viability.