A115 IMPROVEMENT OF RAAV5 TRANSDUCTION EFFICIENCY IN FIBROBLAST-LIKE SYNOVIOCYTES BY DOXORUBICIN

C J Aalbers, S A Loiler, I de Vries, F Mingozzi, M J Vervoordeldonk, P P Tak *AMC/ University of Amsterdam, The Netherlands*

10.1136/ard.2010.129635g

Background We are currently developing local gene therapy for the treatment of rheumatoid arthritis (RA) using interferon β (IFNβ) as therapeutic gene and rAAV5 as vector. We generated a rAAV5 vector that expresses hIFNβ under control of an inflammation inducible promoter (rAAV5.NFkB.hIFNβ). To support development of our clinical gene therapy programme a robust assay is needed to transduce different cell lines with rAAV5.NFkB.hIFNβ. In vitro, rAAV5 does not transduce cells efficiently, including fibroblast-like synoviocytes (FLS), the main target cell for rAAV5 in the joint. Many strategies have been investigated to improve virus transduction and gene expression, among which the use of proteasome inhibitors (PIs).

Aim Our aim is to increase rAAV5 transduction efficiency in primary FLS by using PIs. Since RA FLS are primary cells with a limited lifespan and varying characteristics, 2V6.11 cells were chosen cell line for potency assays and comparability testing between different batches. This 293 cell line expresses the adenovirus E4orf6 gene product (E4 34k) under the control of the ecdysone-inducible promoter.

Methods Primary FLS, isolated from joints of RA patients, and 2V6.11 cells were infected with rAAV5.NFkB.hIFNβ or rAAV5 expressing GFP under the control of a CMV promoter (rAAV5. CMV.GFP) and treated with various concentrations of PIs doxorubicin (Dox) or etoposide in both pretransduction (24 h before) and post-transduction (2–4 h after) conditions. In 2V6.11 cells ponasterone A (pon A) was added to induce E4orf6 gene expression. To activate the NFkB promoter, cells were stimulated with tumour necrosis factor α with or without IL-1β. Supernatants were harvested 48 h after stimulation. Human IFNβ production was measured by ELISA. The number of GFP positive cells was semiquantitatively scored by visual inspection.

Results For primary FLS, Dox was more effective compared to etoposide in increasing gene expression, with an optimal concentration of 0.4 μM and toxic concentration of 3.2 μM. Dox post-treatment was more effective compared to pretreatment. In 2V6.11 cells highest levels of gene expression were achieved with pon A treatment (1 μg/ml), compared to treatment in combination with Dox (0.25 μM to 3.2 μM). Dox concentrations >1 μM with pon A were toxic to 2V6.11 cells

Conclusion Dox post-treatment greatly improves rAAV5 transduction and hIFNβ expression by primary FLS. Recombinant AAV5 transduction of 2V6.11 cells treated with pon A alone provides robust gene expression. We now have excellent tools to evaluate rAAV5 based vectors for the treatment of RA in vitro.

10.1136/ard.2010.129635h