
Proceedings Paper:
Varughese, M.S., Eltumi, H., Kemp, E.H. et al. (1 more author) (2024) 6589 Antisense oligonucleotides for reducing ACTH secretion in a model of Cushing's disease. In: Journal of the Endocrine Society. ENDO 2024 Abstracts Annual Meeting of the Endocrine Society, 01-04 Jun 2024, Boston, USA. The Endocrine Society

https://doi.org/10.1210/jendso/bvae163.1135

Abstract citation ID: bvae163.1135

Neuroendocrinology and Pituitary

6589

Antisense Oligonucleotides for Reducing ACTH Secretion in a Model of Cushing's Disease

Maria Susan Varughese, Hanan Eltumi, PhD, Elizabeth Helen Kemp, PhD, and John D. C. Newell-Price, MD, PhD, FRCP
University of Sheffield, Sheffield, United Kingdom

Disclosure: M.S. Varughese: None. **H. Eltumi:** None. **E.H. Kemp:** None. **J.D. Newell-Price:** None.

Antisense Oligonucleotides for Reducing ACTH Secretion in a Model of Cushing's Disease

Background: Cushing's disease (CD) is a multi-system disorder with high morbidity and mortality characterised by pathologically raised serum cortisol due excess expression of proopiomelanocortin (*Pomc*) and overproduction of ACTH by a pituitary corticotroph adenoma. Trans-sphenoidal adenomectomy is the treatment of choice, but there is a 30% recurrence rate over time. Advances in medical therapy are needed. Antisense oligonucleotides (ASOs) can silence mRNA translation by specific base-pairing. Locked nucleic acid (LNA) and 2'-O-methyl (OMe) ribose terminals make ASOs more effective by improving affinity and resistance to nuclease degradation. **Aims:** To investigate the effectiveness of LNA- and OMe-modified anti-*Pomc* ASOs at reducing ACTH secretion from murine adrenotropic tumour (AtT20) cells. To analyse the nuclease resistance of *Pomc* ASOs and their ability to drive an immune response. **Methods:** Two ASOs (ASO2 and ASO3) were designed against *Pomc* with phosphorothioate backbones modified with either LNA or OMe ribose terminals. These were used in lipofectamine-mediated cell transfections of AtT20 cells and ACTH in the culture medium measured by immunoassay. ASO stability was assessed by nuclease degradation assays and immunogenicity by cytokine ELISAs. **Results:** When compared with untreated

AtT20 cells, LNA- and OMe-modified *Pomc* ASOs at 1 nM significantly suppressed ACTH secretion for up to 120 h and 96 h, respectively ($n = 4$; Unpaired t tests, all P values < 0.05). In contrast, control treatments, including scrambled and mismatched ASOs, did not cause a significant reduction. For comparison, 1 nM of ASO2-OMe, ASO2-LNA, ASO3-OMe and ASO3-LNA lowered ACTH at 24 h by 62%, 71%, 63% and 79%, respectively. Modified *Pomc* ASOs showed 14-31% degradation over a 120-min incubation period with 3'- and 5'-exonucleases, whilst equivalent unmodified *Pomc* ASOs were completely degraded. No secretion of IFN-α, IFN-β, TNF-α, IL-6 or IL-1β was detected following transfection of AtT20 cells with *Pomc* ASOs. **Conclusions:** *Pomc* ASOs caused significant reduction of ACTH secretion from AtT-20 cells. LNA-modified *Pomc* ASOs were more effective than those with OMe modifications, with neither stimulating a detectable immune response. These ASOs hold promise as a systemic therapy for Cushing's disease.

Presentation: 6/3/2024