Melanocortin 2 receptor antagonists in ACTH-dependent hypercortisolism: in vitro study in canine primary adrenocortical cell culture

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Why dogs?
- Spontaneous ACTH-producing pituitary adenomas occur in dogs
- Incidence 1000x higher in dogs than in humans
- Clinical symptoms and signs, diagnostics and medical care of Cushing’s disease are comparable between humans and dogs
- Unique potential for the dog as an animal model

Why MC2R antagonists?
- The search for a more selective medical treatment option to improve effectiveness and tolerability of medical treatment in Cushing’s disease continues
- Hypercortisolism is the result of excessive ACTH binding to the melanocortin 2 receptor (MC2R)
- MC2R has unique ligand selectivity: binds only ACTH
- Effective antagonism of the MC2R would greatly inhibit cortisol production

Objectives
- Test effectiveness of two novel peptides, BIM-22776 (776) and BIM-22A299 (299) (provided by ), as MC2R antagonists, in canine primary adrenocortical cell culture
- Determine effects of peptides on cortisol production and mRNA expression of steroidogenic enzymes, MC2R and MC2R accessory protein (MRAP)

Materials & Methods
- Primary cell suspensions prepared from adrenal cortex of healthy dogs (n=8)
- Cultured in 96 wells plates, 4-7 days, medium refreshed prior to incubations
- Incubations quadruplicate with: 50 nM synthetic ACTH [1-24] (Synacthen®) and 50, 500 and 5000 nM of compounds. Incubations without ACTH as control
- After 24 hours of incubation, cortisol was measured in culture medium (radioimmunoassay), DNA content was measured to correct for differences in number of cells per well, and RNA was isolated for RT-qPCR analysis

Results: cortisol production
- Both peptides significantly inhibited ACTH-stimulated cortisol production (A), with most effect seen at the highest concentration of compound 299 (90.7% inhibition, p<0.001). In non-ACTH-stimulated cells (B), no inhibition in cortisol production was detected with either compound. On the contrary, compound 776 caused a slight but significant increase (p=0.003) in cortisol/DNA ratio.

Summary of Conclusions
- Compound 299 is a potent antagonist of the MC2R in vitro
- Compound 776 has antagonistic properties but is less potent than 299
- Important: selectivity for MC2R compared to other melanocortin receptors?
- Further studies are warranted to evaluate compound 299 in vivo, where dogs with Cushing’s disease could provide a useful animal model