

## ***“A Secretagogue Chemiluminescent Assay”***

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### **Project summary**

Growth hormone secretagogues (GHS) are molecules that stimulate the secretion of human growth hormone from the pituitary. They have proven to be potent agonists of hGH secretion reaching circulating hGH values that are even not easily achievable with rhGH administrations. As such, they are the prime candidate pharmaceuticals to replace rhGH abuse. GHS display large structural diversity and many molecules are under development by the pharmaceutical industry. In order to address the entire family of known and upcoming molecules it can be targeted what all GHS have in common: the interaction with the GHSR1a receptor. Based on this premise we developed and validated a competition assay using a radiotracer, <sup>125</sup>I-ghrelin. Given the difficulties for testing radioactivity at some antidoping laboratories, and the recent implementation of chemiluminescent tube-based immunoassays for hGH we have studied the potential replacement of the radiotracer by a chemiluminescent one. Accordingly, the development of a method using biotinylated ghrelin and streptavidin labeled with a chemiluminescent tag could be effective to achieve this purpose.

The main objectives of this project were:

- Obtention of appropriate chemiluminescent tags bound to streptavidin and synthesis, purification, and characterisation of streptavidin conjugated to acridinium, not commercially available.
- Set-up of a competition assay protocol employing different chemiluminescent tags.
- Comparison of the radiotracer and the chemiluminescent tracers.
- Assessment of the chemiluminescent competition assay in urine samples.

### **Results and Discussion**

The results submitted in this project showed the development of an efficient chemiluminescent method performed in three different chemiluminescent tags: acridinium (AC), alkaline phosphatase (AP) and horseradish peroxidase (HRP). This method, based in the interaction between biotinylated ghrelin and streptavidin labeled with a chemiluminescent tag, was evaluated following different steps. After evaluating biotinylated ghrelin in a competition binding assay, the  $K_i$  obtained was compared with the  $K_i$  values from competition assays performed with ghrelin without any tag, and no interference in the interaction with the receptor was showed. Biotinylated ghrelin appeared then as a suitable ligand.

Subsequently, the optimum time of addition of streptavidin was tested to avoid any effect on the recognition of biotinylated ghrelin by the receptor,

and it was determined its addition 40 min after of the starting of the competition assay. After synthesis and purification of streptavidin labeled with AC, the protocol was optimized for this and the other two chemiluminescent tags (streptavidin labelled with alkaline phosphatase AP and horseradish peroxidase HRP, respectively) through the evaluation of several conditions as: number of washes, membranes obtained from different amount of cells, alternative binding buffers, amount of ST per sample, presence or absence of detergent and process changes of adding of the chemiluminescent substrates, among others. The optimal protocols for each label were tested in a competition assay with different amounts of GHRP-2 in binding buffer and urine and the results showed the best sensitivity with HRP tag. In fact, the presence of GHRP-2 in urine samples obtained 1.5 hours after intravenous treatment with 100 µg of GHRP-2 from in a clinical trial with sedentary health volunteers treated was detected.

In conclusion, the results shown in this project demonstrate the development of a chemiluminescent method based on interaction between biotinylated ghrelin and the GHSR1a receptor, which is more sensitive than other non-radioactive methods described so far but has less sensitivity than the radioactive method described previously by our own group.