“Low molecular weight luteinizing hormone receptor agonists”

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Project Overview

Luteinizing hormone (LH) is a peptide hormone which is abused by athletes to either induce the production of endogenous hormones such as testosterone to hide the use of anabolic agent use which would normally suppress the production of LH. In the case of the low molecular weight LH receptor agonists they would induce the production of endogenous hormones such as testosterone in a similar way as LH. These compounds are banned in the WADA list (S2. Hormones and related substances) as they have a similar biological effect as the use of LH.

A method of detection for these compounds needs to be direct as their effect, the excess production of endogenous hormones, cannot be proven by analysis techniques which test for the endogenous hormones. In this proposal several compounds which have been identified as having high LH receptor agonist activity will be synthesized. An instrumental analysis technique based on liquid chromatography with tandem mass spectrometry will be developed for the analysis of urine samples.

Results and Conclusions

Luteinizing hormone (LH) is a peptide hormone which is abused by athletes to either induce the production of endogenous hormones such as testosterone or it can also be used to hide the use of anabolic agents which would normally suppress the production of LH. LH is a naturally occurring hormone which is secreted by the anterior pituitary gland. In males LH acts on the Leydig cells of the testis, stimulating testosterone production, while in females, LH plays an integral part in the ovulation cycle. In the case of the low molecular weight (LMW) LH receptor agonists they would induce production of endogenous hormones such as testosterone in a similar way as LH. These compounds are banned in the WADA list (S2. Hormones and related substances) as they have a similar biological effect as the use of LH.

The method of detection for these compounds needed to be direct as their effect, the excess production of endogenous hormones, cannot be proven easily by analysis techniques which test for the endogenous hormones. In this project several compounds which had been identified as having high LH receptor agonist activity were synthesised. These compounds fall into two groups, pyrazoles and the Org series. For this project Pyrazole 10, Pyrazole 25, Org 41841 and Org 43553 were synthesised. An instrumental analysis technique was developed using High Resolution Mass Spectrometry coupled with Liquid Chromatography (LC/HRMS) for the analysis of urine samples spiked with the LMW LH receptor agonists. In vitro enzyme analysis was undertaken to identify possible metabolites for the LMW LH receptor agonists. The proposed analysis methodology was validated using the parameters of specificity, linearity, limit of detection (LOD), precision and recovery. The LOD for each compound was determined at 2 ng/mL for Pyrazole 10, Pyrazole 25 and Org 43553 and 5 ng/mL for Org 41841. The analysis technique will be able to be immediately implemented into laboratory screening tests for small molecules using liquid chromatography with mass spectrometry detection. A sample of Org 43553 has been distributed to all WADA laboratories.