"EPO Signal Peptides and the detection of recombinant EPO"

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Project Overview

Reliable, sensitive and specific measurement of the hormone EPO + related analogues, used to illegally enhance sports performance, is a continuing problem for WADA. To date, immunoassay tests for EPO suffer sensitivity and specificity problems due to the multiple forms of EPO normally present in blood and also from newer synthetic short forms that can be used by competitive athletes. This project will validate a novel immunoassay technology for the detection of EPO in human blood through the measurement of the protein's signal peptide. Signal peptides are short, discrete components of hormones that were previously thought to be degraded by cells once the hormone has been produced. However, we have shown that the signal peptide of EPO is not destroyed in human blood and can present as a novel, measurable factor to determine the presence or absence of abnormal EPO administration. The ratio of EPO to its signal peptide in human blood and urine samples will be validated in normal healthy people before and after exercise. We will also characterise the molecular forms of EPO signal peptide in blood and urine and monitor its normal daily circadian rhythm. This project has the potential to offer a novel, simple, robust and specific method to detect illegal EPO, via measurement of the ratio of EPO to EPO signal peptide, for use in competitive sports. We envisage the ratio forming part of individual athletes 'Athlete Biological Passport' or ABP.

Results and Conclusion

Erythropoietin (EPO) and its illegal use to boost performance represents a challenge to sporting regulatory bodies. Further, micro-dosing regimens mean EPO in blood and urine samples can be difficult to detect. The WADA athlete biological passport goes some way to improving EPO detection, but there is room for improvement. We previously made the novel discovery that signal peptide fragments from proteins and peptides are present in human blood and urine and have applied this concept to testing for the signal peptide of EPO in healthy human blood and urine samples. We developed a robust and reliable immunoassay for the detection of human EPO signal peptide (EPOsp). This assay does not cross react with EPO or related peptides and proteins and is not subject to interference from common drugs and medications. Immunoreactive EPOsp in human blood and urine samples eluted on high performance chromatography consistent with a nonapeptide fragment. EPOsp in the blood of 109 healthy control subjects did not correlate with EPO and there was no difference between levels in men and woman. EPOsp levels in blood showed modest but significant correlations with body mass index and systolic blood pressure. The ratio of EPOsp:EPO

was 8.2:1. Blood levels of EPO displayed a diurnal rhythm in which levels rose approximately 50% during the day: EPOsp showed no such change, which meant the EPOsp:EPO ratio was higher in the AM than in the PM. EPOsp was measurable in reasonable amounts in human urine whereas EPO was undetectable. Finally, based on regional venous and arterial sampling in 11 subjects, EPOsp is secreted in greatest amounts from the kidney and heart, consistent with other renal and cardiac data. Thus, we have established a robust working assay for measurement of EPOsp in human samples. We confirm that EPOsp is present in human samples as a distinct entity, separate from EPO, and may potentially be used as a method for the detection of EPO dosing.