PROJECT REVIEW

"The Improved Detection of Recombinant Erythropoietin in Urine Using Immunoaffinity Chromatography"

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Erythropoietin (EPO) improves the oxygen-carrying capacity of blood and the recombinant product has been used by endurance athletes to enhance performance. There is also a strong suspicion that recombinant EPO has also been used to dope racehorses. One of the major requirements of a test for detecting low concentrations of banned substances in blood or urine samples is the ability of the testing procedure to detect the analyte in the presence of many other naturally occurring substances.

This can be achieved by a selective extraction of the analyte of interest from the sample matrix or by a selective detection procedure. The current method for detecting recombinant EPO in urine uses a non specific concentration and extraction procedure followed by gel electrophoresis using a highly specific double blotting procedure to detect EPO in the complex of other proteins present. This procedure works well but is slow and very labor intensive. An alternative approach to detecting recombinant EPO would be to use a selective extraction procedure to remove the EPO from the interfering proteins. Immunoaffinity columns are already routinely used by sports drug testing laboratories to extract and purify banned substances such as anabolic steroids from urine.

Whilst the initial preparation of the antibodies and evaluation of the columns is relatively difficult, such columns once prepared are robust, simple to use, and capable of regeneration so that they can be reused many times. This collaborative project between Charles Sturt University, the Australian Racing Forensic Laboratory and the Australian Sports Drug Testing Laboratory aims to produce antibodies that have a high binding capacity and selectivity for erythropoietin so that they can used in an immunoaffinity column to extract and concentrate recombinant EPO from urine samples. In this way the isoforms of EPO will be able to be separated by gel electrophoresis and detected by single blotting which should lead to a test for EPO that is not only much simpler to perform, and hence cheaper, but also more sensitive. The ability of the antibodies to selectively extract EPO should enable further developments in the detection of EPO to proceed including the extraction of EPO from serum and the detection of EPO by instrumental techniques such as mass spectrometry.

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RESULTS AND CONCLUSIONS

The main aims of this project were to

- produce large quantities of polyclonal antibodies to erythropoietin (EPO) by immunising sheep
- extract these antibodies and use them to prepare immunoaffinity columns for selectively extracting EPO from urine
- investigate the composition of such purified extracts with a view to improving the existing double blotting electrophoresis technique for detecting recombinant EPO in urine.

The objectives have been achieved. Of the six sheep inoculated with recombinant human EPO all but one produced significant quantities of EPO antibodies in their sera. The antibodies from each sheep were characterised by epitope mapping and some significant differences were observed which may be useful in distinguishing various forms of EPO or fragments thereof. Immunoaffinity columns have been prepared using the serum from the sheep with the highest antibody titre. These columns have been found to extract EPO from urine with typical recoveries ranging from 25 to 40%. The purified extracts have markedly lower levels of other urinary proteins. Gel electrophoresis has shown that the isoform distribution of the purified EPO is unchanged by the column purification. This applies to both recombinant and urinary EPO although urinary EPO has only half the recovery of recombinant EPO. The effect of this different recovery behaviour is to make the presence of recombinant EPO in a sample containing both recombinant and urinary EPO more obvious. The unchanged isoform distribution has been confirmed in urine samples which naturally have a greater proportion of more basic isoforms. The unchanged isoform distribution means that further development and simplification of the existing method for detection of doping with recombinant EPO can proceed using the immunoaffinity columns. Over 500 mL of serum is available from each sheep which has the potential to prepare up to 10,000 immunoaffinity columns.