PROJECT REVIEW

"Evaluation of human cryopreserved hepatocytes as an in vitro model for metabolism studies of doping agents"

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In doping controls, knowledge of steroid metabolism is a key issue to ensure adequate detection of steroids in a urine sample. Metabolism studies are usually performed in vivo by collecting urine samples over a certain period of time after administration of the steroid. However, with new designer steroids on the marked lacking a toxicological profile, this approach has become difficult. Moreover, metabolites may be present at low concentrations in urine, making detection and characterisation difficult.

In vitro metabolism studies is an alternative to excretion studies in humans, and human hepatocytes are recognized to be a very close model to the human liver with intact cell membranes and the full compliment of enzymes and cofactors. The limited availability of fresh human hepatocytes has long been the major disadvantage using hepatocytes as a model. The progress in cryopreservation techniques, however, has resulted in an increased accessibility and human cryopreserved hepatocytes are now commercially available.

In the proposed project, the use of cryopreserved human hepatocytes to investigate metabolism of doping agents will be evaluated. Emphasis will be put on anabolic steroids, and the developed model will be tested on two steroids with a well known metabolic profile and one designer steroid with an unknown profile. After hepatocyte incubation and a clean-up procedure, analysis will be performed with liquid chromatography and gas chormatography, combined with mass spectromtery. The in vitro biotransformations and metabolic profiles will be studied in detailed, including the correlation to known in vivo urinary profiles, if available.

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Results and Conclusions:

The present study deals with the usefulness of cryopreserved hepatocytes to access metabolic mixtures of doping substances. The described in vitro methods are simple systems which are fairly easy and fast to perform. The in vitro hepatocyte metabolism has proven to be close to the human metabolism. It does, however, not reflect the whole complexity of human metabolism, particularly in the light of long term metabolites and phase 2 metabolism.

Despite this, incubations with hepatocytes represent a very useful model for identifying and predicting potential metabolites. Major advantages are amongst others:

- A fast access to a metabolic mixture, which can directly be used for detection strategies of doping substances.
- New non-approved substances with unknown toxicological profiles can be used as substates resulting in metabolites as markers for screening and confirmation purposes.
- No ethical approval is necessary
- Easy detection of possible metabolites due to limited matrix interference of the incubation mixture.

Compared to microsomes, the in vivo situation is better reflected with hepatocytes, taking into account drug transport. Furthermore, cryopreserved hepatocytes have several advantages over fresh hepatocytes, first of all a better availability. In addition, cells from several donors can easily be pooled for experiments. Drugs that are metabolized to a high extent in the liver tend to show higher interindividual differences and sometimes genetic polymorphism. Hence, a pooled experiment can better reflect the metabolic picture of the average population.

In this study, human hepatocyte incubations have led to biotransformations generating major metabolites of androstendione and metandienone reported in vivo in humans. Additionally, several metabolites of the synthetic steroid norbolethone were detected, which of only two have been previously described.