PROJECT REVIEW

"Development of ultra sensitive duplex differential immunoassays for detection of doping with insulin analogues"

Dr. Z. WU (Charite Campus Mitte, Germany)

Detection of insulin or insulin analogue doping is still a challenge for doping analysis laboratories. Until now no screening method is available providing the fast and reliable detection of doping with insulin analogue. The insulin analogues are modified insulin preparations to show faster acting or long acting effects compared to regular insulin, which are therefore broadly used in clinical therapy for diabetes and might also be intensively misused in sport and body building. The insulin analogues have amino acid sequences different to regular insulin. Such difference could be recognized by specially selected monoclonal antibodies, which have their binding site or epitopes involved in the positions where amino acid sequences are altered in insulin analogues.

In this project, we will at first generate and select two monoclonal antibodies which bind all insulin analogues significantly differently. Thereafter a differential immunoassay based on immune-PCR Imperacer Technologie from Chimera Biotech will be constructed with these 2 antibodies. The immune-PCR is the ultra sensitive immunoassay using antibody-DNA conjugate. The DNA fragment can specifically amplify millions fold and the signal will be directly read out in a real-time PCR machine. An insulin analogue is detected if the insulin concentrations determined by the two assays are significantly differently. This ultrasensitive real-time immune-PCR duplex assay could directly use small amount of urine samples for doping control. Furthermore, efforts will be made to produce specific monoclonal antibodies to each insulin analogue. Each monoclonal antibody will only bind one insulin analogue but not regular insulin or other analogues.

Differential immunoassays, especially multiplex immune-PCR constructed with these antibodies will be able to identify quickly and unequivocally which insulin analogues are misused.

Results and Conclusions:

In this research project, high affinity anti-insulin monoclonal antibodies (mAbs) including several regular insulin specific mAbs and glargine specific mAbs were produced and they were carefully examined for their binding to insulin and insulin analogues. More than 30 best hybridomas were chosen to be cloned with limited dilution. The antibodies were produced in protein free medium, purified with affinity column and biotinylated. The mAb pairs for the sandwich immunoassays were identified through examining more than 900

antibody combinations. A permissive insulin sandwich assay using mAb 5E10 and a regular insulin preferential assay using mAb 7F3 were constructed. A glargine specific sandwich assay with mAb 6E8 was also established. These three assays are ultra-sensitive (limit of detection <1 pg/ml) and together they can identify any insulin analogue in urine and serum directly with only a few hundred microliters of samples. In a pilot study, these assays showed to be able to identify low amount of insulin analogues in the urine samples of the diabetic patients treated insulin analogues alone or combined with regular insulin preparations.

Furthermore, a regular insulin specific sandwich assay was also constructed with mAb 1B7. This assay together with the permissive insulin assay can identify insulin analogues, bovine and porcine insulin. Due to its high specificity but moderate sensitivity, this assay would be more suitable for the confirmation assay. Additionally, a universal ultrasensitive Immuno-PCR assay format using real-time PCR cycler was constructed, which can easily be converted to the ultra-sensitive duplex assays.