INTRODUCTION

Congenital Adrenal Hyperplasia (CAH) is an autosomal recessive disorder which can appear at birth. It is diagnosed by the newborn screening program at the University Children’s Hospital Zurich (Kinderspital Zürich). In the case of CAH, the biosynthesis of hormones such as cortisol, aldosterone and testosterone are disturbed by a deficiency of enzymes. The whole steroidogenesis involving several metabolites is unbalanced. The current newborn screening method for congenital adrenal hyperplasia, which quantifies 17-hydroxyprogesterone (17-OHP), has a positive predictive value of 2% due to its poor specificity. There could be a possibility to substitute the currently used immuno-assay method which has a lot of cross-reactivity issues, with a tandem mass spectrometry method for dried blood spots (DBS) sample which would allow for a much higher positive predictive value. In order to allow the measurements of the high number of daily samples, the idea is to analyze the DBS by tandem mass spectrometry (TMS) without any preliminary chromatographic step. The goal of the Master’s Thesis was to investigate if there is a possibility to quantify metabolites marker for CAH by TMS without chromatography. The cutoff values for newborns are between 20 and 300 nmol/l in blood. Therefore, the targeted quantification concentration was between 0.6 and 10 nmol/l, since the current extraction method has a 32x dilution factor.

RESULTS & CONCLUSION

The UPLC method allows the separation of the compounds with success (R>1) with a run time of 6.6 minutes. During the signal optimisation, the negative ESI mode trials were unsuccessful and the best results have been found with 1 nmol/l of NH4F as additive in ESI+. The new quantification algorithm allowed to use more intense peaks for the method with no chromatography. All those strategies meant to increase the intensity, led to a remarkable enhancement of 30 times higher signals. It was possible to quantify 17-OHP by MS/MS as low as 4 nmol/l using the TMS method without UPLC, and at 20 nmol/l if it is necessary to discern it from deoxycorticosterone also without chromatography. Even though some repeatability issues were observed, the characterization of the quantification method of 17-OHP, cortisol, androstenedione and progesterone led to encouraging results for both methods. The tests on the alternative TQD showed acceptable indicative recovery and intra-assay precision at 0.6 nmol/l.

OUTLOOK

It has been concluded that the sensitivity is yet too low to quantify the steroids at the aimed level. However, tests on the minimal injection volume has been made and led to the conclusion that the dilution factor of the extraction method could be decreased from 32x to 9x. It could be possible to work at 7x higher concentration by using a new extraction method and by taking two 3.2mm DBS instead of one as it is in the current method, and even at 16x higher levels if taking a 6mm DBS. Another possibility would be to perform further investigation on an alternative instrument. Indeed, the trials on an Agilent TQD 6460 showed very promising results. Finally, a third option would be to measure serum sample instead of dried blood spots. This would allow to take higher sample amount and concentrate it to have higher levels to measure. However, this would induce a huge change in the whole newborn sampling process and sample preparation.

REFERENCES


Determination of Marker Metabolites for CAH from DBS by MS/MS

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Master thesis, Molecular Technologies

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With the best possible conditions of the system, the quantification with internal standards for both MS method with and without UPLC have been characterized by determining their LOD, LOQ, linearity, recovery, intra- and inter-assay precision. Ion suppression has been determined for the method without chromatography.