The influence of severe hepatic dysfunction on the metabolic capacity of the liver in children: Composition and characterization of a liver tissue bank

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Introduction:

The influence of liver disease on the hepatic biotransformation of drugs has mainly been studied in adult populations, whereas for paediatric populations similar data are missing. Information on the impact of hepatic failure on the activity and abundance of the main CYP450 isoforms is however essential for predicting the need for dosage adjustments when a (new) drug is used in a child with liver disease. Therefore, this study aims to fill this knowledge gap by composing and characterizing a liver tissue bank comprising pathological paediatric liver samples.

Methods:

All patients undergoing liver transplantation in a Belgian university hospital at age 12 or lower were included in the study, except when infectious diseases were diagnosed or suspected. Liver tissue samples were snap frozen after explantation, and were processed into subcellular fractions (microsomes and cytosolic fraction) for further analysis. Patient files were consulted for the relevant pre-operative history. The in vitro activities of CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4, will be determined through the incubation of the microsomes with the specific probe substrates phenacetin, tolbutamide, S-mephenytoin, dextromethorphan, chlorzoxazone, and midazolam, respectively. An indirect ELISA will be used for the determination of the abundance of the isoforms. The most important SNPs of the highly polymorphic isoforms will be analyzed using TaqMan® Drug Metabolism Genotyping Assays.

Results:

Up to now, samples from thirty-one children with diverse pathologies (23 with biliary atresia, the others with PFIC II , cystic fibrosis, α1-antitrypsine deficiency, neonatal hemochromatosis, or acute liver failure) were collected and processed. Elaborated patient details will be discussed in the presentation. An incubation protocol and a UPLC-MS/MS method were optimized and validated for the determination of the activities of the aforementioned CYP isoforms. The activities of these isoforms in the 31 microsomal samples were determined, as well as in commercially available adult pools. Some patients showed very low activities compared to a pool of adult microsomes, whereas others showed extremely high activities (up to 300 times the adult activity).

Conclusions:

A liver tissue bank containing microsomes, cytosolic fractions and DNA extracts from 31 paediatric liver patients was collated. As expected, hypervariable activities in this population were detected for all 6 isoforms. Due to the diverse characteristics (age, pathology) of the patients, interpretation of the activity data will be an intricate task. In order to expand the data set, additional data will be collected in the near future, such as information on the most important SNPs leading to altered activity, and on the abundance of the isoforms. Furthermore, the potential inducing or inhibiting pre-operative drug use, as well as the relevant clinical biology results should be taken into account.