ABSTRACT

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Title of diploma thesis: Selection and validation of reference genes for relative mRNA quantification in human liver slices

The use of precision-cut liver slices is often employed *in vitro* system in the biochemical, pharmacological and toxicological studies. This is mainly due to the preservation of the cellular tissue architecture and therefore its functions. The aim of this diploma thesis was to select and validate reference genes for the relative quantification of mRNA in human liver slices. Liver tissues obtained from 3 patients of different age, diagnosis and pharmacotherapy were used as samples. Liver slices, prepared from samples of liver tissue, were exposed to dimethyl sulfoxide (control) or known inducers of cytochrome P450, rifampicin (10 μM) and β-naphthoflavone (10 μM). Subsequently, the stability of the expression of reference genes was verified using RefFinder program, which integrates the programs of the geNorm, BestKeeper, NormFinder and comparative ΔC_T method. The optimal number of reference genes that should be used to evaluate the relative gene expression of target genes was determined using the geNorm program. The most suitable gene combination for the normalization of target genes expression was a pair of YWHAZ and B2M. However, the differences between the stability of each candidate gene were small and all genes could be used for data normalization. The selected reference genes were validated in a practical experiment, in which the mRNA expression of CYP1A2 and CYP3A4 in liver slices incubated with dimethyl sulfoxide, rifampicin or β-naphthoflavone was monitored in the range of 24 hours. The optimal interval for induction studies in human liver slices appears to be 18-24 hours.