Reference gene stability in hepatocyte primary cell cultures of Atlantic cod (*Gadus morhua*)

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Summary

So far no one has described a protocol on how to use Atlantic cod hepatocytes in bioassays, mainly due to the high fat content in these cells, causing the cells to burst during isolation. In this work, we were able to isolate intact liver cells from one mature female individual. The hepatocytes were exposed to different concentrations of PCB 138, which is one of the most widespread PCB congeners and of major concern in the marine environment (AMAP, 1998). Three potential reference genes and six target genes were examined with real-time RT-PCR analyses. The results showed that heat shock cognate 70 was slightly more stable than EF1A and β-actin. We were not able to quantify significant differences between the control and the exposed groups for CYP1A, Vtg, GSH-Px and GR. Further only minor, non-significant differences were found between the control group and the groups of cells exposed to PCB 138 for GST and Mn SOD. The study suggest that both β-actin and EF1A are suitable reference genes in qPCR studies of cod hepatocytes.

Methods

Primary cultures of Atlantic cod hepatocyes were isolated from one adult mature female individual (3.0 kg). The hepatocytes were isolated with a modified version of a method previously described by Bell and coworkers (Bell et al., 1997). The hepatocytes had a viability of 80% and cells were plated on a culture plates (3,8 cm2/well) with a density of 3,5 x 10^6 per well. After 24 h the hepatocytes were exposed for 24 h to different concentrations of PCB 138 (0.1 µM, 1 µM and 10 µM plus one unexposed control group (0,1% DMSO), n=3). Total RNA concentrations were measured using a NanoDrop spectrophotometer and the RNA qualities were evaluated with the Bioanalyzer. Messenger RNA (mRNA) levels were quantified using real-time RT-PCR (LightCycler® 480) with gene specific primer pairs (SYBR Green). The Microsoft Excel applet GeNorm was used to evaluate the stability of the genes (Vandesompele et al., 2002). An overview of the different steps in the method used in this study is presented in Fig.1.

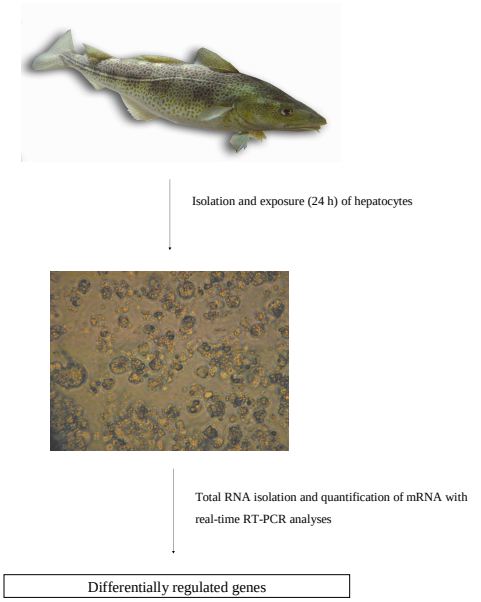

Fig. 1. Schematic overview of the different steps in the method used in this study

Results and discussion

High quality RNA (average RIN value 9.6±0.2) was extracted for gene expression analysis. The transcription levels of three reference genes and six target genes encoding proteins known to be affected by oxidative stress, namely glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione S-transferase (GST) and Mn superoxide dismutase (Mn SOD), in addition to the detoxifying enzyme CYP1A and vitellogenin (Vgt), were quantified. The results showed that heat shock cognate 70 (the non-inducible form of HSP70) was slightly more stable than elongation factor 1A (EF1A) and β-actin, with M-values of 0.152, 0.165 and 0.171, respectively (analyzed by GeNorm). CYP1A is one of the genes known to be affected by PCB 138 (MacFarland & Clarke, 1989), but surprisingly, we were not able to quantify significant differences between the control and the exposed groups. Nor could we measure significant differences in transcription levels between the control and the exposed groups for Vtg, GSH-Px and GR. Further only minor, non-significant differences were found between the control group and the groups of cells exposed to PCB 138 for GST and Mn SOD (Fig.2.).

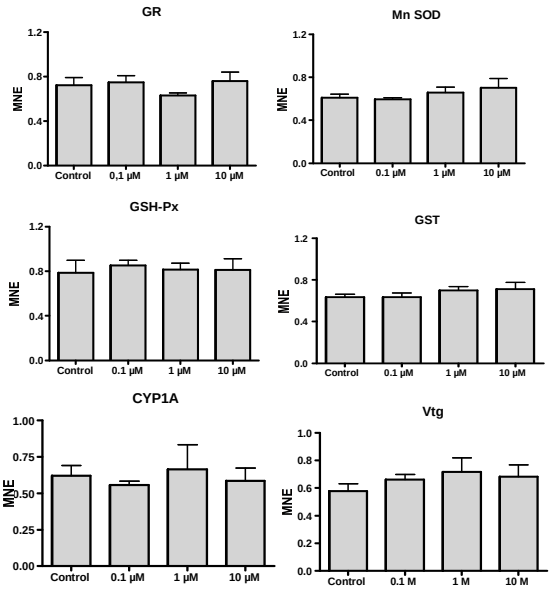

Fig.2. Expression of six potential target genes (MNE = mean normalized expression relative to heat shock cognate 70) in primary culture of cod hepatocytes exposed to different concentrations of PCB 138 and 0,1% DMSO (solvent control)

Conclusions

In this work, two of the most used reference genes EF1A and β-actin were found to be promising reference genes in exposure experiments with cod hepatocytes.

References

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