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An integrated metabolomics and proteogenomics approach reveals metabolic alterations following carbamazepine exposure in the mussel *Mytilus galloprovincialis*

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1. Introduction

The antiepileptic carbamazepine (CBZ) is among the most frequent pharmaceutical active compounds detected in the environment, such as marine ecosystems, with proven adverse effects on organisms, including bivalves [1]. Marine bivalves are particularly exposed due to their filter feeding behaviour, and are of particular ecological, scientific and economic interest. According to the literature, investigations of CBZ effects on those organisms are often hypothesis-driven and could not be exhaustive. In this context, it is necessary to apply holistic approaches to further investigate and understand (sub)individual adverse effects of CBZ on marine bivalves. For that purpose, omics approaches are suitable for measuring molecular changes in a non-targeted way, as an early biological response to contaminant exposure [2]. In this study, we combined the metabolomic and proteogenomic approaches to study underlying mechanisms of CBZ and its potential toxicological effects on the marine mussel *Mytilus galloprovincialis*.

2. Materials and methods

An exposure to CBZ has been performed in controlled laboratory conditions. Mussels (n=90, 5 mussels per aquarium of 2L) were acclimatised for seven days. Then, three exposure conditions were constituted (n = 30 mussels *per* condition) with an environmental relevant CBZ concentration (80 ng/L; low dose), a higher CBZ concentration (8 µg/L; high dose) and a solvent control exposure (20 µL of methanol, vehicle used for the preparation of CBZ solution). During the acclimatisation and exposure periods, filtered seawater was continuously aerated and renewed daily (static renewal) and mussels were fed once daily with the marine green alga *Tetraselmis suecica* at constant density (10,000 cells/mL). Three days following the exposure, mussels were dissected and their sex determined. No mortality was observed during the exposure period. Metabolomics and shotgun proteomics analyses were performed on digestive gland of male mussels by liquid chromatography-high resolution mass spectrometry (solvent control exposure: n=8 males; low dose exposure: n=12 males and high dose exposure n=10 males). To investigate metabolomics and proteomics data, a mid-level data fusion strategy involving MCUVE-PLS (Monte Carlo Uninformative Variable Elimination – Partial Least Squares) [3] and consensus OPLS-DA (Consensus Orthogonal Partial Least Squares-Discriminant Analysis) [4] was implemented in this work. Different bioinformatics tools were used to highlight the relevant biological processes and functions altered by the CBZ exposure from the selected subsets of proteins and metabolites (Gene Ontology classification, KEGG BRITE and Pathway databases, over-representation analysis and global mapping of metabolic pathways).

3. Results and discussion

Both consensus OPLS-DA models comparing the solvent control (SC) to each exposure condition separately, i.e. low dose (SCvsLD) and high dose (SCvsHD), were found optimal with two components (one predictive and one orthogonal) using leave-one-out cross-validation. The SCvsLD model was characterized by R²Y=0.990 and Q²Y=0.851, while the SCvsHD was associated with R²Y=0.985 and Q²Y=0.811. A Shared and Unique Structure (SUS) plot was then used to distinguish variables with common patterns of variations associated with both doses from specific modulations related to a given exposure condition. Based on the SUS plot, most informative variables were selected and submitted to bioinformatics analyses.

The main results concern the involvement of selected proteins and metabolites in the proteins synthesis and lipids degradation, in transport and catabolism processes, and in amino acid and carbohydrate metabolisms (Figure 1). The described results were observed at both doses, but in a higher extent at the highest dose.

More specifically, the up-regulation of lysosomal enzymes (e.g. cathepsins, β -glucuronidase) suggests a disturbance of the lysosomes and more particularly a modification of the lysosomal membrane stability (LMS). These results are in agreement with the literature, some studies have directly measured an alteration of LMS in haemocytes from *M. galloprovincialis*. Such an alteration could lead to the release of hydrolytic lysosomal enzymes in the cytosol and induce oxidative stress and even cell death.

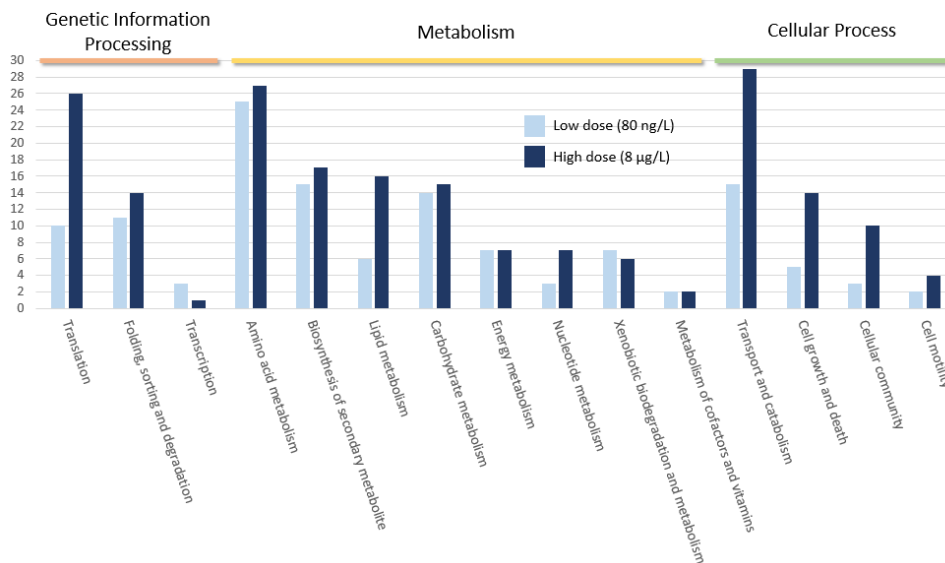


Figure 1: KEGG pathways in which the regulated proteins are involved at the low and high dose of carbamazepine

Our results also demonstrate an up-regulation of peroxysomal and mitochondrial enzymes involved in the β -oxidation of fatty acids (e.g. acyl-CoA oxidase, long-chain acyl-CoA synthetase), as well as numerous modulated metabolites belonging to lipid metabolism. If up-regulation of these enzymes results in an increase in their activity, this could lead to peroxisome proliferation and generate high levels of hydrogen peroxide (reactive oxygen species). Such a phenomenon generally inhibits anti-oxidant defenses, leading to cell membrane damages, to the point of inducing programmed cell death (apoptosis). Our results go in this direction, following the observation of the regulation of enzymes involved in the induction of apoptosis (e.g. TNF ligand-like 1, caspase 2, cathepsin B, mitogen-activated protein kinase kinase 1). To the best of our knowledge, our study is the first to provide evidence of possible CBZ-induced peroxisome proliferation in invertebrates.

4. Conclusions

The data fusion strategy associated to bioinformatics tools were able to highlight relevant correlated subsets of proteins and metabolites, reflecting a general state of stress in the cells of the digestive gland of *M. galloprovincialis* mussels induced by CBZ, even at an environmental dose. This stress results in a disturbance of protein and lipid homeostasis and the induction of oxidative stress, which can lead to programmed cell death. This study supports the interest of combining the metabolomic and proteomic approaches in order to benefit from their complementarity for a better understanding of the effects caused by pharmaceutical active compounds.

5. References

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