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Charge to Move Forward in Volume-Restricted Metabolomics

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Summary

In metabolomics, advanced analytical workflows are needed to study biochemical changes in small-volume biological samples, notably for samples originating from 3D microfluidic cell culture models, individual zebrafish larvae and neonatal clinical blood sampling. Recent work from our lab revealed that capillary zone electrophoresis-mass spectrometry (CE-MS), regardless of utilizing a sheath-liquid or sheathless interface, is a strong analytical tool for probing polar and charged metabolites in biological samples with a good reproducibility. Moreover, in a simulated metabolomics study, CE-MS was able to find the right set of differential metabolites between controls and cases. These studies clearly indicate the value of CE-MS for biomarker discovery and comparative metabolomics studies.

Given our ambition to address volume-restricted biomedical questions with metabolomics, we report in this presentation on the development of new CE-MS-based analytical workflows for the highly efficient and sensitive analysis of polar (endogenous) metabolites in neonatal plasma and individual zebrafish larvae. As only nanoliters of samples are consumed by a single CE-MS analysis, multiple injections/assays can be performed on the same valuable volume-limited sample allowing for technical replicates and/or probing different classes of ionogenic metabolites. We show how these new CE-MS-based workflows can be employed in a reliable way for the quantitative analysis of creatinine, and many more endogenous compounds, in neonatal plasma samples using a starting amount of less than 5 microliter, whereas gold standard clinical chemistry approaches require often a minimum of 100 microliter for only creatinine determination. Hence, the proposed CE-MS-based workflow will contribute to minimizing both the amount and frequency of blood collecting required for diagnostic purposes in a neonatal setting.

We also demonstrate the utility of a new CE-MS workflow for the profiling of metabolites in extracts from individual zebrafish larvae and pools of small numbers of larvae. More than 70 endogenous metabolites could be observed in a pool of 12 zebrafish larvae, whereas 29 endogenous metabolites were detected in an extract from only 1 zebrafish larva. So far, zebrafish has proven to be a very effective model for stress research, in particular for studies on the effects of cortisol, with a clear role of the glucocorticoid receptor during stress. However, the role of the mineralocorticoid receptor (MR) on mediating the effects of cortisol is less known. By using wild-type (WT) and ubiquitous MR-knockout (MRKO) zebrafish larvae exposed to exogenous cortisol treatment, our CE-MS-based metabolomics workflow revealed the implication of metabolic pathways solely activated via MR. Taken together, CE-MS has the potential to identify novel pathways and mechanisms of action in zebrafish larvae and is a viable analytical approach for volume-restricted metabolomics.

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