Effects of perfluorononanoic acid (PFNA) on the metabolic profiling of rat serum by UHPLC-ESI-Q-TOF MSMS

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Human relevant dose of endocrine disrupting chemicals effect on the rat plasma metabolome

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Background

Endocrine disrupting chemicals (EDC) are chemicals disturbing the hormones of the body. Many chemical compounds are under suspicion of being an endocrine disruptor. The effect of a variety of EDC has been tested for changes in male and female hormone composition. In order to understand the effect of EDC on the metabolome an analytical platform has been established. The method focuses separating the compounds from the plasma into three groups: phospholipids, lipids and a fraction containing hormones, organic acids etc. thereby avoiding ion suppression.

The main goal of the present study is to identify if a human relevant dose of EDC will have an effect on the rat metabolome. A human relevant dose of a possible EDC, perflournonanoic acid, was given to a group of rats. To another group PFNA and 12 other EDCs were given. These two groups were compared to a group given only the 12 EDC's and a control group.

By separating the metabolites into three fractions and using high resolution mass spectrometry it is possible to achieve high level of information of how EDC affects the rat metabolome.

Plasma analysis

- 100 µl plasma is extracted with 300 µl icecold acetonitrile and left in the freezer for 20 min
- The sample is centrifuged at 10000 g and supernatant removed
- A SPE hybrid column (Supleco, Sigma-Aldrich, USA) is activated at the supernatant added
- The throughput (T) is collected and the phospholipid eluate with 300 µl 10 % NH₄OH in methanol and collected in another fraction
- Secondly, in 200 µl methanol
- Lastly, in 200 µl acetonitrile
- The heptane is evaporated using a gentle stream of nitrogen and the dried compound resolved in 200 µl 50:50 acetonitrile:isopropanol
- The T is evaporated by a gentle stream of nitrogen and the dried sample is reconstituted with 100 µl water with 5 mM NH₄OH and 0.1 % formic acid

Analytical setup

Dionex 3000 series UHPLC system combined with a Bruker Daltonics maXis qTOF instrument.

A: Water with 5 mM NH₄OH and 0.1 % formic acid
B: Acetonitrile with 0.1 % formic acid

Hydrophilic gradient system

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Plasma analysis data

- The methanol also show a cocktail effect, though the metabolites are not yet identified.
- The heptane shows a 'cocktail effect' – meaning that the animals are affected
- The phospholipids are significantly down-regulated when the rats are given PFNA and cocktail. Furthermore, is there a trend that the animals given both PFNA and cocktail have a larger down regulation that cocktail alone.
- The un-target analysis shows primarily an effect from the cocktail. The main effect shown is a lowered amount of a given metabolite when the cocktail is given compared to control, though in some cases an overexpression is also shown.

Cocktail of EDC

- The phospholipase phase shows some effect at the middle concentration. The main effect shown is a cocktail effect but there is also a trend of an additive effect.
- The analysis also reveals a compound verified by MS/MS to be oleamide. Oleamide is a slippery agent but also an endogenous compound and would therefore normally be discarded as an interesting compound. As the animals has been treated alike and as all blood taken from the animals it is interesting that there is a difference in oleamide between control and closed animals.
- The un-target analysis shows primarily an effect from the cocktail. The main effect shown is a lowered amount of a given metabolite when the cocktail is given compared to control, though in some cases an overexpression is also shown.

Results

- The analysis of the phospholipid phase shows some effect at the middle concentration. The main effect shown is a cocktail effect but there is also a trend of an additive effect.
- The analysis also reveals a compound verified by MS/MS to be oleamide. Oleamide is a slippery agent but also an endogenous compound and would therefore normally be discarded as an interesting compound. As the animals has been treated alike and as all blood taken from the animals it is interesting that there is a difference in oleamide between control and closed animals.
- The un-target analysis shows primarily an effect from the cocktail. The main effect shown is a lowered amount of a given metabolite when the cocktail is given compared to control, though in some cases an overexpression is also shown.

Conclusion

- The phospholipids are significantly down-regulated when the rats are given PFNA and cocktail. Furthermore, is there a trend that the animals given both PFNA and cocktail have a larger down regulation that cocktail alone.
- The heptane phase shows a 'cocktail effect' – meaning that the animals are affected by a low dose cocktail. These compounds are believed to be mono- and di-acylglycerols but this is not yet verified by MS/MS.
- The methanol also show a cocktail effect, though the metabolites are not yet identified.