Poster

[P27-8] P27-8: Assay and monitoring

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[P27-8-2] A HPLC-DAD method for the determination of global methylation in peripheral human blood

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Background

Epigenome is associated as an important link between xenobiotics exposures and changes in gene expression. One of the most studied forms of epigenetic changes is global DNA methylation. HPLC is a robust and reproducible methodology that provides estimates of the amount of 5-methyl-deoxycitine (5mdC) in total DNA, which are expressed as the percentage of the total amount of 2-deoxycitidine (dC). The aim of this study was to develop a method for the determination of dC and 5mdC in human DNA by HPLC-DAD.

Methods

Quantification of global DNA methylation was performed on whole blood. DNA was extracted and concentration was determined with a NanoDrop. 5 g and 10 g of DNA were treated with Rnase A and T1. DNA was incubated with DnaseI for 2 hours and nucleaseP1 for 7 hours at 37˚C. After, nucleotide monophosphates were dephosphorylated with alkaline phosphatase overnight at 37˚C. Samples were centrifuged and 50 μL of the supernatant was injected on HPLC-DAD. Separation was performed in C8 column (150x4.6 mm, 5m) at 20˚C. Mobile phase was a mixture of 25 mM KH_2PO_4 and 0,1% acetic acid and methanol (gradient from 99:1 to 60:40), at 0.6 ml.min^{-1}. Chromatograms were monitored at 280 nm. The relative content of 5mdC was expressed as percentage (%5mdC) with respect to the total amount of cytosine (dC + 5mdC).

Results

The method was linear from 0.195 to 25 g/mL (r=0.99) for dC, and from 0.02 to 3.12 g.mL^{-1} (0.99) for 5mdC. Precision assays presented CV% lower than 9.5% for dC and 6.7% for 5mdC. Accuracy was 98.0–107% for dC and 97.7-110.5% for mdC. The method was applied to 2 samples from healthy subjects in order to establish the DNA amount required for the methodology. The %5mdC was 10.8 and 9.4 for the samples with 10 and 5 g of DNA, respectively.

Conclusions

A HPLC-DAD method for the determination of dC and 5mdC in peripheral blood was developed. The procedure has adequate analytical performance and can be an efficient tool to measure global DNA methylation. Preliminary results indicate the 5 g of DNA is available for the determination of DNA methylation.