Metabolomic Profile of Human Serum to Determine the Age Related Immunosenescence Biomarkers

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Novel aspect:
Comprehensive GC × GC-TOFMS metabolomic approach to determine the age related immunosenescence biomarkers.

Introduction

In the clinics there is an increasing need for reliable biomarkers to assess the risk of immunosenescence in elderly patients. Metabolites are products of biochemical reactions that might be more reproducible and easier to measure compared to proteins and gene entities. However, in the discovery phase high-resolving techniques are required in order to accurately quantify metabolites embedded in complex biological matrices. In the present study, we developed a method using two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC-TOFMS) to monitor approximately 100 endogenous metabolites in human plasma including some exogenous compounds. Finally, we compared metabolomics profiles between different classes of samples using Fisher ratio.

Methods

Clinically fully characterized human serum samples originating from 150 individuals were involved in the current study. The samples were classified and pooled according to specific frailty in elderly people who have experienced different kind of injuries. Thirteen pooled samples were analyzed in duplicates. 200 µl of plasma were spiked with several internal standards solutions: valine $^{13}C_5$, lysine $^{13}C_6$, glutamic acid, $^{13}C_5$, myristic acid d_{27}, stearic acid d_{35}, malic acid d_3, citric acid d_4, caffeine $^{13}C_3$, and cholesterol $^{13}C_3$. Extraction and protein precipitation were performed with a mixture of methanol/ethanol. After centrifugation, the extract was then evaporated to dryness prior to oximation and derivatization. Rtx-5/Rxi-17columns were connected to a Pegasus TOFMS. Masses were acquired in full scan from 40 to 800 m/z.

Preliminary results

The optimized GC × GC-TOFMS method was able to resolve more than 3000 peaks and therefore potential compounds. Among these, approximately 100 metabolites were confirmed with authentic compounds. These target metabolites encompass several classes of compounds such as amino acids, organic acids and fatty acids. In addition, urea, cholesterol, glycerol and some exogenous compounds such as pharmaceuticals were also selected. Thirteen sets of samples were prepared in duplicate. A procedural blank of 200 µl of water and an aliquot of 200 µl of the Standard Reference Material (SRM) Metabolites in Human Plasma (SRM1950) were added to the series of samples for QA/QC purposes. The 100 metabolites were quantified and preliminary data showed that statistically significant variations were observed for many metabolites between the healthy elderly people pooled plasma and the different classes of injured elderly people pooled plasma. The following metabolites were highlighted: 2-hydroxybutyric acid, 3-

hydroxybutyric acid, 3-hydroxyisobutyric acid, 3-hydroxypropanoic acid, 2-hydroxyisovaleric acid, beta-alanine, decanoic acid, fumaric acid, caffeine, glycerol, glyceric acid, salicylic acid, 4-hydroxybenzoic acid, L-cysteine, hexadecenoic acid, L-glutamic acid, linoleic acid and L-histidine.

Moreover, profiling approach using the Fisher ratio method exhibited statistically significant variations in abundance for many unknown metabolites within the set of experimental classes of pooled samples. Additional details of this work will be presented including an attempt of characterization of the chemical structure of these unknown metabolites.