Human Stool Metabolome Differ upon 24 h Blood Pressure Levels and Blood Pressure Dipping Status: A Prospective Longitudinal Study

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Introduction

Dysbiosis of gut microbiota (GM) has been involved in the pathophysiology of arterial hypertension (HT), via a putative role of short chain fatty acids (SCFAs). Its role in the circadian regulation of blood pressure (BP), also called “the dipper profile” (defined as a drop of night-time BP compared with diurnal BP) has been poorly investigated. Sixteen male volunteers and 10 female patients were subjected to 24 h ambulatory BP monitoring and were categorized in nontirexomines (NT) versus HT, as well as in dippers versus non-dippers. In the present study, we have performed a longitudinal study in a general population of volunteers with a focus on the association between GM, GM metabolome, fecal SCFA levels, and 24 h BP levels (including the diurnal and nocturnal BP dip). We used the Human Stool Metabolome (HSM) database (SIB, Sweden). Additionally, we have recruited the female patients (n = 36 participants) in order to test our hypothesis in both genders and to assess the influence of a similar environment on GM composition and stool metabolome.

Materials and methods

Patients. The non-hypertensive male volunteers of our principles (i.e., 2015 male cohort: n = 16) and their female counterparts (n = 10) were contacted to form the “2020 male cohort” and “2020 female cohort”, respectively [1]. After signed informed consent, 24 h ABPM (Pia-BP) data were enrolled in the National Society of Hypertension (ESH) registry (2). A patient was categorized as dipper when his night/day systolic BP (SBP) ratio was ≤ 0.9 or non-dipper when his night/day SBP ratio was ≥ 0.9.

Samples. Faeces were collected at home using stool collection tubes provided with the P3-I Pan Stool DNA Plus Kit (SIGOEN Life Science) for GM composition analysis and Fecal Swab Collection tubes for the anorectal cultures and the metabolomics study. Samples in stool collection tubes were immersed with stool DNA stabilizer solution [3] and stored at −80 °C.

Microbiology. Anaerobic culture of stool samples, 16S Amplicon sequencing and microbiota profiling were performed.

1H-NMR untargeted metabolomics Stools samples were centrifuged and prepared for the NMR analysis of the GM metabolome 19. The 1H-NMR spectra were processed with MestReNova software (v.4.1.1) in order to perform the multivariate analysis. The principal component analysis (PCA) was calculated using the Metaboanalyst web interface (version 5.0, http://www.metaboanalyst.ca/). The OPLS-DA models were used for identifying outliers and distinguishing samples in different clusters. The VIP values for the significant differences in clusters were highlighted (acetate, propionate, and butyrate) and quantified by integration of their signal using macic acid as internal standard. Univariate statistical analysis was performed.

Conclusion

By recruiting 26 patient cohorts with a mean follow-up of 5 years, we found that the fecal metabolome is associated with 24 h BP levels in both genders, with higher SCFA levels in the females of HT patients. Even if no significant change in GM composition was found, our data highlight a novel putative link between the stool metabolome (and specifically SCFAs) and the non-dipping BP profile as recently reported on our study cohort of 44 patients. Moreover, our longitudinal study demonstrates a metabolic dysregulation of the dipping status of patients. Further investigations, including interventional trials aiming at evaluating the hypothetical effect of GM modifications and/or modulation of the fecal metabolome by pre-biotics, pro-biotics, or post-biotics (such as SCFAs per se), are needed to confirm our findings.

Reference


Results

Microbiology

Analysis on anaerobic culture of fecal bacteria and by 16S amplicon sequencing show no significant difference in faeces between HT versus NT patients, as well as between dippers versus non-dippers.

1H-NMR untargeted metabolomics

Multivariate analysis of stool metabolomes did not discriminate male from female groups in the 2020 cohort, even after using super variables methods ([D] = 0.006, p = 0.470). Multivariate analysis principal component analysis (PCA) and orthogonal partial least squares discriminate analysis (OPLS-DA) of stool metabolomes data were not discriminant between HT and NT patients in the 2020 cohort (p = 0.025). The VIP values for the significant differences in clusters were highlighted (acetate, propionate, and butyrate) and quantified by integration of their signal using macic acid as internal standard. Univariate statistical analysis was performed.

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