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Comparative analysis of tryptophan and downstream metabolites of the kynurenine and serotonin pathways in patients with arterial hypertension and coronary artery disease

Additional materials

Metabolome analysis report

Quality control samples were prepared as a mixture of test samples 5 μL each to assess the reproducibility of the method. Biological samples (calibrators or quality control samples) were prepared according to the protocol: 10 μL of a mixture of internal standards and 400 μL of acetonitrile were added to 100 μL of the sample. The resulting mixture was vortexed and centrifuged at 13,000 rpm for 10 minutes. 400 μL of the resulting mixture was transferred to polypropylene tubes and evaporated to dryness in a vacuum evaporator at 37°C. The dry residue was redissolved in 100 μL of 0.02% solution of ascorbic acid in 10% methanol, centrifuged and transferred to a vial for subsequent chromatographic-mass spectrometric analysis.

High performance liquid chromatography and tandem mass spectrometry were performed using an Agilent 1200 liquid chromatograph coupled to a 6450C three quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). Chromatographic separation was performed on a Discovery PFP HS F52.1 × 150,3 μm column (Supelco Inc., USA) using a Waters WAT084560 pre-column (Waters Inc., USA). The column temperature was 40°C and flow rate was 0.4 mL/min. 0.1% formic acid was used as mobile phase A, acentonitrile was phase B. The elution was performed as follows: 0 min – 1% B; 4 min – 10% B; 9 min – 90% B; 10 min – 90% B; 10.1–1% B; 12 min – 1% B. Mass spectrometric determination was performed in the positive electrospray ionization mode. The main parameters of mass spectrometric determination were as follows: gas flow temperature – 300 °C and gas flow rate 8 L/min; spray gas – 20 psi; shell gas temperature – 300 °C; shell gas flow rate – 10 L/min; capillary voltage – 3500 kV. Analytes were determined in the selected reaction monitoring (SRM) mode.

Table 1. Main demographic and clinical characteristics of the subjects

Parameter	Group 1 (control; n=27)	Group 2 (AH; n=58)	Group 3 (CAD; n=46)	p
Male, n (%)	13 (48.1)	33 (56.9)	28 (60.9)	0.943[a]
Age, years	49 (43;51)	62 (51;67)	65 (59;71)	<0.001[a] p_{1-2}<0.001[b] p_{1-3}<0.001[b] p_{2-3}=0.009[b]
BMI, kg/m^2	26.5 (24.8;28.0)	30.8 (29.1;34.4)	30.1 (26.5;33.5)	<0.001[a] p_{1-2}<0.001[b] p_{1-3}=0.021[b] p_{2-3}=0.159[b]
Overweight or obesity, n (%)	18 (66.7)	57 (98.3)	39 (84.8)	<0.001[a] p_{1-2}<0.001[b] p^{1-3}=0.091[b] p^{2-3}=0.103[b]

Table 1 (continued). Main demographic and clinical characteristics of the subjects

Parameter	Group 1 (control; n=27)	Group 2 (AH; n=58)	Group 3 (CAD; n=46)	p
Smoking, n (%)	1 (3.7)	11 (19.0)	10 (21.7)	0.092[a]
Dyslipidemia, n (%)	7 (25.9)	49 (84.5)	45 (97.8)	<0.001[a] p_{1-2}<0.001[b] p_{1-3}<0.001[b] p_{2-3}=0.065[b]
IGT or DM type 2, n (%)	0	8 (13.79)	10 (21.7)	0.035a p_{1-2}=0.294[b] p_{1-3}<0.049[b] p_{2-3}=0.499[b]

[a]Kruskal-Wallis test, [b] post-hoc pairwise comparison of groups using the Dunn's test Holm correction.
The data is presented as Me (Q1 – Q3). AH, arterial hypertension; CAD, coronary artery disease;
IGT, impaired glucose tolerance; DM, diabetes mellitus; CVD, cardiovascular disease

Table 2. Results of laboratory tests and clinical examinations of the subjects

Parameter	Group 1 (control; n=27)	Group 2 (AH; n=58)	Group 3 (CAD; n=46)	p
Glycemia, mmol/L	5.05 (4.55;5.30)	5.50 (5.00;5.90)	5.70 (5.40;6.20)	<0.001[a] p_{1-2}=0.005[b] p_{1-3}=0.051[b] p_{2-3}=0.944[b]
TC, mmol/L	5.31 (4.69;5.95)	5.45 (4.39;6.07)	4.69 (3.94;5.88)	0.156[a]
TG, mmol/L	1.17 (0.82;1.59)	1.33 (1.06;2.02)	1.13 (0.87;1.52)	0.090[a]
LDL cholesterol, mmol/L	2.93 (2.21;3.85)	3.18 (2.36;3.67)	2.78 (1.93;3.64)	0.529[a]
VLDL cholesterol, mmol/L	0.56 (0.35;0.87)	0.58 (0.48;0.87)	0.51 (0.40;0.69)	0.280[a]
HDL cholesterol, mmol/L	1.60 (1.48;1.89)	1.47 (1.23;1.62)	1.50 (1.17;1.80)	0.176[a]
Uric acid, μmol/L	296 (257;366)	342 (281;398)	344 (270;390)	0.127[a]
Creatinine, μmol/L	93.2 (85.9;99.6)	88.7 (80.8;104.0)	99.0 (90.7;107.7)	0.134[a]
TSH, μIU/mL	1.8 (0.7;2.2)	2.0 (1.2;2.8)	1.7 (0.9;2.3)	0.244[a]
SBP mean daily, mm Hg	115 (112;117)	129 (118;137)	132 (115;137)	<0.001[a] p_{1-2}=0.066[b] p_{1-3}=0.001[b] p_{2-3}=0.171b[b]
DBP mean daily, mm Hg	74 (71;79)	78 (69;88)	76 (71;86)	0.448[a]
LVPW thickness, mm	10 (9;10)	11(10;12)	11(10;12)	<0.001[a] p_{1-2}=0.002[b] p_{1-3}=0.001[b] p_{2-3}=0.948[b]
IVS, mm	9 (8;10)	11(10;12)	11(10;12)	<0.001[a] p_{1-2}=0.003[b] p_{1-3}<0.001[b] p_{2-3}=0.512[b]
EDD, cm	4.8 (4.4;5.0)	4.9 (4.6;5.0)	4.9 (4.6;5.3)	0.535[a]
LVEF, %	62 (50;66)	60 (57;62)	57 (54;60)	<0.001[a] p_{1-2}=0.239[b] p_{1-3}<0.001[b] p_{2-3}<0.001[b]
E/A	1.21 (1.11;1.31)	0.80 (0.68;1.10)	0.70 (0.62;1.12)	<0.001[a] p_{1-2}<0.001[b] p_{1-3}<0.001[b] p_{2-3}=0.955[b]

Table 2 (continued). Results of laboratory tests and clinical examinations of the subjects

Parameter	Group 1 (control; n=27)	Group 2 (AH; n=58)	Group 3 (CAD; n=46)	p
LAV, mL	50 (42; 52)	56 (49; 62)	59 (48; 74)	<0.001[a] p_{1-2}=0.067[b] p_{1-3}=0.005[b] p_{2-3}=0.418[b]
RAV, mL	48 (37;51)	50 (45;57)	51 (42;60)	0.064[a]

[a] Kruskal–Wallis test, [b] post-hoc pairwise comparison of groups using the Dunn's test Holm correction.
The data is presented as Me (Q1 – Q3). AH, arterial hypertension; DBP, diastolic blood pressure; E/A, ratio of early (E) to late (A) left ventricular filling velocity; LVPW, left ventricular posterior wall; CAD, coronary artery disease; EDD, end-diastolic dimension; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; VLDL, very low-density lipoprotein cholesterol; IVS, interventricular septum; LAV, left atrial volume; RAV, right atrial volume; TC, total cholesterol; SBP, systolic blood pressure; CVD, cardiovascular disease; TG, triglycerides; TSH, thyroid-stimulating hormone; LVEF, left ventricular ejection fraction.