Biotransformation of Natural Hydroxycinnamic Acids by Gut Microbiota from Normal and Cerebral Ischemia-reperfusion Injured Rats: A Comparative Study

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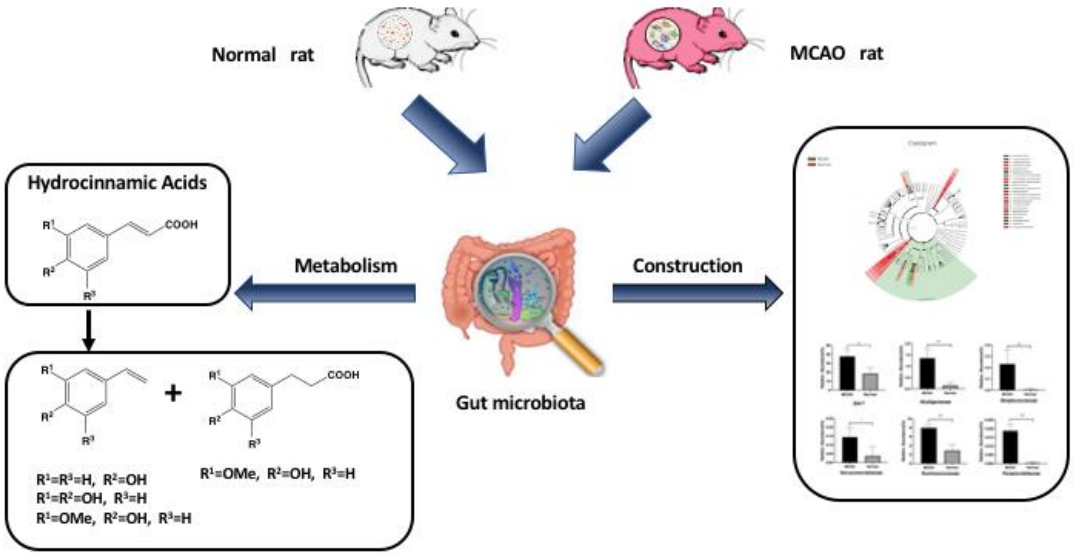

Supporting Information

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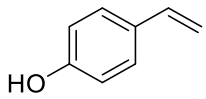
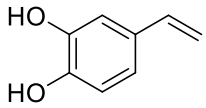
Characterization of the metabolic products of 4-HA, CA and FA

The UHPLC-MS analysis of the metabolic products was performed using a UHPLC (Ultimate 3000, Thermo Fisher Scientific, USA)-Q-Exactive MS (Thermo Fisher Scientific, USA) system. HPLC conditions were as described in the experimental section. HESI negative scan mode was used. The temperature of ion transfer capillary, spray voltage, sheath gas flow rate, auxiliary gas flow rate and S-lens RF level were set to 300 °C, 3.5 kV, 60 L/min, 30 L/min and 55, respectively. The mass spectra were acquired with full MS mode at a resolution of 70000 FWHM with 2.0×10^6 of Automatic Gain Control (AGC) target and 100 ms of maximum ion injection time. The analyses were performed without lock mass. The raw LC-MS data were analyzed with Xcalibur^{TM} 4.0 software (Thermo Fisher, Waltham, MA, USA).

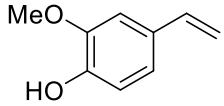
NMR spectra were recorded on Bruker ARX-400 instrument.



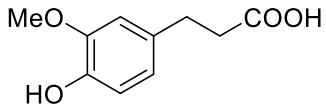
The decarboxylated product of 4-HA, 4-hydrostyrene, was detected in HR-MS. HRMS(ESI) calcd for $C_8H_7O^-$ $[M-H]^-$ 119.0502, found 119.0503. The NMR spectrum of purified compound was not obtained due to its low stability in high concentration.



The decarboxylated product of CA, 3,4-dihydroxystyrene, was obtained as a pale yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (s, 3H), 6.87 (d, J = 1.2 Hz, 1H), 6.70 (d, J = 1.1 Hz, 2H), 6.52 (dd, J = 17.6, 10.9 Hz, 2H), 5.49 (dd, J = 17.6, 1.2 Hz, 1H), 5.00 (dd, J = 10.8, 1.2 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 145.6, 145.3, 136.7, 128.7, 118.0, 115.5, 112.9, 110.4. HRMS(ESI) calcd for $C_8H_7O_2^-$ $[M-H]^-$ 135.0446, found 135.0449.



The decarboxylated product of FA, 4-hydroxy-3-methoxystyrene, was detected in HR-MS. HRMS(ESI) calcd for $C_9H_9O_2^-$ [M-H]$^-$ 149.0608, found 149.0610. The NMR spectrum of purified compound was not obtained due to its low stability in high concentration.



The dihydrogenated product of FA, 3-(4-hydroxy-3-methoxyphenyl)propionic acid, was obtained as a pale yellow powder. ^{1}H NMR (400 MHz, Acetone-d_6) δ 6.16 (d, J = 1.9 Hz, 1H), 6.04 (d, J = 8.0 Hz, 1H), 5.97 (dd, J = 8.0, 2.0 Hz, 1H), 3.12 (s, 3H), 2.09 (t, J = 7.7 Hz, 2H), 1.84 (dd, J = 8.4, 7.1 Hz, 2H).^{13}C NMR (101 MHz, Acetone) δ 174.2, 147.4, 144.7, 131.9, 120.3, 115.3, 112.5, 55.5, 36.2, 30.3. HRMS(ESI) calcd for $C_{10}H_{11}O_4^-$ [M-H]$^-$ 195.0657, found 195.0654.

Table S1. Inhibitory effect of S-(-)-carbidopa and α-methyl-L-tyrosine against the biotransformation of the 3 HAs.

Compound	Inhibitory effect (%)			
	Inhibitor 1. S-(-)-carbidopa		Inhibitor 2. α-methyl-L-tyrosine	
	0.06 mg/mL	0.6 mg/mL	0.06 mg/mL	0.6 mg/mL
4-HA	5.3±0.7	98.8±0.3	81.8±2.8	96.8±0.5
CA	2.7±1.2	90.4 ± 0.9	42.7 ±3.3	84.8 ±2.1
FA	> 99	> 99	> 99	> 99

Table S2. DPPH radical scavenging activity of the metabolic products by gut microbiota of normal rats (Vitamin C: 46.29±1.072)

Compound	Radical scavenging percentage %	
	Before metabolism	After metabolism
4-HA	28.0±1.8	66.8±0.5
CA	60.9±0.6	90.3±0.3
FA	36.2±1.4	63.4±1.3

Table S3. Cell viability of the metabolic products by gut microbiota of normal rats

Compound	Cell viability %	
	Before metabolism	After metabolism
4-HA	84.6±1.9	89.7±7.3
CA	91.2±0.9	103.4±1.5
FA	96.1±6.1	105.1±1.3

Table S4. Cell recovery percentage of the metabolic products by gut microbiota of normal rats

Compound	Cell recovery percentage %	
	Before metabolism	After metabolism
4-HA	3.2±1.1	13.0±0.1
CA	8.4±1.3	27.6±1.8
FA	12.0±1.2	20.6±2.1

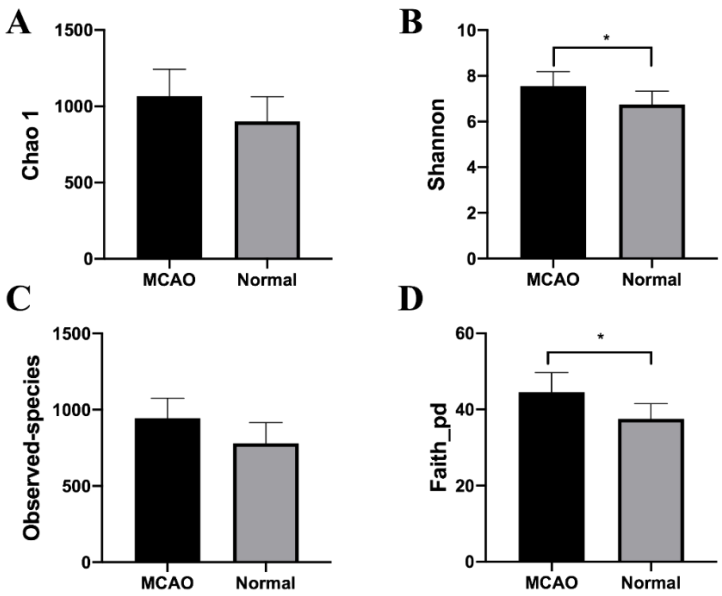


Figure S1 Alpha diversity analysis of the gut microbiota. Microbial alpha diversity between the normal and the MCAO group was evaluated by (A) Chao 1, (B) Shannon, (C)Observed-species, (D)Faith-pd. (* $p<0.05$)

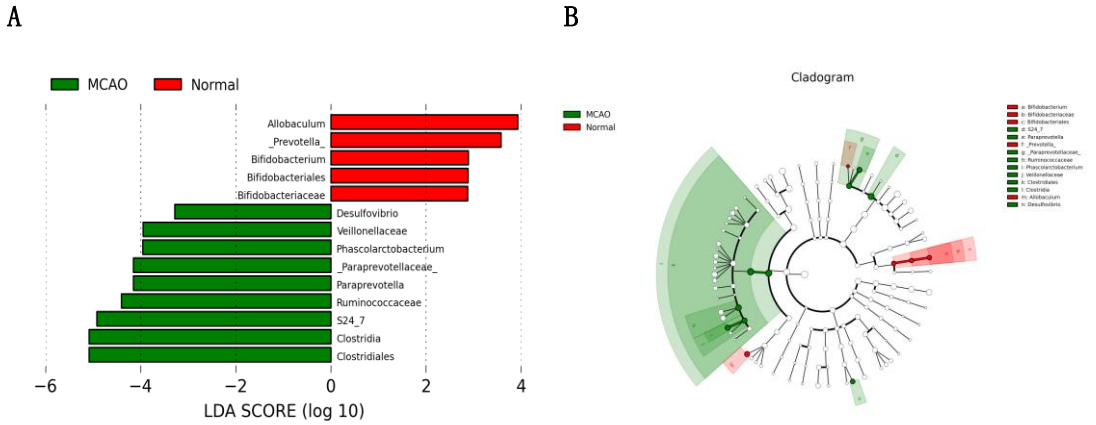


Figure S2. LEfSe analysis result of the gut microbiota from normal and MCAO rats. LEfSe (LDA effect size) analysis showing the OUT biomarkers associated with MCAO and normal groups. (A) LDA score: The green bar chart represents the bacteria that was more abundant in MCAO group and the red bar chart represents the normal group. ($P<0.05$, LDA score>2) (B) LEfSe taxonomic cladogram: The cladogram represents the phylogenetic relationship of important OTUs related to each group.

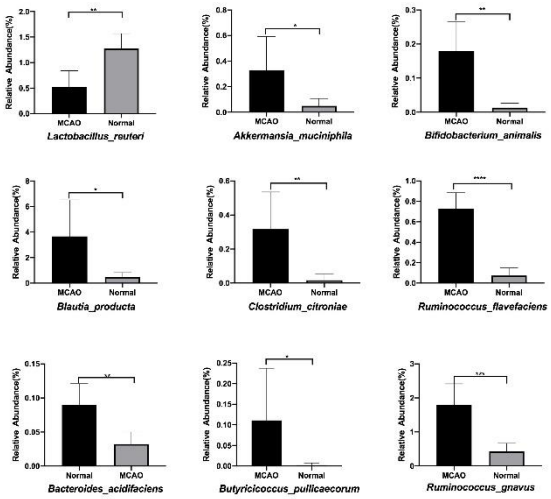


Figure S3. Gut microbiota with significant differences in relative abundance at the specie level between the normal and MCAO group. (*** $p<0.001$, ** $p<0.01$, * $p<0.05$)