Tissue-specific 1H-NMR metabolomic profiling in mice with adenine-induced chronic kidney disease

NMR sample preparation

A modified form of FOLCH extraction protocol was used to extract metabolites from the tissues. Wet weights of all tissue samples were recorded prior to extraction. Tissue samples were immediately homogenized to prevent any possible enzymatic action using 1 mL of ice-cold methanol in a PowerLyzer 24 Homogenizer (QIAGEN Group, Hilden, Germany). The mixture was centrifuged using 13,200 rpm at 4oC for 30 minutes and the resulting supernatant was transferred to a new glass vial consisting 3 mL of ice cold chloroform:methanol (2:1, v/v) mixture. The homogenate was vortexed and left in an ice bath for 15 minutes to allow for phase separation. Next, 1 mL of 0.9% of saline was added, vortexed it for couple of minutes followed by a second incubation in an ice bath for 30-45 min for complete phase separation. The upper aqueous layer was transferred to a new falcon tube. To the remaining organic phase sample, 1 mL of 0.9% of saline was added again followed by vigorous mixing and letting it stand in ice bath (15 minutes) for a second phase separation. This second aqueous phase was combined with the first. The resulting aqueous and organic layers were dried separately. The aqueous layer was dried overnight with a Labconco freezer dryer (Labconco Corporation, MO, USA) and the organic layer was dried via inert nitrogen gas. These two dried powders (aqueous and organic phases) were stored at -80oC until performing NMR experiments.

Proton spectra were collected for both sets of samples (aqueous and organic phases) using a Bruker Avance Neo 14.1 T (Bruker BioSpin Corporation, Billerica, MA) equipped with a 1.7 mm TCl CryoProbe. Aqueous phase samples were dissolved in 45 µL of 50 mM phosphate buffer (at pH 7.2) along with 5 µL of Chenomx standard (Chenomx, Inc., Alberta, Canada). The Chenomx standard had 5 mM of D6-DSS. The buffer mixture was in 100% deuterated environment and also supplemented with 2 mM of EDTA and 0.2% of NaN3. Deuterated chloroform (80 µL) consisting of 10 mM of pyrazine (as internal NMR standard) was used to re-suspend organic phase samples.One-dimensional spectra were acquired using 1D nuclear Overhauser effect spectroscopy (NOESY) pulse sequence using the parameters: 1 s recycle delay(d1), 4 s acquisition time (acq), 128 scans (nt), 12 ppm spectral width (sw), 100 ms mixing time, 1H 90o pulse width (pw), 25 oC temperature, and 8 s dummy scans. Water pre-saturation power was applied during the recycle delays for the aqueous phase samples.