BETaine-Dependent Re-Methylation Abnormalities Contribute to Homocysteine-Mediated Endothelial Dysfunction in Polycystic Kidney Disease

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Vascular abnormalities are the most significant non-cystic complications in PKD and contribute to renal disease progression. Endothelial dysfunction (ED), a systemic pathological state characterized by an imbalance between vasodilating and vasoconstricting substances, is the earliest detectable stage of vascular pathology. A major indicator of this state is decreased nitric oxide bioavailability. In PKD, Homocysteine (Hcy) is elevated, with potential vascular disease. However, the underlying mechanisms leading to increased Hcy in PKD remain unknown. Study Aim: To gain further insight into the molecular mechanisms implicated in Hcy-induced ED, we explored the Hcy pathway using a targeted metabolomics approach in a murine model of PKD.

Figure 1. Homocysteine – Methionine Cycle

- BHMT: Betaine homocysteine methyltransferase
- MAT: S-adenosyl methionine synthetase
- SHMT: Serine hydroxymethyltransferase

Methods

We included 4-week-old male and female, PKD (PCK) and control (SD) rats, (n=12 each). 24-hour urine and blood samples were collected, measured for metabolomics analysis and related chemistries.

Animals were euthanized and the kidneys were either clamp-fixed for metabolomics or preserved in formalin for histological studies.

Hcy, folate, and vitamin B12 levels were determined by ELISA. Hcy-related metabolites were measured in blood, kidney tissue, and urine samples by 1H NMR.

NMR spectra were acquired on a Bruker 600 MHz spectrometer. Spectra were phased and baseline corrected using the TopSpin software. A targeted profiling was accomplished using Chenomx ll.1 software, and compounds were identified by comparing to database ChemSpider and quantified based on internal standard (TSP-d4) peak integral.

Results and Discussion

We observed increased levels in PKD compared to control (SD) rats.

We found that PKD induced a significant increase in urine Betaine levels and decreased Plasma Betaine levels.

We measured a significant increase in tissue methionine and a decrease in tissue homocysteine.

We found a decrease in tissue glutathione.

Summary and Conclusions

- ED is shown by decreased eNOS immunoreactivity.
- Normal vitamin B12 and folate acid levels in PKD animals decreases the probability of a defect in the folate-dependent re-methylation pathway.
- Increased glutathione levels and normal methionine levels in PKD animals support the lack of an abnormality in the transsulfuration pathway.
- Increased urine betaine levels (betaine loss) and decreased betaine levels in the kidney tissue from PKD animals support a defect in the betaine-dependent re-methylation pathway.
- The correlation between urine betaine levels and plasma Hcy levels suggests that abnormalities in the betaine-dependent re-methylation pathway may be implicated in the Hcy-induced ED in early PKD.
- These findings provide novel insights into Hcy-induced ED in PKD and offer potential new targets for therapeutic interventions and novel biomarkers of vascular pathology and renal disease severity in early PKD.

References


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Figure 2. eNOS: Endothelial nitric oxide synthetase is downregulated in PKD. Representative IF staining for eNOS (red) in WT and PCK at end of 4 weeks shows left (a) and quantification: right (b) showing decreased eNOS immunoreactivity in PCK animals at 4 weeks (**p < 0.001, eNOS = quantified in % stained area and adjusted to 0% stained % area).

Figure 3. Metabolite Quantification: (A) Plasma Homocysteine levels were increased in PCK (P<0.001) and decreased in tissue methionine levels, P<0.007. (C) Tissue glutathione levels were elevated in PCK rats compared to SD controls (P<0.007). The top and bottom of the bars are the estimated 95% and 99% confidence intervals. (D) Tissue betaine levels were lower in PKD vs SD controls (P<0.016). The top and bottom of the bars are the estimated 95% and 99% confidence intervals, respectively. The vertical line extends from the 75th percentile to the highest and from the 25th percentile to the lowest data points.

Figure 4. Metabolite Quantification: Tissue methionine levels were not different in PCK (SD) rats compared to SD controls (P>0.05). Histological quantification of renal histology was performed on SD and PCK animals. The top and bottom of the bars are the estimated 95% and 99% confidence intervals. The bars above and below the line are the eNOS and eNOS-/- (mean±SD). The top and bottom of the bars are the estimated 95% and 99% confidence intervals, respectively. The vertical line extends from the 75th percentile to the highest and from the 25th percentile to the lowest data points.