Efficacy of arginine and citrulline for ammonia clearance in urea cycle disorders

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Background

Treatment of the proximal urea cycle disorders CPS1 and OTC deficiency usually includes supplementation of citrulline and arginine, although their role has never been systematically studied. We aim at filling this gap by systematically investigating the effect of arginine and citrulline on the urea cycle function in relevant *in vitro* models.

Methods

Experiments were performed in wildtype HepaRG cells, a hepatoma-derived, human hepatocyte-like cell line expressing the complete urea cycle, and in primary mouse hepatocytes (PMH). HepaRG cells were differentiated into mature hepatocyte-like cells and PMH were freshly harvested from wildtype mouse liver. Cells were treated with ornithine, arginine and/or citrulline (0-4 mM) and isotopically labeled ammonia ($^{15}NH_4Cl$, 1mM) for 24h. Supernatant was analyzed by LC-MS to determine fractionally labeled ^{15}N-urea, while RNA was extracted from cell pellets for RT-qPCR.

Results

The corrected concentration of fractionally labeled ^{15}N-urea was unchanged (1mM ornithine), or increased by 15% (1mM citrulline), 48% (1mM arginine), 79% (each 1mM citrulline and arginine), and by 197% (each 4mM citrulline and arginine) compared to PMH treated with a medium depleted of these amino acids. Similarly, HepaRG cells showed an increase of 12% (1mM citrulline), 27% (1mM arginine), 31% (each 1mM citrulline and arginine) and up to 108% (each 4mM citrulline and arginine) compared to cells in depleted media. Gene expression analysis by RT-qPCR showed a 2-6 fold up-regulation of some of the urea cycle genes after arginine and/or citrulline supplementation in HepaRG cells but not in PMH.

Discussion

We found both arginine and citrulline to increase ureagenesis in both cellular models. Other nutrients tested, such as ornithine, did not show a similar effect. This study provides for the first time a rationale for the use of arginine and citrulline in urea cycle disorders and is the basis for an improved medical therapy of UCD patients. Future work will include cellular models carrying known urea cycle gene mutations and dosage optimization for arginine and/or citrulline in an OTC deficient mouse model.