Synthesis and Evaluation of two new prodrugs for the Treatment of Nephropathic Cystinosis.

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As part of efforts to design, synthesise and evaluate novel prodrugs for the treatment of Nephropathic Cystinosis (NC) two novel antioxidants, NACA and diNACA, (figure 1) were evaluated in our laboratories (1).

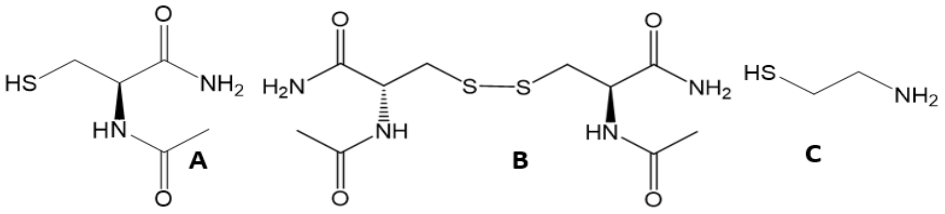

Figure 1**.** **A**: N-acetylcysteine amide (NACA); **B**: (2R,2R')-3,3'-disulfanediyl bis(2-acetamidopropanamide) (diNACA,); **C**: cysteamine.

Cytotoxicity of cysteamine, NACA and diNACA was evaluated in cultured human cystinotic fibroblasts (HCFs) in 96 well plates incubated for 0-72 hours in the presence of 25, 50 or 75 µM each of compound along with an untreated control. Media was removed and cell viability assessed. Cystine-depleting activities of the compounds were screened in HCFs utilising a simple colorimetric assay. HCFs were seeded and allowed to reach approximately 80% confluence before the addition of the test articles: 50 µM of compound in media along with an untreated control. HCFs were incubated, harvested, and cystine reduced to cysteine; the concentration of which was then determined per quantity of protein compared to a cysteine standard. Statistically significant cystine depletion was determined by paired t-test versus untreated control ($p < 0.05$).

Results

Neither compound caused cytotoxicity in HCFs up to 75 µM. NACA and diNACA increased cell viability of HCFs similarly to cysteamine. NACA depletion of cystine was statistically superior to cysteamine at 6, 24 and 48 hours, and diNACA depletion of cystine was superior to cysteamine at 6 and 48 hours.

Conclusions

NACA and diNACA caused no cytotoxicity and resulted in statistically decreases in cystine, relative to untreated control, whereas 50 or 75 µM, but not 25 µM, cysteamine achieved statistical significance at both timepoints. Therefore, further study of NACA and diNACA as potential treatments for cystinosis is warranted.

1. Hector, Cairns and Wall. 'Evaluation of NACA and diNACA in human cystinosis fibroblast cell cultures as potential treatments for cystinosis. Orphanet Journal of Rare Diseases 17(1):231, June 2022, DOI 10.1186/s13023-022-02367-w