Interaction of N-Acetyl-cysteine with OAT1 and OAT3 Transporters

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Purpose

Probenecid increases plasma and brain exposure of the antioxidant N-acetyl-cysteine (NAC) in rats through an unknown mechanism. This novel combination therapy is under Phase I clinical study as a potential treatment for pediatric traumatic brain injury. Our aim was to identify potential NAC-transporter interactions, specifically with the organic anion transporters (OAT)-1 and -3 and the multidrug resistance-associated proteins (MRP)-1 and -4.

Methods

NAC uptake studies were conducted with human embryonic kidney cell lines stably transfected with human OAT3 (HEK-OAT3), human OAT1 (HEK-OAT1), or the empty vector (HEK-EV, control). Time-dependent uptake of 25 μM of NAC traced with 14C–Acetyl-L-Cysteine was conducted over series of time points for each cell line. NAC concentration dependent uptake was determined at 10 minutes (HEK-OAT3) or 25 minutes (HEK-OAT1). Transporter-specific uptake was determined by subtracting non-specific uptake (in HEK-EV cells) from that of HEK-OAT1 or HEK-OAT3. Data were fit to Michaelis-Menten relationships, \( V = \frac{V_{\text{max}} \cdot [S]}{K_m + [S]} \). Inhibition by probenecid was evaluated by incubating cell lines with 25 μM of NAC traced with 14C–Acetyl-L-Cysteine in the presence or absence of 200 μM probenecid. Potential NAC interactions with human MRP1 and MRP4 were evaluated in overexpressing membrane vesicles. MRP1 and MRP4 membrane vesicles and their negative controls were incubated in a reaction mixture containing either 4mM ATP or AMP, 2mM glutathione, 10 μM of E217βG (positive control) traced with 3H-E217βG or 15 μM of NAC traced with 14C–Acetyl-L-Cysteine for designated times.

Results

NAC uptake was linear up to 15 minutes in HEK-OAT3 cells, 42.5 minutes (latest time point measured) in HEK-OAT1 cells and 40 minutes (latest time point measured) in HEK-EV cells. NAC uptake in both transporter overexpressing cell lines was concentration-dependent but did not reach saturation. Probenecid significantly decreased NAC uptake by both OAT1 (59% decrease, 3.88±0.26 to 1.59±0.34 pmol/min/mg of protein, \( n = 3; p < 0.01 \)) and OAT3 (93.9% decrease, 51.08±3.8 to 3.1±0.15 pmol/min/mg of protein, \( n = 3; p < 0.001 \)). There was no difference in NAC uptake by control cells in the presence or absence of probenecid. No appreciable uptake of NAC by either MRP1 or MRP4 was observed.

Conclusion

In vitro uptake and inhibition experiments indicate that NAC is a substrate for OAT1 and OAT3, but not for MRP1 or MRP4. With expanding interest in the use of NAC as an antioxidant therapy for several pathologies, including those within the CNS, the findings in this study have particular relevance for understanding how NAC crosses certain biological barriers, an important aspect of its pharmacokinetics and mechanism of action.

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