Does *N*-acetylcysteine act as an intracellular cysteine precursor in human erythrocytes?

J.E. Raftos,[1] S. Whillier[1] and P.W. Kuchel,[2] [1]Department of Biological Sciences, Macquarie University, NSW 2109, Australia and [2]School of Molecular and Microbial Biosciences, University of Sydney, NSW 2006, Australia.

In sickle cell disease haemoglobin S oxidises at a rate of 6% per day releasing reactive oxygen species that reduce the concentration of the important antioxidant glutathione (GSH). Oral administration of *N*-acetylcysteine (NAC) has been shown to increase the GSH concentrations in sickle red blood cells (RBCs). RBCs with higher GSH concentrations are less likely to become irreversibly sickled and patients with low populations of irreversibly sickled RBCs have fewer vaso-occlusive episodes (Pace *et al.*, 2003). GSH is synthesized from glutamate, cysteine and glycine and in RBCs the rate of synthesis is controlled by feedback inhibition of GSH. When RBCs are depleted of GSH, the supply of cysteine from the plasma becomes rate limiting. It has been assumed that on oral administration NAC enters the RBCs where it is deacetylated to release the cysteine required for GSH production. The aim of this study was to determine whether this was in fact the mode of action of NAC.

In vitro, both extracellular cysteine and NAC can provide a source of cysteine for GSH production in RBCs that have been depleted of 80% of their initial GSH concentration by incubation with 1-chloro-2,4-dinitrobenzene. However, while 1.0 mM NAC was required for the maximum rate of GSH synthesis cysteine at only 60 µM supported synthesis at the same rate (Raftos *et al.*, 2007). To determine why NAC was a relatively inefficient cellular cysteine precursor, we measured the kinetics of the uptake and deacetylation of NAC.

GSH depleted RBCs were incubated in a range of extracellular NAC concentrations up to 10 mM and NAC uptake was measured as the initial rate of increase in total RBC thiols. Uptake did not approach saturation at 10 mM extracellular NAC and the first order rate constant was 2.04 ± 0.07 min^{-1}. When the experiment was repeated with 50 µM DNDS or 5 µM DIDS, NAC uptake was inhibited by 58% indicating that the majority of the NAC enters by low affinity transport via the anion exchange protein. This is not surprising as NAC carries a negative charge at plasma pH. The K_m and V_{max} for deacetyaltion of NAC measured using ^1H NMR in concentrated RBC haemolysates were 1.49 ± 0.16 mM and 1.61 ± 0.018 µmol L^{-1} min^{-1}, respectively (Raftos *et al.*, 2007). Oral NAC at the dosage sufficient to increase GSH concentration in the RBCs of sickle cell patients produces a plasma concentration of only 10 µM while free cysteine increases by 50 µM (Burgunder *et al.*, 1989). At this concentration the measured rates of uptake and deacetylation of NAC are too slow to supply intracellar cysteine rapidly enough to maintain GSH synthesis. We conclude that NAC acts in the plasma by breaking thiol bonds to replace bound cysteine. The freed plasma cysteine can then enter the RBCs to support GSH synthesis.

Burgunder JM, Varriale A & Lauterburger BH. (1989) *European Journal of Clinical Pharmacology*, **36:** 127-31.

Pace BS, Shartava A, Pack-Mabien A, Mulekur M, Ardia A & Goodman SR. (2003) *American Journal of Hematology*, **73** 26-32.

Raftos JE, Whillier S, Chapman BE & Kuchel PW. (2007) *International Journal of Biochemistry and Cell Biology*, **39:** 1698-1706.