

KCI Cotransport Regulation in Red Cells

Clinton H. Joiner, M.D., PhD. Principal Investigator
Kathleen Anderson, PhD. Co-Investigator

Sickle red blood cells (SS RBC) dehydrate quickly upon entering the circulation. This worsens rheological behavior and facilitates Hb S polymerization, which is highly dependent on Hb S concentration. A promising therapeutic approach is to influence membrane ion transporters in ways that increase RBC hydration and decrease sickling. High levels of expression of KCl cotransport (KCC) in SS RBC compared to AA RBC and abnormal response of KCC to cell volume may both contribute to SS RBC dehydration. This project focuses on the post translational control of KCC activity by cell volume and its abnormalities in SS RBC. New techniques combine density shifts after KCC activation with flow cytometric detection of reticulocytes to assess the KCC flux in retics and the final hemoglobin concentration achieved, which reflects the volume set point (VSP) of KCC. Specific Aim 1 compares KCC in SS and AA retics, examining the relationship between VSP and physiological stimuli of KCC—cell volume, pH, cell Mg, and urea—to test hypotheses that the VSP in SS retics is abnormal relative to AA retics and that these abnormalities result from the oxidative damage known to occur to occur in SS RBC. SC and CC retics will also be examined. Specific Aim 2 focuses on the functional properties of several splicing isoforms of KCC1 identified in erythroid cells, testing the hypothesis that these isoforms differ in their response to volume stimuli. KCC1 cDNA constructs will be transiently expressed in cultured human embryonic kidney (HEK) cells using a pcDNA3 vector driven by the CMV promoter. KCC activity will be measured as Cl⁻-dependent, ouabain-and-bumetanide-insensitive ⁸⁶Rb uptake, and the response to volume stimuli (hypotonic swelling and N-ethylmaleimide) assessed. Specific Aim 3 will determine the volume responsive elements in hKCC1. A series of hKCC1 proteins with progressive truncations of the N- and C-terminal cytoplasmic domains will be expressed in HEK cells and analyzed for the basal and volume-stimulated KCC activity. Site-directed mutagenesis will target selected serine/threonine residues in the cytoplasmic domains of hKCC1 to test the hypothesis that one (or more) of these residues is phosphorylated to maintain basal (unstimulated) KCC activity and dephosphorylated to effect KCC activation by volume stimuli. These studies will elucidate the molecular basis of KCC volume regulation and its abnormalities in SS RBC, and lead to improved therapies to mitigate cellular dehydration in sickle cell disease.