**Title:** Protective effect of L-Glutamine (Endari) on free radical scavenging of red blood cell in sickle cell trait individual exposed to physical stress. S. Mukherjee (PI), S.K. Das (Co-Inv.), Henry Okafor (Co-Inv.) and M. del Pillar (Co-Inv.. Emmaus Medical Inc.

**PROJECT SUMMARY:**

Our previous studies have shown that treadmill exercise causes: a) an increase in the lipid peroxidation potential of red blood cell (RBC) membrane; b) an increase in the density of RBC membrane; and c) microvascular shunting in individuals with sickle cell trait (SCT). This change was mediated by oxidative stress. L-glutamine (Endari) is known to increase redox ratio [(NADH): (NAD+ +NADH)] within sickle RBCs. Hence, supplementation with L-glutamine leads to improved transport and utilization of glutamine in sickled RBC The purpose of this research is to study whether the accumulation of free radicals in RBCs of SCT subjects during exercise can be prevented by prior intake of L-Glutamine. The increase in redox ratio within sickle RBC in turn improves the cellular defenses against oxidative stress induced by exercise. Our hypothesis is that the accumulation of excessive amounts of free radicals in red blood cells of SCT subjects during exercise can be prevented by prior intake of L-glutamine. Subjects will be exposed to continuous graded exercise on a treadmill until fatigue (30 minutes maximum), followed by 15 minutes of rest. Electrocardiographic and clinical monitoring will be done during the procedure. Venous blood will be drawn before exercise, at peak exercise, and 15 minutes after completion of exercise. In order to test the hypothesis, we will study the following physical and biochemical parameters of RBC in age-, sex-, race-matched and profession matched subjects from 40 SCT and 40 AA subjects under conditions of exertion and non-exertion and with or without L-Glutamine intake. L-glutamine will be given orally (3 mg.kg body wt.) twice daily for 3 days prior to exercise to 50% of both AA and SCT subjects. To test the hypothesis, we will carry out the following aims: whether L-glutamine intake prevents (1) the exercise-induced changes in venous blood count and blood gas parameter values in SCT subjects; (2) physical stress - induced oxidative stress by regulating the activities of antioxidant enzymes, NOS and redox potential and redox potential NADH NAD in SCT subjects as a result inhibiting lipid peroxidation and (3) exercise-induced loss of RBC deformability in SCT subjects associated with an increase in RBC density by modulating lipid and protein composition, intracellular Ca2+ ion level, Na+-K+ - and Ca2+ -ATPase. If we find that L-Glutamine intake prevents loss of deformability due to decrease in excessive accumulation of free radicals in RBC of SCT subjects during exercise, this observation will open up a new therapeutic approach to counteract the exercise related adverse effects among sickle cell trait subjects.

**RELEVANCE:**

Individuals with SCT develop microvascular complications frequently associated with high altitude and extreme exertions. Our laboratory was the first to demonstrate that under exertion and deoxygenation, RBCs of individuals with SCT exhibit biochemical and physical properties directly associated with lipid peroxidation, membrane protein modification and oxidative stress. L-Glutamine (Endari) reduce the acute complications of SCD due to increase the NAD/NADH redox potential in sickle RBC, and resulting in clinical benefit. This pilot study will greatly accelerate not only efficacy of prevention and /or intervention for physical stress induced microvascular complication in SCT individuals, but also increase our knowledge about mechanism of action of L-glutamine (Endari) by which it protect the loss of deformability. Hence, this study will open up a new therapeutic approach to counteract the exercise related adverse effects among SCT subjects.

**Title:** Protection of mustard gas-induced lung injury/fibrosis associated neuroinflammation by antioxidant liposome. Role – PI, NIH (SC1).

**PROJECT SUMMARY:**

Gulf War syndrome is a chronic and multi-symptomatic disorder affecting returning military veterans of the 1990–91 Persian Gulf War and characterized by memory and concentration problems, depression, confusion, ataxia, pulmonary and autoimmune disorders and other unexplained chronic abnormalities. Enduring brain dysfunction occurs amidst the highly manifested symptoms in veterans with GWS. Furthermore, sulfur mustard (SM) gas causes serious late disabling and progressive pulmonary complications known as mustard lung. We reported earlier that in guinea pigs, intratracheal exposure to SM analog 2-chloroethyl ethyl sulfide (CEES) causes accumulation of high levels of TNF-α associated with the formation of neutrophilic alveolitis, varying degree of interstitial fibrosis and inflammation as a result of stimulation by cytokines and oxidative stress. Recently, we reported that the delivery of N-acetylcysteine (NAC) through drinking water and/or intratracheal infusion of antioxidant liposomes containing tocopherol offered prophylactic protection against CEES-induced lung injury in guinea pigs. We also reported that intratracheal infusion of CEES caused the down-regulation of pro-opiomelanocortin (POMC) in the brain and dose-dependent increases in microglial cell number, microglial activation, an increase in α-synuclein expression and a decrease in dopamine transporter level and brain inflammation. Recent studies by others have established lasting brain dysfunction in animal model of GWS with concomitant augmentation of oxidative stress, inflammation, and declined neurogenesis in the brain, and systemic inflammation. It is interesting to note that known that a proto-oncogene, translocator protein (TSPO) is implicated in neuroinflammation but it is not known whether TSPO is modulated in CEES-induced neuroinflammation. We hypothesize that liposomal antioxidant capable of restoring redox homeostasis in Gulf War Veterans will improve neuroinflammatory diseases associated with lung injury/fibrosis and modulate neuroinflammation. To accomplish this goal, we will address the following three aims: Aim 1: Investigate the mechanistic basis of neuroinflammatory disorders caused by intratracheal CEES-induced lung injury; Aim 2: Establish the molecular link between CEES-induced neuroinflammation and TSPO function; Aim 3: Assess prevention of CEES-induced lung injury-associated neuroinflammation by antioxidant liposomes. The proposed preclinical studies will guide understanding of the immunopathology underlying neuronal disorders associated with lung injury and establish a possible link between peripheral inflammation and CNS, commonly associated with GWS. This study also would enhance the PI’s capacity to transition to non-Score extramural funding support.



