Effects of dietary arginine supplementation on cerebral oxygenation and perfusion in sickle transgenic mice as detected by MRI.

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Introduction: Nitric oxide (NO) is a powerful vasodilator produced by the action of nitric oxide synthase (NOS) on arginine and molecular oxygen to form NO and citrulline. NO may be highly significant in sickle cell disease (SCD) where the vaso-dilatory effects of NO are critical and vasoconstriction can enhance microcirculatory obstruction. Patients with SCD have reduced levels of plasma arginine and we have recently demonstrated that sickle transgenic mice also have diminished levels of plasma arginine (1). Furthermore, using blood oxygen level dependent (BOLD) MRI we demonstrated that transgenic mice expressing high levels of human alpha and beta-S-globin have higher levels of deoxyhemoglobin (deoxyHb) in liver, kidney, and brain compared to control mice (2,3). Arterial spin labeling (ASL) perfusion measurements have shown reduced cerebral blood flow in these same transgenic mouse models. We hypothesize that arginine depletion may play an important role in these cerebrovascular deficits. We report here the effects of arginine supplementation on perfusion and deoxyHb in brain of sickle transgenic mice that express human hemoglobin.

Animal Model: We examined three types of mice expressing exclusively human sickle hemoglobin that have poor cerebral perfusion and oxygenation relative to control mice (2,3). S+S-Antilles mice are moderately severe and express human alpha, beta-S, and beta-S-Antilles and are homozygous for the mouse beta major deletion. NY1KO mice are more severe and express exclusively human alpha and beta-S and varying levels of human gamma. Studies were performed on NY1KO mice with medium and low gamma expression (denoted gamma-M and gamma-L respectively) that have reduced hematocrit (4). Arginine supplementation studies were performed on 8 transgenic mice consisting of 4 S+S Antilles mice and 4 NY1KO mice (2 gamma-M, 2 gamma-L). Mice were anesthetized through a breathing mask with 1.5% isofluorane mixed with a breathing gas of either air or pure oxygen.

MRI: Imaging was performed on a 9.4 Tesla horizontal bore MR imaging systems (Varian Inova). Spin echo images were acquired (echo time=50msec, 7 transverse slices spanning frontal cortex to cerebellum, in-plane resolution of 200 microns, slice thickness 1mm). In order to alter brain deoxyhemoglobin concentrations the breathing gas was cycled from air to pure oxygen and back to air while sequential images were acquired. Parametic maps of deoxyhemoglobin changes were calculated from difference images between normoxia and hyperoxia. ASL perfusion was measured using the FAIR technique in conjunction with 4 shot EPI with in plane saturation of static spins. (TI=1.8s, TR=4s, res=64x64, FOV=3cm)

Arginine Supplementation Protocol: Two baseline scans were taken on each animal before dietary arginine supplementation to determine CBF and BOLD hyperoxia response. The mice were placed on 5% arginine chow and were scanned 3 times after at least 50 days on arginine. Plasma levels of arginine were determined prior to MR measurements. After ~60 days the NY1KO mice were taken off arginine and monitored for an additional 60 days.

Results: Figure 1 shows the evolution of plasma arginine levels and cerebrovascular function in the S+S Antilles mice. The horizontal lines in the figure denote levels of plasma arginine, CBF, and BOLD response that were previously determined in control mice. It is clear that arginine supplementation increased cerebral blood flow and cerebral oxygenation (as indicated by a reduced hyperoxia response). Similar results were found in the NY1KO mice, which showed cerebrovascular improvement by either decreased hyperoxia response (-25% +/- 12 %) or increased cerebral perfusion (+40% +/- 24%). Improved perfusion and oxygenation persisted in the NY1KO mice for more than 60 days after they were taken off the arginine diet. These initial results indicate that dietary arginine supplementation can improve cerebral oxygen delivery capacity in sickle transgenic mice and may be an important treatment option in SCD

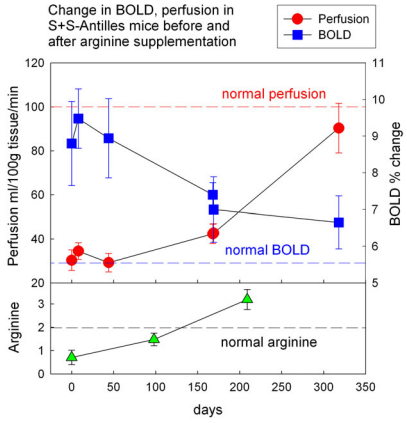

Figure 1: Group perfusion and BOLD hyperoxia responses of S+S Antilles mice post arginine supplementation.

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