This is the background section of an NIH grant application.

Medical Background

1.1.1 Pathophysiology of Sickle Cell-Associated Pulmonary Hypertension

The prevalence of pulmonary hypertension (PAH) in patients with sickle cell disease (SCD) may have been underappreciated in the past. The Sickle-Cell Pulmonary Hypertension Screening Study documented a 32% precedence of (PAH) in adult patients with sickle cell disease.[1] This study and other recent work by the same group have demonstrated that PAH is far more prevalent in this population than was previously assumed. Elucidation of the role of nitric oxide (NO) biology in the development of sickle cell-associated PAH has produced a working model from which future hypotheses can be generated.[1,2,3,4,5]

Hemolysis releases free hemoglobin and arginase, which results in scavenging of endothelial-derived nitric oxide, unavailability of substrate to nitric oxide synthetase, and a paucity of NO available to diffuse to the vascular smooth muscle layer.[6] NO regulates vasodilation and inhibits platelet aggregation, leukocyte adhesion and smooth muscle proliferation. Red cell hemoglobin is largely sheltered from endothelial-derived NO, since diffusion of NO across the erythrocyte membrane is limited, and mechanical factors and fluid dynamics produce an RBC-free zone along the endothelium and an unstirred layer near the erythrocyte.[3] No such protection exists for cell-free plasma hemoglobin resulting from lysis of sickled cells. The heme rapidly scavenges NO in quantities sufficient to deplete local NO and prevent vasodilation. The problem is exacerbated by the dissociation of cell-free hemoglobin into dimers, which are small enough to diffuse into endothelial and smooth muscle intracellular spaces and thereby interfere with NO action upon vascular smooth muscle. In addition, proinflammatory cytokines, endothelin-1, and adhesion molecules are overexpressed by endothelium in the absence of the normal NO inhibition/regulation of the production of these substances.[3]

Most patients are also asplenic, contributing to microthrombosis and erythrocyte adhesion to endothelium. In the lung, repeated minor hypoxic episodes lead to vascular constriction, recruitment of inflammatory cells and vascular proliferation and remodeling.[6] This process proceeds unchecked when sickling and hemolysis decrease the available supply of protective NO. Shear stress and infarction may result.[6] Since small vessels are also affected by these processes, capillary transit times increase, increasing the rate of polymer formation. [7] Endothelial damage over time progresses to endothelial and small muscle hypertrophy and thickening.

Because hypoxic episodes can occur repeatedly at mild and therefore unnoticeable levels of hypoxia, tissue damage may accumulate in the absence of unusual symptoms. By the time pulmonary hypertension presents, usually as dyspnea on exertion, the vascular damage may be irreversible. Echocardiographic changes may be the earliest way to detect impending pulmonary hypertension. [6]

Pulmonary hypertension in patients with sickle cell disease differs from idiopathic pulmonary hypertension in that the pulmonary artery pressure is only moderately elevated, cardiac output is high, and pulmonary vascular resistance is only mildly elevated[5].

Sildenafil increases cGMP levels by inhibiting phosphodiesterase 5, an enzyme found in high concentrations in lung tissue. The drug inhibits degradation of cyclic GMP, promoting smooth muscle relaxation via nitric oxide.

While cardiac catheterization remains the gold standard for measuring pulmonary artery pressures and diagnosing PAH, it is invasive. Thus for initial and routine assessment echocardiography is used. Historically, echocardiographic estimates of pulmonary artery

pressures have been used, but there is increasing attention to monitoring the tricuspid regurgitation (TR) jet. Recent studies in patients with SCD have shown excellent correlation of TR jet velocity with PAH, and increased TR jet velocity has been associated with death in SCD patients.[1]

1.1.2 Alternative Treatments

There is a dearth of alternatives for sickle cell patients with PAH. Current alternative treatments for PAH include oxygen, prostacyclin analogues, ET-1 receptor antagonists, sodium butyrate, arginine butyrate, phosphodiesterase inhibitors, thromboxane inhibitors, anticoagulants and calcium-channel blockers. None has emerged as the standard therapy, and it appears unlikely that any will do so in the near future.

Therapies for SCD include NO, L-arginine, hydroxyurea, dipyridamole and transfusion therapy.[6] These also appear to be of limited benefit for the prevention of PAH.

Therapies that are cumbersome and expensive to administer, such as inhaled NO and IV prostacyclines, are unlikely to ever become standard therapies. We do not anticipate that a competing therapy will emerge and become the standard treatment during the course of the trial.

1.2 Finalization of Protocol

The draft protocol reflects considerable thought on the part of the authors. However, a few points might be worthy of consideration prior to finalization.

Although we recognize this could add appreciably to the cost of the study, inclusion of pharmacokinetic studies should be considered. The pharmacokinetics of sildenafil have not been formally evaluated in children or in the sickle cell population. Pharmacokinetics could be performed on a random subset of patients, stratified by age and gender.

We propose excluding subjects who are actively seeking a bone marrow transplant. Patients who unexpectedly proceed to other therapies such as BMT during the trial should continue to be followed in order to document the reversibility or lack thereof of their pulmonary hypertension, since it will be of interest to try to determine what level of PAH is irreversible.

It would be ideal to extend our current knowledge of the relationship of TR jet velocity in SCD with mortality. PAH in SCD may be more lethal than idiopathic PAH; that is, SCD patients may experience an equivalent risk of death at lower jet velocities than do other PAH patients.[2] One group has suggested that the relationship is linear at lower velocities but becomes logarithmic at about 2.5 m/s. We suggest additional measures of correlation among subjects undergoing both procedures.

1.2.1 Influence of Ancillary Conditions on Measurement of Endpoints

Disease associated impairments may influence outcomes in this trial. For example, anemia may alter exercise capacity. As an acute measure of the severity of SCD, we propose an assessment of hematocrit and white blood cell count, hemoglobin S, and hemoglobin F by high-performance liquid chromatography at the time of each six-minute walk assessment. Although these measures lack sensitivity and specificity as indicators of acute disease, unfortunately at present there are no good markers of disease activity. Additional measures of the severity of underlying disease should include sequelae of SCD, such as recent episodes of pain crises, acute chest syndrome, stroke and priapism.

Ancillary treatments may also influence outcomes. In order to control for the results of such interventions, in addition to recording the use or dosage of hydroxyurea, more precise measures

of the success of treatment should be studied, such as neutrophil count and percent hemoglobin F.

Measures taken to optimize patient stability, such as oxygen therapy and blood transfusion, should be included as covariates. If such supportive care is adjusted during the trial, this should be accounted for by including the treatments undertaken in repeated measures models. Statistical analyses could also evaluate time to addition of a major supportive therapy. Analyses should take into account the patient's disease process: SS, SC or S-beta^0.

2 Literature Cited

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