Hydroxychloroquine and Cognitive Function after Surgery

NCT03025087

Document Date: 11/29/2020

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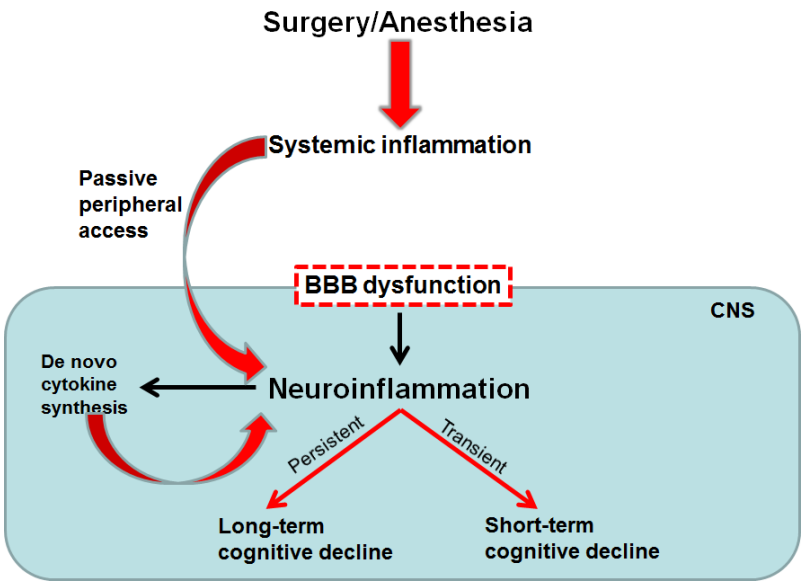
Purpose of the study

As our population ages, the manifestations of systemic atherosclerosis (stroke, cognitive impairment) extend the burden on the healthcare delivery system. The consequences of atherosclerosis are particularly relevant during surgery, where perioperative neurologic events can have a dramatically detrimental effect on the duration and quality of survival. The long-term objective of our multidisciplinary Neurological Outcome Research Group (NORG) is to understand the mechanisms underlying neurologic and neurocognitive dysfunction after surgery and to reduce the incidence of these devastating outcomes.

This study is a pilot investigation intended to gather preliminary information to assess the efficacy of hydroxychloroquine to preserve blood brain barrier integrity, decrease inflammation, and improve cognitive function after surgery including cardiac surgery with cardiopulmonary bypass (CPB). Our study is based on a model (see Figure) that surgical trauma (irrespective of type of surgery) with general anesthesia evokes a systemic inflammatory response.(1) Damage-associated molecular pattern (DAMPs), including HMGB1, are released in the systemic circulation contributing to the permeability of the blood–brain barrier (BBB). Opening of the BBB allows peripheral cytokines and alarmins to enter the brain and the CNS, causing neuroinflammation. In the hippocampus, HMGB1 activates receptor for advanced glycation end products (RAGE) signaling thus perpetuating the inflammatory response and further contributing to neuroinflammation, including de novo synthesis of cytokines like TNF-a and IL-1b. Activation of this positive feedback loop accounts for memory dysfunction and possibly localized synaptic/neuronal deficits.



Preliminary data from our pilot study (Pro00045387) indicates that blood brain barrier disruption occurs in about 60% of cardiac surgery patients and 50% of noncardiac surgery patients. Including an expanded cohort of patients will allow the investigation of differences in the outcomes of interest by surgery type.

The primary purposes of this pilot investigation are to:

1. Obtain data on the pharmacokinetics of hydroxychloroquine during surgery.
2. Determine the effect of hydroxychloroquine upon blood brain barrier (BBB) integrity after surgery.
3. Determine the effect of hydroxychloroquine upon perioperative inflammation.
4. Determine the relationship between hydroxychloroquine, inflammation, BBB disruption and neurological/neurocognitive function after surgery.

Background & Significance

Cognitive decline after surgery has generated great scientific and public interest because brain dysfunction leads to a reduction in quality of life for both the patient and their family. While there has been substantial advancement in perioperative organ protection leading to reductions in mortality associated with surgery, the incidence of cognitive dysfunction has changed little over the last ten years, despite substantial advances in pharmacology and technology. This phenomenon is related in part to the changing surgical population. Although older patients are now able to safely undergo surgical procedures without serious concern over loss of life, these elderly individuals are at a substantially increased risk for central nervous system dysfunction, particularly, cognitive decline. The clinical and financial implications resulting from these problems can be profound, with prolonged hospitalization and increased resource utilization being associated with major and minor neurobehavioral deficits.

Our investigations to date demonstrate that cognitive impairment occurs frequently in the large number of increasingly elderly patients undergoing cardiac operations every year. Post-operative cognitive deficit (POCD) is present in 36% of patients at 6 weeks after surgery and importantly, persists in 42% of patients up to five years after surgery.(2) Similarly after noncardiac surgery, the incidence of POCD was over 50% at 6 weeks after surgery and approximately 45% at 1 year after surgery.(3) Moreover, perioperative cognitive decline predicts long-term cognitive dysfunction, with dysfunction resulting in reduced quality of life.(4,5)

In addition to ischemia due to hypoperfusion and distal embolization, cardiac surgery elicits a systemic inflammatory response that may lead to a disruption of the BBB and neurologic dysfunction.(6) In experimental models, the use of CPB leads to opening of the BBB.(7,8) Disruption of the BBB can be seen on MR imaging (MRI) as enhancement of the subarachnoid space on post-contrast FLAIR images. Under normal conditions, gadolinium-containing compounds do not cross the BBB. Under ischemic conditions, however, an increase in MMP-9 leads to proteolytic breakdown of the BBB integrity that allows passage of gadolinium to the CSF and the parenchyma. Because gadolinium shortens T1, it disrupts the CSF signal suppression of FLAIR, and CSF appears hyperintense. In a recent study of 19 cardiac surgery patients who underwent MRI in the postoperative period, approximately 50% of the patients demonstrated disruption of the blood brain barrier.(9) However, neurological/neurocognitive function was not assessed in this study. Furthermore, preliminary data from our pilot study (Pro00045387) indicates that blood brain barrier disruption occurs in about 60% of cardiac surgery patients and 50% of noncardiac surgery patients and that this increase in permeability may be associated with worsened cognitive function at 6 weeks after surgery.

Hydroxychloroquine (HCQ) is an antimalarial compound that has been reported to have therapeutic effects in a wide array of conditions, including diabetes mellitus, dyslipidemias, coagulopathies, infectious diseases and malignancies. Mechanisms of action responsible for these effects likely include altered signaling through cellular receptors, post-glycosylation modifications of infectious agents, decreases in levels of inflammatory mediators and inhibition of autophagy. Many of the pathways are likely dependent on drug-induced changes in intra-endosomal acidity.(10,11) Therapeutic activity in autoimmune diseases may also be due to its activity as a toll-like receptor 9 (TLR 9) antagonist and through inhibition of quinone reductase 2 (QR2).(12,13)

Although use of the antimalarials in cutaneous lupus dates to the 1890s with J.S.Payne's description of the efficacy of quinine in inducing pallor in a patient with photosensitive malar rash, it was not until the 1950s that their usefulness in cutaneous lupus erythematosus and,

subsequently, SLE became widely recognized.(14) For years chloroquine, followed by the less toxic HCQ, were limited to use in mild RA and for the joint and skin manifestations of SLE. With a broadening understanding of its mechanisms of action, HCQ has become a cornerstone of therapy in SLE, reducing its morbidity and mortality. HCQ, like quinine and the other antimalarials, is a weak base capable of easily gaining entry into cells and gravitating to the acidic compartments of lysosomes and other cytoplasmic vesicles, raising their pH incrementally and altering protein processing. This long-recognized effect, by hindering antigen presentation, receptor recycling, and protein excretion, is thought to lead to a downstream reduction in autoantibody formation and T-cell activation.(15,16) More recently, HCQ has been shown to disrupt even earlier events in the immune activation cascade by inhibiting the function of TLRs 3, 7, and 9 stationed on the lysosomal membranes. By inhibiting the triggering of these TLRs by self DNA, RNA, and immune complexes, HCQ interrupts and deescalates the ongoing inflammatory processes of RA and SLE.(17-19) In vitro, aminoquinoline compounds are specifically effective against □beta-amyloid protein induced neurotoxicity.(20) Hydroxychloroquine suppresses acute-phase reactants, lymphocyte responsiveness, macrophage function, and cytokine release.(20-23)

HCQ is now considered a mainstay of therapy in lupus. Its clinical effects in SLE include improvement in rash and joints, reduction in flares, improved renal outcomes, a reduction in vascular events, and improved mortality.(24-27) It also has efficacy in mild to moderate RA and has been shown in multiple studies to have a protective effect against arterial and venous thrombosis in SLE patients.(28) HCQ has been shown to have a potential antithrombotic role in the antiphospholipid (APL) antibody syndrome, possibly by interfering with the binding of APL antibodies to the endogenous anticoagulant annexin A5.(29) It also has a favorable effect on lipid profiles and glucose metabolism, which may improve the cardiovascular profiles of our patients with these atherogenic diseases.

In animal models, the aminoquinolines have been shown to be protective against BBB dysfunction. In one study, mice were pretreated with either chloroquine or saline for six consecutive days and then given a dose of kainic acid, which is known to induce seizures and cause BBB dysfunction.(30) Animals treated with chloroquine had less IgG and serum protein extravasation into the brain compared with controls, indicating better preservation of the BBB.

In a recently completed study at Duke (unpublished data), adult male Sprague-Dawley rats underwent 60 minutes of deep hypothermic circulatory arrest (DHCA) at 18°C after being pretreated with either Chloroquine (25mg/kg) vs. PBS vehicle, or a selective quinone reductase 2 (QR2) inhibitor (25mg/kg) vs. 50% DMSO vehicle administered intraperitoneally 2 hours before CPB (n=3-4). Brain magnetic resonance imaging (MRI) was performed on postoperative day (POD) 1 using a 7T Bruker Biospec horizontal bore scanner, and neurologic function scores were assessed on POD 1 and 2. Brain samples harvested 48 hours after CPB/DHCA were used to quantify necrotic neurons (acid fuchsin-celestine blue) in 5 representative areas of cortex and CA1 hippocampus. MRI analysis revealed a decrease in blood brain barrier permeability as measured by gadolinium chelate in animals treated with chloroquine or selective QR2 inhibitor compared to their respective control groups ($p < 0.05$). Histologically, animals treated with chloroquine or QR2 inhibitor exhibited lower numbers of cortical and hippocampal necrotic cells compared to vehicle-treated animals ($p<0.05$, respectively). QR2 inhibition also improved neurologic function on POD1 (neurologic score 2.33 ± 0.58 in QR2 inhibitor vs 4.33 ± 0.58 in DMSO vehicle; $p < 0.05$).

As a consequence of its anti-inflammatory, antimicrobial, and BBB preservation properties and its long history of safety, particularly with short-term use, HCQ may be an ideal candidate for evaluation as a neuroprotective agent following surgery.

Design & Procedures

This study is a pilot investigation intended to gather preliminary information to assess the efficacy of hydroxychloroquine to preserve blood brain barrier integrity and improve cognitive function after surgery. In this clinical trial, up to 50 informed and consenting patients ≥50 years of age will be prospectively enrolled over a 1-year period to provide 30 evaluable subjects.

Study Procedure

Patients selected for enrollment will be informed of the study, as well as the risks and benefits of participation. Patients will be invited to participate and a log of all patients and those that accept and reject the invitation will be kept. As part of enrollment, a Mini Mental Status Exam (MMSE) will be performed and all patients scoring < 26 will be excluded to minimize the possibility of early dementia in our patient samples. Similarly patients scoring ≥27 on the Center for Epidemiological Studies – Depression (CES-D) scale (indicating severe depression) will be excluded. If patients develop renal failure (GFR < 30 ml/min/1.73 sq.m) postoperatively (after consent), they will not receive gadolinium based contrast for MRI.

Hydroxychloroquine Administration

The average adult dose of HCQ in the initial management of lupus is 400 mg once or twice daily. Similarly, the initial dose in rheumatoid arthritis is 400-600 mg daily.(31) Recently, HCQ has been administered safely to 19 subjects at a dose of 1000 mg daily as an adjuvant to lung cancer chemotherapy (median follow-up 9.1 months).(32) We therefore propose a dose escalation protocol as follows:

- Phase 1 – 6 evaluable subjects (3 cardiac, 3 noncardiac): 1000 mg HCQ on the day prior to surgery.
- Phase 2 – 6 evaluable subjects (3 cardiac, 3 noncardiac): 1000 mg HCQ on the day prior to surgery followed by 400 mg daily until the day of MRI imaging on postoperative day 1-5.
- Phase 3 – 6 evaluable subjects (3 cardiac, 3 noncardiac): 1000 mg HCQ on the day prior to surgery followed by 400 mg twice daily (800 mg total) until the day of MRI imaging on postoperative day 1-5.
- Phase 4 – 6 evaluable subjects (3 cardiac, 3 noncardiac): 1000 mg HCQ on the day prior to surgery followed by 500 mg twice daily (1000 mg total) until the day of MRI imaging on postoperative day 1-5.
- Phase 5 – 6 evaluable subjects (3 cardiac, 3 noncardiac): 1000 mg HCQ 1-2 hours after separation from CPB or at end of noncardiac surgery followed by the highest tolerated dose from the previous 4 phases divided into 2 equal daily doses until the day of MRI imaging on postoperative day 1-5.

All doses will be provided per oral or per nasogastric tube that is routinely placed after cardiac surgery. A total of 50 patients are proposed to account for drop-outs (drug intolerance, MRI exclusions; unwilling to return at 6 weeks). Subject who do not complete MRI during their hospitalization will continued to be followed at 6 weeks and 1 year, however, they will not be included as an evaluable subject and will need to be replaced to meet the required number of evaluable subject per phase.

A safety review will be done prior to dose escalation. After all subjects in each dose group have completed study procedures through post-operative Day 4-5, all available safety information will be reviewed by the principal investigator (PI) and Dr. Eddie Jooste (Anesthesiology CRU Director). If there are no clinically significant safety concerns at a given dose, the study may continue escalation to the next higher dose.

Safety will be assessed by the following outcome measures:
- Examination of adverse events
  Examination of QTc values
- Examination of abnormal changes in hemodynamic measurements (heart rate, blood pressure)
- Clinically significant abnormal changes in laboratory values compared with baseline (hematology, chemistry, urinalysis)
- Evaluation of concomitant medications and CPB procedure parameters (time on CPB, cross-clamp time, urine output during surgery, any difficulty separating from CPB [such as the need for intra-aortic balloon pump, left ventricular assist device or reinstitution of CPB]) will also be taken into consideration in the evaluation of subject safety.

<u>Neuropsychological Assessment Procedures</u>

Once identified as meeting study criteria and consented for participation, patients will be tested prior to surgery, at 6 weeks (± 2 weeks) and 1 year (± 1 month) post-surgery with the cognitive battery detailed below. All testing will be conducted by experienced psychometricians either in the patient's hospital room or in the Behavioral Testing Laboratory located in Duke South.

1. *Hopkins Verbal Learning Test - Revised (HVLT-R)* - The Hopkins Verbal Learning Test is a word-list learning task used to assess memory. Over three trials, a 12-word list of three semantic categories is presented to the examinee who then recalls as many words as possible, in any order. After a 20-30 minute, a delayed recall and a delayed recognition trial are administered.
2. *Randt Short Story Memory Test* – The Randt requires subjects to repeat a brief paragraph that has been read aloud to them. Verbatim and gist recall is evaluated immediately and after a 30-minute delay. The test is used to assess discourse memory (immediate and delayed) and oral language comprehension.
3. *Modified Visual Reproduction Test from the Wechsler Memory Scale* – This test measures short- and long-term figural memory and requires subjects to reproduce from memory several geometric shapes both immediately and after a 30-minute delay.
4. *Selected subtests from the WAIS-R* –

   a) Digit Span – This is a test of short-term auditory memory and attention that requires subjects to repeat a series of digits that have been orally presented to them both forward and, in an independent test, in reverse order.

   b) *Digit Symbol* – This test measures psychomotor processing speed and attention and requires subjects to reproduce, within 90 seconds, as many coded symbols as possible in blank boxes beneath randomly generated digits, according to a coding scheme for pairing digits with symbols.

   c) *WRAT 3 Reading Subtest* -The WRAT-3 test assesses the basic reading skills. It has two alternative testing forms (blue and tan). One form is administered with the second form available if needed. Each reading test consists of 15 letters and 42 individual words printed on a plastic card. The examinee is asked to read the words loudly while the examiner follows a phonetic guide for each reading item. One point is

given for each correct letter and word. A maximum of 57 points can be earned on either the blue or the tan form. The raw scores are recorded at the bottom of the word reading section.

5. *Trail Making Test, Part A and B* – Trails is a test of processing speed and attention. In Part A, subjects are required to connect a series of numeric circles as quickly as possible. In Part B, subjects connect a series of numeric and alphabetic circles in order (e.g., 1-A-2-B etc.) as quickly as possible.
6. *Grooved Pegboard* – Timed test of motor speed and coordination recommended by consensus panels on the assessment of cognitive decline after surgery. This test is used to control for generalized slowing in performance which might confound performance on some of the other timed neuropsychological tests.
8. *Stroop Test* - The Stroop Task is a psychological test of our mental (attentional) vitality and flexibility. The task takes advantage of our ability to read words more quickly and automatically than we can name colors. If a word is printed or displayed in a color different from the color it actually names; for example, if the word "**green**" is written in **blue ink** we will say the word "green" more readily than we can name the color in which it is displayed, which in this case is "blue." The cognitive mechanism involved in this task is called **directed attention**, you have to manage your attention, inhibit or stop one response in order to say or do something else.

The series of test listed above will be recorded using a digital voice recorder for quality assurance purposes. Quality assurance activities will include reviewing of testing audio files, generating performance review documents, and providing feedback on accuracy and completeness. There is no risk to the study subject since patient identifiers will not be provided. Study subjects will not be identified by name on the recording. Only the study number, test date and initials of the tester will be provided on the digital file label. The link between study number and patient identifiers will be maintained only at the study site. Digital files will be sent to the Duke University Neurocognitive Core Laboratory office located at Duke University Medical Center, Room 215 Baker House, Durham, North Carolina 27710. These files will be stored on locked computers within locked offices to which only authorized research personnel will have access. These files will be destroyed after the primary outcome analysis of this research study is complete.

Delirium Assessment

Subjects will undergo a 3D CAM prior to surgery and nonintubated patients will have the 3D CAM on post-operative days 1, 2, and 3. If the patient is intubated during their post-operative course, a CAM-ICU delirium assessment will be performed in place of the 3D CAM.

Neurological Testing

Neurologic history and physical exam will be completed preoperatively (baseline), six weeks (± 2 weeks), and one-year (± 1 month) after surgery. To allow comparison with stroke trials, the NIH Stroke Scale (NIHSS) will be used. NIHSS, a serial measure of neurologic deficit, is a 42-point scale that quantifies neurologic deficits in 11 categories, and is a sensitive and reliable measure to detect a change in neurologic function. To ensure consistency and to enhance the detection of subtle neurologic changes, each patient will have repeated examinations by the same examiner when possible.

Neuroimaging Procedures

Imaging will be performed on a 3T scanner (Siemans Healthcare, Erlangen Germany) in the Center for Advanced Magnetic Resonance Development in the Department of Radiology.

Patients are expected to undergo postoperative MR imaging once hemodynamically stable, typically around 1-5 days after surgery. Imaging protocol will include standard product pulse sequences, including high resolution structural T1-weighted images co-localized with contiguous multidirection diffusion-weighted imaging with b value of 1000. In addition, contiguous T2-FLAIR images will be obtained prior to and following administration of a standard dose of gadolinium contrast agent (e.g. 0.3 ml/kg gadobutrol or 0.1 mmol/kg gadopentetate dimeglumine). Gadavist (generic name gadobutrol) or MultiHance (generic name gadobenate dimeglumine) will be injected as a rapid intravenous bolus during which a time series of 8 T1 maps acquired with a fast T1-mapping sequence with partial inversion recovery will be performed, following a single pre-contrast T1 map. Total imaging time is approximately 45 minutes. Patients who develop a GFR <30 after surgery will not receive gadolinium but will continue to be followed for adverse events until they are discharged from the hospital, at Week 6 and 1 Year study follow up.
If an orbital radiograph is requested for MRI clearance, it will be completed prior to the scheduled MRI and will be done here at Duke facility. The orbital x-ray will be completed if the subject has no medical records acceptable for clearance.

MR Image review for presence of BBB disruption

A board-certified neuroradiogist, blinded to the clinical and surgical variables, will read the MRI, and also review the precontrast and postcontrast FLAIR images for the presence of HARM (hyperintense acute reperfusion marker) and classify it by location (sulcal, ventricular, and/or generalized) and severity (mild, moderate, or severe), according to the classification scheme described by Merino et al.(9)

Quantitative DCE MRI of BBB disruption

Using T1 time series data, we will calculate the rate at which the contrast agent passes from the vascular compartment into the tissue compartment, Ki, using the Patlak formulation of tracer leakage.(33) Plasma levels of gadolinium will be sampled from a region-of-interest in the sagittal sinus. Parenchymal region-of-interest measurements will be made in the white matter.

Quality of Life Assessment

Quality of life will be measured in conjunction with each cognitive assessment at baseline, 6-weeks (± 2 weeks),, and 1-year (± 1 month) in all enrolled patients. We will use an instrument that includes a measure of functional status derived for use in cardiovascular populations (the Duke Activity Status Index or DASI), psychological measures including depression (the CES-D), the State Trait Anxiety Inventory (STAI), the Hopkins Symptom Checklist (SCL-90), social/role functioning (including employment status), and the Short Form 36 question (SF 36) generic quality of life instrument.

Blood Sampling

Blood samples will be drawn for analysis as a part of this study. One 10 ml sample of peripheral blood will be obtained from each patient prior to surgery and stored. Genomic DNA for analysis will be obtained from this sample and analyzed for the presence of candidate SNPs (single nucleotide polymorphisms) including APOE. 10 ml of blood will also be sampled at baseline, end of CPB, postoperative days 1, 2 and day of the MRI scan, and at 6 weeks (± 2 weeks), and 1 year (± 1 month) follow-up for assessment of inflammatory and proteomic markers. In addition, 10ml of blood will be drawn to assess HCQ drug levels at end of CPB and day of the MRI scan. Subjects will have the option to sign the Genomic and Proteomic Database Repository (IRB#00015651) consent form, allowing the banking of any remaining plasma and DNA samples for future research. If a creatinine level is not drawn for standard of care post-operatively, one 6ml blood sample will be drawn to assess kidney function (GFR ≥ 30 ml/min/1.73 sq.m) for

gadolinium administration safety. Up to 106 ml of blood will be collected during the 12 month study participation period.

Ophthalmic Assessments

Hydroxychloroquine associated retinopathy is rare and thought to be associated with cumulative dosage and with chronic administration. Although toxicity is not expected in the short-term dosing schedule in this protocol, ophthalmic assessment will be performed on every subject consented as an additional measure of safety. Ophthalmic assessments will occur at baseline and at 6 weeks after surgery. Each assessment will take approximately 1.5 hrs and will include visual acuity, optical coherence tomography (OCT) of the macula, and multi-focal electroretinogram (ERG). Ophthalmic assessments will be performed in the undilated eye, but the eye may need to be dilated in the event of minuscule pupils. A report confirming the absence of retinal abnormalities (that are contraindications to hydroxychloroquine administration – see exclusion criteria #18) will be provided to the study team by an ophthalmologist (Dr. Iannaccone) prior to initial dosing. The 6-week report will document serial changes and will be evaluated as part of the safety profile.

ECG for QTc evaluation

Standard of care (SOC) ECG will be used to evaluate QTc prior to HCQ dosing. For males QTc>450msec and for females>470msec will be ineligible to receiving HCQ dosing that day. If no ECG will be obtained per SOC, the research team will perform an ECG at baseline and prior to daily HCQ dosing.

Standard of Care

- Anesthesia (premedication, choice of anesthetic technique and drugs to be administered) per standard of care
- Surgical and postoperative care per standard of care
- Clinical status will be monitored throughout the duration of the procedure
- Standard of Care ECG if performed will be used for evaluation of QTc prior to HCQ dosing throughout the study.

Selection of Subjects

To be eligible for inclusion into the study, each patient will have to fulfill the following criteria:

Inclusion Criteria

1. Male or female, age ≥ 50 years old.
2. Patients scheduled to undergo cardiac surgery (CABG, CABG + Valve, Valve) with CPB or general surgery (e.g. orthopedic, abdominal, urological).
3. Patient has voluntarily signed and dated the study-specific informed consent form approved by Duke University Health System Institutional Review Board (DUHS IRB)

Exclusion Criteria

1. Cardiac surgery scheduled to be performed without cardiopulmonary bypass
2. Patients requiring emergent operation
3. Patients with a history of myocardial infarction within 7 days of surgery
4. Patients with a history of porphyria, psoriasis, chronic dermatitis, or retinal disease
5. Patients receiving preoperative digoxin
6. Patients with symptomatic cerebrovascular disease with substantial residual deficit
7. Patients with a history of alcohol abuse within 2 years of screening
8. Patients with history of psychiatric illness

9. Patients with impaired liver functions (AST, ALT 2 times the upper limit of normal)
10. Patients with impaired renal functions (GFR <30ml/min)
11. Patients with less than a 7th grade education or unable to read and thus unable complete the neuropsychological testing
12. Patients scoring < 26 on a baseline Mini Mental State examination (MMSE) or scoring >27 on the Center for Epidemiological Studies – Depression (CES-D) scale
13. Female subjects of childbearing potential who have had menstrual period within the past two years
14. Patients with bodily implants unsafe for MRI use
15. Patients with a history of claustrophobia
16. Known or suspected hypersensitivity to quinine (chloroquine or hydroxychloroquine)
17. Patient with pre-existing diagnosis of G6PD deficiency
18. Patients who have participated in another interventional clinical study within the previous 30 days
19. Any other concurrent disease or illness that, in the opinion of the investigator, makes the patient unsuitable for the study
20. Major ophthalmologic comorbidities (ex: ruptured globe, retinal vascular occlusive disease, retinal artery occlusion, anterior ischemic optic neuropathy, media opacification due to corneal abnormalities or cataract that prevent ocular and optical coherence tomography examination, glaucoma, age-related macular degeneration, history of intravitreal injections, and macular edema)
21. Patients who have received chemotherapy in the last 12 months
22. Patient with elevated QTc (male>450msec, female>470msec) noted from baseline ECG

Risk/benefit assessment

There is no direct benefit of this study to the enrolled subjects. Data gathered from this pilot study may benefit future patients. Up to 106 ml of blood will be drawn during the 12 month study participation period. The patient's informed consent document discusses this risk, but it is unlikely that this will contribute significantly to the patient's need for blood transfusion. To minimize any potential risk to the patient from genetic data, and to not adversely affect their quality of life in any way, investigators and patients will be blinded to the individual patient's genotype. This information will not be included in the patient chart, will remain absolutely confidential and will not be given to the patient or their family. Samples sent to the IGSP for genotyping will be identified only by a code number whose relation to the patient’s name and other identifiers is available only to the data manager. In any publications, the identity of the patient will remain anonymous. Appropriate medical therapy for any adverse events from study participation is always available.

Following HCQ administration in doses adequate for the treatment of an acute malarial attack, mild and transient headache, dizziness, and gastrointestinal complaints (diarrhea, anorexia, nausea, abdominal cramps and, on rare occasions, vomiting) may occur.(31) In the treatment of lupus as well, adverse events associated with HCQ use is infrequent and generally mild (gastrointestinal, cutaneous).(26,34) When used as an adjuvant to lung cancer chemotherapy, the most commonly observed treatment-related adverse events were rash (37%), nausea (33%), diarrhea (33%), and fatigue (30%). Vomiting, dyspepsia, anorexia, and dry skin occurred in less than 20% of patients.(32) Dose-related retinal damage has been observed in some patients who had received long-term aminoquinoline therapy for lupus or rheumatoid arthritis but is unlikely in our study which is limited to 6 days of therapy at doses well below the cumulative dose/duration reported to be a threshold for injury (5-7 years of therapy or 1000 grams).(35) To optimize safety, ophthalmic assessments will be done prior to dosing in an

effort to ensure subjects do not have existing macular damage and are appropriate to participate. Concomitant hydroxychloroquine and digoxin therapy may result in increased serum digoxin levels; serum digoxin levels should be closely monitored in patients receiving combined therapy. Concomitant hydrocychloroquine and propofol may be associated with QT-prolongation, QTc values should be closely monitored in patients receiving combined therapy. As hydroxychloroquine may enhance the effects of a hypoglycemic treatment, a decrease in doses of insulin or antidiabetic drugs may be required. In summary, we expect that headache, dizziness, diarrhea, anorexia, nausea, and abdominal cramps to be the most likely side effects to short-term HCQ therapy.

The risks of the ophthalmic assessments are very low. Minor fatigue from testing is possible; breaks will be introduced as needed during the testing. If eye dilation is needed, standard dilating eye drops will be employed. Minor discomfort from the lights employed during ophthalmic assessments are possible but unlikely outcomes. Subjects may directly benefit from the study by receiving an ophthalmic exam. The main findings of the eye examination can be given to the subject and appropriate referral, if needed, will be made by the ophthalmologist (Dr. Iannaccone). The study will not provide refractive prescription to the participants.

The MRI procedures follow guidelines set by the FDA with regard to specific absorption ratio (SAR), limits on gradient slew rate (dB/dt), and noise. The presence of non-MR safe metal body implants is determined by the interviewing each potential subject; reviewing the patient's medical history for any operative reports denoting the make, model, and type of any implant; and completing a detailed screening questionnaire with the patient and patient caregiver. MR safe implants will be determined as those listed in the *Reference Manual for Magnetic Resonance Safety, Implants, and Devices, 2012 Edition* (Shellock, Biomedical Research Publishing Group) as being tested safe at 3-Tesla magnet strength. For those subjects determined to have MR safe implants, these must be located below the neck to prevent data acquisition artifacts. Participants and staff are instructed to remove all metal objects, including clothing with metal clasps before entering the magnet room. The magnetic properties of unknown material are tested outside the magnet room with a strong permanent magnet. Participants and staff are also instructed to enter the magnet room slowly and pause at the entrance to determine if any items on their person may be pulling toward the magnet. Participants are required to wear insert earplugs during scanning to reduce noise levels below FDA limits. Some participants may feel uncomfortable or confined once positioned within the bore of the MRI system. This potential reaction is reduced by discussing the procedure before entry into the magnet room, by providing the subject with a mirror through which they can look out into the room, and by communicating with the subject over the intercom. During set-up and anatomical imaging, participants can choose to listen to music or radio over their headphones. Nevertheless, if participants continue to feel uncomfortable, the imaging procedure is terminated and the subject is removed from the magnet.

Evaluation of the BBB on MRI will require intravenous bolus injection of an FDA approved paramagnetic contrast agent, Gadavist (generic name gadobutrol) or MultiHance (generic name gadobenate dimeglumine). Review of the literature shows that administration of Gadavist or MultiHance in humans at a dose of0.3 ml/kg gadobutrol or 0.1mmol/kg gadobenate dimeglumine, has rarely been associated with adverse effects, and up to 0.4 mmol/kg can be given without significant side-effects.(36) The most frequently reported adverse events related to gadolinium-based contrast agents include headache (2%), nausea (2%), injection site reaction (1%), and flushing (1%). The risk for nephrogenic systemic fibrosis among patients with impaired elimination of the drugs is ameliorated in our study by the exclusion of subjects with

GFR < 30 ml/min/1.73 sq.m.Gadavist and MultiHance were identified by The American College of Radiology to fall under the category of Low Risk to the development of nephrogenic systemic fibrosis. No cases of death attributable to administration of this contrast agent have been reported. Intravenous lines inserted as part of gadolinium infusion have the same small risks as any other intravenous lines which will be thrombophlebitis and extravasation of the infused fluid into the subcutaneous tissues. These complications will be watched for and treated, if necessary.

Subject Identification, Recruitment, & Compensation
After obtaining permission from the operating surgeon, surgical subjects will be screened by the study coordinator by reviewing the surgical schedule. Prior to asking any patient for consent to participate, the patient will be approached first by the surgeon or one of the surgical care team to see if the patient is willing to consider being in the study. If so, the patient then will be called by one of the investigators in this study or a member of the Anesthesiology Department who will be responsible for the care of the patient during the surgery, and who understands the study. If the subject is willing to participate and they do not meet any exclusion criteria, they will be contacted by the research coordinator and will discuss their willingness to participate in the research protocol. An IRB approved phone script will be used during this communication. If the subject is in agreement, the coordinator will present the research protocol in its entirety to the prospective participant and will answer any and all questions as they arise. If the subject agrees to participate, the coordinator will ask the subject to sign and date the appropriate consent form at their first visit. A copy of this consent form will be given to the subject and a copy of the consent form will be added to the patient's medical record.

The subject will be asked to sign a separate consent form to allow us to store portions of their collected blood specimens and any data collected obtained under this research study and maintain these samples and data in a database/repository (PRO00015651) for possible use in future research studies relating to surgical outcomes.

Subjects will be reimbursed $50 for participating in each of the two follow-up return visits to Duke Medical Center to defray travel costs and time. Subjects will also receive a $9 parking pass upon each of their return follow-up visits to Duke. Total possible reimbursement per subject/caregiver pair is $177. The study may pay for your hotel accommodation, with prior approval, if required for subject's baseline visit.

Costs to the subject
Subjects will not incur any costs as a result of participation. Costs of all study procedures will be incurred by the study.

Data analysis & Monitoring

Neurocognitive Analyses
Definition of cognitive deficit: Because we are proposing a cognitive test battery that results in many scores per patient, it is essential to assess the results with an analytic approach that makes use of all the information without redundancy. We plan to define cognitive deficit from our raw test scores by using a factor analysis. Factor analysis is a technique that has the objective of representing the large, overlapping set of raw test scores by a smaller set of factors through transformation based on the intercorrelations among the test scores. Each "factor" represents a hypothetical, unobservable characteristic that can be interpreted as a discrete domain of

cognitive functioning. The first factor is based on the computation of principal components and is the linear combination of the items, subject to a normalizing constraint, which accounts for the greatest amount of variance across patients. The second factor is that linear combination, uncorrelated with the first, which accounts for the next largest amount of variance, and so on. An orthogonal rotation will be done for interpretability of the results. The factor analysis can thus identify the independent (orthogonal) domains of cognitive function, as measured by the full battery of cognitive tests, and reduces the number of outcomes to be analyzed. Factor analysis makes use of all the information provided by the patient to return independent, continuous and standardized summary measures for assessment. By measuring each domain with several tests, and combining the scores using the factor analytic solution, we will also enhance the reliability of the overall measurement of each domain. Based on our extensive experience with the test battery, we expect to obtain factor scores for four separate domains of cognitive function.

Our factor analysis will be performed on baseline test scores, and the factor coefficients (weights) of each test on each factor will be used to construct comparable domain scores at each of the follow-up time periods, based on the patients' test scores for that time period. In this manner, the domains will be identified at preoperative baseline and will be consistent at follow-up time points. Numeric change scores will be calculated for each of the factors, and an overall continuous cognitive score will be calculated by averaging the separate scores. We will define our primary endpoint as the 6-week change in the continuous cognitive score, which is the difference between the 6 week and baseline score. Overall POCD, defined as a decline from baseline in any of the domains equal to or greater than one standard deviation of the baseline domain score will also be assessed. The numeric domain scores, which treat cognitive function as a continuum, are sensitive to differences in functional change, including differences in amount of improvement, while the dichotomous POCD endpoint best focuses on the outcome of interest across all cognitive domains.

Baseline characteristics which have been found to be important in cognitive function, including age, sex, baseline cognitive status, years of education, race, duration of surgery and bypass time, depression and anxiety will be examined for differences between the randomized groups. Prior to analysis, assumptions of the regression model will be conducted, including homogeneity, linearity, and independence. The association between the cognitive change score at 6 weeks and blood brain barrier disruption will be assessed using multivariable regression, adjusting for age, level of education, baseline cognitive score, and surgery type (cardiac vs noncardiac). It should be noted, however, that this is a pilot investigation intended to obtain preliminary data for a grant application.

All neurocognitive test instruments will be scored according to the recommendations for the particular tests, with missing item values handled as recommended (e.g., pro-rated sum, average). Baseline and intraoperative variables will be evaluated for balance between the treatment groups. Overall significance level will be set at alpha=0.05, with adjustments for multiple testing made as indicated. All analyses will be conducted using SAS statistical software, widely respected for its power and reliability.

<u>MR Image review for presence of BBB disruption</u>
A board-certified neuroradiogist, blinded to the clinical and surgical variables, will read the MRI, and also review the precontrast and postcontrast FLAIR images for the presence of HARM (hyperintense acute reperfusion marker) and classify it by location (sulcal, ventricular, and/or

generalized) and severity (mild, moderate, or severe), according to the classification scheme described by Merino et al.(9)

Quantitative DCE MRI of BBB disruption

Using T1 time series data, we will calculate the rate at which the contrast agent passes from the vascular compartment into the tissue compartment, Ki, using the Patlak formulation of tracer leakage.(33) Plasma levels of gadolinium will be sampled from a region-of-interest in the sagittal sinus. Parenchymal region-of-interest measurements will be made in the white matter.

Privacy, Data storage & Confidentiality

Primary study data will be images acquired from the neuroimaging procedures and patient case report forms. Preprocessing software will strip any MR image headers that may contain subjects' names, as well as de-skull the images to prevent any visual reconstruction of subject identity, and will rewrite the resulting data as image volumes identified by subject identification number only. The post-processed imaging data will be stored electronically on a secure computer housed at the Center for Advanced Magnetic Resonance Development, as well as archived on DVD storage medium to be placed into a locked cabinet. The data collected in the Case Report Forms will be obtained specifically for research and internal quality assurance purposes. While the hemodynamic, laboratory, and historical data will be collected at the time of the intraoperative study, the information is all part of the routine medical record documenting the patient's care in the perioperative period. The patient's identity will be specifically protected and confidentiality will be maintained by reporting any published material by patient number. All genetic data will remain confidential and will not be available to the patient, the patient's family or physician. All genetic material for patients enrolled into this study will be identified only through unique study identification number. This unique study number will also be linked to a barcode affixed to each study sample, collected according to protocol. For future review or database dissemination, the study number and the barcode will be the only identifying information associated with the subject. The subject's name, social security number, or any other "identifying information" will not be linked to the individual subject. At the time of consent, the patient will be asked to sign an authorization of release to provide us permission to obtain medical information in the event the patient returns to the hospital, health care facility, or family doctor for indications of chest pain, congestive heart failure, heart attack, or stroke during the course of the study. At the one year follow-up visit, the patient will be asked to complete a medical condition questionnaire. This information will be used to determine any changes in health since surgery. Participants will not be identified by name in any analysis of these data, nor any presentation or publication resulting from the analysis of these data.

If the patient is not eligible or does not want to participate in the study, their protected health information (PHI) is shredded and destroyed. For consented patient, PHI is kept in a locked desk or cabinet when not being used by the research staff. The information we review are on individual computers which are password protected. The office doors are kept locked when no one from the research staff is in the office. The only people who have access to the rooms are the PI and research staffs which are listed on the key personnel sheet.

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