Cocoa Flavanols for Modulating the Surgical Immune Response and Accelerating Clinical Recovery

Study Protocol and Statistical Analysis Plan
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1.0 Specific Aims and Major Rational

The primary aim of this mechanistic, double-blind, placebo-controlled proof-of-concept clinical trial is to demonstrate that preemptive oral administration of cocoa flavanol for five days before surgery (equivalent to 50 grams of dark chocolate per day) will attenuate the surgery-evoked increase of HMGB1 in blood plasma and NFkB signaling in innate immune cells shortly after surgery. The HMGB1-NFkB signaling axis is a major pro-inflammatory axis that is activated in response to tissue trauma. Preemptive attenuation of this axis in multiple animal injury models is associated with reduced tissue damage and increased survival. A secondary aim is to capture preliminary patient-centered outcomes data including postsurgical pain and daily functioning for six weeks after surgery, and relate these outcomes to the intake of oral cocoa flavanol and surgery-evoked activation of the HMGB1-NFkB signaling axis.

The protocol for this trial is embedded in previous studies by Drs. Angst and Gaudilliere that examined immune correlates of surgical recovery in patients undergoing major joint replacements.[1,2] These studies have demonstrated that exaggerated activation of NFkB-signaling in innate immune cells in response to surgery predicts delayed resolution of pain and delayed functional recovery after surgery.

2.0 Background and Rational

Over 40 million major surgeries are performed annually in the US alone, while over 250 million surgeries are performed worldwide.[3,4] Recovery after surgery is highly variable and can take much longer than expected. For example, patients undergoing major abdominal surgery can take more than six months to fully recover.[5] Protracted recovery can affect up to 30% of patients and leads to impaired daily functioning, delayed return to work, decreased quality of life, and major socioeconomic costs.[6-8] Reducing “loss of productivity” by one day per major surgery could save the US $8 billion per year for this direct cost alone.[9]

During the last two decades significant effort has been made to enhance recovery after surgery.[10] Despite the implementation of pragmatic and standardized clinical protocols to enhance recovery and shorten hospital length of stay, the utility of these protocols for improving patient-centered recovery cost-effectively remains uncertain.[11-13] Critical elements of recovery that greatly matter to patients and health care providers include the resolution of pain, daily functioning, and loss of postoperative fatigue.[14] A patient-centered and cost-effective focus on postoperative recovery pays tribute to three goals of health care: Improving patients’ experience, improving health, and constraining per capita cost.[15] As such, novel and cost-effective strategies are greatly needed to accelerate patient-recovery after surgery.

Preliminary data by Dr. Angst and his collaborators indicate that administration of a cocoa flavanol extract that is equivalent in dose to the amount of cocoa flavanol contained in about 50 grams of dark chocolate decreases plasma levels of HMGB1. HMGB1 is an archetypical alarmin, i.e., an endogenous mediator that is released upon cellular stress and injury. HMGB1 triggers a pro-inflammatory cascade by binding to toll-like receptors (TLRs) on innate immune and other cells, which results in activation of pro-inflammatory transcription factors (e.g. NFkB) and the subsequent release of major pro-inflammatory cytokines (e.g. TNFα). The prominent role of the HMGB1-TLR axis in inflammatory disease states including surgery, trauma, stroke, and myocardial infarction has recently been highlighted.[16-19] Importantly, dampening activity along this pathway in preclinical injury models has been shown to improve outcomes.[20-22]

The potential of HMGB1 as a therapeutic target in acute inflammatory disease states has recently been emphasized.[23,24] A major challenge is the identification of effective and non-toxic clinical strategies that can safely modulate HMGB1 in humans. This research study will evaluate a safe, highly scalable, and relatively cheap pre-surgical nutritional intervention that has significant potential to do just that, safely modulate HMGB1 and improve clinical recovery after surgery. As such, this proposed research could change clinical practice within years. While studied intervention targets a specific pro-inflammatory pathway implicated in aggravated tissue damage and delayed healing/recovery, the use of broader and less specific anti-inflammatory interventions in the perioperative period including non-steroidal anti-inflammatory drugs and corticosteroids is common clinical practice.

Preliminary data

Drs. Angst and Gaudilliere have studied immune correlates of clinical recovery in patients undergoing hip replacement surgery.[1,2] They have successfully implemented mass cytometry at the "bedside" to characterize the immune response to surgery close to *in-vivo* conditions in peripheral whole blood of patients.[25] These studies have been critically supported by our surgeon-collaborator Dr. Goodman (co-investigator). In close collaboration with Dr. Aghaeepour (co-investigator), the team of investigators has developed computational algorithms that allow extracting biologically meaningful and actionable immune parameters from generated high-dimensional data sets.[26]

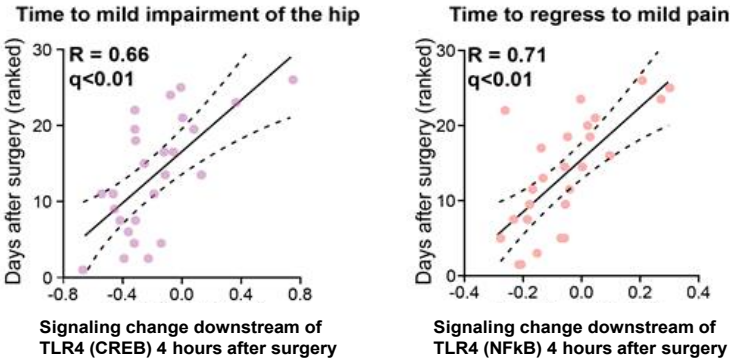

Figure 1: Exaggerated signaling downstream of TLR4 in innate immune cells 4 hours after surgery strongly correlated with the speed at which patients resolved postoperative pain and regained function of the operated hip. Seventy four percent of patients remained mildly impaired at the end of the six-week study period, while 10% remained moderately impaired.

Pertinent to this application are data demonstrating that exaggerated signaling in innate immune cells downstream of toll-like receptor 4 (TLR4) shortly after surgery predicts delayed functional recovery and resolution of pain in patients undergoing hip replacement surgery (Figure 1).[1,2] TLR4 is a major target of HMGB1, which is massively released by tissue trauma.

Data by our collaborator Dr. Sloan (co-investigator) demonstrate that oral administration of a high-dose (1,000 mg) versus a very low dose (10 mg control) of coco-flavanol (CocoaVia®) in 24 healthy adults (age 50-70 years) significantly suppressed plasma levels of HMGB1 (Figure 2).

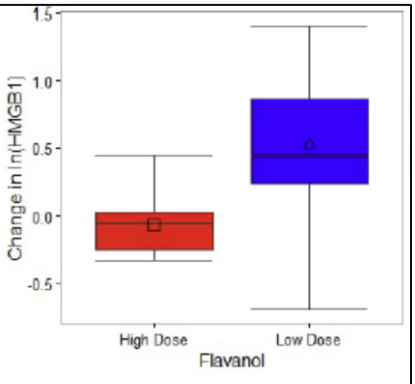

Figure 2: Oral intake of a high dose of cocoa flavanol (weeks) suppressed plasma levels of HMGB1 in 24 healthy mid-aged subjects compared to a very low dose.

Taken together the preliminary data support the main hypothesis that the preemptive administration of cocoa flavanols in patients undergoing surgery will decrease plasma

levels of HMGB1, attenuate signaling activity in innate immune cells downstream of TLR4, and accelerate clinical recovery in patients undergoing joint arthroplasty.

3.0 Study Enrollment and Participation

3.1 Study Enrollment

Participants will be patients undergoing total hip or knee arthroplasty as clinically indicated. Individuals may be referred to this study directly by their orthopedic surgeon, or may be identified in the pre-anesthesia clinic when evaluated for anesthesia care in the context of their scheduled qualifying surgery. A trained study team member, working under the direction of Dr. Angst, will obtain informed consent prior to any study related activity. Screening will be done via chart review as approved by Stanford's IRB, and by direct participant questioning. No advertising materials will be used

3.2 Participant Criteria

Inclusion Criteria

1) 18 - 90 years of age
2) Male or female
3) Planning to undergo total hip or knee arthroplasty, either primary or revision
4) Fluent in English
5) Willing and able to sign an informed consent form and HIPAA authorization and to comply with study procedures

Exclusion Criteria

1) Infectious disease within the last month
2) Immune-suppressant therapy within the last 2 months (e.g., azathioprine or cyclosporine)
3) Chronic medication with potential immune-modulatory effects (e.g., daily oral morphine-equivalent intake > 30 mg)
4) Major surgery within the last 3 months or minor surgery within the last month.
5) History of substance abuse (e.g., alcoholism, drug dependency)
6) Pregnancy
7) Autoimmune disease interfering with data interpretation (e.g. lupus)
8) Renal, hepatic, cardiovascular, or respiratory diseases resulting in clinically relevant impaired function
9) Active malignancy
10) Participation in another clinical trial of an investigational drug or device within the last month that, in the investigator's opinion, would create an increased risk to the participant or compromise the integrity of the study
11) Other conditions compromising a participant's safety or the integrity of the study
12) Allergy to active ingredient of CocoaVia®, the study intervention.
13) Frequent consumption of dark chocolate and flavanol containing foods (e.g. black tea, red wine, apples)

3.3 Enrollment

We plan to enroll 36 participants. All participants will be enrolled at Stanford Health Care.

4.0 Materials and Methods

The proposed study is designed as a randomized, controlled, and double-blind study comparing a daily dose of cacao flavanol versus placebo. Cocoa flavanol is commercially available as a nutrient (CocoaVia®), manufactured by Mars Inc. CocoaVia® is supplied in 125 mg capsules

containing 15% epicatechin, the active anti-inflammatory ingredient. Based on our preliminary data a daily oral dose of 1,000 mg will be used in this study.

After participants have completed the informed consent process they will be randomized to one of two treatment groups - half to group A and half to group B. Group A participants will receive a daily oral dose of 1,000 mg cocoa flavanol (CocoaVia®) for 5 days before surgery, while group B participants will receive a daily oral dose of a placebo for 5 days before surgery.

Patients will be involved in study procedures from the time they consent to participate through postoperative week 6. Procedures to evaluate baseline condition, hospital course, and recovery will take place as follows:

Questionnaires: Before hospitalization participants will complete questionnaires to evaluate expectations prior to surgery (patient expectations), stress level (perceived stress scale), pain and function (WOMAC), anxiety (POMS-anxiety scale), and baseline fatigue and functional impairment (surgical recovery scale; SRS). Participants will complete SRS and WOMAC scales daily while they are in the hospital, and weekly following discharge to track recovery over a six-week course.

Objective measures of function: Physical function will also be measured objectively by directly monitoring participant activity using a wearable device (ActiGraph) providing measurements for physical activity/function and sleep. Participants will be asked to wear the device beginning 1 week before surgery through postoperative week 6.

Blood draws: Blood will be drawn at 4 time points; once before administration of the study intervention (40ml), 1 hour before surgery (30ml), 1 hour after surgery (30ml), and on post-op day 1 (30ml). Blood will be processed for analysis of cell signaling with mass cytometry (NFkB in innate immune cells), HMGB1 plasma concentrations, and epicatechin (flavanol) plasma concentrations. Blood samples will be identified using a study code, and will be analyzed in labs at Stanford University and Stanford Health Care (Stanford Hospital).

Additional data to be collected will be demographic and socioeconomic information, medical history including co-morbidity prior to surgery, and recent medication history. During hospitalization opioid analgesic consumption, pain level, details of surgery and anesthesia, incidence of complications, and time to "discharge ready" will be recorded.

5.0 Statistical Measures

5.1 Outcome Measurements

The primary outcome is the cumulative NFkB signal in innate immune cells 1 and 24 hours after surgery.

Secondary outcomes include HMGB1 plasma levels, functional recovery trajectories (measured objectively with ActiGraph and subjectively with WOMAC function sub-scale), postoperative pain resolution (measured with WOMAC-pain subscale), and postoperative resolution of fatigue and resulting impairment of daily activities (measured with SRS). Trajectories will be reflected by the time to resolve function to mild impairment, the time to resolve pain to mild levels, and the time to resolve fatigue to 50% of maximum postoperative fatigue.

5.2 Analysis Plan

The primary outcome NFkB signaling in innate immune cells between the two study groups will be analyzed with a non-paired t-test. A similar exploratory analysis will be conducted for secondary outcomes. However, the study is powered to detect differences of the primary outcome.

5.3 Sample size

Based on data from previous studies of surgical patients receiving an active (arginine nutritional supplement) versus a control treatment, it is reasonable to assume that intervention-related effect sizes (changes in signaling activity) are about twice as large as pooled standard deviations. This has been noted for signaling molecules downstream of TLR4 in response to arginine supplementation. TLR4-signaling (NFkB) is the primary outcome of this proof-of-concept study. A sample size of 16 patients per group should therefore provide ~80% at $p<0.05$ to detect an intervention-related group differences.

6.0 Data Management

All data will be managed with participant confidentiality as our highest priority. Tissue samples will be labeled using study numbers only. These numbers will be linked to the participant using a study code that will be kept in a separate, secure location. The code linking samples to participant identity will be destroyed at the conclusion of the study.

All paper documents will be kept in a secure locked environment, away from the casual view of individuals not on the study team. All electronic data will be kept on secure, encrypted and password protected computers, and a secure, HIPAA compliant, database.

The Protocol Director, Dr. Angst, will review the data and participant safety in real time; reviewing each subject during active participation when indicated. Given the nature, safety, and scope of this study a Data Monitoring Committee is not required and will not be established.

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