Title: Doxycycline treatment to prevent progressive coronary artery dilation in children with Kawasaki disease.

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I. Purpose and Background

Specific aims and objectives
This research study attempts to reveal whether coronary artery dilation (CAD) progression in patients with Kawasaki disease (KD) could be prevented using a matrix metalloproteinase inhibitor: doxycycline.

We hypothesize a 3 week course of oral administration of doxycycline initiated during the acute phase of KD effectively blocks matrix metalloproteinase-9 (MMP-9) activity in the coronary arteries and therefore prevents the progression of CAD and aneurysm formation in children with KD.

Primary Objective
Demonstrate that doxycycline is effective in preventing progressive CAD and aneurysm formation in children with KD (Step2).

Secondary Objective
Investigate the relationship between levels of specific biomarkers and CAD and improve our understanding of the etiology of KD (Step1).

Background, significance, and rationale
Kawasaki disease (KD) is the leading cause of acquired heart disease in children. Although KD may resolve without treatment, a major sequela may cause serious complications or even death: coronary artery abnormalities (CAA). In susceptible individuals, the immune system triggered by a so far unknown extrinsic factor induces macrophage and lymphocyte activation and inflammation of the coronary arteries. About one in four children with untreated KD will develop irreversible CAA. Even following current therapeutic guidelines and treatment with intravenous gamma globulin (IVIG), 30% of children with KD will have transient or permanent coronary artery dilation. Patients with CAD, especially those refractory to IVIG treatment, may progress to develop aneurysms of the coronary arteries.

Currently, there is no therapy available to prevent the progression of CAD and aneurysm formation. There are solid guidelines about the management of patients with coronary artery dilation in order to avoid ischemic and embolic complications, however, dilation, thrombosis, calcification and subsequent narrowing of the coronary arteries can rarely be prevented. Young children, teenagers and young adults face the results of damaged coronary arteries developing coronary vasculopathy that requires early catheter intervention or sometimes even coronary bypass surgery. There is a gap in the treatment of KD for patients, who develop coronary artery dilation or aneurysm.

Coronary artery abnormalities in KD are caused by vasculitis and destruction of coronary vessel wall. This destruction is mediated by the degradation of certain extracellular matrix elements like elastin and laminin. Elastin and laminin are both enzymatic substrates of the matrix metalloproteinase 9 (MMP-9), which has an increased activity during the acute phase of KD.

MMP-9 is secreted by activated macrophages and lymphocytes that infiltrated the coronary artery walls. The macrophage and lymphocyte infiltration is part of the TNF-alpha mediated generalized immune response triggered by the so far unknown extrinsic factor causing KD. The immune system induced degradation of elastin leads to coronary artery dilation and aneurysm. The development of coronary artery aneurysm produces a high probability of irreversible changes in the coronary arterial wall, leading to subsequent stenotic lesions and significant ischemic complications.

The occurrence of CAA has been linked to elevated circulating levels of MMP-9 and leukocytes in children in the acute phase of KD. Therefore, MMP-9 inhibition may reduce the degradation of elastin and prevent progression of coronary artery dilation during the acute inflammatory process of coronary vasculitis in KD. Among the known inhibitors of MMPs, doxycycline has been studied extensively due to its long established use and safe pharmacologic profile. Doxycycline decreases TNF-alpha mediated macrophage activation and specifically blocks MMP-9 activity. More importantly, *in vivo* administration of doxycycline to mice with a KD model reduces significantly the incidence of coronary artery elastin breakdown and reduces loss of elastin. Therefore, doxycycline can mitigate TNF-alpha-induced MMP-9-mediated coronary elastin breakdown and improve coronary outcome.

Human studies reflect the above findings: doxycycline is effective in reducing aneurysms. In a clinical trial involving adults with abdominal aortic aneurysms, doxycycline administration reduced MMP activity and suppressed neutrophil collagenase activity in the aortic wall. The observation that doxycycline has a selective effect on neutrophil-derived proteases in the aortic wall suggests that it may also be effective in other conditions with vasculitis induced aneurysms, such as KD. Based on the animal studies modeling KD and adult trial showing the beneficial effect of doxycycline on aneurysms, this selective MMP-9 inhibitor offers a promising therapeutic strategy for the management of children with KD.

As of today, clinical studies have not been conducted with doxycycline in children with KD. Doxycycline is a widely used antibiotic with very few adverse effects. In children it may cause discoloration of permanent teeth when administered during tooth development (last trimester to 8 years of age). Therefore, the Food and Drug Administration (FDA) warns against the use of doxycycline in children under the age of 8 years. However, FDA allows children to be treated with doxycycline if there is a strong clinical indication outweighing the risks, such as for the treatment of anthrax.

A systematic review of the literature indicated that doxycycline is safe for use in children, particularly when used for a short duration. Pharmacokinetic and pharmacologic studies conducted by Pfizer and reported in 1967 looking at animal toxicity data and human pharmacodynamic data including administration of doxycycline to 340 children concluded that the adverse reactions in children were similar to that of adults. Subsequent studies revealed that children treated with doxycycline could develop discoloration of permanent teeth. A large independent retrospective study including 140 children treated with doxycycline showed that the degree of teeth discoloration was not universal and it correlated to the length of treatment. In children with a single course of less than 3 weeks of doxycycline treatment, none of the children had notable discoloration. Only prolonged (more than 5 weeks) or repetitive (twice or more) administration of doxycycline resulted in noticeable discoloration.

Current data suggests that doxycycline could be an effective treatment to inhibit MMP-9 activity and prevent coronary dilation in KD with a safe side effect profile if administered for 3 weeks.

The proposed research study will fill in a gap in the treatment protocol of KD. Currently, there is no specific therapy for patients, whose coronary artery dilation progresses despite IVIG or infliximab or combination therapy. Doxycycline administration is aimed to prevent coronary artery dilation in these refractory patients.

In Hawai'i, due to the unique ethnic composition of the population, the prevalence of KD is about 1:1000. That means that annually, about 50-90 children are hospitalized with KD. In the absence of appropriate therapy, as much as 25% of patients could develop coronary artery dilation with or without aneurysms, accounting for the most common acquired heart disease in children in the industrialized world.

Unfortunately, despite the early diagnosis and timely medical intervention, about 15% of children develop CAA. Currently, there is no accepted medical solution for these children. We can only attend to the natural progression of the disease, aiming therapy for the prevention of ischemic and thrombo-embolic complications of coronary artery abnormalities.

Our study assesses the clinical efficacy of doxycycline treatment to prevent coronary artery abnormalities. Since doxycycline is a strong inhibitor of MMP-9 which is thought to be responsible for the destruction of coronary artery adventitia, and doxycycline has been shown efficacious in KD animal model to reduce coronary artery dilation, it is an attractive candidate for clinical evaluation.

This study will serve as a pilot project for a larger multi-center randomized trial. The study results will be summarized and presented in a peer-reviewed journal. The results will be essential in designing a larger trial that can confidently assess the efficacy of doxycycline and promote its widespread use for prevention of coronary artery dilation and aneurysm in KD.

Our **long-term objective** is to develop a multi-center clinical trial to evaluate targeted biomarkers as predictive markers for children with KD, who will develop CAD, and to assess the safety and efficacy of doxycycline in preventing the progression of CAD during the acute phase of KD.

Preliminary studies

The Kawasaki Disease Diagnosis and Treatment Project at KMCWC consists of leading clinical researchers with an established record of clinical excellence and scientific advancement in the field of KD. The members of this group have pioneered diagnostic and therapeutic aspects of KD. They have actively participated in national and international research consortiums and are considered among the few national experts in KD. Clinical researchers in this group have published several articles about KD, including the recognition of elevated levels of MMP-9 during the acute phase of KD. The group has a long-standing relationship with basic science research laboratories at the University of Manoa. The laboratories have professional expertise in performing the proposed diagnostic studies.

Moreover, the group has already treated 12 children with doxycycline, who presented with KD and enlarged coronary arteries at KMCWC. We have explained our concerns about coronary artery aneurysms to the parents of these children, offered the use of doxycycline based on clinical evidence established in adults, and informed them about the potential side effects and the FDA's recommendations. All parents agreed to the use of doxycycline.

II. Criteria for Subject Selection

Number of subjects: 200 subjects

Demographics of subjects: Patient demographics should reflect the demographics of the patients with KD treated at Kapi'olani Medical Center for Women and Children (KMCWC). Patients will not be excluded on the basis of race or gender.

STEP 1

Inclusion criteria

1. Patients aged 1 month to 21 years

2. Confirmed diagnosis of KD by an experienced KD physician based on published guidelines

Exclusion criteria

1. Patients who do not provide informed consent

STEP 2

Inclusion criteria

1. Patients aged 1 month to 21 years
2. Confirmed diagnosis of KD by an experienced KD physician based on published guidelines
3. Dilation of the right coronary artery or the left anterior descending coronary artery (z-score > 2.5) during the acute phase of KD (during the first 4 weeks of the illness from the onset of fever)

Exclusion criteria

1. Patients who do not enroll in Step1
2. Patients whose parents refuse to administer doxycycline.
3. Patients with acute renal failure.
4. Patients with chronic liver and kidney disease.
5. Patients with an allergy to tetracyclines.
6. Patients with sulfite sensitivity.
7. Prior doxycycline use
8. Prior enrollment in Step 2 study

Vulnerable subjects: Children

III. Methods and Procedures

Study design and methods

This is a prospective case-control trial with 2 steps. All patients with the diagnosis of KD by an experienced KD physician based on published guidelines will be eligible to participate in Step 1 of the study.

Patients will be stratified into two groups depending on their echocardiogram results: Group 1 with no CAD (coronary artery diameter z-score $\leq$ 2.5), or Group 2 with the presence of CAD (coronary artery diameter z-score > 2.5). Group 2 patients will then be eligible to participate in Step 2 of the study. Patients, who consent to Step 2 of the study will be randomized into the drug treatment arm (Group 2A) or the control arm (Group 2B). Group 2A will receive a 3 week course of study drug, oral doxycycline. Group 2B will receive identical treatment and placebo. Patients with acute renal or hepatic failure, or known tetracycline allergy, will be excluded from participation in Step 2.

Block randomization will be done to ensure relatively equal numbers of patients in each randomization group to allow for interim analysis once half of the Step 2 subjects have been enrolled. Randomization will be done by the research pharmacist all other study team members and the subjects will be blinded.

Treatment protocol

Children enrolled in the study will undergo standard treatment protocol established by the Kawasaki Disease Diagnosis and Treatment Project at KMCWC reflecting national and international guidelines. Children will receive standard therapy and blood samples will be obtained to confirm diagnosis and follow progression of the disease. Besides receiving IVIG, aspirin and infliximab if necessary, study patients enrolled in Step 2 will be treated with doxycycline or placebo sugar syrup for a period of 3 weeks. We will use the accepted dosage of 4.4 mg/kg/day doxycycline PO divided twice a day. If we notice decreased urine output or

jaundice as an indicator for renal or hepatic failure, or a hypersensitivity reaction or other known side effects of doxycycline, the treatment will be discontinued. At the time of blood sampling an additional tube (3 ml) will be taken from study patients in order to perform laboratory analysis as detailed below. Up to 4 additional blood samples will be attempted to be drawn. Every effort will be made to collect these samples during a routine blood draw to avoid extra sticks. If subjects agree, any remaining blood samples will be stored for future research on Kawasaki Disease. A stool sample, if available during hospitalization, will also be collected (see Amendment 1).

Step 2: Subjects in the control group will receive placebo sugar syrup instead of the doxycycline. The placebo will be administered in the same volume as the doxycycline. Subjects will not be told if they are receiving the placebo or the study drug.

Clinical data collection
A clinical data sheet will be prepared for every patient. Clinical data will be collected as available in the subjects' medical record. Each subject will have clinical data collected at a minimum of 2 time-points.

Data collection will occur at 4 time-points.

1. Baseline – at time of enrollment
2. Visit 1 – approximately 1-2 weeks after diagnosis and initiation of treatment
3. Visit 2 – approximately 3-6 weeks after diagnosis
4. Visit 3 – approximately 8-16 weeks (convalescent phase)

The following data will be collected, as clinically available, from all patients participating in this study:

1. patient demographics (age, sex, ethnic background, height, weight, BSA);
2. vital signs on admission, vital signs at the time of echocardiogram, maximal temperature, time of febrile period;
3. duration of hospital stay, medications administered
4. laboratory data (including, but not limited to: ESR, CRP, WBC, PLT, AST/ALT, Cre, BUN, albumin);
5. echocardiographic measurements (RCA, LAD, aneurysm, LVEDD, LVEF);
6. specific laboratory data from blood samples
7. Microbiome data
8. Feeding information

Additionally, for Step 2 patients a phone call will occur between Visit 1 and Visit 2 to check for medication compliance.

Echocardiography measurements
A complete echocardiogram will be performed as part of current standard diagnostic evaluation of KD for every patient. The echocardiogram will evaluate LVEDD, LVEF in the parasternal long and short axis views. Measurements will be taken of the aortic valve annulus, the aortic sinuses, the sinotubular junction and the ascending aorta in the parasternal long axis view. The coronary arteries will be evaluated and measurements will be taken from the right coronary artery and the left anterior descending coronary artery in the modified parasternal views. Z-score of coronary artery measurements will be calculated based on the body surface area.

Specific laboratory procedures (ELISA assays)
In addition to the established and clinically indicated diagnostic and therapeutic protocol for children with KD, we will collect blood samples along the acute and convalescent phases of KD to evaluate the circulating levels of specific biomarkers: cytokines (including interleukins (IL-1, IL-2, IL-6, IL-10, IL-12), tumor necrosis factor alpha (TNFa), interferon gamma), elastin degradation products, (desmosine, soluble elastin fragments), calponin, transforming growth factor beta (TGFb), MMPs (MMP-1, MMP-2, MMP-3, and MMP-9), tissue inhibitor of metalloproteinase 1 (TIMP-1), pentraxin-3 and vascular endothelial growth factor (VEGF). Based on the

echocardiograms, study subjects will be allocated in Group 1 (no CAD) or Group 2 (CAD). We will compare quantitative biomarker levels in children without CAD (Group 1) and with CAD (Group 2). Other laboratory analysis of collected blood samples may be done to measure biomarker activity.

The laboratory measurements will be conducted in Dr. Vivek Nerurkar's research laboratory located in the Basic Science Building, University of Hawai'i at Manoa. This laboratory has already successfully performed the above experiments.

Subjects will be asked at time of consent if they will allow any extra blood samples to be kept for future research on Kawasaki Disease. If the subject agrees to allow for the storage of their blood sample for future use it will be kept in a secure location with limited access by a repository that is able to store blood samples.

Retrospective data will be collected on previous Kawasaki Disease patients. This will be done through a retrospective chart review. This data will be used to augment the prospective data for the evaluation of the primary objective.

Retrospective data collection will occur at 4 time-points as available.

1. Baseline – at time of diagnosis
2. Visit 1 – approximately 1-2 weeks after diagnosis and initiation of treatment
3. Visit 2 – approximately 3-6 weeks after diagnosis
4. Visit 3 – approximately 8-16 weeks (convalescent phase)

The following data will be collected, as clinically available:

1. patient demographics (age, sex, ethnic background, height, weight, BSA);
2. vital signs on admission, vital signs at the time of echocardiogram, maximal temperature, time of febrile period;
3. duration of hospital stay, medications administered;
   laboratory data (including, but not limited to: ESR, CRP, WBC, PLT, AST/ALT, Cre, BUN, albumin);echocardiographic measurements (RCA, LAD, aneurysm, LVEDD, LVEF).

<u>Data analysis and monitoring</u>
Descriptive statistics will be used to summarize demographic characteristics of study subjects. Study subjects' demographic data will be compared using Chi-squared test.

The efficacy of doxycycline will be assessed by Wilcoxon signed ranks test using paired data of the treatment group and compared to matched control subjects using Mann-Whitney test. Risk ratio and risk reduction rate will be calculated for the side effects and efficacy of doxycycline, respectively.

Based on our pilot data, study power analysis will be performed for a large multi-center trial.

The investigators will monitor the patients in Step 2 for any adverse events. Patients will be monitored for the duration of the study. Adverse events will be reported to the IRB in accordance with IRB reporting requirements. Reports to the FDA of any drug related adverse events will be made in accordance with FDA regulations.

<u>Transition from research participation</u>
Patients receiving the study medication will be monitored and continue to receive standard of care after the completion of their medication course.

<u>Study timeline</u>
The annual incidence of KD in Hawai'i is about 90/100000 in children < 5 years. Every year there are about 50-90 new cases of KD at KMCWC. We expect to enroll the majority of new KD patients in Step 1. About 30% of those children would be eligible for Step 2 enrollment, adding up

to 15-27 children in a year. For Step 2 of this pilot study we aim to collect data from 25 children in the treatment group and 25 children in the control group.

This study is expected to be open for 10 years.

IV. Risk/Benefit Assessment

Risk category: Greater than Minimal risk for treatment group

Potential risk(s)
The study drug may cause permanent tooth discoloration in children (infants and child less than 8 years old), however, this side effect is usually seen in long-term use of the drug. Clostridium difficlie associated diarrhea has also been reported with the use of antibacterial agents. Increase in BUN may occur. Sensitivity to sunlight may also occur. Intracranial hypertension has been associated with tetracycline use and may manifest as a headache, blurred vision, diplopia, and vision loss. Papilledema can be found on fundoscopy. An allergic reaction to the drug may also occur. A decrease in bone growth has been observed in premature infants receiving high doses of this type of antibiotic, however, it is unlikely that this is a risk at the dose of doxycycline you will receive.

Protection against risk(s)
Patients in the treatment group will be closely monitored for any adverse reactions to the study drug. The course of treatment for the study drug will be limited to 3 weeks to decrease the likelihood of tooth discoloration. Patients in the treatment group will also be cautioned against sun exposure.

Potential benefit(s) to the subject
Subjects in the treatment group may experience prevention of the progression of coronary artery dilation and aneurysm formation.

Alternative to participation
Patients who do not wish to participate will continue to receive the standard care given to all KD patients at KMCWC.

V. Subject Identification, Recruitment and Consent/Assent

Method of subject identification and recruitment
Patients will be recruited from the general wards (Carter and Wilcox units) of KMCWC. Patients with potential KD are identified by emergency department physicians and hospitalists. The diagnosis of KD is confirmed by a member of the Kawasaki Disease Diagnosis and Treatment Project at KMCWC. All children with KD will get an echocardiogram with coronary artery measurements. Children with KD eligible for this study will be identified based on their clinical presentation and echocardiographic assessment of the coronary arteries.

Process of consent
All patients (parents) will be asked to provide informed consent prior to enrolling in the study. The informed consent discussions will occur between the patients and their parents and a member(s) of the study team. The study team will explain the aim of this study and the potential benefits and risks of participation in the study and the administered treatment. Patients will be allowed to ask questions and take home the consent document before signing if they would like to discuss participation in the study with other family members or continue to think about their decision to participate. Informed consent discussions will occur in a private area as much as possible. Minor patients will be told about the study and asked to provide assent in accordance with IRB requirements.

No study procedures will occur prior to obtaining patient consent. Patients will only be enrolled if they speak English well enough to understand and read the consent document. Consent will be documented in the patient's electronic medical record.

There will be no cost to the subject to participate in this study and subjects will not be paid for their participation in the study.

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