Timothy G. Egan
5/17/07

IRB Protocol Submission

The Effect of Ultrafiltration versus Loop Diuretics on Systemic Endothelial Activation in Patients Hospitalized With CHF Exacerbation

Principal Investigator: Paolo Colombo, MD. Assistant Professor of Medicine
Co Investigator: Timothy G. Egan, MD.

Lay Abstract

Background: Patients with congestive heart failure (CHF) are often hospitalized for symptoms of fluid overload. These patients have been well documented to have endothelial dysfunction, which is corrected with removal of fluid. Patients with CHF exacerbations are often treated with diuretics to help facilitate fluid removal. Previous studies have shown, however, that use of diuretics leads to activation of neurohormonal markers, which can in turn lead to continued vascular dysfunction. Ultrafiltration is a technique that provides an alternative method of removing sodium and water via hydrostatic filtration of venous blood. However, unlike diuretics, it is not thought to lead to activation of the renin-angiotensin-aldosterone axis.

Study Purpose: In this study, we will compare the effects of ultrafiltration and intravenous diuresis on the activation of the vascular endothelium. We postulate that intravenous loop diuretics (such as furosemide) will have a largely neutral effect, because the positive effect of fluid removal will be offset by the activation of the renin-angiotensin-aldosterone axis. In contrast, we believe that treatment with ultrafiltration will show an improvement in endothelial function, as it will remove fluid without activating the neurohormonal response. To examine this, we will use a minimally invasive technique known as endothelial biopsy to obtain endothelial cells from patients. Subsequent real time PCR (rt-PCR) analysis will be done on these cells to evaluate the expression of three different inflammatory markers – nitrotyrosine, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS). This will be done before and after treatment with either intravenous loop diuretics or ultrafiltration. We will then compare the change in gene expression in these two groups. The technique of endothelial biopsy has been shown in previous studies to be safe and effective in obtaining endothelial tissue. Ultrafiltration has also been shown in previous studies to be a safe and effective alternative to IV diuresis in patients hospitalized for CHF exacerbations.

Study Subjects and Method of Recruitment: We plan to examine a total of 48 adult patients. These patients will be inpatients at CPMC that were hospitalized for CHF exacerbation. Patients will be recruited and enrolled within the first 24 hours of hospitalization. Initially, eligible patients will be approached by their responsible physicians to gauge interest in participation. If they are willing to discuss participation, they will then be contacted by an investigator for further information. All subjects must provide informed consent.

Study Procedures: Patients will undergo endothelial biopsy on two separate occasions during their hospitalization. This procedure is minimally invasive, and has been used in previous studies with minimal complications or discomfort to the patients. Half of the patients will also undergo ultrafiltration as a treatment for fluid overload. Previous studies have shown that ultrafiltration is equivalent to, if not better than, intravenous diuretics at removing fluid in patients hospitalized with CHF, and that there are no significant differences in morbidity and mortality between the two techniques.

Issues: Although ultrafiltration is not typically used as first line treatment in patients with CHF exacerbation, its efficacy and safety as a technique for doing such is documented in previously published in prospective, randomized trials.

IRB Protocol

A. Study Purpose and Rationale

Congestive heart failure (CHF) is an increasingly common problem for patients and physicians in the United States.[1] Among other problems, patients with CHF are known to have impaired endothelial function. Endothelial dysfunction is thought to be both a marker of worsening disease as well as a cause of worsening function.[2] In addition, patients with CHF are often hospitalized with symptoms secondary to fluid overload. Traditionally, these patients are treated with intravenous loop diuretics to facilitate fluid removal and lead to symptomatic improvement. Conversely, however, loop diuretics are also know to directly stimulate the renin-angiotensin-aldosterone axis, which in turn leads to worsening endothelial function. Ultrafiltration is a technique that has previously shown to be an efficacious alternative therapy to removing fluid in patients hospitalized with CHF exacerbations.[3]

A previous study done by this principal investigator demonstrated that patients with decompensated CHF had upregulation of certain inflammatory markers, such as nitrotyrosine, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS), when compared to healthy control subjects. In addition, therapy with inotropes was shown to significantly decrease expression of these inflammatory modulators.[4] In addition, preliminary research in animal models show that dogs have similar upregulation of gene expression of these inflammatory markers states of induced heart failure. However, for these animals, gene expression was similarly upregulated in dogs with normal hearts that had received a fluid load.[5] Thus vascular stretch contributes significantly to endothelial dysfunction.

We postulate that while diuretics have a beneficial effect in that they facilitate the removal of fluid in a patient with decompensated CHF, this benefit will be offset by the activation of the neurohormonal response previously described. As such, we would expect essentially a neutral response in endothelial dysfunction to diuresis – that is, little to no improvement. In contrast, ultrafiltration, while also effective at fluid removal in the decompensated CHF patient, should not have any of the same neurohormonal effects. Because of this we expect to see an improvement in endothelial function as represented by a decrease in gene expression of inflammatory markers. We expect this response similar to that seen with treatment of patients with inotropes.

Our hypothesis, therefore, is that CHF patients treated with ultrafiltration will have significantly improved endothelial function when compared to those treated with IV diuretics. We propose using a vein biopsy technique to compare the effect of IV diuresis to ultrafiltration on the activation of endothelial cells in patients with symptomatic CHF. The vein biopsy technique has previously been shown to be a safe, minimally invasive technique to examine gene expression of certain inflammatory markers in the venous endothelium.[6]

B. Study Design and Statistical Analysis

This study will be a prospective, randomized trial of patients hospitalized for CHF exacerbation, comparing the response of those receiving IV diuresis to those receiving ultrafiltration. We will examine the expression of inflammatory markers in the endothelium (nitrotyrosine, iNOS, COX-2) via rt-PCR. This is quantitated in densometric units. The levels prior to initiating intervention and after removal of 2.5L will be examined. The primary endpoint will be the difference in gene expression levels prior to and after the therapeutic intervention.

Patients will be randomized into two groups via secure computer program. Each group will have 24 subjects. Treatment will not be blinded. We assume approximately 20% of patients in each group will

have treatment failure – either inability to diurese the appropriate volume, or the need of vasoactive drugs. In addition, we expect approximately 10% of patients to have either failure of endothelial biopsy or laboratory error in PCR analysis. Thus, we would be left with 17 patients in each group. This sample size would have an 80% power to detect a difference of one standard deviation between groups to an alpha of 0.05.

C. Study Procedure

1. Hospitalization

While in the hospital, all patients will receive a cardiac diet (2g Sodium) and be fluid restricted to 2L daily. Patients will continue on ace inhibitors and angiotensin receptor blockers as tolerated. Beta-blockers will be continued as tolerated. Patients will be considered to have treatment failure if they require vasoactive drugs. Possible etiologies by which this may occur include hypotension, hypertension, acute renal failure (reflected as rising creatinine or decrease in urine output), or worsening symptoms of heart failure. Throughout their inpatient stay, patients will have daily weights checked via calibrated scales. In addition, they will have strict ins and outs measured by nursing staff, to be recorded every shift.

2. Ultrafiltration

We will use the Aquadex System 100 (CHF Solutions, Minneapolis, MN) for ultrafiltration. Patients will receive ultrafiltration according to the previously described protocol.[7] Venous access will be attained via peripheral or central venous catheters. Patients will be treated will heparin (or if allergic, argatroban), to a goal PTT of 65-85s. The rate of fluid removal will be determined by treating physician.

3. Endothelial Biopsy.

Subjects are first interviewed and given a directed physical exam. For the endothelial biopsy, sterile technique is used to place 18 gauge angio catheter into a superficial forearm vein. A J-shaped vascular guidewire is then placed via the angio catheter into the vein. It is then advanced and retracted approximately 10 times while still in the vein, dislodging endothelial cells and collecting them on the guidewire. The wire is removed and an endothelial dissociation salutation is used to elute cells from the wire tip. This technique is then repeated four more times, each time with a different sterile J-wire. Cells are then centrifugation and purified on magnetic beads coated with mouse monoclonal antibody specific to endothelial cells. Purified endothelial cells are resuspended in 20ml of lysis buffer containing detergent and RNase inhibitor and stored at -80 degrees Celsius.

Patients will undergo endothelial biopsy on two occasions during their inpatient stay. The first will be at the time of randomization, the second will be after removal of 2.5 L of fluid as recorded by nursing staff.

4. Analysis of Gene Expression

Initially, samples are evaluated for purity using reverse transcriptase PCR. Only cells that are positive for endothelial markers such as von Willebrand factor and thrombomodulin and negative for smooth muscle cell a-actine and leukocyte common antigen-1 will be used. In addition, GAPDH, a constitutive house-keeping gene, will be used as an internal control to assure that cDNAs from equal number of cells are used. Real-time PCR (rt-PCR) is then used on the purified endothelial cells to evaluate the expression of the inflammatory markers of interest: nitrotyrosine, iNOS, and COX-2. These levels will be quantified in densitometric units (du).

D. Study Drugs

Patients in the control arm of this study will be given loop diuretics (most commonly furosemide) for management of fluid overload from their CHF exacerbation. The most recent ACC/AHA guidelines recommend diuretics as part of the first line therapy for patients with congestive heart failure and signs and symptoms of fluid overload.[8]

No investigational study drugs will be examined in this study.

E. Study Devices

Patients in the ultrafiltration arm of the study will undergo ultrafiltration via the Aquadex System 100 (CHF Solutions, Minneapolis, MN). Recent data comparing the efficacy of ultrafiltration to intravenous loop diuretics on volume removal for patients hospitalized with CHF exacerbation showed that ultrafiltration was able to remove more fluid after 48hours of therapy, with similar symptomatic improvement. Primary safety endpoints, including change in serum creatinine and hypotension, were not significantly different between the two treatments.[3]

G. Study Subjects

Eligible patients must be over 18 who have been hospitalized with a diagnosis of CHF exacerbation (ICD 9 Code 428.0). These patients must exhibit clinical signs or symptoms of hypervolemia, defined as at least two of the following: rales on pulmonary exam, radiographic appearance of pulmonary edema, jugular venous distension > 7cm, or at least 2+ peripheral pitting edema. Exclusion criteria include patients with acute coronary syndrome, contraindication to anticoagulation, unattainable venous access, chronic renal insufficiency (creatinine > 3mg/dl), hypertension (>150/90), hyperlipidemia (total cholesterol > 300mg/dl), active tobacco use, chronic inflammatory condition, pregnancy, cancer, chronic use of NSAIDs or steroids, and heart transplant. In addition, those requiring use of vasoactive drugs (nitroglycerin, dobutamine, dopamine, milrinone, nesiritide) on admission to hospital will also be excluded.

H. Recruitment of Subjects

Eligible patients admitted to the hospital with CHF exacerbation will be identified by their responsible physician (house staff or attending), who will initially inform the patients of the study. If patients express interest in participation, they will then be approached by an investigator, who will explain the potential risks and benefits. Prior to enrollment, patients will sign a notice of informed consent.

J. Potential Conflict of Interest

There are no potential conflicts of interest to report.

K. Location of the Study

The study will take place on the inpatient ward of Columbia Presbyterian Medical Center.

L. Potential Risk

Potential risks from the placement of angiocath and wire insertion include bleeding, hematoma, thrombophlebitis, and infection. An investigator will follow up with the patients 24 hours after the procedure to evaluate for any potential complications that occurred. In previous experience, however, we have performed this technique in more than 70 patients at Albert Einstein and CPMC, with no complications reported.

M. Potential Benefits

There are no direct benefits for patients in this study

N. Alternative Therapies

The alternative to participating in this study would be to continue treatment for CHF exacerbation under the discretion of the treating physician. This may include use of IV diuretics, vasoactive medications, or ultrafiltration.

O. Compensation to Subjects

Patients will receive $25 compensation for participation in this study, regardless of success or failure of treatment and endothelial cell collection.

---

[1] Koelling TM, Chen RS, Lubwama RN, L'Italien FJ, Eagle KA. The Expanding National Burden of Heart Failure in the United States: The Influence of Heart Failure in Women. Am Heart J 2004; 147; 74-8

[2] Fang ZY, Marwick TH. Vascular Dysfunction and Heart Failure: Epiphenomenon or Etiology Agent? Am Heart J 2002; 143; 383-9

[3] Costanzo MR, Guglin ME, Saltzberg MT, et al. Ultrafiltration Versus Intravenous Diuretics for Patients Hospitalized for Acute Decompensated Heart Failure. J Am Coll Cardiol 2007; 49: 675-83.

[4] Colombo P, Banchs J, Celaj S, et al. Endothelial Cell Activation in Patients With Decompensated Heart Failure. Ciriculation 2005; 111: 58-62

[5] Colombo PC, Rastogi S, Onat D, Sabbah H. Activation of Endothelial Cells in Conduit Veins of Dogs with Heart Failure and Veins of Dogs Following Vascular Stretch by Acute Volume Loading. Abstract; ACC.07

[6] Colombo PC, Ashton AW, Sulejman C. Biopsy Coupled to Quantitative Immunofluorescene: A New Method to Study the Human Vascular Endothelium. J Appl Physiol 2002; 92: 1331-1338

[7] Jaski, BE, Ha J, Denys BG, Lamba S, Trupp RJ, Abraham WT. Peripherally inserted veno-venous ultrafiltration for rapid treatment of volume overloaded patients. J Card Fail 2003; 9: 227-31

[8] Hunt SA, Abraham WT, et al. ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation 2005; 112;154-235