Matrix metalloproteinases 2 and 9 as diagnostic markers in the progression to Chagas cardiomyopathy

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Background Infection with the *Trypanosoma cruzi* parasite is endemic in parts of Central and South America. Approximately 30% of those infected develop Chagas cardiomyopathy, the most common cause of heart failure in this region. No suitable biomarker is available that reflects the evolution of the disease. Although there is substantial evidence of a strong inflammatory reaction following infection that could activate matrix metalloproteinases (MMPs), their role in the development of Chagas cardiomyopathy is unknown.

Methods A cross-sectional study was conducted in Bucaramanga, Colombia, from 2002 to 2006, including 144 patients at different stages of Chagas disease and 44 control patients. The potential enzyme activities of MMP-2 and MMP-9 in plasma samples were determined by gelatin zymography. Clinical data including *T cruzi* serology, electrocardiograms, and echocardiograms were recorded for all patients.

Results Densitometric analysis of potential enzyme activities in plasma samples showed a significant increase of 72-kd MMP-2 ($P < .001$) and 92-kd MMP-9 ($P < .001$) in *T cruzi* seropositive patients compared with control subjects. Matrix metalloproteinase 9 showed significantly increased activity in patients with abnormal electrocardiogram ($P < .004$) and with dilated cardiomyopathy compared ($P < .001$) with controls. Analysis of the MMP-2 and MMP-9 results in relation to clinical data revealed that abnormal heart relaxation correlated positively with high MMP-2 levels in patients with dilated cardiomyopathy ($r = 0.75$, $P < .01$).

Conclusions Plasma MMP-2 and MMP-9 both appear to be useful biomarkers for detecting the advent and progression of cardiomyopathy in *T cruzi*–infected individuals. (Am Heart J 2013;165:558-66.)

Chagas disease is caused by infection with *Trypanosoma cruzi*, which is endemic to the Americas. In Colombia, almost 20% of the population lives in endemic areas, and it has been estimated that close to 5% of the population is infected.[1–3] The Northeast region of Santander, Colombia, is one of the regions with the highest prevalence of Chagas disease in Colombia.[4]

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Chagas disease is characterized by an acute phase, which occurs after infection with *T cruzi*, followed by a chronic phase. Following an asymptomatic period, approximately 20% to 30% of infected people develop cardiomyopathy over the course of 10 to 30 years.[5,6] Epidemiological history, positive serology, and typical electrocardiographic (ECG) abnormalities establish the diagnosis. What determines which infected individuals will progress to overt Chagas cardiomyopathy remains unclear. Once established, Chagas cardiomyopathy is characterized by progressive dilated cardiomyopathy, associated with heart failure, life-threatening ventricular arrhythmias, and death.[7,8] Etiologic treatment of chronic *T cruzi* infection remains controversial, and current guidelines do not recommend treatment in the presence of overt cardiomyopathy; a large clinical trial (BENEFIT) is currently assessing this issue.[9–14] Patients affected with Chagas cardiomyopathy are commonly in the prime socioeconomic age of 20 to 50 years, often unemployed, and require expensive, palliative healthcare that burdens the economies of many Latin American countries.[15,16]

The pathophysiological mechanisms leading to Chagas cardiomyopathy remain unclear. There is substantial evidence of a strong inflammatory reaction during *T cruzi* infection that is triggered by the persistence of parasitemia.[17] This inflammation could induce matrix metalloproteinases (MMPs) production and/or activity. Matrix metalloproteinases are important regulators in a number of normal physiologic processes as embryogenesis, bone remodeling, organogenesis, angiogenesis, and have additional roles in the reorganization of tissues during pathologic conditions such as inflammation, wound healing, and invasion of cancer cells.[18,19] Matrix metalloproteinases have the ability to proteolytically remodel the extracellular matrix, including basement membranes.[20,21] In humans, matrix metalloproteinases are part of a large family of 23 structurally related enzymes called proteinases.[22] Enzymes of this family possess signal peptide, amino terminal propeptide, catalytic Zn^{2+} binding site, and carboxy terminal domains. Matrix metalloproteinase 2 is the most ubiquitous of the MMPs and is expressed in all cells including cardiomyocytes.[23,24] Matrix metalloproteinase 9 is a cytokine-inducible MMP expressed in inflammatory cells.[25] During pathologic processes, however, some MMPs are inappropriately activated and cause cellular dysfunction and tissue destruction.

During the development of heart failure, inappropriate activation of MMPs results in cardiac remodeling and enlargement.[26] As well, the unique additional intracellular action of MMP-2 degrades specific sarcomeric proteins, resulting in diminished contractile efficiency.[24] We demonstrated in isolated rat hearts exposed to proinflammatory cytokines that MMP-2 was activated in the myocardium and caused a subsequent contractile dysfunction, which was reduced by MMP inhibitors.[23] In preclinical models of heart failure, inhibition of MMPs was shown to reduce enlargement of the heart[27] and improve cardiac mechanical function.[28] The importance of plasma MMPs as biomarkers in human studies has already been observed in hypertension,[29] myocardial infarction,[30] hypertrophic cardiomyopathy,[31] and systolic heart failure,[32] among other pathologies.

No clinical study to date has investigated the involvement of MMPs in heart failure due to Chagas cardiomyopathy. The inflammatory reaction stimulated by *T cruzi*[33] and the ensuing enhanced oxidative stress are similar to reactions in other disease states (eg, atherosclerosis), which activate MMPs. Although Chagas cardiomyopathy has its own distinct characteristics, the remodeling process could be similar to that noted in other forms of heart failure in which MMPs are implicated.[34] In acute *T cruzi* infection in mice we observed a significant increase in cardiac MMP-2 and MMP-9 potential enzyme activities and demonstrated that mortality was greatly diminished with MMP inhibitor treatment.[35]

Given the preclinical evidence of a potential role for MMP-2 and MMP-9 in Chagas cardiomyopathy, we hypothesized that they are involved in the progression to heart failure and investigated this in a cohort of Colombian patients.

Methods

A cross-sectional study was conducted at the Fundación Cardiovascular de Colombia (FCV), Bucaramanga, Colombia, from 2002 to 2006. A total of 188 subjects were enrolled. Patient recruitment, clinical evaluation, and sample collection were conducted at the FCV, whereas plasma MMP-2 and MMP-9 potential enzyme activities and data analysis were performed at the University of Alberta, Edmonton, Canada.

This study was approved by the local institutional ethics committees at the FCV and the University of Alberta. All study subjects were older than 18 years and provided written informed consent.

Population

One hundred fourty-four Chagas disease patients in different stages of disease were recruited. The classification used is a combination of the New York Heart Association classification and other clinical information (serology, ECGs, echocardiograms, and chest x-rays), findings commonly used to classify the stage of chronic Chagas infection. All patients had at least 2 different serologic tests that were positive for *T cruzi* infection. Three groups of *T cruzi* seropositive patients were selected, covering all ranges in the evolution of the disease from asymptomatic infection to overt Chagas cardiomyopathy (Table I).

Forty-four *T cruzi* seronegative individuals without a history of myocardial infarction or heart failure were selected from blood donors at the FCV to serve as controls. Exclusion criteria included participants with any chronic disease having <5 years of life expectancy (other than Chagas cardiomyopathy), major surgery before clinical examination, history of acute myocardial infarct or coronary disease, history of congestive heart failure (not related to Chagas disease), pregnancy, or a psychiatric condition, which prevented the ability to collect clinical information.

Procedures

Physical examination. Information pertaining to a complete medical history, the use of prescribed medications (Table II), and a comprehensive physical examination was recorded for each patient. A cardiovascular physical examination, standard 12-lead ECG, and a 2-dimensional echocardiogram were performed on each patient.

Blood samples. All heparin anticoagulated blood samples were centrifuged (3000*g*, 10 minutes, 4°C), and plasma was removed, aliquoted, and frozen at -80°C until used in assays. Plasma samples were shipped on dry ice to the University of Alberta for processing.

Measurement of tumor necrosis factor α. Tumor necrosis factor α (TNFα) plasma concentration was measured by enzyme-linked immunosorbent assay according to manufacturer's recommendations (R&D Systems, Minneapolis, MN).