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Original article

Antagonistic effect of atorvastatin on high fat diet induced survival during acute Chagas disease

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Abstract

Chagasic cardiomyopathy, which is seen in Chagas disease, is the most severe and life-threatening manifestation of infection by the kinetoplastid *Trypanosoma cruzi*. Adipose tissue and diet play a major role in maintaining lipid homeostasis and regulating cardiac pathogenesis during the development of Chagas cardiomyopathy. We have previously reported that *T. cruzi* has a high affinity for lipoproteins and that the invasion rate of this parasite increases in the presence of cholesterol, suggesting that drugs that inhibit cholesterol synthesis, such as statins, could affect infection and the development of Chagasic cardiomyopathy. The dual epidemic of diabetes and obesity in Latin America, the endemic regions for Chagas disease, has led to many patients in the endemic region of infection having hyperlipidemia that is being treated with statins such as atorvastatin. The current study was performed to examine mice fed on either regular or high fat diet for effects of atorvastatin on *T. cruzi* infection-induced myocarditis and to evaluate the effect of this treatment during infection on adipose tissue physiology and cardiac pathology. Atorvastatin was found to regulate lipolysis and cardiac lipidopathy during acute *T. cruzi* infection in mice and to enhance tissue parasite load, cardiac LDL levels, inflammation, and mortality in during acute infection. Overall, these data suggest that statins, such as atorvastatin, have deleterious effects during acute Chagas disease.

Keywords: Chagas disease; Cardiomyopathy; High fat diet; Adipose tissue; Lipidopathy; Metabolic disorder

1. Introduction

Chagas disease is caused by a parasite *Trypanosoma cruzi*, and it is estimated that more than 100 million people are currently at risk of becoming infected [1]. Chagas disease is a major cause of mortality and morbidity in Latin America, largely due to cardiomyopathy that develops in a large segment of the infected population [1,2]. Recent research has demonstrated that *T. cruzi* causes a significant infection in adipocytes and adipose tissue, resulting in changes in adipocyte physiology that affects parasitemia, tissue parasite loads and cardiac pathology [2–4]. A western-style diet that is high in fat and processed foods is becoming prevalent in Latin America, replacing the traditional dietary system, and has been accompanied by an increased incidence of diabetes and obesity in this Chagas disease endemic region. This increase in obesity may have significant effects on the pathogenesis of Chagas disease and has spurred investigation into the

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interaction between diet, host adipocyte physiology, lipid homeostasis, and Chagas disease. However, many aspects of this interaction still remain unknown.

Chagas disease has three described phases during infection – the acute phase, characterized by myocarditis, fever and hepatomegaly, the indeterminate phase that is characterized by a lack of disease symptoms, and the chronic phase, which develops in approximately 30% of infected individuals and is characterized by the development of cardiomyopathy. Previous studies have demonstrated that in a murine model of acute Chagas disease, high fat diet (HFD) has a protective effect during infection, resulting in increased survival [2,5]. In addition, in mice fed a regular diet (RD), *T. cruzi* infection causes significant lipolysis (60%), which may contribute to cardiac lipidopathy and high parasite loads during acute infection [2]. In contrast, HFD ameliorates the effects of infection of adipose tissue and reduces adipocyte lipolysis [2]. Infected mice fed on an HFD also have improved cardiac pathology compared to infected mice fed on a RD. It has also been demonstrated that *T. cruzi* has a high affinity for lipoproteins and that the invasion rate of this parasite increases in the presence of cholesterol [6–8], suggesting that drugs that inhibit cholesterol synthesis, such as statins, may affect infection progression. It has been demonstrated that statins have a potential beneficial effect on the progression of cardiomyopathy in dogs at chronic stages of Chagas disease [10]; however, whether statins affect other stages of Chagas disease has not been explored. This line of inquiry is motivated by the fact that approximately 24.1 million people with diabetes and various associated lipid disorders live in Latin America, the endemic region for Chagas disease, and that many of these patients take statins for hyperlipidemia [9].

In this study we analyzed the effect of HFD in combination of atorvastatin (marketed as Lipitor in the United States) on adipocyte physiology and function, and its relation to cardiac pathology using a murine acute Chagas model that was fed either a HFD or RD. The effect of atorvastatin on adipose tissue was analyzed at the early acute infection stage (d10pi) as we have previously determined that adipose tissue significantly responds to infection at this time point [4]. Treatment with atorvastatin was found to increase lipolysis and caused enhanced lipid accumulation, inflammation and parasite load in the hearts of infected animals. The known effect of atorvastatin to increase LDL receptors in both adipose tissue and heart likely results in a higher parasite load when this drug is used in acute *T. cruzi* infection. In addition, atorvastatin treatment results in an elevated mortality rate during acute infection in the murine CD1 Chagas model. Together, these results indicate that statin drugs may adversely impact the progression of acute Chagas disease.

2. Materials and methods

2.1. Ethical approval

All animal experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Albert Einstein College of Medicine (No. 20130202) and adhere to the National Research Council guidelines (Guide for the Care and Use of Laboratory Animals: Eighth Edition, Washington, DC: The National Academies Press, 2011).

2.2. Experimental animal model

The Brazil strain of *T. cruzi* was maintained by passage in C3H/Hej mice (Jackson Laboratories, Bar Harbor, ME). Male CD-1 mice (Jackson Laboratories) were infected intraperitoneally (i.p.) at 8–10 weeks of age with 5×10^4 trypomastigotes of the Brazil strain [3]. Mice were maintained on a 12-h light/dark cycle. Mice, starting at the day of infection, were randomly divided into four groups (n = 15 per group) and fed on either high fat diet (HFD; 60% fat D12492 Research Diets, Inc., New Brunswick, NJ) or regular diet (RD, 10% fat D12450 Research Diets, Inc., New Brunswick, NJ) or HFD containing atorvastatin (HFDA 100 mg atorvastatin/kg diet) or RD with atorvastatin (RDA 100 mg atorvastatin/kg diet) (Research Diets, Inc., New Brunswick, NJ). Uninfected mice were fed on either HFD (n = 15) or RD (n = 15) or HFDA or RDA and used as respective controls in all the experiments. The experiment was replicated using n = 30 mice in all groups.

Plasma samples were obtained from 75 μl of blood collected from the orbital venous sinus (using isoflurane anesthesia) at 10, 15, 20, 25 and 30 days post infection (dpi). Parasitemia was evaluated by counting in a Neubauer hemocytometer as described previously [3]. At days 10, 20 and 35 days after infection the mice were euthanized and heart and epididymal white adipose tissues (WAT) were harvested for analysis. There was no peripheral parasitemia observed at d 35 after infection and mice appeared normal.

2.3. Immunoblot analysis

Tissue lysates were prepared as previously described [2–5]. An aliquot of each sample (40 μg proteins) was subjected to electrophoresis on a 4–15% gradient SDS-PAGE and the proteins transferred to nitrocellulose filters for immunoblot analysis. LDLr specific rabbit monoclonal antibody (1:1000 dilution, AB52818 Abcam, Cambridge, MA), lipoprotein lipase (LPL) specific mouse monoclonal antibody (1:1000 dilution, AB21356, Abcam), adipose triglyceride lipase (ATGL) specific rabbit monoclonal antibody (1:1000 dilution, AB 109251), hormone sensitive lipase (HSL) specific rabbit monoclonal antibody (1:1000 dilution, AB 45422), lipoprotein lipase (LPL) specific mouse monoclonal antibody (1:1000 dilution, AB 21356) or TNF-α specific rabbit polyclonal antibody (1:1000 dilution, AB6671, Abcam) were used as primary antisera. Horseradish peroxidase-conjugated goat anti-mouse immunoglobulin (1:2000 dilution, Amersham Biosciences, Piscataway, NJ) or horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin (1:5000 dilution, Amersham Biosciences) were used to detect specific protein bands (explained in Figure Legends) using a chemiluminescence system [4]. GDI (1: 10,000 dilution, 71–0300,