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Minocycline attenuates cardiac dysfunction in tumor-burdened mice

Raymond D. Devine [a,b], Clayton M. Eichenseer [a], Loren E. Wold [a,c,d,*]

[a] Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University Wexner Medical Center, Columbus, OH, United States
[b] Molecular, Cellular and Developmental Biology Graduate Program, The Ohio State University, Columbus, OH, United States
[c] Department of Physiology and Cell Biology, The Ohio State University, Columbus, OH, United States
[d] College of Nursing, The Ohio State University, Columbus, OH, United States

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ABSTRACT

Cardiovascular dysfunction as a result of tumor burden is becoming a recognized complication; however, the mechanisms remain unknown. A murine model of cancer cachexia has shown marked increases of matrix metalloproteinases (MMPs), known mediators of cardiac remodeling, in the left ventricle. The extent to which MMPs are involved in remodeling remains obscured. To this end a common antibiotic, minocycline, with MMP inhibitory properties was used to elucidate MMP involvement in tumor induced cardiovascular dysfunction. Tumor-bearing mice showed decreased cardiac function with reduced posterior wall thickness (PWTs) during systole, increased MMP and collagen expression consistent with fibrotic remodeling. Administration of minocycline preserved cardiac function in tumor bearing mice and decreased collagen RNA expression in the left ventricle. MMP protein levels were unaffected by minocycline administration, with the exception of MMP-9, indicating minocycline inhibition mechanisms are directly affecting MMP activity. Cancer induced cardiovascular dysfunction is an increasing concern; novel therapeutics are needed to prevent cardiac complications. Minocycline is a well-known antibiotic and recently has been shown to possess MMP inhibitory properties. Our findings presented here show that minocycline could represent a novel use for a long established drug in the prevention and treatment of cancer induced cardiovascular dysfunction.

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1. Introduction

Cancer is a pervasive disease that was the leading cause of deaths world-wide in 2014 [1], and is predicted to be the leading cause of death in 2015 [2,3]. Of those who develop cancer, approximately half of these patients will develop cachexia. Cachexia is a wasting syndrome, characterized by marked loss of adipose tissue and skeletal muscle mass [4]. Cancer-induced cachexia will be the primary cause of death in a quarter of those affected [5]. While primarily thought of as a syndrome affecting adipose tissue and skeletal muscle, recent investigations have determined that cancer-induced cachexia also negatively affects heart function. In animal models of cancer-induced cachexia, there is a significant decrease in fractional shortening (FS%) and Ejection Fraction (EF) via echocardiography, consistent with heart failure [6]. While no human studies have determined the cardiac functional alterations caused by cancer cachexia, there is histological evidence from patients who died from cancer cachexia that there was marked fibrotic remodeling in the heart [7]. Remodeling in heart failure is an accumulation of multiple factors, but is strongly dependent on the activity of matrix metalloproteinases [8–10].

Matrix metalloproteinases (MMPs) are zinc-peptidases that alter the structure of the extracellular matrix (ECM) by hydrolyzing the peptide bonds holding proteins together [11,12]. MMPs are a normal component of cardiac homeostasis but uncontrolled MMP activity leads to detrimental remodeling that can negatively affect cardiac function [13]. MMPs are endogenously controlled by Tissue Inhibitors of Metalloproteinase (TIMPs) [12]; however, previous research with the c26 mouse model of cancer cachexia showed an insufficient TIMP response to compensate for increased MMPs. This insufficient response leads to MMP-mediated collagen deposition in the heart presenting as fibrosis which can compromise heart structure and negatively impact function. While MMP levels were increased in the c26 cachectic mouse model [14], the degree in which they participate in remodeling remain unknown. In order to determine how increased MMP activity could lead to altered function, we utilized a known inhibitor of MMPs, minocycline.

Minocycline is a semisynthetic tetracycline derivative first synthesized in 1967 by Ledele Laboratories. It quickly gained FDA approval in 1970 as a broad spectrum antibiotic and is still used today for both its antibacterial effects and recently discovered neuroprotective effects. It has been used as an MMP inhibitor in diseases primarily caused through MMP activity in both animal and human studies [15–17]. The mechanism of inhibition remains unknown, however, two theories have been hypothesized. One is that minocycline binds the zinc in the active site of the MMP since minocycline has been shown to have chelating

* Corresponding author at: 603 Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University, 473 W. 12th Ave., Columbus, OH 43210, United States.
E-mail address: Loren.Wold@osumc.edu (L.E. Wold).

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properties, and the other is that it binds allosterically and causes an active site conformational change preventing enzymatic action. Minocycline has been shown to inhibit collagenases as well as gelatinases but seems to have an unexplained increased specificity for MMP-9 [16]. The effects of minocycline on TIMPs are difficult to discern because of different effects based on location. Minocycline has been shown to increase TIMP expression but only in the brain and spinal cord [18]. Minocycline and other tetracyclines have been shown to have no effect on TIMP expression in other tissues. This could be due to changes in minocycline when crossing the blood-brain-barrier, but further work is required. Despite the mechanism of inhibition not being fully characterized, minocycline and other tetracyclines have been shown to be potent MMP inhibitors. Because of its current status as an FDA approved drug and its well documented use and tolerability in humans, minocycline represents a possible treatment strategy to limit the effects of MMPs in cancer-induced cardiac dysfunction.

In the present study, we injected the c26 adenocarcinoma cell line into CD2F1 mice treated with or without minocycline, which was delivered through their drinking water. We hypothesize that minocycline treatment in tumor burdened animals will improve cardiac function by inhibiting MMP activity.

2. Materials and methods

2.1. Animal husbandry

Adult ~10 week old female mice weighing 20–22 g were obtained from Charles River Laboratories (Charles River, Wilmington, MA). Mice were housed 1–3 per cage and were maintained on a 12 h light/dark cycle at 25 °C and were provided ad libitum access to standard rodent chow as well as water. Treated mice were administered minocycline in their drinking water and provided ad libitum access as well. All animal care and use procedures were approved by the Ohio State University Institutional Animal Care and Use Committee. (See Fig. 1).

2.2. Minocycline administration

Mice were administered minocycline orally through supplemented water at the same dosage used previously [19]. Water bottles provided were supplemented with 1 mg/ml minocycline for a dose of 100 mg/kg/day (Sigma, St. Louis, MO). Water bottles were changed every other day throughout the duration of the study. No fluid intake differences were observed between any of the experimental groups (data not shown).

2.3. Model of tumor growth

The c26 line was maintained and injected as previously described [6]. Upon arrival, mice were allowed to acclimate to their environment for one week before being randomly assigned into experimental groups. After acclimatization and group assignment, 5×10^5 c26 cells suspended in PBS were injected subcutaneously between the scapulae region as described previously [6]. Non-tumor bearing mice were subjected to a sham procedure and injected subcutaneously with the same volume of PBS. Female mice are used exclusively in this study since they have been shown to guard their body weight better than male mice and are less prone to an anorexic state than male mice. Cachectic effects in females are more representative of a true cachectic state than their male counterparts [6]. Tumor growth was observed as early as day twelve and mice typically were cachectic by day 21 post injection as determined by body weight as well as body score. Food intake has been shown previously to be reduced in this model; however, this is not the main cause of the wasting condition as pair fed animals did not show as significant of body mass loss [20]. The c26 model shows weight loss beginning at approximately the second week, reaching a wasted state by day 21 correlated with a decreased body score and continues as body score worsens, reaching a wasted state. Minocycline has already been shown in our lab to reverse this wasting condition and the results will not be repeated here [19]. At the time of euthanasia, approximately day 21 post-injection, mice were anesthetized with a ketamine/xylazine cocktail (10/1 mg/ml respectively) at a volume of 0.01 ml per gram of body weight or approximately 0.2 ml. A cardiectomy was performed, the left ventricle was dissected, snap frozen in liquid nitrogen and stored at −80 °C until biochemical analyses. The tumor was also removed and weighed at the end of the study, 21 days, to determine if treatment had any effect on tumor growth. Previous study by our lab has shown minocycline did not have any effect on final tumor weight and will not be repeated here [19].

2.4. Echocardiography

Cardiac function was determined using echocardiography with a 40 mHz VEVO 2100 Ultrasound System (Visual Sonics, Toronto, Ontario, Canada). Mice were anesthetized using isoflurane; 3% in 100% oxygen for induction and 1% in 100% oxygen for maintenance. Animals were placed on a warm table, fur around the chest was removed using a depilatory agent, and temperature was measured via a rectal probe. Prewarmed ultrasound gel was applied to a 15 mHz transducer probe optimized for mouse echocardiography. The transducer was placed on the parasternal short axis to obtain a view of the left ventricle (LV) at the mid-papillary level for image capture and measurements. LV dimensions; LV End Diastolic Dimension, LV End Systolic Dimension (LVEDd and LVEDs) as well as posterior wall thickness during systole and diastole (PWTs, PWTd) were acquired using the leading edge method as recommended by the American Society for Echocardiography [21]. Percent fractional shortening (FS%) was calculated using the following formula; $FS\% = [\frac{LVEDd - LVEDs}{LVEDd}] \times 100$. Ejection Fraction (EF) was also measured and calculated using the following formula; $EF = [\frac{SV}{EDV}] \times 100$. For echocardiography results, an $n = 6$ was used for each experimental group.

2.5. Cardiomyocyte isolation

Cardiomyocytes were isolated as previously described [22]. In brief, hearts were removed and digested using liberase and trypsin. Once digested, the cardiomyocytes were plated on laminin coated glass inserts. Inserts were loaded into a perfusion chamber mounted on an Olympus IX-71 microscope. Cells were stimulated (1 Hz, 3-ms duration) with a Myopacer Field-Stimulator system and function was determined using Sarclen Sarcomere Length Acquisition Module (IonOptix, Milton, MA). Approximately 10–15 cells were measured from each mouse to provide adequate measurements for peak shortening (PS%), cellular equivalent to FS%. Sarcomere contractile velocity was also measured as ±dL/dt and time to 90% shortening and relengthening as TPS90 and TR90, respectively. For Ca^{+2} measurements, a calcium sensitive fluorimetric compound, FURA-2 AM, was loaded onto the cells at a concentration of 5 μM for 20 min. Fluorometric measurements were acquired using a dual excitation single emission system. These transients were analyzed for transient calcium amplitude (Δ340/380), and calcium reuptake (τ). For cardiomyocyte studies, separate mice were used independent of those used for biochemical studies to ensure that there were no artifacts in the biochemical studies caused by digestion of the heart for cardiomyocyte isolation. Cardiomyocyte results use an $n = 3$ for each experimental group, for each mouse 15 cells were used to measure cardiomyocyte function.

2.6. RNA isolation and qPCR

RNA was isolated from frozen tissue using the Trizol/Chloroform (ThermoFisher Scientific, Wilmington, DE) method. Briefly, the tissue was homogenized with a TissueLyser (Qiagen, Boston, MA) with Trizol

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