Adiponectin and Tibialis Anterior Mass are Improved in Cachectic Tumor – Bearing Mice by Rosiglitazone.

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Abstract

Cachexia is a disorder characterized by extreme adipose and muscle loss. Insulin resistance is a characteristic of cachexia, which affects nearly eighty percent of all colon cancer patients. The purpose of this pilot study was to determine the extent that rosiglitazone can alter muscle mass in a murine model for colon cancer cachexia. Rosiglitazone sensitizes peripheral tissues to insulin by activating peroxisome proliferator-activated receptor gamma (PPARγ). Treatment with rosiglitazone increases serum adiponectin levels through the activation of PPARγ. Improved insulin sensitivity should increase glucose uptake into muscle, therefore we hypothesize that treatment with rosiglitazone: 1) increases muscle mass when administered to CD2F1 mice bearing colon – 26adenocarcinoma; and 2) increases serum adiponectin levels. Male CD2F1 mice were divided into two groups and treated daily with rosiglitazone (10mg/kg BW) or phosphate buffered saline (PBS) via intraperitoneal injection. After two weeks of treatment mice were inoculated with C26adenocarcinoma. Three weeks post tumor inoculation, necropsies were performed and liver, gastrocnemius, quadriceps, and tibialis anterior of the right hind limb were snap frozen in liquid nitrogen and later weighed by top loading balance. Mass of the tibialis anterior was significantly elevated in mice treated with rosiglitazone compared to mice treated with PBS ($p<0.05$). Serum adiponectin was significantly elevated in rosiglitazone treated mice ($p<0.05$) as well. These findings suggest that rosiglitazone may elevate muscle mass in a murine cancer cachexia model through increased serum adiponectin. Further work is needed to determine the mechanism of rosiglitazone to sustain muscle, modulate metabolism, and preserve adiponectin levels under the condition of cachexia.

Introduction

Cancer Cachexia

- Energy expensive disorder consisting of severe weight, adipose, and muscle loss
- Induces insulin resistance, anemia, and anorexia
- Responsible for roughly 1/3 of all cancer deaths

Insulin Sensitivity and TNFα

- TNFα is highly expressed in muscle tissue of those with colon cancer (1)
- Abundant expression of TNFα is associated with insulin resistance (1)
- TNFα has been shown to stimulate muscle degeneration and suppress muscle regeneration (2)
- TNFα is negatively associated with GLUT4 mRNA (1)
- GLUT4 translocates to the cell surface of the myocyte upon insulin signaling (3) and is needed for the uptake of glucose into the cell

We theorize that muscle of cancer patients with cachexia is insufficient at utilizing glucose as fuel, due to an increase of TNFα *and a decrease in insulin sensitivity. Therefore, it is important to determine if an insulin sensitizing drug will indeed increase muscle mass in a murine cancer cachexia model.*

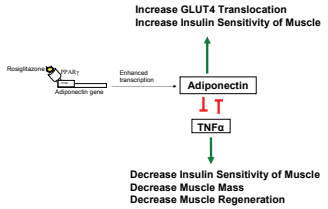
Rosiglitazone

- Pharmacological drug used in the treatment of Type 2 diabetes mellitus
- Sensitizes peripheral tissues to insulin in part by activating PPARγ (4)
- PPARγ is an important transcription factor in the regulation of lipid metabolism (5)
- Rosiglitizone activation of PPARγ results in an increase of adiponectin transcription (6)
- Adiponectin is positively correlated with insulin sensitivity (7)
- Adiponectin and PPARγ have been shown to inhibit TNFα (8)

Hypotheses

- Rosiglitazone increases muscle mass in mice with cancer cachexia
- Rosiglitazone increases serum adiponectin in mice with cancer cachexia

Proposed Mechanism



Materials & Methods

Animals and Treatment. Seven male CD2F1 mice were a gift from Dr. Guttridge's group (The Ohio State University) at 5 weeks of age. Mice (~18g BW) were assigned to 2 groups. Group 1: C26 infected mice with daily IP injections of rosiglitazone, Group 2: (control): C26 infected mice with daily IP injections of PBS. The dose of rosiglitazone (Cayman Chemical, Ann Arbor MI) administered was10mg/kg body weight. Mice were fed a control diet (D12451, 23.6% fat) in pellet form (Research Diets Inc, New Brunswick, NJ). Food intake was measured and 50 grams of food/cage was supplied every other day. Body weight was measured every other day as well. Mice were housed at The Ohio State University under conventional conditions. A constant temperature/humidity and 12 hour light: dark cycle was maintained. Mice were euthanized at three weeks post cancer injection by carbon-dioxide overdose and cardiac puncture.

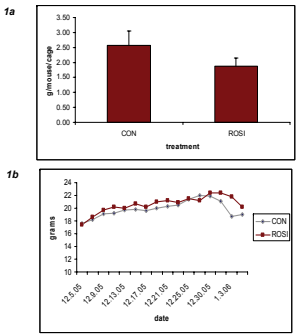
Muscle Mass: In order to determine the effect of rosiglitazone on muscle preservation, snap frozen tibialis anterior, gastrocnemius, and quadriceps from the hind limbs of each mouse was weighed using a top loading scale. All three of these muscle tissues are a mix of both type I and type II muscle fibers. Muscle mass was recorded from the left hind limb muscle. Muscle weight from each treatment group was compared and statistically evaluated. Muscle mass was normalized to body weight and expressed as muscle/body weight. (Tumor Weight measured at necropsy).

Leptin and Adiponectin. Fasted serum levels of leptin and adiponectin were determined by ELISA (Linco Research Inc., St. Charles, MO) according to manufacturer's directions.

PPARγ and PPARα. Protein (50 ug) extract from muscle tissue was subjected to SDS-PAGE and transferred to nitrocellulose membranes. The primary antibody used for PPARγ protein was PPARγ antigoat at a concentration of 1:100 (Santa Cruz, Santa Cruz, CA). The secondary antibody is bovine antigoat at a concentration of 1:5000. After overnight incubation with the primary antibody and 1 hour incubation with the secondary antibody the resolved protein was detected with Supersignal Chemiluminescence System™ (Pierce Biochemicals). PPARα protein was analyzed following the same procedure. Primary used for PPARα (Cayman Chemical, Ann Arbor, MI) at a concentration of 1:200. The secondary used was anti-rabbit at a concentration of 1:1000. Densitometry analysis was performed using the Kodak imager system. PPARγ and PPARα expression was normalized to total β – Actin expression (Cell Signaling Tech, Danvers, MA).

Results

Mouse Data



Tumor Weight

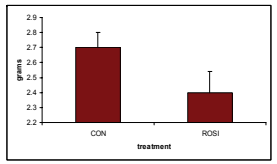


Figure 1: Food Intake, Body Weight, Tumor Weight. a) Food intake between groups was not significantly different. b) Rosiglitazone did not significantly alter total body weight loss or gain. c) Tumor weight was not significantly different between groups. (CON n = 3, ROSI n = 4)

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Muscle Mass

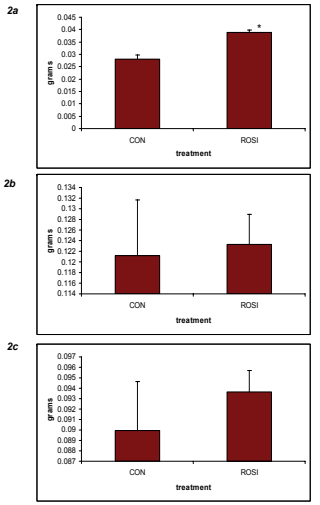

Figure 2: Muscle Mass. a) Daily treatment with rosiglitazone significantly increased ($p<0.05$) muscle mass of the Tibialis Anterior. b) Rosiglitazone treatment did not significantly alter the mass of the quadriceps. Gastrocnemius muscle was not significantly altered with rosiglitazone treatment. (CON n=3, ROSI n=4)

Triglyceride

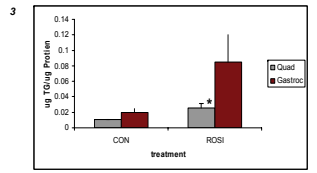

Figure 3: Triglyceride. Triglyceride content was significantly increased in the quad of rosiglitazone treated mice. TG content was not significantly increased in the gastrocnemius, but the same trend was present. This may indicate an improvement in glucose uptake by the skeletal muscle. (CON n=3, ROSI n=4)

Adipocytokines

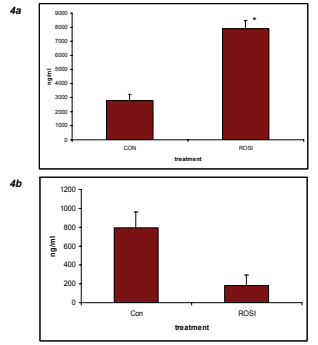

Figure 4: Serum Adiponectin & Leptin. a) Rosiglitazone significantly increased ($p<0.05$) serum adiponectin levels. Adiponectin is important for insulin sensitivity and suppression of TNFα. b) Rosiglitazone did not significantly alter serum leptin levels. (CON n=3, ROSI n=4)

PPARγ & PPARα

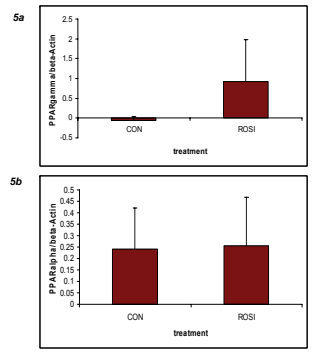

Figure 5: PPARγ & PPARα. a) Treatment with rosiglitazone did not significantly increase PPARγ protein, but a positive trend is present. b) Rosiglitazone treatment did not significantly alter PPARα protein. PPARα is primarily involved in beta – oxidation. (CON n= 3, ROSI n=4)

Summary

- Treatment with Rosiglitazone did not significantly alter body weight, food intake, or tumor weight of cachectic mice.
- Rosiglitazone significantly increased tibialis anterior muscle mass.
- Rosiglitazone did not significantly alter the mass of the quadriceps or gastrocnemius muscles.
- Rosiglitazone significantly increased the triglyceride in the quadriceps, the same trend was observed in the gastrocnemius. This observed increase in muscle TG may indicate an improvement in glucose uptake.
- Serum adiponectin was significantly increased in rosiglitazone treated mice, serum leptin was not significantly altered.
- PPARα and PPARγ protein was not significantly altered by rosiglitazone treatment.