

Serum cardiac troponin I concentrations are transiently increased in rats dosed with rosiglitazone, a peroxisome proliferator-activated receptor γ agonist



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Abstract

Rosiglitazone, a PPAR γ agonist of the thiazolidinedione class, is a major insulin-sensitizing drug widely used to treat type-2 diabetes. Rosiglitazone causes myocardial hypertrophy in rodents and increased risk of myocardial infarction in man. To better characterize its cardiac effects and determine if cardiac troponin I (cTnI) can serve as an early biomarker for cardiac liability, male Wistar rats were orally administered 0, 10 or 80 mg/kg/day rosiglitazone. Myocardial gene expression profiling, hematology, histopathology and clinical chemistry, including measurement of cTnI concentrations with the Singulex Erenna[®] Ultrasensitive Immunoassay, were evaluated after 6, 24, 168 and 336 hours of dosing. Heart weight mildly increased after 168 (~10%) and 336 (~15%) hours of dosing at 80 mg/kg/day in the absence of microscopic changes. At the transcriptomic level, gene categories typically associated with myocardial damage were not over-represented. Most importantly, cTnI transiently increased from 2±2 pg/mL in vehicle-treated rats to 19±4 pg/mL in 5/9 rats after 168 hours of dosing at 80 mg/kg/day, returning to 3±2 pg/mL after 336 hours of dosing underscoring the temporal nature of cTnI increases. This is the first study detecting serum cTnI increases in rats administered rosiglitazone. This effect may be linked to functional changes because rosiglitazone-induced myocardial hypertrophy is postulated to be the result of positive inotropic and lusitropic effects without changes in heart rate, ventricular pressures and hematocrit. In light of reported cardiac events in patients chronically dosed with PPAR γ agonists, our results support cTnI as the earliest biomarker heretofore of cardiac liability associated with these compounds.

Introduction

During the last three years there has been an expansion of highly sensitive biomarker assays. At the forefront are the ultrasensitive assays designed to detect the cardiac troponins (cTn) I and T. These assays have reduced the level of detection (LOD) and level of quantification (LOQ) in some cases to the sub-picogram level. This 30-100 fold increase in sensitivity over previously utilized systems has enabled the identification of accurate and robust baseline cTnI and cTnT values for a variety of species including human, dog, monkey, rat and guinea pig. However, while it has been generally accepted that cTn increases identified by the earlier less sensitive assays are indicative of cellular cardiac necrosis, the biologic significance of previously undetectable cTn increases above baseline remains to be determined. Rosiglitazone, a PPAR γ agonist of the thiazolidinedione class, is a major insulin-sensitizing drug used to treat type-2 diabetes. Chronic administration of rosiglitazone has been shown to produce cardiac hypertrophy in rats and an increased risk of myocardial infarction in man. The current hypotheses for the cardiac hypertrophy in rats include 1. Plasma volume expansion; 2. Compensatory response to fatty acid deprivation and; 3. Intrinsic inotropic and lusitropic effects. A thorough knowledge of the cardiovascular effects of rosiglitazone in rodents may have translational implications and yield insight as to the causative nature of the cardiac events in man. The Singulex Erenna[™] ultrasensitive cTnI assay was used to re-analyze the sera of rats given rosiglitazone for up to 14 days. All samples initially assayed with the Beckman Coulter Access[®] 2 AccuTNI[™] were below LOQ. In contrast, the Singulex Erenna[™] Immunoassay System identified significant cTnI increases.

Materials and Methods

Male Han Wistar rats (CrI:WI(Han)) aged 9 weeks (10/group)
Four sampling time-points: 6 and 24 hours (hr), 7 and 14 days (d)
Dosing regimen: 10 and 80 mg/kg/day, oral
Clinical pathology
Clinical chemistry: Roche Hitachi Modular Analytics System (Roche Diagnostics, Mannheim, Germany)
Hematology: ADVIA[®] 2120 (Siemens Healthcare Diagnostics, Tarrytown, NY)
Cardiac troponin I: AccuTNI[™] Access[®] 2 Immunoassay System (Beckman Coulter, Fullerton, CA), Singulex Erenna[™] Immunoassay System (Singulex, Alameda, CA)
Histopathology: hematoxylin-eosin and phospho-tungstic acid hematoxylin stain
Gene expression: GeneChip Rat Genome 230 2.0 Array[™] (Affymetrix, Santa Clara, CA) on 6 rats/group/time point (rats closest to the average heart weight for the group)
PCA analysis: SIMCA (Umetrics, Kinnelon, NJ)
Transcriptomic data analysis: Ingenuity Pathway Analysis software (Redwood City, CA) for pathways and GoSubTree (Hoffmann-La Roche Inc. Basel, Switzerland) for gene ontology (GO) using a p \leq 0.05 and |F| \geq 1.3 as the criteria for gene selection
Individual gene mining of the most significantly up- and down-regulated genes
Samples frozen at -70C for 2 years, and re-evaluated for cTnI with the Singulex Erenna[™] cTnI assay (individual data represent the average for 2 consecutive assays)

Results

- Hematology**
 - Minimal dose-dependent decrease in hemoglobin on study day (SD) 7 and SD14 at 10 (~-3%) and 80 (~-5%) mg/kg/day.
 - No evidence of hemodilution (no decrease in hematocrit, red blood cell count, total proteins or electrolytes).
- Clinical chemistry**
 - Dose-dependant decrease in triglycerides compared to controls: moderate (~ -20-50%) at 10 mg/kg/day and dramatic (~ -40-70%) at 80 mg/kg/day at all time-points.
 - At the 80 mg/kg/day dose, cTnI levels as measured by the Singulex Erenna[™] assay increased approximately 5 fold in 5 of 9 rats on SD7, returning to control levels on SD14 (Fig.1); All cTnI values BLQ measured by the Beckman Coulter Access[®] 2 AccuTNI[™] assay.
- Organ weights:** higher mean heart weight at 80 mg/kg/day on SD7 (~ +10%; not statistically significant) and SD14 (~ +15%; p<0.01) compared to controls.
- Histology:** no treatment-related findings.
- Gene expression analysis**
 - Principal component analysis: no clustering by dose or time-point (data not shown).
 - Overview: at 80 mg/kg/day, small number of dysregulated probes with more dysregulated probes at 24 hr (356) than at 6 hr (89) and on SD7 (86) and SD14 (218).
 - Gene ontology: noteworthy findings consisted of over-representation of categories associated with blood vessel development at 6 and 24 hr at 80 mg/kg/day and extracellular matrix on SD14 d at 80 mg/kg/day (data not shown).
 - Ingenuity Pathway Analysis: some pathways related with inflammation (I12, I16, arachidonic acid metabolism, leukocyte extravasation signaling, etc.) were over-represented at 24 hr at 80 mg/kg/day but lacked a histopathologic correlate.
 - Individual gene mining: no evidence of cell death, fetal gene program activation, inflammation, or remodeling on SD7 and SD14. Consistent with an water homeostasis imbalance, Aquaporin 1 expression was decreased at 24 hr and on SD7 and SD14 at 80 mg/kg/day.

Results (continued)

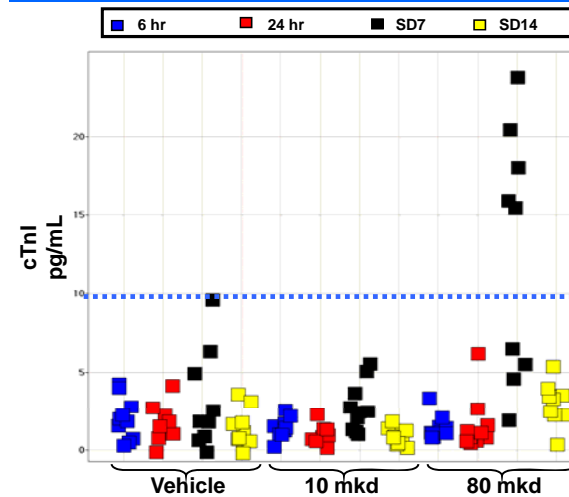


Fig. 1. cTnI concentrations as measured by the Singulex Erenna[®] assay increase above baseline value (dotted line; unpublished data) on SD7 in 5/9 rats given 80 mg/kg/day (mkd) rosiglitazone.

Discussion/Conclusions

To the authors' knowledge, this study is the first to identify cTnI increases in rats treated with a PPAR γ agonist. The toxicologic significance of this cTnI increase in absence of a histomorphologic correlate is uncertain. The advent of ultrasensitive assays will increasingly identify occurrences where cTn elevations are not accounted for by a histomorphologic endpoint: the Pharmaceutical Industry, through the ILSI-HESI and C-Path collaborative forums, is actively engaged in understanding the relationship between increases of cTnI in the previously undetectable range (<30 pg/mL) to established cardiovascular parameters, and to novel biomarkers including cTnI/cTnT complexes, NT-proBNP, and miRNAs.

Transcriptomic changes that robustly identify myocardial damage include over-representation of the categories associated with cell death, the fetal gene program, inflammation, and tissue remodeling, were not seen in this study. In the absence of histomorphologic and transcriptomic changes indicative of myocardial damage, cTnI increases may result from leakage of cTnI from viable cardiomyocytes. This hypothesis could be tested by comparing the concentrations of cTnI and the cTnI/cTnT complexes in rats administered rosiglitazone.

Recently, it was proposed that the rosiglitazone-induced heart weight increase in rats is the result of an increase in contractility (dP/dt_{max}) and lusitropy (dP/dt_{min}), and not the result of hemodilution or changes in other cardiovascular parameters including tachycardia, left ventricular systolic or diastolic pressures [1]. However, transcriptomic data identified alterations of water homeostasis and increased expression of constitutive proteins of the cardiomyocytes and of the interstitial compartment of the myocardium. Thus the increase in heart weight caused by PPAR γ agonists may result from a combination of cardiomyocyte hypertrophy, expanded interstitial tissue elements, and increased intravascular or extravascular fluid. These hypotheses may be tested in studies that concurrently evaluate cardiac functional data, cTnI levels, and water content of the myocardium in the same animals.

In another previous study, increased angiogenesis was identified by immunohistochemistry on SD7 in rats administered troglitazone [2]. This increased angiogenesis coincided temporally with the increase in cTnI found in this study. The relationship between these two observations requires knowledge of the comparative kinetics of angiogenesis in rats given rosiglitazone and troglitazone.

In conclusion, to the authors' knowledge, this is the first study to identify cTnI increases in rats treated with a PPAR γ agonist. These preliminary results are in the process of being confirmed and correlated to novel biomarkers and cardiovascular parameters.

Key references

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