Supraphysiological Testosterone Levels Shorten the QT Interval but do Not Alter Total Anatomic Myocardial Infarct Size in Rabbits with Acute Myocardial Infarction

Michael J. Herring1, Sharon L. Hale1, Jianru Shi1, Peyman Mesbah Oskui1,2, Gregory Kay and Robert A. Kloner1,3
1Heart Institute, Good Samaritan Hospital, Los Angeles, California, USA
2Harbor-UCLA Medical Center, Department of Cardiology, Torrance, California, USA
3Keck School of Medicine at the University of Southern California, Department of Cardiology, Los Angeles, California, USA

Abstract

Introduction: A growing number of men are using exogenous testosterone (T) to treat hypogonadism and to enhance athletic performance. However, some studies suggested that T increased adverse cardiovascular events. Although T has been shown to increase apoptosis, its effect on total acute myocardial infarction (MI) size is largely unknown. We hypothesized that T might increase MI size.

Materials and Methods: Male rabbits received an intramuscular injection of either T (60 mg/kg) or saline one week before receiving 30 minutes of coronary artery occlusion/3 hours of coronary artery reperfusion.

Results: The T levels in the treated group were higher than those of the control group: 15 ± 1 ng/mL T (n=18) versus 1 ± 1 ng/mL control (n=20, P<0.01). Anatomic MI size (tetrazolium staining) expressed as a percentage of the ischemic risk zone (blue dye technique) was similar in both groups: 37 ± 3% in controls and 37 ± 5% in the T group (P= 0.96). T significantly shortened the QTc interval by 9% (P=0.03).

Conclusions: Supra physiological levels of T did not increase infarct size. T shortened the QTc interval, which may create an anti-arrhythmic substrate.

Keywords: Testosterone; QT interval; Ischemia reperfusion; Myocardial infarction; Cardio protection

Introduction

Testosterone (T) was first synthesized in 1935 to treat young men with hypogonadism [1]. Over the past decade, T has become extremely popular in both clinical and athletic practices for both legal and illegal purposes. Due to the increasing number of men using T for medical conditions such as hypogonadism, low libido and weakness, [2] as well as the large number of professional and amateur athletes utilizing testosterone as a performance enhancing drug [3] in order to increase lean body mass, [4-6] the cardiovascular risks from using, and often abusing, exogenous testosterone should be examined. The effect of estrogen on the size of myocardial infarctions has been well documented with little controversy, [7-10] but the role of testosterone has yet to be defined clearly [11]. Some studies suggest positive cardiovascular effects of testosterone with regards to QT segment duration [12-14] and that higher testosterone levels are associated with a lower risk of cardiac mortality [15] and better functional capacity [16]. However, other evidence suggests that testosterone exerts a negative impact on the cardiovascular system with regards to apoptosis [17-20] and adverse cardiac events [21].

Little is known about the effect of testosterone on myocardial infarct (MI) size in the experimental ischemia/reperfusion model. We hypothesized that supra physiological levels of testosterone would shorten the QT interval, worsen cardiac function and increase the size of the zone of no-reflow and the necrotic region in our experimental model.

Methods

The animals used in these studies were maintained in accordance with the policies and guidelines of the Position of the American Heart Association on research animal use (American Heart Association, 1985) and the Guide for Care and Use of Laboratory Animals (2010). The Good Samaritan Hospital Institutional Animal Care and Use Committee approved this protocol.

Pilots

To determine proper dosing of testosterone, 7 pilot rabbits were given intramuscular injections of either 5 mg/kg testosterone cypionate (T, Paddock Labs, Minneapolis, MN, n=1), 15mg/kg T (n=2), 45 mg/kg (n=2) or 50 mg/kg (n=2) on day zero after baseline blood samples were taken. Blood samples were then taken at days 1, 3, 4 and 7 to determine total T concentration. The goal of these pilots was to determine the appropriate concentration and time after the injection that total T blood levels be at least 8 times higher than base line (in order to simulate doses that increased muscle size and strength) [22]. After testing the various concentrations, the dose of 50mg/kg one week before surgery was chosen as it consistently gave us levels greater than 8 times baseline [22].

Surgical preparation

Male New Zealand White rabbits (2.4-3.7kg) were randomized.

*Corresponding author: Michael Herring, B.S., Heart Institute Good Samaritan Hospital1225 Wilshire Blvd. Los Angeles, CA 90017-2395, Tel: (213) 977-4040; Fax: (213) 977-4107; E-mail: mherring26@gmail.com

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was continuously monitored with a probe, and body temperature was
measured with a rectal probe. Tightening this snare induced a CAO. Rectal temperature
was taken 5 minutes prior to CAO. Rabbits were then subjected to 30
minutes of CAO. After 30 minutes of CAO, the snare was loosened
and the rabbits underwent 3 hours of coronary artery reperfusion (CAR).
Hemodynamic and blood gas measurements were taken at
30, 60, 90, and 120 minutes of reperfusion. ECG tracings to measure
QT segment duration and echocardiography images to measure
regional ejection fraction and fractional shortening were obtained
at 175 minutes of reperfusion. At the end of the study, a catheter filled
with heparinized saline was inserted into the left atrial appendage to delineate the area of ischemic risk. The ischemic risk zone
remained pink, while the area not at ischemic risk stained blue.
Under deep anesthesia, rabbits were sacrificed by injecting 12 mEq of
potassium chloride. The heart was then removed and analyzed.

**Blood gas and hemodynamic measurements**

Blood gas and hemodynamic measurements were taken at baseline,
25 minutes of occlusion and 30, 60, 90 and 120 minutes of reperfusion.
Blood gas measures examined were pH, O2 partial pressure, CO2 partial pressure and O2 saturation (NOVA Biomedical, Waltham, MA).

Mean arterial blood pressure was measured via a catheter filled
with heparinized saline inserted into the left carotid artery. Heart
rate was measured on ECG tracings with the pulsatile blood pressure
recording serving as backup. For each measurement, three consecutive
heartbeats were measured and the data were averaged. Data were
measured and analyzed using the Advanced Digital Instruments (ADI,
Grand Junction, CO) system.

**Analysis of no-reflow area, risk zone and necrotic region**

Hearts were cut transversely into 6 to 8 sections. Hearts were
photographed under ultraviolet light to show the area of no-reflow,
and then under standard lighting to show the risk zone. Hearts were
next incubated in a 1% solution of triphenyl tetracarbonyl chloride
for 15 minutes to show the necrotic region. Hearts were subsequently
rephotographed. Measurements were obtained by planimetric tracing
with Image J software to determine areas of interest [23]. Areas were
multiplied by heart slice weight to calculate the weight of each area.
Weights were summed to obtain the weights of the no-reflow, risk, and
necrotic zones.

**Analysis of QT Duration**

QT durations were corrected using Bazett’s formula, or QTc =
QT/√RR, where RR = 60/HR [24]. Bazett’s formula is one of the most
commonly and widely used QT correctional formulae in the clinical
literature. [25-27]. QTc was measured at baseline (n = 14 T, n = 15
control), 25 minutes of occlusion (n = 14 T, n = 18 control) and
175 minutes of reperfusion (n = 15 T, n = 15 control). Three consecutive
heartbeats were analyzed for QT duration at each time point and
averaged.

**Statistics**

Data were collected and analyzed using Excel spread sheets.
Student’s T-tests were performed with Stat Plus Software (Analyst Soft,

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**Table 1:** Blood gas measurements

<table>
<thead>
<tr>
<th>pH</th>
<th>Baseline</th>
<th>30' Rep</th>
<th>90' Rep</th>
<th>120' Rep</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>7.5601 ± 0.018</td>
<td>7.5223 ± 0.016</td>
<td>7.4962 ± 0.017</td>
<td>7.4844 ± 0.018</td>
<td>7.4296 ± 0.017</td>
</tr>
<tr>
<td>25' Occl</td>
<td>7.5441 ± 0.015</td>
<td>7.5076 ± 0.017</td>
<td>7.4847 ± 0.012</td>
<td>7.4667 ± 0.011</td>
<td>7.4501 ± 0.011</td>
</tr>
<tr>
<td>25' Occl</td>
<td>103.0625 ± 5.54</td>
<td>115.1947 ± 7.724</td>
<td>112.02 ± 6.589</td>
<td>114.9475 ± 6.224</td>
<td>129.4111 ± 6.479</td>
</tr>
<tr>
<td>30' Rep</td>
<td>96.2133 ± 1.515</td>
<td>95.4526 ± 2.299</td>
<td>97.145 ± 1.251</td>
<td>95.65 ± 1.771</td>
<td>97.3833 ± 0.654</td>
</tr>
<tr>
<td>60' Rep</td>
<td>98.1571 ± 0.222</td>
<td>97.145 ± 1.251</td>
<td>95.65 ± 1.771</td>
<td>97.3833 ± 0.654</td>
<td></td>
</tr>
<tr>
<td>90' Rep</td>
<td>97.5733 ± 0.423</td>
<td>96.046 ± 1.602</td>
<td>95.3833 ± 0.654</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120' Rep</td>
<td>96.2133 ± 1.515</td>
<td>95.4526 ± 2.299</td>
<td>97.145 ± 1.251</td>
<td>95.65 ± 1.771</td>
<td>97.3833 ± 0.654</td>
</tr>
</tbody>
</table>

Occl indicates occlusion; Rep indicates reperfusion.

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**Experimental protocol**

Baseline hemodynamic, blood gas, and ECG readings were obtained after 15
minutes of CAO. After 30 minutes of CAO, the snare was loosened
and the rabbits underwent 3 hours of coronary artery reperfusion (CAR).

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**Mean arterial blood pressure was measured via a catheter filled
with heparinized saline inserted into the left carotid artery. Heart
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Data were collected and analyzed using Excel spread sheets.
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Results

Animals

A total of 42 animals were included in this protocol. Two animals died before the end of the protocol due to arrhythmias, two animals were excluded due to a small ischemic risk zone, and one animal died as a result of malfunctioning equipment. The remaining 37 animals were studied: Testosterone, n=18; Control, n=19. There was no differences in pH, pO2 and O2 saturation between groups. There was a small but significantly higher pCO2 in the control group as ventilation early in the protocol is difficult to control (P< 0.05); however the absolute values for pCO2 were well within the normal range for both groups (Table 1) [28]. Hemodynamic measurements (Figure 1) were similar in both groups (P = NS for all measures). Testosterone levels in the treated group were higher than in the control group: 14.6±1.3ng/mL T versus 1.4 ± 0.7ng/mL control (P< 0.01).

Baseline body temperature, an independent predictor of infarct size, was 38.15 ± 0.2°C in the T group and 38.18 ± 0.2°C in the control group, respectively. Body weight was 2.9 ± 0.07 kg and 2.9 ± 0.06 kg, respectively. Left ventricle (LV) weight was 4.2 ± 0.2 gram and 4.2 ± 0.1 g, respectively. None of these differences were statistically significant throughout the study (P = NS for all measures).
Risk zone, infarct size and area of no reflow

Areas at risk (AR) as a percentage of the LV (Figure 2A) were similar in both groups: 30.5±2% testosterone and 29.4±3% control (P = 0.75). The infarct sizes, or areas of necrosis (AN) as a percentage of the AR (Figure 2B) were also similar in both groups: 36.9±5% testosterone and 37.1±3% control (P = 0.96). When AN was expressed as a function of AR (Figure 2C), analysis of covariance (ANCOVA) revealed no significant differences between groups (P = 0.83).

Areas of no-reflow as a percentage of the AR (ANRs, Figure 3A) were similar in both groups: 30.1±4% testosterone and 32.6±3% control (P = 0.64). When ANR size was expressed as a function of AR (Figure 3B), ANCOVA revealed no significant differences between groups (P = 0.59).

Cardiac function

LVEF and LVFS were similar in both groups: 54.8±5% T versus 53.5±3% control (P = 0.82) and 40.3±3% T versus 37.9±3% control (P = 0.57) respectively (Figure 4).

QTc

Baseline QTc duration times were 293 ± 9 ms and 322 ± 9 ms for T and control, respectively (P = 0.03). At 25 minutes of occlusion and 180 minutes of reperfusion, there were no differences in QTc (25 minutes of occlusion: 311.8 ± 12.3ms T versus 334.3 ± 10.3ms control, P = 0.17; 180 minutes of reperfusion: 335.2 ± 5.5ms T versus 345 ± 9.8ms control, P = 0.39, (Figure 5).

Discussion

Administration of exogenous T has gained popularity for the treatment of hypogonadism, decreased libido and fatigue and increasing muscle size and strength. Despite warnings about the cardiovascular safety of exogenous T, [21] the number of T prescriptions has nearly tripled over the past decade [2]. Previous studies have suggested that reduced testosterone level causes an increase in experimental MI size, as orchiectomized animals showed larger MI size than both control animals and orchiectomized animals with T supplementation [29,30]. A recent study published in JAMA [31] reported a higher incidence of adverse cardiovascular events in male veterans receiving testosterone therapy for hypogonadism versus control male veterans receiving no testosterone. Although the difference was found to be statistically significant, this was a non-random, retrospective study in which only male veterans with coronary artery disease were included in this study [31]. A prospective, randomized, placebo-controlled study in which cardiovascular outcomes are the primary endpoint will be required to quell the controversy regarding testosterone therapy in the clinical setting. To our knowledge, our study is the first to examine the effect of supra physiological levels of testosterone on infarct size in an acute model. The present study suggests that supra physiological levels of testosterone did not adversely affect the outcome of myocardial infarction when compared to physiological levels of testosterone in an experimental model. Our data suggested that administration of exogenous testosterone had no effect on acute MI size, the extent of no-reflow or post-MI cardiac function. In fact, exogenous testosterone shortened the QTc interval. Testosterone did not cause abnormal blood gas levels or abnormal hemodynamic readings.

Our findings concerning QTc reflect those of Bai, Brouilette and coworkers, [13,14] suggesting that testosterone shortens QTc segment duration. This may have an important clinical implication as a shortened QTc segment has been associated with a lower incidence of arrhythmias [24]. Also, while prolongation of the QTc carries the majority of the pro-arrhythmic risks and it is associated with at least 10 genetic mutations [32], short QT syndrome also comes with risks. Short QT intervals have been shown to cause arrhythmias, but the mechanism of this disease is limited in scope and tends to affect infants, children and young adults [33]; individuals unlikely to be receiving testosterone therapy.

Despite the evidence suggesting that T increases apoptotic cell death, [17-20] the present study suggests that the use of exogenous testosterone at supra physiological levels is safe regarding MI size, area of no-reflow and post-MI cardiac function. This study also suggests that supra physiological levels of testosterone may in fact be beneficial in the scope of creating an anti-arrhythmia setting by shortening the QTc interval. However, more research is needed to determine the effect of testosterone on arrhythmias.

Limitations

While we focused on the effect of testosterone on acute myocardial infarctions, we did not examine other effects of testosterone. Such effects include lean body mass [22] (although there was no difference in weight between groups) and inflammatory biomarkers [34]. The purpose of our pilot studies was to determine a dose of testosterone that attained a supra physiological level as described in the literature.
[22]. Once this dose was obtained, we did not think it was necessary to carry out a concentration – response study or to study other time points. The main purpose was to induce an experimental myocardial infarction at the time testosterone levels were elevated, and we achieved that goal.

Acknowledgements
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Reference