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

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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 (50 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

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Measurement	Time	Testosterone (Average ±SEM)	Control (Average ±SEM)	P Value
pH	Baseline	7.5601 ± 0.018	7.5249 ± 0.02	0.22
	25' Occl	7.5441 ± 0.015	7.5078 ± 0.017	
	30' Rep	7.5223 ± 0.016	7.4847 ± 0.012	
	60' Rep	7.4962 ± 0.017	7.4793 ± 0.012	
	90' Rep	7.484± 0.018	7.4667 ± 0.011	
	120' Rep	7.4396 ± 0.017	7.4501 ± 0.01	
pO2	Baseline	130.8938 ± 13.172	144.995 ± 10.846	0.43
	25' Occl	103.0625 ± 5.54	115.1947 ± 7.724	
	30' Rep	109.34375 ± 5.297	108.42 ± 7.931	
	60' Rep	111.675 ± 6.317	111.15 ± 9.053	
	90' Rep	120.62 ± 5.238	127.02 ± 6.589	
	120' Rep	114.94375 ± 6.224	129.4111 ± 6.479	
pCO2	Baseline	38.8063 ± 2.886	41.12 ± 2.965	0.02
	25' Occl	32.3125 ± 2.56	39.1211 ± 2.75	
	30' Rep	32.2313 ± 2.705	40.56 ± 2.603	
	60' Rep	32.4938 ± 2.245	40.945 ± 3.797	
	90' Rep	32.44 ± 2.061	37.36 ± 1.482	
	120' Rep	32.62 ± 1.961	36.2471 ± 1.55	
SO2	Baseline	96.7125 ± 1.658	97.99 ± 0.662	0.76
	25' Occl	96.2133 ± 1.515	95.4526 ± 2.29	
	30' Rep	97.84 ± 0.319	95.885 ± 1.521	
	60' Rep	97.5733 ± 0.423	96.045 ± 1.602	
	90' Rep	98.1571 ± 0.222	97.145 ± 1.251	
	120' Rep	95.65 ± 1.771	97.3833 ± 0.654	
Occl indicates occlusion; Rep indicates reperfusion.				

Table 1: Blood gas measurementspCO2 levels were higher in the control versus the T group, yet all levels were within the normal range.

using a random number generator to receive either an intramuscular (IM) injection of T (50mg/kg, n=20) or saline (n=22) 1 week before surgery. Only one dose of testosterone was given in an attempt to determine the effectiveness of testosterone as an acute therapy of acute MI. On the day of surgery, rabbits were anesthetized by an IM injection of ketamine (50mg/kg, JHP Pharmaceuticals, Rochester, MI) and xylazine (10 mg/kg, Lloyd Labs, Shenandoah, IA).A second dose was given 5 to 10 minutes before surgical preparation. Intravenous (IV) sodium pentobarbital (Lund beck Inc., Deerfield, IL) was given during the procedure as necessary to ensure deep anesthesia. Tracheotomies were performed and rabbits were ventilated mechanically with oxygen-enriched air. Catheters filled with heparinized saline were inserted into the left jugular vein for anesthesia administration and into the left carotid artery for hemodynamic measurements and blood sample extraction in order to measure blood gas levels.ECG electrodes were inserted into the muscle of each hind leg and the right foreleg of the rabbits. The heart was exposed by opening the chest at the fourth left inter-costal space. A pericardial cradle was created if access to the myocardium was obstructed by the lung. Coronary artery occlusion (CAO) was accomplished by threading a 4-O silk suture around the circumflex artery near the left atrial appendage and creating a snare by threading the ends of the suture through a piece of plastic tubing. Tightening this snare induced a CAO. Rectal temperature was continuously monitored with a probe, and body temperature was maintained with a heating pad.

Experimental protocol

Baseline hemodynamic, blood gas, and ECG readings were taken 5 minutes prior to CAO. Rabbits were then subjected to 30 minutes of CAO by tightening the snare and securing it with a clamp. Hemodynamic, blood gas, and ECG readings were obtained after 25 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 and 1 mg/kg of 4% Thioflavin S solution was injected to define the area of no-reflow. Thioflavin S binds to endothelial cells, making it a marker for perfusion, causing the area of perfusion to fluoresce green-yellow under ultraviolet light, thus the area of no-reflow appears non-fluorescent (dark).After Thioflavin S circulated, the coronary artery was re-occluded and 4 milliliters of a 50% solution of Unisperse blue dye (Ciba-Geigy, Hawthorne, NY) were injected 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 tetrazolium 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/$\sqrt{RR}$, 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 175minutes 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,

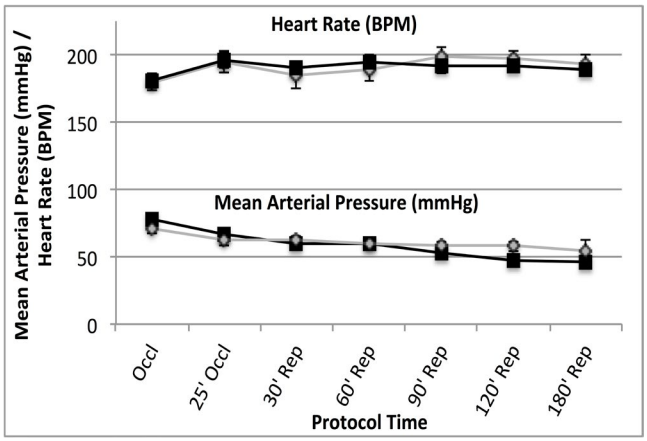

Figure 1: Hemodynamic measurements of Mean Arterial Pressure (mmHg) and Heart Rate (BPM).P = NS for all measures.

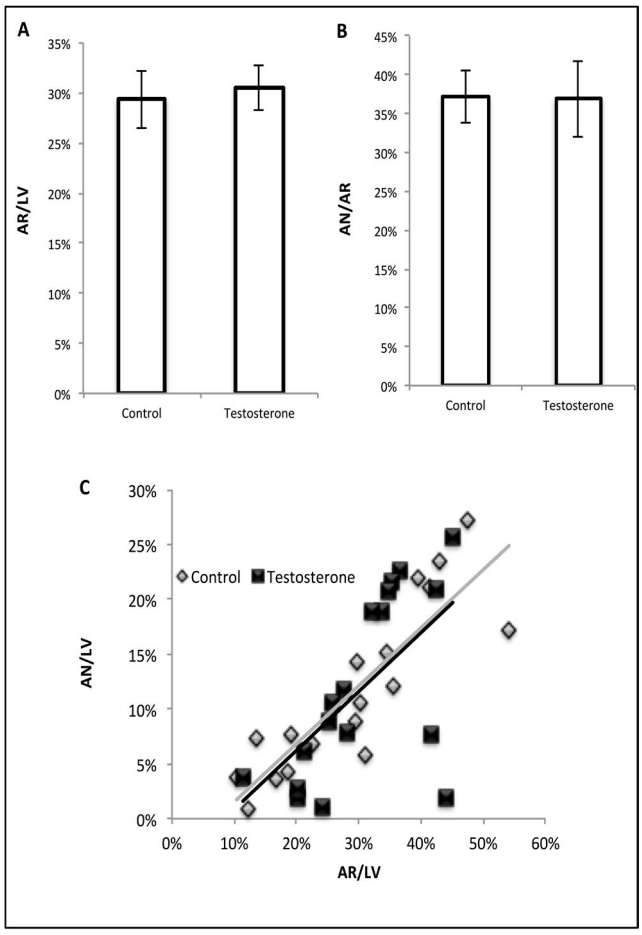

Figure 2: Area at risk and area of necrosis analysisA, the size of the area at risk for each group.B, the size of the area of necrosis as a percentage of the area at risk for each group.C, the relationship between the size of the risk area and the size of the area of necrosis for each group (P=NS). AR denotes area at risk; AN denotes area of necrosis.

Alexandria, VA).Repeated measures analysis of variance and analyses of covariance were performed with SAS (Version 9, Cary, NC). Data are expressed as mean ± SEM.

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, pO_2 and O_2 saturation between groups. There was a small but significantly higher pCO_2 in the control group as ventilation early in the protocol is difficult to control (P< 0.05); however the absolute values for pCO_2 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).

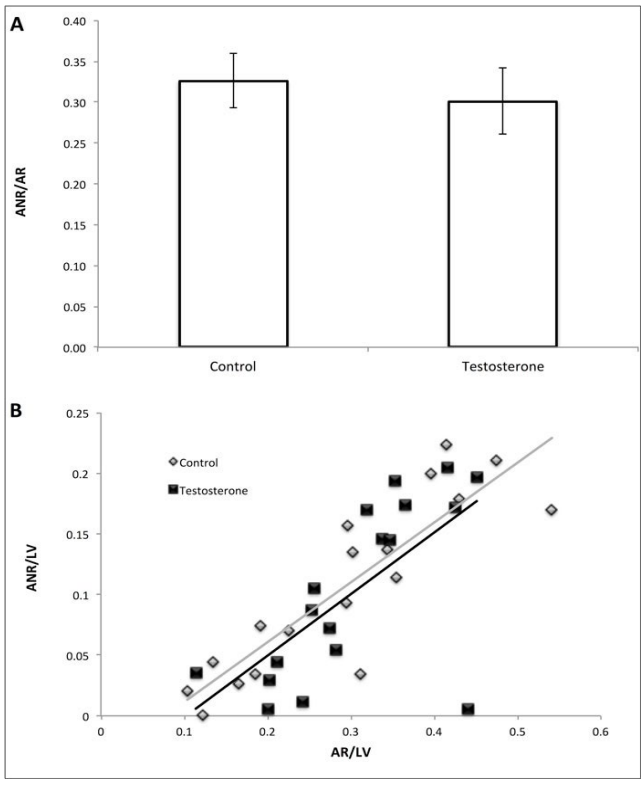

Figure 3: Area of no-reflow analysisA, the size of the area of No-reflow as a percentage of the area at risk for each groupB, the relationship between the size of the area at risk and the size of the area of no reflow for each group (P=NS). AR denotes area at risk; ANR denotes area of no-reflow.

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).

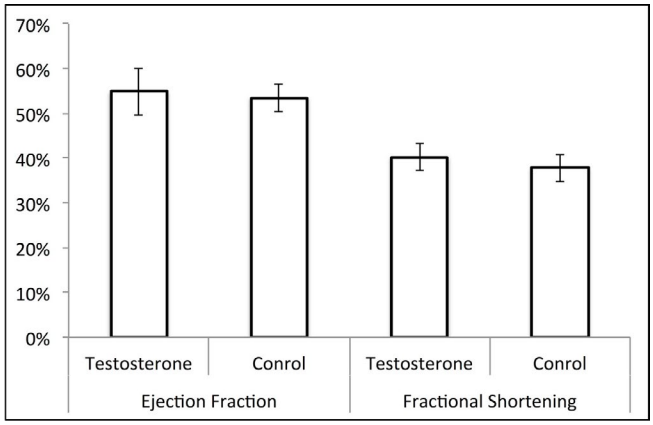

Figure 4: Post-MI cardiac function measurements for each group.Regional ejection fraction and fractional shortening comparisons (P=NS).

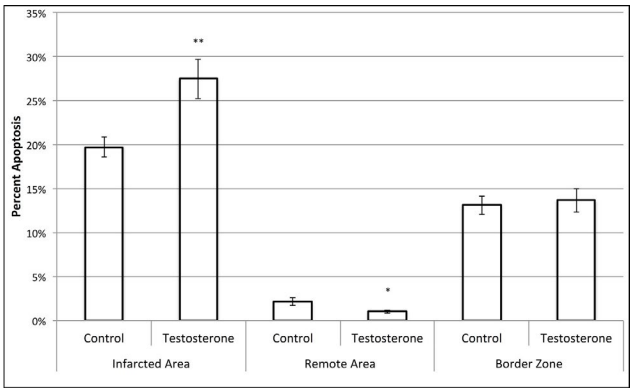

Figure 5: Corrected QT segment duration at baseline, 25 minutes of occlusion, and 180 minutes of reperfusion (*, P< 0.05 compared to control).

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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