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Original Research

Testosterone and Voluntary Exercise Promote Angiogenesis in Hearts of Rats with Diabetes by Enhancing Expression of VEGF-A and SDF-1a

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ABSTRACT

Objectives: Impaired angiogenesis in cardiac tissue is a major complication of diabetes. This study was aimed to evaluate the effects of testosterone and voluntary exercise on vascular endothelial growth factor-A (VEGF-A), stromal cell-derived factor 1a (SDF-1a) and myocardial capillary density in heart of rats with diabetes.
Methods: Type 1 diabetes was induced by intraperitoneal injection of 55 mg/kg of streptozotocin in 80 male Wistar rats. After 42 days of treatment with testosterone (2 mg/kg/day) or voluntary exercise alone or in combination, angiogenesis was determined in the hearts by immunostaining for PECAM-1/CD31. The expressions of VEGF-A and SDF-1a levels in heart were also determined by the ELISA method.
Results: Our results showed that capillary density, VEGF-A and SDF-1a levels in the heart were significantly decreased in castrated rats with diabetes, whereas these effects were reversed by testosterone and exercise. Furthermore, simultaneous treatment of castrated rats with diabetes with testosterone and exercise had a synergistic effect on capillary density, VEGF-A and SDF-1a levels in the heart. In the group with diabetes, either testosterone or exercise increased capillary density, VEGF-A and SDF-1a protein levels in heart tissue. However, the effects of combination therapy in rats with diabetes with testosterone and exercise on capillary density, VEGF-A and SDF-1a levels in the heart was synergistic.
Conclusions: Our findings suggest that testosterone and exercise can promote neoangiogenesis in rats with diabetes and in castrated rats with diabetes. The proangiogenesis effect of testosterone and exercise is associated with the enhanced expression of VEGF-A and SDF-1a in heart tissue.

Mots Clés :
Diabète
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RÉSUMÉ

Objectifs : Une angiogenèse altérée au sein du tissu cardiaque est une complication majeure du diabète. Cette étude avait pour but d'évaluer les effets de la testostérone et de l'exercice volontaire sur le facteur de croissance de l'endothélium vasculaire de type A (VEGF-A), sur le facteur dérivé de cellules stromales de type 1-alpha (SDF-1a), et sur la densité capillaire myocardique dans le cœur de rats diabétiques.
Méthodes : Le diabète de type 1 a été induit par injection intrapéritonéale de 55 mg/kg de streptozotocine chez 80 rats mâles Wistar. Après 42 jours de traitement avec la testostérone (2 mg/kg/jour) ou d'exercice volontaire, uniquement ou en combinaison, le degré d'angiogenèse a été déterminé dans le cœur par immunomarquage de PECAM-1/CD31. Les niveaux d'expression de SDF-1a et VEGF-A dans le cœur ont également été déterminés par la méthode ELISA.
Résultats : Nos résultats ont montré que la densité capillaire, les niveaux cardiaques de VEGF-A et de SDF-1a ont été significativement diminués chez les rats diabétiques castrés, alors que ces effets ont été inversés par la testostérone et l'exercice. En outre, le traitement simultané des rats diabétiques castrés par la testostérone et l'exercice a eu un effet synergique sur la densité capillaire, et sur les niveaux cardiaques de VEGF-A et de SDF-1 a dans le cœur. Dans le groupe diabétique, la testostérone ou l'exercice a augmenté

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la densité capillaire ainsi que les niveaux des protéines VEGF-A et SDF-1 dans le tissu cardiaque. Cependant, les effets de la thérapie combinée de la testostérone et l'exercice sur la densité capillaire, et les niveaux cardiaques de VEGF-A et SDF-1a chez les rats diabétiques étaient synergiques.
Conclusions : Nos résultats suggèrent que la testostérone et l'exercice peuvent promouvoir la néoangiogenèse chez les rats atteints de diabète et chez les rats diabétiques castrés. L'effet proangiogénique de la testostérone et de l'exercice physique est associé à une expression accrue de VEGF-A et SDF-1 dans le tissu cardiaque.

Introduction

The main cause of mortality and morbidity in subjects with diabetes is cardiovascular disease (1). Hyperglycemia in diabetes causes endothelial dysfunction by its various glyco-oxidative products and decreases angiogenesis in cardiac tissue of subjects with diabetes (2). It is also well known that the cardiovascular system is 1 of the important targets of androgen action. Several studies have demonstrated that testosterone deficiency is common in men with diabetes, regardless of the type (3). It has also been reported that insulin sensitivity decreases in castrated male rats and is corrected by physiologic testosterone replacement therapy (4). Some studies have also demonstrated that androgens may modulate endothelial functions and angiogenesis (5,6). Therefore, the combination of impaired glucose tolerance and testosterone deficiency in diabetes increases the risk for cardiovascular disease.

The protective role of regular exercise on the cardiovascular system in diabetes is well known (7). The positive effects of physical activity in reducing cardiovascular-based mortality in persons with type 1 diabetes have been shown (8). Exercise can be divided into 2 types: voluntary and involuntary. Voluntary exercise is considered mild to moderate exercise, and many beneficial effects have been reported for such exercise (9). Several studies in humans and animals suggest that voluntary exercise is a useful therapeutic approach to cardiovascular diseases (10,11). Although the cardiovascular system is an important target of androgen action and exercise, the molecular mechanism of the action of testosterone and voluntary exercise in the hearts of those with diabetes remains largely unexplored.

Vascular endothelial growth factor-A (VEGF-A) and stromal cell-derived factor-1a (SDF-1a) play key roles in the process of angiogenesis. VEGF is the main signalling system that regulates the proliferation and migration of endothelial cells, forming the basis of many vessels and their receptors (12). SDF-1a, also known as CXCL12, induces angiogenesis both by stimulating local endothelial cell proliferation and by recruiting proangiogenic cells from the bone marrow (13).

In this study, we aimed to investigate the effects of testosterone replacement therapy and exercise, alone or in combination, on angiogenesis in the heart and its corresponding mechanisms in rats with diabetes and castrated rats with diabetes.

Methods

Animals and study design

This research was carried out in accordance with the National Research Council's protocol for the care and use of laboratory animals. We obtained 80 Wistar male rats (200 to 250 g) from the laboratory animal house of Tabriz University of Medical Sciences. They were kept in standard laboratory conditions under a natural light-and-dark cycle. The animals were housed in standard conditions (temperature 22°C, light from 8.00 AM to 8.00 PM) and had free access to tap water and food pellets. All the experimental procedures were conducted according to protocols approved by the Animal Care Committee of the Tabriz University of Medical Sciences.

The animals were divided into 8 groups (n=10):

1) Having diabetes + placebo group (Dia)
2) Having diabetes + testosterone group (Dia-T)
3) Having diabetes + exercise + placebo group (Dia-E)
4) Having diabetes + testosterone + exercise group (Dia-T-E)
5) Having diabetes + castrated + placebo group (Dia-Cas)
6) Having diabetes + castrated + testosterone group (Dia-Cas-T)
7) Having diabetes + castrated + exercise + placebo group (Dia-Cas-E)
8) Having diabetes + castrated + testosterone + exercise group (Dia-Cas-T-E)

Castration and hormone replacement therapy

Sexually adult male rats were anesthetized with ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (5 mg/kg). Then a horizontal incision was performed in the scrotum, the testes were tied off and removed with a cut distal to the ligature, and the incision was sutured. To avoid disruption of hormonal influences, testosterone replacement began immediately after surgery (14). Testosterone propionate (Unigen, Life Science, Fremont, California, USA) dissolved in dimethyl sulfoxide (DMSO) and administered subcutaneously to rats at a physiologic dose (2 mg/kg) once daily. Rats in the Dia, Dia-E, Dia-Cas and Dia-Cas-E groups were injected with the same amount of DMSO vehicle.

Induction of diabetes

Type 1 diabetes was induced by a single intraperitoneal injection of streptozotocin (55 mg/kg) (Sigma, St. Louis, Missouri, USA) to all animals. Streptozotocin was dissolved in 10 mM sodium citrate, pH 4.5, with 0.9% NaCl. Diabetes was verified 72 hours later by evaluating blood glucose levels using a glucometer (Elegance, model CT-X10, Frankenberg, Germany). Rats with blood glucose levels≥300 mg/dL (16.67 mmol/L) were considered to have diabetes.

Voluntary exercise

Rats in exercise groups were housed individually in cages that were equipped with stainless-steel vertical running wheels (Tajhiz Gostar, Tehran, Iran) and were allowed free access to the wheel 24 hours per day for 6 weeks. Rats exercised in the running wheel according to their physiologic threshold for physical activity. Such voluntary exercise is considered to be mild to moderate exercise (9). Daily running distances were recorded by a permanent sensor installed on the running wheel.

Tissue processing and protein measurement

At the end of study, hearts were excised and frozen immediately with liquid nitrogen. Tissue samples from the left ventricles were used for VEGF-A and SDF-1a measurement. Samples were stored in –70°C temperatures until SDF-1a and VEGF-A measurement. Then samples were homogenized in PBS (pH 7.2 to 7.4) and centrifuged for 20 minutes at the speed of 3000 rpm at 4°C. Resulting supernatants were removed, and target proteins were extracted. VEGF-A (Hangzhou Eastbiopharm, Hangzhou, China) and SDF-1a