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The Selective Androgen Receptor Modulator Ostarine Increases the Extracellular Matrix in the Myocardium Without Altering it in the EDL Muscle

Fanka Gerginska[1], Slavi Delchev[1], Veselin Vasilev[2], Katerina Georgieva[2], Nikolay Boyadjiev[2]*

[1]Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University-Plovdiv, Plovdiv, Bulgaria;
[2]Department of Physiology, Faculty of Medicine, Medical University-Plovdiv, Plovdiv Bulgaria

*Corresponding author e-mail: Fanka.Gerginska@mu-plovdiv.bg

The selective androgen receptor modulators (SARMs) are androgen receptor (AR) ligands that bind to AR and exhibit a pronounced anabolic effect. They are used in sports to improve physical performance. Their adverse side effects are not well studied. The aim of this study was to investigate the effect of SARMs on the myocardium and a skeletal muscle. Male Wistar rats were divided into 2 groups: control (SARM–) and group receiving SARM (Ostarine for 8 weeks) (SARM+). At the end of the experiment, Azan staining on sections of myocardium and extensor digitorum longus muscle (EDL) was applied to account for the collagen distribution. The heart weight in SARM+ group was higher than controls. The amount of extracellular matrix (ECM) in the SARM+ myocardium was increased, while in EDL it was not altered. The observed increase of the heart weight and the amount of ECM can be taken as an indication for side effect of SARM.

Key words: SARM, collagen, myocardium, EDL, side effects

Introduction

SARMs are characterized by predominant anabolic effects and relatively limited androgenic ones. SARMs accomplish their effects by genomic mechanism. They are widely used in sports to improve physical performance and athletic achievements [7]. In addition, they have potential use in patients with a number of diseases – amyotrophic lateral sclerosis (Lou Gehrig's disease), dermatomyositis, osteoporosis, breast cancer, sarcopenia, various types of cachexia, benign prostatic hyperplasia and hypogonadism [2, 3, 11]. Some of the most commonly used SARMs include Ostarine and Andarine [6]. Their adverse side effects are not well studied. The aim of this study was to investigate

the effect of SARMs on the extracellular matrix (ECM) of myocardium and skeletal muscles.

Material and Methods

Young, sexually mature male Wistar rats, weighing 140-200 g, aged three to four months, were used in the experiment. The animals were divided into two groups: a control group without SARM (SARM–, n = 6) and a group receiving SARM (SARM+, n = 6). Throughout the experiment, rats were housed in individual metabolic cages, which made it possible to determine the amount of food eaten and the amount of SARM (Ostarine) consumed, taken orally once daily 5 times a week at a dose of 0.4 mg / kg with supplemental food. Water and food were given ad libitum. A light/dark cycle and a temperature of 23±1° C were maintained for 12 h. The experimental protocol was approved by the Ethical Committee on Human and Animal Experimentation of the Medical University-Plovdiv, and the Commission for Ethical Treatment of Animals at the Bulgarian Food Safety Agency. The rats were reared and all experimental procedures were performed according to the recommendations of the European Commission for the protection and humane treatment of laboratory animals. At the end of the experiment, all rats were decapitated after anaesthesia with Ketamine at a dose of 180 $mg \times kg^{-1}$ i.p. and Xylazine at a dose of 15 $mg \times kg^{-1}$ i.p., having previously measured their body weight. A guillotine for small experimental animals (HUGO SACHS ELECTRONIC D-79232 March F.R., Germany) was used for decapitation.

After dissection, heart and muscles of each animal were weighed and the ratio of organ weight to body weight was calculated. Materials from the wall of the left ventricle of the heart and the whole EDLs were fixed in neutral formalin 10% for 24 hours and embedded in paraffin. Paraffin sections, 5 μm thick, after deparaffinization and rehydration were stained with Azan by Heidenhain (1915). With the help of image analysis software ("DP-Soft" 3.2, Olympus, Japan) using a measuring grid (19×25 fields) at ×200 magnification, the relative percentage distribution of collagen fibres in the ECM of muscles was calculated according to the formula $x = (n/475).100$, where n is the number of squares containing collagen fibres and 475 is the total number of squares [9]. Student's t-test was applied for statistical processing, and a P value ≤ 0.05 was considered statistically significant.

Results

Heart weights in the SARM+ group were higher than SARM– (strong tendency was found out, P=0.06), **(Fig. 1)**. Statistical analysis of the heart/body weight ratio did not reveal an effect of the application of SARM (P>0.05). The weight of EDL didn't show differences between the groups. In the myocardium of the control animals, collagen fibres of the endomysium were distributed in fine longitudinal stripes between the cardiomyocytes, and those of the perimysium were circularly arranged around the blood vessels in the perivascular spaces **(Fig. 2A, B)**. A significant increase in the amount of collagen fibres between the cardiomyocytes and around the conducting coronary arteries and arterioles was found in the myocardium of animals receiving

SARM **(Fig. 2C, D)**. Collagen fibres, which are missing in the control group, were also noted around smaller arterioles in myocardium of SARM+ animals. The amount of ECM in EDL of the SARM+ animals was not altered **(Fig. 2E, F)**.

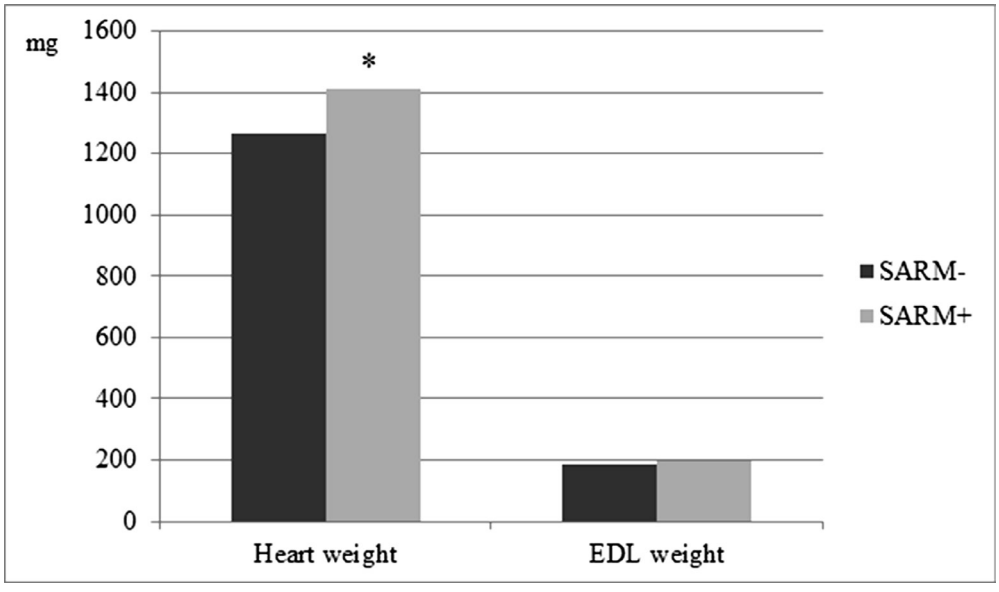

Fig. 1. Heart and EDL weight (mg) at the end of the experiment. *P = 0.06 v/s SARM–.

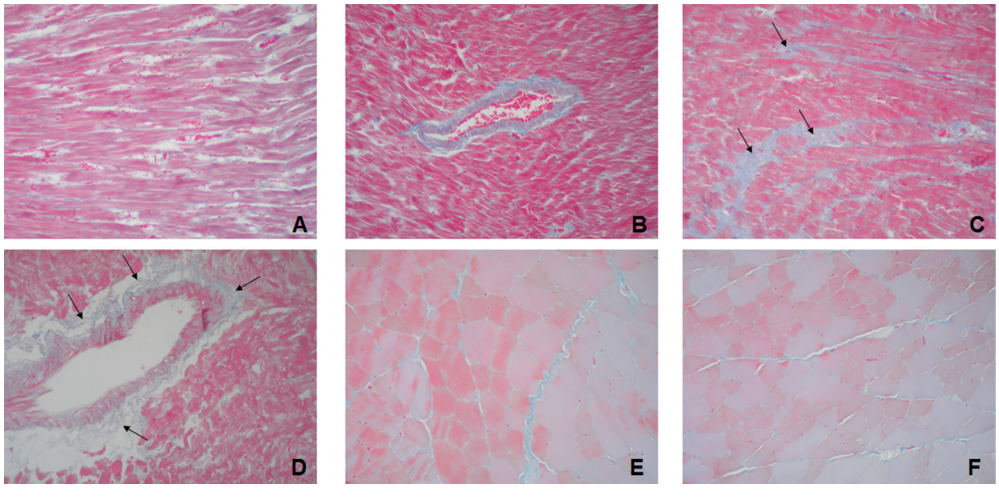

Fig. 2. Sections of myocardium (A-D) and EDL (E, F) of animals from the experimental groups. *Arrows* – collagen fibres in myocardium of SARM+ group (C, D). Azan staining (*Magn.* ×200).

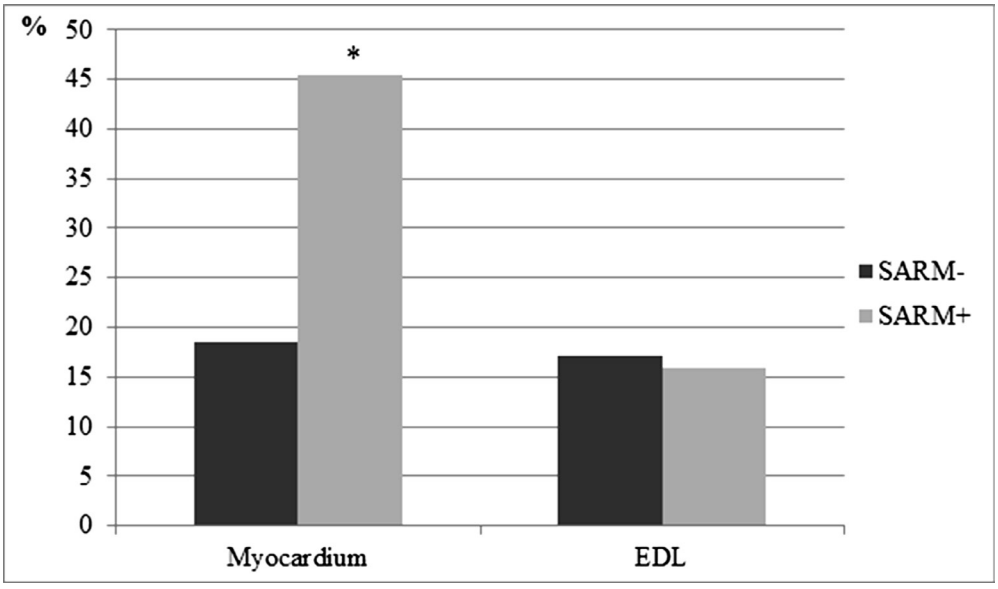

Fig. 3. Relative percentage distribution of collagen fibres in the myocardium and EDL of the experimental groups in Azan stained sections. *P <0.001 v/s SARM–.

Discussion

Myocardial collagen is a complex three-dimensional matrix that surrounds and connects individual cardiomyocytes, thus facilitating their contraction and promoting normal systolic performance [1, 4]. The results of our experiment show that the application of SARM for an 8-week period affected the amount of collagen in the endomysium. Interstitial fibroblasts are mainly responsible for the synthesis of type I and type III collagen fibres present in the endomysium [4]. The dose and duration of Ostarine we used have activated processes of enhanced collagen synthesis by fibroblasts. The increase in collagen fibres around arterial vessels in the myocardium of SARM receiving animals would be difficult to assess according this duration of administration. Increases in perivascular collagen have also been reported in anabolic steroid treated rats [10].

As a potential mechanism to explain the predominant increase in collagen around conducting coronary arteries and arterioles, the ability of vascular smooth muscle cells to produce collagen (mainly type III) may be considered. The Ostarine administration probably activated AR in the nuclei of these cells [7]. If this trend persists with prolonged usage, then certainly the perivascular accumulation of collagen would lead to limited vasodilation, disorders of coronary blood flow and a reduced oxygen supply to cardiomyocytes. Similar changes are common in pathological myocardial hypertrophy [8]. Initial signs of hypertrophy were found in animals of the SARM-treated group, such as an increase in heart weight. Comparable data on cardiac hypertrophy after the use of anabolic steroid with SARMs like properties have been found by other authors [5].

Conclusion

The observed increase in heart weight and amount of the extracellular matrix in the myocardium after SARMs treatment can be considered as an initial myocardial fibrosis and potential side effect. Further research would help to clarify the nature of the side effects of these drugs.

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