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Testosterone Propionate Promotes Angiogenesis and Nerve Regeneration in Extensor Digitorrum Longus Muscle Grafts

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Abstract: Androgens are renowned for improving the process of skeletal muscle regeneration. They are potent agents for quick reinnervation of nerves and angiogenesis in muscle grafts. So far several studies have reported their anabolic roles in clinical transplantations. In the current study, effects of testosterone propionate (TP) onextensordigitorrumlongus muscle grafts were studied. It was noticed that TP supplementation caused significant increase in blood vessels and nerves diameter along with their walls thickness up to 2nd week. Then there was a gradual decrease observed in the above mentioned pattern till end of 4thweek of the hormone supply. It was found that over dosage caused negative effects on the process of regeneration in EDL muscle grafts and resulted in shrinkage of nerves and vascular supply.

Keywords: Androgens, Skeletal Muscle Regeneration, TP Supplementation, Angiogenesis, Reinnervation.

1. INTRODUCTION

Effects of androgens on skeletal muscle development and growth are well known (Serra *et al.*, 2013). The effect is so pronounced that before the awareness of their detrimental effects, the hormones had been a very potent booster for athletes as an adjunct to weight training, exercise, and diet programs to perk up vigor and physique and to induce motivation (Frankle *et al.*, 1984; Strain, 2011). The anabolic steroids are synthetic derivatives of testosterone which are considered ideal to enhance the anabolic rather than the androgenic actions of the hormone. The anabolic effects are considered to be those promoting protein synthesis, muscle enlargement and erythopoiesis (Mottram and George, 2000). It is hypothesized that skeletal muscle fiber regeneration recapitulates and highlights of the normal ontogenetic development of muscle fibers (Sinha*et al.*, 2014).

Synthetic anabolic steroids are based on the chief male hormone testosterone and unite to receptors in reproductive tissue, muscles and fat (Matsumoto *et al.*, 2008). Testosterone Propionate (TP) is a very strong androgenic complex having a high anabolic effect. Users of this compound gain prevailing strength fast without excessive gain in body weight. TP is the acetate form of trenbolone and hence its effect lasts only for a short duration leading to the need for frequent administration. However, TP has significant androgenic side effects both in men and women (Chance *et al.*, 2000; Pope *et al.*, 2000).

During muscle regeneration, angiogenesis plays a pivotal role in adult and also in physiologic processes such as wound repair and in tissue responses to ischemia. The potential for androgens to affect basic processes related to angiogenesis has been demonstrated in vitro. It has been reported that androgens augment angiogenic events in male endothelial cells, including migration, proliferation, tubulogenesis and the expression of various pro-angiogenic factors (Ng *et al.*, 2003; Death *et al.*, 2004; Sieveking *et al.*, 2010; Cai *et al.*, 2011).

Regarding the role of anabolic hormones on nerve regeneration, Kujawa and coworkers (1991) demonstrated that testosterone differentially regulates the regenerative properties of injured hamster facial motoneurons. They suggested means of gonadal steroids action on injured nervous system as partly through the differential parameter of the regenerative properties of the injured cell, seemingly via hormone receptor-mediated action at the level of the neuronal genome. Likewise, Tanzer and Jones (2004) identified effective temporal window of TP exposure adequate to enhance regenerative properties of injured facial motoneurons and functional recuperation from facial paralysis induced by facial nerve injury. They pointed to a vital 6-h interval immediately after injury when TP enhances nerve outgrowth distances and augments behavioral recovery. This information gives a clue to the need of hormone supply at specific stage in the process of myotube and skeletal muscle fiber regeneration. The

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present study was designed to see anabolic effects of testosterone propionate supplementation on the processes of revascularization and reinnervation in extensor digitorumlongus muscles' orthotransplants in rats. The outcome of present experiments contributes to improve in clinical muscular transplantations.

2. MATERIALS AND METHODS

2.1 Experimental animals

For research the present purpose twenty seven adult, *Rattusnorvegicus*, males were used. Weight range of the rats in the beginning of experiment was 121 to 210g. They were placed in animal house with an approximate circadian dark/light rhythm of 12 hours and fed on lab. Prepared food composed of poultry feed, fish meal, wheat flour, molasses and water (Ali and Shakoori, 1990). Moreover, the animals were provided with regular drinking supply of water.

2.2 Surgical protocol and hormonal administration

The rats were ether anaesthetized and othotopictransplantaton of both EDL muscles was performed under controlled conditions. The operated animals were supplied with 0.06% terramycin as postoperative dosage for 3-4days suspended in water. Details of surgical method have been reported elsewhere (Qazi and Mufti, 1997). Categorization of rats was done in to two groups, TP and CTP, after muscle grafting. The TP animals were injected with testosterone propionate solution in corn oil in an amount of 1.0mg/100g body weight (bw). Whereas the CTP rats were provided with 0.2ml of corn oil/100g bw and served as vehicle injected controls. Daily administration of drug as well as the vehicle was done intraperitoneally starting from the day of muscle transplantation.

a. Grafts' recovery and processing

The operated rats were anaesthetized and the EDL muscle grafts were recovered weekly up to one month postgrafting duration. The regenerates were separated from nearby tissues removed out by cutting the tendinous connections and to avoid drying, they were instantaneously immersed in 0.9% saline solution. After that both tendons ends were removed and the mid portions were processed for histological and morphometric analyses. After 4-6 hours Bouin's fixation of the tissues, they were further processed for paraffin embedding and sectioning. The transverse and longitudinal sectioning was done at 8µm on a Cambridge rotary microtome. Later on the sections were stained with haematoxyline and eosin. Photomicrographs of representative sections were taken on a camera fitted microscope. Morphometric analysis of the regenerated nerves and blood vessels within the EDLmuscle regenerates was accomplished Microscopically employing calibrated ocular micrometer. The data were compared applying student's t-test.

3. RESULTS

It was observed that during first week, the testosterone propionate in treated EDL muscle grafts showed 38.22%, 24% and 74.8% increases, respectively in diameter of arteries, veins and nerves than the control **(Fig.1)**. Whereas from 2nd to 4th weeks post grafting, over all gradual decrease in diameters of blood vessels and of nerves was recorded **(Table 1)**. As during 2nd week, arteries and nerves exhibited diameter reduction of 9.7% and 3%, respectively while veins showed 27% enhanced diameter **(Fig.1)**.

In 3rd week's cross sections, little bit change in the pattern was noticed because irrespective of blood vessels, nerves were with 6.6% large diameter, while 33% and 55% diameter reduction presented by arteries and veins, respectively. After 4th week, sharp reductions in diameters of TP treated grafts' were 51%, 67% and 41.43% for arteries, veins and nerves respectively as compared to the control group **(Table1)**. Thickness of blood vessels and nerves' walls of one-week old TP treated arteries were 27.44% higher than the control ones **(Fig.1)**. Same pattern was exhibited by veins and nerves with 8.75µm and 5.42µm increased walls' thickness over the respective control values **(Fig.1)**.

At 2nd week, there was decrease in arteries walls' thickness up to 10.5µm. whilst no change was observed for veins in both controland experimental groups. The TP treated nerves of this group showed 3.37µm thickened walls and the difference was significant at 5% probability level **(Fig. 1)**.

In the 3rd week, blood vessels' walls showed reduction in thickness down to 5.83µm and 13.75µm for veins and arteries, respectively comparable to the value presented by 2nd week old transplants **(Table1)**. Whereas nerves showed minor increase in thickness of walls of about 0.42µm. But in 4th week, only 1.52µm increased width of veins walls was noticed, whereas the arteries and nerves showed walls thickness decreases of 10.48µm and 5.25µm, respectively **(Table 1)**.

In the light of obtained results from this study, it is concluded that the TP dosage showed its positive effects for angiogenesis and nerves regeneration in EDL muscle grafts during the early phase of muscle regeneration. But after certain time limit, it's over dosage appeared to interfere with further growth of regenerated nerves and the process of angiogenesis. Accordingly, shrinkage of blood vessels and nerves was observable in the later stages of the TP treated muscle orthotransplants **(Table-1)**.

Table 1: Effect of Testosterone Propionate Supplementation (1.0mg/100gb.w/day) on the processes of Revascularization; Arteries (A), Veins (B) and Reinnervation (C)

Experiment	Stages of Regeneration (Weeks)											
	1st			2nd			3rd			4th		
	A	B	C	A	B	C	A	B	C	A	B	C
TP	47.93±30.2 (12.1±7.46)	50.42±33.4 (5±3.0)	41.25±26.04 (3.33±1.84)	71.3±3.64 (33±8.12)	78±17 (10±5.7)	89.4±31.85 (4.6±1.2)	63.75±5.46 (23.33±0.5)	53.75±12.54 (10.1±0.653)	63.75±2.044 (7.5±0.42)	72.5± 12.2 (20.5±4.42)	74.6±1.62 (7.1±2.54)	64.2±5.1 (8±1.7)
TP	66.25± 17 (15.42±4.42)	62.5±31.4 (13.75±6.3)	72.1± 14.5 (8.75±3.2)	65± 13.01 (22.5±4.4)	98.75±50.8 (10±0.723)	87.1±25.8 (7.97±2.73)*	47.92±5.52 (9.58±2.73)	34.6± 3.0 (4.17±1.04)	67.93±7.43 (7.917±1.504)	35.033±5.72 (10.02±2.2)	24.6±8.62 (3.33±8.62)	37.6±4.0 (2.75±0.145)

*The values are means ± S.E.M of triplicates. The values show diameter whereas within the brackets show reading of walls' thickness. The value with asterisk is significantly different from respective control at $p \leq 0.05$ (Student's t-test).

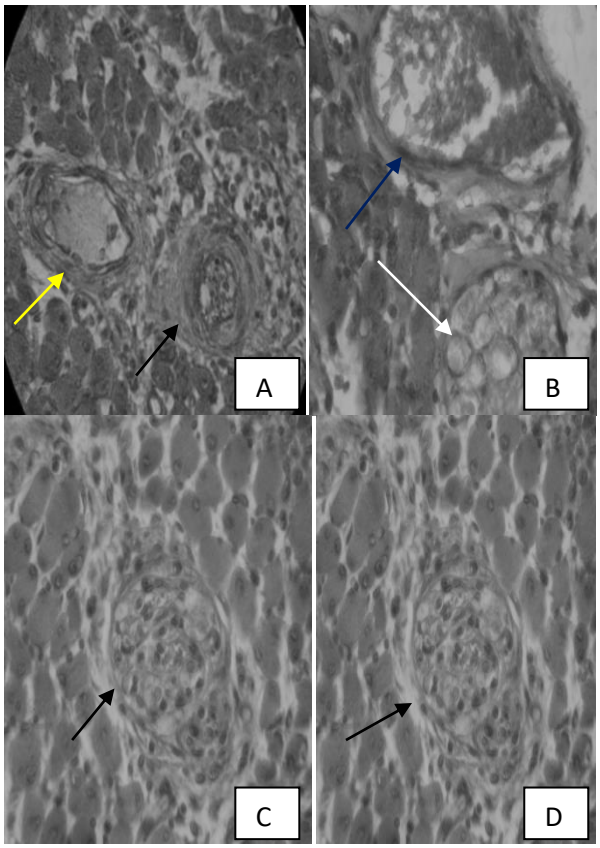

Fig. 1: Cross section of one-week old EDL muscle transplant. A= CTP artery with black arrow and vein with yellow. B= TP treated artery with white arrow and vein with blue. C = a well regenerated TP treated artery is indicated by black arrow. D=TP treated regenerated nerve is presented by black arrow. All the sections represent H & E staining. All micrographs have 400X magnification.

4. DISCUSSION

The hormone supplementation caused significant increase in diameter of arteries right at the beginning of muscle regeneration but continuous administration of testosterone propionate (TP) resulted in decrease in the diameter and also caused variations in blood vessels and nerves' walls thickness. Androgens are recognized to modulate the skeletal muscle proliferation and differentiation processes (Fu *et al.*, 2011). Skeletal muscle is the main target of androgens (Michel and Baulieu, 1976; Mackrell *et al.*, 2015). It has been documented that testosterone stimulates human muscle growth and strength, chiefly by increasing the protein synthesis (Rooyackers and Nair, 1997). In this regard, this hormone is found beneficial for satellite cell activation and proliferation and the regeneration of mice muscle (Neal *et al.*, 2012; Serra *et al.*, 2013).

Moreover, the androgens not only promote the muscle grafts' regeneration but they also enhance the process of reinnervation and angiogenesis (Hansen-Smith and Carlson, 1979; Carlson, 1982). However, in the present investigation these anabolic effects were more pronounced in early weeks of the muscle regeneration and later on vascular shrinkage was observed. Testosterone injection basically promotes angiogenesis by enhancing metabolic factors like collagen fibers production and this hormone also reduces any impairment related to angiogenesis (Chen *et al.*, 2012; Gonçalves *et al.*, 2014). Testosterone has ability to activate the androgen receptors of nerves and resultantly enhances the speed of reinnervation in muscle grafts (Kuiper *et al.*, 1997; Lund *et al.*, 2006; Bielecki *et al.*, 2016).

In relation to the effects of androgen on the process of muscle regeneration Qazi and Mufti (2001) reported that presence of testosterone propionate reversed the atrophy and degeneration of regenerated muscle fibers of freely EDL muscle grafts, observed in orchidectomized rats. The hormone replacement promoted the development process so that from 2nd week onward (up to 4th week), average cross-sectional area of regenerated muscle fibers turned significantly higher than the hormone deprived grafts. Revascularization of skeletal muscle grafts is pivotal for the removal of degenerated mass and development of regenerating muscle fibers (Qazi *et al.,* 2011). But continuous supply of testosterone propionate causes toxic effects too (Beg*et al.,* 2008). As in present study during last week reductions in vascular and nerves size and walls thickness were noticed. In short, the current study indicates that testosterone enhances the processes of reinnervation and angiogenesis in muscle grafts, especially during the early stages of skeletal muscle regeneration. Further research is required to channelize the potent effects of androgen to achieve better skeletal muscle regeneration for clinical applications.

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