Basic Medical Research

Effect of selective androgen receptor modulator RAD140 on prostate and testosterone levels in Wistar strain rats with bilateral orchidectomy

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ABSTRACT

BACKGROUND Selective androgen receptor modulators (SARMs) have been investigated as a potential treatment for hypogonadism, a condition characterized by low testosterone levels in men. The idea is to provide therapeutic benefits similar to traditional testosterone replacement therapy. However, research in this area is still in its early stages, and more extensive studies are needed to establish the efficacy of SARM. This study aimed to determine the impact of SARM RAD140 on testosterone levels, fibromuscular stroma, and prostate mass in rats undergoing bilateral orchidectomy.

METHODS This was an *in vivo* study using posttest-only control group design in rats (*Rattus norvegicus*). The positive and negative control groups consisted of rats with and without bilateral orchidectomy, respectively. The treatment groups were rats given SARM RAD140 with and without orchidectomy. Testosterone levels, histopathology, and prostate mass were examined at the end of week 6, and the quantitative data were analyzed using one-way ANOVA.

RESULTS This study found no difference in prostate mass (0.598 [0.05] g *versus* 0.590 [0.07] g, $p = 0.984$), fibromuscular stroma ratio (0.483 [0.094] *versus* 0.463 [0.057], $p = 0.984$), and testosterone level (0.006 [0.005] ng/dl *versus* 0.014 [0.004] ng/dl, $p = 0.098$) compared to positive control with orchidectomy and SARM RAD140 administration 6 weeks after treatment.

CONCLUSIONS There were no differences in testosterone levels, prostate mass, or the ratio of fibromuscular stroma to epithelium area in rats undergoing bilateral orchidectomy and placebo surgery with the administration of SARM RAD140.

KEYWORDS androgen, epithelium, prostatic neoplasms, testosterone, therapeutic use

Selective androgen receptor modulators (SARMs) show promise as potential therapies for hypogonadism, a medical condition in which men exhibit abnormally low testosterone levels. This hormonal deficiency can trigger various symptoms that impact the quality of life, including persistent fatigue, diminished sex drive, erectile dysfunction, reduced muscle mass and strength, and mood swings. SARMs are targeted therapies that aim to selectively bind to androgen receptors (ARs), potentially mitigating these symptoms by mimicking the beneficial effects of testosterone with possibly fewer side effects than traditional hormone replacement therapies.[1,2]

SARMs are of interest treating hypogonadism because they selectively target ARs in specific tissues such as muscle and bone, promoting anabolic effects, including increased muscle mass and bone density, while minimizing androgenic side effects. The aim is to provide therapeutic benefits similar to traditional testosterone replacement therapy (TRT) with

potentially fewer adverse effects on tissues such as the prostate and hair follicles.[2–5] Some clinical trials have explored the use of SARMs in hypogonadism, primarily focusing on assessing their safety, tolerability, and effectiveness in increasing muscle mass and improving physical function. However, research in this area is still in its early stages, and more extensive studies are needed to establish the long-term safety and efficacy of SARMs compared with existing treatments such as TRT.[6,7]

SARMs are divided into two types: steroidal and non-steroidal antiandrogens. Several first-generation compounds are being studied in phase I trials as promising treatments for hypogonadism, particularly for the extraprostatic symptoms of cancer-related cachexia, and degenerative damage to skeletal muscle and/or bone.[8,9] SARMs specifically activate tissue-selective androgenic signaling outside the prostate gland. This study aimed to determine the effect of SARM RAD140 on testosterone levels, prostate mass, and the prostate tissue-related ratio of the fibromuscular stroma-epithelium area in rats undergoing bilateral orchidectomy.

METHODS

This was an *in vivo* study using a posttest-only control group design. This study used male Wistar white rats (*Rattus norvegicus*) aged 3 months and weighed 200–250 g. Sampling was performed using the simple random sampling technique. Using Federer's formula to determine the research sample size, it was found that a size of six rats was required for each group. The rats were divided into six groups: negative control (placebo surgery), positive control (bilateral orchidectomy without SARM RAD140 [Ellipses Pharma, London]), SARM RAD140 (3 mg/kg) without orchidectomy, SARM RAD140 (10 mg/kg) without orchidectomy, SARM RAD140 (3 mg/kg) after orchidectomy, and SARM RAD140 (10 mg/kg) after orchidectomy. All rats in groups received daily SARM RAD140 at the beginning of the study for the 6-week study period. The SARM RAD140 was suspended in 0.5% methylcellulose and administered orally. The effects of SARM RAD140 administered to rats after orchidectomy were determined by measuring the fibromuscular stroma-epithelium area, prostate mass, and testosterone levels at the end of the 6th week. This research has received ethical approval from the Ethics Commission of General Hospital Dr. Saiful Anwar Malang (No: 400/299/K.3/102.7/2022).

Tissues used for light microscopy analysis were fixed in 10% normal buffer formalin, sliced at a thickness of 3 µm, stained using hematoxylin and eosin and observed using a conventional light microscope at 400× magnification. Prostate tissue was weighed using an analytical balance (g). Relative prostate weight was calculated as the ratio of prostate weight to body weight by dividing prostate weight by animal body weight. Rat serum hormone levels were measured using a testosterone enzyme-linked immunosorbent assay (ELISA) kit (DRG Testosterone ELISA EIA-1559, DRG Instruments GmbH, Germany). One-way analysis of variance (ANOVA) was used for quantitative data analysis to determine the significance of the fibromuscular stroma-epithelium area ratio, prostate mass, and testosterone levels after SARM therapy.

RESULTS

The ANOVA test results revealed a non-significant value of $p>0.05$, indicating no differences in testosterone levels in the negative control (placebo surgery) and SARM RAD140 (3 and 10 mg/kg) without orchidectomy groups compared to the positive control (bilateral orchidectomy without SARM RAD140) and SARM RAD140 (3 and 10 mg/kg) after orchidectomy

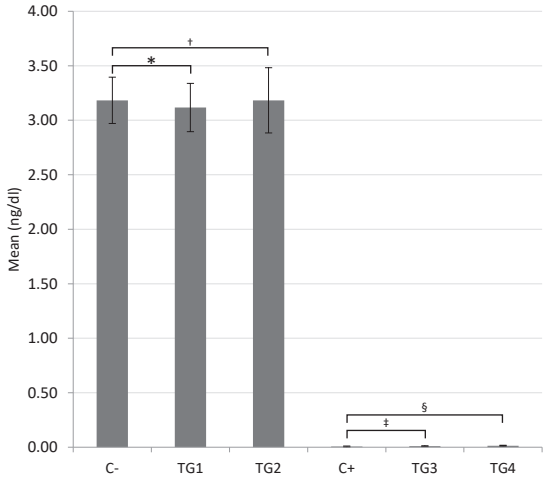

Figure 1. Average prostate gland testosterone levels. No significant with negative control: *p = 0.99; †p = 1.0; no significant with positive control: ‡p = 0.78; §p = 0.09. C−=negative control; C+=positive control; TG1=SARM RAD140 (3 mg/kg) without orchidectomy; TG2=SARM RAD140 (10 mg/kg) without orchidectomy; TG3=SARM RAD140 (3 mg/kg) after orchidectomy for 6 weeks; TG4=SARM RAD140 (10 mg/kg) after orchidectomy for 6 weeks

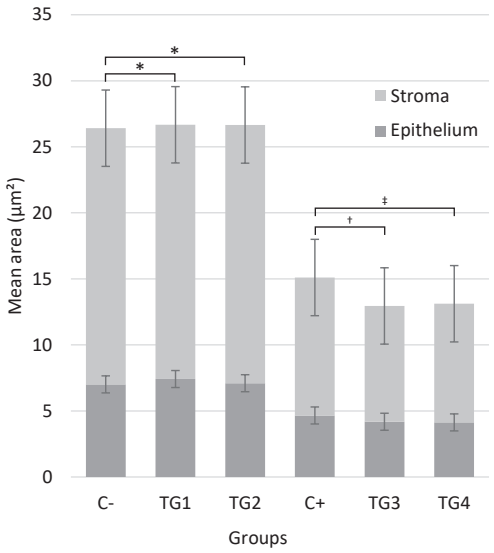

Figure 2. Mean ratio of fibromuscular stroma-prostate gland epithelium area. No significant with negative control: *p = 1.0; no significant with positive control: †p = 1.0; ‡p = 0.99. C−=negative control; C+=positive control; TG1=SARM RAD140 (3 mg/kg) without orchidectomy; TG2=SARM RAD140 (10 mg/kg) without orchidectomy; TG3=SARM RAD140 (3 mg/kg) after orchidectomy for 6 weeks; TG4=SARM RAD140 (10 mg/kg) after orchidectomy for 6 weeks

groups. There was no significant difference (p = 0.78) between the positive control group and SARM RAD140 (10 mg/kg) 6 weeks after orchidectomy group (Figure 1).

With regards to the ratio of the fibromuscular stroma-epithelium area, there was no difference (p>0.05) in the ratio of epithelium and stroma between the negative control (placebo surgery) and SARM RAD140 (3 and 10 mg/kg) without orchidectomy groups. The positive control (bilateral orchidectomy without SARM RAD140) and SARM RAD140 (both 3 and 10 mg/kg) groups were not significantly different (p>0.05) with regards to fibromuscular stroma-epithelium area (Figure 2).

Prostate mass was calculated for the negative control group (0.993 [0.12] g), SARM RAD140 (3 mg/kg) without orchidectomy group (0.970 [0.09] g), SARM RAD140 (10 mg/kg) without orchidectomy group (0.967 [0.13] g), positive control group (0.967 [0.13] g), SARM RAD140 (3 mg/kg) after orchidectomy group (0.598 [0.05] g), and SARM RAD140 (10 mg/kg) after orchidectomy group (0.590 [0.07] g). Based on the results of the ANOVA test, there was no difference (p>0.05) in prostate mass between the negative control (placebo surgery) and SARM RAD140 (3 and 10 mg/kg) groups without orchidectomy. With regards to prostate mass, there was no significant difference between the positive control group (bilateral orchidectomy without SARM RAD140) and the SARM RAD140 (3 and 10 mg/kg) groups.

DISCUSSION

In this study, no significant differences were found in testosterone levels in the white rat model with or without orchidectomy and SARM administration. Felix-Patrício et al[10] found that hormone depletion and replacement were effective in experimental animals that underwent orchidectomy. This was verified by analyzing serum testosterone levels. The rats in the orchidectomy group had undetectable testosterone levels, whereas statistically, the rats that underwent orchidectomy with hormone replacement therapy had the same hormone levels as the placebo group.[10] Rats that underwent orchidectomy and were administered SARM did not have significant differences in testosterone levels. Conversion of testosterone to dihydrotestosterone (DHT) increases its effect on several androgenic tissues. Non-steroidal SARMs do not serve as substrates for 5α-reductase.[11] The selectivity of SARMs for specific tissues may be linked to the tissue-specific presence of coregulation proteins. Likewise, variations in the effect of SARMs compared to the effects of testosterone can be traced to the inability of non-steroidal SARMs to undergo aromatization. Nejishima et al[12] revealed that a SARM called S-40542 had a minimal impact on testosterone and luteinizing hormone levels in the bloodstream. Our findings are consistent with the results of previous research, which showed that administration of SARM RAD140 to male cynomolgus monkeys aged 3–4 years caused a decrease in testosterone levels to 200–300 ng/dl, while normal levels were 600–800 ng/dl.[13] Other studies that used SARM MK-4541 in rats also showed a significant decrease in testosterone levels at 100 and 200 mg/kg doses.[14]

The present study found no significant differences in the fibromuscular stroma-epithelium area between the rats with placebo surgery and those without orchidectomy and SARM administration. The regular structure of prostate epithelium cells is maintained through balanced interactions with smooth muscle cells. Stromal remodeling is triggered when androgen levels are reduced experimentally, such as through orchidectomy. This remodeling results in the replacement of smooth muscle cells with fibroblasts

or myofibroblasts.[15] The increased fibromuscular area of the prostate gland in the orchidectomy groups found in this study is in line with the work of Kajiwara et al[15] who investigated the effects of orchidectomy-induced stromal remodeling and epithelium-stromal interactions that cause aberrant activation of human prostate-like epithelium structures. In the study conducted by Kajiwara et al,[15] rats underwent orchidectomy at 12 weeks of age and were implanted with a DHT pellet 14 days post-castration. Over time, the orchidectomy group exhibited a progressive increase in the percentage of fibrotic areas and a disruption of the prostatic epithelium structure, including the loss of basement membranes. However, these detrimental effects were mitigated by the administration of androgen replacement therapy.[15] Zhang et al[16] conducted a study that focused on the rise in the estrogen-to-androgen (E/T) ratios found in the bloodstream and within the prostate of older men. This increase in the E/T ratio coincided with elevated expression of estrogen receptors in the stromal tissue. The heightened estrogenic effects, which are mediated through the stromal tissue, were observed to be associated with the development of prostatic stromal hyperplasia.[16] Felix-Patrício et al[10] also concluded that orchidectomy experimental animals experienced a significant increase in stromal collagen compared to the control experimental animals ($p<0.001$). Androgen/ARs signaling had a significant impact on various aspects of prostate development, including proliferation, differentiation, morphogenesis, and the ongoing maintenance of prostate function. The ARs in stromal tissue, which affect the growth of epithelium cells, are influenced by several factors such as insulin-like growth factor-1, placental growth factor, and secreted phosphoprotein-1. The combined findings from several studies on stromal cell lines suggest that the ARs in the stroma may play a crucial role in prostate development.[17] Information from other studies examining the effect of SARMs, especially SARM RAD140, on experimental animals after orchidectomy is limited. Further studies are required to compare the results of this study with those of other studies.

The present study found no significant difference in prostate mass between rats that underwent placebo surgery and those that were administered SARM RAD140 without orchidectomy. In 2017, Unwalla et al[18] introduced a novel SARM with a unique cyanopyrrole structure. This compound promotes muscle growth with minimal androgenic effects in a rat model subjected to orchidectomy. Miller et al[19] investigated RAD140, a powerful anabolic SARM, and found that it exhibited antagonistic effects on the prostate and seminal vesicles, suggesting that it is a promising candidate for treating conditions such as benign prostatic hyperplasia, while also promoting muscle and bone growth. A 2012 study by Nejishima et al[12] also reported that S-40542 binds to ARs with high affinity. S-40542 enhanced transcriptional activity at relatively high concentrations. This compound also demonstrates AR antagonistic activity that depends on its concentration when 5α-DHT is present at a concentration of 1 nM. When S-40542 and flutamide were repeatedly administered, both effectively reduced prostate weight to a similar degree in a dose-dependent manner. Al-Shahat et al[20] examined 84 adult male albino rats and found a decrease in the height of the epithelium cell layer and apoptosis of the epithelium lining of the prostatic acinus. Increased interacinar fibromuscular stroma was observed. Bilateral orchidectomy results in prostate atrophy. Orchidectomy can halt testosterone production and reduce its conversion into DHT. These hormonal changes lead to a decline in the expression of vascular endothelial growth factor in the prostate, leading to decreased blood supply to the prostate tissue. The sharp reduction in androgen levels, including testosterone and DHT, following orchidectomy results in a significant decrease in blood flow to the prostate parenchyma.[21] The administration of SARM RAD140 did not cause an increase in the size of the seminal vesicles and the prostate.[22] This study has some limitations. The intricate biological effects of steroid hormones and SARMs vary depending on the binding affinity and degree of agonism and antagonism to ARs in various types of tissues. Therefore, it is crucial to explore high throughput screening methods further to identify SARMs with favorable biological and pharmacokinetic characteristics.[23] In conclusion, there were no differences in testosterone levels, prostate mass, or the ratio of fibromuscular stroma to epithelium area in rats undergoing bilateral orchidectomy and placebo surgery with the administration of SARM RAD140.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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