Chapter 11

Testosterone and Erectile Function: A Review of Evidence from Basic Research

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Additional information is available at the end of the chapter

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Abstract

Androgens are essential for male physical activity and normal erectile function. Hence, age-related testosterone deficiency, known as late-onset hypogonadism (LOH), is considered a risk factor for erectile dysfunction (ED). This chapter summarizes relevant basic research reports examining the effects of testosterone on erectile function. Testosterone affects several organs and is especially active on the erectile tissue. The mechanism of testosterone deficiency effects on erectile function and the results of testosterone replacement therapy (TRT) have been well studied. Testosterone affects nitric oxide (NO) production and phosphodiesterase type 5 (PDE-5) expression in the corpus cavernosum through molecular pathways, preserves smooth muscle contractility by regulating both contraction and relaxation, and maintains the structure of the corpus cavernosum. Interestingly, testosterone deficiency has relationship to neurological diseases, which leads to ED. Testosterone replacement therapy is widely used to treat patients with testosterone deficiency; however, this treatment might also induce some problems. Basic research suggests that PDE-5 inhibitors, L-citrulline, and/or resveratrol therapy might be effective therapeutic options for testosterone deficiency-induced ED. Future research should confirm these findings through more specific experiments using molecular tools and may shed more light on endocrine-related ED and its possible treatments.

Keywords: testosterone, erectile dysfunction, endothelial function, testosterone replacement therapy, basic science

1. Introduction

Androgens are essential for male physical activity and normal erectile function [1–5]. Thus, age-related androgen deficiency, known as late-onset hypogonadism (LOH), is a risk factor for erectile dysfunction (ED) [1, 2]. Several studies have reported that androgen replacement therapy mitigates the symptoms of LOH and ED. In this context, bioidentical or synthetic testosterone

facilitates erectile function by maintaining an adequate supply of nitric oxide (NO), penile structure, and the endothelial functioning of the corpus cavernosum. Thus, reduced NO bioavailability is believed to be the main cause of ED in individuals with testosterone deficiency [6]; however, the pathophysiological mechanisms underlying this process remain unclear and require further study. This chapter summarizes relevant basic research reports examining the effects of testosterone on erectile function.

2. Testosterone and erectile function

Androgens are well established as being essential for erectile function, and their deficiency is considered a risk factor for ED. LOH is a result of the normal aging process and is responsible for androgen deficiency [7, 8]. In recent years, epidemiologic studies have suggested that metabolic syndrome and diabetes mellitus are also associated with the development of androgen deficiency [9–12].

Erectile function is regulated by complex mechanisms [13]. When sexual stimulation occurs, NO is released in the penis, causing corporal smooth muscle relaxation through the activation of the cGMP/protein kinase G signaling cascade. ED results when the relaxant system is weakened; therefore, many studies have focused on smooth muscle relaxation. In contrast, in the flaccid state, corporal smooth muscle contraction is controlled by constrictors such as noradrenaline. Recent studies have indicated that the balance between smooth muscle relaxation and contraction is disturbed by abnormal activation of the RhoA/Rho-kinase signaling pathway. In some syndromes causing ED, such as diabetes mellitus or metabolic syndrome, the RhoA/Rho-kinase signaling pathway is enhanced [14–16]. Additionally, enhancement of the RhoA/Rho-kinase signaling pathway is known to occur in aged individuals, and a Rho-kinase inhibitor (Y-27632) has been shown to improve erectile function in aged rats [17, 18]. As contractility may play a significant role in erectile function, its role in ED should be considered along with contraction. Thus, the balance between smooth muscle contraction and relaxation is important for normal erectile function.

3. Testosterone deficiency and ED

Most animal studies have shown that castration causes ED by reducing arterial inflow [19]. Further, endothelial nitric oxide synthase (eNOS) and neuronal NOS (nNOS) are important in erectile functioning. In castrated animals, testosterone administration restores the erectile response and NOS expression in the penis [20]. Li et al. showed that testosterone deficiency decreases eNOS activity (phosphor-eNOS/eNOS ratio) by upregulating reactive oxygen species production [21], and the decreased eNOS activity decreases cGMP levels in the penis.

Some studies found that testosterone changes phosphodiesterase type 5 (PDE-5) expression in the penis. Traish et al. showed that castration decreased PDE-5 activity in rabbit penises [22], whereas Zhang et al. showed that testosterone deficiency decreased PDE-5 expression in the rat penis and that testosterone administration increased PDE-5 expression [23]. These

results suggest that testosterone is essential not only for regulating eNOS activity but also for regulating PDE-5 activity. Traish et al. also suggested that while these actions may seem paradoxical, in which androgens are upregulating both signal initiators (NOS) and signal terminators (PDE-5), they may be interpreted to be part of a homeostatic mechanism that maintains a relatively constant ratio of critical pathway enzymes [3]. They also postulated that PDE-5 expression may be controlled by NO. Androgen-mediated upregulation of NOS may lead to increased NO synthesis, which may then upregulate PDE-5 expression and activity. Conversely, androgen deprivation-mediated NOS downregulation also results in the downregulation of PDE-5 expression and activity. More studies are needed to define this delicate and crucial mechanism of testosterone action.

Testosterone also affects the smooth muscle of the corpus cavernosum. Reilly et al. showed that castration reduced the number of α-adrenergic-1 receptors on smooth fascia [24]. They also showed that testosterone modulated the adrenergic response of the corpus cavernosum vascular smooth muscle [25]. Their results indicate that when testosterone levels decrease, smooth muscle contractility also decreases. On the other hand, Wingard et al. showed that castration increased the levels of Rho-A and Rho-kinase proteins in rats. RhoA, a small monomeric GTPase, activates the Rho-associated protein kinase, a serine/threonine kinase, which phosphorylates the myosin-binding subunit of myosin light chain phosphatase, thereby deactivating it and promoting contraction [26]. Their results indicate that when testosterone levels decline, smooth muscle contractility increases, leading not only to the development of ED but also to the hypertension. Thus, although testosterone deficiency might increase contraction, additional research is required to fully elucidate its impact on smooth muscle contraction.

Interestingly, testosterone also directly affects smooth muscle relaxation. Yue et al., using an isometric tension study, showed that testosterone relaxed the smooth muscle of rabbit coronary arteries and aortas [27]. Others also showed that testosterone induces the relaxation of isolated human corpora cavernosa strips by activating smooth muscle ATP-sensitive K^+ channels [28]. These findings suggest that testosterone, in addition to its known endothelial action, might regulate erectile function locally by acting on human corpus cavernosum smooth muscle. These results indicate that testosterone might affect both the genomic and nongenomic actions of erectile function.

Some studies demonstrated that testosterone also impacts the structure of the penis. One group showed that castrated rats show smooth muscle loss and fibrosis [29], and another group reported that castration increases the collagen content of the internal pudendal arteries and decreases α-actin expression [30]. These testosterone effects suggest that testosterone deprivation results in programmed trabecular smooth muscle cell death (apoptosis) and increased development of extracellular matrix [22]. Traish et al. also proposed that testosterone deprivation is associated with the accumulation of fat-containing cells (fibroblasts or preadipocyte-like cells), especially in the subtunical region of the corpus cavernosum, contributing to impaired veno-occlusion [31]. Interestingly, Wang et al. showed that castration attenuates erectile function and induces corporeal fibrosis by inhibiting autophagy and promoting apoptosis of the corpus cavernosum smooth muscle cells in rats [32]. Their study has limitations, but they highlighted the important role of androgens in maintaining the structural integrity and functioning of the corpus cavernosum. This resulted from androgens mediating

the counter-regulation of autophagy and apoptosis through regulation of the BECN 1-Bcl-2 (key dual regulators of autophagy and apoptosis) interaction [33, 34].

4. Testosterone and neurogenic factors

ED has relationships between not only cardiovascular diseases but also neurological diseases. Yang et al. found the hazard risk for Alzheimer's disease and non-Alzheimer dementia to be greater in patients with ED [35]. They also found that log-rank test revealed that patients with ED had significantly higher cumulative incidence rates of dementia than those without. Yang et al. found the incidence density rate of Parkinson's disease (PD) was higher in the ED cohort than in the non-ED cohort [36]. Balsamo et al. reported that men with multiple sclerosis had high risk of ED [37]. Interestingly, testosterone deficiency is often observed in these neurological disease patients relative to age-matched controls [38–40].

In basic study, there are some reports on the relationship between testosterone deficiency and neurogenic factors. Baba et al. reported the mean number of NOS-containing nerve fibers in the corpora cavernosa and in both dorsal nerves of castrated rats [41]. Others also showed that castration decreased nNOS protein expression in the corpus cavernosum [32]. However, reports regarding nNOS responses differ significantly; some studies show increased activity but no change in protein expression [42] in rats, whereas others report no effects in rabbits [43]. Thus, more research into the relationship between nNOS and testosterone is required.

On the other hand, Suzuki et al. measured the ICP during electrical stimulation of the preoptic area and cavernous nerve in castrated male rats with and without testosterone replacement [44]. They showed the actions of testosterone and its metabolites on both the central and peripheral neural pathways are crucial for maintaining and restoring erectile capacity. Syme et al. reported that castration resulted in a decreased erectile response to electrostimulation following nerve grafting due to decreased graft neuronal nitric oxide synthase-positive axonal regeneration [45]. Armagan et al. indicated that testosterone had a neuroprotective role in the nerve fibers of the dorsal nerve and testosterone deficiency led to different forms of nerve degeneration resulting in anatomic alterations [46]. Baba et al. also reported that castration decreased the number of nicotinamide adenine dinucleotide phosphate diaphorase-staining nerve fibers not only in corpus cavernosum but also in dorsal nerve [47]. These results indicate that testosterone deficiency would cause neurogenic dysfunction of erectile tissues; however, future study needs to unravel the mechanism of testosterone action to the nerve systems.

5. Testosterone and metabolic syndrome

Obesity has become a major public health issue that is associated with increased mortality primarily due to increased risks of cardiovascular disease and type 2 diabetes mellitus

(T2DM) [1, 48, 49]. Obesity is also considered a strong risk factor for ED [4, 5]. In men, visceral adipose tissue causes arteriosclerosis and vessel endothelial dysfunction [12]. Therefore, men with T2DM have a high incidence of ED [5, 48, 50, 51].

In recent years, epidemiologic studies have suggested that obesity is also associated with multiple alterations in the gonadal endocrine system, including low testosterone levels [1, 48, 52, 53]. Low testosterone levels have also been reported in animals with T2DM, including two seminal research papers that reported testosterone replacement therapy (TRT) in such animal models [54, 55]. Davis et al. administered TRT to obese Zucker rats, resulting in improved cholesterol parameters and insulin sensitivity [54]. On the other hand, others administered TRT to rabbits with high-fat diet-associated hypogonadotropic hypogonadism [55]. TRT partially ameliorated the animals' blood glucose levels and improved CC sensitivity to acetylcholine and eNOS.

We also reported that T2DM increased inflammatory biomarker (inducible NO synthase, interleukin-6, and tumor necrosis factor alpha) mRNA expression levels in the CC, but TRT decreased them [56]. Ota et al. reported an in vitro study that demonstrated testosterone prevented inflammation caused by hydrogen peroxide in blood vessel cells by upregulating the sirtuin-1 (Sirt1)/eNOS pathway [57, 58]. In one of our studies, testosterone administration upregulated Sirt1 and eNOS mRNA transcription, possibly preventing CC inflammation in T2DM rats (**Figures 1** and **2**). Interestingly, serum asymmetric dimethylarginine (ADMA) levels were also increased in T2DM rats, and rats receiving TRT were observed to have decreased ADMA levels. ADMA is an endogenous arginine compound that rises in individuals demonstrating some disease states [59]; in particular, several reports have suggested a potential relationship between ADMA levels and ED [60, 61]. ADMA has NOS inhibitory activity, and the elevation of ADMA levels contributes to decreased NO bioactivity and decreased endothelial functioning of vessel tissues. Zhang

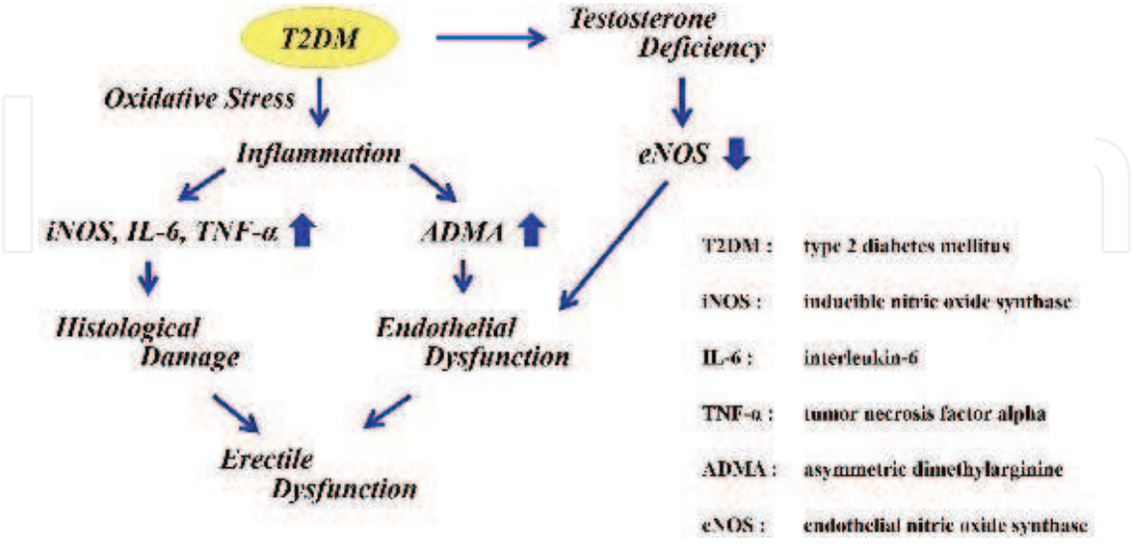

Figure 1. The mechanism of erectile dysfunction caused by T2DM.

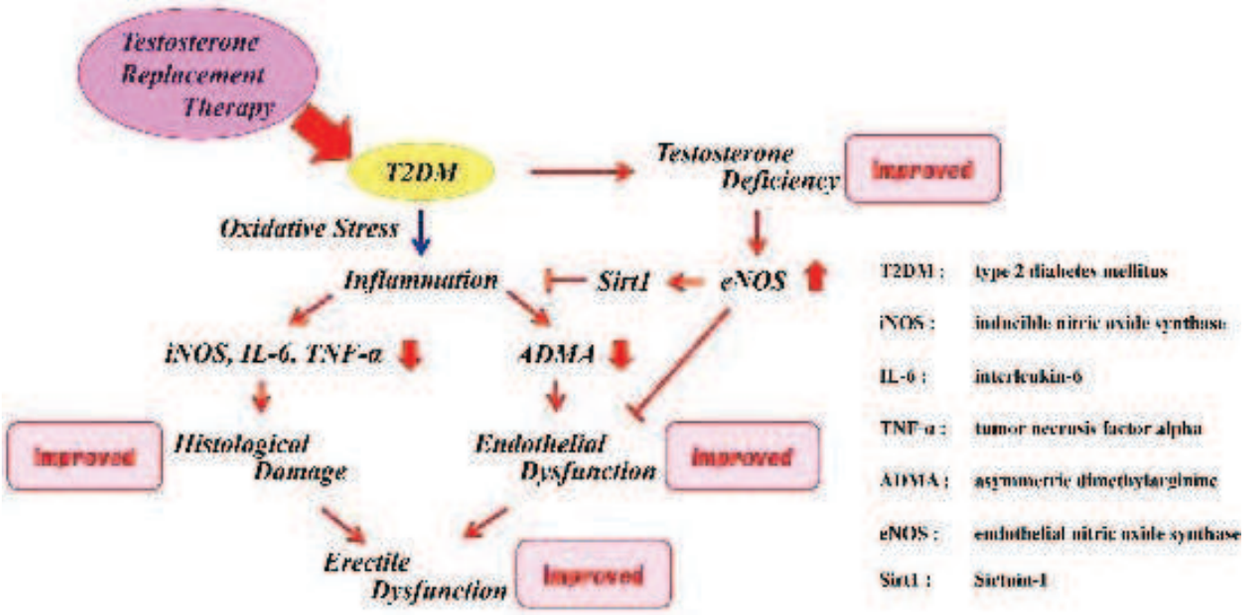

Figure 2. The mechanism of ART for T2DM.

et al. also reported that testosterone treatment improved nNOS activity using streptozotocin-induced diabetic rat [62].

6. TRT limitations

TRT is widely used to effectively treat patients with testosterone deficiencies. It has also been applied to animal models for investigating the mechanisms of testosterone action. However, Burns-Cox et al. pointed out that testosterone (testosterone enanthate) injections cause extremely high levels of testosterone after a few days [63]. Similarly, we injected testosterone enanthate into rats, and the animals demonstrated serum testosterone level increases that rose in a dose-dependent manner (**Figure 3**).

Amano et al. reported that the therapeutic administration of testosterone ointment to patients with LOH successfully kept testosterone at normal levels [64]. We administered low-dose testosterone (similar to applying testosterone ointment) to rats, as previous report [65], 4 weeks after castration. Interestingly, this TRT did not improve erectile functioning over the first 4 weeks of administration. However, after 8 weeks of TRT, partial ED improvements were observed (**Figure 4**). Baba et al. reported that delayed TRT improved ED, in rats, for 4 weeks [41]. However, they used high-dose testosterone administrations and the testosterone levels were ≥10 times normal. These results suggest that low-dose testosterone treatments may require longer treatment periods to overcome testosterone deficiency. Currently, testosterone undecanoate, a drug that is applied over a long period (about 3 months), is widely used in European countries. The medication has been shown to be a safe and effective treatment for patients with testosterone deficiencies [66]. However, some countries have not approved

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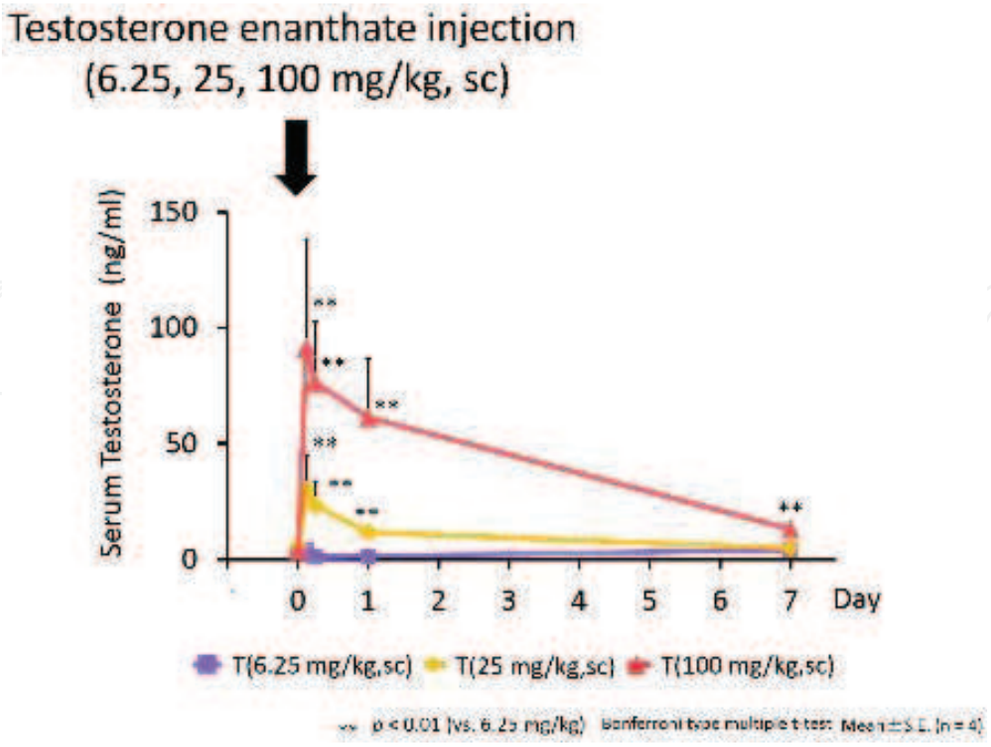

Figure 3. Testosterone levels after testosterone injection in rat.

the medication, and there are no basic science reports addressing its use. These results demonstrate the need to investigate differences between various testosterone administration methods.

Erectile functioning is a complex process, with an underlying mechanism that is affected by several factors [4, 67–69]. Recent studies have suggested that one of these factors may be endogenous estrogen levels [70–77]. For example, Baser et al. suggested that serum estrogen levels are correlated with aging in men and that estrogen may, therefore, play an important role in the expression of the symptoms of aging [70]. Further, Greco et al. reported that tadalafil treatment suppresses estrogen levels in some obese men and improved their erectile function domain scores [71]. Another group reported high estrogen levels in elderly patients with ED and sexual disinterest; therefore, they suggested that pathophysiological estrogen-testosterone imbalance is involved in these conditions among elderly men [72, 73]. In a basic study, Goyal et al. reported that estrogen caused developmental disorders of the rat penis and that it decreased penile testosterone levels [74, 75]. Others reported that estrogen caused pathophysiological changes in the corpus cavernosum and a decline in erectile function in rats [76]. These authors also reported that estrogen induction enhanced corpus cavernosum smooth muscle contraction and decreased smooth muscle relaxation in rabbits [77]. We reported the use of TRT in a rat model of testosterone deficiency induced by estrogen injections. Interestingly, TRT is not an effective ED treatment in the high-estrogen level model [78]. Thus, attention needs to be given not only to the testosterone levels but also to the levels of other hormones.

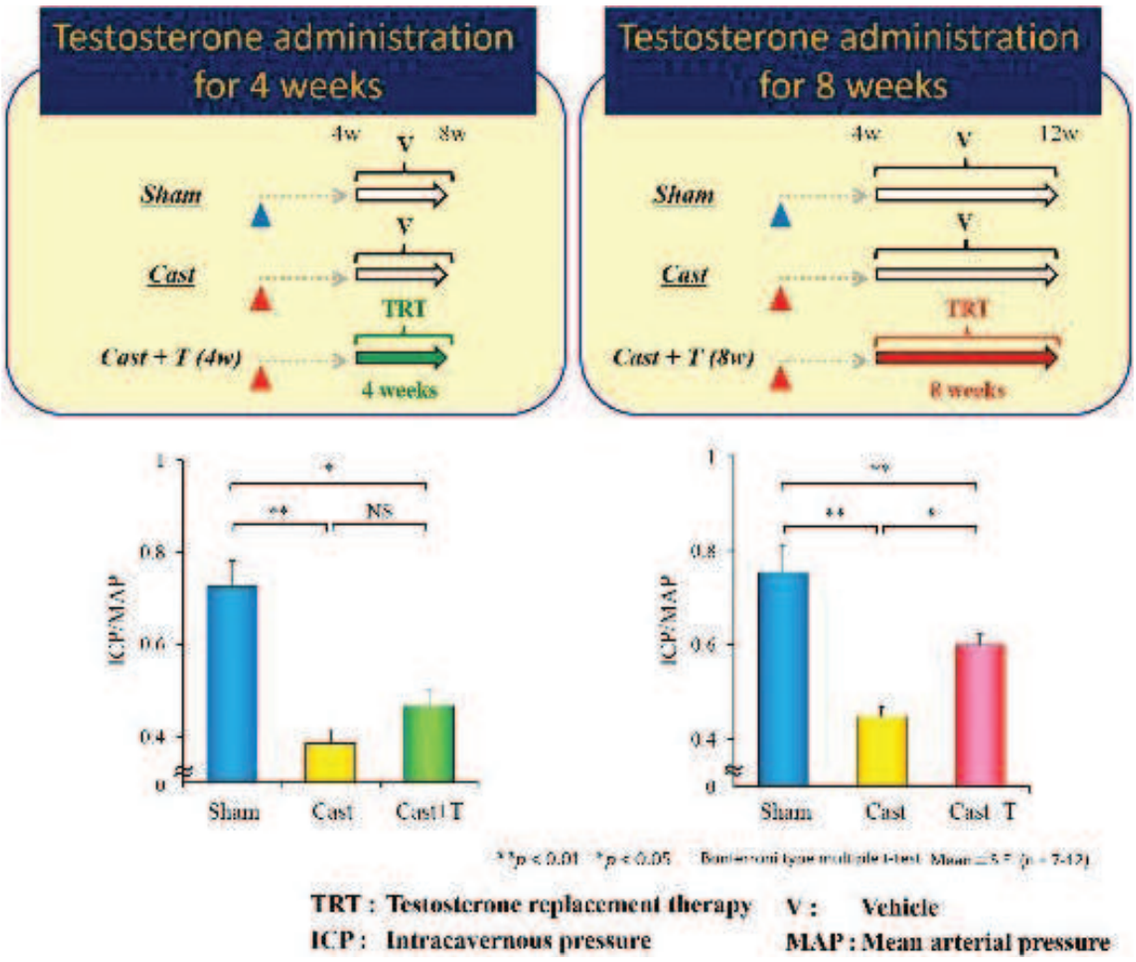

Figure 4. Different effects of TRT periods for delayed treatment.

7. New approaches for testosterone deficiency treatment

Although TRT is an effective treatment for testosterone deficiency, some reports have reported a new treatment approach based on the use of testosterone deficiency models that consider the mechanism of testosterone action on erectile function. PDE-5 inhibitors are the first choice for ED patients, but they are not always effective in patients with testosterone deficiencies [79, 80]. One of the reasons for the lack of efficacy might be the PDE-5 expression changes induced by testosterone. A combination therapy involving both testosterone and PDE-5 inhibitors is one choice, but it is the one that is being vigorously debated, with strong reasons being presented for and against its use. PDE-5 inhibitors are also effective, but regardless of their pharmacokinetics or the regimen used, none has been shown to cure ED [2].

Moody et al. showed that L-arginine administration also improves ED in castrated rats [81]. Similarly, we demonstrated that L-citrulline supplementation improves erectile function and penile structure in castrated rats [82]. L-arginine and L-citrulline are amino acids present in free form in the human body. When L-citrulline is orally administered, it is converted to L-argininosuccinate and, subsequently, to L-arginine by renal argininosuccinate lyase [83]. L-arginine is then converted to NO and L-citrulline by NOS [84]. Orally administered L-arginine is known to be extensively metabolized by autochthonous gut bacteria and by arginases in the

gut and liver [3]. However, oral L-citrulline administrations were shown to avoid such metabolism [85]. Accordingly, oral L-citrulline supplementation was reported to increase L-arginine levels more efficiently than oral L-arginine administration; L-citrulline also increased NO production [86]. In addition, we conducted a similar study using an acute arteriogenic ED model [87]. In that study, oral L-citrulline supplementation improved erectile function and increased NO production, without side effects (e.g., decreased mean arterial pressure) [87].

Fukuhara et al. showed that resveratrol and vardenafil improved erectile responses in rats with streptozotocin-induced diabetes [88]. Recently, Dalaklioglu et al. also reported that resveratrol improved sildenafil-induced corpus cavernosum relaxation in both diabetic and nondiabetic aged rats, probably by potentiating NOS activity [89]. Oral supplementation might improve the vasculogenic condition, considering our previous study, though this is just speculation and needs to be examined.

8. Conclusions

Testosterone levels affect several organs, including the functioning of male erectile tissue. Many studies have described the mechanism of testosterone deficiency effects on erectile function as well as the impact of TRT. Testosterone affects NO production and PDE-5 expression in the corpus cavernosum through molecular pathways. It also preserves smooth muscle contractility by regulating both contraction and relaxation. Further, testosterone maintains the structure of the corpus cavernosum. TRT is widely used to treat patients with testosterone deficiencies; however, the present discussion has also documented some problems associated with this therapeutic approach. Basic research has also identified other potentially effective therapeutic methods for treating testosterone deficiency. Among these, PDE-5 inhibitors, L-citrulline, and resveratrol might be options for treating testosterone deficiency-induced ED. Future research should confirm these findings in more specific experiments that use molecular tools. Such additional research may shed more light on possible treatments for endocrine-mediated ED and its treatment.

Conflict of interest

The authors declare no conflict of interest.

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