Review

Tadalafil and steroid hormones interactions in adipose, bone and prostate tissues: focus on translational perspectives

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Abstract: Tadalafil is a selective phosphodiesterase type-5 (PDE5) inhibitor that is approved for the treatment of men with erectile dysfunction (ED) and/or benign prostate hyperplasia (BPH) associated symptoms. Besides its classical actions on PDE5 within the genitourinary tract, where the specific enzyme expression is maximal, it may exert different systemic effects. This is mainly due to the pleiotropic distribution of PDE5 enzyme throughout human (and animal) body, where it can exert protective effects in different clinical conditions. Recently, it has been demonstrated that tadalafil may display novel actions on androgen receptor (AR) expression and activity, cytochrome P19a1 (Cyp19a1) and estrogen receptor β (ERβ) expression in different in vitro systems, such as adipose, bone and prostate cancer cells where it can act as a selective modulator of steroid hormone production. This may determine novel potential mechanism(s) of control in pathophysiologic pathways. In this review we summarize basic research and translational results applicable to the use of tadalafil in the treatment of different clinical conditions.

Keywords: Tadalafil; prostate cancer; aromatase; adipocytes; bone; androgen receptors; obesity; osteoporosis

1. Introduction

The phosphodiesterase (PDE) enzyme superfamily consists of 11 isoforms (PDE1–PDE11) that modulate the intracellular concentrations of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) by catalyzing their degradation to inactive 5’ nucleotide monophosphates. Interestingly, cAMP and cGMP are ubiquitous second messengers, which regulate multiple functions in virtually all eukaryotic cells. The deregulation of these factors has been involved in several pathophysiological processes and diseases, including cancer [1]. Consequently, PDEs regulate many physiological processes whilst their altered expression, localization and function are implicated in the pathogenesis of several diseases, [2-3] where these enzymes have been shown to be often over expressed and/or aberrantly activated, thus promoting the onset and progression of tumors [4].

In the last decades, numerous pharmaceutical compounds that selectively inhibit the catalytic activities of PDEs have been developed for the treatment of various diseases, but only PDE5 inhibitors (PDE5i) reached clinical application mainly for treating male erectile dysfunction (ED) [5]. PDE5i inhibit cGMP-dependent PDE5 in the penile corporal smooth muscle, and their efficacy is based on the ability to block the cGMP break-down, produced by the nitric oxide (NO)-dependent activation of guanylyl cyclase. Initial studies in transgender males demonstrated that small PDE5 amounts are present in different extra-genital tissues from humans, such as skeletal muscle, heart, lung, and adrenal gland [6]. Successive preclinical and clinical observations, coming from our and other research units, demonstrated that tadalafil modulates the aromatase (ARO) activity and the expression and function of the androgen receptor (AR) in lines of bone, breast, prostate, adipose and...
muscle cells, suggesting a possible direct interaction with steroid hormones [3,7-9]. Thus, the aim of this review will be to summarize the putative effect of tadalafl on ARO activity and on steroid hormone receptor(s) expression by using both in vitro and in vivo different cellular models and to translate them into possible clinical applications.

2. PDE5i and adipocytes

Controversial data confirming a role for PDE5 in adipocyte biology in vitro have been reported [1]. Serendipity, a clinical observation coming from our pilot study suggested that the administration of tadalafl in men with ED was associated with an increase of serum testosterone/estriadiol (T/E2) ratio, mainly due to significant reduction of E2 levels [3]. In that pilot study, where both lean and obese subjects were investigated, no clear-cut explanation for the observed serum E2 decrease was found as to why this effect might have occurred; even if a possible variation of the ARO activity was hypothesized, in that moment any potential effect of PDE5i on ARO were unknown [7]. On the basis of this clinical data we subsequently found for the first time that PDE5 mRNA is present in human adipocytes and that selective PDE5 inhibition significantly stimulated ARO mRNA expression in mature adipocytes in vitro, upon short-time exposure with parallel increase in E2 concentrations in the supernatant [2]. E2 is synthesized by cytochrome P450-ARO which converts androgens into estrogens; indeed, changes at the level of estrogen biosynthesis are closely related to modifications in the transcription of ARO, and may play an important role, with regard to cardiovascular and metabolic disease prevention [2]. These findings showed for the first time that acute PDE5i exposure is able to increase ARO mRNA expression which should translate to beneficial clinical effects. In fact, these results, in addition to our previous clinical observations [3], led us to speculate that, at least in theory, a tadalafl-related stimulation of ARO activity could positively modulate the serum T/E2 ratio in vivo during chronic treatment with PDE5i, and this might represent a possible mechanism influencing fat-mass content and hormonal functions [2]. Importantly, in a mouse model of diet-induced insulin-resistance, chronic treatment with the PDE5i sildenafil caused a significant improvement in insulin sensitivity [4]. Moreover, successive studies investigated the expression and activity of PDE5 in different sites of human adipose tissue (i.e. visceral vs. subcutaneous) and also in different metabolic animal conditions, and revealed that tadalafl counteracted high fat diet-associated visceral adipose tissue alterations, by restoring insulin-sensitivity and prompting preadipocytes differentiation towards a metabolically healthy phenotype where brown fat-specific genes (such as uncoupling protein-1; UCP1) are mostly expressed. [5]. Finally, clinical studies using chronic tadalafl administration in men with ED demonstrated that it was able to improve insulin secretion [6] and to reduce visceral fat mass in non-obese men with prostate dysfunctions [7]. In addition, also skeletal muscle it has been demonstrated to be a target tissue for tadalafl, and experimental data support its pharmacological actions on modulating glucose metabolism i.e. through direct control on insulin signaling, on improving sex hormones profile and body composition, and on ARO expression, as well as increasing exercise capacity due to its cardiovascular and vasodilatory effects, as demonstrated by in vitro and in vivo studies [9]. Thus, we can speculate that the stimulation of the NO/cGMP signal transduction system through PDE5 blockade can provide new, effective and reliable ‘target’ for deranged adipose tissue pathways, suggesting potential role in the treatment of some forms of abdominal fat accumulation and mild obesity. (Fig. 1).
3. PDE5i and bone

Over the last two decades many physiological studies have demonstrated a tight association between NO, PDEs and bone cells homeostasis [10]. Preclinical studies showed that mice lacking NOS presented an osteoporotic phenotype, and both preclinical and clinical studies showed that the treatment with NO donor drugs improved bone mineral density and reduced fracture risk [11-14]. Moreover, murine studies showed that a high function of the cGMP-dependent protein kinase G (PKG), which is the downstream target of NO and inactivated by PDEs, determines a high bone mass phenotype [15, 16], suggesting a key role for the NO-cGMP-PKG axis in regulation of bone remodeling, and suggesting that the inhibition of PDEs may represent a protective factor against bone loss. In a recent interesting study, Kim et al demonstrated that the expression of PDE5A was significantly higher in the bone of old mice than that in young ones, as well as the expression of molecular components of the NO-cGMP-PKG axis, and demonstrated that the administration of tadalafil and vardenafil, increased bone mass through central and peripheral actions. In particular, they observed that PDE5i act directly on osteoblasts by modulating the expression of specific genes involved in the osteoblastogenesis (Ogn and Bsp were up-regulated; Bmp2 was suppressed), in a time- or dose-dependent manner, or both [10]. Finally, since PDE5A-positive sympathetic neurons were found to innervate bone [17, 18], Kim et al studied the osteoblast precursors of tadalafil- and vardenafil-treated mice, and demonstrated that both drugs suppressed specific sympathetic neurons-regulated genes involved in osteoblast precursor proliferation (Per1, Per2, Bmal1, Myc, and Ccnd), and exerted a direct anabolic action favoring the mineralization process and new bone formation [10]. Thus, since the 47% of men over 50 years are clinically positive for osteopenia [19], the authors concluded that the use of PDE5i in aging men, to treat ED and/or BPH associated symptoms and low urinary tract symptoms (LUTS), may protect them from bone loss [10]. Moreover, in a rat model of glucocorticoid-induced osteoporosis, markers of oxidative stress and bone atrophy were significantly reduced by treatment with the PDE5i, zaprinast and avanafil [20], while the PDE5 inhibition, with vardenafil, udenafil, and tadalafil, increased bone angiogenesis and bone formation rate, as well as improved oxidative stress markers and resorption markers, in osteoporotic ovariectomized rats [21].
observations were found by Pal et al in a model of mouse calvarial osteoblasts treated with sildenafil and vardenafil, which increased surface referent bone formation, serum bone formation marker P1NP, expression of vascular endothelial growth factor and its receptor 2 in bones and osteoblasts, and increased skeletal vascularity [22]. Finally, we demonstrated that human osteoblasts (SAOS-2) express significant levels of both PDE5 mRNA and protein, and that their exposure to increasing concentrations of tadalafil [10(-8)-10(-7) M] decreased PDE5 mRNA and protein expression. Also, in this cellular model, we demonstrated that tadalafil inhibited ARO mRNA and protein expression leading to an increase in T levels in the supernatants, and that interestingly, tadalafil increased total AR mRNA and protein expression and decreased ERα, with an increased ratio of AR/ER, suggesting preferential androgenic vs estrogenic pathway activation. [23]. These results are consistent and confirm that tadalafil decreases ARO expression and increases AR protein expression in human SAOS-2 cells, strongly suggesting a new control of steroid hormones pathway by PDE5i, representing the first evidence of translational actions of PDE5i on AR, which leads to hypothesize a growing relevance of this compound in men with ED and prostate diseases long-term treated with tadalafil for sexual rehabilitation [23]. Unfortunately, no targeted results from clinical studies are available at present. Certainly, to confirm the hypothesis coming from preclinical observations, ad hoc designed clinical studies having as primary endpoint the effects of PDE5i on bone metabolism and skeletal preservation (Fig. 1), in aging male, are deemed necessary.

4. PDE5i and prostate

PDE5i are largely used as daily treatment for BPH-related LUTS where they reduce spontaneous contractility of the glands, thereby reducing the muscle tone of the genitourinary tract [8]. The reported up-regulation of PDE5 in hyperplastic human prostate could provide a rationale for the high efficacy of PDE5i in treating patients with LUTS/BPH with/without ED [27]. By contrast, a specific inhibitory growth pattern on prostate tissue has not been clearly documented. Immunohistochemical studies have shown PDE5 immunolocalization mainly in the fibromuscular stroma and vascular (endothelial and stem) cells in the rat and human prostate from BPH subjects [25], as well as in glandular and subglandular areas of human prostate cancer (Pca) patients [26].

PCA is a leading cause of death in adult male and often castration-resistant prostate cancer (CRPC) has a lower therapeutic response to conventional chemotherapy [24], and the expression of PDE5 and cGMP-signaling pathway in normal and cancerous prostate tissues and their possible involvement in carcinogenesis still remain controversial. Although PDE5i are largely used after oncological curative treatments for Pca, PDE5 immunolocalization studies in prostate adenocarcinomas have not been exhaustively reported in the literature. In order to properly localize PDE5 expression, Bisegna et al. [28] recently found PDE5 overexpression in the stromal compartment of hyperplastic prostate samples. Interestingly, their immunohistochemical study showed a 22% of PCA samples expressing PDE5 in the epithelial compartment compared to normal (8%) or hyperplastic samples (11%), and that such positivity was not correlated with the Gleason grading system. Moreover, recent data suggest that sildenafil and vardenafil may induce PDE5-independent apoptotic sensitization to doxorubicin (or other topoisomerase II inhibitors), thus suggesting a combinatorial treatment as an important strategy for anti-CRPC development [29].

This prompted us to investigate the effects of tadalafil on the expression of AR and Cyp19a1, and its potential impact in modulating the antiproliferative activity of androgen deprivation therapy (ADT) in human PCA androgen-sensitive human PCA cell line (LnCAP) cells. We demonstrated for the first time that tadalafil can modulate AR expression in prostate cancer cells in vitro. We also showed that tadalafil induces the stabilization and reduces the degradation of AR, that is more efficiently accumulated within the nucleus [30]. Interestingly, this effect could lead to a potential anti-cancer action of tadalafil by enhancing the therapeutic effect of ADT. In this PCA cell-line, the acute exposure of LNCaP to tadalafil did not affect cell viability, as well as proliferation rate. Tadalafil up-regulates AR protein expression and transcriptional activity, without affecting neither
metabolism nor proliferation of PCa cells [30]. AR is the classical target for PCa prevention and treatment, but more recently estrogens and their receptors have also been implicated in both development and tumor progression. Increasing evidence demonstrate that local estrogen signaling mechanisms are required for prostate carcinogenesis and tumor progression [31]. Cyp19a1 (ARO) converts androgens into estrogens, and the role of estrogens in the pathophysiology of PCa is not well established. Bonkhoff confirmed the hypothesis according to which estrogens may play a major role in the regulation of prostate growth in men [32]. In detail, estrogen ERβ is the most prevalent ER in the human prostate, while the estrogen receptor alpha (ERα) is restricted to basal cells of the prostatic epithelium and stromal cells. In high grade prostatic intraepithelial neoplasia, the ERα might be upregulated while a partial loss of the ERβ might occur, suggesting a potential action as tumor suppressor. The ERβ is generally retained in hormone naïve and metastatic PCa, but it is partially lost in castration resistant disease [33]. Studies conducted in hypogonadal ARKO mouse models, when exposed to E2, have demonstrated a pivotal role of estrogen in the proliferative response of the prostatic stroma and epithelium [34]. Finally, induction of prostate carcinoma requires the combined actions of both T plus E2, since both androgens and estrogens have the potential to initiate changes in the prostate independently, but they cannot individually produce malignancy [35]. Moreover, in PCa cell lines, the AR antagonist bicalutamide (BCT) increased ARO expression and ERβ transcriptional activity; indeed, in CRPC ARO expression was significantly increased in tumor samples. Our study carried on with LnCAP cells, demonstrated for the first time that chronic exposure (48hrs) to BCT produced a significant increase in ARO mRNA, that was reverted by co-treatment with tadalafil [30]. The inhibition of PDE5 has been shown to induce anticancer effects [36] both in pre-clinical [37, 38] and clinical experiences [39]. Indeed, anastrozole and selective aromatase inhibitors (AIs) had been proposed for the treatment of men with advanced prostate cancer, but nowadays, results are still inconsistent [40]. Attia and Ederveen demonstrated that high expression of ERβ in PCa cells increases cell apoptosis and decreases cell proliferation exerting a potential interesting pharmacological role in neoplastic lesions [41]. We herein speculate that the local increase in estrogen levels might activate ERβ intracellular pathway and that chronic exposure to BCT may induce loss of ERβ due to induction of Cyp19a1. Tadalafil potentiated the antiproliferative activity of BCT in LnCAP cells [30] and co-treatment with tadalafil was able to block these effects, thus leading to hypothesize the maintenance of androgen responsiveness to anti-androgen therapy. (Fig. 1)

5. Tadalafil and translational perspectives

In the last decades, numerous pharmaceutical compounds that selectively inhibit the catalytic activities of PDEs have been developed for the treatment of various diseases, with PDE5i largely used for treating andrological disorders, such as ED, BPH associated symptoms and LUTS. It is actually known that the PDE5 enzyme is widely distributed in human tissues, including fat, bone and genitourinary tract, and that it is involved in the pathophysiological process of numerous diseases, including cancers. It has also been demonstrated that PDE5i can exert protective effects in different clinical conditions, apart from ED, such as myocardial infarction, endothelial dysfunction, platelet aggregation, insulin resistance and, finally on skeletal muscle functions. However, to date, the molecular mechanisms underlining the interactions between tadalafil, steroid hormones and skeletal muscle metabolism and differentiation are still not clear, as well as the mechanisms by which PDE5i might positively influences hormone metabolism and physical activity. Since these considerations, in this review we have summarized basic research and translational results applicable to the use of tadalafil in the prevention and/or treatment of obesity, bone loss and prostate cancer, in addition to the conventional therapies (Fig. 1).

Adipocytes

Several preclinical studies investigated the expression and activity of PDE5 in visceral and subcutaneous adipose tissue and revealed that tadalafil counteracted high fat
diet-associated visceral adipose tissue alterations, by restoring insulin-sensitivity, insulin secretion, and favoring brown fat-specific genes expression in animal models, as well significantly stimulated ARO mRNA expression in mature adipocyte \textit{in vitro}. On the other hand, several clinical studies showed as tadalafil is able to improve insulin secretion and reduce visceral fat mass, both in obese and non-obese men, suggesting that the NO/cGMP signal transduction system can represent a new pharmacological target for dysmetabolic adipose tissue, and that its stimulation through PDE5 blockade may have a potential role in the treatment of abdominal fat accumulation and obesity.

\textit{Osteoblasts}

Preclinical studies regarding tadalafil and bone tissue interaction showed that PDE5 inhibition favors osteoblast proliferation and differentiation, as well as mineralization and bone formation rate, probably through a direct control of steroid hormones pathway, as suggested from the observation that tadalafil decreases ARO expression and increases AR protein expression in human SAOS-2 cells \textit{in vitro}, speculating a potential protective effect of tadalafil, on bone loss.

\textit{Prostate cancer cells}

\textit{In vitro} and \textit{in vivo} studies showed that PCa cells overexpress PDE5, and that PDE5i, which are largely used after oncological curative treatments for PCa, exert a PDE5-independent apoptotic sensitization to doxorubicin or other topoisoasmerase II inhibitors. This observation in addition to the recent demonstration that tadalafil modulates ARO and AR expression in a model of LNCaP, improving the local androgens/estrogens imbalance, suggests that tadalafil may counteract tumor progression and enhance the therapeutic effect of ADT, representing an important strategy for anti-CRPC development.

6. Conclusions

In this review we have summarized the putative effect of tadalafil on ARO activity and on steroid hormone receptor(s) expression by analyzing both \textit{in vitro} and \textit{in vivo} different cellular models to translate them into possible clinical applications. We conclude that tadalafil can operate as selective modulator of ARO expression and functions, depending on the tissue and organ involved; it has stimulatory effects on adipocytes and PCa cell lines, whereas it has inhibitory actions on bone tissues. This dimorphism needs further to be investigated based upon sex differences in clinical context. Finally, monomorphism of action on facilitating translocation of AR into the nucleus independently from the system studied, opens new strategies for the chronic use of tadalafil when indirect androgen action is needed in clinical context, i.e. CRPC and LOH.

\textbf{Abbreviations:}

- AR: Androgen Receptor
- PCa: Prostate cancer
- Cyp19a1: Aromatase
- LnCAP: Androgen-sensitive human PCa cell line
- BCT: Bicalutamide
- T: Testosterone
- ADT: Androgen deprivation therapy
- CRPC: Castration-resistant prostate cancer
- cAMP: Cyclic adenosine monophosphate
- cGMP: Cyclic guanosine monophosphate
- PDE: Phosphodiesterase
- PDE5i: PDE5 inhibitor
- ED: Erectile dysfunction
- LUTS: Lower urinary tract symptoms
- AIs: Aromatase inhibitors
References


41. Attia, D.M.A.; Ederveen, A.G.H. Opposing roles of ERα and ERβ in the genesis and progression of adenocarcinoma in the rat ventral prostate. Prostate 2012, 72, 1022. doi: 10.1002/pros.21507