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REVIEW

Oral phosphodiesterase-5 inhibitors and sperm functions

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This review aims to elucidate the possible effects of phosphodiesterase-5 (PDE5) inhibitors on sperm functions. PDEs hydrolyze cyclic nucleotides, and together with adenylyl and guanylyl cyclase, which catalyze the formation of cAMP and cGMP, regulate the levels of these second messengers in cells. cGMP-specific PDE5 is one of the PDEs that have been intensively studied because of its fundamental pharmacological relevance, as oral PDE5 inhibitors are used successfully in treating erectile dysfunction. In addition, they have shown diverse beneficial actions in different disease categories. Specific relevance of the cGMP system in reproductive functions has been recently proposed. Its use was shown to be devoid of effects on semen volume, concentration, sperm membrane integrity or sperm penetration assay. Most available studies demonstrated a significant increase in sperm motility and viability both *in vivo* and *in vitro*, which seems to be enhanced at low doses and reduced at high concentrations. Also, these molecules showed a role in capacitation and a debated one concerning acrosome reaction. However, due to the relative short period since the launching of oral PDE5 inhibitors, more investigations should be carried out in wider scales to assess their effect(s) on variant sperm function that could be beneficial as potential therapeutic approaches.
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Introduction

Mammalian phosphodiesterases (PDEs) are divided into 11 families: some specifically hydrolyze cAMP (PDEs 4, 7 and 8) or cGMP (PDEs 5, 6 and 9), whereas others hydrolyze both nucleotides (PDEs 1, 2, 3, 10 and 11).[1] All PDEs contain a conserved catalytic domain of ~270 amino acids at its carboxy terminus containing two Zn^{2+}-binding motifs that bind Zn^{2+} and Mg^{2}; binding of Zn^{2+} activates its enzymatic activity.[2] Regulatory domains and motifs that vary among the PDE families are often found near the amino terminus.[3] PDE5 contains a catalytic domain that hydrolyzes cGMP and a regulatory domain that contains two cGMP-binding PDEs, *Anabaena* adenylyl cyclases, *Escherichia coli* FhlAs (GAFs) (A and B) and a phosphorylation site for cyclic nucleotide-dependent protein kinases. Binding of cGMP to GAF-A increases cyclic nucleotide-dependent protein kinase phosphorylation and improves the catalytic site's affinity for cGMP or inhibitors. GAF-B contributes to dimerization, inhibition of cGMP binding to GAF-A and sequestration of the phosphorylation site.[4] The domain organization of PDE5 is similar to PDEs 2, 6, 10 and 11.[5]

Oral PDE5 inhibitors were first presented by launching sildenafil citrate in March 1998, followed by vardenafil and tadalafil. These were prescribed worldwide and have been successful in erectile dysfunction (ED) patients with underlying diabetes, cardiovascular disease, minor depression, spinal cord injury and multiple sclerosis. Promising results have also been raised for ED patients with treated prostate cancer, renal failure, Parkinson's disease, spina bifida and multiple organ transplant recipients.[6] In addition, they were used in diverse disorders: endothelial dysfunction, pulmonary hypertension, Raynaud's phenomena, esophageal motility, prostatic hyperplasia, and so on.[7–11]

In the testis, cGMP signal transduction pathways are involved in a variety of local functions based on autocrine or paracrine effects suggested to influence sperm motility, development of germ cells, relaxation of peritubular lamina propria, testosterone synthesis in Leydig cells and dilatation of testicular

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vessels. As a whole, it is suggested that cGMP-mediated processes influence both the potentia coeundi within the penis and the potentia generandi at various levels within the testis.[12] Data on PDE expression in testis are restricted predominantly to cAMP-hydrolyzing PDEs, such as PDE1C, PDE4A, PDE4C, PDE7B and PDE8A, and also provide useful information about PDE localization in male germ cells and spermatozoa.[13] Transcripts of PDE10 were found in the human testis,[14] cGMP-hydrolyzing PDE5 was localized in peritubular myoid cells of rats[15] and regulation of epididymal duct contractility by PDE3 has been suggested.[16]

PDE11, the latest isoform of the PDE family that raised interest recently,[17] contains one gene, PDE11A, with four splice variants (PDE11A1–4). It was suggested that PDE11A3 is found in the testis, whereas PDE11A4 is found in the prostate. Weeks *et al.*[18] showed that PDE11A4 displays *K*(m) values of 0.97 μmol for cGMP and 2.4 μmol for cAMP, and maximal velocities 4- to 10-fold higher for cAMP than for cGMP. Given the homology between PDE11 and PDE5, the biochemical potencies of tadalafil, vardenafil and sildenafil were compared for PDE11A4 and PDE5A1. PDE5A1/PDE11A4 selectivity was 40-, 9300- and 1000-fold, respectively, suggesting that none of these compounds crossreact with PDE11A4.

The influence of oral PDE5 inhibitors on sperm functions was lately proposed.[19] Several isotypes of PDEs, such as PDE1 and PDE4, have been found in human spermatozoa.[20] Lefièvre *et al.*[21] showed that human sperm PDEs are associated with the plasma membrane and the particulate fraction with more affinity for cAMP than for cGMP. It is indicated that PDE1A is localized in the equatorial segment of the sperm head as well as in the mid and principal pieces of the flagellum, and that PDE3A is localized in the post-acrosomal segment of the sperm head. In human ejaculated spermatozoa, Richter *et al.*[22] obtained strong specific bands for the PCR products of PDE1B, PDE3B, PDE4A, PDE4B and PDE8, whereas amplification products of PDE1A/C, -2, -3A, -4C and -5 were observed as weak signals. Cheng and Boettcher[23] emphasized that the rate of hydrolysis of cyclic nucleotides in spermatozoa is much faster (9- to 600-fold) than the rate of cyclic nucleotide formation, suggesting that the PDEs have a dominant role in the control of the concentration of cyclic nucleotides in spermatozoa.

Semen parameters

Various studies were carried out to assess the influence of oral PDE5 inhibitors on semen parameters, some of which were double-blind, randomized, crossover, placebo-controlled ones. Aversa *et al.*[24] showed that after audiovisual sexual stimulation, 100 mg sildenafil caused no changes in seminal parameters compared with placebo in 20 healthy volunteers in a double-blind, randomized, placebo-controlled, crossover, two-period study. Purvis *et al.*[25] randomized 17 healthy males to a single 100 mg dose of sildenafil or a placebo followed for 4 h. The amount of sildenafil, or its metabolite UK-103320, in the ejaculate was found to be $<2\times10^{-4}$% of the dose at 1.5 h. Sildenafil showed nonsignificant effect on sperm motility, count, density, abnormal sperm forms, living sperm percentage, ejaculate volume or viscosity. In an open-label pilot study, Jannini *et al.*[26] investigated the effect of 50 mg orally administered sildenafil in a group of sexually healthy men who planned sexual intercourse to perform a post-coital test (one or two tests). They did not find any effect of sildenafil administration on sperm motility; sperm concentration; total number of ejaculated spermatozoa; or percentage of nonlinear, progressive, motile spermatozoa.

du Plessis *et al.*[27] subjected 20 healthy volunteers to 50 mg of sildenafil or placebo orally and added 8-bromo-cGMP (20 μmol) to semen samples collected after 1 h in a prospective double-blind, placebo-controlled, crossover, two-period study. Neither sildenafil nor 8-bromo-cGMP treatments had any effect on both macroscopic and microscopic seminal parameters, except a significant increase in the population of rapid cells. Hellstrom *et al.*[28] assessed the effects of placebo vs 10 or 20 mg tadalafil daily for 6 months on 421 males. Tadalafil demonstrated no adverse effects on sperm concentration, sperm count/ejaculate, sperm motility, normal morphology or serum reproductive hormones (testosterone, luteinizing hormone and follicle-stimulating hormone) tested at baseline, 3 and 6 months. Francis[17] showed that tadalafil administered to healthy volunteers did not alter the semen analysis parameters or blood hormonal parameters.

Ali and Rakkah[29] studied whether chronic sildenafil treatment modifies seminal parameters in 50 insulin-dependent and 50 non-insulin-dependent diabetic patients with and without neuropathy. In both groups, a significant decrease in total sperm output and sperm concentration was elicited. Sperm motility and semen volume were increased, whereas sperm morphology and quality of sperm motility remained unaffected. Non-neuropathic diabetic patients showed nonsignificant difference in all parameters compared with either untreated groups or controls. Comparison between neuropathic and non-neuropathic diabetic patients showed nonsignificant difference. A chronic neurophysiological effect of sildenafil was suggested on male fertility profile exclusively in diabetic neuropathic condition with improved testicular function.

Grammeniatis *et al.*[30] noted that semen samples from infertile men treated with 10 mg vardenafil daily for at least 45 days presented a significantly

larger total number of spermatozoa, quantitative sperm motility, qualitative sperm motility and percentage of morphologically normal spermatozoa compared with their samples collected before vardenafil administration. It is suggested that vardenafil stimulated the prostatic secretory function, thereby increasing the quantitative and qualitative motility of spermatozoa. Jarvi *et al.*[31] demonstrated that vardenafil (20 mg) and sildenafil (100 mg) administered daily at their maximum recommended dose for 6 months, compared with placebo, had no adverse effects on sperm concentration, total sperm count per ejaculate, sperm morphology, sperm motility or levels of reproductive hormones.

Assisted reproductive techniques

Some men may have difficulties in producing spermatozoa on demand to produce semen sample before egg collection. Tur-Kaspa *et al.*[32] and Kalsi *et al.*[33] described the use of sildenafil in temporary ED in couples undergoing assisted reproductive techniques. Kaplan *et al.*[34] showed that sildenafil may reverse secondary ejaculatory dysfunction during infertility treatment. Lenzi *et al.*[35] suggested that sildenafil is effective in increasing compliance of male patients and in improving some sperm parameters, above all the number of sperms penetrating the cervical mucus. Jannini *et al.*[26] showed that 50 mg sildenafil effectively reversed stress-induced transitory ED before semen collection for intrauterine artificial insemination or planned intercourse for a post-coital test. Furthermore, sildenafil improved the percent of linear sperm progressive motility and the number of spermatozoa penetrating the cervical mucus.

Sperm motility

Most seminal PDE inhibitor effects were concentrated on sperm motility ever since the documented influence of pentoxifylline and caffeine.[36,37] PDE inhibitors specific to PDE1 and PDE4 were known to alter sperm motility in humans where PDE4 inhibitors enhanced sperm motility over controls.[12] Localization of protein kinase A (PKA) is regulated by A-kinase-anchoring proteins, which control the intracellular distribution of PDE. Bajpai *et al.*[38] suggested that AKAP3 binds both PKA and PDE4A and functions as a scaffolding protein in spermatozoa to regulate local cAMP. Cai *et al.*[39] showed that the expressions of soluble adenylyl cyclase and cAMP were significantly decreased and that of PDE4C was significantly increased in asthenozoospermia compared with controls with no differences in cGMP. Yunes *et al.*[40] showed that pentoxifylline had overcome altered motion characteristics and defective phosphorylation of sperm tail proteins in asthenozoospermic semen samples.

In vivo *effects*

Lefièvre *et al.*[41] postulated that sildenafil triggers human sperm motility via its inhibitory action on PDE activity, other than type 5, with a resultant rise in cAMP levels. They showed that sildenafil (100 μmol) inhibited PDE activity of Percoll-washed spermatozoa when cAMP or cGMP was used as the substrate. Because the IC_{50} of sildenafil obtained for PDE5 was much lower than that obtained with sperm PDE, they suggested that PDE5 represents a small fraction of the whole PDE activity of spermatozoa. Sildenafil caused dose-dependent increases in sperm cAMP with increased tyrosine phosphorylation of two fibrous sheath proteins (p105/81) with increased sperm velocity, amplitude of lateral head displacement and hyperactivation (30–180 min).

Jannini *et al.*[26] observed a significant increase in the linear progressive motility due to sildenafil 50 mg administration post-coital test with positive effects on sperm number and motility in the cervical mucus. du Plessis *et al.*[27] demonstrated various increased kinematical parameters on 20 healthy volunteers after treatment with 50 mg of sildenafil, with a significant increase in the population of rapid cells in semen samples collected 1 h later. Pomara *et al.*[42] observed a significant increase in sperm progressive motility after 50 mg sildenafil dose, compared with baseline, and a significant decrease after 20 mg tadalafil dose evaluated after 1 or 2 h in a prospective randomized, double-blind, crossover study on 18 young infertile men.

It is suggested that the administration of PDE5 inhibitors has a role in the reduction of ejaculation-associated stress, resulting in an ejaculation with higher sexual satisfaction and a subsequent increased number of good quality spermatozoa in the semen. Sofikitis and Miyagawa[43] suggested that higher the sexual stimulation, larger the prostatic secretory function with an overall result of better sperm motility, and larger the vas deferens loading during ejaculation. Thus enhancement of the concentrations of prostatic secretions, providing an environment ideal for sperm motility and transport, in the seminal samples collected after the administration of PDE5 inhibitors may explain the higher sperm motility profiles in semen samples.

In vitro *effect*

Various studies were carried out concerning this point. Andrade *et al.*[44] mixed semen or washed sperm with various doses of sildenafil and analyzed for motility for 30 min. A 200 $\mu g\,ml^{-1}$ dose of sildenafil had no effect on sperm motility, but a 2000 $\mu g\,ml^{-1}$ dose significantly reduced motility by

about 50%, suggested to be due to a decline in pH. Cuadra *et al.*[45] showed that when incubated in different concentrations of sildenafil (0–40 nmol l^{-1}), sperm progressive motility and hyperactivation were stimulated to a greater extent than in controls at 4 h, followed by a decrease with a dose-dependent effect. A study by Glenn *et al.*[46] on 57 male patients showed that the number and velocity of progressively motile sperms were significantly increased between 15–135 min when incubated with sildenafil (0.67 μmol) at 37 °C for 3 h. Burger *et al.*[47] demonstrated that sildenafil incubation (125, 250 and 750 ng ml^{-1}) did not affect sperm motility and viability of washed human spermatozoa compared with Ham's control, whereas pentoxifylline (3 mM) incubation enhanced both significantly.

Mostafa[48] followed-up for 3 h the *in vitro* sperm motility effect of tadalafil solutions (4.0, 1.0, 0.5 mg ml^{-1}) on 70 asthenozoospermic semen specimens. Specimens treated with 4 mg ml^{-1} solution showed a significant decrease compared with controls, and those treated with 1.0 mg ml^{-1} solution showed a significant increase. Also, Mostafa[49] followed the *in vitro* sperm motility effect of sildenafil solutions (4.0, 2.0, 1.0, 0.5 and 0.1 mg ml^{-1}) on 85 asthenozoospermic specimens for 3 h. Sildenafil solution exhibited a concentration-related stimulatory effect on ejaculated sperm motility, being optimal at 1.0 mg ml^{-1}. Mostafa[48,49] speculated that the concentration of either *in vitro* sildenafil or tadalafil plays an important role in the degree of sperm enhancement.

Normal mammalian sperm motility seems to be governed predominantly by the cAMP/PKA pathway and calcium signaling pathway, whereas mechanisms involving heterotrimeric and small G-proteins have also been entailed the regulation of sperm motility.[50] It should be emphasized that cAMP may also act through PKA-independent pathways. Burton *et al.*[51] speculated that cAMP may activate a cyclic-nucleotide-gated ion channel in spermatozoa and/or cAMP-mediated guanine nucleotide exchange factors in testes, providing these ways as alternative pathways for the PKA-mediated regulation of flagellar motility. The dual effect of *in vitro* sildenafil or tadalafil on sperm motility with regard to its concentration in the semen could be explained by one or more of these pathways.

Membrane integrity

On normal donors and infertile men, Burger *et al.*[47] studied the effect of sildenafil on human sperm membrane integrity washed using Percoll (80%) gradient, suspended in Ham's F-10 and incubated with sildenafil (125, 250 and 750 ng ml^{-1}); pentoxifylline (3 mM) was used as a positive control and Ham's F-10 as a reagent control. Neither sildenafil nor pentoxifylline affected membrane integrity (by hypo-osmotic swelling test) as compared with Ham's controls.

Sperm penetration assay

On normal donors and infertile men, Burger *et al.*[47] showed that spermatozoa incubated with sildenafil and pentoxifylline demonstrated nonsignificant change in sperm penetration assay from controls. du Plessis *et al.*[27] demonstrated that oral 50 mg sildenafil and 8-bromo-cGMP treatments on semen collected after 1 h increased sperm–zona-pellucida-binding results concluding that it can be used to enhance sperm binding to the oocyte.

Capacitation

Capacitation is a series of transformations that spermatozoa undergo in the female genital tract in order to bind to zona pellucida, initiate acrosome reaction and fertilize an egg. It is regulated by signal transduction systems involving cAMP as a second messenger acting through the activation of PKA and indirectly regulates protein tyrosine phosphorylation. Evidence was provided for the involvement of PDEs in capacitation, as they modulate cyclic nucleotides by catalyzing their degradation, and PDE inhibitors specific to PDE1 and PDE4 were known to alter capacitation. Fournier *et al.*[52] showed that although cAMP regulation by PDE1 may occur early during capacitation, downstream events appear to prevent full capacitation from occurring prematurely. Baxendale and Fraser[53] indicated that the intracellular location of specific PDEs has important functional significance during capacitation and fertilization.

Lefièvre *et al.*[41] showed that sildenafil causes dose-dependent increases in sperm cAMP and capacitation associated with increased levels of tyrosine phosphorylation of two fibrous sheath proteins (p105/81). They suggested that sildenafil triggers human sperm capacitation via its inhibitory action on PDE activity, other than type 5, with a resultant rise in cAMP. Lefièvre *et al.*[54] showed that PKA activity was higher in capacitating than in non-capacitating spermatozoa, and that intracellular cAMP was decreased but PDE activity remained constant during human sperm capacitation. It was suggested that the net cAMP level is under AC control as PDE activity is constant during sperm capacitation. Moreover, low levels of cAMP are sufficient for capacitation and PKA activation, and/or cAMP in spermatozoa did not reflect the effective intracellular cAMP levels present in specific compartments of these cells.

Acrosome reaction

Interesting studies have indicated that the sperm acrosome reaction rate is greatly influenced by cGMP synthesis.[55] A complex crosstalk phenomenon between the cAMP- and cGMP-generating systems regulating sperm function occurs in human spermatozoa.[56] Spermatogenesis and sperm–egg interaction appear to be positively affected by sperm GC activation, whereas experimental observations indicate that excessive amounts of certain GC activators might exert opposite, antireproductive effects through an increase in oxidative stress and lipid peroxidation on sperm membranes.[57,58]

Inhibition of sperm PDE has been shown to increase cAMP and acrosome reaction. Using type-selective inhibitors, Fisch *et al.*[20] showed that PDE4 inhibitors did not affect acrosome reaction, whereas PDE1 inhibitors selectively stimulated it. This supports the hypothesis that PDE subtypes affect sperm function by regulating separate pools of cAMP, and that PDE inhibitors result in the buildup of intracellular cyclic nucleotides affecting acrosome reaction. Cuadra *et al.*[45] investigated whether sildenafil has an effect on acrosome parameters in spermatozoa washed by two-layer colloid wash and resuspended in modified human tubal fluid with 5% serum albumin incubated in different concentrations of sildenafil followed for 48 h. Sildenafil stimulated sperm acrosome reaction by 50% above controls, suggesting a role in preventing premature acrosome reaction associated with failed fertilization.

Lefièvre *et al.*[41] showed that sildenafil did not trigger the acrosome reaction in capacitated spermatozoa and that induced acrosome reaction was associated with increased cAMP and PKA activity but not PDE activity.[21] It was suggested that the net cAMP level is under AC control, as PDE activity was constant during sperm acrosome reaction. du Plessis *et al.*[20] determined the effect of acute *in vivo* 50 mg sildenafil or placebo on 20 healthy volunteers, and *in vitro* 8-bromo-cGMP was added to semen samples collected 1 h after that. Neither sildenafil nor 8-bromo-cGMP treatments had any effect on acrosome reaction.

Glenn *et al.*[46] determined whether sildenafil influences the acrosome reaction by fluorescein isothiocyanate-labeled peanut agglutinin staining in 57 male patients. Spermatozoa were divided into 90% (best fertilizing potential) and 45% (poorer population) fractions by density centrifugation and incubated with sildenafil (0.67 μmol) for up to 180 min. In both populations, sildenafil caused a significant increase in the proportion of acrosome-reacted sperm (22.1% vs 11.8% in controls) in the good quality fraction as well as (16.6% vs 9.4% in controls) in the poorer quality fraction, concluding that the use of sildenafil citrate may adversely affect male fertility.

It is concluded that the use of oral PDE5 inhibitors is devoid of deleterious effects on semen volume, concentration, sperm membrane integrity or sperm penetration assay. Most studies demonstrated a significant increase in sperm motility and viability both *in vivo* and *in vitro* that seems to be enhanced at low doses and reduced at high concentrations. Also, these molecules showed a role in capacitation and a debated one concerning acrosome reaction. Due to the relative short period since the launching of oral PDE5 inhibitors, more investigations should be carried out in wider scales to assess their precise effect(s) on variant sperm functions.

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