Phosphodiesterase Type 5 Inhibitors, Sport and Doping

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Abstract

Phosphodiesterase type 5 inhibitors (PDE5i) (*e.g.,* sildenafil, tadalafil, vardenafil, and avanafil) are drugs commonly used to treat erectile dysfunction, pulmonary arterial hypertension, and benign prostatic hyperplasia. PDE5i are not prohibited by the World Anti-Doping Agency (WADA) but are alleged to be frequently misused by healthy athletes to improve sporting performance. *In vitro* and *in vivo* studies have reported various effects of PDE5i on cardiovascular, muscular, metabolic, and neuroendocrine systems and the potential, therefore, to enhance performance of healthy athletes during training and competition. This suggests well-controlled research studies to examine the ergogenic effects of PDE5i on performance during activities that simulate real sporting situations are warranted to determine if PDE5i should be included on the prohibited WADA list. In the meantime, there is concern that some otherwise healthy athletes will continue to misuse PDE5i to gain an unfair competitive advantage over their competitors.

Introduction

Phosphodiesterases (PDE) are a family of enzymes (from PDE1 to PDE11) with different selectivity for cyclic nucleotides, sensitivity to inhibitors and activators, physiological roles, and tissue distributions. PDE catalyze the hydrolysis of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) to the corresponding 5-nucleotide monophosphate, modulating their intracellular levels and hence affecting different cell functions in many tissues. Phosphodiesterase type 5 inhibitor (PDE5i) drugs, such as sildenafil, tadalafil, vardenafil, and avanafil, have different pharmacokinetic properties that preferentially inhibit the PDE5 albeit PDE6, PDE9, and PDE11 also are inhibited to a lesser extent (26,31). PDE5i are approved for treating erectile dysfunction (ED), pulmonary arterial hypertension, and benign prostatic hyperplasia. Furthermore, other therapeutic applications have been proposed (*e.g.*, heart failure, cardiomyopathy, stroke, metabolic diseases) because of their cardiovascular and metabolic effects (9,26).

PDE5i work by influencing nitric oxide (NO)-related cardiovascular, endocrine and metabolic pathways. NO impacts cardiovascular hemodynamics, energy metabolism, hormones, and mitochondrial biogenesis through cGMP-dependent and -independent mechanisms (*e.g.*, Ca^{++}, prostaglandins) (13,15,17). PDE5i enhances the cGMP-dependent effects of NO by increasing the intracellular levels of NO-induced cGMP in different tissues. Particularly, PDE5i influences the pathway downstream of NO: (a) NO activates soluble guanylate cyclase producing cGMP, an intracellular transduction mediator of NO; (b) intracellular cGMP is physiologically decreased by the degradative action of intracellular PDE5; (c) PDE5i inhibits the PDE5 action thus increasing cGMP bioavailability; and (d) increased cGMP availability amplifies the cGMP-related pleiotropic effects of NO.

In addition to the widespread therapeutic use of PDE5i for ED, there appears to be widespread abuse for recreational purposes in healthy men (7,25,33). Based on anecdotal reports and "doping control forms" data (36,39), many healthy athletes (not affected by ED) participating in sports requiring endurance and/or competing in hypoxic conditions (*i.e.*, cycling, running, rowing, and so on) misuse PDE5i to improve sporting

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performance, as PDE5i medications are not prohibited by the World Anti-Doping Agency (WADA).

Unfortunately, as is true for other substances already prohibited by WADA, there are no studies documenting a specific performance-enhancing effect of PDE5i at either therapeutic or supratherapeutic doses during real sporting competitions. However, *in vitro* and *in vivo* studies describing the effects of PDE5i related to physical performance enhancement provide a stronger case for prohibiting PDE5i than some of the currently prohibited drugs. PDE5i have great potential to enhance exercise capacity due to their cardiovascular and vasodilatory effects increasing oxygen transport to the exercising muscles, in addition to the numerous neuroendocrine, muscular, and metabolic effects summarized below.

PDE5i and Exercise Performance in Healthy Individuals

The capacity of PDE5i to enhance exercise tolerance in humans affected by cardiovascular diseases is well-documented (9,23,28,47). However, there is a paucity of information on the effects of different PDE5i in either normoxia or hypoxia on maximum aerobic ($\dot{V}O_{2max}$) and anaerobic capacity in healthy individuals of different ages (*i.e.*, older humans might have a reduced PDE5i-related sympatholytic effect) (42). This includes athletes involved in different sports.

In normoxia, a single dose of tadalafil (20 mg) in a healthy athlete did not substantially influence performance indicators, such as the ventilatory threshold, $\dot{V}O_{2max}$, exercise tolerance, or the cardiopulmonary response, during a maximal standardized exercise test (12). Moreover, the same dose of tadalafil did not influence the mean and peak power output values during a 30-s Wingate anaerobic power test, but did significantly decrease the time to peak power and increased blood lactate concentrations during recovery. The observed association between a reduced time to peak power and higher blood lactate concentration (24) could be related to a possible effect of PDE5i in stimulating anaerobic glycolysis (46), and this may benefit performance in sports requiring a rapid attainment of maximum power output.

Sildenafil is one of the first drugs to show increased exercise capacity in healthy individuals during severe hypoxia both at sea level and at high altitude at therapeutic doses. In healthy cyclists and triathletes at simulated high altitude, sildenafil (50 mg–100 mg) increased stroke volume, cardiac output, and arterial oxygen saturation (SaO_2) during set-work-rate exercise and significantly lowered 6-km time-trial time by 15% in a double-blind study versus placebo (27). In the same study, there were subjects who appeared to be sildenafil responders and nonresponders with improved time-trial performances of 39% ($P < 0.05$) and 1.0%, respectively (27). If confirmed, these individual differences in PDE5i response could explain the observed discrepancies in the studies evaluating PDE5i-related effects. In healthy mountaineers and trekkers breathing a hypoxic gas mixture with 10% fraction of inspired oxygen at low altitude (Giessen, 155-304 m), sildenafil (50 mg) significantly increased SaO_2 during exercise and reduced systolic pulmonary artery pressure at rest and during exercise (20). Sildenafil also significantly increased maximum workload and maximum cardiac output compared with placebo (20). At the Mount Everest base camp (elevation 5380 m), sildenafil (50 mg) reduced systolic pulmonary artery pressure (at rest and during exercise) and increased maximum workload and cardiac output (20). Other studies have been unable to replicate these effects on performance. During acute exposure to hypobaric hypoxia (elevation, 4000 m) at rest and during maximal and submaximal (60% $\dot{V}O_{2max}$) exercise, sildenafil (100 mg) did not impact performance in healthy men or women (53). Moreover, no effects of sildenafil (50 mg) were observed on cardiovascular hemodynamics, arterial oxygen saturation, peak exercise capacity, and 15 or 6 km time-trial performance in endurance-trained subjects of either sex at simulated moderate altitude (~2100 and 3900 m) (30,34). These negative findings may be due to methodological factors, such as altitude (*i.e.*, PDE5i may have greater effects above 4000 m), duration of exposure to acute or chronic hypoxia, possible variability of individual response to PDE5i, or individual hormonal status (*i.e.*, in men affected by ED, serum testosterone levels influence the responsiveness to PDE5i) (5,32,49).

Alveolar hypoxia, either natural (*e.g.*, high altitude) or artificial (*e.g.*, hypoxic tents), may negatively influence exercise capacity, because hypoxia reduces alveolar partial oxygen pressure (pPO_2) and SaO_2, increases pulmonary arterial pressure and enhances the right heart. PDE5i may increase exercise capacity in hypoxic conditions due to the vasodilatory function and modulatory effects on central nervous system and sympathetic system (*i.e.*, functional sympatholytic effects), heart rate, myocardial contractility, and alveolar-capillary membrane conductance (23,29,35,42,45,51). It is likely that the improvement in exercise capacity and cardiac output observed in healthy subjects during exercise under hypoxic conditions after PDE5i administration is due to the blunting effect of PDE5i on pulmonary hypertensive response to hypoxic exercise and to reduced right ventricular afterload, which is a critical factor limiting exercise capacity in hypoxia (20,27). Moreover, the possible presence of responders and nonresponders to PDE5i may explain the variability in pulmonary hemodynamic, pPO_2 and SaO_2 responses, and in the adaption to acute or chronic hypoxic conditions.

The reported effects of PDE5i on human physiology under hypoxic conditions could explain the mechanism(s) by which PDE5i can enhance athletic performance at very high altitude and/or the response to exercise training performed in natural or artificial hypoxia.

Intriguingly, PDE5i (*e.g.*, sildenafil) could be a prophylactic medication for swimming-induced pulmonary edema (SIPE) in swimmers (38,40). SIPE occurs during immersed exercise, in susceptible healthy individuals, because of higher pulmonary artery and wedge pressures, and sildenafil (50 mg) administration may reduce the pulmonary pressures and prevent the hemodynamic pulmonary edema (38,40).

Although the existing data are contradictory, NO donors *per se* (*e.g.*, beetroot juice, and so on) could mitigate the ergolytic effects of hypoxia on cardiorespiratory endurance (18,19,37,41,44,50). Unfortunately, there is no adequate information regarding the possible role of other drugs and/or supplements increasing NO availability and influencing the individual responses to PDE5i in athletes (1,6,11,16).

PDE5i and Hormones Adaptation to Exercise

In animals, prolonged sildenafil administration increases testosterone production by stimulating Leydig cell steroidogenesis (3). In humans with ED, chronic PDE5i administration also has been shown to increase serum testosterone and the testosterone to estrogen ratio, due to increased sexual intercourse, PDE5i-related antiestrogen effects, and/or to direct effect at testicular levels (8,22,43,52). In healthy men, a single dose of tadalafil (20 mg) amplified the physiological cortisol and testosterone responses to a maximal exercise-related stress in normoxia (Fig. 1), decreasing the testosterone to cortisol ratio (13). Interestingly, when compared with placebo, a slightly prolonged tadalafil administration (20 $mg \cdot d^{-1}$ for 2 d) reduced the ACTH, cortisol, corticosterone, and free cortisol index responses to a maximal exercise and increased beta-endorphin and dehydroepiandrosterone sulfate (DHEAS) to cortisol ratio during recovery by influencing the 11b-hydroxysteroid dehydrogenases activity (14,17). In fact, after tadalafil (20 $mg \cdot d^{-1}$ for 2 d), higher postexercise tetrahydrocortisol-to-cortisol ratio and tetrahydrocortisone-to-cortisone ratio were observed (14). Recently, chronic administration of vardenafil reduced dehydroepiandrosterone (DHEA) levels and increased DHEAS to DHEA ratio in men with type 2 diabetes, confirming a PDE5i-related modulation of steroidogenic enzymes by tissue changes in cAMP and cGMP (48). The fact that there were no effects on cardiorespiratory and performance parameters in these studies does not exclude potential endocrine effects of PDE5i during a specific competition and/or training. A laboratory exercise cannot reproduce all the factors influencing the final result during real competition and many confounding factors exist making the link to performance enhancement and PDE5i difficult to confirm. Based on the available studies (13,14,17,48), we believe in athletes that a) acute tadalafil administration could amplify the positive psychophysical effects of acutely increased endogenous cortisol and testosterone during sport competition (*e.g.*, the response to exercise-stress is increased by tadalafil) (13) and b) chronic tadalafil administration, by decreasing the adrenal steroids response to exercise-stress, could be useful during training because of reduced cortisol-related protein catabolism, increased testosterone-related anabolic effects, improved recovery from exercise, and reduced risk of overtraining (14,17,48).

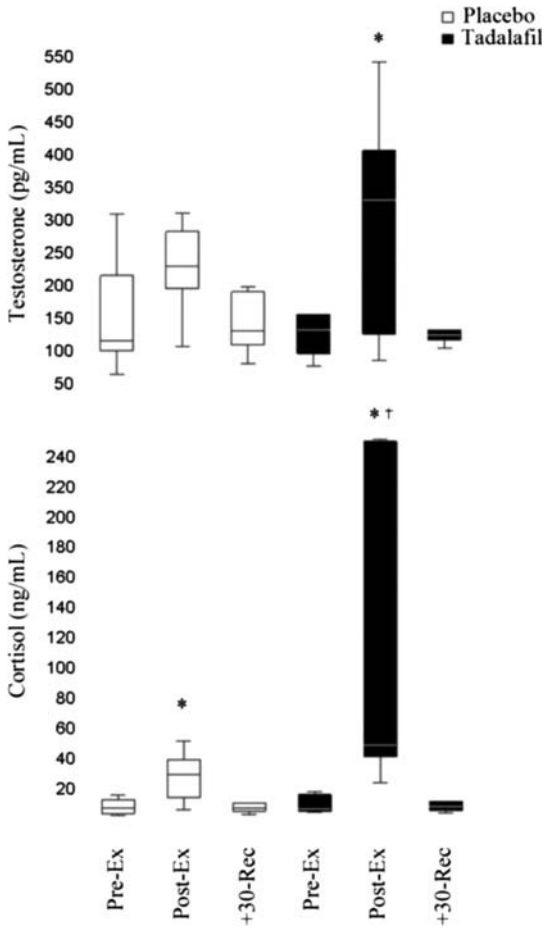

Figure 1: Box plot of salivary testosterone and cortisol concentrations before (Pre-Ex), at the end (Post-Ex), and 30 min after (30-Rec) a maximal exercise test on cycle ergometer after placebo (*open box*) and tadalafil (20 mg; *dark box*) administration in healthy male athletes. *Lower* and *upper edges of each box* represent the first and third quartile of observed data. The *line-partitioning box* corresponds to median observation and whiskers give range of data. $*P < 0.05$ vs respective Pre-Ex; $\dagger P < 0.05$ vs placebo (Adapted from Di Luigi L et al., *J Clin Endocrinol Metab.* 2008;93:3510–4).

PDE5i, Muscle, and Metabolism

PDE5i also may act directly on skeletal muscle cells. *In vitro*, an acute tadalafil exposure influenced the metabolism of murine C2C12 skeletal muscle cells (46), indicating that cGMP signaling may play a role also in the regulation of energy homeostasis. Specifically, acute treatment with 0.5 μM tadalafil improved glucose metabolism through the induction of anaerobic glycolysis with an accompanying decrease of aerobic metabolism (46). Studies performed using isolated human skeletal muscle cells, either myoblasts or myotubes, showed that tadalafil did not affect lactate, but enhanced citrate synthase activity involved in Krebs cycle while simultaneously increasing free fatty acid release. At the same time, tadalafil was able to activate the main insulin-dependent intracellular steps dedicated to cell metabolism regulation, such as Ras-Raf mitogen-activated protein kinase, protein kinase B/Akt, glycogen synthase kinase 3-β (downstream target of phosphatidylinositol 3-kinase), and the transcription factor c-Myc (downstream target of glycogen synthase kinase 3-β), all paths directly engaged in the control of intracellular nutrient fate and utilization (10). Tadalafil, like insulin, seems to target and potentiate part of the energy management and metabolic control in human skeletal muscle cells (Fig. 2). Recent studies have shown that prolonged tadalafil administration improved free fatty mass content in nonobese men, probably via enhanced insulin secretion and estradiol reduction (4,10,22). PDE5i also may amplify the action of endogenous NO on muscle satellite cells (2). Furthermore, after prolonged tadalafil administration, the endothelial function increased and correlated directly with insulin and inversely with estrogen serum levels (4). Moreover, the exposure of C2C12 cells to increasing tadalafil concentrations (10^{-7} to 10^{-6} M) significantly increased total androgen receptor (AR) mRNA and protein expression as well as myogenin protein expression after 24 and 72 h, suggesting a translational action of PDE5i on AR and on muscle cells (4). After 24-h treatment with upregulation of AR expression, a significant increase

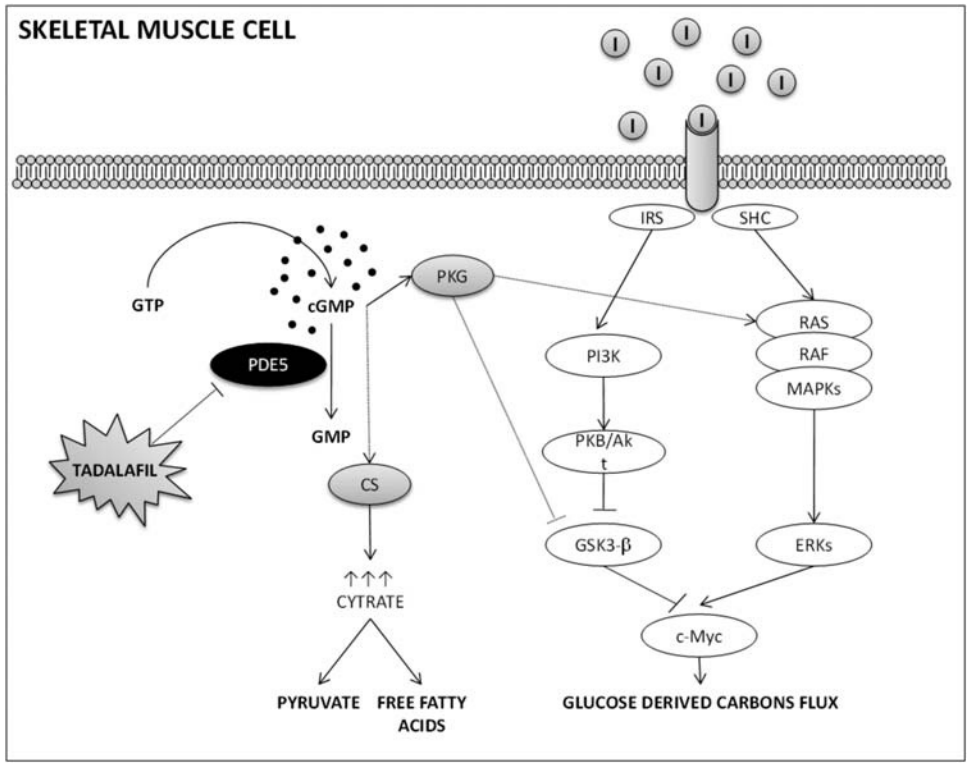

Figure 2: Metabolic effects of tadalafil in human skeletal muscle cells. Tadalafil-induced PDE5 inhibition stabilized cGMP levels, increased the activity of citrate synthase (CS) and simultaneously induced free fatty acids release; likely, this effect relies on a citrate shunt/accumulation. Insulin (I)-responsive steps were phosphorylated after cell exposure the drug: in particular, Ras-Raf mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinases (ERK) and phosphatidylinositol 3-kinase (PI3K) downstream paths, such as protein kinase B/Akt (PKB/Akt), glycogen synthase kinase 3-β (GSK3-β) and the transcription factor c-Myc were all involved in cellular response.

of testosterone concentrations in the supernatant of 10^{-6} M tadalafil-treated cells (2.3 ± 0.5-fold) compared with untreated cells was found ($P < 0.05$) (4).

Conclusions

PDE5i administration-related effects on cellular and body physiology, observed both in animal models and in some healthy individuals and specific circumstances (*e.g.*, hypoxia), could potentiate sport performance. The possible effects of PDE5i on exercise physiology are related both to the type of PDE5i use (*e.g.*, molecules, doses and length of administration) and to various individual factors (*e.g.*, age, hormone status, individual responsiveness, oxygen availability, and so on). In our opinion, PDE5i should be included in the list of prohibited substances for athletes, rather than waiting for challenging investigations on the possible performance enhancing effects of PDE5i during real-life sporting competition (*i.e.*, in different sports and experimental conditions), for the already observed effects of PDE5i on exercise physiology/performance and possible serious health risk related to their misuse (21).

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