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Research report

Repeated and chronic administration of Vardenafil or Sildenafil differentially affects emotional and socio-sexual behavior in mice

H. Dadomo[a,b,*], S. Parmigiani[a,b], Y. Nicolini[a], S. Freschini[a], L. Gioiosa[a], T.S. Patrelli[c], P. Palanza[a], R. Volpi[b]

[a] Neuroscience Department, University of Parma, Italy
[b] Department of Clinical and Experimental Medicine, Unity of Andrological Medicine, University of Parma, Italy
[c] Department of Clinical and Experimental Medicine, Gynecological and Obstetrical Sciences, University of Parma, Italy

HIGHLIGHTS

- We test the behavioral differential effect of Vardenafil and Sildenafil.
- We administered for 5 weeks, Sildenafil or Vardenafil, to subordinate mice.
- We measured the aggression, anxiety, exploration and sexual behavior.
- Although structurally similar Vardenafil act only on sexual behavior.
- Sildenafil restore aggression, social exploration, decreases anxiety.

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ABSTRACT

Selective phosphodiesterases (PDEs) inhibitors have been widely studied as therapeutic agents for treatment of various human diseases, including cardiotonics, vasodilators, smooth muscle relaxants, antidepressants, antithrombotics, antiasthmatics, and agents for improving learning and memory. Although Sildenafil® and Vardenafil® have similar chemical formulae, the same target and interact with many of the same residues at the active site of phosphodiesterse-5 (PDE-5), they exhibit both in vitro and in vivo some important functional differences that could differentially affect behavior. Therefore we assessed whether repeated and chronic administration of Vardenafil and Sildenafil at a dose based upon human treatment can differentially affect aggressive, social, emotional and sexual behavior. To this aim, the effects of Sildenafil (10 mg/kg) or Vardenafil (2 mg/kg) (t.i.w., for 5 weeks) were observed in CD1 subordinate male mice in a low aggression and social subordination context. The results show that Sildenafil increased competitive aggression, environmental and social exploration, and reduced anxiety like behaviors as compared to controls, whereas Vardenafil had a significant major effect on appetitive and consummatory aspect of sexual behavior. This demonstrates that Sildenafil and Vardenafil, although being structurally and functionally similar, are characterized by different neuro-behavioral actions and can have differential therapeutic potentials.

1. Introduction

Cyclic nucleotide phosphodiesterases (PDEs) are key enzymes controlling cellular concentrations of cAMP and cGMP [1–4]. Family-selective PDE inhibitors have been widely studied as therapeutic agents of various human diseases, as cardiotonics, vasodilators, smooth muscle relaxants, antidepressants, antithrombotics, antiasthmatics, and drugs for improving learning and memory [5–11]. Therefore, development of compounds that selectively block the catalytic function of particular PDEs is of great interest for the pharmaceutical industry. In particular, the potential impact of such compounds has already been demonstrated with the therapeutic and marketing success of three PDE5-selective inhibitors, Sildenafil (Viagra™), Vardenafil (Levitra™) and Tadalafil (Cialis™), which have been used to treat male erectile dysfunction [12]. A growing body of attention has recently been given to the new generation of PDE5 inhibitors showing the same or different scaffolds from the current drugs, but different pharmacokinetic profiles [13,14]. In this regard, Sildenafil and Vardenafil have similar chemical formulae, the same target and interact with many of the same residues at the active site

* Corresponding author at: Department of Neuroscience, University of Parma, Viale delle Scienze 11/A, 43100 Parma, Italy. Tel.: +39 0521 905636; fax: +39 0521 905657.
E-mail addresses: harold.dadomo@unipr.it, harold.dadomo@gmail.com (H. Dadomo).

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of PDE5, as shown by the crystal structures of the isolated PDE5 catalytic domain in complex with Sildenafil and Vardenafil [15]. Despite this, the pharmacokinetic and pharmacodynamics analyses showed that these PDE5 inhibitors have similar efficacy and tolerance but exhibit some functional differences both in vitro and in vivo [16–21]. For instance, Vardenafil shows a tighter binding with PDE5 and a different folded configuration compared to Sildenafil [22,23,15]. These differences are likely to contribute to the different properties of these drugs.

Centrally, both Sildenafil and Vardenafil are capable of crossing the blood brain barrier and affecting central PDE-5, which is expressed in different brain areas [24–27] underlying modulation of behaviors, such as rage, emotion and sexual drive. In a recent animal study [28], we reported that Sildenafil counteracts the inhibitory effects of chronic social subordination on modulation of competitive aggression by restoring both aggressive and sexual behavior in subordinated male mice. In a number of studies, Vardenafil and Sildenafil have been used as they could produce the same identical effects as PDE5 inhibitors; however in view of their subtle difference in structure and consequently different affinity to PDE5, we hypothesize that they are capable of producing differential effects on behavior. To this aim, we examined here the effects of a repeated and chronic Vardenafil or Sildenafil treatment on a broad range of behavioral responses of male mice in a stable dyadic context. This stable dyadic context has been shown to be a condition producing a stable dominant-subordinate hierarchy with a low level of stress and capable of magnifying the behavioral effects of this type of drugs on behavior [28,29]. We selected a single dose treatment equivalent to a human safe chronic dose that is 50 mg (0.84 mg/kg) for Sildenafil [31] and 10 mg (0.17 mg/kg) for Vardenafil [31]. Thus, we injected subordinate male mice with Sildenafil 10 mg/kg and Vardenafil 2 mg/kg; this dosage were extrapolated from human equivalent dose (HED), using the allometric dose translation of body surface area (BSA) [30]. Males were then subjected to different behavioral tests, namely the elevated plus maze (EPM), the social open field (SOF), the free-exploratory test, sexual behavior test.

2. Materials and methods

2.1. Experimental subjects

Experimental subjects were male mice derived from Charles River Italia (Calco, Italy), but born and reared in our colony room at the Laboratory of Behavioral Biology at University of Parma at 22 ± 2 °C in a 12 h light–dark cycle, food (4rf21 standard diet, Mucedola, Italy) and water ad libitum (lights on 07:00–19:00). After weaning (25–28 days) mice were housed in same-sex groups of sibling (5 ± 1 per cage) in Plexiglas cages (45 cm × 25 cm × 20 cm) with wood shaving bedding. When 6 months old, 108 adult male mice were housed in sibling pairs in Plexiglas cages (45 cm × 25 cm × 20 cm). Siblings Mice were paired on weight basis (±1 g) to ensure similar body masses at the start of the experiment, and weighed thrice a week throughout the study. For each male-male pair, dominant or subordinate status was determined. The status determination was carried out in the baseline period, at day 1, 5, 10 and 15 in the 30 min immediately after the wood-bedding change. During these periods we measured the agonistic and social behaviors displayed. Specifically the number of biting attack (head and ventrum), upright posture, freezing behavior, following behavior, chasing behavior were observed.

In this sibling dyads context, intensity of aggression is low and frequency of attacks is a good and sufficient measure of aggression level [28,29]. Only males that at day 1,5,10, and 15 displayed stables submissive behaviors (i.e. upright posture, flight behavior, squeaking behavior) were categorized as subordinate animals as opposed to the males that stably displayed dominance related behaviors in the same days and pairs (i.e. biting attacks, following, chasing) that were categorized as dominant animals.

A stable social rank was observed in all dyads during the 2-week drug-free period (i.e. baseline). Fifty-four subordinate mice were randomly selected as experimental animals (SILD mice $n = 20$; VARD mice $n = 18$; SALINE mice $n = 16$). As the rank relationship in each animal dyad is context dependent [31], to avoid differential manipulation effects on aggressive behavior, also the dominants animals (DOM) in each dyad were injected with saline [28]. From day 15 to 50, animals were intra-peritoneally injected every other day [34]. During this period we observed aggressive behavior in each dyad every 5 days at bedding change and carried out a series of behavioral tests: elevated plus maze (EPM) at day 37, social open field test (SOF) at day 39 free-exploratory test at day 41 and socio sexual behavior analysis at day 49.

The experimenters conducting the behavioral tests and measuring the frequency of aggressive behaviors were blind to the treatment.

Throughout the study food and water were available ad libitum. Housing and experiments were conducted in accordance with the animal experimentation European Communities Council Directive of 24 November 1986 (86/EEC) and approved by the Italian Institute of Health.

2.1.1. Drugs preparation

Sildenafil (Pfizer, Italy) and Vardenafil (Bayer, Italy) solutions were prepared according to [34]. Briefly, Sildenafil citrate (100 mg) and Vardenafil (20 mg) tablets were grounded into a fine powder using a mortar and pestle. As the good solubility in water of the two drugs is respectively 3.5 mg/ml for Sildenafil and 1.1 mg/ml, for Vardenafil, both the drugs were dissolved in 100 ml saline solution (0.9% NaCl) and passed through a 40 micron filters (PTFE Filters, Cole-Parmer, Generalcontrol, Milan, Italy) to eliminate residues of excipients. The final concentration, 1 mg/ml for Sildenafil and 0.2 mg/ml for Vardenafil, was checked by high-performance liquid chromatography (HPLC; see later drug analysis). The resulting solution was kept at 4 °C. Drug solutions were prepared every 4 days and the stability of solution were spectrometrically performed (see later).

Drug solutions were brought to room temperature 2 h prior to injections.

The structural differences between Vardenafil and Sildenafil complexes in both PDE-5 protein conformation and inhibitor configuration, and the Vardenafil affinity 10–40-fold with PDE-5 greater than Sildenafil lead us to use a 1:5 dosage ratio between Sildenafil and Vardenafil as also observed in literature [12,15,34,35].

The dosage for mice were calculated using allometric dose translation of BSA by the formulae:

Mice dosage (mg/kg) = human dosage (mg/kg)/(mice K.M./human K.M.; 29). The final dosage of the two drugs are 10 mg/kg of Sildenafil or 2 mg/kg of Vardenafil. Both Sildenafil and Vardenafil solutions were injected intraperitoneally (i.p.) in a volume of 1 ml per 100 g of mouse body weight. From day 15 to 50, animals were intra-peritoneally injected every other day [34] and to avoid acute effect of the drugs on behavior, behavioral tests were conducted in the day free to any manipulation or injection as used our previous study [28]. We have not differentiated treatment frequency between the two drugs because the effects of the chronic treatment of Vardenafil and Sildenafil are comparably persistent [36].

2.1.2. Drug analysis

Sildenafil and Vardenafil were measured by HPLC [37,38]. In brief, samples (50 μl) were mixed with 150 μl of 0.01 M NaOH. After adding 1 ml of diethyl ether (Sigma Aldrich, Milan, Italy), the vials were shaken for 5 min and centrifuged (5 min 10,000 × *g*). The lower aqueous layer was frozen on dry-ice and the residual transferred to a clean tube and dried by vacuum. The residue was dissolved in 50 μl of acetonitrile: water (30:70; v/v) and 20 μl was subjected to HPLC separation on a Symmetry C18 3.5 μm column (2.1 mm × 150 mm) Waters, Milford, MA, USA. The mobile phase A was 0.05 M phosphate buffer pH 6.0 containing 10 mM tetrabutyl ammonium bromide (Merck, Darmstadt, Germany) and mobile phase B was a mixture of acetonitrile: water (80:20; v/v). The gradient conditions were 0–7 min: fixed at 50% B; 7–12 min: linear gradient 50–80% B; 12–17 min: fixed at 80% B; 17–17.5 min: back to 50% B and equilibration for 12 min. Effluent was monitored by UV detection at 300 nm.

2.1.3. Assessment of stability of solutions

The stability of the two solutions was tested with HPLC (see below). The obtained UV–vis absorption spectra remained unaffected at least 4 days latter to its preparation. The UV–vis absorption spectra were recorded every 8 h during a period of 4 days. The solutions were stored at 1–4 °C and 30 min before analysis they were put at room temperature (i.e. 22 °C) to simulate the experimental conditions. In all cases, the absorption spectra and the concentration of Sildenafil and Vardenafil remained unaffected.

2.1.4. Assessment of aggressive behavior

On the first day of drug treatment (day 15) and every 5 days after bedding change males were observed for 30 min by a trained observer, blind to the treatment, for frequency of biting attack and agonistic behaviors displayed. No animals show the defensive type of aggression. Changing the wood-shaving bedding is a standard procedure to elicit territorial aggression aimed at re-establishing dominance in a clean and non-scent marked environment in male mice [32,38,39]. Aggressive behavior was assessed during the entire treatment period till the end of the experiment (i.e. for 50 days). All observations were carried out starting at 10:00 am.

2.1.5. Behavioral tests

Researchers who conducted the behavioral tests and those that scored the video were blind to the animal treatments. All behavioral tests were carried out starting 15:30 pm to 18:30 pm as standard procedure used in our laboratory [31].