Aggressive behavior increases after termination of chronic sildenafil treatment in mice

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Abstract

Recent reports to the U.S. Food and Drug Administration Adverse Event Reporting System implicate sildenafil citrate in adverse emotional and aggressive behaviors. Sildenafil citrate (Viagra) is widely prescribed for erectile dysfunction and acts by inhibiting phosphodiesterase Type-5, resulting in accumulation of cyclic-guanosine monophosphate (cGMP). Cyclic-GMP is synthesized by guanylyl cyclase that is directly activated by the messenger molecule, nitric oxide (NO), formed throughout the CNS by the enzyme nitric oxide synthase (NOS). Elevated concentrations of cGMP have been associated with increased aggressive behavior. In addition, the potential effect of cGMP accumulation on NO-mediated behavioral and neuroendocrine function through possible feedback mechanisms remains unspecified; however, neuronal NOS (nNOS) inhibition by pharmacologic agents or ablation of the gene encoding nNOS increases aggressive behavior in male mice. We tested the hypothesis that sildenafil citrate may increase aggression via its actions on cGMP and potential feedback inhibition of NO concentrations. Male C57BL/6 mice were injected with saline vehicle (0), 2, 5, 8, or 10 mg/kg of sildenafil citrate thrice weekly for 4 weeks. Latency to display aggressive behavior, frequency, and duration of aggressive behavior were recorded during neutral-arena aggression tests. No change in agonistic behavior was observed in mice during treatment with sildenafil citrate. However, sildenafil-treated mice given the highest dose were generally more aggressive 1 week post-cessation of drug treatment as compared to vehicle-treated mice. Additional investigation into potential withdrawal effects or abuse doses seems warranted.

Keywords: Sildenafil; Aggression; Mouse; Nitric oxide; Cyclic-GMP

1. Introduction

In 1998 the Food and Drug Administration (FDA) approved the use of sildenafil citrate (Viagra) for treatment of erectile dysfunction (ED) [1–3]. Sildenafil is now available in more than 90 countries, and by 2001 more than 15 million men had taken the drug [4]. The drug acts by inhibiting phosphodiesterase Type-5 (PDE-5), an enzyme found in the corpus cavernosum, allowing cyclic-guanosine monophosphate (cGMP) to accumulate, and causing relaxation of the smooth muscle with concomitant increased blood flow to the penis, leading to an erection [4,5].

The localization of PDE-5 to areas of the brain provides for the possibility of central nervous system (CNS) effects of sildenafil administration. Several direct effects of sildenafil administration on the CNS have been reported in both rodents and humans. For example, oral sildenafil administration to rats has been shown to increase brain levels of cGMP and evoke neurogenesis [6]. Another study examined potential central effects of sildenafil on attention and memory function in humans. Although no overt effects on behavior were observed, sildenafil treatment caused an enhanced ability to focus attention [7]. In addition, sildenafil...
is capable of centrally altering copulatory behavior in rats, perhaps via a dopaminergic pathway [8,9]. Finally, sildenafil administration significantly increases sympathetic nerve activity in humans [10].

Various adverse effects (i.e., headache, dyspepsia) have been attributed to the inhibition of PDE-5 by sildenafil in smooth muscle of cerebral or other vascular vessels, esophageal sphincter, and nasal mucosa, and by inhibition of PDE-6 (another isoenzyme form of PDE) in smooth muscle of the retina [11–14]. In addition, central nervous system (CNS) adverse effects also have been noted in clinical trials [15,16]. These effects include dizziness, depression, insomnia, abnormal dreams, and “nervousness”; a causal relationship between these effects and sildenafil has not yet been established. Finally, several anecdotal reports and case studies have described neurological and emotional disturbances including: amnesia or loss of consciousness, and increased aggressive behavior (e.g. rape, suicide, attempted suicide, or murder) associated with sildenafil use [17,18]. Supporting these reports, a recent study examining the FDA database, Adverse Event Reporting System, also provides evidence suggesting an association between sildenafil and various CNS effects. The authors note, however, that the mechanisms which could be responsible for such alterations remain to be determined [19,20].

One possible mechanism by which sildenafil citrate could directly affect CNS function is through its inhibition of PDE-5 and the subsequent accumulation of cGMP. Alternatively, accumulation of cGMP has the potential to negatively feedback on upstream mechanisms involved in nitric oxide (NO) production. cGMP acts as a second messenger in neuron–neuron communication and in cell–cell signaling from pre- to post-synaptic elements and vice versa, as well as between presynaptic fibers or between postsynaptic structures [21–23]. Increased cGMP elevates aggression in mice [24], dogs [25], and elevated cGMP concentrations have been observed in humans classified as aggressive [26]. In vitro, cGMP provides negative feedback on the NO–cGMP pathway, specifically on NOS activity [27]. During neuronal excitation, nNOS catalyzes the reaction by which NO is formed from arginine [5]. NO then activates guanylyl cyclase (GC) to synthesize cGMP [28,29]. Increased concentrations of cGMP might, over time, feedback on nNOS to reduce the production of NO, mimicking the situation in nNOS−/− mice. The potential effect of cGMP accumulation on NO-mediated behavioral and neuroendocrine function through possible negative feedback mechanisms remains unspecified; however, previous studies suggest that neuronal nitric oxide synthase (nNOS) inhibition by pharmacologic agents, or ablation of the gene encoding nNOS, causes increases in aggressive behavior in male mice [30,31].

Taken together, these studies suggest that increased levels of cGMP, in addition to potential decreases in NO levels in the CNS, profoundly affect aggression in male mice. Thus, we hypothesized that elevations in cGMP levels occurring during treatment with sildenafil citrate could contribute to an increase in aggressive behavior through these two independent mechanisms. We tested the hypothesis that repeated administration of sildenafil citrate, due to its actions on cGMP accumulation and subsequent potential effect on NO concentrations, would increase aggressive behavior in mice.

2. Methods

2.1. Animals

Adult male C57BL/6 mice were single-housed in polycarbonate cages (27.8×7.5×13 cm) under constant temperature (21±4 °C) and relative humidity (50±10%), in a photoperiod of 16 h of light per day (LD16:8). Mice were given ad libitum access to food (Harlan Teklad 8640 Rodent Diet, Indianapolis, IN) and filtered tap water. The effects of sildenafil citrate on aggressive behavior in male mice were examined using the neutral arena aggression model. Prescribed human doses of sildenafil citrate range from ~1.5 to 2.0 mg/kg (and not more than 3 times/week). We injected adult male C57BL/6 mice i.p. with either saline vehicle (0), 2.0 mg/kg, or 10 mg/kg of sildenafil thrice weekly for 4 weeks (n=12–15 per dose). Animals were weight-ranked at the beginning of the study to ensure similar starting body masses between groups. Two blocks (n=6–8 mice/group/block) were run in the study to better accommodate the logistics of repeated dosing and behavioral testing.

2.2. Preparation of dosing solutions

Dosing solutions were prepared following methods similar to those previously reported [32]. Briefly, sildenafil citrate (50 mg) tablets were ground into a fine powder using a mortar and pestle. The resulting powder was then mixed with saline and passed through 40 μm filter paper. The resulting solution was kept chilled at 4 °C. Dosing solutions were brought to room temperature prior to injections.

2.3. Aggression in neutral arena

An adult male stimulus mouse and one mouse from one of the experimental groups were simultaneously introduced into a clean polycarbonate cage (38.5×26.5×30.7 cm). The floor of the cage was covered with 2–3 cm of fresh corn-cob bedding. The latency to first aggressive encounter, the total duration of aggressive encounters, and the total number of aggressive encounters initiated by the experimental male was recorded. Aggression tests lasted 10 min and stimulus males were not used more than once per day. Aggression testing was conducted after one (week 1) and four (week 4) weeks of sildenafil citrate treatment, and again one week after the cessation of treatment (week 5).
All procedures were performed following the Guide for the Care and Use of Laboratory Animals and were approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee.

2.4. Analysis

A mixed model ANCOVA (SAS, Cary, NC) was run with block as a random factor, and dose and week as fixed effects with repeated measurements on week for vehicle and drug-treated mice. As a significant block effect was detected in latency to attack (two-tail test; latency ($F[1,75]=4.95$, $p=0.0292$) and total duration of attacks ($F[1,75]=7.45$, $p=0.0079$), the analyses for latency, frequency, and duration were run separately for the two blocks. The alpha level was set at $\alpha=0.1$ (one-tail for drug effect) for the overall ANCOVA due to the small sample sizes in each block ($n=6–8$). This analysis was run in order to determine any significant differences due to drug effects controlling week as a covariate. In addition, post hoc multiple comparison tests were run using a Tukey–Kramer adjustment. Mean differences were considered statistically significant if $p<0.05$.

3. Results

Analysis of the data did not show a significant dose or week effect in the overall ANCOVA with the 2, 5, or 8 mg/kg doses (unpublished data). Therefore, only data for the highest dose (10 mg/kg) are presented below. Statistical analysis did not reveal a direct effect of sildenafil administration on aggression in neutral arena aggression tests for either of the blocks ($p>0.1$). However, week of testing significantly affected the latency to attack in both block 1 ($F[1,55]=6.25$, $p=0.015$) and block 2 ($F[1,58]=2.97$, $p=0.09$). In both blocks, post hoc analysis revealed that in week 5 (after cessation of treatment) latency to attack was significantly reduced in the sildenafil-treated (10 mg/kg) animals as compared to week 1 (block 1, $p=0.0039$; block 2, $p=0.0007$) and week 4 (block 1, $p=0.01$; block 2, $p=0.0025$) (Table 1 and Fig. 1). No significant differences between weeks were observed in the vehicle-treated animals (Table 1 and Fig. 1). A significant treatment by week interaction was observed for latency to attack by week of testing showed that latencies to attack were shorter for sildenafil-treated mice during week 5 ($F[1,58]=3.01$, $p=0.0882$). Post hoc testing showed that latencies to attack were shorter for sildenafil-treated mice during week 5 ($F[1,58]=3.01$, $p=0.0882$). Post hoc testing showed that latencies to attack were shorter for sildenafil-treated (10 mg/kg) mice than for vehicle-treated mice only during week 5 ($p=0.0022$) (Fig. 1). There was not a significant treatment by week interaction in block 1. Week of testing significantly affected the frequency of attack for the 10 mg/kg sildenafil-treated mice in both block 1 ($F[1,55]=6.25$, $p=0.066$) and block 2 ($F[1,58]=2.97$, $p=0.10$). In both blocks, post hoc analysis revealed that in week 5 (after cessation of treatment), frequency of attack was significantly increased in the sildenafil-treated (10 mg/kg) animals compared to week 1 (block 1, $p=0.0099$; block 2, $p=0.0099$; block 2, $p=0.0099$)

### Table 1

<table>
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<tr>
<th>Treatment (mg)</th>
<th>Week 1 Mean (SEM)</th>
<th>Week 4 Mean (SEM)</th>
<th>Week 5 Mean (SEM)</th>
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<tr>
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<td>367.5(80.6)</td>
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<td>8.3(2.8)</td>
<td>4.3(2.8)</td>
</tr>
<tr>
<td>10</td>
<td>0.8(1.9)</td>
<td>2.6(1.8)</td>
<td>7.9(1.8)*</td>
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<tr>
<td>Duration (s)</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>72.7(28.3)</td>
<td>42.5(28.3)</td>
</tr>
<tr>
<td>10</td>
<td>5.9(19.2)</td>
<td>23.7(18.5)</td>
<td>92.6(18.5)*</td>
</tr>
</tbody>
</table>

* $p<0.05$.
p=0.0013) and week 4 (block 1, p=0.05; block 2, p=0.014) (Table 1 and Fig. 2). No significant differences between weeks were observed in the vehicle-treated animals (Table 1 and Fig. 2). A significant treatment by week interaction was observed for frequency of attack for block 2 (F[1,58]=3.34, p=0.0727). Post hoc testing showed that frequency of attack was increased for sildenafil-treated (10 mg/kg) mice as compared to untreated mice during week 5 (*p<0.05) (Fig. 2). There was not a significant treatment by week interaction in block 1.

There was a significant main effect of week of testing for the total duration of attack in block 1 (F[1,55]=4.40, p=0.04). Post hoc analysis showed that duration of attack during week 5 (after cessation of treatment) was greater than either week 1 (p=0.002) or week 4 (p=0.011) for sildenafil-treated (10 mg/kg) mice (Table 1). A significant treatment by week interaction was observed in block 2 (F[1,58]=5.99, p=0.0174). In sildenafil-treated (10 mg/kg) mice, post hoc analysis revealed that week 5 duration of attack was greater than either week 1 (p=0.0019) or week 4 (p=0.011) (Fig. 3). Again, post hoc analysis did not show any significant differences between weeks in the vehicle-treated mice (Table 1 and Fig. 3). Post hoc analysis also showed that sildenafil-treated (10 mg/kg) animals attacked for a longer duration than vehicle-treated animals only during week 5 (p=0.0093) (Fig. 3). There was not a significant treatment by week interaction for block 1.

4. Discussion

This study was conducted in order to examine whether administration of sildenafil citrate would affect aggression in male mice. We hypothesized that sildenafil citrate-induced accumulation of cGMP, or subsequent feedback of cGMP on NO production, would increase mouse aggression. However, no direct effects of sildenafil administration were observed on aggression in male C57BL/6 mice. Importantly, sildenafil has no direct affect on NO or cGMP production per se. For this reason, the effect of sildenafil on aggression, during administration of the drug, would be directly dependent on its effect on cGMP levels. The literature suggests that increased cGMP levels are positively correlated with increased aggression [24–26]. Because sildenafil acts through PDE-5 to increase cGMP levels, we expected sildenafil administration to increase aggressive behavior. Possibly, the inability of sildenafil administration to increase aggression is due to the simultaneous effect of cGMP feedback on eNOS and nNOS enzymes in the CNS. eNOS, when ablating in mice (eNOS/-/-) causes a dramatic overall decrease in aggression [33], whereas mice with ablation of the gene coding for the formation of nNOS (nNOS-/-), or those that have been treated with 7-nitroindazole (7-NI; 50 mg/kg, i.p.), a selective nNOS inhibitor, display increased aggression as compared to wildtype control animals [30,31,33]. These competing effects on NO production could explain the lack of a direct effect of administration of sildenafil on aggression.

Sildenafil-treated mice were more aggressive in the neutral arena paradigm one week after the cessation of treatment (week 5), at the highest dose (10 mg/kg), as compared to weeks 1 and 4. Mice in block 2, treated with the highest dose of sildenafil were also more aggressive than the vehicle-treated mice during week 5. This effect may reflect possible up or down regulation at several points in the NO-cGMP pathway, causing a withdrawal effect and subsequent increases in aggression. The cessation of sildenafil treatment may result in alterations in the expression of various enzymes and receptors associated with cGMP, or in receptor sensitivity on target tissues. Aggression in mice observed after the cessation of sildenafil treatment may closely resemble conditions found in nNOS deficient mice. It appears that administration of sildenafil, and the subsequent accumulation of cGMP, was inadequate to dramatically alter aggressive behavior. Subtle changes in NO production evoked by chronic administration of sildenafil, through a negative feedback mechanism, may have been revealed after cessation of drug treatment. During drug administration, NO production may have been down-regulated through negative feedback, but this change may not have made a significant impact on aggressive behavior because of the exaggerated accumulation of cGMP through inactivation of PDE-5. After the drug was removed, down-regulation of NO production resulting from negative-feedback, in combination with plummeting cGMP

![Fig. 3. Least squares means for duration of attack (s) for male mice treated with sildenafil citrate or vehicle. Duration of attack was significantly increased in the 10 mg/kg treated animals during week 5 as compared to week 1 and week 4 in both blocks 1 (data not shown) and 2 (*p<0.05). In block 2, duration of attack was also significantly increased in the 10 mg/kg sildenafil-treated mice, as compared to untreated mice during week 5 (*p<0.05).](image-url)
levels, may create a neurophysiological condition similar to nNOS deficient mice leading to increased aggression. Alternatively, accumulation of cGMP through PDE-5 may have altered some other factor (e.g. 5-HT expression), modifying aggressive behavior. Finally, the interaction between repeated exposures to aggressive situations, in combination with the administration and withdrawal of sildenafil citrate, may be responsible for the effects seen here. Additional studies to examine these relationships, as well as studies utilizing NOS knockout animals to determine the role of these enzymes in the effects reported here, are required.

In conclusion, administration of sildenafil citrate did not directly affect aggressive behavior in mice. A significant increase in aggressive behavior was observed in mice the week immediately following cessation of 4 weeks of treatment with sildenafil, but only at the highest dose. This finding suggests that future studies investigating possible withdrawal and abuse effects of the drug are warranted. However, as in all nonhuman animal studies, it is important to note that the applicability of the murine model of aggression to human aggression remains unclear [34]. Therefore, additional mechanistic studies in mice and epidemiological studies in men are needed to determine the behavioral effects associated with discontinuation of sildenafil citrate.

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References


