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ORIGINAL ARTICLE

Developmental toxicity of orally administered sildenafil citrate (Viagra) in SWR/J mice

Faisal Mohamed Abou-Tarboush *, Mohamed Fathy Abdel-Samad, Mokhlid Hamed Al-Meteri

Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

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Abstract Normal adult inbred SWR/J mice were used to investigate the teratogenic and other possible toxic effects of various dose levels of sildenafil citrate (Viagra) on fetuses. Multiple dose levels of 6.5, 13.0, 19.5, 26.0, 32.5 or 40.0 mg of sildenafil citrate/kg body weight (which correspond to the multiples of 1, 2, 3, 4, 5 or 6 of human 50 mg Viagra, respectively) were orally administered into pregnant mice on days 7–9, 10–12 or 13–15 of gestation. On day 17 of pregnancy, all fetuses were removed and examined for toxic phenomena (embryo-fetal toxicity) and for external, internal and skeletal malformations. A total of 285 pregnant mice were used in the present study.

None of the dams treated with sildenafil citrate at any of the oral dose levels used in the present study died during the experimental period and all dams treated with the drug failed to reveal overt signs of maternal toxicity. Moreover, the results of the present study clearly demonstrate that none of the multiple oral dose levels of the drug at any time interval used has induced any external, internal or skeletal malformations in the fetuses obtained from treated females.

However, the dose level of 40 mg/kg body weight of sildenafil citrate has a growth suppressing effect on alive fetuses when it was administered at all the time intervals used in the present study.

* Corresponding author. Tel.: +966 14675762; fax: +966 14678514.
E-mail address: ftarbush@yahoo.com (F.M. Abou-Tarboush).

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Furthermore, the dose levels 26.0, 32.5 and 40 mg/kg of the drug have embryo-fetal toxicity when the drug is applied on days 13–15 of gestation. The possible mechanisms involved in the embryo-fetal toxicity and fetal growth suppressing effects of sildenafil citrate were discussed.

The results of this study have important implications for the widespread use of this drug.

1. Introduction

Sildenafil citrate (Viagra) is an oral medication used to treat male erectile dysfunction by the inhibition of phosphodiesterase-5 in the corpus cavernosum and subsequent facilitation of penile erection (Vatansever et al., 2003). Since its introduction in 1998, sildenafil citrate has been used to treat over 27 million men with this problem worldwide (Boyce and Umland, 2001; Glenn et al., 2009). Moreover, the drug is used increasingly by men of reproductive age and there is now robust evidence that its use in recreation has gained credence in young healthy males as a sexual enhancer as well in older men requiring it for impotence problems (Aldridge and Measham, 1999; Smith and Romanelli, 2005). Furthermore, its use is also rapidly increasing in the population of young men who suffer from impotence related to medical conditions, such as diabetes and spinal cord injuries (Monga et al., 1999).

Sildenafil citrate has been used successfully in males to remediate problems associated with impaired neural and/or hemodynamic response to sexual stimulation (Krenzelok, 2000). In addition, it is effective in the treatment of pulmonary hypertension in hemoglobinopathies (Derchi and Forni, 2005). Moreover, sildenafil citrate could be an alternative in the treatment of intrauterine growth retardation (IUGR) and premature delivery (Villanueva-Garcia et al., 2007).

The widespread use of sildenafil citrate is of concern, because it is a selective type 5 phosphadiesterase (PDE) inhibitor, and PDE inhibitors have been shown to affect sperm function and embryo development (Tournaye et al., 1993; Scott and Smith, 1995; Glenn et al., 2007, 2009). Although a few studies (Abbott et al., 2004; Product Monograph, 2006; Villanueva-Garcia et al., 2007) have conducted to investigate sildenafil citrate's teratogenic effect in experimental animals, all of those studies indicated that it is not a teratogenic agent. However, other studies (Refuerzo et al., 2006; Glenn et al., 2009) indicated that sildenafil citrate could affect fetal size and early embryo development, respectively. Therefore, the aim of the present study was to investigate the teratogenic, toxic and growth suppressing effects of various dose levels of sildenafil citrate on the embryos and fetuses of SWR/J mice when administered into pregnant females during different days of gestation.

2. Materials and methods

Inbred normal SWR/J male and female mice, 8–10 weeks old and weighing 25–30 g were used in the present study. Animals were kept and bred under controlled room temperature of 22 ± 1 °C, a relative humidity of 45 ± 5% and a light/dark cycle of 10/14 h. Rodent chow (commercially available in Saudi Arabia) and water were offered *ad libitum*.

In each box, 3–4 nulliparous females were caged together with a single male. The day the vaginal plug was detected was considered as day 0 (D0) of gestation and the pregnant females were placed in separate cages. A total of 285 pregnant females were used, and were divided into four groups (I–IV), 15 females each. The females of each of the three groups were orally treated on days 7–9, 10–12 or 13–15 of pregnancy with multiple dose levels of 6.5, 13.0, 19.5, 26.0, 32.5 or 40.0 mg/kg body weight of sildenafil citrate (Pfizer Inc., USA) dissolved in sterile normal saline. The fourth group of pregnant females served as a control group and received 0.4 ml of the vehicle alone (sterile normal saline).

On day 17 (D17) of gestation, pregnant females from all groups were killed by cervical dislocation, the abdominal wall of each female was opened and both uterine horns were promptly exposed to their full extent. The number of resorbed and intact fetuses was counted and recoded. The uterine horns were then opened to determine the number of alive and dead fetuses. Spontaneous movement, reddish color, size and/or movement induced with a forceps on the neck or the head of the fetus were the criteria used to distinguish between alive and dead fetuses. The relative positions of fetuses and resorption of dead ones were also recorded. Alive fetuses were carefully examined under a stereoscopic microscope for gross malformations and were accordingly classified as normal or abnormal. Normal and abnormal alive fetuses were removed onto paper towels, dried up and weighed. Twenty fetuses/treatment dose levels were cleared and stained according to the method of McLeod (1980) for the study of skeletal abnormalities. Twenty fetuses/control groups were similarly prepared for skeletal malformation examination.

2.1. Statistical analysis

The data obtained were statistically analyzed using a 2×2 contingency table (χ^2) for the actual number of resorptions observed, and the significance of the difference between means of sildenafil citrate-treated and control group was calculated by Student's *t*-test (Sokal and Rohlf, 1981).

3. Results

None of the dams treated with sildenafil citrate at any of the dose levels used in the present study died during the experimental period and all the dams treated with the drug failed to reveal overt signs of maternal toxicity.

Data in Table 1 show a significant ($p < 0.05$) increase in the percentage of resorption and a simultaneous reduction in the mean alive fetal body weight at the dose level of 40 mg/kg body weight when the drug was applied on days 7–9 of gestation.

Data in Table 2 show a significant ($p < 0.05$) reduction in the mean alive fetal body weight at the dose level of 40 mg/kg body weight when the drug was administered on days 10–12 of pregnancy.

Data in Table 3 show a significant ($p < 0.05$) increase in the percentages of resorptions at the dose levels of 26.0, 32.5 and 40.0 mg/kg body weight when the drug was applied on days

Table 1 Effect of the administration of various doses of sildenafil citrate (Viagra) applied to SWR/J female mice on days 7–9 of pregnancy on the fetuses.

Dose used (mg/g)	No. of dams used	No. of implantation sites	No. of fetuses/dam (Mean ± SE)	No. of alive fetuses/dam (Mean ± SE)	No. of resorptions (1%)	Alive fetal body wt. in g (Mean ± SE)	Abnormalities observed
Control	15	164	10.93 ± 0.42	10.60 ± 1.06	5 (3.05)	0.82 ± 0.017	None
6.5	15	168	11.20 ± 0.35	10.73 ± 0.85	7 (4.17)	0.83 ± 0.020	None
13.0	15	160	10.67 ± 0.11	10.20 ± 0.81	7 (4.32)	0.84 ± 0.018	None
19.5	15	169	11.27 ± 0.43	10.60 ± 0.80	10 (5.92)	0.86 ± 0.017	None
26	15	168	11.20 ± 0.80	10.33 ± 0.69	13 (7.74)	0.79 ± 0.022	None
32.5	15	166	11.07 ± 0.63	10.27 ± 0.71	12 (7.23)	0.78 ± 0.021	None
40.0	15	165	11.00 ± 0.78	10.07 ± 0.69	14 (8.48)*	0.63 ± 0.023*	None

* Differences are statistically significant from the control group at $p < 0.05$.

Table 2 Effect of the administration of various doses of sildenafil citrate (Viagra) applied to SWR/J female mice on days 10–12 of pregnancy on the fetuses.

Dose used (mg/g)	No. of dams used	No. of implantation sites	No. of fetuses/dam (Mean ± SE)	No. of alive fetuses/dam (Mean ± SE)	No. of resorptions (1%)	Alive fetal body wt. in g (Mean ± SE)	Abnormalities observed
Control	15	164	10.93 ± 0.42	10.60 ± 1.06	5 (3.05)	0.82 ± 0.017	None
6.5	15	167	11.13 ± 0.39	10.67 ± 0.85	7 (4.19)	0.83 ± 0.019	None
13.0	15	168	11.20 ± 0.86	10.53 ± 0.84	10 (5.95)	0.79 ± 0.030	None
19.5	15	166	11.07 ± 0.85	10.67 ± 0.77	6 (3.61)	0.79 ± 0.022	None
26.0	15	163	10.87 ± 0.85	10.33 ± 0.56	8 (4.91)	0.77 ± 0.023	None
32.5	15	161	10.73 ± 0.72	10.00 ± 0.81	11 (6.83)	0.78 ± 0.031	None
40.0	15	159	10.60 ± 0.53	9.80 ± 0.80	12 (7.55)	0.71 ± 0.028*	None

* Differences are statistically significant from the control group at $p < 0.05$.

Table 3 Effect of the administration of various doses of sildenafil citrate (Viagra) applied to SWR/J female mice on days 13–15 of pregnancy on the fetuses.

Dose used (mg/g)	No. of dams used	No. of implantation sites	No. of fetuses/dam (Mean ± SE)	No. of alive fetuses/dam (Mean ± SE)	No. of resorptions (1%)	Alive fetal body wt. in g (Mean ± SE)	Abnormalities observed
Control	15	164	10.93 ± 0.42	10.60 ± 1.06	5 (3.05)	0.82 ± 0.017	None
6.5	15	167	11.13 ± 0.39	10.27 ± 0.83	13 (7.78)	0.85 ± 0.021	None
13.0	15	168	11.20 ± 0.86	10.60 ± 0.84	9 (5.36)	0.84 ± 0.022	None
19.5	15	169	11.27 ± 0.87	10.53 ± 0.61	11 (6.51)	0.78 ± 0.028	None
26	15	164	10.93 ± 0.85	10.00 ± 0.31	14 (8.54)*	0.80 ± 0.029	None
32.5	15	162	10.80 ± 0.49	9.93 ± 0.42	13 (8.02)*	0.77 ± 0.024	None
40.0	15	163	10.87 ± 0.75	9.93 ± 0.57	14 (8.59)*	0.69 ± 0.019*	None

* Differences are statistically significant from the control group at $p < 0.05$.

13–15 of gestation. Moreover, there is a significant ($p < 0.05$) reduction in the mean alive fetal body weight when the drug was administrated on the same days.

However, none of the drug dose levels used has induced any external, internal or skeletal malformations in any of the fetuses obtained from sildenafil citrate-treated females at any day of gestation used in the present study.

4. Discussion

None of the dams treated with sildenafil citrate at any of the oral dose levels used in the present study died during the experimental period and all dams treated with the drug failed to reveal overt signs of maternal toxicity. Moreover, the results of the present study clearly demonstrate that the multiple oral dose levels of sildenafil citrate ranging from 6.5 to 40.0 mg/kg body weight into SWR/J pregnant mice on days 7–9, 10–12 or 13–15 of gestation did not induce any external, internal or skeletal malformations in the fetuses obtained from such treated females. Therefore, these results are in agreement with what is known about this drug from the few studies that have been documented (Abbott et al., 2004; Product Monograph, 2006; Villanueva-Garcia et al., 2007).

However, the dose level 40 mg/kg body weight of sildenafil citrate has growth suppressing effect on fetuses obtained from pregnant females when it was administrated on all days in the present study. Furthermore, the dose levels 26.0, 32.5 and 40 mg/kg of the drug have embryo-fetotoxicity when it was applied on days 13–15 of pregnancy. Such results are also consistent with the results of Refuerzo et al. (2006) and Glenn et al. (2009) who found that sildenafil citrate resulted in a decrease in fetal size, fertilization rates and embryo development in animal models (rats and mice).

Alternations in intracellular Ca^{2+} distribution may be the key to the effects of sildenafil citrate observed in the present study. There is strong evidence that free intracellular Ca^{2+} controls cell division in embryos (Webb and Miller, 2003). Moreover, Blancato and Seyler (1990) concluded that both Ca^{2+} flux and distribution to particular sites are required for normal embryo development. In addition, calcium depletion by PDE inhibitors has been reported by Ghalayini (2004), and the calcium channels facilitating both flux and distribution are tightly regulated by cAMP-dependent phosphorylation and minor changes in concentrations of either cAMP or cGMP have major impact on embryonic cell proliferation (Fischmeister and Hartzell, 1991; Grealy and Sreenan, 1999).

Another cause of the toxic and growth suppressing effects of sildenafil citrate on embryo development may be due to PDE inhibitor effects on the embryos' DNA synthesis and repair (Glenn et al., 2009). Souness et al. (1992) showed that inhibitors of PDE types 3 and 4 impair DNA synthesis and proliferation in pig aortic smooth muscle may be via the elevation of intracellular cAMP. Pentoxifylline (a PDE type 4 inhibitor) has also been shown to inhibit DNA repair in vitro during the S and G_2 phases in a human ovarian cell line (Schiano et al., 1991).

Apoptosis is a further mechanism by which PDE inhibitors may suppress the growth of the embryos and/or destroy them (Glenn et al., 2009). The mechanism by which this occurs has been studied in eosinophils and pulmonary endothelial cells and is related to a sustained increase in intracellular cGMP concentration (Wang et al., 2005; Zhu et al., 2005).

Finally, sildenafil citrate can enhance nitric oxide (NO) production in embryos and such action could cause damage to such embryos and reduce their survival if NO is generated in excess (Moncada and Higgs, 1991; Barroso et al., 1998; Glenn et al., 2009). However, further studies are needed to elucidate the mechanism(s) by which the impairment occurs.

In conclusion, the present study demonstrates that sildenafil citrate (Viagra) has embryo-fetotoxicity and growth suppressing effects when applied during development in an animal model. This has important implications for the widespread use of this drug. However, there is still limited information about the efficacy of sildenafil citrate for the treatment of IUGR and premature delivery.

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