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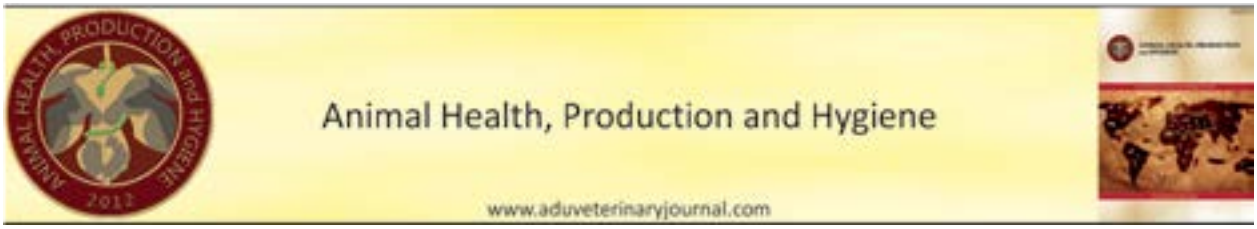

Research Article

Effect of Sildenafil Citrate on Uterine Artery Doppler Indices in Non-pregnant Rabbits

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ABSTRACT

The effect of different doses of sildenafil citrate (SC) administrations on arteria (a.) uterine blood flow was investigated in healthy female rabbits. Twelve healthy non-pregnant New Zealand rabbits were used in the present study. In the first experimental group (n=6), 5 mg SC thawed in 0.9% NaCl solution was administered orally. Arteria uterina was examined with Pulsed Doppler ultrasonography at pre and post (60 minutes) SC administration. Heart rate (HR), pulsatility index (PI) and resistance index (RI) values were recorded from the a. uterina visualized with cervical channels. Similarly, the study was repeated in second experimental group (n=6) by using 10 mg SC for per animal. In the first experiment, the mean HR, PI and RI values were recorded as (223 ± 7.80 & 239 ± 15.00), (1.60 ± 0.08 & 1.63 ± 0.16) and (0.73 ± 0.02 & 0.73 ± 0.03) before and after SC administration, respectively. In the second experiment, the mean HR, PI and RI values were observed as (210 ± 15.00 & 223 ± 14.00), (1.46 ± 0.23 & 1.79 ± 0.08) and (0.68 ± 0.05 & 0.77 ± 0.01) before and after SC administration, respectively.

No significant difference was between pre and post-administration Doppler parameters in both experiments (P>0.05). Consequently, no enhancing effect on the uterine perfusion was observed in oral 5 and 10 mg SC administrations per rabbit.

Keywords: Doppler, uterine artery, sildenafil citrate, rabbit

Gebe Olmayan Tavşanlarda Sildenafil Sitratın Uterin Arter Doppler Bulgularına Etkisi

ÖZET

Sağlıklı dişi tavşanlara farklı dozlarda uygulanan Sildenafil sitratın (SS) arteria (a.) uterinadaki kan akımı üzerine etkisi incelendi. Çalışmada 12 adet sağlıklı, gebe olmayan Yeni Zelanda ırkı dişi tavşan kullanıldı. Birinci deneme grubuna (n=6) 5 mg SS 0,9% NaCl solüsyonu içerisinde çözdürülerek oral yolla uygulandı. Sildenafil sitrat uygulamasından hemen önce ve 60 dk sonrasında a. uterina Pulsed Doppler ultrasonografi ile incelendi. Servikal kanallar ile birlikte görüntülenen a. uterinadan kalp atım hızı (HR), pulsatil indeks (PI) ve rezistans indeks (RI) değerleri kaydedildi. Benzer şekilde, ikinci deneme grubunda da (n=6) hayvan başına 10 mg SC ile çalışma tekrarlandı. Birinci denemede, SC uygulama öncesi ve sonrasında ortalama HR, PI ve RI değerleri sırasıyla (223±7,80 ve 239±15,00), (1,60±0,08 ve 1,63±0,16), (0,73±0,02 ve 0,73±0,03) olarak kaydedildi. İkinci denemede ise 10 mg SC uygulama öncesi ve sonrasında ortalama HR, PI ve RI değerlerinin sırasıyla (210±15,00 ve 223±14,00), (1,46±0,23 ve 1,79±0,08), (0,68±0,05 ve 0,77±0,01) olduğu görüldü. Her iki deneme içinde uygulama öncesi ve sonrası Doppler değerlerinde farklılığa rastlanmadı (P>0,05). Sonuç olarak, tavşan başına 5 ve 10 mg dozda oral SC uygulamasının uterus perfüzyonu üzerinde arttırıcı bir etkisi olmadığı görüldü.

Anahtar kelimeler: Doppler, arteria uterina, sildenafil sitrat, tavşan.

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Introduction

Phosphodiesterase-5 (PDE-5) is responsible for the degradation of the cyclic guanosine monophosphate (cGMP) to guanosine monophosphate. Therefore, inhibiting PDE-5 delays the breakdown of cGMP and induces vasodilatation (Ballard et al., 1998). Sildenafil Citrate, a specific PDE-5 inhibitor, has been shown to potentiate the effects of nitric oxide (Ramesar et al., 2010). Nitric oxide is a key signaling agent involved in smooth muscle cells and activates the cGMP/protein kinase G pathway within smooth muscle relaxation (Hale et al., 2010). Sildenafil citrate acts as a potent vasodilator that enhances and prolongs the action of cGMP by selectively inhibiting PDE-5, which enzymatically converts the intracellular second messenger cGMP into its inactive form (Pellicer et al., 2011). Uterine blood flow has an important role in many reproductive cases, whether it is before and after pregnancy. Thus, its manipulation has been investigating as an additional therapy in lots of cases. In recent years, indications of SC in women have been explored after detection of the arterial dilatation in reproductive tract (Zoma et al, 2004; Hale et al., 2010; Satterfield et al., 2010)nulliparous women. Fifteen women were randomized in a double-blind fashion to receive either placebo or sildenafil (25 or 100 mg**.** In this direction, some investigations have been performing on infertile women (Malinova et al., 2013) especially need improving endometrial thickness (Takasaki et al., 2010). But, the most common usage of SC in pregnancies has been reported in intrauterine fetal growth retardation in women (Villanueva-Garcia et al., 2007; Von Dadelszen et al., 2011; there is no effective therapy for severe early-onset intrauterine growth restriction (IUGRTrapani et al., 2016). Also, similar studies reported in experimental animal models (Sanchez-Aparicio et al., 2008; Satterfield et al., 2010; Pellicer et al., 2011)starting from day 35 of gestation to delivery. Fetuses were delivered by cesarean section. Fetal asphyxia was induced by clamping the umbilical cord at birth for 5 minutes. Results: Sildenafil protected the pups against induced asphyxia at birth in a dose-dependent manner (eg, partial pressure (tension. Accordingly, it was seen that the improving a. uterina blood flow (Ramesar et al., 2010; Batissaco et al., 2013) and abnormal umbilical artery waveforms (Stanley et al., 2012).

There is limited knowledge regarding the effect of SC on uterine perfusion in rabbits. In this study, the effect of two different doses of SC on the uterine artery Pulsed Doppler ultrasonography findings was investigated in non-pregnant rabbits.

Material and Method

All experiments were performed on 12 healthy, sexually mature; New-Zealand white does weighing 3.6 - 4.1 kg. They were housed individually in wire mesh cages under controlled light (14 h light/10 h dark) and temperature (18 to 24 ˚C) conditions. All does were with free access to water and standard pellets. Animal handling and all procedures were performed in accordance with applicable regulations and guidelines and with the approval of the Animal Research Ethics Committee of Aydin Adnan Menderes University (64583101/14/145). Before the start of the experiments, the does were accustomed to the environment and trained to medical manipulations during three weeks.

In the first experimental group (n=6), 5 mg SC thawed in 5 ml 0.9% saline solution was administered peros way via naso-esophageal catheter. Similarly, the study was repeated second experiment group (n=6) by using 10 mg SC thawed in 5 ml 0.9% saline solution for per animal. In both groups, a. uterina was examined with Pulsed Doppler ultrasonography with an 8-MHz microconvex transducer (MyLab Vet30- Esaote®, Genova, Italy) just before drug administration. Same examinations were repeated 60 minutes after from drug administration. Pelvic transversal scanning was performed for Doppler measurements. Heart rates, PI and RI were recorded from the most cranial part of the artery visualized with cervical channels. After visualizing the a. uterina via color Doppler, Pulsed-wave Doppler examinations were performed. Recordings were obtained for at least regularly three consecutive arterial waveforms. Waveforms were disregarded during doe's movements or cardiac arrhythmias. In order to evaluate the blood flow waveform patterns of the a. uterina, the HR, PI, and RI were measured. Due to the possible thermal and cavity side effects of Doppler sound waves on tissues, Pulsed-wave examinations did not exceed 30 seconds and were recessed for 1 minute. Regarding to stress factors of the study group, all examinations lasted between 9:00-12:00 a.m. and 50-60 minutes totally in silent and dimly lit room, and were performed by the same trained operator.

Average data presented as mean ± SEM. The Mean Doppler findings between pre and post administrations were compared using paired samples t-test and the differences between Doppler findings of first and second experimental groups were evaluated with independent samples t-test. Differences were considered statistically significant at P levels of less than 0.05.

Results

During the study, the does tolerated the ultrasonographic examinations well. The a. uterina was successfully visualized and Doppler trace was recorded in all examined animals. The uterine artery blood flow was characterized low resistance and showed systolic waveform in non-pregnant does. Any pathologic finding (arrhythmia, reverse flow, end-diastolic notch etc.) was not recorded.

Table 1 presents the mean HR, PI and RI values of both experimental groups. In statistical analysis, there was no significant

Table 1: The mean HR, PI and RI values of both experimental groups.

Doppler parameters	Group 1 (n=6)		Group 2 (n=6)	
	Pre 5mg SC administration (Mean±SEM)	Post 5mg SC administration (Mean±SEM)	Pre 10mg SC administration (Mean±SEM)	Post 10mg SC administration (Mean±SEM)
HR (bpm)	223 ± 7.80	239 ± 15.00	210 ± 15.00	223 ± 14.00
PI	1.60 ± 0.08	1.63 ± 0.16	1.46 ± 0.23	1.79 ± 0.08
RI	0.73 ± 0.02	0.73 ± 0.03	0.68 ± 0.05	0.77 ± 0.01

Sildenafil citrate (SC), heart rate (HR), pulsatility index (PI), resistance index (RI) (P>0.05).

difference between pre and post-administration Doppler findings in both experimental group (P>0.05). Also, the results of both experimental groups were found similar (P>0.05). Although, there were a tendency to increase uterine perfusion after SC administrations especially in 10 mg dose, the differences did not reach statistical significance.

Discussion

Uterine blood flow has an important role in many reproductive cases either before or after pregnancy. Thus, the manipulation of blood flow has investigated as an additional therapy in lots of cases. It has been reported that the support the uterine perfusion with SC may improve implantation (Sher and Fisch, 2002; Simon and Laufer, 2012)with normal ovarian reserve and at least two consecutive prior IVF failures attributed to inadequate endometrial development. Intervention(s and fetal growth restriction (Wareing et al., 2005). Also, it can be used in postoperative uterine adhesions (Batukan et al., 2007) as well as Ashermann syndrome (Zinger et al., 2006)Pfizer, Inc., New York, NY.

Although SC is used in females of different species, little reproductive findings obtained from females have been in rabbit model (Lopez-Tello et al., 2017)which had increased systolic peak and time-averaged mean velocities at the MCA. Furthermore, fetuses in the SC group had significantly higher biparietal and thoracic diameters and longer crown–rump lengths than fetuses in Group R. Hence, the SC group had a reduced IUGR rate and a higher kit size at birth compared with Group R. In conclusion, SC may provide potential benefits in pregnancies with placental insufficiency and IUGR, partially counteracting the negative effects of food restriction on placental development and fetal growth. However, the present study also found evidence of a possible blood overflow in the brain that warrants further investigation. López-Tello et al., 2017. In our study, SC was given orally in healthy non-pregnant rabbits and the acute effect on the arteria uterina blood flow was investigated. A Previous study has reported that SC provides 40% bioavailability after oral administration (Villanueva-Garcia et al., 2007). Sildenafil citrate was administered 0.7 mg/kg IV and 1.4 mg/kg PO in previous rabbit studies (Ockaili et al., 2002; Das et al., 2004). These doses were equivalent to clinical dose of 100 mg for 70 kg human patient (Das et al., 2004). The based on these reports, we preferred to use 5 and 10 mg doses per rabbit.

It was shown that SC causes significant increase in uterine blood flow of non-pregnant females in the luteal phase (Hale et al., 2010; Dzieciol et al., 2014, 2015). Taking into account these findings, it would be seen that the different application procedures cause similar results. The results of our study show that no changes of a. uterina blood flow parameters was in response the SC administration in rabbit model in 5 and 10 mg experimental groups. In our study, all the rabbits were in follicular phase of estrus cycle since there was no stimulation (mating or GnRH injection) to induce ovulation subsequently to start luteal phase. Disregarding the doses and routes, unaltered a. uterina blood flow might be related with basal progesterone level of the non-pregnant follicular phase of the rabbits. In further studies, SC use can be done in experimental luteal stage following GnRH injection or artificial cervical stimulations. In this way, it can be examined that whether or not a relation between the level of SC impact and progesterone level. Another result of our study was the well toleration of the SC by study material, and no pathologic finding (reverse flow, end-diastolic notch etc.) seen during Doppler examinations. It was not recorded any general complication after oral administrations. In conclusion, although there was no difference in uterine artery blood flow after SC administration of 5 and 10 mg doses, it is considered that needs further studies reexamining different doses and routes on rabbits in luteal phase.

Conflict of interest

The authors declare that they have no competing interests.

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