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Effect of viagra on retinal vein diameter in AMD patients

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Abstract

The aim of the present study was to investigate the effect of sildenafil citrate (viagra) on retinal venous diameter in patients with age-related macular degeneration (AMD). We investigated 14 male patients in a double-masked, randomized, placebo-controlled, crossover study. In each subject, one eye with typical non-exudative AMD fundus features was studied. Each of the subjects received 100 mg dose of sildenafil or matching placebo on two separate study visits. Monochromatic fundus photographs were obtained in the study eye before dosing and then 30, 90, 180 and 300 min later. Measurements of the diameter of the major retinal veins from digitized negatives were carried out using ''Vessel map'' static vessel analysis program (IMEDOS GmbH, Weimar, Germany). Statistical analysis of the data comparing the effect of sildenafil and placebo on venous diameters was performed using analysis of variance (ANOVA) for repeated measures. An analysis of variance (ANOVA) comparing the effects of sildenafil citrate and placebo on retinal vein diameters showed a significant interaction between time and treatment ($P = 0.03$). In comparison to placebo, sildenafil citrate produced a statistically significant vasodilatation of major retinal veins of 4.7% at 90 min ($P = 0.004$), 5.5% at 180 min ($P < 0.0001$) and 5.8% at 300 min ($P < 0.0001$). At 30 min there was a 2.2% difference, which was not statistically significant ($P = 0.14$). Our results suggest that in patients with age-related macular degeneration, sildenafil citrate (viagra) produces a statistically significant vasodilatation of major retinal veins that is similar to what has been reported in normal subjects. Whether this vasodilatation is associated with changes in retinal blood flow needs to be further investigated.

Keywords: sildenafil citrate (viagra); retinal vein diameter; age-related macular degeneration (AMD)

1. Introduction

Sildenafil citrate (viagra), the first oral drug approved for the treatment of erectile dysfunction, selectively inhibits phosphodiesterase 5 (PDE5), the isozyme that metabolizes cyclic guanosine monophosphate (c-GMP) in the corpus cavernosum (Boolell et al., 1996). In addition to the corpus cavernosum smooth muscle, PDE5 is also found in other human tissues including vascular smooth muscle (Pfizer, Investigative Brochure). Endothelium-derived relaxing factors, such as nitric oxide (NO) (Furchgott and Vanhoutte, 1989; Ignarro et al., 1987) produce smooth muscle relaxation and dilatation of blood vessels due to the increase in levels of cGMP (Furchgott and Vanhoutte, 1989; Gruetter et al., 1981; Ignarro and Kadowitz, 1985). By blocking PDE5 sildenafil increases the levels of c-GMP and thus greatly enhances the dilating effects of NO on the vascular smooth muscle.

This vasodilatory quality of sildenafil is of great interest because of the potential use of this type of compound in the treatment of vascular occlusive disease. Indeed several investigators have studied the effects of sildenafil on the retinal vessel diameter in normal subjects (Grunwald et al., 2002; Pache et al., 2002; Polak et al., 2003). Vasodilatation induced by sildenafil in the human retina was reported by some studies (Pache et al., 2002; Polak et al., 2003), but not all (Grunwald et al., 2002).

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In this study we investigate the effect of sildenafil on the retinal vessel diameter in patients with age-related macular degeneration (AMD). Previous reports of Friedman et al. (1989, 1995), Grunwald et al. (1998, 2005) and Pauleikhoff et al. (1990) have suggested that impairment of the choroidal circulation may play an important role in the etiology of this disease. Little is known however, about the retinal vasculature changes in this disease. Sato et al. (2005) has shown using laser Doppler velocimetry that pulsatily in retinal arteries is higher in patients with AMD than healthy controls. The exact implications of this finding are not known but suggest that perfusion abnormalities go beyond the choroid in patients with age-related macular degeneration. Because the vasculature of the fundus seems to be affected by AMD it is important to study whether sildenafil affects the vasculature of AMD patients. A previous study of Metelitsina et al. (2005) did not show any statistically significant effect of sildenafil on the choroid of AMD patients. In this study we investigate the effect of sildenafil on the retinal vasculature of AMD patients.

2. Materials and methods

Fourteen male AMD patients (13 Caucasians and 1 African-American) with a mean age of 75 ± 7 years ($\pm$1SD) were included in this double-masked, randomized, placebo-controlled, crossover study. AMD features of enrolled patients were similar or worse than those present in eyes graded as AMD category 3 in the AREDS study. Only one eye of each patient was included in the study. Study eyes had clear ocular media, intraocular pressure (IOP) of 21 mm Hg or less, pupillary dilatation of 5 mm or more, steady fixation, no intraocular diseases other than AMD and no choroidal neovascularization (CNV). Right eyes were chosen in ten patients and left eyes in the other four. All study eyes had large drusen and eight eyes had retinal pigment epithelium hyperpigmentary changes. Five patients had small areas of extrafoveal geographic atrophy in the study eye or the fellow eye. Two patients had exudative AMD with a disciform scar present in the fellow eye. External and slit lamp examinations were normal except for the presence of mild lens nuclear sclerosis in nine study eyes and intraocular lens implants in three eyes.

A history of well-controlled systemic hypertension was reported in nine patients. All of them were taking antihypertensive medications. None of the 14 subjects enrolled in the study had a history of systemic hypotension or serious heart condition or was receiving nitrate therapy. All study subjects took the same medications throughout the length of the study, and none of the study participants was under fasting conditions.

The study was carried out after the approval from University of Pennsylvania Institutional Review Board. The tenets of Declaration of Helsinki were followed. All subjects signed an appropriate IRB approved consent form after the detailed explanations of the study procedures. We have previously reported our findings on the effect of sildenafil citrate on the choroidal blood flow in this cohort of patients (Metelitsina et al., 2005).

The study design included two separate study visits, with all patients being randomized to receive a single oral dose of either 100 mg of sildenafil citrate (Viagra; Pfizer Inc, New York) or matching placebo on the first study visit. The alternative drug was tested on the second study visit. Placebo pills were identical to the sildenafil ones, but they did not contain the active component. The same protocol was performed on both study days, which were separated by a wash out period of three or more days. In order to prevent bias, both patients and investigators were masked to the treatment modalities.

After pupillary dilation was achieved with tropicamide 1% (Alcon, Fort Worth, TX) and phenylephrine hydrochloride 10% (Sanofi Winthrop, New York, NY), monochromatic fundus photographs ($\lambda = 570$ nm) were obtained with a Zeiss fundus camera (Oberkochen, Germany) on Kodak Plus-X pan film (Rochester, New York, USA). Photos of the study eye of each patient were obtained while the subjects were seated in the darkened room. Intraocular pressure (IOP), brachial artery systolic and diastolic blood pressure (BPs and BPd) and heart rate (HR) were obtained immediately after the photographs were taken. Tono-pen XL (Mentor Ophthalmics, Norwell, MA) and automated sphygmomanometer (Accutorr 1A, Datascope, Paramus, NJ) were used to measure IOP and BP, respectively. The mean brachial artery pressure (BPm) was calculated according to the following formula:

$$\mathrm{BPm} = \mathrm{BPd} + 1/3(\mathrm{BPs} - \mathrm{BPd})$$

Perfusion pressure (PP) for the study eye was estimated according to the following formula:

$$\mathrm{PP} = 2/3\mathrm{BPm} - \mathrm{IOP}$$

All tests mentioned above were performed prior to the administration of the drug, and then 30, 90, 180 and 300 min, thereafter. These times were chosen to coincide with the maximal serum concentration levels of sildenafil, which are reached in 30–60 min. Plasma half-life of sildenafil is about 4 h (Marmor and Kessler, 1999).

Approximately ten fundus photographs were obtained at each time point for each patient. Photographic negatives were scanned and digitized, using a Nikon SF-2000 35 mm film scanner (Tokyo, Japan). Out of the ten digital images, five photographs with the sharpest focus were selected for further analysis in a masked fashion.

Retinal vein diameter was measured with ''Vessel map'' static vessel analysis program software (IMEDOS GmbH, Weimar, Germany). This program enables the determination of retinal vessel diameter from digitized photographs. The vessel diameter is determined segment by segment along the length of a blood vessel in a predefined measurement window. The instrument creates an intensity or brightness profile perpendicular to the vessel. The width of the vessel is defined as the distance from wall to wall through the midpoint of the vessel.

Two reference points were identified on vessel bifurcation for each photograph (see Fig. 1). This allowed us to overlap all images of one subject assuring that all fundus details from one picture corresponded to the same fundus details in all images of the same patient. This preliminary step enabled

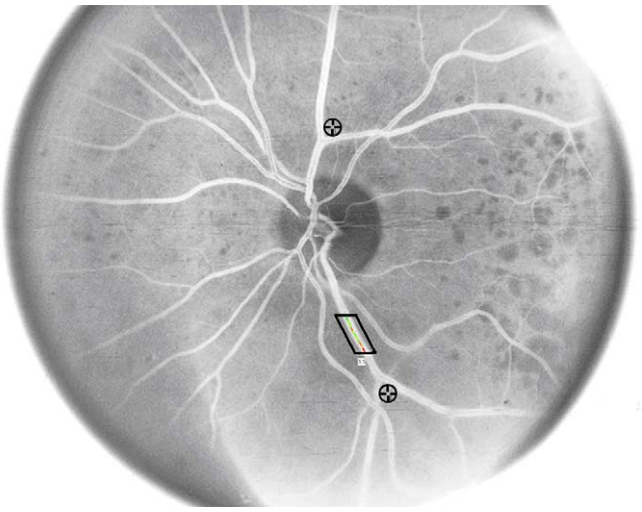

Fig. 1. Monochromatic fundus photograph of a typical study eye of an AMD patient with multiple large drusen. Measurement of the inferior temporal vein segment was performed using the ''Vessel map'' software. Rectangle shows segment of the vessel chosen for measurement. Circles show two reference points that are used by the software to overlap multiple images.

multiple measurements of the same vessel simultaneously on multiple images.

A predefined measurement window of about −1/2 disk diameter ensured that the length of the vessel segment measured was the same for all images of the same subject (Fig. 1). The same segment of the vessel chosen for analysis (Fig. 1) was evaluated in all photos of the same patient. To make sure that the location of the measurement window was consistent on all images, a transparent plastic template with an outline of the disk and the major vessels was superimposed on all photographs before measurements.

Measurements were performed on straight segments of the vessels within 1.5 disk diameters from the edge of the optic nerve head. We avoided vascular bifurcations and arteriovenous crossings. Major veins were chosen for analysis. In ten patients, two major veins were measured. In four patients only one major vein was analyzed because a second vein in proper focus was not available in all photographs. Thus a total number of 24 veins were included in our analysis. All circulatory measurements are shown in arbitrary units (AU).

Statistical analysis of the data comparing the differences in retinal vessel diameters before and after administration of sildenafil and placebo was performed using analysis of variance (ANOVA) for repeated measures. To compare the differences between these two groups at each time point (baseline, 30, 90, 180 and 300 min), alpha level adjustments for multiple comparisons by means of Bonferroni approach were also carried out. Because we had five time points comparisons, we considered $P = 0.01$ $(0.05/5 = 0.01)$ as statistically significant. Regression analyses between vein diameter changes from baseline and pressure changes (IOP, PP and BP) from baseline were also performed and a P value of 0.05 was considered to be statistically significant. Since the diameters from two veins were measured for most of the patients, the generalized estimating equations (GEE) were used to adjust the correlation from multiple veins from the same eye. SAS 9.1 software (Cary, North Carolina, USA) was used for the analysis of the data.

3. Results

Mean retinal venous diameters at baseline, 30, 90, 180 and 300 min for sildenafil and placebo treatments are shown in (Table 1, Fig. 2). An analysis of variance (ANOVA) comparing the effects of sildenafil citrate and placebo on retinal veins diameters showed a significant interaction between time and treatment ($P = 0.03$), therefore, comparisons of both treatments at each time point were performed.

In comparison to placebo, sildenafil citrate caused a statistically significant vasodilatation of major retinal veins at 90 min ($P = 0.004$), 180 min ($P < 0.0001$) and 300 min ($P < 0.0001$). Compared with the mean of placebo treatment, the mean venous diameter after sildenafil citrate treatment was 4.7% larger at 90 min, 5.5% larger at 180 min, and 5.8% larger at 300 min. There was no statistically significant difference between placebo and sildenafil at 30 min ($P = 0.14$).

Because our previous study (Metelitsina et al., 2005) had demonstrated a statistically significant decrease in BPm of 15.2% ($P = 0.006$) and PP of 22.3% ($P = 0.006$) at 30 min after administration of sildenafil citrate, we investigated whether changes in BPm and PP are associated with an increase in venous diameter after sildenafil treatment. Our analysis, however, showed no statistically significant association between changes in retinal vein diameters and changes in BPm ($P = 0.07$) or PP ($P = 0.25$). Also no statistically significant association between changes in retinal vein diameter and changes in IOP ($P = 0.67$) after sildenafil were detected (Table 2).

4. Discussion

In this current study we investigated the effect of sildenafil on retinal vessel diameter in patients with AMD. Sildenafil citrate produced statistically significant dilatation of major retinal veins at 90, 180 and 300 min in this group of patients.

The effect of sildenafil on the retinal vessel diameters of AMD patients has not been studied before. Our previous study on the effects of sildenafil on the choroidal circulation of AMD patients did not show any statistically significant

Table 1
Venous diameters after administration of sildenafil citrate and placebo at all study time points

Time (min)	Venous diameter (μm)		Difference (mean (SE))	P value[a]
	Sildenafil citrate (mean (SE))	Placebo (mean (SE))		
0	230 (9)	228 (9)	2 (3)	0.50
30	237 (9)	232 (9)	5 (3)	0.14
90	244 (9)	233 (9)	11 (3)	0.0004
180	248 (9)	235 (9)	13 (3)	<0.0001
300	239 (9)	226 (9)	13 (3)	<0.0001

[a] Correlation from multiple veins of the same eye was adjusted.

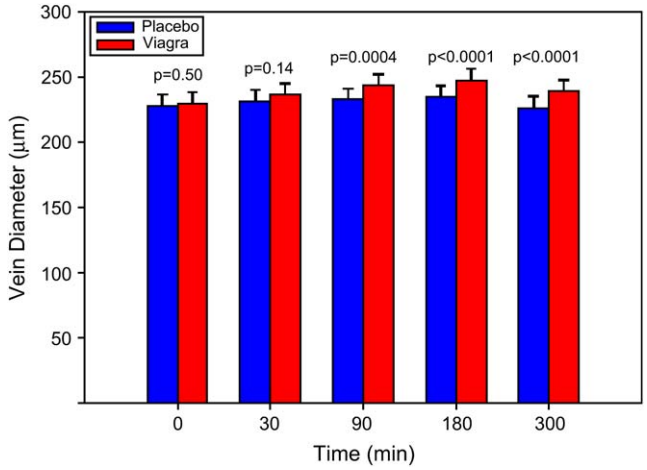

Fig. 2. Average retinal venous diameters in µm at baseline, 30 min, 90 min, 180 and 300 min after treatment with placebo and sildenafil citrate (viagra). Error bars correspond to ±1 standard error (SE).

differences in the choroidal blood flow parameters after administration of sildenafil or placebo at any of the time points (Metelitsina et al., 2005). The differences between the findings obtained in these two studies may be due to the fact that the retinal and choroidal circulations are two different vascular beds that may have different concentration of target receptors. The significance of this retinal vasodilatation following sildenafil treatment in AMD and normal patients is not known at this time. This issue should be further clarified in the future when the effect of sildenafil on retinal blood flow is elucidated.

We also attempted to measure the effect of sildenafil citrate on retinal arteries. We were able to measure only 11 arteries of 11 patients. One of the major problems was that the light reflex present in the center of the arterioles introduced errors in the diameter measurements and this prevented us from getting an accurate estimation of arterial diameters in many instances.

All previous studies of the effect of sildenafil on the retinal vasculature were performed in normal volunteers. Polak et al. (2003) showed a significant 4.7% increase in the retinal venous diameter at 80 min after 100 mg of sildenafil citrate. The findings from this study and the timing of the changes observed are very similar to our current data. Maximum serum levels of sildenafil are reached in 30–60 min (Marmor and Kessler, 1999) and it is interesting that we found statistically significant venous dilatation starting at 90 min and the veins remained dilatated even at 300 min.

Table 2
Association between change in retinal venous diameter and change in mean intraocular pressure (IOP), blood pressure (BPm), and perfusion pressure (PP)

Predictors	Slope (β)[a]	*P* value[b]
IOP	0.60	0.67
BPm	0.29	0.07
PP	0.33	0.25

[a] Estimated from the model: diameter change = intercept + β × pressure change.

[b] Correlations between repeated measurements from same subjects are adjusted.

Data of Pache et al. (2002) in a study without a control group also showed a 5.8% increase in venous and arterial diameter after administration of 50 mg of sildenafil. The peak of the changes, however, was observed at 30 min and by 120 min both arterial and venous diameters had returned to baseline values. In contrast to this study, Polak et al. (2003) did not observe any statistically significant effect on the retinal arterial diameters, and no effect of sildenafil was found on a flicker-induced vasodilatation in retinal arteries or veins.

Our previous study in normal male volunteers (Grunwald et al., 2002) did not reveal any statistically significant effect in retinal venous and arterial diameters after administration of sildenafil. This study, however, used an older method that had only an 80% power to detect 6.5% difference between the two groups. The lower sensitivity of this older method may explain the discrepancy with the other above-mentioned studies.

In conclusion, our results show a statistically significant vasodilatation of major retinal veins at 90, 180 and 300 min after administration of a 100 mg of sildenafil citrate in AMD patients. This effect is similar to what has been previously reported in normal subjects. Whether increased retinal venous diameters are associated with increased retinal blood flow in AMD patients and whether this effect may be of any therapeutic value are questions that need further investigation.

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