Published in final edited form as:

Exp Eye Res. 2013 June ; 111: 67–70. doi:10.1016/j.exer.2013.03.016.

Sildenafil Stimulates Aqueous Humor Turnover in Rabbits

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Abstract

Sildenafil citrate increases ocular blood flow and accelerates the rate of anterior chamber refilling after paracentesis. The latter effect could have resulted from a reduction in outflow facility or from an increase in aqueous humor (AH) production. In this study, we used scanning ocular fluorophotometry to examine the effects of sildenafil on AH turnover, and thus, AH production in eyes of live normal rabbits. For this, the rate of aqueous humor flow (AHF) was quantified with a commercially available fluorophotometer that measured the rate of fluorescein clearance from the anterior segment, which predominantly occurs via the trabecular meshwork. After ≈ 2 hrs of control scans to determine the baseline rate of AHF, the rabbits were fed 33 mg of sildenafil and allowed ≈ 45 min for the drug to enter the systemic circulation. Thereafter, fluorescence scans were retaken for an additional 90–120 min. Sildenafil ingestion increased AHF by about 36%, from 2.31 $\mu\text{L}/\text{min}$ to 3.14 $\mu\text{L}/\text{min}$ ($P < 0.001$, as two-tailed paired data, $n = 20$ eyes). This observation indicates that sildenafil citrate, which is a phosphodiesterase type-5 inhibitor currently marketed as a vasodilator (e.g., Viagra, Revatio), stimulates AHF in rabbits. Our results seem consistent with reports indicating that the drug dilates intraocular arteries and augments intraocular vascular flow. These physiological responses to the agent apparently led to increased fluid entry into the anterior chamber. As such, the drug might have utility in patients with ocular hypotony resulting from insufficient AH formation.

Keywords

Viagra; PDE5 inhibition; aqueous humor dynamics; aqueous humor production albino rabbits

Sildenafil citrate (i.e., Viagra) is a systemic vasodilator due to its selectivity as a cGMP-specific phosphodiesterase type 5 (PDE5) inhibitor ($\text{IC}_{50} \approx 4 \text{ nM}$) (Laties and Fraunfelder, 1999; Marmor and Kessler, 1999). This agent was originally designed to treat cardiac ischemic conditions and is presently administered as an effective treatment for erectile dysfunction, as well as, other vascular diseases, including pulmonary hypertension (Jackson *et al.*, 1999; Konstantinos and Petros, 2009). PDE5 inhibitors promote smooth muscle relaxation and subsequent blood vessel dilation by increasing cGMP levels.

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In the eye, sildenafil is recognized as an inhibitor of PDE6 (IC₅₀ ≈40 nM), which is solely found in the retina and is a critical enzyme in the regulation of the phototransduction cascade (Laties and Zrenner, 2002). Separately, the drug has been reported to increase ocular blood flow (Harris *et al.*, 2008), presumably due to its activity as a PDE5 inhibitor. In general, sildenafil may raise blood flow velocity in the retrobulbar and choroidal circulation (Harris *et al.*, 2008), and elevate blood flow to the ciliary body via an increase in the flow of the posterior ciliary artery and its pre-capillary arterioles (Koxsal *et al.*, 2005). Such flow could result in a higher leak of plasma-like fluid from the fenestrated capillaries of the ciliary body, and subsequent leakage of such fluid into the anterior chamber (AC).

Consistent with the latter possibility, we reported that sildenafil elevated intraocular pressure (IOP) and AC protein concentration in a sheep animal model (Gerometta *et al.*, 2010), effects consistent with a stimulation of plasma-like fluid entry into the eye, and increased the rate of IOP recovery after paracentesis in sheep and rabbit animal models (Gerometta *et al.*, 2012).

IOP recovery subsequent to paracentesis reflects the refilling of the AC with “secondary” aqueous. The fact that such IOP recovery, with concomitant aqueous humor (AH) refilling, was saliently enhanced by sildenafil in two different animal models, implied that the vasodilator not only provided more fluid for secondary aqueous formation after paracentesis, but might also stimulate AH turnover in the normal eye. However, we did not measure AH turnover directly in our previous studies (Gerometta *et al.*, 2010, 2012), and there are no reports in the literature on the effects of the PDE5 inhibitors on AH turnover and/or the rate of AH formation. Both the accelerated rates of IOP restoration after paracentesis in animals administered sildenafil (Gerometta *et al.*, 2012), as well as, the findings that sildenafil increases vascular flow in the eye due to dilations of intraocular arteries (Koxsal *et al.*, 2005; Harris *et al.*, 2008), suggest that the vasodilator may stimulate the turnover of AH of the normal eye. The aim of this study was to directly test this hypothesis.

In this work, our general protocol was to determine the aqueous flow in rabbits by fluorophotometry before and after the animals orally ingested sildenafil. We present evidence indicating a higher rate of AH turnover in normal rabbits systemically administered the PDE5 inhibitor.

All animal experiments were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) guidelines. Ten adult albino rabbits of either sex weighing 2.5–3 kg were purchased via the Mount Sinai Animal Facility that obtains the animals from biological suppliers throughout the Northeast. The rabbits were well cared for by animal facility personnel under veterinary supervision and transported, individually, to our laboratory when needed for experiments. In the laboratory, the rabbits were examined non-invasively with the fluorescein corneal depot method (Brubaker, 1989) on separate days.

Each rabbit bilaterally received one drop of topically applied 0.5 % proparacaine hydrochloride (Alcon Laboratories, Fort Worth, TX, USA) onto the central cornea followed by consecutive, 50 µL instillations of a 2 % fluorescein Na solution (Alcon) that were administered every 5 min to both eyes over 6 applications between 5:00 and 5:30 PM on the evening previous to the fluorophotometric experiment. Blinking was prevented between drops, and for 5 min after the final drop, with a lid speculum. Then, the lids, eyelashes, conjunctival sac, and fur around the eye were washed with saline to remove excess fluorescein.

The next morning (usually ≈ 9–10 AM), the rabbit was snugly wrapped in a diaper that was firmly secured about the animal with Velcro straps so that only its head protruded

freely. The rabbit was then placed on a platform stage with one of its eyes aligned with the objective of a FM-2 Fluorotron (TM) Master Ocular Fluorophotometer (OcuMetrics, Mountain View, CA, USA) in a darkened room and given ≈ 30 – 40 min to acclimate to this condition (no systemic anesthesia was administered). Thereafter, 2 or 3 consecutive scans of the visual axis were rapidly taken (≈ 1 min per scan) of the eye facing the objective, after which, the rabbit was repositioned to align the contralateral eye with the objective of the fluorophotometer. Scans were then taken of the fellow eye.

Each scan provided a measurement of the fluorescein concentrations in the cornea and anterior chamber. Fig. 1A shows a representative scan obtained from the scanning fluorophotometer. The instrument made point measurements (along the visual axis of the eye) of the fluorescein concentration using a stepper motor that moved the focal point in 148 steps from a focus beyond the back of the eye toward the photometer lens. As such, the x-axis is labeled 0 mm at the approximate point at which a measurement was made within the back of the eye. No fluorescence was recorded between 0 and 11.5 mm, indicating the absence of detectable fluorescence from the vitreous and eye lens of the rabbit. The plateau seen between 11.5 and 15.5 mm represents the fluorescein concentration measured from the AC, which in this example was 280 ng/mL. The peak centered about 19.5 mm from the back of the eye represents the fluorescein concentration in the cornea, with the circle atop the peak denoting the position at which the maximum concentration was measured, which was 1455 ng/mL.

Most rabbits were docile and did not move during the brief (≈ 1 min) duration of the individual scans. On occasion, an animal would blink or move slightly during a scan in progress. In such cases, the resulting scan was immediately deleted and a new scan promptly performed. In principle, our experiments aimed to obtain as many reliable scans as possible during an initial ≈ 120 -min control period, after which, the rabbit was fed sildenafil and a new series of scans lasting ≈ 90 – 120 min in duration were initiated approximately 45 min after the animal ingested the drug.

Tablets (100 mg) of sildenafil citrate (Vorst[®], Laboratorios Bernabo, Argentina) were purchased from a local pharmacy in Argentina and brought to New York for the presently described experiments. We decided to use such tablets because they were used in our earlier studies on sheep and rabbits (Gerometta *et al.*, 2010, 2012). Each 100 mg tablet was pulverized with a mortar and pestle, divided into thirds, with the resulting fraction then stirred into a mashed, well-ripened banana. The rabbits, from whom food was withheld for about 8 hours prior to the experiments, vigorously ate the banana containing ≈ 33 mg sildenafil citrate at the appropriate point of the protocol.

The sequentially compiled scans that were obtained from the ocular fluorophotometer (OcuMetrics) provided measurements of the changes in the corneal and AC fluorescein concentrations over time. From these data, aqueous humor flow (AHF) through the AC was calculated from the rate of fluorescein clearance from the anterior segment minus an assumed correction for fluorescein diffusional loss from the anterior segment of $0.25 \mu\text{L}/\text{min}$ (Brubaker, 1989), using the following equation: $\text{AHF} = [\Delta M/(\Delta t \times C_{\text{ac}})] - 0.25 \mu\text{L}/\text{min}$, where ΔM is the loss of mass of fluorescein from the combined cornea and anterior chamber during an interval Δt , and C_{ac} is the average concentration of fluorescein in the anterior chamber during the same interval (estimated from the initial and final concentrations). The mass of fluorescein in each compartment was determined by multiplying the fluorescein concentration (directly obtained from the ocular fluorophotometer) by the compartment volume. The volumes of the rabbit cornea and AC were assumed to remain constant throughout the protocol at $80 \mu\text{L}$ and $200 \mu\text{L}$, respectively (Minkowski *et al.*, 1984; Toris, 2008).

The significance of experimentally elicited changes in aqueous humor flow were analyzed using Student's t-test as paired data, with $\alpha = 0.05$ chosen as the level of significance. Comparisons of the aqueous humor flow measured in the right eye (oculus dexter, OD) versus that measure in the left eye (oculus sinister, OS) were made using Student's t-test as unpaired data.

Data from a representative experiment are plotted in Fig. 1B, which illustrates the changes in corneal and AC fluorescein concentrations that were obtained from sequential scans taken with the fluorophotometer, both before and after the rabbit was administered sildenafil. Time 0 in the figure represents 9:00 AM on the morning after the animal was pre-treated with topical instillations of fluorescein at 5:00 PM on the previous evening. Control scans of the rabbit eye began at $\approx 9:40$ AM, after allowing the rabbit time to acclimate to the conditions of being tightly held in a diaper before the ocular fluorophotometer for about 20–30 min. Control scans were taken at various time points over a 120-min period, while post-sildenafil scans were taken over a 90-min period. From the clearance in the mass of fluorescein from the corneal and AC compartments over the respective 120-min and 90-min periods, aqueous flow rates of 1.97 and 2.81 $\mu\text{L}/\text{min}$ were calculated for this experiment.

Table 1 compiles the AHF rates of the 20 eyes from the 10 rabbits that were assayed in this study. The mean control flow rate was 2.31 $\mu\text{L}/\text{min}$ prior to the administration of sildenafil, and 3.14 $\mu\text{L}/\text{min}$ ($\approx 36\%$ increase) after the rabbits ingested the compound.

As seen in the table, this method exhibits some variability, at least in our hands, in that the contralateral, fellow eyes of each animal did not express an identical rate of aqueous flow, and did not exhibit a similar percentage change in the increase in the rate of AHF after sildenafil administration. The stimulation in AHF elicited by sildenafil in the individual eyes ranged from $\approx 12\%$ to 64% .

Nevertheless, when the flow rates from the 10 right eyes are averaged and compared to the mean of the 10 rates obtained from the 10 left eyes, the means are virtually identical, i.e., the mean of the 10 OD control values in Table 1 is $2.29 \pm 0.11 \mu\text{L}/\text{min}$ ($\pm$ SEM), while that of the 10 OS control values is $2.32 \pm 0.12 \mu\text{L}/\text{min}$ ($\pm$ SEM; $P > 0.87$, as unpaired, two-tailed data). Likewise, averaging the 10 post-sildenafil OD values for AHF in Table 1 gives a mean of $3.13 \pm 0.16 \mu\text{L}/\text{min}$, while the mean of the post-sildenafil OS values is $3.14 \pm 0.15 \mu\text{L}/\text{min}$ ($P > 0.94$, as unpaired, two-tailed data). As such, on average, the response of the AHF rate to sildenafil ingestion was largely self-consistent with a $\approx 36\%$ increase. The individual variability exhibited by contralateral eyes of the same animal is most likely due to inherent limitations in the method. Yet, it was clear, that sildenafil evoked an increase in AHF in all cases.

In this study, AHF was measured non-invasively in rabbits using the fluorescein dilution technique, whereby the cornea serves as a depot, from which the dye diffuses into the AC and is cleared from the anterior segment by the flow of aqueous. The changes in corneal and AC fluorescein concentrations with time were quantified with the software of a commercially available scanning ocular fluorophotometer. Fluorophotometry measurements are based on methods developed in humans (Jones and Maurice, 1966), and later automated with a computerized fluorophotometer (McLaren and Brubaker, 1985).

Using this methodology, we determined that oral sildenafil ingestion augmented the rate of AHF in all rabbits that were administered the PDE5 inhibitor. Our present results buttress an interpretation that we presented earlier to explain the observation that oral administration of sildenafil increased the rate of IOP restoration in sheep and rabbits subjected to a rapid depressurization of the eye by paracentesis of the AC (Gerometta *et al.*, 2012). In this latter study, IOP was measured, paracentesis performed on one eye, and AC refilling followed by

continuous IOP measurements as the AC formed. After IOP stabilization, sildenafil was orally administered. Forty-to-sixty min later, AH was withdrawn from the contralateral eye. The point at which IOP recovered was used to determine the refilling time. We estimated AH refilling rates by dividing the volume of the paracentesis by the IOP recovery time. After sildenafil, such AH refilling rates were markedly larger. With rabbits administered sildenafil, for example, the time necessary for IOP restoration was approximately halved, and the estimated AH refilling rates therefore doubled, to values larger than the AH formation rate attributed to secretion by the ciliary epithelium (Gerometta *et al.*, 2012).

The aforementioned accelerated rates of IOP restoration after paracentesis in animals administered sildenafil in our earlier study (Gerometta *et al.*, 2012) suggested that the vasodilator might increase the turnover of AH (independently of paracentesis), and the data obtained in this study are consistent with this suggestion. These most recent experiments provide important information because it was also hypothetically possible that sildenafil might inhibit the AH outflow facility by an unknown mechanism, a possibility diminished by the results of the present study.

Sildenafil relaxes the pre-capillary arterioles. Thus, relaxing the ciliary pre-capillaries may increase the leak across the fenestrated capillaries in the ciliary body stroma. The fluid leaked from the fenestrated capillaries is about 75–100 $\mu\text{L}/\text{min}$ (Bill, 1975), most of which is reabsorbed due to the oncotic pressure created by vascular proteins, thereby leaving only about $\approx 2\text{--}3$ $\mu\text{L}/\text{min}$ to be transported by the ciliary epithelium (CE) into the posterior chamber. A small additional leak across the fenestrated capillaries elicited by sildenafil could be sufficient to increase the AHT by the 0.83 $\mu\text{L}/\text{min}$ (i.e., 36% more) recorded in the present study, presumably via CE transport, although a small leak directly from the ciliary body stroma into the AC via the iris root cannot be completely discarded.

At steady state with IOP constant, AH inflow = AH outflow. This flow can be considered the AH turnover. Upon an increase in inflow, which we interpret that sildenafil has elicited, the IOP would immediately rise, and this increased pressure would accelerate AH outflow, resulting in a new steady state with a higher IOP and a larger AH turnover, without any changes in AH outflow facility. The magnitude of the IOP increase would directly correlate with the extent of the AH inflow. Should the baseline value of the outflow facility be sufficiently large, a large increase in AH inflow could result in a relatively small change in IOP, which is a consequence of the relative rates of inflow and outflow. The resulting increase in both inflow and outflow would be detected as an increase in AHF with the non-invasive fluorescein technique, which only estimates the rate of outflow of AH from the AC and cannot determine the proportion of AH entering the AC from the posterior chamber versus the possible direct entry of fluid into the AC via the anterior surface of the iris, as we discussed earlier in detail (Gerometta *et al.*, 2010). After sildenafil ingestion, fluid may enter the AC via the latter pathway in sheep given our finding of an increased protein concentration in the AC of sheep administered sildenafil. We are unaware of any reports indicating that sildenafil disrupts the blood-aqueous barrier.

AH turnover can also be viewed as a measurement of the time needed to replace the AH in the AC. From the AHF values, a half time ($T_{1/2}$), or half-life of elimination, of 60.1 min was calculated to replace half of the fluorescein concentration of the anterior chamber under control conditions, with $T_{1/2} = 44.2$ min after the rabbits were administered sildenafil. The $T_{1/2}$ calculation assumes that the volume of the AC remains constant and requires that the AH outflow carries a component (i.e., fluorescein) that is not contained in the inflow. As such, the half-time of elimination of fluorescein from the AC was calculated from the clearance rate (i.e., the AHF) and the AC volume (V_{ac}) using the equation: $T_{1/2} = (\ln 2 \times V_{ac})/\text{AHF}$, where $\ln 2$ is the natural log of 2, and V_{ac} is assumed to be 200 μL in rabbit.

Overall, therefore, from the combination of the enhanced rate of fluorescein clearance after sildenafil ingestion, with the enhanced rate for AH entry after paracentesis in animals treated with sildenafil (Gerometta *et al.*, 2012), it seems clear that the PDE5 inhibitor stimulates fluid entry into the AC.

In the present calculations of AHF and $T_{1/2}$, we assumed that the AC volume remained constant during the experiments, which is plausible because a substantial pressure elevation would have had to occur in the eye subsequent to sildenafil ingestion in order to suspect that possible AC volume changes had come into play. The control, baseline IOP of normal rabbits does not change upon treating the animals with the PDE5 inhibitor (Gerometta *et al.*, 2012), indicating that the augmented AHF observed subsequent to sildenafil ingestion (Table 1) was matched by sufficient AH outflow to preclude a detectable pressure elevation.

In contrast, sildenafil doubles IOP in sheep (Gerometta *et al.*, 2010), and elicits ocular hypertensive effects in some studies in man (Gerometta *et al.*, 2011), although the magnitude of the IOP elevation in man, when observed, is more limited and transitory than that obtained with sheep. Nevertheless, we predict *a priori* that the PDE5 inhibitor will evoke increases in AH turnover in both sheep and humans because of the indications that the drug increases vascular flow in the eye. The effects of sildenafil on AHF in human subjects can be determined with experiments analogous to those presented herein with rabbits.

We presently hypothesize that sildenafil could have utility as an agent enhancing fluid entry into the AC of patients who experienced AH loss during eye surgery, as well as, to patients with ocular hypotony that results from insufficient AH formation (Pederson, 1996; Fine *et al.*, 2007). Such individuals may require a rapid restoration of IOP, which could be effected with drugs that stimulate fluid inflow, such as the PDE5 inhibitors.

Acknowledgments

This work was supported by National Eye Institute Grants EY00160 and EY01867, and by an unrestricted grant from Research to Prevent Blindness, Inc., New York, NY. The authors are grateful to Dr. Janet Serle, M.D. for access to the ocular fluorophotometer used in this study, and to Dr. Rong-Fang Wang, M.D., who provided essential instruction and preliminary discussion of our protocols.

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Research Highlights

- Sildenafil ingestion increased aqueous humor (AH) production in live rabbits
- AH production was measured with a scanning ocular fluorophotometer
- AH production increased by 36% after the rabbits ingested sildenafil
- $T_{1/2}$ for AH replacement was about 60 min under control conditions
- $T_{1/2}$ for AH replacement was about 44 min after sildenafil ingestion

Fig. 1.

A) Illustration of a typical fluorescence scan obtained during this study from a rabbit eye after topical fluorescein instillations. A commercially available ocular fluorophotometer was used to produce the depicted scan showing the fluorescein concentrations in the anterior segment. See text for additional details. B) Representative experiment showing fluorescein concentrations in one eye of a rabbit cornea (triangles) and anterior chamber (squares) before (solid symbols) and after (open symbols) oral sildenafil administration. See text for additional details.

Table 1

Effect of Oral Sildenafil on Aqueous Humor Flow (AHF) in Rabbits

Rabbit No.	Eye	AHF ($\mu\text{L}/\text{min}$)		Percentage Increase
		Control Rate	Rate after Sildenafil Ingestion	
1	OD	1.97	2.81	43
	OS	2.12	2.72	28
2	OD	2.45	3.15	29
	OS	2.35	3.07	31
3	OD	2.26	2.53	12
	OS	2.12	2.58	22
4	OD	2.34	3.48	49
	OS	2.13	3.16	48
5	OD	2.13	2.57	21
	OS	2.08	2.69	29
6	OD	1.93	2.74	42
	OS	1.99	3.27	64
7	OD	2.53	3.19	26
	OS	2.34	3.27	40
8	OD	3.06	4.20	37
	OS	2.67	3.19	19
9	OD	2.04	3.33	63
	OS	2.13	3.26	53
10	OD	2.23	3.27	47
	OS	3.27	4.23	29
Mean:		2.31	3.14 *	36
SEM:		0.08	0.11	3.3
(n):		20	20	20

* Value significantly larger than pre-sildenafil rate with $P < 0.001$ as paired, two-tailed data.