Sildenafil Accelerates Anterior Chamber Refilling after Paracentesis in Sheep and Rabbits

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Purpose. Sildenafil increases ocular blood flow. Thus, the authors investigated if it also increases anterior chamber (AC) refilling after paracentesis.

Methods. Corriedale sheep and albino rabbits were used as animal models. Intraocular pressure (IOP) was measured, paracentesis performed on one eye, and AC refilling followed by observation using oblique illumination. IOP measurements continued as the AC formed. After IOP stabilization, sildenafil (100 mg) was orally administered. Forty to 60 minutes later, AH was withdrawn from the contralateral eye. The point at which IOP recovered was used to determine refilling time. Paracentesis volumes were either 60, 120, or 300 μL in sheep, and 50 or 100 μL in rabbits.

Results. IOP recovered in approximately 49, 56, and 50 minutes after the 60, 120, and 300 μL withdrawals in sheep. The refilling times of the contralateral eye after sildenafil ingestion were approximately 19, 26, and 37 minutes for the respective AH withdrawals. With rabbits, IOP recovered in approximately 13 minutes after the 50 and 100 μL AH withdrawals. After sildenafil, the IOP recovery times of the fellow eye were approximately 6 minutes. AH refilling rates were estimated by dividing the paracentesis volume by IOP recovery time. After sildenafil, such rates were larger than the AH formation rate attributed to secretion by the ciliary epithelium.

Conclusions. Sildenafil accelerates the rate of AC refilling and might have beneficial utility as an agent enhancing fluid entry into the AC of patients who experienced AH loss during eye surgery, as well as in some cases of ocular hypotony. (*Invest Ophthalmol Vis Sci.* 2012;53:565–573) DOI:10.1167/iovs.11-8275

Sildenafil citrate (i.e., Viagra) is a potent cGMP-specific phosphodiesterase type 5 (PDE5) inhibitor (IC50 ≅ 4 nM) that is commonly administered to patients as an effective treatment for erectile dysfunction,[1,2] as well as for various vascular diseases, including pulmonary hypertension.[3] As a relatively potent systemic vasodilator, sildenafil was originally designed to treat cardiac ischemic conditions.[3,4] Endothelium-derived relaxing factors (e.g., NO) diffuse into the smooth muscle and increase cGMP levels, which in turn produces muscle relaxation and dilation of blood vessels. The PDE5 inhibitors potentiate the muscle-relaxant effects of NO and cGMP.[1,2]

Given the widespread application of this agent, and the fact that sildenafil is also relatively selective for PDE6 (IC50 ≅ 40 nM), which is solely found in the retina and is a critical enzyme in the regulation of the phototransduction cascade,[5] several studies examined the effects of the PDE5 inhibitor on ocular blood flow.[6–9] In general, sildenafil increases blood flow velocity in the retrobulbar and choroidal circulation.[9] It also appears that sildenafil may increase the blood flow to the ciliary body via an increase in the flow of the posterior ciliary artery.[7] Such flow could result in a higher leak of plasma-like fluid from the fenestrated capillaries of the ciliary body (CB). Augmented fluid accumulation in the CB stroma may provide (1) additional fluid for secretion across the ciliary epithelium (CE), (2) additional fluid for ultrafiltration between the CE cells (especially if the CB stroma becomes turgid and CE tight-junction permeability increases), and/or (3) a leakage of fluid directly into the anterior chamber (AC) across the front face of the iris, given the absence of an anatomic barrier at this surface.[10,11]

In sheep, sildenafil elevated intraocular pressure (IOP) and AC protein concentration,[12] effects consistent with a stimulation of plasma-like fluid entry into the eye. Moreover, sildenafil increased IOP in some studies with human subjects.[1,2,13]

In this work, we demonstrate that oral administration of sildenafil increases the rate of IOP restoration in sheep and rabbits subjected to a rapid depressurization of the eye evoked by paracentesis of the AC. We did these experiments to test our hypothesis that the combination of sildenafil (to increase CB stromal pressure) and paracentesis (to reduce AC pressure) could provide rates of aqueous humor (AH) refilling well beyond the capacity of CE transport.

We recently demonstrated the effectiveness of using Corriedale sheep (*Ovis aries*) as an animal model for glucocorticosteroid-induced ocular hypertension.[14] From this experience, we found that the docile nature of these animals, which readily submitted to manipulations such as those required for in vivo outflow facility measurements,[15] rendered this species an ideal model for both examining the mechanisms underlying corticosteroid-induced glaucoma and testing possible IOP-lowering agents.[15,16] In addition, sheep have eyes with dimensions similar to those of human eyes (e.g., an anterior–posterior axis of approximately 27 mm and an equatorial axis of approximately 30 mm). As in other ruminants, sheep have a trabecular meshwork and an aqueous plexus that is equivalent to Schlemm's canal. As such, we began our characterizations on the ocular hypertensive effects of sildenafil with this animal model,[12] a topic that we further elaborate on with the present studies.

We also conducted analogous experiments with rabbits to determine whether the PDE5 inhibitor also accelerates the rate of AH refilling in a species with which the drug, in itself, does not elicit the ocular hypertensive effect that is readily obtained with sheep (our unpublished observations). Moreover, rabbits are particularly sensitive to paracentesis and exhibit an acute ocular inflammation in response to this procedure that includes a dis-

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ruption in the barrier properties of the ciliary epithelium,[17–19] and rapid entry of protein from the ciliary body stroma into the posterior chamber (PC),[20] responses suggesting that rabbit IOP might be restored promptly secondary to paracentesis. Nevertheless, we are unaware of any reports quantifying the time necessary for IOP restoration and/or AH refilling rate after paracentesis in rabbits. As such, it was uncertain a priori whether sildenafil ingestion would lead to a measurable stimulation in the rate of IOP restoration secondary to AH withdrawal from the AC of rabbits. In this work, we demonstrate that sildenafil indeed increases the rate of IOP recovery secondary to paracentesis in two very different animal models.

Materials and Methods

Animals: Care, Husbandry, and General Experimental Procedure

All animal experiments were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) guidelines. For protocols involving sheep, a total of 14 healthy female sheep (Corriedale breed) between 12 and 24 months of age, and weighing 35 to 40 kg, were selected from a local ranch in Corrientes, Argentina for this study. The eyes and general health of the animals were considered normal by an ophthalmologist and a veterinarian, respectively. Sheep were tagged for individual identification on their ear lobes and herded from pasture as needed for the experiments. In general, the protocols entailed (1) measurement of IOP by applanation tonometry; (2) paracentesis of the anterior chamber of one eye to promptly depressurize the globe by rapid fluid removal; (3) sequential monitoring of IOP to determine the time point at which IOP recovered to its initial, control value; (4) oral administration of the vasodilator, sildenafil; and (5) a repeat of the above steps on the contralateral eye to determine the rate of IOP recovery in the fellow eye after sildenafil ingestion. Because sildenafil was administered systemically, approximately 45–60 minutes were allowed to lapse between the ingestion of the drug by the animal and the initiation of paracentesis and IOP monitoring of the second eye. Earlier observations indicated that sheep IOP is increased within this time after oral sildenafil administration.[12] This result was reproduced in the present study. For all maneuvers involving sheep, the animals were guided into a funnel corral ending in a loose-fitting yoke,[14] an arrangement allowing for movement and holding of the head by one person while another either measured IOP, administered the drugs, and/or removed AH. Between these procedures, the sheep were free to pasture.

For protocols involving rabbits, 12 adult albino rabbits of either sex weighing 2.5–3 kg were purchased from a local supplier in New York and kept at the Mount Sinai Animal Facility. They were well cared for by animal facility personnel under veterinary supervision and transported to our New York laboratory when needed for experiments. In the laboratory, the rabbits were allowed to roam freely within large cardboard boxes between the steps of the experimental protocols, which were virtually identical with those described earlier for sheep. At the appropriate times necessary for measuring IOP or performing paracentesis, the rabbits were wrapped within a diaper with only their head exposed and firmly held by one individual while a second investigator completed the necessary maneuvers on the conscious animal. Care was taken not to squeeze or apply force to the animal while it was being held. The rabbits acclimated to this procedure and learned to relax while being held as the protocols progressed, which generally took approximately 2 to 3 hours for each rabbit.

Drug Administration

With sheep, oral ingestion of the vasodilator entailed the placement of a 100 mg tablet of sildenafil citrate (Vorst; Laboratorios Bernabo, Buenos Aires, Argentina) in the animal's mouth, followed by pouring water into it to force the animal to swallow. The docile nature of sheep rendered them compliant to this procedure. Tablets of sildenafil were purchased without prescription from a local pharmacy in Argentina. Such tablets were also brought to New York and given to the rabbits. However, for rabbits, each 100 mg tablet was pulverized with a mortar and pestle, divided into thirds, with each resulting fraction then stirred into a mashed, well-ripened banana. The rabbits, from whom food was withheld for approximately 8 hours before the experiments, vigorously ate the banana containing approximately 33 mg sildenafil citrate at the appropriate point of the protocol.

Measurement of IOP of Conscious Sheep and Rabbits under Local Anesthesia

Sheep were led to a funnel corral and their heads were suitably oriented within a neck yoke to enable an investigative ophthalmologist (R.G.) to measure IOP with a Perkins tonometer. Before the IOP measurement, 2 drops of topical proparacaine (Alcaine, 0.5%; Alcon, Buenos Aires, Argentina) followed by 2 drops of 0.25% fluorescein were instilled. Measurements were taken on each eye, alternating first one eye and then the other. The Perkins tonometry readings were converted to mm Hg as described in detail previously.[14]

The IOP of rabbits was recorded from measurements made with an applanation tonometer (Tono-Pen XL; Mentor O&O, Norwell, MA). For this, the eyelids were gently opened, 2 drops of topical 0.5% tetracaine HCl were applied, and the Tono-Pen reading was taken without applying pressure to either the ocular surface or the head of the rabbit. As with sheep, the IOP was measured in one eye and then the other, so that throughout the experiment a record of the simultaneous pressures in both eyes was continuously recorded.

Use of the Perkins tonometer with sheep and the Tono-Pen (Mentor O&O) with rabbits was merely coincidental and related to logistic considerations at the time the experiments were carried out in Corrientes and New York, respectively. Because our primary interest was the comparison of pressures (i.e., paired data before and after paracentesis, with and without systemic sildenafil administration) rather than the absolute IOP values, we were able to accomplish our objectives using these different devices.

Paracentesis of Anterior Chamber and Recovery of IOP

After topical application of a local anesthetic to the ocular surface (either 2 drops of 0.5% proparacaine, or 2 drops 0.5% tetracaine), a custom-built eye speculum for each species was inserted, and the cornea was impaled near the angle (i.e., approximately 2 mm from the limbus), with a 28-gauge needle attached to a 1-mL syringe. Care was taken to avoid touching the iris or lens by entering the eye at an angle perpendicular to the visual axis. Approximately 60, 120, or 300 μL of aqueous were drawn, in separate experiments, from the anterior chambers of sheep, whereas 50 or 100 μL of AH were aspirated from rabbit eyes. The volumes of AH withdrawn were a fraction of the total aqueous humor in these animals, that is, approximately 900 μL in sheep and approximately 200 μL in rabbits.

The paracentesis was completed within 5 seconds, which immediately reduced IOP and rendered the globe somewhat flaccid in the case of the largest volume withdrawal in sheep, and with all volumes withdrawn from the much smaller rabbit eye. As fluid entered the eye, repressurizing the globe, the point at which the IOP could again be quantified with the Perkins and Tono-Pen (Mentor O&O) devices was determined and rate of IOP recovery was then plotted.

Data Analysis

The significance of experimentally elicited changes in IOP and IOP-recovery time were analyzed using Student's *t*-test as paired and unpaired data, respectively, with $\alpha = 0.05$ chosen as the level of significance.

Results

IOP Recovery after Paracentesis in Sheep

The IOP in both eyes of the normal sheep used in this study was measured before any treatment to establish the baseline

values. The measured Perkins tonometry readings and the equivalent IOP as determined from a calibration curve indicated baseline pressures between approximately 9 and 11 mm Hg, values similar to those obtained earlier.[12,14–16] On determining bilateral control values, either 60, 120, or 300 μL of AH were rapidly withdrawn from the right eye of 10 sheep (representative experiments shown in Fig. 1). Subsequent refilling of the AC was followed by observation using oblique illumination and IOP was continuously monitored as the AC formed. Sheep IOP recovered to initial values in 49, 56, and 50 minutes after the respective AH withdrawals (Table 1).

We can calculate an average for the AH refilling rate by merely dividing the volume of the paracentesis by the time needed for IOP restoration to control values. Such rates were 1.2, 2.2, and 6.0 μL/min for each of the respective withdrawals (Table 1).

After the IOP of the right eye recovered and stabilized, the sheep were orally administered 100 mg sildenafil (Fig. 1), which increases their IOP between 1.6- and approximately 2-fold within 1 hour.[12] In sheep, the ocular hypertensive effect of the PDE5 inhibitor persists for approximately 3 hours.[12] Under this ocular hypertensive condition, AH was withdrawn from the contralateral eye and the time necessary for IOP recovery was again determined. Such recovery times were significantly shorter than those exhibited by the right eye before systemic treatment with sildenafil (Fig. 1). Subsequent to the withdrawal of 60, 120, or 300 μL of AH from the left eye, IOP returned to the elevated baseline pressures in approximately 19, 26, and 38 minutes, representing estimated AH refilling rates of 3.2, 4.7, and 8.1 μL/min that are significantly higher than those obtained before sildenafil administration (Table 1).

Four additional sheep were used in a permutation to the above protocol. In this set of experiments, after the initial control IOP readings were recorded, 300 μL of AH was withdrawn from the left eye and the animal was immediately given 100 mg of sildenafil (Fig. 2). Under this condition, the IOP of the depressurized left eye and that of the control fellow eye slowly increased in tandem as the ocular hypertensive effect of sildenafil ensued. Approximately 50 minutes after the paracentesis of the left eye, the two eyes exhibited the same IOP, indicating that the AH refilling had been completed. On average, such AH refilling took approximately 44 minutes, representing a mean AH inflow rate of 7.0 μL/min (Table 2).

In these experiments, the sheep were subjected to paracentesis volumes representing a relatively small proportion of their total aqueous. We removed between 1/15 and 1/3 of the 900 μL AH volume that we determined in preliminary work to exist within the eyes of this species. The exhibited reductions in IOP after such paracentesis were relatively similar for the three withdrawal volumes that we applied (Table 3). On paracentesis, the initial IOP measurements represented declines in pressure between 2.3 and 3.1 mm Hg in control eyes (i.e., the right eyes before sildenafil treatment; Table 3, left-side columns). The subsequent administration of sildenafil did not affect the ocular depressurization observed with paracentesis of 60 μL (Table 3, right-side columns). With the larger volume withdrawals, there was a tendency for the depressurization to be greater after the postsildenafil withdrawals of 120 and 300 μL, with which IOP declined by approximately 4.3 mm Hg. This decline was 48% larger ($P < 0.001$, as unpaired two-tailed data) than the 2.9 mm Hg reduction that was obtained from the fellow eye before sildenafil treatment in the case of the 120 μL paracentesis (Table 3). The low number of experiments for the 300 μL withdrawal protocol precludes statistical rigor for determining if sildenafil affected the degree of depressurization on paracentesis, although this point is outside the scope of our current emphasis (i.e., to characterize the time necessary for IOP recovery and the influence of sildenafil on this parameter).

IOP Recovery after Paracentesis in Rabbits

Twelve healthy rabbits were used. Their control IOPs as obtained from Tono-Pen (Mentor O&O) readings were 12.2 ± 0.8 mm Hg (oculus dexter [OD]; $n = 12$) and 11.8 ± 0.6 mm Hg (oculus sinister [OS]; $n = 12$; $P > 0.45$). In all eyes subjected to the removal of 50 μL of AH, the IOP was reduced to 3 mm Hg, which was the lowest value that could be reliably recorded with the Tono-Pen in our experience. In all rabbit eyes from which 100 μL were removed, the IOP was reduced to essentially zero because the eye was too flaccid to obtain a Tono-Pen reading. In these cases, as the eye filled with "secondary" aqueous, the first Tono-Pen reading that could be recorded was 3 mm Hg. As with sheep, the IOP recovery time was determined from the point of paracentesis ($t = 0$) to the time at which the control, baseline IOP was restored.

Representative experiments with rabbits are illustrated in Figure 3. On the withdrawal of 50 μL AH, approximately 13.5 minutes, on average, were needed for IOP recovery, signifying a mean AH refilling rate of 3.9 μL/min (Table 4). After the rabbits were fed sildenafil, the time necessary for IOP restoration shortened to approximately 6 minutes with the average AH refilling rate increased to approximately 8 μL/min. Virtually identical recovery times were obtained when the volume of the paracentesis was increased to 100 μL, both before and after sildenafil ingestion (i.e., 13 and 6 minutes, respectively; Table 4). However, these recovery times reflect higher estimated rates (~2-fold) for AH refilling (Table 4). Overall, these data indicate that although AH refilling occurs relatively promptly in rabbits, the inflow mechanisms involved can still be accelerated by the ingestion of sildenafil.

Discussion

IOP recovery subsequent to paracentesis reflects the refilling of the AC with "secondary" aqueous. The rate of such IOP recovery, with concomitant AH refilling, was stimulated by sildenafil in two different animal models, implying that the vasodilator not only provides more fluid for secondary aqueous formation after paracentesis, but may also increase AH turnover in the normal eye. Although we have not measured AH turnover directly, both the accelerated rates of IOP restoration after paracentesis in animals administered sildenafil, as well as the fact that sildenafil increases vascular flow in the eye due to dilations of intraocular arteries,[9] suggest that the vasodilator should increase the turnover of AH in the AC.

The measurement of AH turnover in the presence of systemic sildenafil administration is an important parameter that should be characterized in future studies to corroborate our findings and interpretations. This could be done in animal models, as well as in human subjects, noninvasively using the fluorescein depot method.[21–23] The rate of AH turnover in the AC could be monitored under control conditions, followed by sildenafil ingestion to determine whether turnover is indeed increased, as reflected by an enhanced rate of fluorescein clearance from the AC. We posit that such result is very likely attainable, and would occur independently of paracentesis. At steady state with IOP constant, AH inflow = AH outflow. This flow is the AH turnover. On an increase in inflow, which we predict sildenafil will elicit, 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

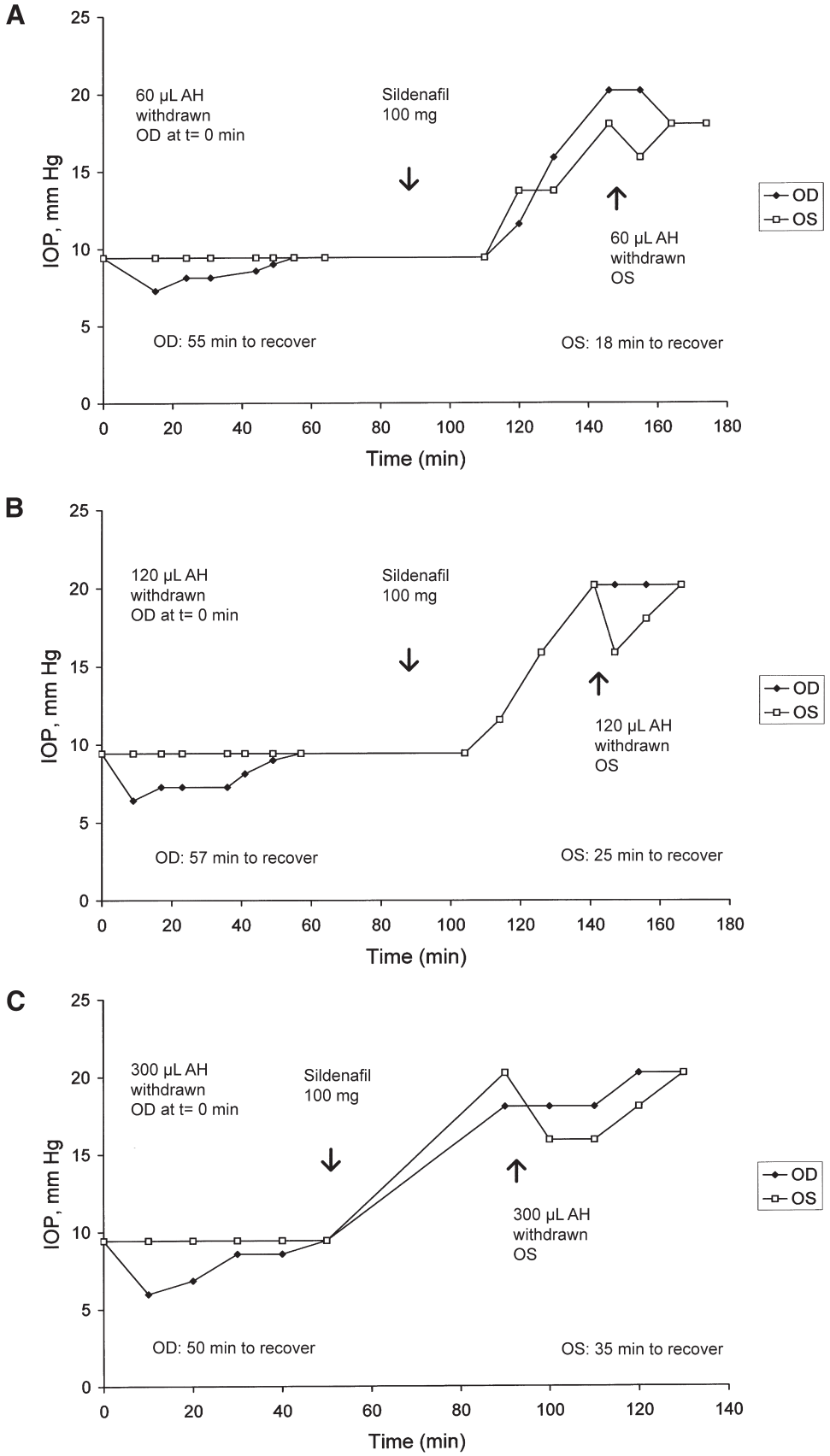

Figure 1. Representative results from the paracentesis of either 60 µL (**A**), 120 µL (**B**), or 300 µL (**C**) and sildenafil ingestion on sheep IOP. Values from right eye (oculus dexter; OD) are plotted with solid symbols; those from fellow eye (oculus sinister; OS) with open symbols.

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 AH turnover with the noninvasive fluorescein technique.

TABLE 1. Time Necessary to Restore IOP after Paracentesis in Sheep; Effect of Sildenafil on IOP Recovery

	Right Eye before Sildenafil		Left Eye after Sildenafil	
Sheep Number	**Recovery Time (min)**	**AH Refilling Rate (μL/min)**	**Recovery Time (min)**	**AH Refilling Rate (μL/min)**
Paracentesis Volume 60 μL				
1	56	1.1	20	3.0
2	55	1.1	18	3.3
3	46	1.3	17	3.5
7	40	1.5	20	3.0
Mean	49.3	1.2	18.8*	3.2*
SEM	3.8	0.1	0.8	0.1
n	4	4	4	4
Paracentesis Volume 120 μL				
4	58	2.1	27	4.4
5	58	2.1	26	4.6
6	57	2.1	25	4.8
8	50	2.4	25	4.8
Mean	55.8	2.2	25.8*	4.7*
SEM	1.9	0.1	0.5	0.1
n	4	4	4	4
Paracentesis Volume 300 μL				
9	50	6.0	35	8.6
10	50	6.0	40	7.5
Mean	50	6.0	37.5*	8.1†
SEM	0	0.0	2.5	0.5
n	2	2	2	2

Sheep were subjected to paracentesis of the right eye, followed by oral administration of sildenafil and the paracentesis of the left eye as described in text and shown in Figure 1.

* Significantly different from respective presildenafil value of fellow eye with $P < 0.05$, as unpaired, two-tailed data.

† Marginally larger than respective presildenafil value of fellow eye with $P = 0.065$, as unpaired, two-tailed data.

Conversely, it is also hypothetically possible that sildenafil inhibited the AH outflow facility by an unknown mechanism. In such a case, and presuming that the PDE5 inhibitor had no stimulatory effect on inflow, the refilling of the AC with "secondary" aqueous after paracentesis plus sildenafil ingestion would still occur faster than the rate of refilling after paracentesis in the absence of systemic PDE5 administration. Our results cannot directly exclude this possibility. If such mechanism comes into play, a reduction in outflow facility would be quantifiable. Putative changes in outflow facility evoked by sildenafil could be determined in live animal models administered the drug,[15,22,24] as well as in human subjects noninvasively.[23] However, we consider this prospect unlikely based on contemporary knowledge of the physiological effects of sildenafil.

In our present experiments with paracentesis, the calculated AH refilling rates represent an average for the entry of "secondary" aqueous after the removal of AH from the AC. At the moment of paracentesis (i.e., $t = 0$), the hydrostatic pressure difference between the capillaries of the ciliary body stroma and the AC would be maximal, resulting in a higher rate of ultrafiltration from the vasculature that should gradually decline as the IOP recovers. Moreover, simultaneous with AC refilling there is an increased outflow of AH consequent to the buildup in pressure in the AC, which would also contribute to minimizing our estimate of the AH refilling rate. As such, our results represent an average that may be the result of a very complicated refilling curve.

Interestingly, it was observed with rabbits that paracentesis, in itself, evoked a large IOP overshoot above the control level as the pressure was restored (Fig. 3), a phenomenon in rabbits attributed to the base of the iris bowing forward, thereby closing the angle and blocking the outflow.[25] We merely recorded this excess in pressure and calculated the time point at which the IOP was approximately equal to that of the control level for determining the recovery time. Since the protocol was designed so that AH was withdrawn from each eye only once, the IOP overshoot evoked by paracentesis was a unilateral effect that did not influence the IOP of the fellow eye. In our data, elevated IOPs evoked by paracentesis gradually declined to control levels within 90 minutes ($n = 24$ eyes).

The postparacentesis overshoot in IOP to a level above that of the control value was observed only with rabbits, a species with which paracentesis also induces miosis, which allows for the above-noted pupillary block.[25] In contrast, we never ob-

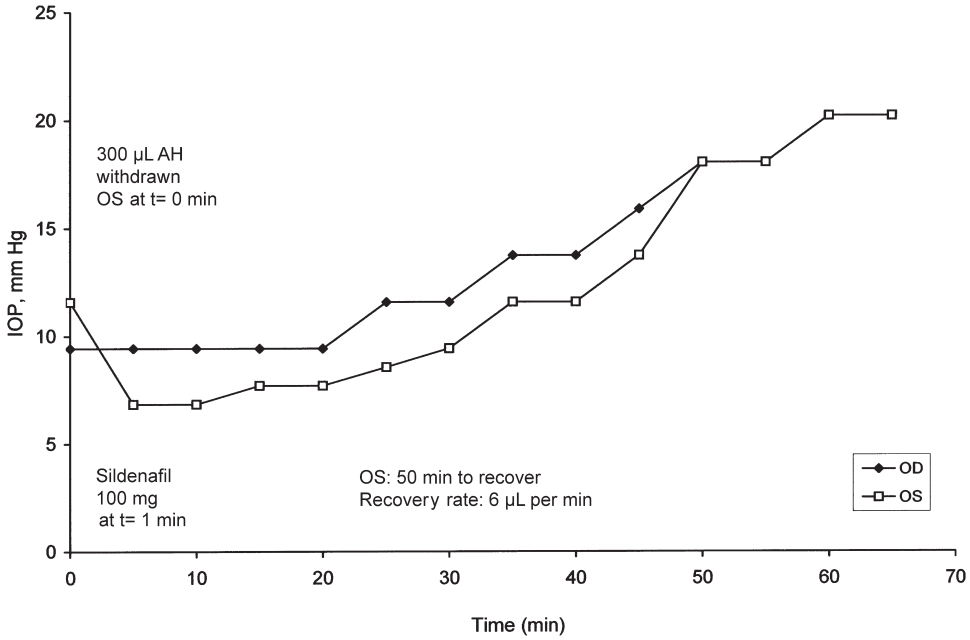

FIGURE 2. Representative experiment in which 300 μL of AH was withdrawn from the left eye of a sheep and the animal was immediately treated with sildenafil. Values from OD are plotted with solid symbols; those from OS with open symbols.

Table 2. Time Necessary to Restore IOP in Sheep Subjected to a Unilateral AH Withdrawal of 300 μL from Left Eye Followed by the Immediate Administration of Sildenafil to the Animal

Sheep Number	Recovery Time (min)	AH Refilling Rate (μL/min)
11	40	7.5
12	50	6.0
13	35	8.6
14	50	6.0
Mean	43.8	7.0
SEM	3.8	0.6
n	4	4

Paracentesis and sildenafil treatment were conducted as described in text and shown in Figure 2.

served miosis with sheep, and observed that the relatively large, rectangular-shaped pupils of this animal exhibit a mostly fixed shape, with minimal responses to ambient light.

An additional comparative difference between the two animal models was that sildenafil ingestion did not directly increase the IOP of rabbits. Neither the elevated IOP produced secondarily by paracentesis, nor the baseline IOP of the contralateral eye not yet subjected to paracentesis, was affected by sildenafil (Fig. 3). With three other rabbits that were not subjected to paracentesis and solely fed sildenafil, no significant effects on IOP were observed (data not shown). As such, sildenafil accelerated the AH refilling rate independently of whether it also increased baseline IOP, as saliently occurs with sheep (Figs. 1 and 2).[12]

Thus, an explanation is necessary for the absence in rabbits of an IOP-elevating effect by the drug, which nevertheless stimulated the AH refilling rates after paracentesis. We presume that if the drug indeed increased AH turnover in the normal rabbit eye, it concomitantly increased the pressure-dependent outflow via the trabecular meshwork in this species, thereby not producing a detectable change in IOP. Our present emphasis is the finding that sildenafil increased the AH refilling rate in a species in which such refilling after paracentesis occurs rapidly. After the removal of 50 and 100 μL AH, IOP recovered, on average, within 14 minutes (Table 4), resulting in estimated AH-refilling rates of 3.9 and 8.3 μL/min, respectively (Table 4). These rates are markedly higher than the 2 to 3 μL/min rate of AH formation attributed to secretion by the CE in rabbit.[26–28] After sildenafil treatment, the time necessary for IOP restoration was approximately halved, and the estimated AH refilling rates therefore doubled.

As classically accepted, the formation of AH first entails the leak of fluid from the fenestrated capillaries of the ciliary processes into the surrounding ciliary body (CB) stroma. Be-

Table 3. Reductions in IOP (mm Hg) Elicited by Paracentesis in Sheep before and after Sildenafil Ingestion

	Right Eye before Sildenafil			Left Eye after Sildenafil		
Sheep Number	Control IOP OD	Paracentesis 60 μL IOP OD	Delta IOP	Baseline IOP OS	Paracentesis 60 μL IOP OS	Delta IOP
1	9.4	7.3	2.1	20.2	18.0	2.2
2	10.5	8.3	2.2	18.1	15.9	2.2
3	9.4	7.3	2.1	20.2	18.0	2.2
7	11.0	8.4	2.6	18.1	15.9	2.2
Mean	10.1	7.8	2.3	19.2	17.0	2.2
SEM	0.4	0.3	0.1	0.6	0.6	0.0
n	4	4	4	4	4	4
	Right Eye before Sildenafil			**Left Eye after Sildenafil**		
Sheep Number	Control IOP OD	Paracentesis 120 μL IOP OD	Delta IOP	Baseline IOP OS	Paracentesis 120 μL IOP OS	Delta IOP
4	10.5	7.5	3.0	18.0	13.7	4.3
5	11.0	8.0	3.0	20.2	15.9	4.3
6	9.4	6.4	3.0	20.2	15.9	4.3
8	9.4	6.8	2.6	18.0	13.7	4.3
Mean	10.1	7.2	2.9	19.1	14.8	4.3*
SEM	0.4	0.4	0.1	0.6	0.6	0.0
n	4	4	4	4	4	4
	Right Eye before Sildenafil			**Left Eye after Sildenafil**		
Sheep Number	Control IOP OD	Paracentesis 300 μL IOP OD	Delta IOP	Baseline IOP OS	Paracentesis 300 μL IOP OS	Delta IOP
9	9.4	6.8	2.6	20.2	15.9	4.3
10	11.0	7.5	3.5	18.1	13.7	4.4
Mean	10.2	7.2	3.1	19.2	14.8	4.4
SEM	0.8	0.3	0.4	1.1	1.1	0.1
n	2	2	2	2	2	2

Values are the IOP readings (mm Hg) that were recorded from the 10 sheep whose IOP recovery times are compiled in Table 1. IOP OD, intraocular pressure of right eye (oculus dexter); IOP OS, intraocular pressure of left eye (oculus sinister). Compiled IOP values after paracentesis were recorded 9 to 15 minutes after fluid withdrawal from the eye (see Fig. 1 for representative IOP plots).

* Significantly larger than the change in IOP elicited by the 120 μL paracentesis of the fellow eye before the systemic administration of sildenafil ($P < 0.001$, as unpaired two-tailed data). All reductions in IOP elicited by paracentesis were significant with $P < 0.05$, as paired one-tailed data.

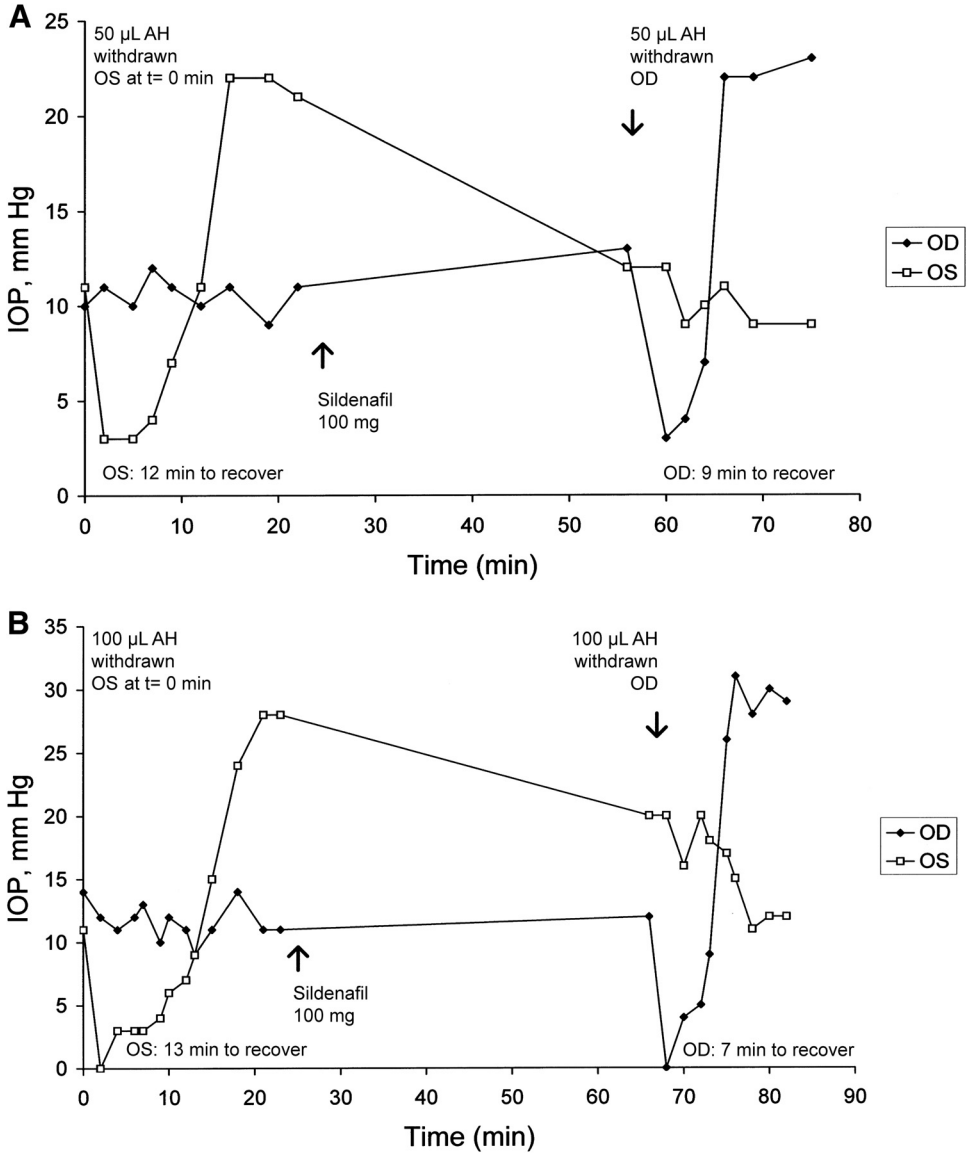

FIGURE 3. Representative results from the paracentesis of either 50 μL (**A**), or 100 μL (**B**) and sildenafil ingestion on rabbit IOP. Values from OD are plotted with solid symbols; those from OS with open symbols.

cause of the fenestrations, the leaked fluid contains proteins that subtract from the oncotic pressure within the capillaries, thereby reducing fluid reabsorption into the capillaries. The leaked fluid (not reabsorbed by the ciliary capillaries) is estimated to be approximately 4% of the rate of 75 to 100 μL/min blood flow in the CB capillaries.[10] AH formation across the ciliary epithelium (CE) then results from two driving forces: hydrostatic pressure gradients between the CB stroma and the PC, and osmotic forces produced by active electrolyte transport across the CE.[10,29] The first mechanism has been referred to as ultrafiltration and the second as secretion.[30] Ultrafiltration across the CE is currently thought to represent only a minor component of AH production when the blood–aqueous barrier (BAB) is intact,[29] so that most AH results from secretion across the CE into the PC and subsequent entry into the AC via the pupil.[10,29]

As originally considered by Bill,[10,30] we proposed that fluid could also directly enter the AC across the anterior face of the iris,[12] given the absence of an anatomic barrier between the CB stroma and the AC via the iris root. This flow could occur when there is a pressure difference between the CB stroma and the AC, and putatively, sildenafil could induce, or increase, such a pressure difference by increasing the rate of leakage of protein-rich fluid from the capillaries. Consistent with this possibility, sheep given sildenafil exhibit an increased protein concentration in the AC.[12] Sildenafil could also increase ultrafiltration across the CE, particularly when the permeability of the CE tight junctions is increased, as occurs secondary to paracentesis in rabbits, cats, and monkeys.[17,31,32]

To the best of our knowledge, there are no indications that sildenafil disrupts the BAB. We are unaware of patients, who take sildenafil for various vascular diseases, reporting incidents of proteinaceous "flare" in their visual axis. The most commonly recognized adverse effect of sildenafil in the eye is a transient blue tinge to vision and increased sensitivity to bright lights that has been attributed to an inhibition of PDE6, a critical enzyme in the regulation of the phototransduction cascade. As such, we suggest that the increase in AC protein content elicited by sildenafil in the sheep animal model probably resulted from an increase in flow of plasma-like fluid directly from the CB stroma to the AC via the iris in accord with Freddo's model.[11] Moreover, such putative increased inflow of fluid directly into the AC of sheep treated with sildenafil, and other longer-lasting PDE5 inhibitors,[12] likely accounts for the IOP elevation observed with this species (Figs. 1 and 2). Presently, the only protocol that we are aware of that could be used

Table 4. Time Necessary to Restore IOP after Paracentesis in Rabbits; Effect of Sildenafil on IOP Recovery

Rabbit Number	Left Eye before Sildenafil		Right Eye after Sildenafil	
	Recovery Time (min)	AH Refilling Rate (μL/min)	Recovery Time (min)	AH Refilling Rate (μL/min)
Paracentesis Volume 50 μL				
1	14	3.6	5	10.0
2	12	4.2	9	5.6
3	10	5.0	6	8.3
4	18	2.8	5	10.0
Mean	13.5	3.9	6.3*	8.5*
SEM	1.7	0.5	0.9	1.0
n	4	4	4	4
Paracentesis Volume 100 μL				
5	12	8.3	8	12.5
6	9	11.1	6	16.7
7	10	10.0	5	20.0
8	10	10.0	6	16.7
9	11	9.1	6	16.7
10	13	7.7	7	14.3
11	22	4.5	5	20.0
12	18	5.6	6	16.7
Mean	13.1	8.3	6.1*	16.7*
SEM	1.6	0.8	0.4	0.9
n	8	8	8	8

Rabbits were subjected to paracentesis of the left eye, followed by oral administration of sildenafil and the paracentesis of the right eye as described in text and shown in Figure 3.

* Significantly different from respective presildenafil value of fellow eye with $P < 0.01$, as unpaired, two-tailed data.

to directly test this interpretation is to examine the integrity of the CE tight junctions morphologically in animals given sildenafil or tadalafil. If such junctions remain unperturbed in the presence of the PDE5 inhibitors, ultrafiltration of plasma-like fluid between the CE cells is unlikely.

Separately, we do not have evidence indicating whether paracentesis, in itself, disrupts the BAB in sheep; nor are we aware of published reports on the rate of AH formation in this species. Indirectly, we can estimate the sheep AH turnover to be approximately 3 μL/min from a control IOP of 10 mm Hg and an outflow facility of approximately 0.3 μL/min per mm Hg.[15] As such, we can only speculate that the spontaneous rate of AH refilling observed with sheep after the paracentesis of 60 and 120 μL (i.e., 1.2 and 2.2 μL/min; Table 1) may possibly reflect secretion by the CE. However, after the paracentesis of 300 μL, as well as after the sheep ingested sildenafil, the postparacentesis AH refilling rates ranged between 3.2 and 8.1 μL/min (Table 1), or rates generally larger than those reported for AH formation attributed to secretion by the CE, i.e., usually 2 to 3 μL/min.[26] Hypothetically, in these latter conditions, either an increased inflow into the AC via the anterior aspect of the iris occurred, and/or ultrafiltration across the CE increased, if the resistance of the tight-junction barrier declined. Nevertheless, it was clear that the postparacentesis rates of AH refilling after the ingestion of sildenafil were markedly higher than published estimates for AH secretion by the CE.

Although experimental paracentesis in rabbits has been extensively done to characterize the mechanisms underlying paracentesis-induced disruption of the BAB, which is thought to be mediated primarily by prostaglandins,[17,19,20,25,31] we are unaware of reports quantifying the time necessary for IOP restoration and/or the AH refilling rate. We presume that the rapid refilling rates that we estimated for secondary aqueous, which is plasmoid in nature in rabbits, cats, and monkeys,[17,31–33] resulted from a substantial increase in ultrafiltration across the CE. The technique of fluorescein angiography has been used to qualitatively visualize the entry into the eye of systemically administered fluorescein in rabbits and monkeys subsequent to experimental paracentesis.[17,32] Fluorescein initially entered the eye from the capillaries of the ciliary body stroma into the PC, followed by entry into the AC via the pupil, such that most fluorescein entered the AC via transpupillary flow.[17,32] Interestingly, in pigmented rabbits, a diffusion of fluorescein from the anterior surface of the iris into the AC, which occurred sequentially to the fluorescein entry via the pupil, was also observed.[17,33] We posit that sildenafil may have increased the flow via both of these pathways in the experimental paracentesis of the rabbit eye due to its intraocular vascular dilating effects.

Given the above indications that sildenafil should increase the AH turnover of the normal eye (presumably due to increased flow in the CB stroma-to-iris pathway), as well as the rate of AH refilling after paracentesis (due primarily to an increased ultrafiltration), we suggest that the drug be considered as a potential prophylactic agent to augment the rate of AH refilling after eye surgeries of the anterior segment. In some cases, the angle collapses, leading to a potential complication of damage to the corneal endothelial cells due to mechanical interaction with the iris. Presently, many surgeons inject saline into the AC and/or with viscoelastic material to maintain normal ocular dimensions until CE secretory function and AH volume are fully restored. However, it can take 4 to 8 hours for freshly secreted AH to completely replace the saline in the AC.[34] One surgeon recommended withdrawing the aqueous at the beginning of surgery and returning it at the end of the procedure.[35] Sildenafil could be tested as an important adjunct in these types of procedures. It may also have utility in treating cases of ocular hypotony that result from insufficient AH formation.[36,37]

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