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Surgical lowering of elevated intraocular pressure in monkeys prevents progression of glaucomatous disease

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Abstract

Recent reports from large clinical trials have clearly demonstrated that lowering intraocular pressure (IOP) in persons with ocular hypertension has a beneficial effect on reducing the progression of glaucomatous disease. Few studies of this effect have been conducted in controlled laboratory settings, however, and none have been conducted using non-human primates, the model of experimental glaucoma considered most similar to the human disease. Using data collected retrospectively from a trabeculectomy study using 16 cynomolgous monkeys with experimental ocular hypertension, we evaluated both the threshold of elevated IOP required to cause clinically observable damage to the optic nerve head and also if lowering IOP below this threshold prevents further damage. An index of the level of elevated IOP experienced by experimental eyes (the Pressure Insult) was calculated as the slope of the difference in cumulative IOP between experimental and control eyes during 4 intervals of time over the course of the experiment, while damage to the optic nerve head was evaluated by measuring the Cup:Disc ratio for each eye from stereoscopic photographs taken at the end of each interval. An increase in the Cup:Disc ratio was significantly associated with both the maximum IOP obtained in the experimental eye during each interval ($r = 0.573$, $P < 0.001$) and the Pressure Insult ($r = 0.496$, $P < 0.001$). Pressure Insult values less than 11 mmHg-Days/Day were not associated with glaucomatous damage in monkey eyes, whereas values greater than 11 showed a significant correlation with increasing Cup:Disc ratios ($P < 0.001$). Trabeculectomy to reduce the Pressure Insult below 11 was correlated with an attenuation of the rate of progression of the Cup:Disc ratio in eyes that had exhibited damage before surgery. These results contribute further to our understanding of this model of experimental glaucoma by demonstrating a threshold at which IOP needs to be elevated to stimulate damage, while also providing corroborating evidence that lowering IOP in ocular hypertensive monkeys can attenuate the progression of glaucomatous disease.

Keywords

glaucoma; trabeculectomy; intraocular pressure; cup:disc ratio; pressure insult threshold

1. Introduction

Glaucoma is a common blinding disease that is characterized by the progressive loss of retinal ganglion cells and degeneration of the optic nerve. Elevated intraocular pressure (IOP) is the

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most significant risk factor for developing glaucoma (Quigley et al., 1994), but as recently as the last decade there were no controlled studies that demonstrated that lowering IOP was beneficial as a treatment (Rossetti et al., 1993). Long term, multi-center clinical trials involving large numbers of patients, have now shown that lowering IOP with medications is strongly associated with slowing progression of the disease or preventing conversion of glaucoma suspects (Heijl et al., 2002; Kass et al., 2002; Leske et al., 2003). Few studies examining the beneficial effects of lowering IOP have been conducted under a controlled laboratory setting using animal models of experimental glaucoma. Morrison and colleagues used timolol, or more recently cyclodialysis, to lower IOP in a rat model of ocular hypertension and showed a correlation between reduced IOP and the amount of damage sustained by the optic nerve after a set period of time (Johnson et al., 2006; Morrison et al., 1998). Surprisingly, no significant studies involving IOP and glaucoma progression have been done using monkeys with experimental glaucoma, even though this model is considered most similar to the human disease. Recently, we conducted a trabeculectomy study involving 16 monkeys with experimental ocular hypertension in one eye. The purpose of the study was to test the efficacy of adjunctive gene therapy using a recombinant adenovirus expressing human p21^{WAF-1/Cip-1} to reduce scarring post-operatively and maintain a target IOP (Heatley et al., 2004). Since this study involved first elevating IOP followed by reducing it with trabeculectomy surgery, it was possible to assess the progression of glaucomatous damage both before and after treatment.

2. Materials and methods

2.1 Animals

Sixteen *Macaca fascicularis* of approximately 8 years of age were used in this study. Animals were handled in accordance with the Association for Research in Vision and Ophthalmology Statement on the use of animals for research and the guidelines for the use of non-human primates established by the University of Wisconsin and approved by the Institutional Animal Care and Use Committee. A complete description of the study design and the methods used to induce ocular hypertension are published elsewhere (Heatley et al., 2004). Briefly, ocular hypertension was induced in one eye of each animal by laser scarification of the trabecular meshwork. When an elevated IOP was reached and remained stable for at least 1 month, the animals underwent a partial thickness trabeculectomy including adjunctive use of 1 of 4 treatments to reduce post-surgical wound healing. These treatments were Balanced Saline Solution (BSS), Mitomycin C (MMC - 0.5 mg/mL), a recombinant adenovirus with no transgene, and a recombinant adenovirus carrying human p21^{WAF-1/Cip-1} under the control of the immediate early promoter of Cytomegalovirus. Complete details of the surgical protocol and the application of these reagents are described elsewhere (Heatley et al., 2004).

2.2 Clinical evaluation of IOP and glaucomatous progression

Both eyes of each animal were examined clinically before and after surgery. Glaucomatous damage to each experimental eye was evaluated by calculating the Cup:Disc ratio (CDR) for each eye from stereoscopic photographs of the optic nerve. Previous studies have shown that the CDR increases with the loss of axons in this model of experimental glaucoma (Varma et al., 1992). Optic nerve photographs were taken on animals under ketamine and pentobarbital anesthesia using a Zeiss fundus camera (Thornwood, NY). Baseline photographs were made upon arrival of the animals into quarantine at the University of Wisconsin and again just prior to surgery. A minimum of three pairs of 35 mm stereoscopic photographs was subsequently taken of each eye at regular intervals (each between 85 and 90 days) post-operatively. At the end of the study, the photographs were randomized as to treatment and time and the vertical CDR was read by a trained masked observer (GH) in two independent sittings. There was no significant difference between the test-retest values obtained at each sitting ($P = 0.365$, paired

sample *t*-test). The mean ($\pm$ SD) CDR for non-experimental eyes was 0.25 ± 0.05 ($n = 16$) with a coefficient of variation of 0.21. The mean ($\pm$ SD) CDR for experimental eyes was 0.44 ± 0.23 ($n = 16$) with a coefficient of variation of 0.32. There was a highly significant difference between the coefficients of variation between the 2 groups (*t*-test, $P < 0.001$), indicating that while experimental eyes showed variation in the CDR during the course of the experiment, the CDRs of the control eyes were consistent over the same period.

Starting when the animals arrived for this study, IOP was measured every 7 to 10 days by 'minified' Goldman applanation tonometry using a Haag-Streit slit lamp. Animals were anesthetized with ketamine for these measurements. Although in the past, we have not detected a significant deviation in IOP measured under low dose anesthesia (relative to manometer readings), it is possible that our measurements in this study were influenced by the ketamine, which often leads to an increase in IOP. Some IOP measurements were also taken using a Tonopen XL (Mentor Ophthalmics Inc, Norwell, MA) and converted to actual mm Hg based on a standard calibration curve (Peterson et al., 1996). At the end of the study, IOP data for each eye were plotted against time and the cumulative IOP (cIOP) was calculated as the area under the curve using the trapezoidal rule ('trapz' routine, MATLAB Inc., Natick, MA). The difference in cIOP between the two eyes was calculated by subtracting the cIOP of the control fellow eye of the same monkey at each time point ($\Delta\text{cIOP} = \text{cIOP}_{\text{exp}} - \text{cIOP}_{\text{con}}$).

The data comparing the change in IOP in the experimental eye and the amount of optic nerve damage sustained by that eye were analyzed in 4 time intervals over the course of the experiment. The first interval was defined as the pre-operative period from when IOP first started to elevate to the day of the trabeculectomy. This period had a mean interval length of 115.7 ± 49.2 days (SD). The subsequent 3 post-operative intervals were determined as relatively equal periods of time (88.9 ± 7.7 days, mean interval length $\pm$ SD) and set to when optic disc photographs were taken. Each interval contained 8 to 10 IOP measurements. An index of the amount of increased pressure in experimental eyes during each interval was obtained by graphing the ΔcIOP against time and then calculating the linear slope of the best-fit line through the data points in each interval. This index was designated the Pressure Insult (PI) and defined in units of mmHg-Days/Day. Optic disc photography was timed to coincide with the end of each interval so the change in CDR accurately reflected the amount of damage sustained during that interval. The only exception was the first interval, where the amount of damage sustained was measured using baseline photographs and comparing them to the images collected just prior to trabeculectomy. Maximal damage to the optic nerve was judged when the CDR reached 0.8-0.9. In total, there were 64 intervals examined (4 for each of 16 animals). Ten of these intervals were excluded in the analysis of PI and the change in CDR because maximal damage had already been sustained in the eye. Therefore during subsequent intervals, these eyes had no potential to exhibit any further progression of damage.

3. Results

3.1 Summary of the variables obtained from each interval

The study period was divided into 4 intervals (1 presurgery and 3 postsurgery) as described in the methods. Table 1 shows the duration (in days), the maximum IOP, and the change in CDR obtained in the experimental eyes of each monkey during each interval of this study. Regression analysis for each variable showed that there was no significant correlation between the duration of each interval and either the change in CDR ($r = 0.068$, $P > 0.5$) or the maximum IOP obtained during that interval ($r = 0.217$, $P > 0.2$). A significant correlation was observed between the change in CDR during each interval and the maximum IOP recorded for that interval ($r = 0.573$, $P < 0.001$).

3.2 Determination of the Pressure Insult

An index of the amount of elevated IOP sustained by each experimental eye was determined by calculating the slope of the Δ IOP during each of the 4 time intervals defined in the experiment (see Methods for this definition) and called the Pressure Insult (PI – Table 1). Although it is possible to assess the development of glaucomatous damage relative to individual variables, the PI encompasses the duration and level of IOP during each interval, and is corrected for the control fellow eye of each individual animal. Because the PI is a function of all the different variables of this study, we focused on it for our analyses of the relationship between progressive damage to the optic nerve and the lowering of IOP. Figure 1A shows the entire IOP history for both eyes of one monkey used in this study (monkey 10855) to illustrate how the PI was determined for each interval. This animal had a spike of elevated IOP following meshwork ablation and sustained elevated IOP at the time of surgery. Immediately after trabeculectomy, the IOP of the experimental eye transiently elevated, which is not uncommon (Liebmann et al., 1990), after which it dropped to the level of the control fellow eye where it remained during the course of the experiment. The Δ IOP was calculated for each time point where IOP was measured and this was plotted against time in the same graph. The PI for each interval was taken as the slope of the best fit line through the Δ IOP data points within each interval. In the case shown in Figure 1A, the PI was 13.5 (mmHg·Days/Day) during interval I, when IOP was greater in the experimental eye, and was decreased to 2.4, -0.2, and 0.5 during intervals II, III, and IV, respectively, indicative of a successful operation that had controlled the IOP.

3.3 Assessment of glaucomatous damage

Progressive damage to the optic nerve was measured by determining the CDR of each eye at the end of each interval. The change in CDR, relative to the last measurement of the previous interval, was then calculated (Table 1). A previous study of experimental glaucoma in cynomolgus monkeys had found a correlation between the change in CDR and axonal loss (Varma et al., 1992). Three patterns of CDR changes were observed in the 16 animals examined in this study. The first pattern was an increase in the CDR during interval I, but no further progression in the post-operative intervals (Figure 1B). The second pattern was an increase in the CDR during successive intervals of the experiment until a maximum ratio was obtained and the eye could sustain no further damage (Figure 1C). The third pattern was no change in the CDR during any of the intervals (representative disc photos not shown).

3.4 Relationship Between the PI and the CDR

The PI and the change in CDR (Δ CDR) for each interval were graphed (Figure 2). Data for intervals where the eyes had already reached maximal damage were excluded from this analysis because there was no potential for a further increase in the CDR (see Methods). This data set was evaluated in three ways. First, using a simple regression analysis, there was a significant correlation between the PI and the change in CDR ($r = 0.496$, $P < 0.001$). Second, we examined the mean PI for each of 3 groups based on the level of damage to the optic nerve head observed during each interval. Nerves that exhibited no damage (Δ CDR = 0) had a mean ($\pm$ SD) PI = 2.8 ± 11.7 . Nerves that appeared to have sustained early damage (Δ CDR = 0.1 to 0.2) had a mean PI = 7.6 ± 6.6 , while nerves with moderate to extensive damage (Δ CDR > 0.2) had a mean PI = 18.6 ± 3.2 . Third, in order to estimate a critical PI value, we evaluated the mean Δ CDR of eyes grouped around different individual PI values. The largest increases in glaucomatous damage were first apparent in eyes that had a PI greater than 11 mmHg·Days/Day. This value was designated the Pressure Insult Threshold (PIT). The mean ($\pm$ SD) Δ CDR for values below the PIT was $0.02 (\pm 0.003)$, while the mean Δ CDR above the PIT was significantly higher (0.29 ± 0.07 , $P < 0.001$, t -test). It is noteworthy that 1 monkey showed no increase in the CDR (10989) even though it sustained the highest PI in the study (PI=38.7).

Once a PIT was determined, we observed that the history of PI values fit into 3 general groups. The first group had a pressure insult greater than the threshold ($PI > PIT$) during the first interval, but a pressure insult less than the threshold ($PI < PIT$) after surgery. The second group had a $PI > PIT$ for the first interval, and at least the second interval after surgery. The third group never had a $PI > PIT$ at any interval. Table 2 shows the breakdown of the monkeys in each group and compares how their CDR changed during each interval. Six monkeys fit into the first group ($PI_{pre} > PIT > PI_{post}$). Two of the 6 animals had sustained maximal or nearly maximal damage during the first interval. Three of the animals, however, showed only partial damage to the optic nerve during the first interval, but no further damage after surgery. One of the monkeys in this group (11182) showed improvement of the CDR after IOP lowering, suggesting that the CDR estimate after the initial interval was overestimated due to hypercompliance of the optic nerve head (see Discussion). Four monkeys fit into the second group ($PI > PIT$ for at least the first and second intervals). Three of them sustained maximum damage to the optic nerve during the course of the experiment, while the optic nerve of the fourth monkey (10989) was completely resistant to the elevation in IOP. The remaining 6 monkeys never achieved a $PI > PIT$ during any interval. None of these animals exhibited any damage to their optic nerves.

We also evaluated if there was a correlation between the reagent used to prevent wound healing (BSS, MMC, recombinant adenovirus with and without the p21 therapeutic transgene) and the ability to prevent further damage to the optic nerve after surgery. Eyes that showed progressive damage after surgery were more likely to have been treated with reagents that did not prevent wound healing (BSS and adenovirus without transgene, 3/4 animals; MMC 1/4 animals), and were thus more likely to exhibit failed glaucoma surgery and higher IOPs (see Heatley et al., 2004).

4. Discussion

For many years, common sense has supported the notion that lowering IOP will reduce glaucomatous damage in humans. This intuitive conclusion was supported by a variety of observations, including population studies that showed that elevated IOP was the most important risk factor for developing glaucoma; numerous case studies that showed an effective attenuation of disease progression in patients with lowered IOP; and laboratory animal models in which experimental glaucoma could be induced by ocular hypertension. Controlled large multi-center clinical trials have now conclusively shown that IOP lowering therapy is beneficial in reducing the onset and progression of disease, but this relationship has not been tested extensively in a laboratory setting. This study provides data showing that there is a correlation between the average level (PI) of elevated IOP in a monkey eye and the level of damage sustained during a defined interval. Evaluation of these data also suggests that a threshold of pressure insult must be reached in a healthy young monkey eye in order to achieve glaucomatous damage during an interval of approximately 90 days. In our study, we observed a statistical likelihood of an increase in the CDR when the IOP of the ocular hypertensive eye was at least 11 mm Hg/day higher (on average) than the IOP of the fellow control eye. Lowering the IOP below this threshold with trabeculectomy was correlated with a reduction in disease progression in eyes that had sustained partial optic nerve damage pre-operatively. Eyes in which trabeculectomy failed to reduce the IOP to a level below the threshold exhibited continued progression of damage until they reached a maximum cupping of the optic nerve. These results quantitatively demonstrate that lowering IOP below a specific level has a therapeutic benefit to treat glaucomatous damage in this animal model.

Interestingly, one monkey (10989) showed complete resistance to elevated IOP, even though it was exposed to the highest elevation of pressure. The presence of an IOP-resistant monkey in such studies is rare, but not unique. In our experience, these monkeys appear with a frequency

of about 1:20 animals (using cynomolgous monkeys). Since the pool of monkeys used for our studies are from out-bred populations, the presence of these animals may reflect a segment of the human population with a higher tolerance to ocular hypertension. We have not examined the underlying cause for this dramatic resistance, however.

Although this study supports the concept of lowering IOP to treat glaucoma, there are several caveats that limit comparisons made between this model and the human disease. The first of these is the nature of the model, in which young animals (approximately 8 years of age) experience relatively high IOPs and damage is generated over an accelerated time period relative to the human disease. With these young healthy animals, it is likely that a relatively high PIT must be achieved to result in damage, whereas in older humans with disease, a low PIT may be equally damaging over much longer intervals. An interesting counter argument to this is that the monkeys in this study are more susceptible to the damaging effects of elevated IOP. It seems reasonable to consider that over relatively short time periods, such as those examined here, the average human eye would be able to sustain IOPs only 11 mm Hg above normal. In these kinds of arguments, it is important to consider that elevations in IOP and the assignment of a PIT may be both species specific and unique to individual eyes, taking in to account a variety of variables such as the strength and composition of the connective tissue in the optic nerve head. Additionally, the assignment we have made of a PIT should be considered in the context of the period of time we examined during each interval. Examination of the PIT over longer or shorter intervals may lead to different findings.

A second caveat is the limited sensitivity of using the change in CDR as a measure of damage. Although a previous study has shown a correlation between an increase in the CDR and the loss of axons (Varma et al., 1992), this study was limited by having samples with predominantly mild or severe axon loss. It is possible that the relationship between the CDR and axon loss is more complex for eyes with intermediate amounts of damage. Newer optic nerve head and nerve fiber layer imaging technologies, such as scanning laser polarimetry and optical coherence tomography, are potentially more sensitive at measuring damage. These technologies are currently used in the clinic and are being adapted for use on the non-human primate eye (Weinreb et al., 2002). Application of these technologies may yield a better estimate of the PIT, show a greater effect of lowering IOP, and may reveal that individual monkeys have unique PITs. This would clearly be the case for monkey 10989, for example.

A third caveat is that the CDR may also reflect a change in optic nerve head compliance. Because these animals were originally in a surgery study where the principal outcome measure was the change in the natural history of the IOP, we did not artificially lower the IOP of each eye before taking measurements. Thus, hypercompliant deformation of the optic nerve could have contributed to an increase in the CDR. The hypercompliant component would be expected to reverse after the reduction in IOP to more normal levels, so in our experimental paradigm, we may have overestimated glaucomatous damage in some samples. If hypercompliance were a significant issue, then we would have expected to measure improvements in the CDR of those eyes that underwent successful IOP lowering. In fact, we did observe this in one animal (monkey 11182). All other animals with damage after the first interval, however, showed no change or a further progression of damage, depending on the success in lowering IOP. It is possible that prolonged deformation due to hypercompliance is a contributory factor in the development of plastic deformation associated with axon loss and scarring of the laminar connective tissue (Bellezza et al., 2003; Burgoyne et al., 1995).

A fourth caveat is that the measurements of IOP over time were limited. Although regular IOP measurements were taken on both eyes of each animal during the period of this study, it is not possible to precisely predict the IOP history of each eye. The methods we used to monitor IOP involved sedating the animals, which can alter the IOP, and did not account for variations in

IOP regulated by the diurnal cycle of each animal (note that IOPs were taken at the same time of day for each animal during the course of this study) or for potential changes in corneal thickness that may have occurred in the same eye during the course of the experiment. More precise measurement of IOPs can only be obtained using remote sensing of implanted devices, which was not part of the surgery protocol of this study.

Lastly, by combining all the data pre and post surgery, we were able to provide an initial estimate of the pressure threshold (PIT) leading to damage, but because of the relatively small data set used for analysis, the sensitivity of this study was not able to distinguish if the threshold of damage is different in eyes pre and post-surgery. It is possible that eyes that convert to glaucoma and begin to sustain damage become weakened and hypersensitive to PIs below their original threshold. In a clinical setting this could mean that extra efforts must be made to reduce IOPs even further than “normal” to reach this new lowered threshold.

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Fig. 1.

(A) IOP history of monkey 10855 and the calculation of the Pressure Insult. The complete IOP history of both eyes of animal 10855 is illustrated in this graph. Laser ablation of the trabecular meshwork of the experimental eye (open circles) produced an elevation of intraocular pressure that was alleviated by trabeculectomy surgery (day 0). During the remainder of the experiment, the IOP of the operated eye remained similar to the IOP of the fellow control eye (grey-filled circles). These data were used to calculate the cumulative IOP (cIOP) for each eye and the change in cIOP ($\Delta cIOP$) was determined by the formula $\Delta cIOP = cIOP_{exp} - cIOP_{con}$. The graph of $\Delta cIOP$ over time (black-filled circles) indicates that the experimental eye was exposed to increasing cIOP during the pre-operative interval (I). The $\Delta cIOP$ line plateaus during the

post-operative intervals (II, III, and IV) indicating that both eyes had similar IOPs during this period. To estimate the amount of Pressure Insult that the experimental eye received during each interval, the slope of the best fit line (shown for each interval) through the Δ cIOP data was calculated during this period. In this case, monkey 10855 exhibited slopes of 13.5, 2.4, -0.2, and 0.5 during intervals I, II, III, and IV, respectively. (B) Optic disc photographs of monkey 10855. Five photographs, taken at successive approximately 90 day intervals are shown. The first photo on the left of the series represents the baseline picture before IOP was elevated. The second photo from the left was taken just before trabeculectomy and the remaining photos were taken post-operatively. The number shown under each photograph represents the change in Cup:Disc ratio (Δ CDR) between the labeled photograph and the one just to the left of it. This animal exhibited a Δ CDR of 0.33 during the pre-operative interval, but no further change after surgery. (C) Optic disc photographs taken of monkey 10512. This animal exhibited nearly maximal damage during the first interval (Δ CDR = 0.5) with minimal progression post-operatively.

Fig. 2.

A scatter plot showing the relationship between the Pressure Insult (PI) and the Change in Cup:Disc ratio (Δ CDR) in experimental monkey eyes. Data from intervals where the optic nerves had already reached maximal damage (10/64 intervals) were excluded since there was no expectation that a high PI would cause any further change in the CDR. The PI threshold (PIT) for damage under these experimental conditions was determined to be 11 mmHg·Days/Day (see Results section). Data points shown as open circles have a PI equal to or less than the PIT. Data points with a PI greater than the PIT are shown as closed circles. The mean ($\pm$ SD) Δ CDR for eyes below the PIT is 0.02 ± 0.003 , and 0.29 ± 0.07 for eyes above the PIT. This represents a significant chance of damage occurring if the PI exceeds the threshold ($P < 0.001$, t -test).

Table 1

Summary of data collected from each interval for each monkey in this study. Interval I is shown first, followed by intervals II, III, and IV. The change in Cup:Disc Ratio (Δ CDR) refers to the actual change measured in the optic nerve head relative to the CDR measured at the end of the previous interval. Peak IOP refers to the maximum IOP recorded for the experimental eye during each interval. The Pressure Insult (PI) was calculated as described in the Materials and Methods and reflects the difference in IOP experienced by the experimental eye, relative to the control fellow eye of the same animal, during the period of each interval. One monkey (11182) showed an improvement of the CDR in the experimental eye after the second interval. Since this was also associated with a decrease in the PI, the first interval CDR may also reflect a hypercompliant deformation of the optic nerve.

Monkey Number	Duration (Days)	Δ CDR	Peak IOP	PI
10989	49	0	65	37.8
	89	0	41	13.7
	92	0	40	11.5
	78	0	36	8.6
11064	48	0.1	30	9.64
	89	0	34	1.44
	92	0.1	20	-1.73
	78	0	23	-0.63
10647	49	0.7	66	30.3
	89	max	50	9.3
	92	max	30	7.4
	78	max	23	3
10512	48	0.5	39	18.23
	89	0.1	25	4.99
	92	max	17	0.25
	78	max	33	9.15
10970	104	0.18	58	15.5
	86	0	57	10.9
	83	0.1	38	9.6
	98	0	35	10.6
10855	104	0.33	50	13.5
	86	0	31	2.4
	83	0	25	-0.2
	98	0.1	21	0.5
10537	104	0	40	10.1
	86	0	28	4.3
	83	0	30	1.9
	95	0.18	33	4.9
10584	104	0	44	11
	86	0	34	9.5
	83	0	21	2.4
	98	0	17	-0.5
11316	130	0.4	64	20.8
	97	0.1	60	18.9
	83	max	61	15
	84	max	59	17
11168	130	0.58	67	17.2
	97	0.17	28	11.5
	83	0	31	4.9
	84	max	30	2.2
11182	130	0.55	54	12.6
	97	-0.50	26	3.6
	83	0.2	17	-1
	84	0.2	24	-0.7
11231	172	0	30	2.7
	104	0	22	-2.9
	82	0	19	-3
	86	0	14	-9.2
11338	172	0	64	15.6
	104	0.67	49	20.4
	82	max	47	10.5
	86	max	33	2.4
11361	172	0.43	61	15.7
	104	0	44	7.2
	82	0	29	3.2
	86	0.2	23	2.6
11138	172	0	34	4.8
	104	0	23	1.5
	82	0	29	1.8
	86	0	27	3.2
11301	172	0	23	1.2

Monkey Number	Duration (Days)	Δ CDR	Peak IOP	PI
	104	0	20	-5.4
	82	0	12	-19.7
	86	0	9	-41.5

Evaluation of the Pressure Insult (PI) and the change in Cup:Disc Ratio (CDR) of experimental eyes during pre- and post-surgery intervals. Animals were grouped according to their PI history; including animals that had a PI > the Pressure Insult Threshold (PIT = 11 mmHg/day/day) during the first interval followed by a PI < PIT after surgery (Class 1), animals that had a PI > PIT for at least the first and second intervals (Class 2), and animals that always had a PI < PIT even before surgery (Class 3). Following this grouping, the animals then were scored for disease progression, including those who achieved a maximum CDR prior to surgery, those who had initial damage prior to surgery and then no progression of damage after, those that had progressive damage both before and after surgery, and those that never exhibited signs of damage. Evaluation of the change in CDR during each interval shows that surgically lowering the PI below the threshold (Class 1) had a beneficial effect in reducing progression of damage in 4/6 monkeys. The two animals in which no effect was observed likely had sustained nearly maximal damage to their nerves during the first interval. Conversely, if surgery was unable to lower the PI below the PIT (Class 2), animals were more likely to progress to maximal damage (3/4 animals). Further demonstration of the importance of the PIT value was observed in Class 3 animals, in which no animals sustained optic nerve damage if the PI < PIT during all intervals.

Table 2

Monkey Number	PI History (Class)	Maximum Damage in First Interval	No Further Progression after Surgery	Progression of Damage after Surgery	No Damage during any Interval
10855	1		+		
11182	1		+/-	+/-	
11361	1		+		
10970	1		+		
10512	1	+		+/-	
10647	1	+			
11316	1			+	
11168	2			+	
11338	2			+	
10989	2			+	
10584	3				+
10537	3				+
11064	3				+
11138	3				+
11231	3				+
11301	3				+