

Experimental Glaucoma and Optic Nerve Transection Induce Simultaneous Upregulation of Proapoptotic and Prosurvival Genes

Hani Levkovitch-Verbin, Rima Dardik, Shelly Vander, Yael Nisgav, Maya Kalev-Landoy, and Shlomo Melamed

PURPOSE. To investigate changes in gene expression induced by elevated intraocular pressure (IOP) and complete optic nerve transection (ONT) over time.

METHODS. A gene array of 18 signal transduction pathways was used to examine the changes in RNA profiles of retinas post-ONT in rats. Among the seven genes that were determined to be upregulated, four were confirmed to have higher expression by semiquantitative RT-PCR analysis: *Ei24* and *Gadd45a* (both associated with apoptosis induced via the p53 pathway), *IAP-1* (inhibitor of apoptosis protein 1), and *Cdk2* (cell cycle regulation and apoptosis). Their mRNA levels were then studied by quantitative RT-PCR in experimental glaucoma and ONT over time. Levels of the corresponding proteins were evaluated by Western blot analysis and immunohistochemistry.

RESULTS. Proapoptotic genes from the p-53 pathway (*Ei24* and *Gadd45a*), *Cdk2* and the prosurvival gene *IAP-1* (a caspase inhibitor) were simultaneously and significantly upregulated early after ONT, returning to baseline at 2 weeks. In experimental glaucoma, *Gadd45a* was significantly upregulated 1 week after induction of increased IOP and stayed upregulated for 2 months and long after IOP returned to baseline. The prosurvival gene *IAP-1* was simultaneously upregulated but returned to baseline earlier than the proapoptotic gene. *Ei24* and *Cdk2* were only slightly upregulated in glaucoma. Western blot analysis demonstrated upregulation of *Gadd45a* and *IAP-1* proteins. Immunohistochemistry localized these changes to the retinal ganglion cell layer.

CONCLUSIONS. Members of the p-53 signal transduction pathway are significantly involved in glaucoma and ONT. The endogenous caspase inhibitor *IAP-1* is upregulated simultaneously, possibly as part of an intrinsic neuroprotective mechanism. Changes in glaucoma are gradual and last long after IOP returns to normal. (*Invest Ophthalmol Vis Sci.* 2006;47:2491-2497) DOI:10.1167/iovs.05-0996

Glaucoma is a neurodegenerative disease characterized by specific changes in the optic nerve and retina, resulting in apoptosis of retinal ganglion cells (RGCs).^{1,2} Models of exper-

imental glaucoma allow identification of genes and proteins that are involved in different stages of the disease.³⁻⁷ Extensive data have been collected to understand how RGCs degenerate in glaucoma. Gene array analysis recently revealed up- and downregulation of many genes in response to elevated intraocular pressure (IOP).⁸⁻¹⁰ Ahmed et al.¹⁰ analyzed early (8 days) and late (35 days) changes in gene expression of retinas from glaucomatous eyes of rats, by using the episcleral vein injection of hypertonic saline. They found altered expression of 81 genes, approximately half of which were associated with either apoptosis or neuroinflammatory responses. Farkas et al.⁸ found increased mRNA levels of iron-regulating proteins, suggesting the involvement of iron and copper metabolism and associated antioxidant systems in the pathogenesis of glaucoma. Nevertheless, dynamic changes in gene expression over prolonged periods in experimental glaucoma and after optic nerve transection (ONT) have not yet been characterized in depth.

In an attempt to identify the signal-transduction pathways that are activated in response to optic nerve injury, we used a specific gene array containing 96 genes encoding proteins involved in 18 different signaling pathways. Among the investigated signal-transduction pathways were p53, NFκB, CREB, and TGFβ. Using the translimbal photocoagulation laser model for glaucoma in rats, this study focused on genes involved in signal transduction, for the study of changes in gene expression in glaucomatous eyes and in eyes that had undergone ONT.

METHODS

Animals

Wistar rats (375–425 g) were used in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research in protocols approved and monitored by the Animal Care Committee of the Tel-Aviv University School of Medicine. The animals were housed in a 14-hour light and 10-hour dark cycle with standard chow and water ad libitum.

Experimental Glaucoma

Elevated IOP was induced in one eye of 34 animals using the laser photocoagulation model for experimental glaucoma as described earlier.¹¹ Briefly, the outflow channels of the eye were lasered through the peripheral cornea. Treatment was repeated at 1 week if the difference in IOP between the two eyes was less than 6 mm Hg. IOP was measured with a handheld tonometer (Tonopen XL; Medtronic, Jacksonville, FL) weekly.

Optic Nerve Transection

ONT was performed unilaterally in 18 rats under anesthesia. Using an operating microscope, we incised the superior conjunctiva and transected the intraorbital optic nerve 1 to 2 mm behind the globe, taking care not to damage its blood supply. The retinas were examined ophthalmoscopically, to assure blood vessel patency.

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TABLE 1. Primer Sequences Used for Real-Time RT-PCR Analysis

Gene	Forward Primer	Reverse Primer
<i>Gadd45a</i>	TAACTGTGGCGGTGTACGAG	GCAACAGAAAGCACGAATGA
<i>IAP-1</i>	TGTGCATCTGGGCGCTG	CTGACCGTCCCTGTAGTCTCAAC
<i>ei24</i>	AAGCTTCTTGTGGGGCACTA	CTGCACTCCTGCACAAGGTA
<i>cdk2</i>	GAGGTGGTGACCCTCTGGTA	GTCACCATTTGGGCAAGAT
<i>p53</i>	GTCCGAGAGCTGAATGAGG	TTTTATGGCGGGACGTAGAC
<i>b-Actin</i>	GCTACAGCTTCACCACCACA	TCTCCAGGGAGGAAGAGGAT

Gene Array Analysis

Total RNA was extracted from retinas dissected 6 hours after ONT ($n = 4$; RNeasy mini kit; Qiagen, Valencia, CA). RNA was then reverse transcribed into cDNA, by using random hexamers (Promega, Madison, WI) and MMLV-reverse transcriptase (Promega, Madison, WI) in the presence of ^{32}P - α -dCTP (GE Healthcare, Piscataway, NJ). Radiolabeled cDNA was then hybridized to a gene array (Cat. number MM-008; Signal Transduction Pathway Finder; Superarray Inc., Frederick, MD), according to the manufacturer's instructions. The results were assessed by autoradiography and densitometric analysis (Scion Image software; Scion Corp., Frederick, MD).

Semiquantitative Reverse Transcription-Polymerase Chain Reaction

The gene array found that seven genes were upregulated. To confirm these results, semiquantitative RT-PCR was performed with cDNA samples from retinas excised 1 day after ONT (see Table 1 for primer sequences). PCR reactions were performed in 25 μL total volume containing 2 μL cDNA, 0.5 μM each primer, 0.25 mM each dNTP, and 0.5 unit *Taq* polymerase (Supernova; JMR Holdings, Kent, UK) with the buffer supplied by the manufacturer by 30 cycles of 45 seconds at 94°C, 45 seconds at 55°C, and 1 minute at 72°C, terminated by a 5-minute extension step at 72°C.

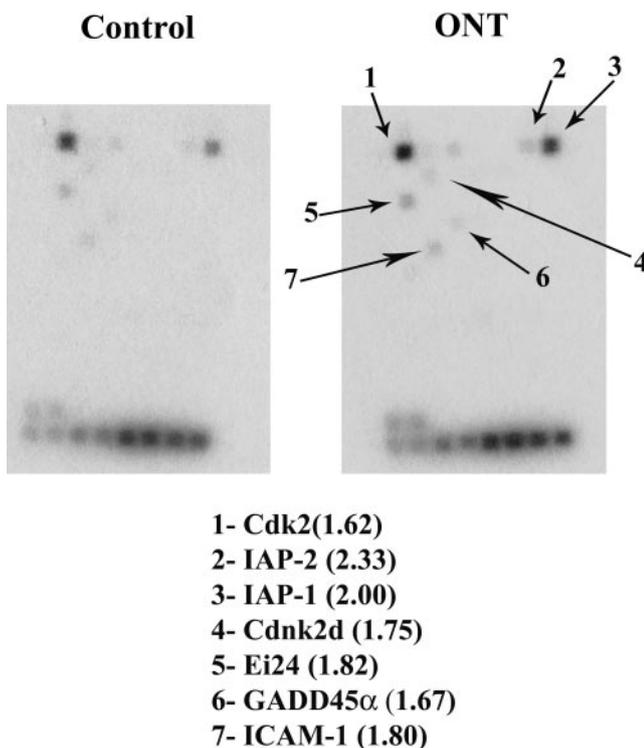


FIGURE 1. Gene array analysis 6 hours after ONT. Arrows: upregulated genes listed under the arrays. The numbers in the parentheses indicate enhancement (\times -fold) by the ONT procedure for each gene.

Quantitative RT-PCR

Semiquantitative RT-PCR had confirmed the upregulation of four genes. These genes were further analyzed by quantitative real-time RT-PCR using RNA retinal samples from eyes at various time points after either ONT (from 3 hours to 14 days) or IOP elevation (from 1 to 60 days). Because the *Gadd45a* and *Ei24* genes are members of the p-53 pathway, the level of p-53 was also investigated. Quantitative RT-PCR was performed (Quanti Tect SybrGreen RT-PCR system; Qiagen) on a sequence detector (Prism 7700; Applied Biosystems [ABI], Foster City, CA). Real-time RT-PCR reactions were performed with 40 amplification cycles of 20 seconds at 95°C and 1 minute at 60°C. A standard curve for each gene was created using three 10-fold dilutions of a cDNA sample produced from a retina of an untreated animal. β -Actin was used as a reference gene (see Table 1 for primer sequences used in real-time RT-PCR assays). Each sample was analyzed in triplicate by at least three separate PCR reactions. The results were analyzed using the sequence detector software (Ver. 1.6; ABI). For each sample, we calculated the normalized ratio (i.e., the number of copies of a given gene divided by the number of copies of β -actin in each sample). The results of real-time PCR experiments are expressed as the ratio between the experimental and the control eye calculated by using the normalized ratios.

Western Blot Analysis

Cell extracts from retinas of separate cohorts of animals were centrifuged for 5 minutes at 14,000g at 4°C. The Triton X-100-soluble fraction (supernatant) containing membrane and cytoplasmic proteins, as well as the Triton X-100-insoluble fraction (pellet) containing cytoskeletal and nuclear proteins were separated by SDS-PAGE, electrotransferred onto a polyvinylidene difluoride (PVDF) membrane (Immobilon-P; Millipore, Bedford, MA), and blocked in TBS containing 5% skim milk for 16 hours at 4°C. The membranes were then incubated with either rabbit anti-Gadd45 α (Santa-Cruz Biotechnology, Inc., Santa Cruz, CA) or goat-anti-IAP-1 (Santa-Cruz Biotechnology) for 2 hours, followed by incubation with the appropriate secondary antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) and detection by a chemiluminescent substrate (ECL; GE Healthcare).

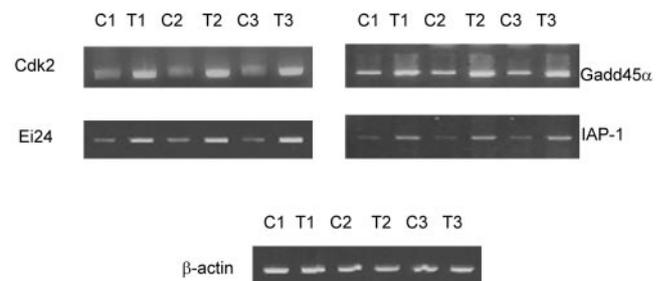


FIGURE 2. Semiquantitative RT-PCR analysis of *Gadd45a*, *Cdk2*, *Ei24*, and *IAP-1* genes in ONT. β -Actin was used as a reference gene. One day after ONT, there was a significant upregulation of all four genes in each experimental eye (T) compared with the control fellow eye (C) although β -actin expression was similar in control and experimental eyes.

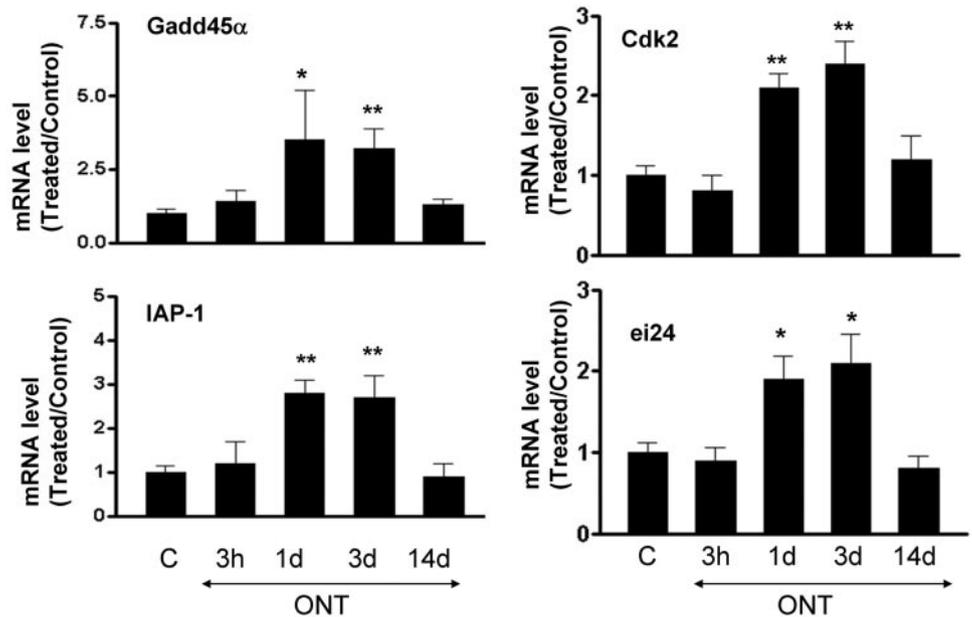


FIGURE 3. Quantitative real-time PCR analysis of *Gadd45a*, *IAP-1*, *Ei24*, and *Cdk2* gene expression after ONT. All four genes were significantly upregulated at 1 and 3 days after ONT and returned to normal after 14 days. Results are expressed as the ratio between the experimental and the control eyes. *n* = three to four rats at each time point. Each gene was tested three times and each sample was run in triplicate. **P* < 0.05; ***P* < 0.01. C, control naïve animals (*n* = 4).

Immunohistochemistry

Eyes were enucleated and cryopreserved in sucrose and optimal cutting temperature compound (OCT; Sakura Finetek, USA, Inc.; Torrance, CA). Cryosections 10-μm-thick were stained with a rabbit polyclonal anti-Gadd45α antibody (Santa-Cruz Biotechnology) at a 1:50 dilution. Negative control experiments included nonimmune serum of the same species as the primary antibody at the same protein concentration and incubation buffer alone.

For immunohistochemistry we used equatorial sections containing the optic nerve head as standard anatomic reference. At least four sections from each eye were viewed by two experienced, masked observers on a semiquantitative scale at one of nine levels by comparison with digital grading of standard slides for each antibody. Images of representative slides were captured digitally at standardized microscope and camera settings (Olympus Optical Co., Tokyo, Japan).

RESULTS

cDNA Hybridization to Gene Expression Arrays

cDNA prepared from RNA extracted from retinas 6 hours after ONT and from their control fellow eyes were hybridized to gene arrays containing 96 marker genes associated with 18 signal-transduction pathways. Examination of the gene expression pattern revealed that seven genes were upregulated more than 1.5-fold compared with baseline levels (Fig. 1). These genes were inhibitor of apoptosis protein-1 (*IAP-1*) and inhibitor of apoptosis protein 2 (*IAP-2*), both associated with inhibition of apoptosis; *Ei24* and *Gadd45a*, both associated with induction of apoptosis via the p53 pathway; cyclin-dependent kinase inhibitor 2D (*Cdkn2d*; P19) and cyclin-dependent kinase 2 (*Cdk2*), both associated with regulation of the cell cycle and apoptosis; and intercellular adhesion molecule-1 (*ICAM-1*), associated with cell adhesion and inflammation.

The gene array was used as a semiquantitative approach for identifying potentially essential genes in the pathogenesis of RGCs in glaucoma.

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Verification by Semiquantitative RT-PCR Analysis

To verify the changes observed in response to ONT in the gene array, we tested the seven altered genes by semiquantitative RT-PCR in retinas 1 day after ONT (*n* = 6). Significant upregulation of four of these seven genes was confirmed: *Ei24*, *Gadd45a*, *IAP-1*, and *Cdk2* (Fig. 2).

Time Course Analysis of Changes in *Gadd45a*, *Ei24*, *IAP-1*, and *Cdk2* Genes by Quantitative RT-PCR

ONT Model The levels of the upregulated genes as well as the β-actin levels (reference gene) were tested by RT-PCR at 3 hours and 1, 3, and 14 days after ONT (Fig. 3). The control and experimental eyes were compared for the ratio between the gene of interest and β-actin levels for each of the four genes in each animal at the indicated time points. Retinas of untreated naïve animals (*n* = 4) were also tested for the ratio between

TABLE 2. IOP History in Rats with Experimental Glaucoma

Time Point (d)	Mean IOP			Peak IOP		
	Glaucoma	Control*	<i>P</i>	Glaucoma	Control*	<i>P</i>
1	34.5 ± 7.6	23.5 ± 4.1	0.09	38.3 ± 10.8	25.1 ± 3.3	0.11
8	38.3 ± 1.4	25.6 ± 0.5	0.0001	46.1 ± 3.6	28.2 ± 0.8	0.001
16	30.9 ± 1.4	22.2 ± 1.9	0.0003	41.1 ± 4.9	25.2 ± 2.4	0.0012
30	30.7 ± 2.3	23.3 ± 1.7	0.002	44.9 ± 7.6	26.1 ± 2.0	0.003
64	26.7 ± 1.9	23.6 ± 0.9	0.01	36.8 ± 4.6	30.4 ± 3.6	0.01

Results are shown as the mean ± SD.
* The animal's fellow eye.

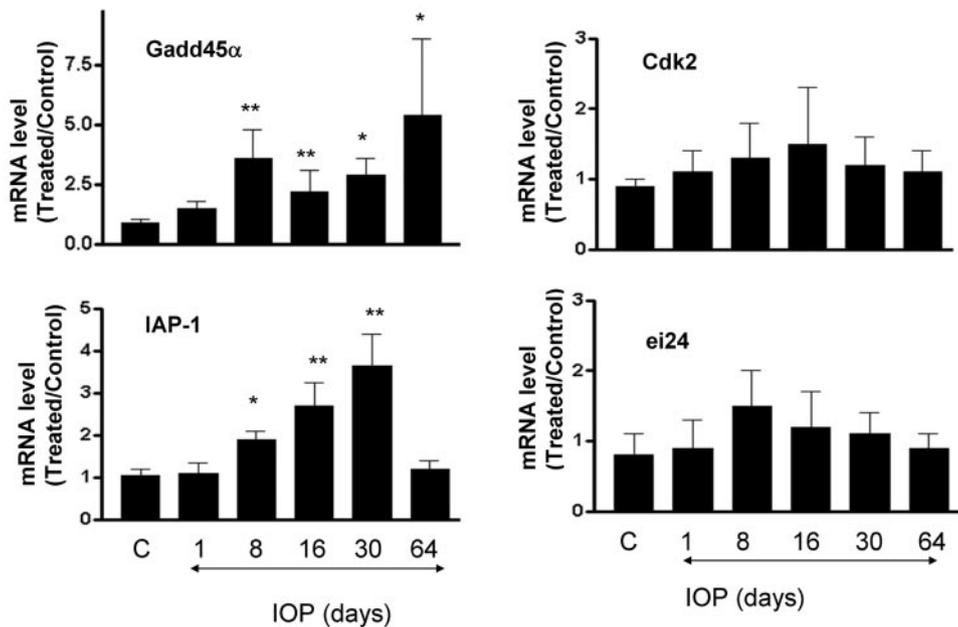


FIGURE 4. Quantitative real-time PCR analysis of *Gadd45a*, *IAP-1*, *Ei24*, and *Cdk2* gene expression in experimental glaucoma. The proapoptotic gene *Gadd45a* and the prosurvival gene *IAP-1* were significantly upregulated 1 month after the induction of elevated IOP. At 2 months, however, only *Gadd45a* was still upregulated. * $P < 0.05$; ** $P < 0.01$. C, control naïve animals ($n = 4$).

the gene of interest and β -actin level for each of the four genes. No change was observed early after ONT (3 hours), but the *Gadd45a*, *Cdk2*, *Ei24*, and *IAP-1* genes were significantly upregulated on days 1 and 3 after ONT (Fig. 3). Their levels returned to normal after 14 days.

Glaucoma Model. Elevated IOP was induced in 34 animals, and all of them demonstrated increased IOP (Table 2). IOP returned to baseline 3 weeks after the first laser treatment, as expected in this model. Retinas from experimental and control fellow eyes were tested for expression of the above four genes at 1, 8, 16, 30, and 64 days after the first laser treatment. Real-time RT-PCR analysis showed enhanced expression of the proapoptotic gene *Gadd45a* as early as 24 hours after IOP elevation, as seen with ONT (Fig. 4). *Gadd45a* was still significantly upregulated at 64 days, although IOP returned to baseline at 3 weeks. Also, as has been detected in ONT, we found simultaneous overexpression of the antiapoptotic gene *IAP-1*. Its level, however, gradually increased from days 8 to 30 after IOP elevation and returned to baseline on day 60. Sixty days after the induction of elevated IOP, the level of the caspase inhibitor *IAP-1* was back to normal whereas the proapoptotic gene *Gadd45a* was still upregulated. The levels of *Ei24* mRNA and *Cdk2* increased on day 8 but not significantly, unlike the findings after ONT (Fig. 4).

Analysis of p53 Gene Expression in ONT and Glaucoma Models. Both *Gadd45a* and *Ei24* are regulated by the tumor-suppressor gene *p53*. Consequently, we compared expression of *p53* mRNA between control retinas and retinas of eyes after ONT or IOP elevation. No significant alteration in the level of *p53* was detected at days 1 ($n = 3$) and 3 ($n = 3$) after ONT and at days 8 ($n = 3$) and 30 ($n = 3$) after induction of elevated IOP (Fig. 5).

Western Blot Analysis

The IAP-1 level was analyzed in the Triton X-100-soluble fraction containing membrane and cytoplasmic proteins, whereas *Gadd45a*, a predominantly nuclear protein, was studied in the Triton X-100-insoluble fraction containing nuclear proteins. Laser-treated retinas demonstrated a significant increase in the levels of both *Gadd45a* (2.1 ± 0.3 -fold; $n = 4$) and IAP-1 (2.2 ± 0.1 -fold; $n = 4$) proteins at 8 days (Fig. 6). These results support the RT-PCR data and demonstrate that

Gadd45a and *IAP-1* genes are activated and translate to proteins in elevated IOP conditions.

Immunohistochemical Analysis

Intense labeling for *Gadd45a* and *IAP-1* was detected specifically in the RGC layer. The intensities of *Gadd45a* and *IAP-1* immunolabeling were compared on a nine-level scale. The mean intensity of *Gadd45a* at 2 days after ONT was 6.62 ± 0.11 ($n = 3$) compared with 3.41 ± 1.13 ($n = 3$) in the fellow control eyes ($P = 0.007$). The mean intensity in the glaucomatous eyes at 8 days after the first laser was 4.23 ± 1.52 ($n = 6$) compared with 1.33 ± 0.94 ($n = 6$) in the fellow control eyes ($P = 0.003$; Fig. 7). The staining intensity for IAP-1 at 4 days after ONT increased in 1.6-fold compared with fellow control eyes ($n = 4$; Fig. 8).

DISCUSSION

In the present study, we found that glaucoma and ONT induce simultaneous upregulation of proapoptotic and prosurvival genes and that the p-53 pathway is significantly involved in optic nerve damage. We demonstrated that *Gadd45a* and *Ei24* genes, members of the p-53 pathway, are upregulated in both glaucoma and after ONT. In addition, an endogenous neuro-

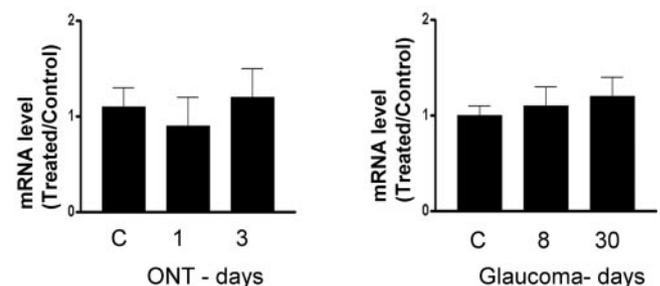


FIGURE 5. Quantitative real-time PCR analysis of *p53* gene expression in ONT and experimental glaucoma models. No significant changes in the expression of the p53 gene were detected at 1 and 8 days after ONT or at 8 and 30 days after induction of elevated IOP. C, control naïve animals ($n = 4$).

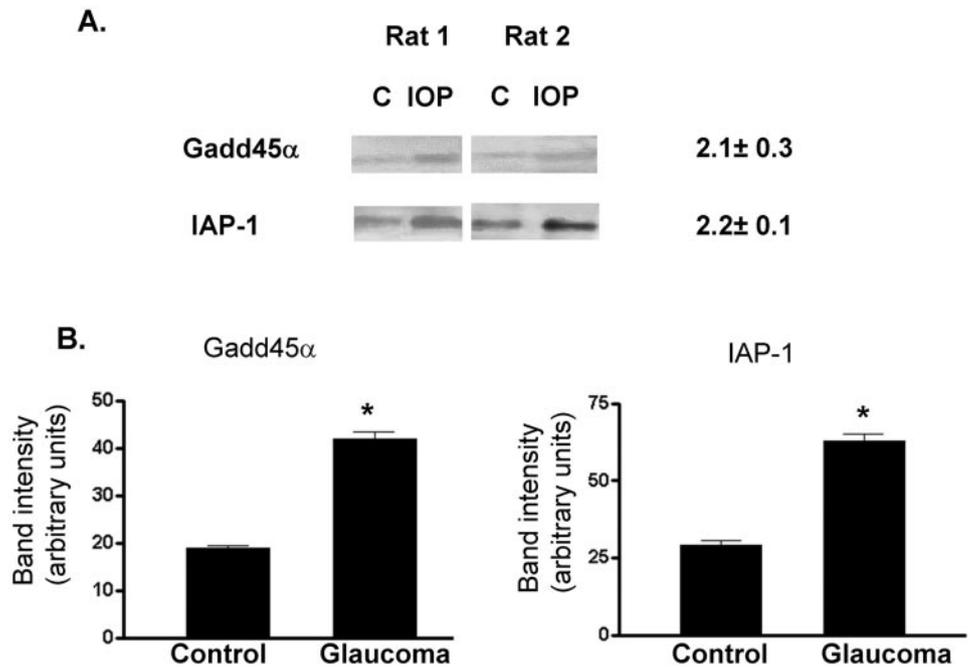


FIGURE 6. Western blot analysis of *Gadd45a* and *IAP-1* in glaucomatous eyes. *Gadd45a* and *IAP-1* protein levels were significantly high in glaucomatous eyes compared with control fellow eyes (C) at 8 days after the induction of elevated IOP. (A) Representative blots of two of four animals tested. (B) Densitometric analysis of *Gadd45a* and *IAP-1* protein levels in glaucomatous versus control fellow eyes. The results are expressed as mean \pm SD of band intensities. * $P < 0.001$.

protective gene, the caspase inhibitor IAP-1, was significantly upregulated in both models.

Several signal transduction pathways have previously been shown to be activated in glaucoma and ONT, including the MAP-kinase pathway,^{4,12,13} proteolytic enzymes of the caspase family,^{6,14-16} TNF α , NF κ B,^{17,18} and others. The role of p53-mediated cellular processes in RGC apoptosis due to optic nerve damage and glaucoma has not yet been fully characterized. An association between allelic variants of the *p53* gene and human primary open-angle glaucoma has been examined in different groups, and variable results have been reported.¹⁹⁻²¹

p53 is a tumor-suppressor gene involved in regulation of the cell cycle in response to DNA damage. It plays an important role in cell cycle control and in the induction of apoptosis²² and acts as a transcriptional activator, regulating the expression of genes involved in growth arrest (e.g., *p21*, *Gadd45*, *14-3-3-d*), DNA repair (*p53R2*), and apoptosis (*BaxE*, *Apaf-1*).^{22,23} Mutations in the *p53* gene are among the most commonly diagnosed genetic disorders, occurring in as many as 50% of patients with cancer. In the present study, we found that *Gadd45a*, the growth arrest and DNA damage-inducible gene, was significantly upregulated in glaucoma and after ONT. In glaucoma, it continued to be upregulated, even long after

the IOP had returned to normal. *Gadd45a* is a predominantly nuclear protein widely expressed in normal tissues.²⁴ It is involved in p53-mediated apoptosis and suppression of cell growth, as well as in DNA repair.²⁵⁻³² *Gadd45a* expression was also reported to be upregulated in the retinas of rats undergoing apoptosis due to exposure to neurotoxic injury by *N*-methyl-D-aspartic acid (NMDA).³³

Similarly to *Gadd45a*, *Ei24* is also a gene induced by p53 in response to DNA damage.³⁴⁻³⁷ We now demonstrated that *Ei24* is significantly upregulated after ONT, simultaneously with *Gadd45a*. In glaucoma, however, it is only mildly upregulated at 8 days after the first laser treatment and then declines to baseline, whereas *Gadd45a* continues to be significantly upregulated.

Differences in *Ei24* expression between glaucoma and ONT could be due to differences in the severity of damage. For example, RGCs die rapidly in ONT and so more RGCs are involved in apoptosis at any particular moment. It is also possible that each model is different and that *Gadd45a* plays the more significant role in glaucoma. Nevertheless, the induction of both *Gadd45a* and *Ei24* expression in rat retinas in response to ONT and elevated IOP, as observed in the present study, may suggest p53-mediated apoptosis to be one of the pathways activated by optic nerve damage.

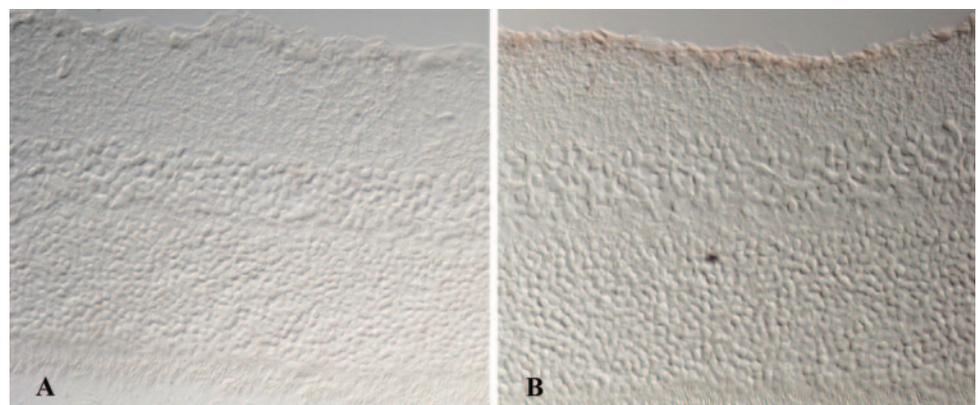


FIGURE 7. Immunohistochemistry for *Gadd45a* at 8 days after the induction of elevated IOP showed significant staining in the RGC layer in the glaucomatous eye (B) and none in the control fellow eye (A).

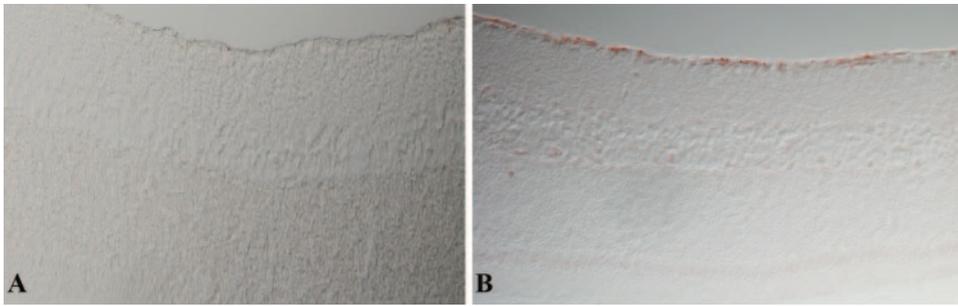


FIGURE 8. Immunohistochemistry for *IAP-1* at 4 days after ONT showed significant staining in the RGC layer in the glaucomatous eye (**B**) and none in the control fellow eye (**A**).

Cdk2 is the third proapoptotic gene that was significantly upregulated after ONT. This gene participates in cell-cycle regulation at the G₁/S interphase.³⁸⁻⁴¹ Of interest, we observed significant upregulation of *Cdk2* gene expression in the ONT model, but only mild upregulation in the glaucoma model. This finding suggests that although equally capable of inducing eventual RGC death, the two models might differ in their effect on the genes involved in cell-cycle regulation.

We also found that the caspase inhibitor *IAP-1*, a powerful prosurvival gene, is upregulated in both glaucoma and after ONT. Most studies have shown downregulation of antiapoptotic genes as a response to injury, thereby explaining why the cells eventually die. Antiapoptotic (prosurvival) factors, such as the *Bcl2* gene, were found to be downregulated after elevation of IOP,⁴² and neuroprotective compounds were shown to act by stimulating prosurvival pathways (e.g., the PI3-K/Akt pathway).^{43,44} In contrast, Manabe and Lipton⁴⁵ studied a model of NMDA-induced RGC death and found activation of an apoptotic pathway mediated by P-38, as well as an antiapoptotic pathway mediated by PI-3 kinase-Akt. Our group has demonstrated that both ONT and IOP elevation induce activation of c-Jun,⁴ which is known to be involved in both apoptotic and antiapoptotic processes.

Our current finding that a caspase inhibitor gene is upregulated by elevated IOP may indicate that RGCs exhibit an intrinsic neuroprotective mechanism for counteracting apoptosis and for potentially improving RGC survival.⁴⁶

The IAPs are a family of ubiquitous intracellular proteins protecting cells from apoptosis induced by a variety of stimuli.^{47,48} IAPs are capable of direct interaction and inhibition of caspases 3, 7, and 9, thereby partially mediating the apoptosis-inhibitory function of IAPs.⁴⁹⁻⁵¹ Recently, McKinnon et al.⁵² found that gene therapy delivering the human x-linked inhibitor of apoptosis (*XIAP*; a member of the inhibitors of apoptosis proteins [IAPs] family compounds) promotes optic nerve axon survival in a rat chronic ocular hypertension model, suggesting that caspase inhibition may play a protective role in glaucoma.

This study demonstrates that changes in ONT returned to normal at 2 weeks when most RGCs had already died.⁵³ However, changes in glaucoma persisted long after IOP had returned to normal, with proapoptotic genes being upregulated for a longer time than the prosurvival gene *IAP-1*. In the translimbal photocoagulation model for glaucoma, IOP returned to baseline by 3 weeks after the first laser treatment. The proapoptotic gene *Gadd45* was upregulated for at least 2 months after the first laser treatment (i.e., more than 1 month after normalization of IOP). The prosurvival gene *IAP-1* was still upregulated at 1 month after the first laser treatment, and its level declined back to baseline at 2 months. These data indicate that RGCs may degenerate and die, even long after IOP has returned to normal. There is no way to determine whether this is a result of delayed degeneration of RGCs that had been partially injured or, alternatively, is a process of secondary degeneration.

In this study, we used a small gene array to screen for the major signal-transduction pathways that are involved in apoptosis. Further investigation is needed to shed light on the intrinsic mechanisms of apoptosis and neuroprotection in glaucoma.

In summary, we found that the *p53* pathway plays a role in RGC death in glaucoma and that elevated IOP simultaneously induces an intrinsic neuroprotective mechanism that includes upregulation of the caspase inhibitor *IAP-1*. The activation of the proapoptotic *p53* pathway lasted long after the apoptosis inhibitor *IAP-1* had returned to baseline levels. The fact that upregulation of proapoptotic factors lasted longer than that of the prosurvival factors may determine the fate of the cell to undergo apoptosis and degeneration. Modulating the intrinsic neuroprotective mechanisms may be beneficial for enhancing the survival of RGCs in glaucoma.

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