Effect of 2′-Benzoyl-oxycinnamaldehyde on RPE Cells In Vitro and in an Experimental Proliferative Vitreoretinopathy Model

Jae Jun Lee,[1,2] Jong Kuk Park,[2,3,4] Yun-Taik Kim,[3] Byoung-Mog Kwon,[5] Shin Goo Kang,[6] Young Do Yoo,[4,7] Young Suk Yu,[8,9] and Hum Chung[8,9]

Purpose. To investigate whether 2′-benzoyl-oxycinnamaldehyde (BCA) induces apoptosis in human retinal pigment epithelial (hRPE) cells and has an antiproliferative effect in a proliferative vitreoretinopathy (PVR) model in the rabbit.

Methods. Fifty percent growth inhibition doses of hRPE cells at 50%, 75%, and 100% confluence were determined by MTT assay. Apoptosis in hRPE cells induced by BCA was shown by DAPI staining. Expression of p53, p21, Bcl-2, GADD45, cyclin D, phospho-MAP kinase, cdk2, and Akt1 at various concentrations of BCA in cultured hRPE cells was examined by immunoblot analysis. In the efficacy study, 2.0×10^5 rabbit RPE cells were injected into the vitreous cavity after gas compression, and the eyes subsequently received either sham injections or 600 μM BCA. Fundus examination was performed before and 1, 7, 14, 21, and 28 days after BCA injection. The toxicity studies were conducted by the same protocol as used for the efficacy evaluation but without the RPE cell injection. Simultaneous electroretinograms were recorded on days 1, 7, 14, 21, and 28 after exposure to the drug.

Results. BCA treatment induced apoptosis in hRPE cells. Furthermore, an increase in p53 expression, phosphorylation of Bcl-2, and downregulation of Akt1 expression were observed in BCA-induced apoptotic cells. BCA effectively prevented the proliferation of rabbit RPE cells in the experimental PVR model. BCA exhibited a wide safety margin, showing no evidence of causing retinal toxicity, even at the 600-μM concentration.

Conclusions. The results of this study suggest that BCA effectively inhibits proliferation of RPE cells and has a very wide safety margin, indicating a potential therapeutic usefulness in treating PVR. (*Invest Ophthalmol Vis Sci.* 2002;43:3117–3124)

From the [1]Department of Ophthalmology, St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea; the [3]Department of Life Science, Sogang University, Seoul, Korea; the [5]Korea Research Institute of Bioscience and Biotechnology, Yusong, Taejon; the [6]Department of Ophthalmology, Eulji University School of Medicine, Seoul, Korea; the [7]Department of Internal Medicine and the [4]Genomic Research Center for Lung and Breast/Ovarian Cancers, Korea University College of Medicine, Seoul, Korea; the [8]Department of Ophthalmology, College of Medicine, Seoul National University, Seoul, Korea; and the [9]Seoul National University Hospital Clinical Research Institute, Seoul, Korea.

[2]Contributed equally to the work.

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Corresponding author: Hum Chung, Department of Ophthalmology, Seoul National University Hospital, 28 Yeongun-dong Chongro-ku, Seoul, 110-744, South Korea; chungh@snu.ac.kr.

Proliferative vitreoretinopathy (PVR), the principal cause of failed retinal reattachment surgery, is characterized by migration and proliferation of cells that form cellular membranes in the vitreous and on the retina.[1,2] Cell types identified in PVR membranes include retinal pigment epithelial (RPE) cells,[3] Müller cells,[4] astrocytes,[5] fibroblasts, and inflammatory cells.[6] A variety of antiproliferative and anti-inflammatory agents, such as daunomycin,[7] aclacinomycin A,[8] fluorouracil,[9] paclitaxel,[10] retinoic acid,[11] corticosteroid,[12] camptothecin,[13] daunorubicin,[14] and genistein[15] have been tested for their potential to reduce the development of traction retinal detachment (TRD) in experimental models of PVR. Most of the drugs have found limited clinical application because of toxicity, a relative lack of efficacy, and the need for multiple injections or for a vitrectomy procedure with vitreous infusion.

2′-Benzoyl-oxycinnamaldehyde (BCA; Fig. 1) is a derivative of 2′-hydroxycinnamaldehyde which was originally isolated from the stem bark of *Cinnamomum cassia* Blume, and inhibits farnesyl-protein transferase.[16–18] BCA has been shown to inhibit growth of various tumor cells in vitro and in vivo. In the present study, we investigated whether BCA can induce apoptosis in human RPE (hRPE) cells and effectively inhibit proliferation and migration of hRPE cells in vitro. Also, the safety of intravitreous BCA in rabbit eyes and its potential antiproliferative effect in a PVR rabbit model were studied.

Materials and Methods

Human RPE Cell Culture

hRPE cells were obtained from eyes of human donors aged between 15 and 50 years. Eyes were enucleated within 24 hours after death and cultured in Dulbecco's modified Eagle medium (DMEM; Gibco/BRL, Grand Island, NY), supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL) and maintained at 37°C and 5% CO_2. hRPE cells from passages 4 to 6 were used in the experiments.

Rabbit RPE Cell Culture

We used New Zealand albino rabbits to provide a uniform source of tissue-cultured RPE cells. We grew RPE as a monolayer in DMEM containing 20% fetal bovine serum and antibiotics (penicillin G sodium, streptomycin sulfate) in an incubator with 5% CO_2 at 37°C. The medium was then changed to DMEM supplemented with 10% fetal bovine serum and antibiotics, as in hRPE cultures. Rabbit RPE cells from 2 to 4 passages were used in the experiments. Cells were harvested by incubating with 3.5 mL 0.05% trypsin for 4 minutes, and the cells were collected in stop medium. The dispersed cells were centrifuged at 1000 rpm for 5 minutes and resuspended in 1 mL phosphate-buffered saline (PBS). After the cells were counted, the suspension was diluted to a final concentration of 2.0×10^5 cells in 0.1 mL PBS. A trypan blue test indicated that 99% of the cells were viable just before injection.

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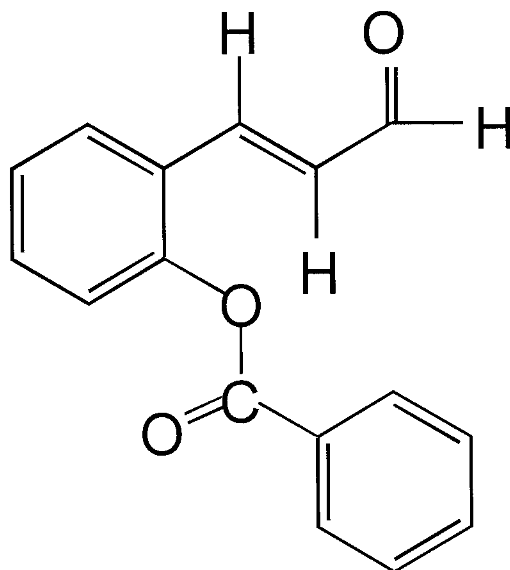


Figure 1. Molecular structure of BCA.

Drug Sensitivity Assay

Cells (2×10^3, 3×10^3, and 5×10^3) were seeded onto 96-well plates and were treated with various concentrations of BCA (4, 8, 16, and 32 μM) after 20 hours of incubation. Seventy-two hours later, 50 μL of 3-(4,5-dimethylthiazol-2-yle)-2,5-diphenyltetrazolium bromide (MTT) solution (2 mg/mL) was added to each well, and the plate was then incubated at 37°C for 4 hours. Dark-blue formazan crystals formed by living cells were dissolved in 150 μL of dimethyl sulfoxide (DMSO), and absorbance of individual wells at 545 nm was determined with a microplate reader (model 450; Bio-Rad, Hercules, CA). The 50% inhibition dose (IC_{50}) was calculated from a dose–effect analysis performed on microcomputer (Biosoft, Cambridge, UK).

DAPI Staining

hRPE cells were seeded into 100-mm tissue culture dishes containing a slide glass that had been precoated with 1 mg/mL poly-L-lysine (Sigma, St. Louis, MO). After 20 hours, the cells were treated with 5, 10, and 40 μM BCA and were harvested at various times. The cells on the slide glass were washed with PBS, fixed with methanol, and stained with 1 μg/mL 4′,6′-diamino-2-phenylindole (DAPI; Sigma) in PBS for 30 minutes. After the cells were washed with PBS and mounted, the apoptotic cells were counted under a fluorescence microscope (Axioplan2; Carl Zeiss, Oberkochen, Germany).

Immunoblot Analysis

Cells were treated with various doses of BCA, and cell lysates were prepared in 50 μL RIPA buffer (150 mM NaCl, 1.0% Nonidet P-40, 0.5% deoxycholic acid, 0.1% SDS, and 50 mM Tris [pH 8.0]) containing phosphatase inhibitors (1 mM sodium orthovanadate, 30 mM NaF, 1 mM phenylmethylsulfonyl fluoride, and 30 mM NaPPi). Protein concentration was determined by the Bradford analysis,[19] and equal amounts of proteins (50 μg/mL) were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Subsequently, the membranes were probed with p53 (Dako, Glostrup, Denmark), p21 (Santa Cruz Biotechnology, Santa Cruz, CA), Bcl-2 (Dako), GADD45 (Santa Cruz Biotechnology), cyclin D (Santa Cruz Biotechnology), phospho-MAP kinase (Santa Cruz Biotechnology), Cdk2 (Santa Cruz Biotechnology), and Akt1 (Santa Cruz Biotechnology) antibodies and developed with peroxidase-labeled anti-rabbit antibody according to the manufacturer's instructions (Amersham, Little Chalfont, UK).

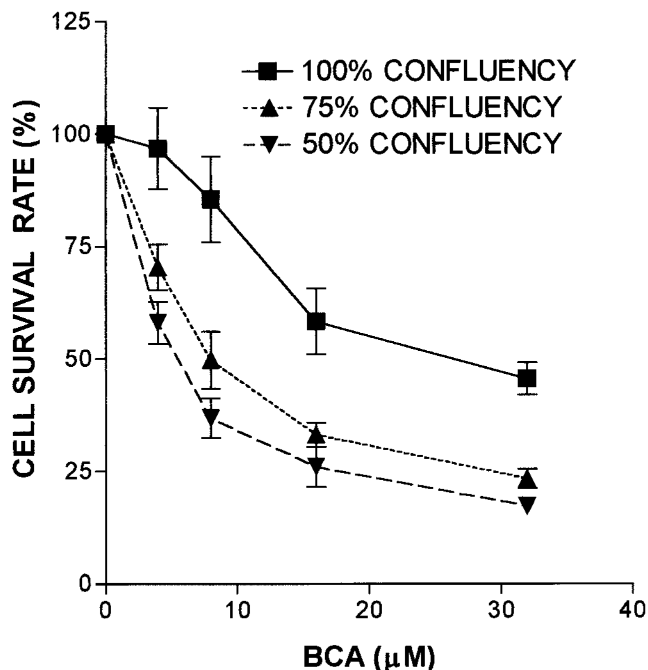


Figure 2. The effect of the extent of confluence on cell survival after treatment with BCA. RPE cells were seeded on a 96-well plate, and the cells were maintained at approximately 50%, 75%, or 100% confluence. The cells were then treated with 4, 8, 16, and 32 μM BCA for 72 hours, and cell survival was measured by MTT assay.

Cell Motility Assays

hRPE cell motility was measured by a permeable membrane assay (0.8-μm pore; Transwell; Costar, Cambridge, MA), a modification of the method of Hinton et al.[20] The lower chamber was coated with fibronectin (25 μg/mL), and DMEM (10% FBS, 0.6 mL) was added. Subconfluent RPE cells (5×10^4) were seeded in each upper chamber

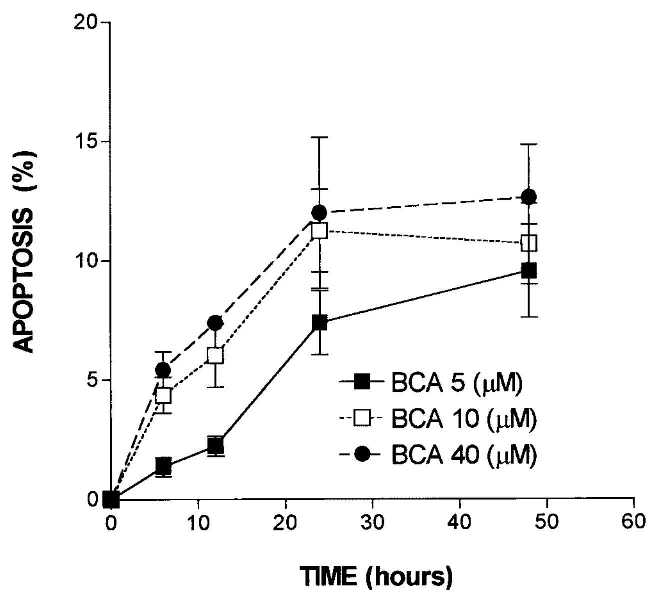


Figure 3. BCA-induced apoptosis. RPE cells were treated with 5, 10, and 40 μM BCA and harvested at various times. After staining with DAPI, apoptotic cells were counted under a fluorescence microscope. The apoptosis rate is presented as the percentage of apoptotic cells.

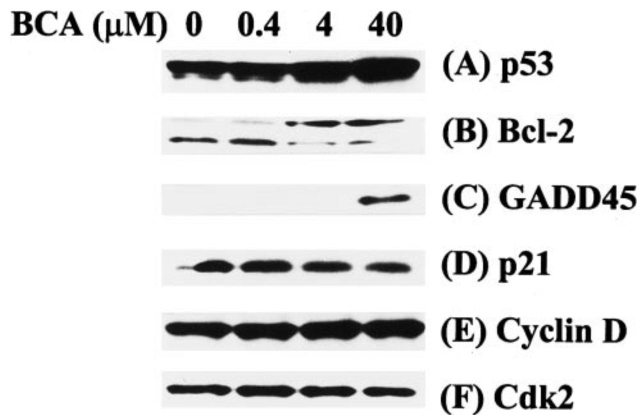


FIGURE 4. Western blot analyses of (A) p53, (B) Bcl-2, (C) GADD45, (D) p21, (E) cyclin D, and (F) Cdk2 in BCA-treated RPE cells. Cells were treated with 0.4, 4, and 40 μM BCA for 24 hours. After nonconfluent cells were harvested, cellular proteins were extracted. Equal loading of the protein was confirmed by ponceau-S staining.

after cells were washed twice with serum-free DMEM. The hRPE cells were pretreated with 1.25, 1.8, and 2.5 μM BCA or with DMSO (Sigma), a solvent of BCA, for 30 minutes at room temperature.

Animals and Anesthesia

All investigations in animals were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the NIH Principles of Laboratory Animal Care (revised 1985). Twenty-two New Zealand albino rabbits weighing 1.5 to 2.5 kg were used in the present study. Rabbits were anesthetized with intramuscular injections of ketamine HCl (25 mg/kg) and xylazine (5 mg/kg), and pupils were dilated with topical 1% tropicamide and 2.5% phenylephrine.

Gas Compression and Gas–Fluid Exchange

The technique of gas-mediated vitreous compression as part of the refined experimental model of PVR has been described.[21,22] Perfluorocarbon gas (0.3 mL) was injected by anterior chamber paracentesis to reduce the possibility of ocular damage caused by an acute increase in pressure. Three days later, the gas had expanded to fill 80% to 90% of the vitreous cavity. Gas–fluid exchange was accomplished at 5 days after gas injection, by using a 5-mL syringe filled with sterile balanced salt solution (BSS). A 30-gauge needle was inserted into the vitreous cavity inferiorly, and approximately 0.2 to 0.3 mL BSS was injected. Gas was observed to escape readily through the inserted needle. This process of slow injection of BSS and gas escape was repeated until gas could no longer be seen in the eye.

Effect of BCA on Experimental PVR

Toxicity Study. Ten days after gas–fluid exchange, a 30-gauge needle was inserted 4 mm posterior to the corneoscleral junction in the superotemporal quadrant with the assistance of a microscope. Nine rabbits were assigned to two groups. Four eyes were injected with 300 μM BCA in group 1, and five eyes were injected with 600 μM

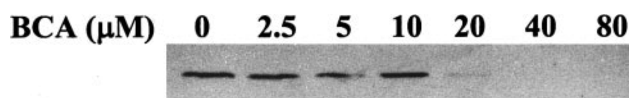


FIGURE 5. Western blot analysis of Akt in BCA-treated RPE cells. Cells were treated with 2.5, 5, 10, 20, 40, and 80 μM BCA for 24 hours. After nonconfluent cells were harvested, cellular proteins were extracted. Equal loading of the protein was confirmed by ponceau-S staining

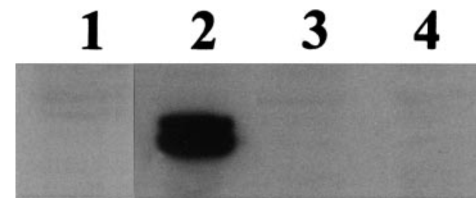


FIGURE 6. Immunoblot of phospho-p44/42 MAP kinase. RPE cells were seeded on 100-mm dishes and starved for serum for 24 hours. Samples were harvested after the following treatments. *Lane 1*: no serum; *lane 2*: 10% serum added for 10 minutes; *lane 3*: serum added for 10 minutes after cells were pretreated with 10 μM BCA for 1 hour; *lane 4*: serum added for 20 minutes after cells were pretreated with 20 μM PD98059 for 1 hour.

BCA in group 2. Indirect ophthalmoscopy, fundus photography, and scotopic electroretinogram (ERG) recordings were performed before injection of BCA, and also on days 1, 7, 14, 21, and 28 after the injection. ERG was performed with a commercial apparatus (Neuropack-II plus; Nihon, Kohden, Japan). Maximal b-wave amplitude was used for evaluation of the results. The eyes were enucleated 4 weeks after the BCA injection. Anterior segments of the eyes were removed, and small sections of tissue including retina, choroid, and sclera were fixed in 2% glutaraldehyde for 24 hours. The specimens were rinsed in several changes of PBS for 1 hour. The tissues were postfixed in 2% osmium tetroxide, washed in distilled water, dehydrated in a graded ethanol series, placed in propylene oxide, embedded in epoxy resin, and examined with light and electron microscopes.

Efficacy Study. Ten days after the gas–fluid exchange, a 26-gauge needle was inserted 4 mm posterior to the corneoscleral junction in the superotemporal quadrant with the assistance of a microscope. With the bevel of the needle directed upward to avoid damaging the retina with the injection stream, 2.0×10^5 tissue-cultured homologous rabbit RPE cells, which were suspended in 0.1 mL sterile PBS, were injected just in front of the optic nerve head—slowly, to prevent retinal damage. The animals were immediately placed on their backs for 1 hour to allow the cells to settle over the vascular wings of the retina. In group 3, one eye in each rabbit (n = 12) was injected with 200 μg BCA in 0.1 mL DMSO 1 day after RPE cell injection to achieve a final intraocular concentration of approximately 600 μM BCA. In group 4, one eye in each rabbit (n = 12) was injected with 0.1 mL DMSO as the control. Each eye was examined by indirect ophthalmoscopy, and fundus photographs were taken at 7, 14, 21, and

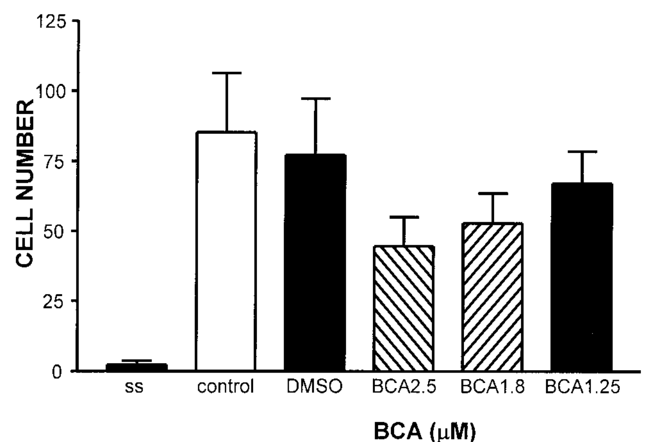
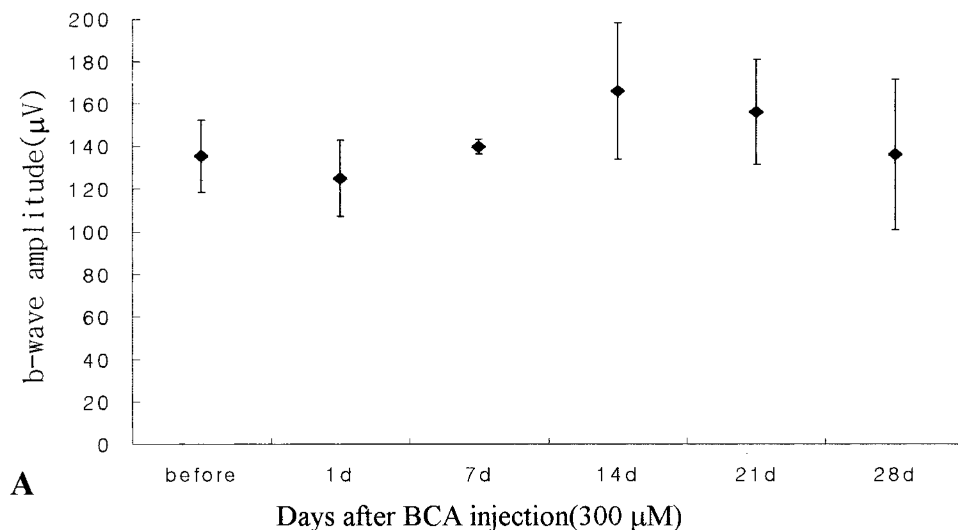


FIGURE 7. Assay of RPE cell motility. RPE cells were placed in the upper chambers and treated as follows: serum-free medium (ss), medium containing only 10% fetal bovine serum (control), pretreated with BCA before adding 10% fetal bovine serum (DMSO), and pretreated with BCA at 2.5, 1.8, and 1.25 μM. The cells on the bottom of the lower chamber were counted under a microscope and examined for motility.



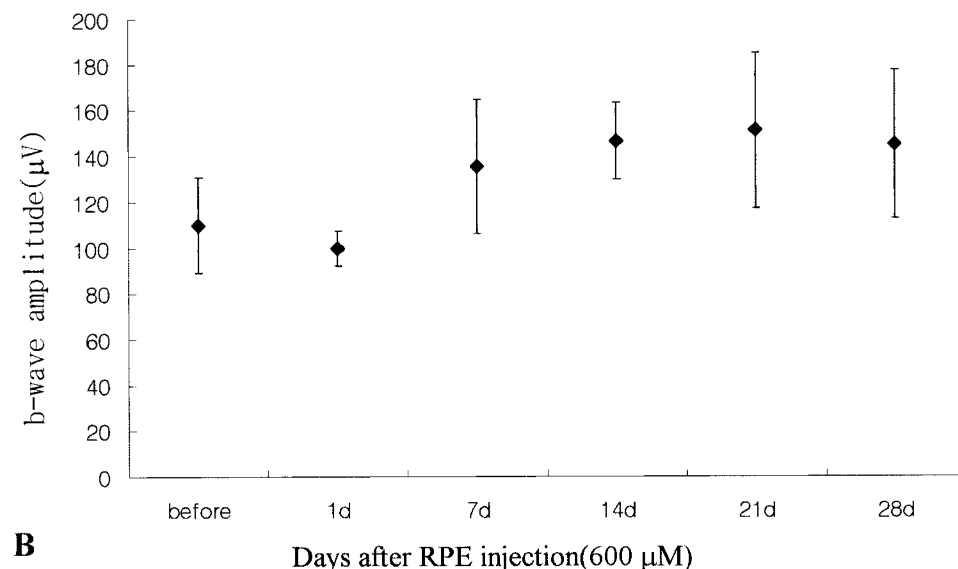


FIGURE 8. Electroretinography of rabbits treated with BCA. Mean b-wave amplitude and 95% confidence interval in rabbits treated with (**A**) 0.1 mL 300 μM BCA or (**B**) 0.1 mL 600 μM BCA in one eye indicated that no electrophysiological damage was caused by either dose at any point. $n = 4$ at both doses.

28 days after the injection of rabbit RPE cells. PVR was clinically graded from stages 0 to 5, according to a classification published by Fastenberg et al.[23] The rabbits were killed with intracardiac injection of lidocaine 4 weeks after injection of rabbit RPE cells, and the eyes were enucleated and immersed in a fixative solution containing 4% paraformaldehyde. Corneal buttons were removed to allow faster infiltration of the fixative. The eyes were left in fixative for at least 24 hours, rinsed in PBS and bisected circumferentially 4.5 mm posterior to the corneoscleral limbus. After dehydration in graded alcohol, the specimens were embedded in paraffin, and sections were stained with hematoxylin and eosin.

Statistical Analysis

χ^2 and the Fisher exact test were used for statistical analyses.

RESULTS

Effect of BCA on hRPE Cells In Vitro

We investigated whether BCA induces cell death by assaying the growth-inhibiting activity of BCA with the MTT method. We seeded various concentrations of hRPE cells into a 96-well plate, thereby producing an approximately 50%, 75%, or 100% confluent status of hRPE cells. The cells were then treated with various concentrations of BCA and the percentage of surviving cells was determined by MTT assay. As shown in Figure 2, 50% inhibition of cells occurred with 5.1 μM BCA in hRPE cells at 50% confluence, with 8.54 μM BCA at 75% confluence, and with 23.1 μM BCA at 100% confluence. Cells at 50% confluence were 2.7-fold more sensitive to BCA than cells at 100% confluence. BCA-induced cell death was observed with DAPI staining. Thus, the cells were harvested at indicated times after treatment of various BCA concentrations and stained with DAPI. BCA treatment induced chromosome condensation (data not shown), which was indicative of apoptosis. hRPE cells treated with 5, 10, and 40 μM BCA at 48 hours were 9.2%, 10.7%, and 12.7% apoptotic, respectively (Fig. 3). However, BCA treatment did not induce DNA fragmentation in these cells. Such a finding was also observed in some gastric cancer cells (data not shown).

Many factors are involved in drug-induced apoptosis, and the p53 protein is one of the best-known proteins responsive to genotoxic stress. Upregulation of p53 occurred after treatment with BCA, and the increase was dependent on the concentration of BCA (Fig. 4A), suggesting that BCA-induced apoptosis is p53-dependent. Phosphorylation of Bcl-2 occurred 24 hours after treatment with 4 μM BCA, even though 0.4 μM BCA treatment did not affect its phosphorylation (Fig. 4B). Bcl-2 phosphorylation and consequent inactivation may contribute in part to BCA-induced apoptosis. Induction of GADD45, which has been known to be regulated by p53, was also detected in BCA-treated RPE cells (Fig. 4C). However, expression of p21 was not affected by BCA (Fig. 4D), nor was expression of cyclin D and Cdk2 (Figs. 4E, 4F, respectively). We further examined expression of Akt by immunoblot analysis and found that the expression was abruptly decreased by treatment with 20 μM BCA for 24 hours (Fig. 5). When the BCA concentration was increased from 10 μM, at which dose the expression of Akt was not affected, to 20 μM, cell morphology changed from fibroblast-like into a rounded shape, and the cells began to float (data not shown). This result demonstrated that downregulation of Akt is important for BCA-induced cell death.

At this juncture, we hypothesized that BCA might inhibit the activity of proteins involved in proliferation-signaling pathways; therefore, we investigated the effect of BCA on activation of mitogen-activated protein (MAP) kinase. Addition of serum to serum-starved hRPE cells induced phosphorylation of MAP kinase (Fig. 6), and pretreatment of hRPE cells with BCA completely inhibited phosphorylation. Because the positive control PD98059, a MEK inhibitor, also blocked activation of MAP kinase, the result suggests that BCA inhibits cell proliferation by blocking activation of MAP kinase. It is known that the migration of RPE cells is induced by addition of serum and is mediated by activation of MAP kinase.[24] Furthermore, because BCA blocked the phosphorylation of MAP kinase (Fig. 6), we next examined the possibility of inhibition of serum-induced migration of RPE cells by BCA. As shown in, BCA at low concentration of 2.5 μM suppressed serum-induced cell migration (Fig. 7); however, cell death was not observed at this concentration of BCA (data not shown). All evidence suggests that BCA inhibits cell proliferation and migration by blocking activation of MAP kinase.

Effect of BCA on Experimental PVR

Toxicity Study. Electroretinographic results are shown in Figure 8. Figure 8A demonstrates the mean b-wave amplitude ratio (with 95% confidence interval) of five rabbits treated with 300 μM BCA in one eye. The mean b-wave amplitude ratio decreased to approximately 90% on day 1, increased thereafter to a level of approximately 140% on day 7, transiently tended to increase on days 14 and 21, and finally returned to the control level. Figure 8B demonstrates the mean b-wave amplitude ratios (with 95% confidence interval) in four rabbits treated with 600 μM BCA in one eye. The mean b-wave amplitude ratio tended to decrease to approximately 90% on day 1 and then increased to a level of approximately 140% during the remainder of the experiment, indicating no electrophysiologically detectable damage during this experimental period.

Histologic observations are presented in Figures 9 and 10. The outer nuclear layer and photoreceptor outer segments were well preserved during the 4 weeks after intravitreous injection of BCA, showing no evidence of retinal toxicity of BCA (Fig. 9). Electron microscope studies of eyes treated with 600 μM of BCA revealed no damage to any layer of retina 4 weeks after intravitreous injection of BCA (Figs. 10A, 10B).

FIGURE 9. Light microscopy of the rabbit retina 28 days after intravitreous injection of BCA (600 μM). The outer limiting membrane and outer nuclear layer appeared to be normal with well-preserved photoreceptor outer segments. Toluidine blue staining; magnification, ×400.

Efficacy Study. Both eyes of 24 rabbit eyes were excluded because of cataract and vitreous hemorrhage during the follow-up period. TRD always developed around the optic nerve head. In the treated eyes, there was no difference in stages of PVR between at days 7 and 28 after injection of BCA. The incidence of TRD was reduced from the control of 63.6% to a frequency of 27.3% in the treated eyes on day 7 after injection of rabbit RPE cells, which was a reduction in TRD rate with statistically borderline significance (Fig. 11; $P = 0.087$). The incidence of TRD was reduced from the control of 72.7% to a frequency of 27.3% in the treated eyes on days 14, 21, and 28 after rabbit RPE injection—a statistically significant reduction in TRD rate (Fig. 11; $P = 0.033$). The PVR stages (0–5) of treated eyes were significantly lower ($P = 0.037$) than those of control eyes on days 14, 21, and 28 after injection of rabbit RPE cells (Table 1; Wilcoxon rank sum test).

Discussion

Retinal detachment complicated by PVR remains a significant problem, because of the development of contractile membranes that keep the retinal break open, form new retinal breaks, and disrupt the adhesion between the sensory retina and RPE. Because of the importance of vitrectomy surgery in reducing traction on the retina, removing vitreous opacities, and providing access to the vitreous cavity and retina in a variety of surgical procedures, vitrectomy will continue to be the primary approach for treatment of PVR. Nonetheless, additional methods are needed to prevent formation of epiretinal membranes and contraction after vitreous surgery.

For the past 10 years, numerous chemotherapeutic agents have been tried for amelioration of PVR.[24,25] Most studies, however, have not been successful. The procedures in some studies involve vitrectomy, including an intravitreous infusion of those agents. Hence, in the present study, we explored the possibility that BCA would prevent PVR with a single intravitreous injection without vitrectomy. We recently developed a chemotherapeutic drug, BCA, with less in vivo toxicity than any other agent and which effectively inhibits tumor growth in

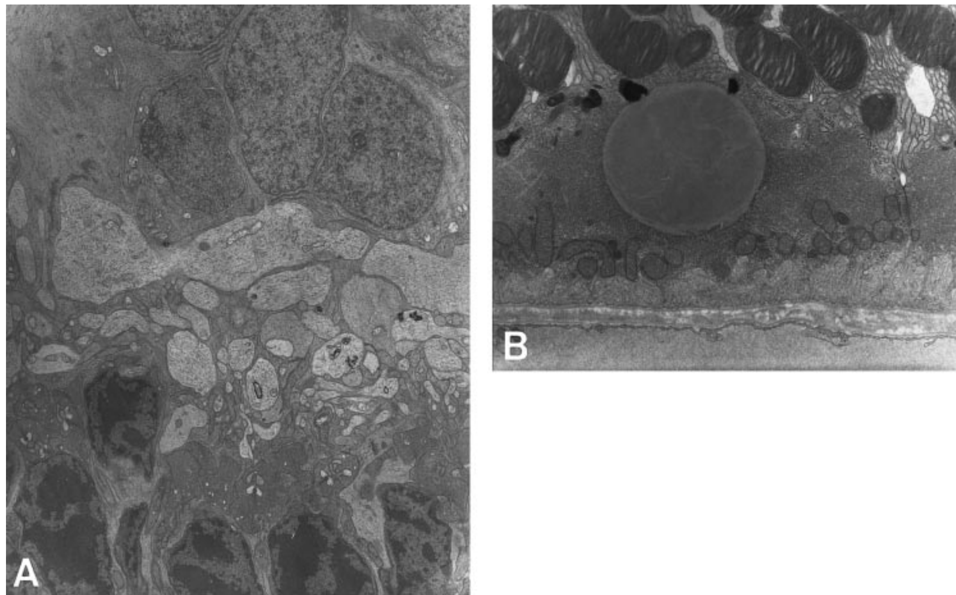


FIGURE 10. Electron microscopy of rabbit retina 28 days after injection of intravitreous BCA (600 μM). (**A**) The inner nuclear, outer plexiform, and outer nuclear layers were well preserved. (**B**) The endoplasmic reticuli, mitochondria, and microvilli of the RPE cells appeared normal. Magnification: (**A**) ×4100; (**B**) ×7300.

vitro and in vivo,[17] and found it to inhibit proliferation of RPE cells and induce apoptosis in vitro. The exact mechanism of BCA-induced apoptosis is not clear; however, we observed the accumulation of p53 and GADD45 during apoptosis and the induced phosphorylation of Bcl-2. Induction of Bcl-2 phosphorylation at Ser-70 and Ser-87 has been known to be essential for apoptosis induced by the microtubule-damaging drug.[26] Although BCA also induced phosphorylation of Bcl-2, the mechanism by which it induced apoptosis seemed to be different from that of microtubule-damaging agents or DNA-damaging agents, based on the following consideration. Paclitaxel, a well-known microtubule-damaging agent, induces cell-cycle arrest,[27] and many DNA-damaging agents also induce cell-cycle arrest. However, when the RPE cells were treated with 40 μM BCA for 24 hours, during which period cell death was initiated, no cell-cycle arrest at any phase of cell division was detected (data not shown), suggesting that BCA may not be a DNA-damaging agent.

Akt plays a central role in promoting the survival of cells, and its activation is important for antiapoptotic signaling.[28] In the present study, expression of Akt was found to disappear at the concentration of 20 μM BCA (Fig. 5). However, expressions of other genes such as cyclin D, Cdk2, and p21 were not changed under the above control (Fig. 4). As described earlier in the result section, morphologic transformation of fibroblast-like shape into round shape occurred at 20 μM BCA. This morphologic change seemed to be a process of cell death, and the cells undergoing their morphologic change began to float and finally died. Our observations suggest that downregulation of Akt is one of the apoptotic processes induced by BCA.

Proliferation and migration of hRPE cells is an important step in PVR. hRPE cells are detached from the monolayer and then migrate into the vitreous cavity and settle down on the retina to form a periretinal membrane.[1] In this process of hRPE cell migration, two factors, PKC and MAP kinase, are known to mediate signal transduction. Calphostin C or PD98059, which are PKC and MAP kinase inhibitors, respectively, inhibit the migration of hRPE cells.[2,29] Hence, inhibition of either hRPE migration or proliferation should be an ideal goal in drug development for PVR. Growth factors such as platelet-derived

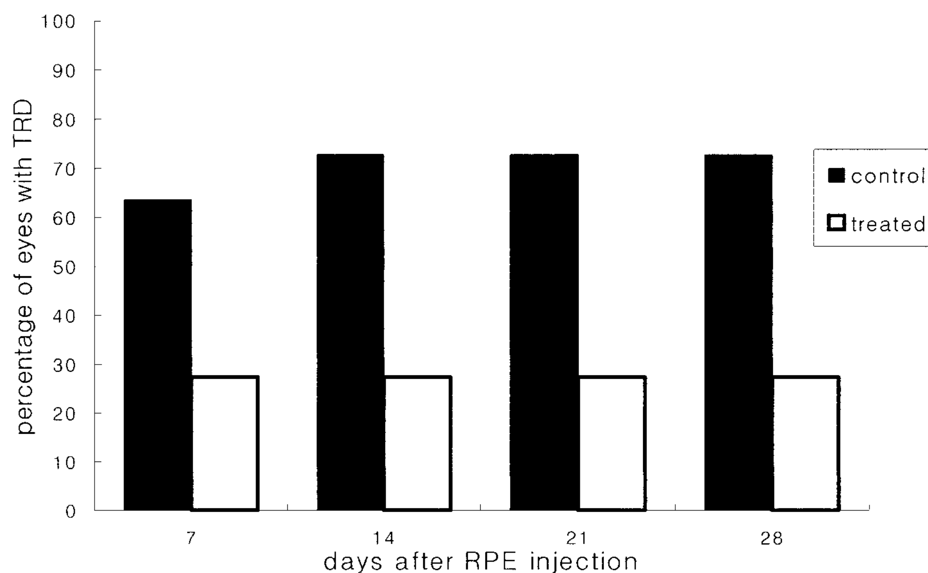


FIGURE 11. Frequency of development of TRD in eyes injected with 2.5×10^5 RPE cells. Treated eyes received 600 μM BCA, and control eyes received the same volume of PBS 24 hours after injection of rabbit RPE cells. The TRD ratios in the treated eyes were significantly decreased at 14, 21, and 28 days ($P < 0.05$, χ^2 test).

Table 1. Stages of PVR 4 Weeks after Intravitreous Injection of RPE Cells in Control Eyes and Eyes Treated with BCA

	Dose of BCA	
Stage of PVR	600 μM, BCA (*n* = 11)	Control Eyes (*n* = 11)
0 No pathologic change	2	0
1 Intravitreal membrane	1	0
2 Focal traction	5	3
3 Localized detachment of medullary ray	1	3
4 Extensive retinal detachment	0	4
5 Total retinal detachment	2	1

Significant differences ($P = 0.037$) were observed between treated and control eyes. Wilcoxon rank sum test.

growth factor (PDGF) also stimulate proliferation and migration of RPE cells,[21] and an elevated concentration of PDGF in the vitreous of eyes affected by PVR has been observed.[30] Blockade of MAP kinase by antisense oligonucleotide inhibits PDGF-BB–mediated migration of vascular smooth muscle cells.[31] PDGF has been known to activate phosphoinositide 3-kinase (PI3K), which acts as an upstream mediator of Akt.[32] Because BCA decreased the expression of Akt, BCA may inhibit growth factor–mediated cell proliferation, migration, and survival through downregulation of Akt in vitro. This results show that Akt is one of the major targets for treatment of PVR by BCA. Many agents have been known to inhibit migration of RPE cells,[33–35] and in the current study BCA inhibited activation of MAP kinase and migration of RPE cells (Fig. 6, 7). When pretreated with a low concentration of BCA for 30 minutes, BCA inhibited serum-induced migration of hRPE cells, and cell death did not occur. Because MAP kinase is activated by serum or growth factors such as PDGF and this activation is inhibited by BCA, the inhibition of cell migration by BCA may have been mediated by blockade of activation of MAP kinase.

We used rabbit eyes as an animal model for study of intraocular proliferation and demonstrated the possibility that BCA can be used as an agent for treatment and prevention of PVR in vivo. The use of gas compression provides an efficient, easy method of simulating vitrectomy which, when followed by injection of relatively small amounts of homologous RPE, provides the same results in treating PVR as mechanical vitrectomy and injection of RPE cells. These animal models closely mimic the human disorder, in that the clinical condition of PVR is mimicked by partially detached and collapsed vitreous and preretinal membrane formation.[21] The present animal studies showed that intraocular administration of 600 μM BCA (as a single dose 1 day after the injection of RPE cells) effectively reduced both the incidence and severity of experimental PVR.

Toxicity studies after administration of 600 μM BCA in rabbit eyes that had undergone vitreous gas compression but no injection of RPE cells did not reveal any evidence of retinal toxicity by both electrophysiologic and retinal histologic studies until 4 weeks after the drug administration. The dose of 600 μM in this study is 100 times as high as the IC_{50} of BCA. This result suggests that BCA has a very wide safety margin and a promising clinical application in the prevention of PVR.

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