Attenuated Glial Reaction in Experimental Proliferative Vitreoretinopathy Treated with Liposomal Doxorubicin

Hsi-Kung Kuo,[1,2,3] *Yi-Hao Chen,*[1,2,3] *Pei-Chang Wu,*[1,2] *Yi-Chen Wu,*[1] *Faye Huang,*[2,4] *Chun-Wei Kuo,*[5] *Li-Hua Lo,*[5] *and Jentaie Shiea*[5]

Purpose. We investigated the therapeutic effect of liposomal doxorubicin (Lipo-dox) on experimental proliferative vitreoretinopathy (PVR).

Methods. The toxicity of Lipo-dox was determined in vitro in cultured rabbit retinal pigment epithelium (RPE) cells by tetrazolium-based (MTT) assay for cell viability performed 48 and 96 hours after treatment, and in vivo by electroretinography and histopathology. The therapeutic effect of intravitreous injection of Lipo-dox was evaluated in a rabbit model of PVR induced by injection of rabbit RPE cells after gas compression of the vitreous. The presence of PVR was determined by indirect ophthalmoscopy on days 1, 7, 14, 21, and 28 after injection. Western blot and immunofluorescence studies were performed to evaluate the expression of the glial markers vimentin and glial fibrillary acidic protein (GFAP). A pharmacokinetic study also was performed and analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS).

Results. The 50% inhibitory concentrations (IC_{50}) of doxorubicin (Doxo) and Lipo-dox in RPE cells were 0.01–0.1 and 0.1–1.0 μg/mL, respectively. Lipo-dox (10 μg/mL) did not reduce the amplitude reduction in the ERG study or produce obvious retinal toxicity. Lipo-dox still could be detected in the vitreous 7 days after injection. The Lipo-dox (10 μg/mL)-treated eyes showed lower grade PVR than did the untreated eyes. Lipo-dox also decreased the retinal expression levels of vimentin and GFAP.

Conclusions. Lipo-dox can attenuate the severity of experimental PVR, and reduces the glial cell expression of intermediate filaments in PVR retinas. Lipo-dox has a wider safe dosage range and a longer half-life in the vitreous than does primary Doxo. (*Invest Ophthalmol Vis Sci.* 2012;53:3167–3174) DOI:10.1167/iovs.11-7972

From the Departments of [1]Ophthalmology, and [4]Plastic and Reconstruction Surgery, Kaohsiung Chang-Gung Memorial Hospital, Kaohsiung, Taiwan; [2]Chang-Gung University College of Medicine, Kaohsiung, Taiwan; and the [5]Department of Chemistry, National Sun Yat-Sen University, Kaohsiung, Taiwan.

[3]These authors contributed equally to the work presented here and should therefore be regarded as equivalent authors.

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Corresponding author: Pei-Chang Wu, Department of Ophthalmology, Kaohsiung Chang Gung Memorial Hospital, 123 Ta-Pei Road, Niao-Sung District, Kaohsiung 83301, Taiwan; Telephone 886-7-7317123, ext. 2801; Fax 886-7-7352775; wpc@adm.cgmh.org.tw.

Rhegmatogenous retinal detachment (RD) is a severe sight-threatening disease. Failure of retinal reattachment most commonly is due to development of proliferative vitreoretinopathy (PVR),[1–4] a process characterized by uncontrolled cellular proliferation throughout the detached retina. This proliferation results in the formation of contractile periretinal membranes with subsequent tractional retinal detachment and severe impairment of vision.[5,6]

The principal cells involved in PVR are retinal pigment epithelium (RPE) cells, retinal glial cells, macrophages, fibrocytes, and myofibrocytes.[7–10] There is histological evidence of RPE-like cells in PVR membranes, but Müller glial cells likely also have a critical role in this process. Intraretinal changes, such as neuronal remodeling and intraretinal gliosis, also could interfere with the maintenance or restoration of functional synapses after detachment.[11] In an experimental primate model of RD induced by subretinal injection of sodium hyaluronate, intraretinal cell proliferation began within a day of detachment.[12] Reactive gliosis also has a crucial role in retinal neuronal damage after RD.[12–14]

Various antiproliferative and antiinflammatory agents, including daunomycin and corticosteroids, have been tested for their potential to reduce the development of tractional RD in cultured cells, experimental models of PVR, and clinical trials.[15–22] Although most of these drugs have short half-lives and, therefore, must be injected multiple times, this problem may be addressed with two new strategies: encapsulating the drug within liposomes and implanting intravitreal sustained-released devices that release small amounts of the drug over long periods of time. Liposomes release their aqueous contents slowly, protecting the enclosed substance from degradation and clearance, and avoiding the toxicity of the high peak concentrations that occur after injection of free drugs.[23,24] The advantages of using liposomes inside the eye are longer clearance time, less toxicity, and take-up by phagocytic cells, including the RPE.[23] Liposomal doxorubicin (Lipo-dox) recently has been made commercially available as an anticancer drug and may produce a long-acting antiproliferative effect.[23,25,26] Lipo-dox, thus, shows potential as a clinical adjuvant for PVR treatment. We investigated the efficacy of Lipo-dox in reducing experimental PVR induced by intravitreal RPE cells and its influence on the intermediate filament expression of glial cells.

Methods

In Vitro Study

Primary Culture of Rabbit RPE Cells. Pigmented rabbit eyes were enucleated, and the anterior segment, vitreous, and retina removed completely. Trypsin (0.25%) with EDTA (0.02%) was added to the remaining eye cup and incubated at 37°C for 25 minutes. The RPE cells thus loosened from Bruch's membrane then were removed with a pipette. The cell suspension was centrifuged at 200 × *g* for 10 minutes

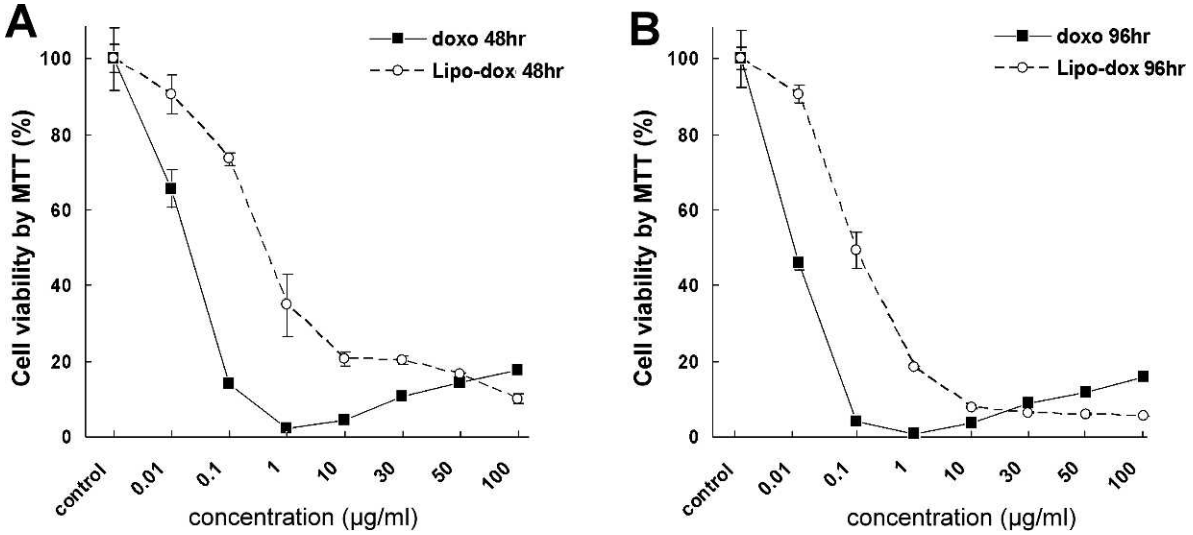

Figure 1. Evaluation of retinal pigment epithelium cell viability by the MTT cytotoxicity assay at 48 (**A**) and 96 (**B**) hours after treatment. The IC_{50} concentrations of Doxo and Lipo-dox were 0.01–0.1 and 0.1–1.0 μg/mL, respectively. The slope of the dose-response curve of Lipo-dox declined slowly, indicating a broad therapeutic range. In contrast, the curve for the primary form of doxorubicin had a sharp slope with a narrow therapeutic range.

and the pellet washed twice with culture medium consisting of Dulbecco's modified Eagle's medium with L-glucose (4500 mg/mL), 25 mM HEPES buffer, and L-glutamine, and supplemented with 20% fetal calf serum, antibiotics, and antimycotics. The cells were incubated at 37°C under 5% CO_2 with medium changes every 4 days, and subcultured when they reached confluence. Early-passage cells (up to the 10th passage) were used in the following experiments.

Preparation of Drugs for In Vitro Studies. Doxorubicin (Doxo; Adriblastina, Pfizer, Italy) and Lipo-dox (Tung Yang Chemical Industries Co., Taiwan) were prepared fresh in sterile culture medium at desired concentrations (0.01, 0.1, 1.0, 10, 30, 50, and 100 μg/mL) for in vitro studies.

Evaluation of Cell Proliferation by the Tetrazolium Dye (MTT) Cytotoxicity Assay. RPE cells were trypsinized, centrifuged, and resuspended in culture medium in 96-well plates. Each well contained 2×10^4 cells/0.1 mL medium. After 24 hours, the medium was replaced with fresh medium, and 10 μL of drug solution at each concentration were added. After 48 or 96 hours of incubation, the culture medium was removed and the cells washed with phosphate-buffered saline (PBS). The MTT assay then was performed as per manufacturer's protocol (MTT cell proliferation kit; Roche, Indianapolis, IN). Results were expressed as the percent optical density (OD) relative to the untreated controls and indicated the cell viability. Each experiment was performed in triplicate and repeated 3 times.

In Vivo Study

Animals. All animal management in this study complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Pigmented rabbits weighing between 2 and 2.5 kg were used. All animals were sedated by intramuscular injection of ketamine-xylazine (10 mg/kg body weight). The eyes were dilated with 1 drop each of 0.5% tropicamide and 2.5% phenylephrine HCl. The animals were sacrificed by intravenous injection of an overdose of pentothal.

Toxicity Study. To evaluate the retinal toxicity of the drug, bilateral electroretinograms (ERG) were obtained on the day before the injection, and on days 7 and 14 after the injection. Based on in vitro studies to determine 50% inhibitory concentrations (IC_{50}, 4 rabbits per dose), 3 doses were chosen to achieve different vitreal concentrations (1, 10, and 100 μg/mL) of Lipo-dox. One eye was treated and the other left untreated as the control eye. The intravitreal injection (IVI) was performed through the pars plana 2 mm posterior to the limbus following anterior chamber paracentesis for intraocular pressure control. ERGs were obtained using an UTASE-E 3000 unit with a Ganzfeld flash (LKC Technologies, Gaithersburg, MD). The pupils of the rabbits' eyes were dilated and the rabbits kept in a dark room for 30 minutes for dark adaption. The rabbits then were anesthetized and corneal contact lens electrodes placed on the corneal surfaces over a layer of 1% methylcellulose gel. The standard white flash stimulation of the retina on a scotopic background was performed and the scotopic 0-dB ERGs recorded. To minimize variability, the ratios of the scotopic b-wave amplitudes of the treated eyes to those of the control or pre-injection eyes were used as indices of retinal function. Two weeks later, the rabbits were sacrificed, and their eye globes immediately enucleated, fixed with formaldehyde, stained with hematoxylin and eosin, and examined by light microscopy.

Efficacy Study. *PVR Induction***.** Pigmented rabbits were used to study the drug efficacy. PVR was induced by injection of rabbit RPE cells after gas compression of the vitreous (gas vitrectomy).[20,27,28] Each eye of every experimental rabbit was injected intravitreally (after anterior chamber paracentesis) through the pars plana (gas vitrectomy) with 0.2 mL pure SF6 (100%). Seven days later, each eye was treated again with 1×10^5 RPE cells in 0.1 mL Hanks' buffered salt solution (HBSS) injected into the vitreous cavity after anterior chamber paracentesis. PVR was induced in both eyes of every animal, one eye receiving treatment and the other eye serving as control.

Drug Injection and Observation. The study eyes (right eye of each rabbit) received 1 IVI that achieved a vitreous concentration of 10 μg/mL of Lipo-dox. The dose was chosen according to the IC_{50} concentration and the toxicity study. The left eye was injected with 0.1 mL HBSS as a control. All animals received follow-up examinations by slit-lamp biomicroscopy and indirect ophthalmoscopy to evaluate the development and progression of PVR on days 1, 7, 14, 21, and 28 after the drug injection. The animals were sacrificed after 28 days. The severity of the PVR was graded on a scale of 0–5.[20,29] Briefly, stage 0 represented normal; stage 1 intravitreous membranes; stage 2 focal traction, localized vascular engorgement, and elevation; stage 3 localized medullary ray detachment; stage 4 total medullary ray detachment and peripheral retinal detachment, and stage 5 total retinal detachment and retinal folds. The same procedure was used to prepare the samples for the study of the glial cell marker expression except that the animals were sacrificed on days 1 and 7 after injection.

Western Blot Analysis of the Intermediate Filament Proteins in Glial Cells. Total protein was extracted from dissected retina

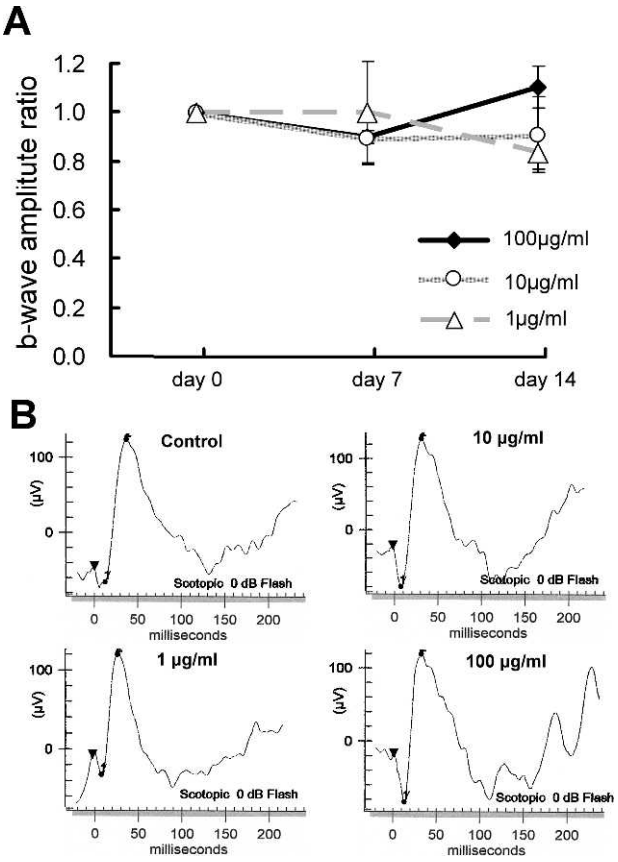

Figure 2. Bilateral electroretinograms were obtained on the day before the injection, and on days 7 and 14 after the injection to evaluate the retinal toxicity of the drug. To minimize variability, the ratios of the b-wave amplitudes of the Lipo-dox injected eyes to those of the normal control of pre-injection eyes were used as indices of retinal function. (**A**) In the 1 µg/mL group, the b-wave ratio remained unchanged at 1.00, and mildly decreased to 0.84 on days 7 and 14, respectively. These values did not differ significantly from those of the normal control eyes on the same days. In the 10 µg/mL group, the b-wave ratio decreased mildly to 0.89 and 0.91 on days 7 and 14, respectively. In the 100 µg/mL group, the b-wave ratio decreased mildly but significantly to 0.90 on day 7. On day 14, the ratio showed a mild increase to 1.1. (**B**) Representative scotopic ERGs from each group on day 14. There were no significant differences between the groups.

samples using tissue protein extraction reagent (Pierce Co., Rockford, IL). The extracted samples were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels and then transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked overnight at 4°C with 5% dry milk in PBS with 0.1% Tween, and then incubated for 2 hours at room temperature with primary antibodies, including anti-actin (Millipore, Billerica, MA), anti-vimentin (DAKO, Carpinteria, CA), and anti-glial fibrillary acidic protein (GFAP, Millipore). The membranes then were washed and incubated for 90 minutes at room temperature with 1:20,000 horseradish peroxidase conjugated anti-mouse or anti-rabbit IgG as the secondary antibody. The bound antibodies were detected with a Western blot analysis detection system (ECL Plus; Amersham Biosciences Inc., Piscataway, NJ).

Immunofluorescence Analysis of the Expression of Glial Cell Markers. Primary antibodies raised against vimentin (DAKO) and GFAP (Millipore) were used to visualize these intermediate filaments within glial cells in the retina. Tissue sections were incubated with primary antibodies overnight at 4°C, washed with PBS, and then incubated with a rhodamine-conjugated secondary antibody (Invitrogen, Carlsbad, CA) for 40 minutes, washed with PBS, and finally

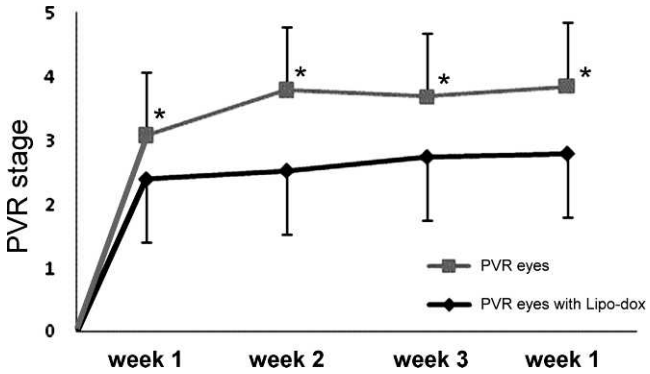

Figure 3. Efficacy study of liposomal doxorubicin for experimental PVR. The PVR grades of the study eyes versus those of the control eyes were: 2.395 ± 1.075 vs. 3.079 ± 1.004 at week 1, 2.526 ± 1.328 vs. 3.789 ± 1.357 at week 2, 2.737 ± 1.408 vs. 3.684 ± 1.455 at week 3, and 2.789 ± 1.437 vs. 3.842 ± 1.344 at week 4. The difference was statistically significant at all time points. *Statistical significance ($P < 0.05$). The bar indicates the SEM.

incubated with DAPI (Invitrogen, Carlsbad, CA) for 3 minutes. The slides were observed and photographed by fluorescence microscopy. The fluorescence densities of the glial markers vimentin and GFAP were compared with those of their respective backgrounds, and the ratios (referred to in the following as "fluorescence ratios") expressed as percentages of the retinal area in the acquired images.

Pharmacokinetic Study

Sample Preparation. The eyes of each rabbit received an IVI to achieve 10 µg/mL of Lipo-dox (right eye) and 10 µg/mL of Doxo (left eye) in the vitreous. The rabbits were sacrificed on days 1, 3, and 7 after injection, and their eyeballs enucleated and the vitreous collected. The vitreous samples (150 µL) were pretreated by adding 600 µL of 100% acetonitrile followed by vortex mixing at high speed for 1 minute at room temperature. The mixtures then were centrifuged in a micro-ultracentrifuge (CS C100 EX; Hitachi, Tokyo, Japan) at 45,000 revolutions per minute (rpm) for 30 minutes at 4°C. The supernatants (450 µL) were transferred to clean tubes and dried in a centrifugal vacuum concentrator (CS C100 EX; Hitachi). For HPLC injection, the samples were re-dissolved in 120 µL of 20% acetonitrile containing 0.1% trifluoroacetic acid (TFA) and vortex-mixed for 1 minute. Insoluble particles were removed by ultracentrifugation at 45,000 rpm for 30 minutes at 4°C.[27] The supernatants were collected and frozen at −80°C for analysis by chromatography.

Liquid Chromatography Coupled with Tandem Mass Spectrometry (LC-MS). A 150 × 0.3 mm ZORBAX 300SB-C18 column (3.5-µm particle size) was used to separate the supernatant samples, with a flow rate of 10 µL/minute delivered by an Agilent 1100 capillary HPLC pump (Agilent Technologies, Santa Clara, CA). Mobile phase A consisted of 0.1% TFA in H_2O, while mobile phase B consisted of 0.1% TFA in acetonitrile. The effluent from the LC column was directed to the LC system, which was interfaced with the TOF Q mass analyzer (Bruker Daltonics micro-TOF Q II, Bremen, Germany) through the ion-sampling capillary.

Statistical Analyses

The IC_{50} was defined as the concentration of the drug that reduced cell proliferation by 50%. For in vivo studies, the Wilcoxon signed-rank test was used to compare the degree of PVR between the 2 groups. A paired *t*-test was used to determine the statistical significance between groups of the expression levels of glial proteins as determined by immunofluorescence and Western blotting. Statistical significance was defined as a *P* value less than 0.05.

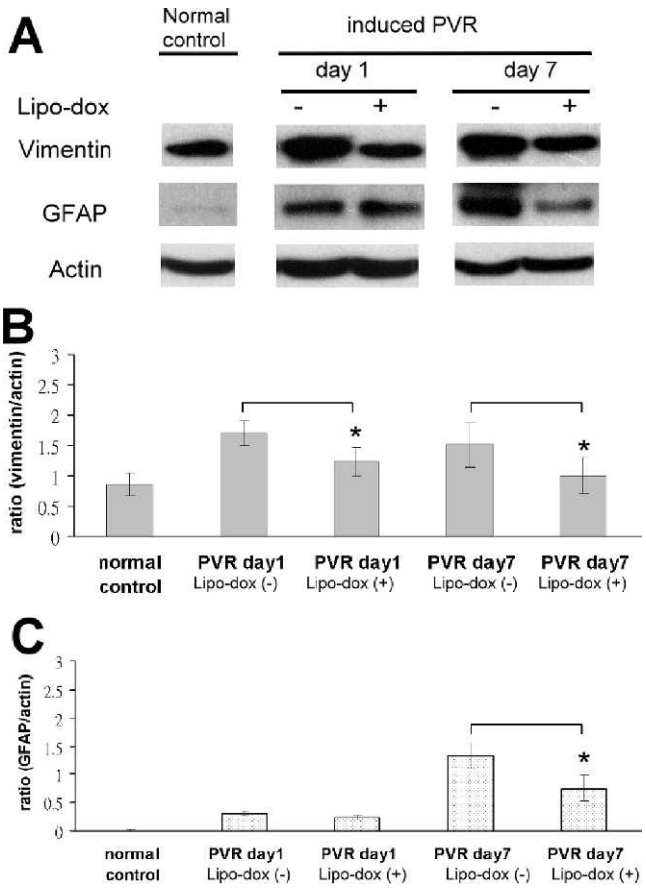

FIGURE 4. Western blot analysis of glial markers after induction of experimental PVR. (**A**) On day 7, the vimentin and GFAP expression levels were decreased by Lipo-dox treatment. (**B**) Densitometry analysis showed that vimentin expression was significantly decreased in Lipo-dox-treated eyes compared to that in untreated eyes on days 1 and 7. (**C**) Densitometry analysis showed that GFAP expression was decreased significantly in Lipo-dox treated eyes compared to that in untreated eyes on day 7. *Statistical significance ($P < 0.05$).

RESULTS

In Vitro Study

Comparison of Reduction of RPE Cell Proliferation by Doxo and Lipo-Dox. Treatment of RPE cells with various concentrations of Doxo and Lipo-dox for 48 or 96 hours produced a dose-responsive reduction in cell proliferation as measured by the MTT assay. The IC_{50} concentrations of Doxo and Lipo-dox were 0.01–0.1 and 0.1–1.0 μg/mL, respectively. Dose-response curves were plotted in Figure 1. The slope of the dose-response curve of Lipo-dox declined slowly, indicating a broad safe therapeutic range. In contrast, the curve for the primary form of doxorubicin had a very sharp slope, indicating a narrow therapeutic range.

In Vivo Studies

Lipo-Dox Toxicity Study. There were 3 treatment groups with different vitreous concentrations (1, 10, and 100 μg/mL) of Lipo-dox after IVI. In the 1 μg/mL group, the b-wave ratio was unchanged at 1.00 on day 7 and mildly decreased to 0.84 on day 14. These values were not significantly different from those of the normal control eyes on the same days (Fig. 2, $P = 0.995$ and 0.165 for days 7 and 14, respectively). In the 10 μg/mL group, the b-wave ratio was decreased to 0.89 and 0.91 on days 7 and 14, respectively. These values did not differ significantly from those of the normal control eyes on the same days ($P = 0.303$ and 0.605 on days 7 and 14, respectively). In the 100 μg/mL group, there was a mild but statistically significant decrease of the b-wave ratio to 0.90 on day 7 ($P = 0.038$), while on day 14 the ratio was increased mildly to 1.1 ($P = 0.303$). Light microscopy showed no obvious retinal degeneration on either day in any group. Based on the aforementioned results, an intravitreal concentration of 10 μg/mL was chosen for the efficacy study.

Efficacy of Lipo-Dox for Experimental PVR. We used 20 rabbits to study the efficacy of the drugs. One rabbit died and was excluded. The PVR grades of the study eyes versus those of the control eyes were: 2.4 ± 1.1 vs. 3.1 ± 1.0 at week 1, 2.5 ± 1.3 vs. 3.8 ± 1.4 at week 2, 2.7 ± 1.4 vs. 3.7 ± 1.5 at week 3, and 2.8 ± 1.4 vs. 3.8 ± 1.3 at week 4. The PVR was significantly less severe in the study eyes that received IVIs to achieve a vitreous concentration of 10 μg/mL of Lipo-dox at all time points assessed (Fig. 3, $P = 0.030$, 0.001, 0.021, and 0.008, respectively).

Glial Response after Induction of Experimental PVR. Vimentin was detectable in the normal eye by Western blot analysis; its expression increased quickly and was notably higher by day 1 after induction of PVR (Fig. 4). The vimentin-to-actin protein expression ratio was significantly greater in the induced PVR eyes than in the normal eyes on days 1 and 7 after induction. GFAP was almost undetectable in the normal eye by Western blot analysis, but its expression increased gradually beginning on day 1 after induction and continued to increase up to day 7 after induction. The GFAP-to-actin protein expression ratio was significantly greater in the induced PVR eyes than in the normal eyes on day 7 after induction. Immunostaining for vimentin and GFAP showed similar results (Fig. 5). The vimentin expression, by the fluorescence ratio within the retinal area, was significantly greater in the induced PVR eyes than in the normal eyes on days 1 and 7 after induction. The GFAP expression, by the fluorescence ratio within the retinal area, was significantly greater in the induced PVR eyes than in the normal eyes on day 7 after induction.

Comparison of the Glial Response between the Lipo-Dox-Treated Eyes and the Untreated Eyes. Western blot analysis showed significantly less vimentin expression in the Lipo-dox-treated eyes than in the untreated eyes on days 1 and 7 after PVR induction (Figs. 4A, 4B). In the immunofluorescence analysis, the treated eyes also expressed significantly less vimentin than the untreated eyes at 1 and 7 days after treatment based on analysis of the vimentin fluorescence ratio within the retinal area (Figs. 5A, 5C). The results were similar for GFAP: the Lipo-dox treated eyes expressed less GFAP than did the untreated eyes by Western blot (Fig. 4C) and immunofluorescence (Figs. 5B, 5D) analyses, although the difference was statistically significant only at 7 days after treatment in both cases.

Pharmacokinetic Study by LC-MS/MS. An ion peak of m/z 544.2 was determined to be the standard peak for Doxo in the LC-MS spectrum. In vitreous specimens of Lipo-dox-treated eyes, the ion peak (m/z 544.2) representing doxorubicin was detected on days 1, 3, and 7. In Doxo-treated eyes, the peak was detected only on days 1 and 3, but not on day 7. Therefore, Lipo-dox has a longer half-life in the eye than does Doxo (Fig. 6).

DISCUSSION

In our study, the slowly released liposomal doxorubicin effectively reduces the severity of induced PVR. Hashizoe et al. investigated the use of a scleral plug made of a biodegradable polymer containing doxorubicin implanted at

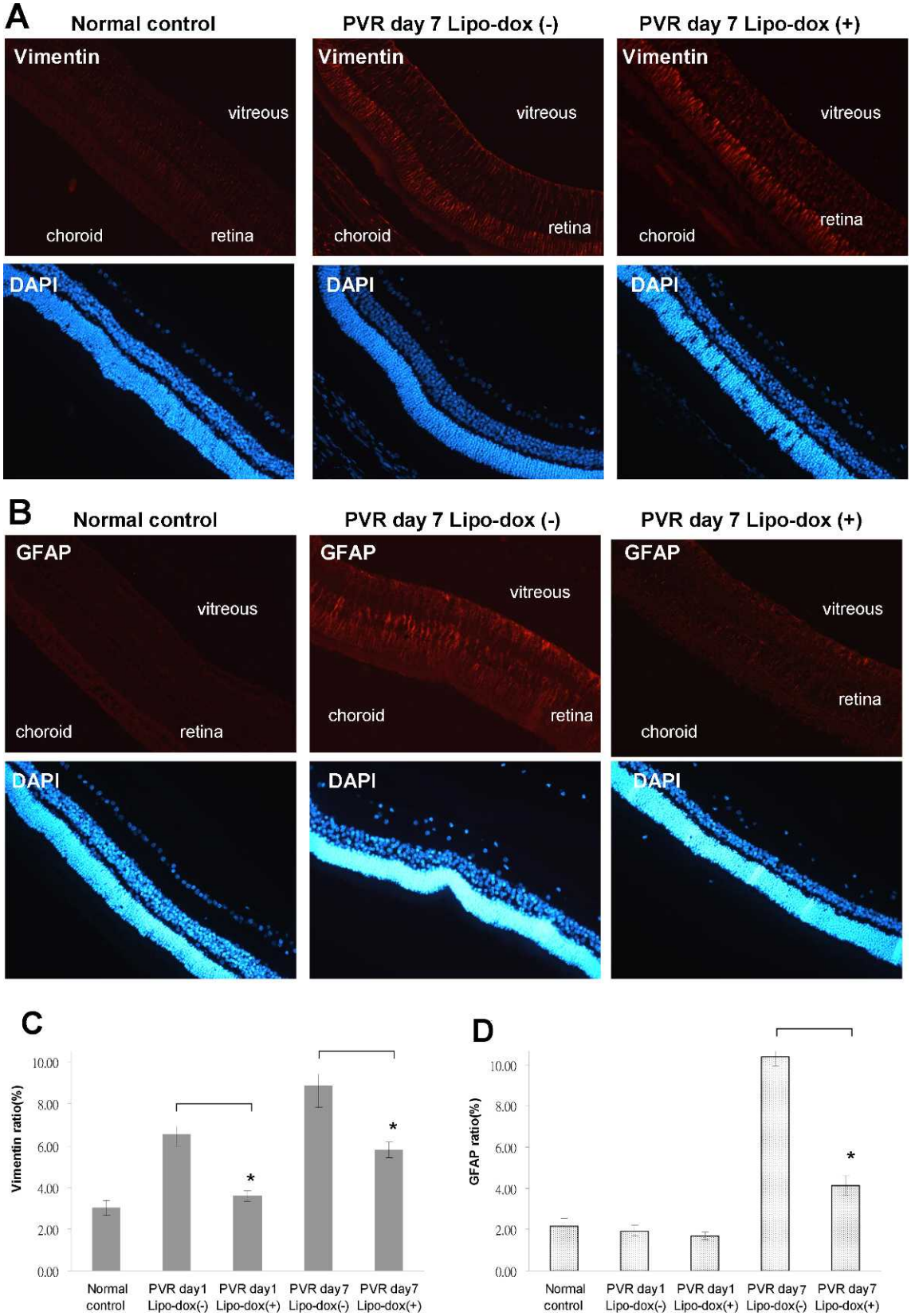

Figure 5. Immunostaining for glial response after induction of experimental PVR. The vimentin and GFAP expression levels, represented by the fluorescence ratio within the retinal area, increased after the induction of PVR (**A**, **C**). Quantitative analysis of vimentin expression showed a statistically significant difference between Lipo-dox-treated eyes and untreated eyes on days 1 and 7 after PVR induction (**B**, **D**). For GFAP expression, a statistically significant difference existed between Lipo-dox -treated and untreated eyes on day 7, but not day 1 after PVR induction. *Statistical significance ($P < 0.05$).

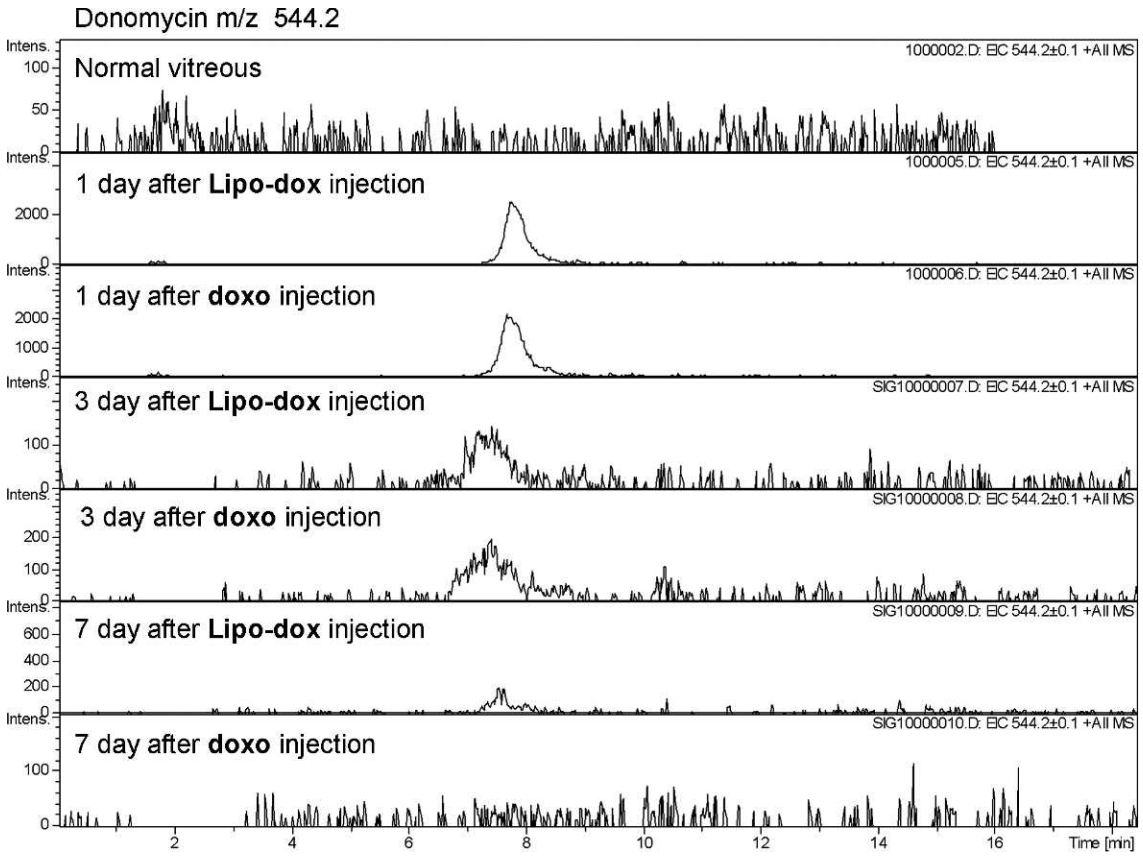

FIGURE 6. A pharmacokinetic study using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). An ion peak (m/z 544.2) representing the doxorubicin signal was detected in days 1, 3, and 7 vitreous specimens from the Lipo-dox-treated eyes. For the Doxo-treated eyes, the doxorubicin signal was detected only in days 1 and 3, but not in day 7 specimens.

the pars plana, and showed that it decreased effectively the incidence of experimental PVR.[30] For future clinical applications, the intravitreous injection technique is simpler and more practical, and might even produce fewer complications than scleral plug implantation.

Glial cells are an integral component of PVR lesions. Retinectomy specimens obtained at retinal relaxing surgery for PVR demonstrated marked upregulation of the glial cell markers, GFAP and vimentin.[11] In an experimental model of PVR induced by IVI of fibroblasts, cells in the epiretinal membrane showed vimentin and GFAP immunoreactivity.[31] Contemporary neuroscience has developed the concept of glial cell activation: activated glial cells secrete mediators that produce responses from target cells and have an active role in the induction of the pathologic lesions.[3,32,33] In experimental RD induced by subretinal injection of sodium hyaluronate, glial cell proliferation begins within a day of detachment and glial cell numbers reach a maximum after 3 to 4 days.[12,13] Mice deficient in GFAP and vimentin demonstrated attenuated reactive responses of retinal glial cells and monocyte infiltration, and subsequently less photoreceptor damage after experimental retinal detachment.[14,34]

Topoisomerase II is essential for DNA replication, chromosome condensation, and chromosome segregation.[35,36] Topoisomerase II inhibitors are important drugs used for treatment of many neoplasms, and include epipodophyllotoxins (etoposide and teniposide), anthracyclines (doxorubicin, daunorubicin, epirubicin, and idarubicin), and mitoxantrone. Doxorubicin is the most commonly used anthracycline and is superior to other anthracycline analogues for the treatment of solid tumors.[37] Cardiotoxicity is the most serious toxicity of anthracycline-based chemotherapy. Acute myeloid leukemia is another serious complication, but it is rare.[38] For ocular use, the concern of systemic toxicity might be eliminated by administering a lower dose with local distribution of the drug in the ocular chamber. Liposomal encapsulation of doxorubicin alters the pharmacokinetic properties of the drug by increasing its distribution in tumors, prolonging its circulation, and reducing the free-drug concentration in plasma. These effects may increase the antitumor activity and improve the tolerability of the anthracyclines.[37] A previous multicenter clinical trial of adjunctive primary daunorubicin in the treatment of proliferative vitreoretinopathy identified no severe adverse effect related to daunorubicin.[21] However, even given the reduced toxicity of Lipo-dox, local and systemic side effects still should be monitored carefully in further studies of Lipo-dox for treating PVR.

Lipo-dox is a drug similar to Doxil and Caelyx.[26] It reduces the harmful side effects of doxorubicin by stably encapsulating the doxorubicin inside liposomes, modifying its biodistribution and pharmacokinetic profile.[39] Lipo-dox consists of a liquid suspension of single unilamellar vesicles with an approximate size of 80–110 nm in a histidine-buffered sucrose solution. The osmolarity of Lipo-dox is at 330 mOs, the pH is 6.5, and the color is dark red. The active ingredient in Lipo-dox is doxorubicin hydrochloride (molecular weight 580). The doxorubicin concentration is 2 mg/mL, and the encapsulation efficiency is more than 95%. In vitro investigation of the release of Lipo-dox into plasma showed that Lipo-dox is very stable in plasma, with only about 10% of the doxorubicin leaking out from the liposomes. The median lethal dose (LD50) of Lipo-dox

in mice given a single intravenous injection is approximately double that of doxorubicin.[40]

Clinically, the PVR process often follows rhegmatogenous RD. The RPE cells are released slowly into the vitreous from the retinal tear, and the PVR incidence is around 5% to 10% after 1 to 2 months.[41,42] To date, there still is no experimental PVR model identical to the clinical PVR process with its relatively low incidence and slow progress. In our study, we used an experimental PVR model in which RD was induced by injection of RPE cells into the vitreous cavity. After gas vitrectomy, the intravitreally injected RPE cells attached to the retinal surface, transformed to fibroblasts or myofibroblasts, and then induced retinal surface contraction, causing tractional retinal detachment. Once retinal detachment occurred, the retinal glial cells were activated and produced a gliosis reaction.[42–44] The expression levels of vimentin and GFAP were increased in the non-treated eyes at week 1, and this increase was attenuated by the use of Lipo-dox in the treated eyes. This suggests that Lipo-dox decreased the severity of PVR by affecting RPE and the glial response.

In our study, Lipo-dox reduced effectively the subsequent PVR response when given by IVI on the same day as RPE cells to induce PVR. Further study is warranted to clarify whether Lipo-dox has an effect when there is a delay between the cell and drug injections. Sunalp et al. found that 10 nmol (3 μg/mL) Adriamycin (doxorubicin) per eye controlled PVR if injected at the same time as the cells.[45] However, PVR was not affected if there was a time lag between the cell and drug injections. This suggests that co-administration of a cytotoxic agent may kill the PVR induction cells, and thereby blunt the severity of the initial PVR insult. In other words, the drug is able to attenuate the proliferation stage of the wound healing process but does not reverse established scarring. Therefore, cytotoxic agents are most applicable for prophylactic use in high-risk patients to reduce the cellular proliferation liable to produce more aggressive gliosis.

The cell toxicity of Adriamycin (3 μg/mL) in the study of Sunalp et al.[45] was relatively high compared to that of Lipo-dox 10 μg/mL in our in vitro study. In addition, the previous study found toxic effects of Adriamycin (3 μg/mL) by ERG and histology, whereas we observed none for Lipo-dox (10 μg/mL). Therefore, the use of Lipo-dox could reduce the toxicity of the free form of doxorubicin and, thus, increase its safety range. The longer duration of action of Lipo-dox might be advantageous in the treatment of PVR, which classically progresses over a number of weeks.

In summary, our study shows that Lipo-dox has lower toxicity and longer duration of action compared to simple doxorubicin in ocular tissue. In addition, Lipo-dox attenuates the retinal glial response in experimental PVR. These results suggest that Lipo-dox has potential for clinical efficacy in PVR treatment and warrants further investigation.

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