Celecoxib Inhibits Proliferation of Retinal Pigment Epithelial and Choroid-Retinal Endothelial Cells by a Cyclooxygenase-2-Independent Mechanism

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ABSTRACT

Age-related macular degeneration (ARMD) is a leading cause of blindness. The major reason for severe vision loss in ARMD is choroidal neovascularization due to an elevation in the expression of angiogenic factors such as vascular endothelial growth factor (VEGF). Drugs with anti-VEGF and antiproliferative activities can be beneficial for the treatment of this disorder. We have previously demonstrated that celecoxib [a selective cyclooxygenase (Cox)-2 inhibitor] inhibits VEGF expression in retinal pigment epithelial cells. In this study, we investigated the antiproliferative effects of celecoxib in adult retinal pigment epithelial (ARPE-19) and choroidal endothelial (RF/6A) cells. The results indicate that celecoxib 1) causes a dose-dependent antiproliferative effect in ARPE-19 and RF/6A cells (IC₅₀ of 23 and 13 μM, respectively); 2) leads to a G₂-M phase cell cycle arrest in these cell types; and 3) inhibits VEGF-induced proliferation of RF/6A cells (IC₅₀ of 20 μM). In addition, 4) the concentrations of celecoxib required for antiproliferative effects are lower than those required for the cytotoxicity. These effects of celecoxib are by mechanisms independent of its Cox-2 inhibitory activity because rofecoxib (another Cox-2 inhibitor) had no effects on the proliferation or cell cycle distribution of the two cell types, and flurbiprofen (an inhibitor of Cox-1 and Cox-2) had weak antiproliferative effects on ARPE-19 cells, with IC₅₀ of 90 μM. In summary, celecoxib has potent antiproliferative effects in RF/6A and ARPE-19 cells; thus, it can be a potential new treatment in proliferative disorders of the choroid-retina such as choroidal neovascularization in age-related macular degeneration.

Age-related macular degeneration (ARMD) is a leading cause of blindness (Seddon and Chen, 2006). ARMD is broadly divided into two categories, wet and dry ARMD. Although approximately 80% of the cases with ARMD involve the dry form, most of the severe vision loss problems are associated with the wet form of ARMD (Ferris et al., 1984). The pathophysiology of the wet form of ARMD involves choroidal neovascularization and in some cases proliferation of the retinal pigment epithelium.

Current therapies for ARMD are limited in their efficacy, and they lead to a modest improvement in the vision. Therefore, there is an ardent need to investigate new approaches for the treatment of ARMD, most importantly to prevent the vision loss due to neovascularization. Antiproliferative drugs that act on specific phase of the cell cycle and that target the proliferating cells could be a beneficial approach for treatment of this advanced aggressive form of ARMD. Such drugs have been used in the treatment of neovascularization associated with malignant disorders. It is probable that a similar approach with or without currently existing treatment regimens might be useful in improving the therapy of choroidal neovascularization associated with ARMD.

Cyclooxygenase (Cox) is an important enzyme involved in the process of inflammation. This enzyme has two major isoforms, Cox-1 and Cox-2, which are thought to be the basal and the inducible isoforms, respectively, and two lesser known variants, termed Cox-3 and PCox-1a (Simmons et al., 2004). Cox inhibitors in general and selective Cox-2 inhibitors in particular have been investigated as potential antitumor drugs (Sarkar et al., 2007). Among the Cox-2 inhibitors, celecoxib has been most widely investigated as an antitumor agent. Indeed, celecoxib has been shown to have antiproliferative effects in several cancer cell types and also in human umbilical vein endothelial cells (HUVEC) cells (Lin et al., 2004).
were supplemented with L-glutamine and penicillin-streptomycin. The RF/6A cells were grown in Ham’s F-12 with 10% fetal calf serum, and they were due to inhibition of Cox-2 or due to a Cox-2-independent mechanism. We used ARPE-19 and RF/6A cell lines, which are models for retinal pigment epithelial and choroidal endothelial cells. We investigated whether the antiproliferative effects of celecoxib were due to inhibition of Cox-2 or due to a Cox-2-independent mechanism. We used ARPE-19 and RF/6A cell lines, which are models for retinal pigment epithelial and choroidal endothelial cells, respectively. For investigating the Cox-2 dependence, we investigated the effects with another Cox-2 inhibitor rofecoxib, and the nonselective Cox inhibitor flurbiprofen.

Materials and Methods

Celecoxib [4-(5-[4-methylphenyl]-3-(trifluoromethyl)pyrazol-1-yl)benzenesulfonamide] and rofecoxib [4-(4-methylsulfonylphenyl)-3-phenyl-5H-furan-2-one] were purchased from ChemPacific Corporation (Baltimore, MD). The antiproliferative BrdU ELISA kit was purchased from EMD Biosciences (San Diego, CA). The culture medium Dulbecco’s modified Eagle’s medium/F-12 and F-12, trypsin-EDTA, penicillin-streptomycin, and fetal calf serum were purchased from Invitrogen (Carlsbad, CA). The PGE2 ELISA kit was purchased from Cayman Chemical (Ann Arbor, MI). Recombinant human VEGF-165 was purchased from R&D Systems (Minneapolis, MN). ARPE-19 (ATCC CRL-2302, a spontaneously arising human retinal pigment epithelial cell line) and RF/6A (ATCC CRL-1758, spontaneously transformed monkey choroid/retinal endothelium) cells were obtained from American Type Culture Collection (Manassas, VA). All other chemicals, including flurbiprofen [2-(3-fluoro-4-phenyl-phe- nyl)-5H-propanoic acid], were of analytical grade, and they were obtained from Sigma-Aldrich (St. Louis, MO).

Cell Culture. The ARPE-19 cells were grown in Dulbecco’s modified Eagle’s medium/F-12 with 10% fetal calf serum and supplemented with l-glutamine and penicillin-streptomycin. The RF/6A cells were grown in Ham’s F-12 with 10% fetal calf serum, and they were supplemented with l-glutamine and penicillin-streptomycin. The medium was changed every other day. For ARPE-19 cells, cells with passage number between 21 and 30 were used for all the experiments. For RF/6A cells, cells of passage number between 40 and 60 were used for all the experiments.

Antiproliferative Effects of Drugs on ARPE-19 and RF/6A Cells. The antiproliferative effects of the drugs in ARPE-19 and RF/6A cells were performed as described by Raghava and Kompella (2007), with some modifications. In brief, for both cell types, 25,000 cells/well were plated in 96-well plates. One day after plating, the medium was removed, and the cells were incubated with serum-free medium with or without the drug (1 nM–100 μM) for 24 h. Along with the drug treatment, BrdU label was added to the culture medium. After the treatments, the cells were processed for the antiproliferative effects using BrdU ELISA with a kit supplied from the manufacturer and as per the manufacturer’s instructions. In addition, the effects of VEGF-165 on proliferation of RF/6A and ARPE-19 cells were examined after incubating the cells for 24 h with 0.05 to 200 ng/ml VEGF-165. The antiproliferative effects of celecoxib in presence of VEGF-165 were examined by coincubating the cells with 50 ng/ml VEGF-165 and 0 to 100 μM celecoxib.

Cytotoxicity Assessment Using the MTT Assay. For both cell types, approximately 10,000 cells per well were plated in 96-well plates and grown until confluence. After confluence, the medium was removed, and the cells were treated with drug solutions in serum-free medium for 24 h. After the treatments, the medium was removed, and cells were incubated in serum-free medium containing MTT for a period of 3 h. At the end of 3 h, the medium was removed, and the formazan crystals formed were dissolved in dimethyl sulfoxide. Absorbance was measured at 540 nm.

Pharmacodynamic Modeling of Toxicity and Antiproliferative Effects. The data obtained from the cytotoxicity and antiproliferative effects (percentage of control) was plotted against the drug concentrations. The data were fit to either the inhibitory or sigmoidal inhibitory models with or without the baseline effects. The model equations used are described below. The model selection was based on the precision of the parameter estimates (mean ± S.E.M.), and the Akaike information criteria (AIC), which is a goodness-of-fit metric. When comparing two or more similar models, the lower the AIC value, the better the model fits the data. The IC50 values were obtained from the model fits. The curve-fitting and parameter estimation was performed using WinNonlin 1.5 (Pharsight, Mountain View, CA).

Inhibitory E\(^\text{max}\) model:
\[
E = E_{\text{max}} \times (1 - (C/C + IC_{50}))
\]

Inhibitory E\(^\text{max}\) model with a baseline effect:
\[
E = E_{\text{max}} - (E_{\text{max}} - E_{0}) \times (C/C + IC_{50})
\]

Sigmoidal inhibitory E\(^\text{max}\) model:
\[
E = E_{\text{max}} \times (1 - (C/C + IC_{50}))
\]

Sigmoidal inhibitory E\(^\text{max}\) model with baseline effect:
\[
E = E_{\text{max}} - (E_{\text{max}} - E_{0}) \times (C/C + EC_{50})
\]

where E is pharmacodynamic response, E\(^\text{max}\) is maximum pharmacodynamic response, C is concentration; IC\(_{50}\) is concentration for 50% inhibitory effect, E\(_{0}\) is baseline pharmacodynamic response, and γ is the curve shape factor.

Cell Cycle Analysis. Approximately 50,000 cells/well were plated in six-well plates. The cells were allowed to attach and grow for 24 h in medium containing serum. The cells were then treated with the drugs in serum-free medium (1 nM–25 μM) for 24 h. After the drug exposure, the medium was removed; then, the cells were trypsinized and pelleted with centrifugation. The pellet was resuspended in 1 ml of phosphate-buffered saline, and then 3 ml of ice-cold absolute ethanol was added dropwise. The cells were incubated on ice for 1 h, and they were stored at −20°C until analysis. The ethanolic cell suspension was centrifuged again, washed once with phosphate-buffered saline, and resuspended in 1 ml of Telford’s reagent overnight. The cell cycle phases were analyzed by FACSCalibur (BD Biosciences, San Jose, CA).

PGE\(_2\) Secretion from ARPE-19 and RF/6A Cells. The cells were grown in 48-well plates. After reaching confluence, the cells were incubated with the drugs (celecoxib, rofecoxib, and flurbiprofen) at varying concentrations (1–25 μM) for a period of 24 h in serum-free medium. The lipopolysaccharide (LPS)-stimulated PGE\(_2\) secretion was examined in ARPE-19 cells. The cells were treated for 24 h with 1 to 25 μM of the drugs in serum-free medium with 1 μg/ml LPS. At the end of the incubation period, the medium was removed, and the PGE\(_2\) secreted in the medium was analyzed using an ELISA kit. The PGE\(_2\) secreted was normalized to the protein content of the
cells measured using a bicinchoninic acid protein assay kit (Pierce Biotechnology, Rockford, IL).

**Statistical Analysis.** The statistical criteria used for the model selection have been described above. To compare cell cycle analysis and PGE₂ secretion between controls and drug-treated cells, a one-way analysis of variance was used. Post hoc comparisons were made using Tukey’s post-hoc analysis. The statistical comparisons were made using SPSS 11.0 (SPSS Inc., Chicago, IL). All results were considered statistically significant at \( P \leq 0.05 \).

**Results**

**Pharmacodynamic Model Selection.** The model selection criteria indicated that the sigmoidal \( E_{\text{max}} \) model is the best fit model for antiproliferative effects and cytotoxicity. As an example, for the antiproliferative effects of celecoxib in RF/6A cells, the final model selection diagnostic comparisons between the four models examined are given in Table 1. Based on the AIC criteria, the sigmoidal inhibitory \( E_{\text{max}} \) model without a baseline effect is the best model as far as statistical fits to the data are concerned. The IC₅₀ values in this study were calculated using this model. The final parameter estimates for the model are shown in Table 1.

**Celecoxib Has Antiproliferative Effects in RF/6A and ARPE-19 Cells.** The antiproliferative effects of celecoxib on RF/6A and ARPE-19 cells are shown in Fig. 1. The data were fit to a sigmoidal inhibitory \( E_{\text{max}} \) pharmacodynamic model. The IC₅₀ values for the antiproliferative effects of celecoxib in RF/6A and ARPE 19 cells are 12.17 ± 0.9 and 23.4 ± 8.8 μM, respectively. Neither rofecoxib nor flurbiprofen had some antiproliferative effects in ARPE-19 cells up to 100 μM. It was found that no cytotoxic effects in ARPE-19 cells, with an IC₅₀ close to 100 μM (Fig. 3C).

**Celecoxib Is Less Cytotoxic to Nonproliferating Cells.** The MTT assay for cell survival is a measure of the cytotoxic effects of celecoxib in RF/6A and ARPE-19 cells (Fig. 1, B and D). With a 24-h exposure of celecoxib, no cytotoxicity was observed at concentrations up to approximately 20 μM. The IC₅₀ values for the cytotoxic effect in RF/6A and ARPE 19 cells are 30.2 ± 1.3 and 49.1 ± 2.6 μM, respectively. Rofecoxib and flurbiprofen significantly inhibited the PGE₂ secretion from RF/6A and ARPE-19 cells, at concentrations of 10 μM and above (Table 4). LPS (1 μg/ml) stimulated the PGE₂ secretion from ARPE-19 cells by 2.5-fold, and the LPS-stimulated PGE₂ secretion was significantly inhibited by all three drugs at the concentrations tested (Table 2).

**Celecoxib at Higher Concentrations Causes a G₂-M Phase Cell Cycle Arrest.** The cell cycle analysis after 24 h of exposure to celecoxib ranging in concentrations from 10 nM to 25 μM showed a dose-dependent G₂-M phase cell cycle arrest at doses ≥10 μM (Fig. 4). There was no significant difference in the cell cycle distribution at lower doses of celecoxib. Neither rofecoxib nor flurbiprofen had any effect on the cell cycle distribution of ARPE-19 cells for up to 25 μM concentration. A slight reduction in the S phase of RF/6A cells with a corresponding increase in the G₂-G₁ phase was observed with flurbiprofen at a concentration of 25 μM (Fig. 4F).

**Celecoxib Inhibits VEGF-Stimulated Proliferation of RF/6A Cells.** We examined the proliferation of RF/6A cells that were supplemented with recombinant human VEGF-165 at concentrations ranging from 0.05 to 200 ng/ml. VEGF-165 induced proliferation of RF/6A cells at concentrations >10 ng/ml (Fig. 5A). When the cells were incubated with 50 ng/ml VEGF-165 in the presence of varying concentrations of celecoxib, no proliferation of RF/6A cells was seen. The IC₅₀ value for the antiproliferative effects of celecoxib in the presence of VEGF was 20.4 ± 1.1 μM (Fig. 5). There was no statistically significant difference in the IC₅₀ for antiproliferative effects of celecoxib in RF/6A cells in the presence or absence of VEGF-165. ARPE-19 cells did not respond to the stimulation by VEGF. There was no cell proliferation up to a VEGF-165 concentration of 200 ng/ml.

**Celecoxib, Rofecoxib, and Flurbiprofen Inhibit PGE₂ Secretion from ARPE-19 and RF/6A Cells.** The PGE₂ secretion in ARPE-19 cells and RF/6A cells was 1688 ± 866 and 715 ± 519 pg/mg tissue, respectively. Celecoxib, rofecoxib, and flurbiprofen significantly inhibited the PGE₂ secretory from RF/6A and ARPE-19 cells, at concentrations of 10 μM and above (Table 4). LPS (1 μg/ml) stimulated the PGE₂ secretion from ARPE-19 cells by 2.5-fold, and the LPS-stimulated PGE₂ secretion was significantly inhibited by all three drugs at the concentrations tested (Table 2).

**Discussion**

In the present study, we demonstrate for the first time that celecoxib, a clinically used Cox-2 inhibitor, has antiproliferative effects on choroid-retinal endothelial cells and retinal pigment epithelial cells. We also demonstrate that the antiproliferative effects of celecoxib on these cell types are independent of its Cox-2 inhibitory action. In addition, celecoxib can inhibit VEGF-165-induced proliferation of choroid-retina endothelial cells. Celecoxib also causes a G₂-M phase cell cycle arrest in the endothelial and RPE cells, with greater antiproliferative and cell cycle arrest potency in endothelial cells compared with the RPE cells. VEGF is thought to be a primary growth factor responsible for the proliferation of endothelial cells and neovascularization in several pathologies, including cancer, diabetic retinopathy, and ARMD (Jonas and Neumaier, 2007). VEGF is known to be a survival factor for endothelial cells both in vitro and in vivo (Gerber et al., 1998a,b; Benjamin et al., 1999). VEGF,

**TABLE 1**

Pharmacodynamic model selection for antiproliferative effects of celecoxib on RF/6A cells

<table>
<thead>
<tr>
<th>Modela</th>
<th>Parameter Estimate</th>
<th>Correlation Coefficient</th>
<th>AICb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Inhibitory ( E_{\text{max}} )</td>
<td>100.5 ± 3.3</td>
<td>8.1 ± 1</td>
<td>N.A.</td>
</tr>
<tr>
<td>2. Inhibitory ( E_{\text{max}} ) with baseline effect</td>
<td>100.2 ± 2.3</td>
<td>10.7 ± 2.2</td>
<td>N.A.</td>
</tr>
<tr>
<td>3. Sigmoidal inhibitory ( E_{\text{max}} )</td>
<td>94 ± 2.8</td>
<td>12.7 ± 0.9</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>4. Sigmoidal inhibitory ( E_{\text{max}} ) with baseline effect</td>
<td>93.9 ± 2.6</td>
<td>12.9 ± 1.1</td>
<td>2.2 ± 0.4</td>
</tr>
</tbody>
</table>

N.A., not applicable.

a Numbers 1 to 4 refer to equations under Materials and Methods.

b Statistical goodness-of-fit criteria used to select among models for a particular data set. The lower the AIC value, better is the statistical fit to the data.
through VEGF receptor 2 signaling, can induce proliferation and migration of endothelial cells. VEGF also prevents the induction of apoptosis in endothelial cells, and this is mediated through the phosphatidylinositol 3-kinase/Akt pathway (Gerber et al., 1998b).

We have previously demonstrated that celecoxib inhibits VEGF secretion from RPE cells and that it also inhibits diabetes-induced VEGF secretion from the retina (Amrite et al., 2006). In the present study, we found that celecoxib has an antiproliferative effect in the choroid-retinal endothelial cells, with an IC_{50} of ~13 μM. This value is in agreement with the IC_{50} value of 10.6 ± 6.3 μM observed in HUVEC cells, another type of endothelial cell (Niederberger et al., 2004). Thus, celecoxib can have a dual beneficial effect in the choroid-retinal endothelial cells, with an IC_{50} of 10.6 ± 6.3 μM observed in HUVEC cells, another type of endothelial cell (Niederberger et al., 2004). The concentration required for statistically significant antiproliferative effects of celecoxib on ARPE-19 cells is much higher (~5 μM). In addition, the slope for VEGF inhibition is much shallower than the slope for the antiproliferative effects. Thus, two different mechanisms could be involved in the anti-VEGF and antiproliferative effects of celecoxib on the ARPE-19 cells.

The possible mechanisms (Fig. 6) for the antiproliferative effect could be inhibition of Akt signaling in these cells by celecoxib. Celecoxib is shown to inhibit Akt signaling in several cell types, including endothelial cells (Hsu et al., 2000; Arico et al., 2002; Lai et al., 2003; Kulp et al., 2004; Lin et al., 2004; Nam et al., 2004; Wu et al., 2004; Yang et al., 2004; Zhang et al., 2004; Basu et al., 2005; Barnes et al., 2007). In addition to inhibiting Akt signaling, celecoxib can induce apoptosis in the cells through interaction with extracellular signal-regulated kinase 2 and endoplasmic reticulum calcium ATPase signaling pathways (Hsu et al., 2000; Johnson et al., 2001).
The retinal pigment epithelial proliferation is involved in late age-related macular degeneration and in proliferative retinopathy (Spaide, 2006). Celecoxib inhibited proliferation of RPE cells in our in vitro studies. For both the cell types, the antiproliferative IC$_{50}$ values are less than the IC$_{50}$ for cytotoxicity. Thus, celecoxib is more toxic to proliferating cell types than cells that are nondividing. However, it is important to note that the cytotoxic and antiproliferative potencies of celecoxib on these cell types differ only by 2-fold. Thus, with drug level fluctuations associated with an immediate release dosage form (Amrite et al., 2007), it is possible that there could be significant toxicity to normal cells, and this might be a limitation in developing celecoxib as an antiproliferative therapeutic agent for choroidal neovascularization. In addition, the mechanisms involved in both the antiproliferative and the cytotoxic effects might be similar.

We also found that rofecoxib, another Cox-2-selective inhibitor with Cox-2 selectivity greater than that of celecoxib and Cox-2 inhibition potency similar to that of celecoxib, has no antiproliferative effects on the choroid-retinal endothelial or the ARPE-19 cells up to a concentration of 100 μM, which is more than 4 to 7-fold higher concentration than the antiproliferative IC$_{50}$ concentration of celecoxib in these cell lines. It has been demonstrated previously in HUVEC cells that celecoxib is much more potent than rofecoxib in its antiproliferative effects (Lin et al., 2004; Niederberger et al., 2004). In addition, we demonstrate here that flurbiprofen, a nonselective Cox inhibitor with Cox-2 inhibitory potency similar to the selective Cox-2 inhibitors rofecoxib and celecoxib, did not have a significant antiproliferative effect in the choroidal endothelial cells. Furthermore, in vivo studies indicate that celecoxib inhibits corneal neovascularization (Leahy et al., 2002), but flurbiprofen is unable to do so (Riazi-Esfahani et al., 2006). These observations suggest that not all Cox-2 inhibitors can have antiangiogenic potential.

Although the Cox-2 IC$_{50}$ value for celecoxib is in the range of 40 to 70 nM (Smith et al., 1998), the observed IC$_{50}$ value for the antiproliferative effects is much higher (~10–25 μM).
for the two cell types. At these concentrations, there is a significant inhibition of Cox-2 by the drugs tested, as evidenced by the PGE$_2$ secretion from the two cell types with or without the treatment with the Cox inhibitors. These huge differences in IC$_{50}$ values also point to a Cox-2-independent mechanism for the antiproliferative effects of celecoxib. With all the three drugs tested, the decrease in PGE$_2$ secretion occurs at concentrations much higher than the reported Cox-1 and Cox-2 IC$_{50}$ values of the drugs. However, the IC$_{50}$ values reported in the literature were for the purified enzyme and not in a cell culture system (Barnett et al., 1994; Smith et al., 1998; Prasit et al., 1999). The unbound concentrations within the cell could be much lower than the concentrations in the culture medium; hence, this discrepancy in IC$_{50}$ values can arise. In addition, the two cell types examined, ARPE-19 and RF/6A, are pigmented and they contain melanin. We have demonstrated that there is a significant binding of celecoxib to melanin (Cheruvu et al., 2007). This binding can further reduce the available unbound celecoxib. The effect of celecoxib is not likely due to physicochemical property differences between the three drugs. All three drugs are similar in their water solubility and lipophilicity (Table 3). Hence, the observed differences are not likely due to differential drug levels achieved by the three drugs within the cells. In addition, it is important to note that all the three drugs reduced PGE$_2$ secretion from RF/6A and ARPE-19 cells, indicating that at the concentrations tested, the drugs were able to inhibit the Cox enzymes (Table 4).

We observed that celecoxib causes a G$_2$-M phase cell cycle arrest in the retinal endothelial cells and the retinal pigment epithelial cells. There is also an S phase cell cycle arrest at the highest concentrations tested (25 $\mu$M). The effect is observed at celecoxib concentrations greater than 10 $\mu$M. In contrast, rofecoxib and flurbiprofen have no such effect on the cell cycle. The potency of the cell cycle arrest with celecoxib is greater in RF/6A cells compared with the ARPE-19 cells. Celecoxib has been shown to selectively inhibit the growth of malignant cells by causing a G$_2$-M phase cell cycle arrest (Dvory-Sobol et al., 2006).

The in vitro antiproliferative IC$_{50}$ for celecoxib on these cell
types is in the range of tens of micromolar. The therapeutically achievable $C_{\text{max}}$ levels lie in the 3 to 8 $\mu$M range in the plasma (Davies et al., 2000). The tissue levels could be slightly higher. In this study, there was an acute exposure of celecoxib to the cells for 24 h. A more sustained exposure over a longer period might have antiproliferative effects at lower concentrations of celecoxib. This is supported by the fact that celecoxib exerts antiangiogenic and antiproliferative effects in vivo in cancers in several animal models and some human studies (Basu et al., 2005; Soo et al., 2006). In addition, celecoxib has been demonstrated to be an effective chemotherapeutic for colon polyps and in clinical trials as an adjunct therapy for several cancers (Arber et al., 2006; Bertagnolli et al., 2006; Gadgeel et al., 2007; Heath et al., 2007). Development of more permeable analogs of celecoxib with similar antiproliferative effects could be another strategy to achieve the therapeutically effective levels in the body.

Although this study illustrates some beneficial effects of

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**Fig. 4.** Effect of drugs on cell cycle of ARPE-19 and RF/6A cells. A, effect of celecoxib on cell cycle of ARPE-19 cells. B, effect of celecoxib on cell cycle of RF/6A cells. C, effect of rofecoxib on cell cycle of ARPE-19 cells. D, effect of rofecoxib on cell cycle of RF/6A cells. E, effect of flurbiprofen on cell cycle of ARPE-19 cells. F, effect of flurbiprofen on cell cycle of RF/6A cells. Data are expressed as mean ± S.D. for $n = 3$. * significantly different from controls; $P < 0.05$. 

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Celecoxib in proliferative retinal disorders, some limitations of this study have to be taken into consideration. The study investigated the effects of the drug on individual cell types without the consideration of the extracellular matrix and the tissue milieu. This can have significant effects on cell signaling and cell survival; hence, the results observed in vitro cannot be directly extrapolated in vivo. It is likely that mechanisms other than those proposed are involved in the antiproliferative effects. Toxic effects need to be given special attention as the antiproliferative and cytotoxic potencies of celecoxib on these cell types were close.

Thus, in summary, celecoxib can have antiproliferative effects in ARPE-19 and RF/6A cells, and it can cause cell cycle arrest in the $G_2$-M phase in these cells. This, together with its anti-inflammatory and anti-VEGF effects, provides a strong rationale for potential use of celecoxib by itself or as an adjunct to currently existing therapies in the proliferative disorders of the eye.

**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LPS-Stimulated PGE$_2$ Secretion in ARPE-19 Cells (% of Untreated Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS</td>
<td>244 ± 56*</td>
</tr>
<tr>
<td>LPS + celecoxib 1 µM</td>
<td>95 ± 32†</td>
</tr>
<tr>
<td>LPS + celecoxib 10 µM</td>
<td>53 ± 23‡</td>
</tr>
<tr>
<td>LPS + celecoxib 25 µM</td>
<td>N.D.‡</td>
</tr>
<tr>
<td>LPS + rofecoxib 1 µM</td>
<td>85 ± 22†</td>
</tr>
<tr>
<td>LPS + rofecoxib 10 µM</td>
<td>47 ± 11†</td>
</tr>
<tr>
<td>LPS + rofecoxib 25 µM</td>
<td>N.D.*</td>
</tr>
<tr>
<td>LPS + flurbiprofen 1 µM</td>
<td>73 ± 18†</td>
</tr>
<tr>
<td>LPS + flurbiprofen 10 µM</td>
<td>48 ± 7†</td>
</tr>
<tr>
<td>LPS + flurbiprofen 25 µM</td>
<td>N.D.‡</td>
</tr>
</tbody>
</table>

N.D., not detected.
* Significantly different compared with untreated controls.
† Significantly different compared with LPS treatment.

Fig. 5. VEGF-165 induces proliferation in RF/6A (B) but not ARPE-19 (A) cells at concentrations greater than 10 ng/ml. Data are expressed as mean ± S.D. for $n = 6$ to 8. Celecoxib inhibits proliferation of ARPE-19 (C) and RF/6A (D) cells in presence of 50 ng/ml VEGF-165 stimulus.
Table 3

Properties of drugs used in the study

<table>
<thead>
<tr>
<th>Drug</th>
<th>Log D, pH 7°</th>
<th>Solubility at 25°C</th>
<th>Cox-1 IC₅₀</th>
<th>Cox-2 IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/ml</td>
<td>µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celecoxib</td>
<td>4.21</td>
<td>7 (Seedher and Bhatia, 2003)</td>
<td>15 (Smith et al., 1998)</td>
<td>0.04 (Smith et al., 1998)</td>
</tr>
<tr>
<td>Rofecoxib</td>
<td>1.34</td>
<td>8.19 ± 0.03 (Desai et al., 2003)</td>
<td>&gt;15 (Prasai et al., 1999)</td>
<td>0.02 (Prasai et al., 1999)</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>4.12</td>
<td>9.9 (Li and Zhao, 2003)</td>
<td>0.04 (Barnett et al., 1994)</td>
<td>0.51 (Barnett et al., 1994)</td>
</tr>
</tbody>
</table>

* From Scinder Scholar 2006.

Table 4

Celecoxib, rofecoxib, and flurbiprofen inhibit PGE₂ secretion from ARPE-19 and RF/6A cells at concentrations used in the study

The data are presented as mean ± S.D. for n = 4. Levels below the limit of detection of the assay (36 pg/ml) were considered as zero.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PGE₂ Secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ARPE-19</td>
</tr>
<tr>
<td>Control</td>
<td>1688 ± 866</td>
</tr>
<tr>
<td>Celecoxib 1 µM</td>
<td>1540 ± 712</td>
</tr>
<tr>
<td>Celecoxib 10 µM</td>
<td>413 ± 827**</td>
</tr>
<tr>
<td>Rofecoxib 1 µM</td>
<td>982 ± 1330</td>
</tr>
<tr>
<td>Rofecoxib 10 µM</td>
<td>2125 ± 227</td>
</tr>
<tr>
<td>Rofecoxib 25 µM</td>
<td>N.D.</td>
</tr>
<tr>
<td>Flurbiprofen 1 µM</td>
<td>1513 ± 1164</td>
</tr>
<tr>
<td>Flurbiprofen 10 µM</td>
<td>1065 ± 1965*</td>
</tr>
<tr>
<td>Flurbiprofen 25 µM</td>
<td>508 ± 1017**</td>
</tr>
</tbody>
</table>

N.D., not detected.

* Detected in only one of the four samples.

† Statistically significant difference with respect to untreated control cells (P ≤ 0.05).

Acknowledgments

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References


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