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Short communication

Inhibition of cyclooxygenase-2, but not cyclooxygenase-1, reduces prostaglandin E_2 secretion from diabetic rat retinas

Surya P. Ayalasomayajula[a,1], Aniruddha C. Amrite[a], Uday B. Kompella[a,b,*]

[a]*Department of Pharmaceutical Sciences, University of Nebraska Medical Center, 985840 Nebraska Medical Center, Omaha, NE 68198-5840, USA*
[b]*Department of Ophthalmology, University of Nebraska Medical Center, 985840 Nebraska Medical Center, Omaha, NE 68198-5840, USA*

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Abstract

Up-regulation of cyclooxygenase-2 occurs in retinal cells during the early onset of diabetic retinopathy. Under these conditions, prostaglandin production is elevated, which in turn leads to an increased expression of vascular endothelial growth factor (VEGF)—a growth factor implicated in vascular leakage and neovascularization. In this ex vivo study, we tested whether cyclooxygenase-1 or cyclooxygenase-2 is responsible for diabetes-induced secretion of prostaglandin E_2 from isolated rat retinas. Celecoxib, a selective cyclooxygenase-2 inhibitor, significantly inhibited prostaglandin E_2 secretion, whereas SC560 [5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethylpyrazole], a selective cyclooxygenase-1 inhibitor, had no inhibitory effect. This result suggests that the enzymatic activity of cyclooxygenase-2, but not cyclooxygenase-1, results in prostaglandin E_2 secretion under diabetic conditions.

Keywords: Cyclooxygenase-2; Celecoxib; Prostaglandin E_2; Diabetic retinopathy; VEGF

1. Introduction

Diabetic retinopathy is a disease of the small blood vessels in the retina of the eye. Diabetic individuals are usually susceptible to this disease, which could lead to blindness due to retinal neovascularization. In retinal diseases, ischemia is a common precursor to neovascularization. It was shown that early pro-inflammatory genes are predominantly expressed in ischemic retina. One of these genes expressed at high levels during the early stages of the disease is cyclooxygenase-2, whose expression is induced by cytokines, mitogens, and endotoxins (Dubois et al., 1998). Cyclooxygenase-2 expressed in ischemic retina exerts angiogenic effects in corneal neovascularization (Leahy et al., 2002). Administration of aspirin, a nonselective inhibitor of cyclooxygenase-1 and cyclooxygenase-2, reduced retinal vascular leakage and abnormalities in diabetic rats (Qaum et al., 2001) and dogs (Kern and Engerman, 2001), and reduced the incidence of retinopathy in diabetic human subjects (Powell and Field, 1966). Taken together, these findings suggest that the inhibition of cyclooxygenase expression could ameliorate diabetic retinopathy.

Prostaglandins, the products of cyclooxygenase pathway, are pro-angiogenic factors that are implicated in vascular permeability and angiogenesis. They are important mediators of normal and abnormal growth control in many tissues. Prostaglandins are also expressed at the sites of inflammation and their production is increased by 40% in diabetic rat retinas (Johnson et al., 1999). Prostaglandin E_2, one of the most studied prostaglandins, is expressed in many tissues and has been implicated in a myriad of diverse pathological conditions. Experiments with cyclooxygenase-1-deficient and cyclooxygenase-2-deficient mice showed that both these isozymes are capable of producing prostaglandin E_2 (Kirtikara et al., 1998). Increased expression of prostaglandin E_2 leads to up-regulation of vascular endothelial growth

* Corresponding author. Department of Pharmaceutical Sciences, University of Nebraska Medical Center, 985840 Nebraska Medical Center, Omaha, NE 68198-5840, USA. Tel.: +1 402 559 5320; fax: +1 402 559 5368.
[1] Current address: MDS Pharma Services, Lincoln, NE 68502, USA.
E-mail address: ukompell@unmc.edu (U.B. Kompella).

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factor (VEGF) expression (Cheng et al., 1998). VEGF is expressed at high levels in diabetic retinas and is involved in the pathogenesis of diabetic retinopathy (Adamis, 2002). It is also a potent inducer of vascular hyperpermeability and neovascularization (Miller et al., 1997). Elevated expression of VEGF correlates with diabetic blood–retinal barrier breakdown and ischemia-related neovascularization in animal models and in humans (reviewed in Adamis, 2002). Thus, inhibition of prostaglandin production reduces the expression of VEGF and, therefore, can be an effective therapeutic approach for diabetic retinopathy. Recently, we have shown that celecoxib, a selective inhibitor of cyclooxygenase-2, inhibits diabetes-induced retinal VEGF expression and vascular leakage in vivo (Ayalasomayajula and Kompella, 2003). The objective of this study was to determine whether cyclooxygenase-1 or cyclooxygenase-2 is responsible for diabetes-induced secretion of prostaglandin E_2 from isolated rat retinas. We show here that in diabetic rat retinas, prostaglandin E_2 production is significantly reduced in the presence of celecoxib, but no such effect was found in the presence of SC560 [5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethylpyrazole]. These results suggests that cyclooxygenase-2 inhibition, but not cyclooxygenase-1 inhibition, reduces prostaglandin E_2 production in diabetic rat retinas. Therefore, cyclooxygenase-2-selective inhibitors are appropriate drug candidates for the therapy of diabetic retinopathy.

2. Materials and methods

2.1. Chemicals

Celecoxib was a gift from Pharmacia (St. Louis, MO). SC560 was purchased from Cayman Chemical (Ann Arbor, MI). Components of assay buffers and streptozotocin were purchased from Sigma (St. Louis, MO).

2.2. Animal study

Sprague–Dawley rats weighing 150–160 g were purchased from SASCO (Wilmington, MA). After fasting the animals for 24 h, diabetes was induced by administering a single intraperitoneal injection of streptozotocin (60 mg/kg). Citrate buffer (pH 4.0) was used as the vehicle for nondiabetic controls. The blood glucose levels were determined 24 h later using onetouch ultra® glucometer (LifeScan, Milpitas, CA) and animals with blood glucose levels >250 mg/dl were deemed diabetic. On day 14, the animals were sacrificed and retinas were isolated from both nondiabetic and diabetic rats.

2.3. Prostaglandin E_2 assay

Retinas were incubated in 100 μl of assay buffer (pH 7.4) at 37 °C, in the presence or absence of 1 μM celecoxib or SC560. The secreted prostaglandin E_2 levels in the supernatants at the end of 2 h were quantified using the Prostaglandin E_2 Express EIA kit (Cayman Chemical).

2.4. Statistical analysis

Data are expressed as mean±S.D. Comparison of mean values was done using analysis of variance (ANOVA) followed by Tukey's post-hoc analysis. Differences were considered statistically significant at $P<0.05$.

3. Results

3.1. Celecoxib treatment inhibits cyclooxygenase-2-induced prostaglandin E_2 expression

Retinas, isolated from diabetic rats, were assayed for prostaglandin E_2 production after incubating in the assay buffer for 2 h. The secreted prostaglandin E_2 levels in diabetic retinas were significantly higher when compared to nondiabetic controls (32.1±4.7 vs. 11.1±1.6 pg/mg, $P<0.05$). Coincubation with celecoxib (11.76±4.5 pg/mg, $P<0.05$), but not SC560 (30±1.2 pg/mg), significantly reduced diabetes-induced retinal prostaglandin E_2 secretion (Fig. 1). However, both celecoxib and SC560 had no affect on basal prostaglandin E_2 secretion from the isolated nondiabetic rat retinas. Also, retinal expression of cyclooxygenase-2, (Ayalasomayajula and Kompella, 2003) but not cyclooxygenase-1, is elevated during diabetes, indicating that the inhibition of cyclooxygenase-2 alone might be sufficient to reduce retinal vascular leakage (Fang et al., 1997).

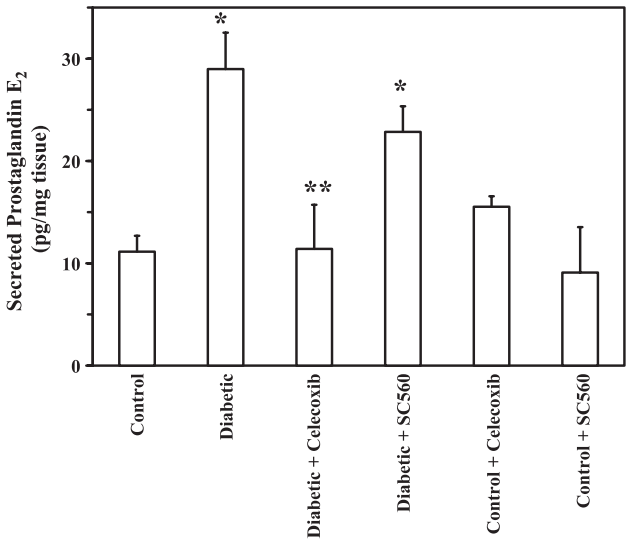

Fig. 1. Cyclooxygenase-2, but not cyclooxygenase-1, is involved in increased prostaglandin E_2 secretion from isolated diabetic rat retinas. Prostaglandin E_2 levels were measured as mentioned in Materials and methods and expressed as mean±S.D. for $n=4$. *Nondiabetic control vs. diabetic; **diabetic control vs. diabetic celecoxib-treated.

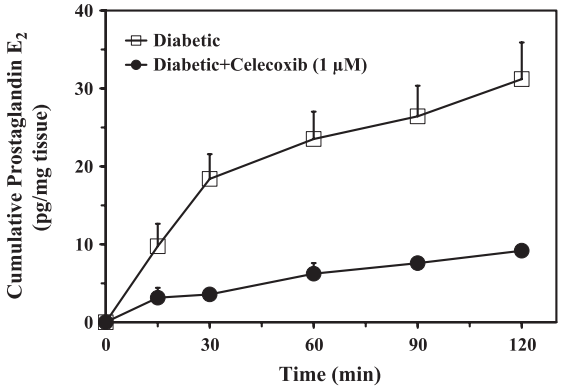

Fig. 2. Celecoxib (1 μM) inhibits the rate of prostaglandin E_2 synthesis in diabetic rat retinas. The cumulative prostaglandin E_2 secreted from diabetic rat retinas was estimated using EIA. Data are expressed as mean±S.D. for n=4.

3.2. Celecoxib inhibits the rate of prostaglandin E_2 synthesis in diabetic rat retinas

To further determine the celecoxib-mediated inhibition of prostaglandin E_2 synthesis, a time course experiment was performed and prostaglandin E_2 secretion was analyzed in isolated diabetic rat retinas. Celecoxib, 1 μM, was added at time 0 after isolating 14-day retinas. Production of prostaglandin E_2 was observed after 15 min of incubation in assay buffer. Celecoxib significantly reduced the rate of prostaglandin E_2 secretion from 0.23±0.09 to 0.07±0.02 pg/mg tissue/min (Fig. 2). This result shows that celecoxib significantly inhibits the production and secretion of prostaglandin E_2 in diabetic cells.

4. Discussion

The onset of diabetes in rats is characterized by pathological changes in the retinal vasculature and a significant increase in the production of pro-inflammatory proteins such as cyclooxygenase-2 and prostaglandins. During early stages of diabetic retinopathy, inflammatory symptoms such as blood vessel dilation, altered blood flow, exudation of fluids, and plasma proteins occur together with the aggregation of platelets along the retinal vasculature (Garner, 1994). These events lead to induced expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) (Miyamoto et al., 1999) and vascular cell adhesion molecule-1 (VCAM-1) (Joussen et al., 2001) and the breakdown of blood–retinal barrier, causing leukocyte-mediated injury to the vascular endothelial cells (Joussen et al., 2001). In fact, the rate of proliferation and death of retinal vascular endothelial cells is much higher in diabetic rats than in nondiabetic controls (Sharma et al., 1985). The aggregation of platelets and leukocytes, and endothelial cell injury result in the development of ischemia, leading to the release of inflammatory proteins. The elevated expression of these inflammatory mediators is associated with an increase in VEGF expression and occurrence of retinal neovascularization. A 94% decrease in VEGF production and vascular density was observed in cyclo-oxygenase-2-deficient fibroblasts, indicating that cyclo-oxygenase-2 modulates intratumoral vascular density and tumor growth (Williams et al., 2000). Celecoxib treatment also resulted in significant reduction in Lewis lung carcinoma progression and a 92% reduction in VEGF production. Because anti-inflammatory agents such as aspirin are known to exert their effects by inhibiting prostaglandin production, it can be suggested that prostaglandins might be playing a key role in the pathogenesis of diabetic retinopathy.

We observed a higher synthesis of prostaglandin E_2 in diabetic rat retinas when compared to the normal rats (Fig. 1). This is consistent with the earlier observations that retinal cyclooxygenase-2 (Carmo et al., 2000) and VEGF (Qaum et al., 2001) expressions are elevated within a week of inducing diabetes in rats. Increased expression of cyclooxygenase-2 leads to an increased production of prostaglandins, which induce VEGF expression. Results presented here show that this signaling pathway can effectively be blocked by administering celecoxib, making it a potential candidate for therapeutic purposes. We show here that diabetes-induced prostaglandin E_2 expression was significantly inhibited by the treatment of 1 μM celecoxib (Fig. 1). This inhibition of prostaglandin E_2 likely contributes to the reduced expression of VEGF. In a recent study, we found that celecoxib distributes to the retina following oral administration and inhibits retinal VEGF expression (Ayalasomayajula and Kompella, 2003). Our findings in this study will reinforce the potential value of celecoxib in treating diabetic retinopathy.

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