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Arachidonic Acid Metabolism in Human Trabecular Meshwork Cells

Robert N. Weinreb,* Jon R. Polansky,† Jorge A. Alvarado,† and Murray D. Mitchell*

Prostaglandins and other eicosanoids in the trabecular meshwork may play important physiological and pharmacological roles in the aqueous outflow pathway. In the present studies, we employed [^{14}C]-arachidonic acid to explore potentially important pathways for the production of eicosanoids in cultured human trabecular meshwork cells (HTM). In these cells, we demonstrated that prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ are major cyclooxygenase products, with some 6-keto-$PGF_{1\alpha}$ also detected. The amount of radiolabelled PGE_2 formed was substantially higher than the $PGF_{2\alpha}$ formed in the early time periods. The amount of $PGF_{2\alpha}$ in the culture media increased at a time when the amount of PGE_2 was declining, suggesting a possible metabolic conversion between the prostaglandins. HTM produced a range of products of the lipoxygenase pathway. Products co-eluting with 5, 12, and 15-hydroxyeicosatetraenoic acids (HETEs) were detected, with 12 and 15-HETEs predominating. A large amount of radiolabelled product was detected also in peaks co-eluting with leukotriene B_4 (LTB_4) and an LTB_4 degradation product. Biosynthesis of lipoxygenase products was markedly inhibited by BW 755c and partially inhibited by dexamethasone. These data emphasize that HTM cells are capable of converting arachidonic acid into a wider variety of biologically active products than previously recognized. Invest Ophthalmol Vis Sci 29:1708–1712, 1988

Arachidonic acid metabolism provides many important biological products that are known to exert a wide range of different cellular effects. The effects observed may depend upon the type of eicosanoid produced, as well as the individual cell type and tissue involved.[1] Products derived from arachidonic acid metabolism are thought to influence specific functions of inflammatory cells and appear to play equally important roles as physiologic autocoids in a number of tissues throughout the body.[2] Substantial attention has been directed to investigate the possible roles of prostaglandins as participants in ocular inflammation.[3,4] Recent reports also have emphasized that they can reduce intraocular pressure in the eyes of monkeys[5–7] and normal human volunteers.[8]

Because of the potential role of prostaglandins and other eicosanoids as regulators of physiologic and/or pharmacologic responses in the aqueous outflow pathway, we have been evaluating the biosynthesis of these substances by the human trabecular meshwork (HTM). Employing specific radioimmunoassay methods, we reported previously the production of prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ in HTM cells and a dose-related inhibition of the formation of these substances by dexamethasone.[9] Subsequently, we described the production of products of the lipoxygenase pathway by HTM cells.[10] Recently, Gerritsen et al[11] extended these observations, but questioned whether HTM synthesized $PGF_{2\alpha}$.

In the current study, we have examined the time course of the formation of radiolabelled metabolites of [^{14}C]-arachidonic acid by differentiated cultured HTM cells.

Materials and Methods

Cell Culture and Radiolabelling Methods

HTM cells were established in serial cell culture by methods we have reported previously.[12] Third and fourth passage trabecular cells from 14-, 30- or 57-year-old patients were employed in the present studies. Cell ampules were removed from frozen stock, plated at approximately 20,000 cells/35 mm dish. After the cells reached confluency, the cultures were maintained for an additional 1–2 weeks. Under these conditions, the trabecular cell lines have been shown to retain the differentiated ultrastructural features of trabecular meshwork cells in situ through at least five passages in culture.[13,14] The cells were obtained under the guidelines and with the approval of the Human

From the Departments of Ophthalmology, *University of California, San Diego and †University of California, San Francisco, California.

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Reprint requests: Robert N. Weinreb, MD, University of California, San Diego, Department of Ophthalmology (T-014), La Jolla, CA 92093.

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To evaluate the range of eicosanoids formed by the HTM cells, 5×10^5 cpm of [^{14}C]-arachidonic acid (58.4 Ci/mmol) (Amersham; Arlington Heights, IL) was added to each 35 mm dish in 1.5 ml of fresh culture media (Dulbecco's Modified Eagle's Medium, with 10% fetal calf serum). Media samples were collected at specific times following addition of the radiolabelled arachidonic acid. In addition, dexamethasone (10^{-7} M, 24–48 hr preincubation) (Sigma, St. Louis, MO) or BW 755c (50 μg/ml, 5 min preincubation) (Burroughs Wellcome, London, England) was added to the culture media. The incubation continued in the presence of both of these substances throughout the duration of the experiment. The results presented are representative of experiments on three different HTM cell lines (14, 30, 57 years) and also of another experiment on the same cell line. The identified products were present in all cell lines with reproducible quantitative differences.

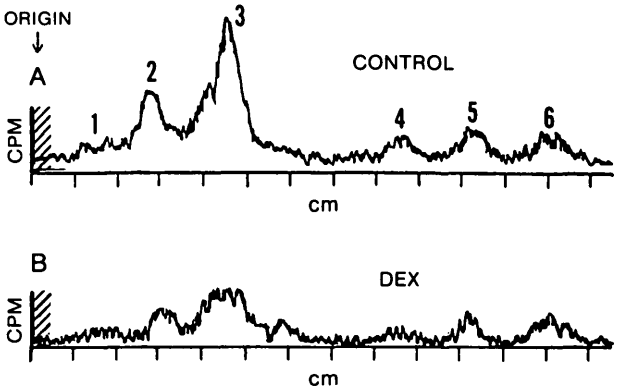

Fig. 1. Thin layer chromatograms of human trabecular meshwork cell radiolabelled eicosanoids following 18 hr incubation with [^{14}C]-arachidonic acid. (**A**) No treatment and (**B**) pretreatment for 24 hr with 10^{-7} M dexamethasone. Peak 1 corresponded in mobility to 6-keto-$PGF_{1\alpha}$ peak 2 with $PGF_{2\alpha}$ and peak 3 with PGE_2. Peaks 4 and 5 probably represented partially extracted lipoxygenase products and peak 6 represented arachidonic acid. The standards for PGE_2, PGD_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ were run as controls for the R_f values. HTM cells were from a 14-year-old patient.

Analytical Methods

Thin layer chromatography (TLC) was employed as the initial analytical method to separate the radiolabelled eicosanoids. For cyclooxygenase products, extraction was conducted using ten volumes of acidified ethyl acetate; for lipoxygenase products, ten volumes of ethyl acetate at neutral pH was employed.[15] These extraction methods result in recovery of radiolabelled products of greater than 90%. Chromatography was conducted on silica gel G TLC (Analtech, Newark, DE) plates using the following solvent systems—for cyclooxygenase products: organic phase of ethyl acetate:2,2,4-trimethylpentane:acetic acid: water (110:50:20:100, by vol.); for lipoxygenase products: ether:petroleum ether:acetic acid (50:50:1, by vol.).

Although separation of cyclooxygenase products was readily achieved using TLC, the separation of lipoxygenase products was not. To improve the separation of lipoxygenase products, high pressure liquid chromatography (HPLC) was performed in the reverse phase using a C18 MicroBondapak column (Waters, Milford, MA) according to the method of Mitchell et al.[16] A gradient was employed consisting of a mixture of methanol, water, acetic acid in various volumes as described above. In this system, metabolites of arachidonic acid elute from the column in order of decreasing polarity (ie, prostaglandins first, followed by leukotrienes, HETEs, and finally, unchanged arachidonic acid).

Radiolabelled eicosanoids were identified on the TLC plates using a Berthold radio-chromatogram scanner. Quantitative evaluations were performed using liquid scintillation spectrometry following HPLC and TLC.

Results

Radiolabelled metabolites of arachidonic acid produced by the cultured HTM cells were first examined 18 hr after a media change. Figure 1A and B represents chromatographic profiles of the radiolabelled prostaglandins from this experiment. PGE_2 (peak 3) was the largest peak on the chromatograms, comigrating with the PGE_2 standard (R_f = 0.32). Peak 2 corresponded in mobility to $PGF_{2\alpha}$ standard (R_f = 0.20) and was present in higher amounts than peak 1, which corresponds in mobility to 6-keto-$PGF_{1\alpha}$ standard (R_f = 0.12). The noncharacterized peaks could represent unidentified prostaglandins or their metabolites. Peaks 4 and 5 probably represented partially extracted lipoxygenase products and peak 6 represented arachidonic acid. These data clearly demonstrate the formation of $PGF_{2\alpha}$. Although the 6-keto-$PGF_{1\alpha}$ peak was small, its presence could be confirmed by scintillation spectroscopy. These data are consistent with our prior observations on the relative amounts of cyclooxygenase products synthesized by HTM cells after an 18 hr incubation (PGE_2 > $PGF_{2\alpha}$ > 6-keto-$PGF_{1\alpha}$).[9]

Dexamethasone (10^{-7} M) pretreatment decreased formation of radiolabelled prostaglandins by 33–35% (Fig. 1B). The effect of steroid treatment did not appear to be due to an effect on prostaglandin secretion, since no accumulation of radiolabelled prostaglan-

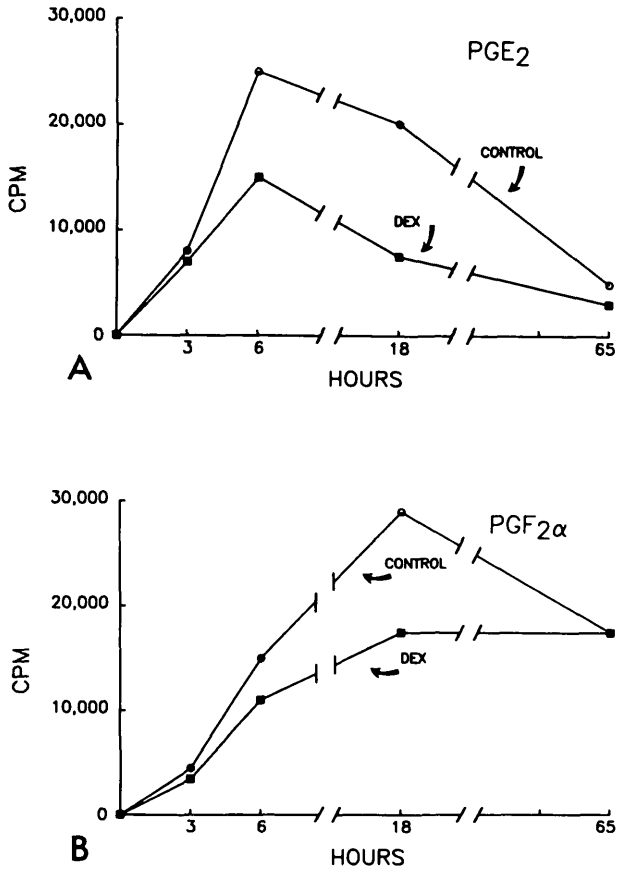

Fig. 2. Time course of conversion of [^{14}C]-arachidonic acid into prostaglandins after media change on confluent HTM cultures from a 30-year-old patient. (A) PGE_2 and (B) $PGF_{2\alpha}$. No treatment control (O). Pretreatment for 24 hr with 10^{-7} M dexamethasone (■).

dins was observed when the cell layer was examined.

Time course studies of the conversion of [^{14}C]-arachidonic acid into PGE_2 and $PGF_{2\alpha}$ then were conducted. The amount of PGE_2 decreased between 6 and 18 hr (Fig. 2A). In contrast, $PGF_{2\alpha}$ increased during this time, peaking at 18 hr (Fig. 2B). The effect of dexamethasone was most apparent at 18 hr. Conversion of PGE_2 to $PGF_{2\alpha}$ in parallel incubations in the absence of cells was less than 10%, determined by both radioimmunoassay and TLC.

We next investigated the radiolabelled products of arachidonic acid metabolism by the lipoxygenase pathway. Initial observations at 18 hr demonstrated two products with chromatographic mobilities similar to those of 5- and 12-HETE standards on TLC (data not shown). We used HPLC to improve resolution of lipoxygenase product formation. Time course studies demonstrated production of radiolabelled eicosanoids co-migrating with LTB_4, an LTB_4 degradation product, 15-HETE, 12-HETE and possibly a small amount of 5-HETE. These products were present in largest amounts at the 3–6 hr time period and the levels decreased rapidly thereafter (Figs. 3, 4).

As shown in Table 1, dexamethasone (10^{-7} M, 24 hr pretreatment) and BW 755c (50 μg/ml, 5 min pretreatment) (Fig. 5) decreased the size of peaks identified with LTB_4, as well as the HETEs at both 1 and 6 hr. A major effect of BW 755c could be observed at 1 hr, while the effect of dexamethasone was less prominent at 1 hr. BW 755c appeared to provide a greater degree of blockade of the lipoxygenase products than did dexamethasone. In control incubations without cells, lipoxygenase products were not observed. Similar effects of dexamethasone and BW 755c were obtained in a repeat experiment on this cell line (30 years), and from other trabecular cell lines (14 and 57 years).

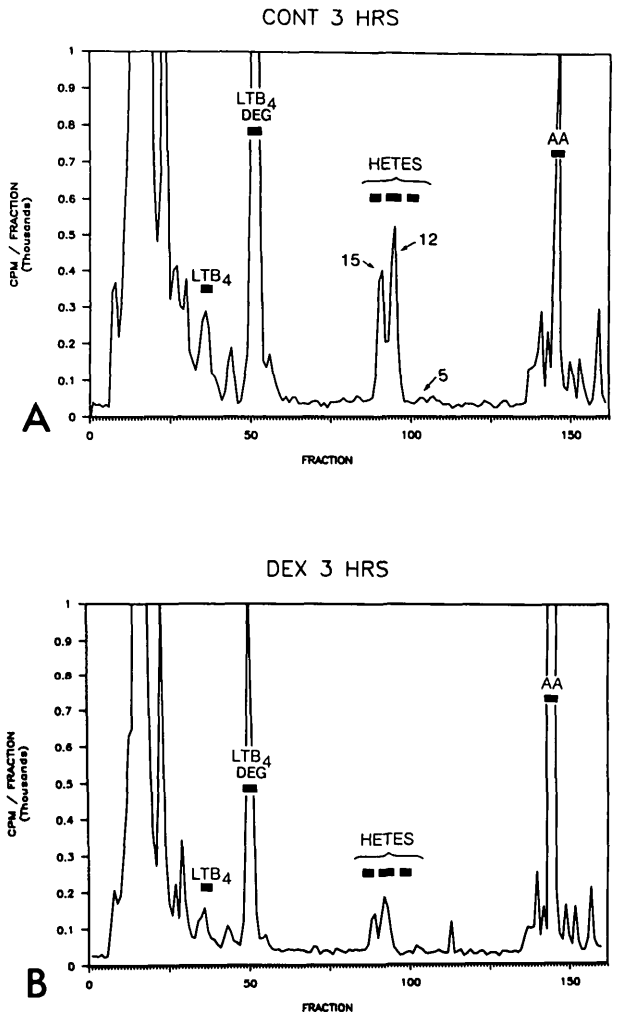

Fig. 3. High pressure liquid chromatographic separations of radiolabelled metabolites of the lipoxygenase pathway detected 3 hr after a media change. (A) No treatment control and (B) pretreatment for 24 hr with 10^{-7} M dexamethasone. The position of unlabelled standards is indicated by arrows on the profiles. HTM cells were from a 30-year-old patient.

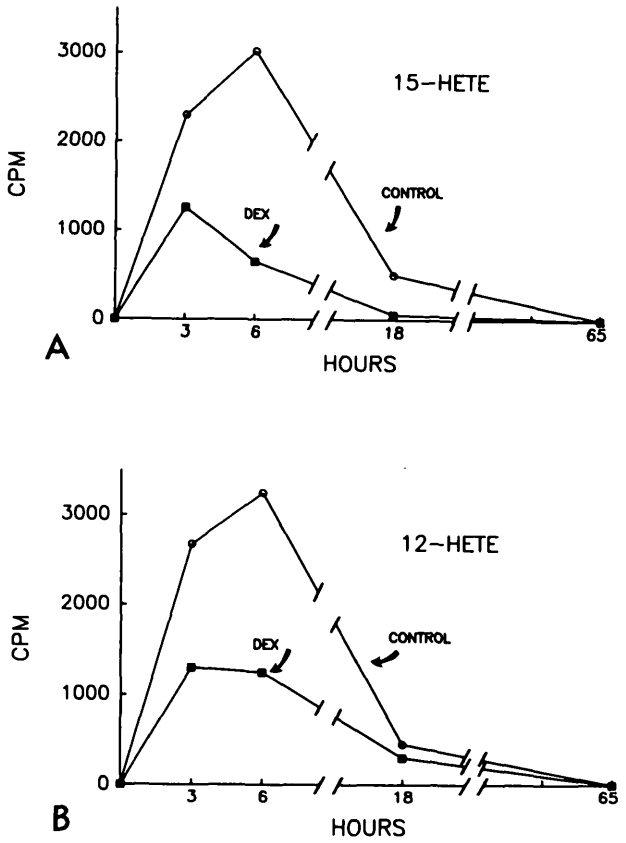

Fig. 4. Time course of appearance of radiolabelled lipoxygenase products after media change on cultured HTM cells. (**A**) 15-HETE and (**B**) 12-HETE. No treatment control (O). Pretreatment for 24 hr with 10^{-7} M dexamethasone (■). HTM cells were from a 30-year-old patient.

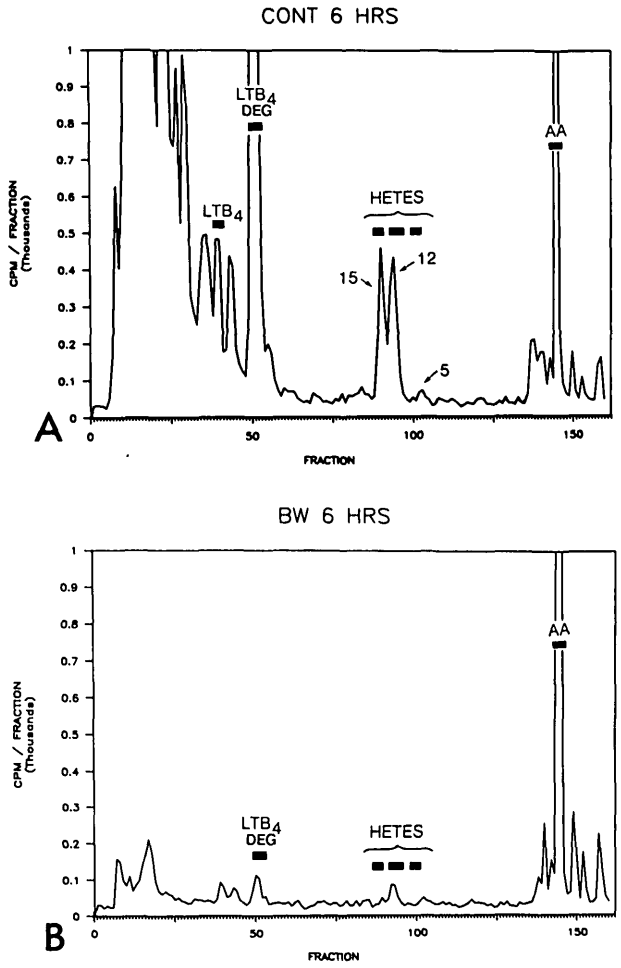

Fig. 5. High pressure liquid chromatographic separations of radiolabelled metabolite of the lipoxygenase pathway at 6 hr. (**A**) No treatment control and (**B**) pretreatment for 5 min with 50 μg/ml BW 755c. HTM cells were from a 30-year-old patient.

Discussion

Our results confirm that PGE_2 is the predominant prostaglandin synthesized by HTM cells in culture. In separate experiments, we confirmed that PGE_2 is also the major prostaglandin produced by both HTM tissue maintained in organ culture[10] and cell-free tissue homogenates (data not shown).

Table 1. BW755c and dexamethasone effects on HTM lipoxygenase pathways*

	LTB_4	*LTB_4†*	*15-HETE*	*12-HETE*	*5-HETE*
1 Hour					
CONT	566	1351	408	478	320
DEX	498	1180	343	372	158
BW755	375	170	121	190	ND‡
6 Hours					
CONT	2580	7551	1359	1300	139
DEX	521	2811	305	397	ND‡
BW755	42	391	132	219	91

* CPM in fractions from HPLC effluents were determined in HTM cultures treated with dexamethasone (DEX) and BW755c according to *Methods*.

† LTB_4 degradation product according to *Methods*.

‡ Nondetectable.

Of particular interest, our time course studies demonstrate that the proportion of $PGF_{2\alpha}$ relative to PGE_2 increased with time. This effect was confirmed in subsequent studies in which prostaglandins were measured by radioimmunoassay. These data may help to reconcile our previous findings with those of Gerritson et al,[11] who concluded that there was no evidence for $PGF_{2\alpha}$ production after 2 hr in their experiments with HTM cells in culture. Our current findings suggest that there may be an enzymatic pathway for conversion of PGE_2 to $PGF_{2\alpha}$ in HTM cells. This could be confirmed by determining the presence of the enzyme PGE_2-9-keto-reductase. If present, this pathway could be of special interest because of the intraocular pressure lowering effects of $PGF_{2\alpha}$ in normal human volunteers.[8]

The formation by HTM cells of small amounts of 6-keto-$PGF_{1\alpha}$, and substantial amounts of lipoxygenase products which comigrate with leukotriene

B_4, 15-HETE and 12-HETE is also of considerable interest. The formation of 6-keto-$PGF_{1\alpha}$ indicates that HTM cells can synthesize prostacyclin. The relative amount of prostacyclin made by these cells is much lower, however, than that of vascular endothelium in which it is the predominant eicosanoid.[17] As with other cells in culture, the prostaglandins synthesized were found predominantly in the culture medium rather than in the cell layer. The inhibition of the biosynthesis of lipoxygenase products by addition of BW 755c and by dexamethasone supports the view that the formation of those products is due to cellular metabolism of arachidonic acid. Definitive identification of these products and other potentially important eicosanoids will require the application of mass spectrometric techniques.

The maximal steroid-induced inhibition of eicosanoid synthesis was achieved by 10^{-7} M dexamethasone, a concentration sufficient to saturate trabecular cell glucocorticoid receptors.[18] The inhibition of both cyclooxygenase and lipoxygenase pathways by dexamethasone suggests that glucocorticoids may act to inhibit the release of arachidonic acid in HTM. Thus, HTM cells may be similar to other cell types in which phospholipase inhibitory protein(s) may be induced by glucocorticoids.

The methods we have described will permit an evaluation of the effects of various hormones and drugs on HTM cell eicosanoid production. Alterations in the endogenous production of eicosanoids may take place in aging and glaucoma and we can evaluate whether mediators of inflammation and neurotransmitters may alter outflow from the trabecular meshwork or uveoscleral pathway by influencing the bioynthesis and/or release of certain HTM eicosanoids.

Key words: trabecular meshwork, prostaglandins, eicosanoids, glucocorticoid, glaucoma

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