Localisation of thromboxane A₂ receptors and the corresponding mRNAs in human eye tissue

Zheng Chen, Shiv Prasad, Max Cynader

Abstract

Thromboxane A₂ (TxA₂) receptors in human eye sections were identified and localised using a potent TxA₂ specific agonist, ¹²⁵I-iodinated 5-heptenoic acid 7-[3-[3-hydroxy-4-[4-(iodo-¹²⁵I)phenoxy]-1-butenyl]-7-oxabicyclo[2.2.1]hept-2-yl]-, [1S-[1a,2a,(Z),3B(1E,3S*),4a]]-C₂₃ H₂₉ IO₅ (¹²⁵I-BOP) in a binding assay. TxA₂ receptors were concentrated in several specific loci within ocular tissues, including the corneal epithelium, the ciliary processes, retina, and posterior ciliary arteries. In addition, we have used the method of in situ hybridisation to observe the distribution of TxA₂ receptor mRNA. The distributions of both receptor binding sites and receptor mRNAs showed a close correlation. These studies employed film autoradiography which does not permit cellular resolution. In order to obtain enhanced cellular resolution and more detailed information about the localisation of the receptors and their corresponding mRNAs, emulsion autoradiography was used after ligand binding and in situ hybridisation. This approach showed further that TxA₂ receptors are mainly concentrated on non-pigmented epithelial cells of the ciliary processes, on photoreceptors within the retina, and on endothelial cells of the posterior ciliary arteries. These results may be helpful for understanding the pathophysiological effects of TxA₂ in the human eye.

(*Br J Ophthalmol* 1994; 78: 921–926)

Thromboxane A₂ (TxA₂) is a potent vasoconstrictor and platelet aggregating agent which is thought to be involved in the pathophysiology of cardiovascular disease¹ and some circulatory disorders^{2–4} such as thrombosis, atherosclerosis, angina, coronary artery vasospasm, and acute myocardial infarction. In the eye, it has been reported that TxA₂ plays a role in ocular traumatic reactions,⁵ ocular inflammation,⁶ and in the pathophysiology of cystic macular oedema (CMO).⁷ Although the precise mechanism of action in these situations is still not fully elucidated, it is believed that these activities are receptor mediated.⁸

Because both TxA₂ and its precursor, prostaglandin H₂, show similar pharmacological properties, they are presumed to share a common receptor, which has been named the TxA₂/PGH₂ or TxA₂ receptor. The existence of TxA₂ receptors in platelets is extensively substantiated by a variety of radioligand binding studies and platelet aggregation bioassays.^{9–15} The evidence for putative receptors in smooth muscle derives mainly from studies of smooth muscle constriction in tissues including dog saphenous vein, rabbit aorta, and guineapig trachea.¹⁶ There are

also physiological studies evaluating the functions of TxA₂ in smooth muscle from different organs such as guineapig lung, ileum, fundic strip, trachea, rat stomach, and also in cat and dog iris sphincter muscle strips.¹⁷ However, there have been no direct binding studies of TxA₂ receptors in whole eye sections. TxA₂ is an important substance in a variety of pathophysiological conditions and has the potential to play a therapeutic role in both systemic diseases and in ocular disorders. Previous physiological studies have shown that platelet and vessel TxA₂ receptors have species differences.¹⁸ Therefore, it is important to study TxA₂ receptors in the human eye. This study examines the distribution of TxA₂ receptors and their messenger RNAs in sections through the human eye using autoradiographic binding assays and in situ hybridisation methods.

Material and methods

IN VITRO LIGAND BINDING AND AUTORADIOGRAPHY

Human cadaveric eyes were obtained within 24 hours after death from the Eye Bank of British Columbia. The eyes from three different individuals were used: a 60-year-old woman who died of breast cancer, a 47-year-old man who died of a skull fracture, and a 44-year-old woman who died of liposarcoma. The eyes used in this study had no documented ocular diseases. They were frozen in isopentane cooled to –80°C, and stored at –20°C until used. Sections were cut on a cryostat (Cambridge Instruments, Nussloch, Germany) at a thickness of 20 µm, and mounted onto 1.7% gelatin subbed glass slides. For the ligand binding assay, sections were preincubated at room temperature for 60 minutes in 50 mM Tris HCl (pH 7.4) containing 100 mM sodium chloride, 3 mM calcium chloride, and 5% (w/v) bovine serum albumin (BSA). The sections were then incubated in the same buffer containing 1 mM of ¹²⁵I-iodinated 5-heptenoic acid 7-[3-[3-hydroxy-4-[4-(iodo-¹²⁵I)phenoxy]-1-butenyl]-7-oxabicyclo[2.2.1]hept-2-yl]-, [1S-[1a,2a,(Z),3B(1E,3S*),4a]]-C₂₃ H₂₉ IO₅ (¹²⁵I-BOP, Cayman Chemical) for 90 minutes at room temperature, and washed at 4°C for 40 minutes in the same buffer with 1% BSA.

Autoradiography was performed by apposing the sections to tritium sensitive film (Hyperfilm-³H: Amersham) for 7–10 days in the dark. Non-specific binding was determined on sections incubated under the same conditions as described above, but which contained in addition either 1–10 µM non-radioactive I-BOP (TxA₂ agonist: Cayman Chemical), or U-46619 (TxA₂ agonist: Cayman Chemical), or SQ 27427

Department of
Ophthalmology,
University of British
Columbia, Vancouver,
Canada
Z Chen
S Prasad
M Cynader

Correspondence to:
Dr Zheng Chen, Department
of Ophthalmology, 2550
Willow Street, Eye Care
Center, University of British
Columbia, Vancouver, BC,
Canada V5Z 3N9.

(TxA₂ antagonist: kindly provided by Allergan Pharmaceuticals).

IN SITU HYBRIDISATION

The donor eyes from a 47-year-old man who died of tongue cancer and a 52-year-old woman who died of breast cancer were obtained 4–6 hours after the patients died. As soon as they were obtained, the eyes were put into diethyl pyrocarbonate (DEPC) treated tubes and frozen using 2-methylbutane at -80°C . They were then kept in a -80°C freezer until sectioning. For sectioning, the 16 μm thick sections were cut in an anterior posterior horizontal plane on a cryostat and mounted onto double subbed glass slides coated with 0.5% gelatin and 0.02% poly-

lysine (Sigma). After air drying, sections were fixed for 20 minutes at room temperature with 4% paraformaldehyde, then rinsed in PBS and postfixed in 4% paraformaldehyde for 5 minutes, then rinsed in PBS again. The sections were dehydrated through a series of ascending concentrations of fresh ethanol (50% EtOH, 70% EtOH, 95% EtOH, 100% EtOH, 100% EtOH) for 5 minutes each, and finally sections were air dried up to 2 hours.

A complementary DNA probe encoding the TxA₂ receptor (kindly supplied by Masakazu Hirata, Department of Pharmacology, Kyoto University Faculty of Medicine, Japan) from human placenta was used for hybridisation. In order to remove the vector DNA, the cDNA insert was gel purified from a 1% low melting

Figure 1 Autoradiographic views of ^{125}I -BOP binding sites in eye sections. Sections were incubated with 1 nM ^{125}I -BOP alone (A) or in the presence of 1 μM ^{125}I -BOP (B), or with 1 μM unlabelled SQ27427 (C) to determine non-specific binding. In (A) increased optic density represents a greater number of receptors. The arrows illustrate dense concentrations of binding sites within the corneal epithelium, ciliary processes, posterior ciliary artery, and neural retina. Other zones of apparent concentration within the lens and choroid were not displaceable (B and C) using unlabelled competitors (BOP: (B) 1 μM unlabelled SQ27427: (C)). (D) Shows haematoxylin and eosin staining of the section whose autoradiogram is shown in (A). (CE, cornea epithelium; ISM, iris sphincter muscle; CP, ciliary processes; CM, ciliary muscle; L, lens; CH, choroid; NR, neurosensory retina; PCA, posterior ciliary artery.)

Table 1 Binding densities of ¹²⁵I-BOP in eye tissues (fmol/mg tissue)

Eye tissues	M (SEM)
Ciliary processes	0.12 (0.03)
Corneal epithelium	0.06 (0.02)
Posterior ciliary arteries	0.07 (0.02)
Retina	0.03 (0.01)

point agarose gel¹⁹ by restriction digestion with the enzymes that flank the cDNA insert. This insert was then labelled with [α -³²P] dATP (NEN) by using the random primer labelling method of Feinberg and Vogelstein²⁰ and unincorporated nucleotides were removed using push column purification (Stratagene).

The prehybridisation buffer consisted of 50% formamide, 25% PIPES (piperazine-*N,N'*-bis-[2-ethanesulphonic acid]), 5×Denhardt's solution (1×Denhardt's solution is 0.02% BSA, 0.02% Ficoll, and 0.02% polyvinyl pyrrolidone), 1% sodium dodecyl sulphate (SDS), salmon sperm DNA (250 µg/ml), and tRNA (250 µg/ml). Prehybridisation was performed at 37°C for 1 hour with 200 µl of prehybridisation buffer for each eye section. Hybridisation was carried out in the same buffer with the addition of 10% dextran sulphate. For each eye section, 200 µl of buffer containing 5000 cpm/µl of labelled probe was used. Incubation was performed in a humid chamber at 37°C for 18 hours. Following hybridisation, sections were washed in 4×SSC (1×SSC: sodium chloride 150 mM; sodium citrate 15 mM) for 10 minutes at room temperature, 4×SSC for 30 minutes at 37°C and then 2×SSC, 1×SSC, 0.5×SSC for 45 minutes each at 37°C. The sections were then washed in 0.1×SSC for 10 minutes at room temperature, air dried and apposed to x ray film (Hyperfilm: Amersham) for 7 days in the dark.

For a negative control, adjacent sections mounted on separate slides were treated with RNase A (20–40 µg/ml) for 60 minutes at 37°C before hybridisation as described above.

Quantitative analysis of the distribution of

performed using a computerised densitometry system (NIH Image: Macintosh). For all three eyes, the images of the sections were captured and the mean autoradiographic densities in different zones representing the ciliary processes, corneal epithelium, posterior ciliary arteries, and retina were measured respectively using the densitometric program and calibrated against the density of co-exposed ¹²⁵I-plastic standards ([¹²⁵I] Micro-scales, Amersham). This enabled us to compare directly the density of images from different films and also to convert grey scales into values of radioactivity per milligram tissue.

LIQUID EMULSION AUTORADIOGRAPHY

The sections derived from ligand binding autoradiography experiments were fixed by using paraformaldehyde powder at 80°C as described by Palacios *et al.*²¹ Sections were then dipped into 45°C Kodak NTB2 emulsion (diluted 1:1 with DDH₂O), air dried for 2 hours at room temperature and stored at 4°C in lightproof slide boxes. After appropriate exposures (14–21 days), the sections were developed using Kodak D19 developer, fixed (Kodak fixer), rinsed in DDH₂O, lightly counterstained with haematoxylin and eosin, dehydrated, and coverslipped with permount.

Results

Representative autoradiograms illustrating the distribution of ¹²⁵I-BOP binding sites in the human eye are shown in Figure 1. Quantitative densitometric analyses showed that the highest densities of specific binding sites were located in the corneal epithelium, in the ciliary processes, in blood vessels surrounding the optic nerve, and in the retina (Table 1, Fig 1A). The density of binding was reduced dramatically in the presence of 1 µM non-radioactive BOP (Fig 1B), SQ27427 (Fig 1C), and U46619 (data not shown), indicating that specific labelling occurred in these structures. Although both the lens

Fig 2A

Fig 2B

Figure 2 Autoradiographic visualisation of TxA₂ receptor specific mRNA in the eye. Note the concentration of mRNA in the corneal epithelium, ciliary processes, and retina. Sections were hybridised with 5000 cpm/µl of ³²P-labelled TxA₂ receptor cDNA probes (A). The labelling disappeared after pretreatment with RNase (B).

and choroid showed moderate levels of signal, these were not displaceable and not significantly higher than those of the corresponding non-specific binding levels shown in Figure 1C.

In situ hybridisation investigations of the localisation of mRNAs coding for TxA_2 receptors were broadly consistent with the results obtained from the ligand binding assays (Fig 2A). Within the anterior segment, the highest density of hybridisation sites were found in the corneal epithelium and in the ciliary processes. Although hybridisation to blood vessels was not detected in the posterior segment of the eye, a high density of reaction product was seen in the retina.

Figure 3 illustrates the results of liquid emulsion autoradiography using ^{125}I -BOP as a ligand. The results at low power (not shown) were the same as those illustrated in Figure 1. The high magnification views of Figure 3 show that the ciliary process binding sites identified at

low power in Figure 1 were concentrated on non-pigmented epithelial cells within the processes rather than on pigmented cells (Fig 3A, B). For the blood vessels at the optic nerve head, emulsion autoradiography showed that the binding was concentrated on the endothelial cells of the ciliary artery rather than on nearby smooth muscle cells (Fig 3C, D), and within the retina a high density of binding sites were seen only within the photoreceptor layer (Fig 3E, F).

Discussion

The localisation of TxA_2 receptors has been examined in human eye sections using film autoradiography, liquid emulsion autoradiography, and in situ hybridisation techniques. The results from the three different methods are similar, consistent, and complementary to each other.

In the autoradiographic binding studies, the

Figure 3 Microautoradiograms of ^{125}I -BOP binding in anterior segment. Bright field (A) and dark field (B) views of the same section after incubation with 1 nM ^{125}I -BOP alone. Silver grains were located mainly on non-pigmented epithelial cells within the ciliary processes. In the posterior segment bright field (C) and dark field (D) autoradiograms show silver grains decorating the endothelial cells (E, F) of the ciliary artery. Note the absence of silver grains on the immediately adjacent smooth muscle cells (C). In the retina only the photoreceptor layer is labelled (F) and the ganglion cell layer, bipolar cell layer, and inner or outer plexiform layers illustrated in (E) are essentially unlabelled. (NPE, non-pigmented epithelium; PE, pigmented epithelium; EC, endothelial cells of artery; SMC, smooth muscle cells of artery; GCL, ganglion cell layer; BCL, bipolar cell layer; IPL, inner plexiform layer; OPL, outer plexiform layer; PL, photoreceptor layer.)

binding sites representing the TxA₂ receptor were primarily localised to the corneal epithelium, to the ciliary processes of the ciliary body, to the retina, and to the vessels surrounding the optic nerve. These binding sites could be displaced using unlabelled I-BOP, U-46619 and SQ27427 attesting to the specificity of the binding in these structures. The film autoradiographic method suffers from limited spatial resolution and a relatively high noise/signal ratio. Its spatial resolution is limited to about 100 µm. The liquid emulsion method gives more detailed information about the location of binding sites which enable a more precise assessment of possible function of the TxA₂ sites.

The localisation of the TxA₂ receptor in human eye was examined not only at the protein level but also at the mRNA level. The distribution of mRNA encoding the TxA₂ receptor, revealed by in situ hybridisation, showed a good correlation with that of the corresponding receptor distribution within the cornea, ciliary processes, and retina. The correlation was not exact, however. TxA₂ receptor mRNA was not found concentrated within the posterior ciliary artery, on which the ¹²⁵I-BOP showed a high density of binding, but the mRNA was highly concentrated within corneal epithelium cells which show comparable levels of ¹²⁵I-BOP binding. These differences may be due to poor penetration of the various ligands in different ocular tissues, individual variation, or some unexplained variation in the localisation of receptors and their mRNAs. One possibility is that receptor turnover in the ciliary arteries is very slow, so there would be only a low concentration of mRNA at any given time, while receptors would be present in higher numbers. Conversely, receptor turnover in the corneal epithelial cells may be unusually rapid, so there may be relatively low numbers of receptors present at any given time, but the tissue might still show a high concentration of mRNA.

The blood supply of the eye is derived from two separate systems, the retinal vessels and the uveal vessels. The posterior ciliary arteries belong to the uveal vessels and provide the blood supply to the choroid and retina. During the past decade, anterior ischaemic optic neuropathy has been recognised as a commonly seen clinical entity. Optic disc cupping and visual field defects are the cardinal features of glaucoma and low tension glaucoma. There is evidence that the optic nerve head changes are due to vascular disturbances in the anterior part of the optic nerve,^{22,23} and that the posterior ciliary arteries are the main source of blood supply to the anterior part of optic nerve. Thus, the present results, showing that TxA₂ receptors are highly concentrated in the wall of the posterior ciliary arteries, suggest that this receptor system might be involved in the pathophysiological mechanism of glaucomatous optic neuropathy.

At a microscopic level, the binding sites were found in endothelial layers but under high power, the adjacent smooth muscle cells lining the posterior ciliary arteries were found not to contain many receptors. It has been established that many eicosanoids have powerful effects on the vasculature, causing vasodilatation or vaso-

constriction was well as increased permeability in the eye.²⁴ Most of them are potential vasodilators,²⁵ whereas TxA₂ causes vasoconstriction in practically all vascular beds. It has been shown that the level of TxA₂ in plasma was increased in patients with essential hypertension as well as biosynthesis of TxA₂ in spontaneously hypertensive rats.²⁶ The selective TxA₂ receptor antagonist BM13177 also can induce a strong decrease of arterial blood pressure with a maximum reduction of 21.6% in the third minute after intravenous injection.²⁷ In the past few years, the study of TxA₂ in vascular tissues has been based mainly on vascular smooth muscle constriction assays. There have been few direct binding studies examining the TxA₂ receptor localisation on endothelial cells. Direct functional studies have been reported recently by Sung *et al.*²⁸ They demonstrated the existence of TxA₂ receptors in hypertensive membrane preparations of bovine pulmonary artery endothelial cells using a ligand binding assay with a potent TxA₂ specific antagonist, ¹²⁵I-PTA-OH. They found that U-46619 suppressed prostacyclin-PGI₂ (the most potent vasodilator and platelet inhibitor) production in cultured endothelial cells. Furthermore, the suppression of PGI₂ by U-46619 could be blocked by the TxA₂ specific antagonists, I-PTA-OH or SQ29548. Thus, it has been hypothesised that the vasoconstriction effect of TxA₂ may be caused by suppressing the release of vasodilators from vascular endothelial cells, resulting in an enhanced vasoconstriction of blood vessels. Our results indicating TxA₂ receptor binding in endothelial cells of posterior ciliary artery support this hypothesis and may also help us to understand the pathological mechanism of glaucomatous optic neuropathy. Further studies in glaucomatous eyes with a focus on the optic nerve head and associated blood vessels are required to address the question of whether there are any changes in the TxA₂ receptor binding sites in glaucomatous eyes.

Compared with classic prostaglandins, the binding sites for the TxA₂ receptor in the anterior segment were mainly located on non-pigmented epithelial cells of the ciliary processes, whereas PGE₂ and F_{2α} binding is mostly concentrated within the ciliary muscle.²⁹ The non-pigmented epithelial cells of the ciliary processes are not only acting in aqueous humour production but are also involved in the maintenance of the blood-aqueous barrier (BAB). Although PGE₂ induces most of the features of ocular inflammation, it has been found that levels of TxB₂ (the metabolic product of TxA₂) are significantly increased in vitamin E unsupplemented uveoretinitic rats.³⁰ The role of TxA₂ in the conjunctiva and alkaline burn reaction of the eye has also been examined since changes in the aqueous humour production and the breakdown of BAB are used extensively to indicate intraocular inflammatory reactions. The existence of TxA₂ receptors in non-pigmented epithelial cells of the ciliary processes implies a possible role of TxA₂ in some reactions of ocular inflammation including the breakdown of BAB and alteration of intraocular pressure.

The second messenger system for TxA₂ receptors has been studied extensively.^{8,31,32}

TxA₂ acts as an agonist at a specific, G protein coupled cell surface receptor which is linked to phospholipase C. Activation therefore results in a rise of IP₃ and diacylglycerol and consequent increase in intracellular free calcium concentration. Since both Ca²⁺ and GTP are involved in signal transduction processes of photoreceptors, our results, which show that TxA₂ receptors are concentrated in retinal photoreceptors, imply a possible and rather unexpected role of TxA₂ receptors in the phototransduction process. The exact pathophysiological implications and mechanisms of this process need to be explored further.

- Mitchell JAR. Clinical aspects of the arachidonic acid-thromboxane pathway. *Br Med Bull* 1983; 39: 289-95.
- Whittle BJR, Moncada S. Pharmacological interactions between prostacyclin and thromboxanes. *Br Med Bull* 1983; 39: 232-8.
- Lefer AM. Eicosanoids as mediators of ischemia and shock. *Fed Proc* 1985; 44: 275-80.
- Lewy RI, Wiener L, Walinsky P, Lefer AM, Silver MJ, Smith DSJB. Thromboxane release during pacing-induced angina pectoris: possible vasoconstrictor influence on the coronary vasculature. *Circulation* 1980; 61: 1165-71.
- Song LY. Experimental study of the pathological mechanism of thromboxane A₂ in alkaline ocular burn. *Chung-Hua Yen Ko Tsa Chin* 1990; 26: 301-3.
- Woodward DF, Nieves AL, Williams LS. Thromboxane A₂-mimetics are potent microvascular permeability factors in the conjunctiva. *J Pharmacol Exp Ther* 1990; 255: 23-7.
- Stjerschantz J, Nilsson SF, Astin M. Vasodynamic and angiogenic effects of eicosanoids in the eye. *Prog Clin Biol Res* 1989; 312: 155-70.
- Halushka PV, Mais DE, Mayeux PR, Morinelli TA. Thromboxane, prostaglandin and leukotriene receptors. *Annu Rev Pharm Tox* 1989; 10: 213-39.
- Masuda A, Mais DE, Oatis JE Jr, Halushka PV. Platelet and vascular thromboxane A₂/prostaglandin H₂ receptors. Evidence for different subclasses in the rat. *Biochem Pharmacol* 1991; 42: 537-44.
- Naka M, Mais DE, Morinelli TA, Hamanaka N, Oatis JE Jr, Halushka PV. 7-[(1R,2S,3S,5R)-6,6-dimethyl-3-(4-iodobenzenesulfonylamino) bicyclo[3.1.1]hept-2-yl]-5(Z)-heptenoic acid: a novel high-affinity radiolabeled antagonist for platelet thromboxane A₂/prostaglandinH₂ receptor. *J Pharmacol Exp Ther* 1992; 262: 632-7.
- Okwu AK, Ullian ME, Halushka PV. Homologous desensitization of human platelet thromboxane A₂/prostaglandinH₂ receptor. *J Pharmacol Exp Ther* 1992; 262: 238-45.
- Ushikubi F, Nakajima M, Hirata M, Okuma M, Fuliwara M, Narumiya S. Purification of the thromboxane A₂/prostaglandin H₂ receptor from human blood platelet. *J Biol Chem* 1989; 264: 16496-501.
- Hirata M, Hayashi Y, Ushikubi F, Yokota Y, Kageyama R, Nakanishi S, et al. Cloning and expression of cDNA for a human thromboxane A₂ receptor. *Nature* 1991; 349: 617-20.
- Mais DE, Kochel PJ, Saussy DL Jr, Halushka PV. Binding of an ¹²⁵I-labeled thromboxane A₂/prostaglandin H₂ receptor antagonist to washed canine platelets. *Mol Pharmacol* 1985; 28: 163-9.
- Halushka PV, Mais DE, Garvin M. Binding of a thromboxane A₂/prostaglandinH₂ receptor antagonist to guinea pig platelets. *Eur J Pharmacol* 1986; 131: 49-54.
- Jones PL, Peesapati V, Wilson NH. Antagonism of the thromboxane-sensitive contractile systems of the rabbit aorta, dog saphenous vein and guinea-pig trachea. *Br J Pharmacol* 1982; 76: 423-38.
- Coleman PA, Humphrey PPA, Kennedy I, Levy GP, Lumley P. Comparison of the actions of U-46619, a prostaglandin H₂-analogue, with those of prostaglandin H₂ and thromboxane A₂ on some isolated smooth muscle preparations. *Br J Pharmacol* 1981; 73: 773-8.
- Narumiya S, Okuma M, Ushikubi F. Binding of a radioiodinated 13-azapinane thromboxane antagonist to platelets: correlation with antiaggregatory activity in different species. *Br J Pharmacol* 1986; 88: 323-31.
- Sambrook J, Fritsch EF, Maniatis T. Gel electrophoresis of DNA. In: Sambrook J, Fritsch EF, Maniatis T, eds. *Molecular cloning*. 2nd ed. New York: CSH Laboratory Press, 1989: 6.30-3.
- Feinberg A, Vogelstein B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 1983; 132: 6-13.
- Herkenham M. Influence of tissue treatment on quantitative receptor autoradiography. In: Huston JP, ed. *Molecular neuroanatomy*. Amsterdam: Elsevier, 1988; 3: 118.
- Hayreh SS. Pathogenesis of optic nerve damage and visual field defects in glaucoma. *Doc Ophthalmol Proc Ser* 1980; 22: 89-110.
- Weinreb RN. Why study the ocular microcirculation in glaucoma? *J Glaucoma* 1992; 1: 145-7.
- Stjerschantz J, Nilsson SFE, Astin M. Vasodynamic and angiogenic effects of eicosanoids in the eye. *Prog Clin Biol Res* 1989; 312: 155-70.
- Hayashi S, Park MK, Kuehl TJ. Effects of prostaglandins and arachidonic acid on baboon cerebral and mesenteric arteries. *Prostaglandins* 1986; 32: 587-96.
- Taube CH, Hoffmann P, Forster W. Enhanced thromboxane production in the aorta of spontaneously hypertensive rats in vitro. *Prostaglandins, Leukotrienes Med* 1982; 9: 411-3.
- Kohler AH, Kohler AR, Taube CH, Mest HJ. Decrease in arterial blood pressure in spontaneously hypertensive rats by the TxA₂ receptor. *Biomed Biochem Acta* 1988; 47: 269-73.
- Sung CP, Arleth AJ, Berkowitz BA. Endothelial thromboxane receptors: biochemical characterization and functional implications. *Biochem Biophys Res Commun* 1989; 158: 326-33.
- Matsuo T, Cynader M. Localisation of prostaglandin F₂ alpha and E₂ binding sites in the human eye. *Br J Ophthalmol* 1992; 76: 210-3.
- Pararajasegaram G, Sevanian A, Rao NA. Suppression of S antigen-induced uveitis by vitamin E supplementation. *Ophthalmol Res* 1991; 23: 121-7.
- Ashby B. Effect of thromboxane antagonists on prostaglandin regulation of platelet adenylate cyclase. *Second Messengers and Phosphoproteins* 1988-9; 12: 241-50.
- Kawahara Y, Yamanishi J, Furuta Y, Kaibuchi K, Takai Y, Fukuzaki H. Elevation of cytoplasmic free calcium concentration by stable thromboxane A₂ analogue in human platelets. *Biochem Biophys Res Commun* 1983; 117: 663-9.