A method of lowering intraocular pressure (IOP) employs a gene that encodes a cyclooxygenase (COX) enzyme. Two isoforms of COX (COX–1 and COX–2) produce prostaglandins PGE2 and PGF2α. The method of treatment entails administering to the eye of a mammal in need thereof a gene construct encoding a polypeptide having COX activity. In a preferred embodiment the COX enzyme is COX–2. It is also preferred that the gene be inducible with a specific small molecule or cytokine associated with the onset or progression of glaucoma.
<table>
<thead>
<tr>
<th>Code</th>
<th>Country</th>
<th>Code</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>Albania</td>
<td>ES</td>
<td>Spain</td>
</tr>
<tr>
<td>AM</td>
<td>Armenia</td>
<td>FI</td>
<td>Finland</td>
</tr>
<tr>
<td>AT</td>
<td>Austria</td>
<td>FR</td>
<td>France</td>
</tr>
<tr>
<td>AU</td>
<td>Australia</td>
<td>GA</td>
<td>Gabon</td>
</tr>
<tr>
<td>AZ</td>
<td>Azerbaijan</td>
<td>GB</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>BA</td>
<td>Bosnia and Herzegovina</td>
<td>GE</td>
<td>Georgia</td>
</tr>
<tr>
<td>BB</td>
<td>Barbados</td>
<td>GH</td>
<td>Ghana</td>
</tr>
<tr>
<td>BE</td>
<td>Belgium</td>
<td>GN</td>
<td>Guinea</td>
</tr>
<tr>
<td>BF</td>
<td>Burkina Faso</td>
<td>GR</td>
<td>Greece</td>
</tr>
<tr>
<td>BG</td>
<td>Bulgaria</td>
<td>HU</td>
<td>Hungary</td>
</tr>
<tr>
<td>BI</td>
<td>Benin</td>
<td>IE</td>
<td>Iceland</td>
</tr>
<tr>
<td>BR</td>
<td>Brazil</td>
<td>IL</td>
<td>Israel</td>
</tr>
<tr>
<td>BY</td>
<td>Belarus</td>
<td>IS</td>
<td>Iceland</td>
</tr>
<tr>
<td>CA</td>
<td>Canada</td>
<td>IT</td>
<td>Italy</td>
</tr>
<tr>
<td>CF</td>
<td>Central African Republic</td>
<td>JP</td>
<td>Japan</td>
</tr>
<tr>
<td>CG</td>
<td>Congo</td>
<td>KE</td>
<td>Kenya</td>
</tr>
<tr>
<td>CH</td>
<td>Switzerland</td>
<td>KG</td>
<td>Kyrgyzstan</td>
</tr>
<tr>
<td>CI</td>
<td>Côte d’Ivoire</td>
<td>KP</td>
<td>Democratic People’s Republic of Korea</td>
</tr>
<tr>
<td>CM</td>
<td>Cameroon</td>
<td>KR</td>
<td>Republic of Korea</td>
</tr>
<tr>
<td>CN</td>
<td>China</td>
<td>KZ</td>
<td>Kazakhstan</td>
</tr>
<tr>
<td>CU</td>
<td>Cuba</td>
<td>LC</td>
<td>Saint Lucia</td>
</tr>
<tr>
<td>CZ</td>
<td>Czech Republic</td>
<td>LI</td>
<td>Liechtenstein</td>
</tr>
<tr>
<td>DE</td>
<td>Germany</td>
<td>LK</td>
<td>Sri Lanka</td>
</tr>
<tr>
<td>DK</td>
<td>Denmark</td>
<td>LK</td>
<td>Liberia</td>
</tr>
<tr>
<td>EE</td>
<td>Estonia</td>
<td>LS</td>
<td>Lesotho</td>
</tr>
<tr>
<td>LT</td>
<td>Lithuasia</td>
<td>LU</td>
<td>Luxembourg</td>
</tr>
<tr>
<td>LV</td>
<td>Latvia</td>
<td>MC</td>
<td>Monaco</td>
</tr>
<tr>
<td>MD</td>
<td>Republic of Moldova</td>
<td>MG</td>
<td>Madagascar</td>
</tr>
<tr>
<td>MK</td>
<td>The former Yugoslav</td>
<td>ML</td>
<td>Mali</td>
</tr>
<tr>
<td>MN</td>
<td>Mongolia</td>
<td>MR</td>
<td>Mauritania</td>
</tr>
<tr>
<td>MW</td>
<td>Malawi</td>
<td>MX</td>
<td>Mexico</td>
</tr>
<tr>
<td>NE</td>
<td>Niger</td>
<td>NL</td>
<td>Netherlands</td>
</tr>
<tr>
<td>NO</td>
<td>Norway</td>
<td>NZ</td>
<td>New Zealand</td>
</tr>
<tr>
<td>PL</td>
<td>Poland</td>
<td>PT</td>
<td>Portugal</td>
</tr>
<tr>
<td>RO</td>
<td>Romania</td>
<td>RU</td>
<td>Russian Federation</td>
</tr>
<tr>
<td>SD</td>
<td>Sudan</td>
<td>SE</td>
<td>Sweden</td>
</tr>
<tr>
<td>SG</td>
<td>Singapore</td>
<td>SI</td>
<td>Slovenia</td>
</tr>
<tr>
<td>SK</td>
<td>Slovakia</td>
<td>SN</td>
<td>Senegal</td>
</tr>
<tr>
<td>SZ</td>
<td>Swaziland</td>
<td>TD</td>
<td>Chad</td>
</tr>
<tr>
<td>TG</td>
<td>Togo</td>
<td>TJ</td>
<td>Tajikistan</td>
</tr>
<tr>
<td>TM</td>
<td>Turkmenistan</td>
<td>TR</td>
<td>Turkey</td>
</tr>
<tr>
<td>TT</td>
<td>Trinidad and Tobago</td>
<td>UA</td>
<td>Ukraine</td>
</tr>
<tr>
<td>UG</td>
<td>Uganda</td>
<td>US</td>
<td>United States of America</td>
</tr>
<tr>
<td>UZ</td>
<td>Uzbekistan</td>
<td>VN</td>
<td>Viet Nam</td>
</tr>
<tr>
<td>YU</td>
<td>Yugoslavia</td>
<td>ZW</td>
<td>Zimbabwe</td>
</tr>
</tbody>
</table>
USE OF AN INDUCIBLE GENE FOR THE ENZYME
CYCLOOXYGENASE TO LOWER INTRAOCULAR PRESSURE

TECHNICAL FIELD

The present invention relates to methods of lowering intraocular pressure (IOP), such as in the treatment of glaucoma. The invention particularly relates to the use of cyclooxygenase (COX) enzymes, which are involved in the production of prostaglandins, in such treatment.

BACKGROUND OF THE INVENTION

Glaucoma

Glaucoma is a progressive disease which leads to optic nerve damage and, ultimately, total loss of vision. The causes of this disease have been the subject of extensive studies for many years, but are still not fully understood. The principal symptom of and/or risk factor for the disease is elevated intraocular pressure or ocular hypertension due to excess aqueous humor in the anterior segment of the eye. The anterior segment of the eye consists of anterior and posterior chambers. The anterior chamber lies in front of the iris and contains aqueous humor which helps support the cornea. The posterior chamber lies behind the iris and encompasses the crystallin lens of the eye.

The causes of aqueous humor accumulation in the anterior segment are not fully understood. It is known that elevated intraocular pressure ("IOP") can be at least partially controlled by administering drugs such as beta-blockers and carbonic anhydrase inhibitors, which reduce the production of aqueous humor within the eye, or agents such as miotics and sympathomimetics, which increase the outflow of aqueous humor from the eye.
Most types of drugs conventionally used to treat glaucoma have potentially serious side effects. Miotics such as pilocarpine can cause blurring of vision and other visual side effects, which may lead either to decreased patient compliance or to termination of therapy. Systemically administered carbonic anhydrase inhibitors can also cause serious side effects such as nausea, dyspepsia, fatigue, and metabolic acidosis, which side effects can affect patient compliance and/or necessitate the termination of treatment. Another type of drug, beta-blockers, have been associated with serious pulmonary side effects attributable to their effects on beta-2 receptors in pulmonary tissue. Sympathomimetics, on the other hand, may cause tachycardia, arrhythmia and hypertension.

*Prostaglandins*

Recently, certain prostaglandins and prostaglandin derivatives have been described in the art as being useful in reducing intraocular pressure. Typically, however, prostaglandin therapy for the treatment of elevated intraocular pressure is attended by undesirable side-effects, such as irritation and hyperemia of varying severity and duration. There is therefore a continuing need for therapies which control elevated intraocular pressure associated with glaucoma without the degree of undesirable side-effects attendant to most conventional therapies.

Prostaglandins are metabolite derivatives of arachidonic acid. Arachidonic acid in the body is converted to prostaglandin G2, which is subsequently converted to prostaglandin H2. Other naturally occurring prostaglandins are derivatives of prostaglandin H2. A number of different types of prostaglandins have been discovered including A, B, D, E, F, G, I and J-Series prostaglandins (EP 0 561 073 A1). Two naturally-occurring prostaglandins which have been shown to lower IOP are PGF2α (an F-series prostaglandin) and PGE2 (an E-series prostaglandin) which
have the following chemical structures:

![Chemical structures](image)

The relationship of PGF$_{2\alpha}$ receptor activation and IOP lowering effects is not well understood. It is believed that PGF$_{2\alpha}$ receptor activation leads to increased outflow of aqueous humor. Regardless of the mechanism, PGF$_{2\alpha}$ and certain of its analogs have been shown to lower IOP (Giuffre, *The Effects of Prostaglandin F$_{2\alpha}$ the Human Eye*, Graefe's Archive Ophthalmology 222:139-141 (1985); and Kerstetter et al., *Prostaglandin F$_{2\alpha}$-1-Isopropylester Lowers Intraocular Pressure Without Decreasing Aqueous Humor Flow*, American Journal of Ophthalmology 105:30-34 (1988)). Thus, it has been of interest in the field to develop synthetic PGF$_{2\alpha}$ analogs with IOP lowering efficacy.

Synthetic PGF$_{2\alpha}$-type analogs have been pursued in the art (Graefe's Archive Ophthalmology 229:411-413 (1991)). Though PGF$_{2\alpha}$-type molecules lower IOP, a number of these types of molecules have also been associated with undesirable side effects resulting from topical ophthalmic dosing. Such effects include an initial increase in IOP, breakdown of the blood aqueous barrier and conjunctival hyperemia (Alm, *The Potential of Prostaglandin Derivatives in Glaucoma Therapy*, Current Opinion in Ophthalmology, 4(11):44-50 (1993)).

Similarly, the relationship between EP receptor activation and IOP lowering effects is not well understood. There are currently four recognized subtypes of the EP receptor: (EP$_1$, EP$_2$, EP$_3$, and EP$_4$ (Ichikawa, Sugimoto, Negishi, *Molecular aspects of the structures and functions of the prostaglandin E receptors*, J. Lipid Mediators Cell Signaling, 14:83-87 (1996)). It is known in the art that ligands capable

It has now been postulated that ocular hyperemia, such as that attendant to the topical administration of the prostaglandins described above, is mediated by a sensory nerve response on the surface of the eye [1]. The prostaglandins PGF$_{2a}$ and PGE$_2$ are naturally formed by different tissues in the eye and are components of normal aqueous humor. Nevertheless, both are associated with acute inflammation and are considered early mediators of an induced inflammatory response. Still, co-administration of these natural prostaglandins to reduce IOP has been proposed. See, U.S. Patent No. 5,565,492.

Many synthetic prostaglandins purporting to avoid or reduce one or more of the side effects attributable to the natural prostaglandins have also been shown to lower IOP by varying degrees. See, for example, U.S. Patent Nos. 5,321,128; 5,698,733; 5,700,835; and 5,721,273.
The cornea, which is reportedly capable of producing both PGF$_{2\alpha}$ and PGE$_2$, appears also to have the ability to convert topically applied PGF$_{2\alpha}$ into PGE$_2$ to elevate aqueous humor levels of this important prostaglandin. In fact, prostaglandins are believed to be produced in all tissues surrounding the anterior chamber of the human eye including the iris/ciliary body, lens epithelial pocket and trabecular meshwork. Constitutive prostaglandin synthesis (non-inducible and providing relatively constant prostaglandin levels in normal aqueous humor) by these tissues may be an important factor in the normal control of IOP, and the loss of prostaglandin synthetic capability at or near the anterior chamber could result in an increase in IOP. Based in part on these observations, it is suggested that tissues in contact with the anterior chamber are likely accustomed to rapid changes in, and probably accommodate to, elevated levels of prostaglandins in aqueous humor.

Since prostaglandins, both naturally occurring and synthetic, exogenously applied to the cornea, lower IOP in the glaucoma patient, the availability of a critical concentration of a naturally occurring prostaglandins at or near the "target site(s)" of action is likely diminished over time during the course of the disease. This is one basis for the current prostaglandin therapy for the treatment of glaucoma. The presumed target(s) of prostaglandins are postulated to be related to an altered outflow mechanism associated with possible structural modifications through the uveal-scleral tract or trabecular meshwork. For purposes of this discussion, the mode of action of prostaglandins is of secondary importance to that of sustaining the critical concentration of prostaglandins, at or near the target site in the anterior chamber, adequate to lower and control IOP. Current prostaglandin therapies require chronic topical dosing and are, to varying degrees, attended by one or more of the side-effects discussed above.
Cyclooxygenases

Prostaglandins, including both PGF$_{2\alpha}$ and PGE$_2$, are products of a common biosynthetic pathway, generally known as the arachidonate cascade. The lead enzyme in this pathway, cyclooxygenase (COX), utilizes arachidonic acid and other 20 carbon polyunsaturated fatty acids as substrates to form endoperoxides, ultimately leading to the formation of prostaglandins, including PGE$_2$ and PGF$_{2\alpha}$. COX exists in at least two isoforms referred to in the literature as COX-1 and COX-2 [2]. The COX-1 isoform is considered to be a constitutive enzyme, being relatively non-inducible and remaining at relatively constant levels in the anterior chamber.

Unlike COX-1, expression of the COX-2 gene is under the regulation of a number of known effectors including transforming growth factor (TGF-(b)) [3-5], interleukin-1 (IL-1) [3,4,6] and tumor necrosis factor (TNF-(a)) [7]. Expression of the COX-2 gene is particularly responsive to what seems to be a synergistic control of two key cytokines, TGF-(b) and IL-1 [3]. The isolation of the entire COX-2 gene and its 5'-flanking region from a human bacteriophage P1 genomic library has been reported and may be achieved by the methodology of Tazawa et al. [8]. The gene containing 10 exons is 7.5 kb in length and is located on human chromosome 1. The transcriptional start site was mapped at 134 bases upstream from the ATG start codon. The nucleotide sequence of the 1.8kb promoter region contains a TATA box and a number of potential regulatory elements including CRE, NF-kappa B, Sp1 and AP2 sites. Studies of the promoter activity showed that the first 460 nucleotides of the 5'-flanking region efficiently drove transcription of a luciferase reporter gene in human umbilical vein endothelial cells upon stimulation [8].

The natural promoter region for the COX-2 gene has recently been isolated and sequenced [9], thus permitting the possibility of assembling gene constructs made from this or other effective promoters coupled to the structural gene for the enzyme. The in vitro expression of recombinant human cyclooxygenase isoenzymes
has been reported in COS-7 cells transfected with COX cDNA [10] and also in transiently transfected COS-1 cells [11]. Moreover, a recombinant prostaglandin synthesis gene (COX cDNA) has been expressed in vitro in bovine pulmonary artery endothelial cells which resulted in increased intracellular COX protein and increased PGE$_2$ synthesis. Human COX-2 cDNA, as reported by Hla et al., has been deposited in the GenBank data base (accession no. M90100). Rabbits intravenously transfected with a COX gene plasmid construct had increased plasma levels of prostacyclin and PGE$_2$, and their lungs produced increased amounts of these same eicosanoids [12]. This technique was used to protect the rabbits from endotoxin induced injury to the lungs due to the hyperexpression of prostaglandins.

Although various regulatory and structural features of COX genes have now been identified, an approach for using this knowledge to reduce IOP has not heretofore been elucidated. An object of the present invention therefore is to use genetic engineering techniques to provide a COX-based therapy in the treatment of glaucoma thereby avoiding certain limitations and side-effects associated with currently available prostaglandin therapies.

**SUMMARY OF THE INVENTION**

The present invention is directed to a method of treating, ameliorating or preventing the occurrence of glaucoma or elevated intraocular pressure (IOP) in the eye of a mammal. The method comprises administering to the eye a therapeutically effective amount of a polynucleotide (including native DNA and cDNA) that encodes a polypeptide having cyclooxygenase activity.

One method of administering an aforementioned polynucleotide to the eye comprises applying the polynucleotide topically to the exterior of the eye, e.g., to the cornea, under conditions effective to transform the contacted eye tissue. A second method of administration comprises applying to the eye, e.g., by surgical techniques,
corneal or other ocular tissues previously transformed with a polynucleotide of the invention. A third method of administration comprises introducing the polynucleotide into the anterior segment of the eye, such as by injecting cells previously transformed with the polynucleotide into the anterior segment. Yet another method of introducing a polynucleotide of the invention into cells or tissues adjoining the anterior chamber or ciliary body of the eye is by providing the polynucleotide as a single or double stranded DNA or RNA of a virus, which is used to transfect ocular tissues either proximal to, or forming the anterior chamber of the eye.

In any of the above methods, an aforementioned polynucleotide can be applied to or introduced into the eye in combination with a transformation facilitating agent, such as a liposome, a cationic lipid, a calcium salt, LIPOFECTIN or any of a number of other agents used for enhancing transfection efficiency in mammalian cells and tissues. The polynucleotide can also be provided as "naked DNA", that is, without a transformation vehicle, without completely compromising transformation efficiency. For purposes of this description, transformation means altering a cell by the introduction of new or additional genomic material to the cell. The use of a transformation facilitating agent generally enhances transformation of ocular tissue with the polynucleotide by facilitating its passage through the lipid bilayer of the cell membrane. This increases the copy number of COX genes adjacent to the anterior chamber, thereby increasing capability of prostaglandin production by cells found near the anterior chamber.

A related aspect of the present invention is a pharmaceutical composition, which comprises a pharmaceutically acceptable carrier and a therapeutically effective amount of an aforementioned polynucleotide capable of encoding a polypeptide having cyclooxygenase activity. Preferably, the polynucleotide would encode a full-length COX enzyme, which is expressed by cells transfected with the polynucleotide.
DETAILED DESCRIPTION OF THE INVENTION

The present invention involves a method of treating the eyes of a mammal suffering from a glaucomatous condition, wherein the intraocular pressure (IOP) of the eye is or is likely to become elevated above its "normal" state. The present method can be employed to reduce, or ameliorate elevated IOP and to prevent or impede increases in normal IOP, thereby controlling or slowing the progression of the disease.

A method of the present invention comprises administering to an eye of the mammal a therapeutically effective amount of a polynucleotide that encodes a polypeptide having cyclooxygenase activity. The polypeptide can be a full-length COX enzyme, such as COX-1 or COX-2; however, the entire protein is not required as long as the desired COX activity is afforded by the selected polypeptide, e.g., by providing the active site of the enzyme. The active site of the COX-2 enzyme providing cyclooxygenase activity is identified by Hla et al. [6] (the human COX-2 cDNA produced enzymatically active COX). The precise nucleotide sequence employed will generally be a matter of convenience, for instance, due to the availability of a cDNA encoding the full-length protein. Preferred is the human osteosarcoma COX-2 cDNA described in U.S. Patent No. 5,543,297, the disclosure of which is incorporated herein by this reference. Isolation of the human COX-2 genome is also described by Appleby et al. [29]. Although the present invention can be used in veterinary applications, the treated subject will generally be a human being.

An aforementioned polynucleotide is typically provided within an expression vector, e.g., as a plasmid or viral nucleic acid. An "expression vector", as used herein, refers to a vector that facilitates expression of an instant polynucleotide, e.g., by virtue of providing a regulatory sequence (i.e. a promoter) operably linked upstream of the polynucleotide. Examples of such expression vectors are described
in U.S. Patent No. 5,543,297, previously incorporated by reference. Additional vectors are described in U.S. Patent No. 5,770,580, the complete disclosure of which is incorporated herein by this reference. U.S. Patent No. 5,770,580 discloses ocular gene therapies, including the control or suppression of glaucoma, but does not disclose the use of COX genes in that regard.

It is generally preferred that a transformation facilitating agent is used in combination with a polynucleotide of the invention so as to increase the amount of polynucleotide in the transformed cells. Suitable transfection and transformation facilitating agents are typically cationic in nature, which increases their affinity for negatively charged nucleic acids. The agents also frequently have lipophilic tails, which increase their affinity for the lipid bilayer, thereby facilitating penetration of the polynucleotide through the cell membrane. Whenever a transformation facilitating agent is employed with the present invention the agent is selected so as to enhance the level of transformation that occurs over that obtained by applying a polynucleotide without the facilitating agent. Exemplary agents include liposomes, cationic lipids (including cationic detergents), calcium salts, such as calcium phosphate, dimethylsulfoxide with polycation, as well as commercially available agents such as LIPOFECTIN, DOTMA, DOTAP (Bethesda Research Laboratories; Gaithersburg, MD) and other commercially available cationic agents that facilitate transformation. Representative cationic lipids and methods for intracellular delivery of nucleic acids are described by U.S. Patent No. 5,264,618, the disclosure of which is incorporated herein by reference.

It should be noted that in this sense, a virus can also be considered a transformation facilitating agent, due to its ability to inject nucleic acids into the interior of a cell. Preferred vectors of this type include adenoviruses, which are double-stranded DNA viruses, and retroviruses, as when it is desired to integrate the transforming DNA into the genome of the cell. Exemplary of these methods are those described in U.S. Patent Nos. 5,645,829; 5,656,465; and 5,240,846, the
disclosures of which are incorporated herein by reference. In order to enhance gene
transfer, the virus can also be used in combination with a cationic molecule, such as
polylysine or conjugate thereof, as described by U.S. Patent No. 5,547,932, the
disclosure of which is incorporated herein by reference.

Still other methods of transforming a cell with an instant polynucleotide include
electroporation and microprojectile bombardment as described by Rodriguez [28].
These methods are obviously less desirable for in vivo transformations. Alternatively,
the polynucleotide can be applied as "naked DNA", i.e., independent of a
transformation facilitating agent. If muscle tissue (e.g. the ciliary muscle) is to be
transformed, administration of "naked DNA" is preferred.

Administration of an instant composition to the eye can comprise applying an
aforementioned polynucleotide directly to the exterior of the eye, i.e., topically. With
this mode of administration, cells of the eye, e.g., epithelial cells of the cornea, can
"take up" the applied polynucleotide where it can be processed by cellular organelles
and enzymes to express the encoded protein translation product of the
polynucleotide. Prostaglandins produced by the cell can permeate adjoining tissue,
i.e., the stroma and endothelial layers, and enter the anterior chamber thereby
reducing IOP. In this mode, it is generally preferred that the polynucleotide is co-
administered to the eye with an aforementioned transformation facilitating agent.
Alternatively, the polynucleotide can be applied through the action of a virus particle,
which transfsects the contacted cells, resulting in replication and translation of the
polynucleotide. In the above modes of administration, it is generally preferred that
transformation is focused to the cells of the uveal-scleral tract or trabecular
meshwork, where the outflow of aqueous humor is most likely to be attenuated in the
diseased eye.

Another mode of administration comprises directly introducing an
aforementioned polynucleotide into the anterior chamber of the eye, e.g. by injection.
The polynucleotide can be taken up to some extent by cells surrounding the chamber, with those cells thereby being able to express elevated levels of prostaglandin. The polynucleotide can be provided either as "naked DNA" or in combination with a transformation facilitating agent. Alternatively, the polynucleotide can be provided through a drug delivery device or as part of an artificial lens, e.g., around the perimeter of the lens, and implanted in either the anterior or posterior chamber of the eyes by surgical techniques.

Still another mode of administration comprises applying to the eye previously transformed cells or tissue. For instance, corneal tissue transformed ex vivo with a polynucleotide of the present invention can be surgically provided either as a corneal transplant or as a "patch" adjacent to the original corneal tissue. Transformation of the corneal tissue with a subject polynucleotide can be performed conventionally, using a transformation facilitating agent as mentioned above, microprojectile bombardment, electroporation, and the like. Sources of corneal tissue are readily available commercially. Alternatively, cells transformed with polynucleotide can be introduced directly into the anterior chamber, e.g., by way of injection.

During a typical treatment regimen, repeated administrations of an aforementioned polynucleotide will likely be required, especially in the event the transformation is not stably maintained. However, because the eye is an immune-privileged environment, reasonable half-lives of the COX gene and, if applicable, its expression vector should result and repeated administrations should be well tolerated. In such event, the administration can be repeated periodically, according to a physician's instructions, to maintain a targeted reduction in intraocular pressure.

A polynucleotide of the present invention preferably comprises a regulatory sequence that controls transcription of a nucleotide sequence encoding the COX polypeptide. The regulatory sequence is typically linked operably to the nucleotide sequence and is usually located immediately upstream of the encoding sequence.
Enhanced expression of a polypeptide having COX activity can be achieved merely by virtue of the increased intracellular copy number of COX-encoding polynucleotides provided according to the present invention. For instance, the regulatory sequence can comprise an endogenous COX promoter requiring no exogenous agent to initiate transcription. Alternatively, the regulatory sequence is inducible in the presence of a cytokine, preferably one associated with a glaucomatous condition, or other chemical agent. Thus, transcription of the COX encoding sequence can be induced by an endogenous biomolecule, such as transforming growth factor beta (TGFbeta) or by the administration of a suitable drug or chemical modulator, e.g., in the form of eyedrops.

As used herein, the term "regulatory element" or "promoter", and the like, refers to a DNA sequence typically, but not exclusively, located 5' (upstream) of the transcription initiation site of a structural gene. The regulatory element modulates the specificity and/or level of transcription of the gene. The regulatory element can contain positive and/or negative element(s), which influence the level of expression observed.

A number of promoters that can be used to regulate COX expression have been identified. For instance, a TGF-beta 1 responsive element in the mouse HSP47 gene has been identified [13]. TGF-beta 1 is reported to increase the promoter activity of a region -5.5 kb upstream of the HSP47 gene 4-6 fold, with the regions -3.9 to -2.7 bp and -280 to -50 bp shown to be involved in the activation in response to TGF-beta 1. TGF-beta stimulates PGE2 production and enhances IL-1-stimulated PGE2 production in cultured mouse cells [19]. The activation of prostaglandin G/H synthase (PGHS-2 or COX-2) by IL-1, TGF-alpha, forskolin, and phorbol 13-myristate 12-acetate is also enhanced by TGF-beta [19]. The region from -371 to -213 bp of the PGHS-2 promoter reportedly accounts for some 90% of the activity of this promoter[19]. A promoter positively activated by TGF-beta 1 is a preferred embodiment of the present invention.
Similarly, tumor necrosis factor alpha (TNF-alpha) is reported to induce COX-2 [26]. Positive regulatory regions at -186 to -131 bp and -512 to -385 bp upstream of the mouse COX-2 gene were identified. These regions contain putative NF-IL6, AP2, and NF kappa B motifs [26]. Additional inducing agents are contemplated for use with the present invention. For example, lipopolysaccharide has been shown to potentiate transcription of COX-2 genes [14, 15, 27]. Interleukin 1 (IL-1) is reported to produce a 10-fold induction of COX-2 [22] and to have a biphasic effect on COX-2 expression, with a NF-IL-6 binding motif being important to achieving maximal promoter activity [16]. Coordinate roles of IL-1, NF-kappaB, and NF-IL-6 are suggested [16, 25]. The dihydroxy bile acids Chenodeoxycholate and deoxycholate reportedly increase PGE\(_2\) production 10-fold [17]. The compounds 12-0-tetradecanoylphorbol-13-acetate (TPA) [24, 27] and 8-bromo-cAMP reportedly increase PGHS-2 promoter activity [24]. Superinduction of IL-1beta-induced COX-2 by cycloheximide is reported to increase transcription levels [18, 23]. Other inducing agents can be readily determined and may be preferred depending on the indications for the patient and a physician's recommendations.

Prostaglandin formation according to the present invention may be further enhanced by topical ocular application of an appropriate formulation of a suitable substrate, such as arachidonic acid (20:4) or the ethyl ester of arachidonic acid (ethyl-20:4). The concentration of the arachidonic acid or its ethyl ester is preferably in the range of 0.1 micromolar (0.000003%) to 10 micromolar (0.0003%), with a most preferred concentration of 1 micromolar (0.00003%).

A suppressing agent, i.e., one that can down-regulate the native COX-2 promoter, can be employed whenever over-expression of an instant polynucleotide occurs. Examples of such suppressing agents include non-steroidal anti-inflammatory drugs (NSAIDS), such as aspirin[24], indomethacin [20], glucocorticoids [21] including dexamethasone [14], phenylarsine oxide [22], actinomycin D [23], and the tyrosine kinase inhibitor herbimycin A [15].
A pharmaceutical composition for use with the present invention is also contemplated. The composition comprises a pharmaceutically acceptable carrier and a therapeutically effective amount of a polynucleotide of the invention, which encodes a polypeptide having COX activity. Preferably, the polynucleotide comprises an inducible regulatory sequence operably linked to a nucleotide sequence encoding the polypeptide. For example, the polynucleotide can encode a full-length COX enzyme. Suitable carriers include those conventionally available, such as BSS® and BSS-PLUS®. In addition to conventional carriers, an aforementioned transformation facilitating agent can be provided in the composition in order to enhance cell transformation, transfection and expression of polypeptide having COX activity.

The present invention has been described with reference to certain embodiments for purposes of clarity and understanding. It should be appreciated that various improvements and modifications can be practiced within the scope of the appended claims and equivalents.

REFERENCES

The pertinent disclosures of the following references are incorporated herein by reference.

WHAT IS CLAIMED IS:

1. A method of treating, ameliorating or preventing elevated intraocular pressure in an eye of a mammal comprising administering to the eye a therapeutically effective amount of a polynucleotide that encodes a polypeptide having cyclooxygenase activity.

2. The method of claim 1, wherein the mammal is a human.

3. The method of claim 1, wherein said administering comprises applying the polynucleotide topically to the eye.

4. The method of claim 1, wherein the polynucleotide is applied in combination with a transformation facilitating agent.

5. The method of claim 4, wherein the transformation facilitating agent comprises a liposome, a cationic lipid, a calcium salt, a combination of a polycation and dimethylsulfoxide, a cationic detergent, or LIPOFECTIN.

6. The method of claim 4, wherein the transformation facilitating agent comprises a virus.

7. The method of claim 1, wherein said administering comprises introducing the polynucleotide into the anterior chamber of the eye.

8. The method of claim 7, wherein the polynucleotide is introduced in combination with a transformation facilitating agent.

9. The method of claim 8, wherein the transformation facilitating agent comprises a liposome, a cationic lipid, a calcium salt, or LIPOFECTIN.
10. The method of claim 8, wherein the transformation facilitating agent comprises a virus.

11. The method of claim 7, wherein the polynucleotide is introduced by injecting cells transformed with the polynucleotide into the eye.

12. The method of claim 1, wherein said administering comprises applying to the eye corneal tissue transformed with the polynucleotide.

13. The method of claim 1, wherein the polynucleotide comprises an inducible regulatory sequence operably linked to a nucleotide sequence encoding said polypeptide.

14. The method of claim 13, wherein said regulatory sequence comprises a cytokine responsive promoter.

15. The method of claim 14, wherein the cytokine responsive promoter is up-regulated by TGF beta or IL-1.

16. The method of claim 1, wherein the polynucleotide encodes a full-length COX enzyme.

17. The method of claim 16, wherein the encoded COX enzyme is COX-2.

18. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a polynucleotide that encodes a polypeptide having cyclooxygenase activity.
19. The composition of claim 18, wherein the polynucleotide encodes COX-2.

20. The composition of claim 19, further comprising a transformation facilitating agent.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 7 C12N15/03 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>
| X        | WO 96 40720 A (UNIV ROCHESTER)  
19 December 1996 (1996–12–19)  
page 37, line 11 - line 26; claims 1,4,7 | 18-20 |
| X        | WU ET AL.: "injury-coupled induction of endothelial eNOS and COX-2 Genes: a paradigm for thromboreistant Gene Therapy"  
PROC. ASSOC. AM. PHYSICIANS,  
vol. 110, no. 3, 1998, pages 163–170,  
XP000891324 | 18 |
| A        | abstract  
page 166, right-hand column, line 25 - page 167, right-hand column, paragraph 1 | 1 |

Further documents are listed in the continuation of box C.  
Patient family members are listed in annex.

**Date of the actual completion of the international search**

29 March 2000

**Date of mailing of the international search report**

06/04/2000

Name and mailing address of the ISA  
European Patent Office, P.B. 5818 Patentissan 2  
NL – 2280 HV Rijswijk  
Tel. (+31–70) 340-0400, Tx. 31 651 epo nl,  
Fax (+31–70) 340-3816

Authorized officer  
Niemann, F
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>
| A        | US 5 770 580 A (LEDLEY FRED D ET AL)  
abstract  
column 9, line 53 – column 10, line 23  
column 18, line 27 – line 43  
column 33, line 16 – line 31 | 1-20                  |
| A        | US 5 698 733 A (HELLBERG MARK R ET AL)  
16 December 1997 (1997-12-16)  
cited in the application  
the whole document | 1                     |
| A        | WAX ET AL: "A rationale for gene targeting in glaucoma therapy"  
J. OCUL. PHARMACOL.,  
vol. 10, no. 1, 1994, pages 403-410,  
XP000891389  
abstract  
page 404, line 1 – line 28  
page 408, line 45 – page 409, line 3 | 1-13                  |
INTERNATIONAL SEARCH REPORT

Box I  Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [x] Claims No.: 1-17 because they relate to subject matter not required to be searched by this Authority, namely:
   Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

2. [ ] Claims No.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims No.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(e).

Box II  Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims No.:  

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims No.:  

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>WO 9640720 A</td>
<td>19-12-1996</td>
<td>AU 6029396 A</td>
<td>30-12-1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 5837479 A</td>
<td>17-11-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 1681195 A</td>
<td>01-08-1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2181170 A</td>
<td>20-07-1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0871723 A</td>
<td>21-10-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 9519182 A</td>
<td>20-07-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 684050 B</td>
<td>04-12-1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 4670993 A</td>
<td>31-01-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2139948 A</td>
<td>20-01-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0653943 A</td>
<td>24-05-1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 7508988 T</td>
<td>05-10-1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 9401139 A</td>
<td>20-01-1994</td>
</tr>
<tr>
<td>US 5698733 A</td>
<td>16-12-1997</td>
<td>AU 701808 B</td>
<td>04-02-1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 3639895 A</td>
<td>26-04-1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0783308 A</td>
<td>16-07-1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 10506893 T</td>
<td>07-07-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 9610407 A</td>
<td>11-04-1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 9821180 A</td>
<td>22-05-1998</td>
</tr>
</tbody>
</table>