Based on research for compounds that can display a corneal epithelial migration promoting effect in ophthalmology, the present invention provides P2Y receptor agonist corneal epithelial migration promoters, such as phosphoric acid compounds having an adenosyl group, uridylyl group, xanthosyl group, guanosyl group, or thymidyl group, or their salts, with excellent corneal epithelial migration promoting effects.
CORNEAL EPITHELIAL MIGRATION PROMOTER


TECHNICAL FIELD

[0002] The present invention pertains to a type of corneal epithelial migration promoter that contains P2Y receptor agonist as the effective component.

BACKGROUND

[0003] The cornea is transparent tissue with no blood vessels, a diameter of about 1 cm and a thickness of about 1 mm. The transparency of a cornea has an important influence on visual function. Various physiological and biochemical phenomena of the cornea mainly function to maintain this transparency.

[0004] Corneal epithelial defects caused by corneal ulcers, exfoliation of corneal epithelium, keratitis, dry eyes, and various other diseases can be repaired naturally if no mixed infection occurs. However, if repair is delayed or not made for certain reasons, corneal epithelial migration takes place, such that the normal epithelium construction is adversely affected, and the structure and function of the parenchyma and endothelium are also harmed. In prior art, the principal therapy was the so-called passive method, in which the surface of the cornea is protected from external stimulation so that the epithelium again extends naturally to cover the damaged portion. In recent years, with developments in cell biology, factors pertaining to split, movement, fusion, migration, etc., have been clarified, and it has been reported that compounds that can promote corneal epithelial migration play an important role (Ringan, 46, 738-743 (1992); Ganka Shujutsu, 5, 719-727 (1992)).

[0005] On the other hand, many authors have reported research on P2Y receptor agonists which are the effective component in the present invention. For example, U.S. Pat. No. 5,292,498 described use of uridine 5'-triphosphate (UTP), adenosine triphosphate (ATP), etc., in maintaining secretion of mucus as a characteristic feature in treating lung diseases. WO 97/29756 stated that UTP or other phosphoric acid nucleoside P2Y receptor agonist are effective in treating tympanitis. WO 98/34593 stated that UTP or other P2Y receptor agonists have a tear secreting function, and can be used in treating dry eyes and diseases of the nasolacrimal duct. However, no research yet exists on the corneal epithelial migration effect of P2Y receptor agonists.

[0006] Discovery of new applications of said P2Y receptor agonists is of great interest. Also, in ophthalmology, searching for compounds that can display a corneal epithelial migration promoting effect is a very important topic.

DISCLOSURE OF THE INVENTION

[0007] The present inventors have searched for various compounds and performed tests on their pharmacological functions, and have found that P2Y receptor agonists have a corneal epithelial migration promoting effect. As a result, the present invention was reached.

[0008] The present invention provides a type of corneal epithelial migration promoter which contains as its effective component a P2Y receptor agonist; that is, a compound represented by following formula [I] (hereinafter referred to as “these compounds” if not specified otherwise).

[0009] The present invention also provides a corneal epithelial migration promoting method characterized by the fact that in the present invention also pertains to the use of P2Y receptor agonists for manufacturing a corneal epithelial migration promoter.

\[ \text{O} \quad \text{O} \quad \text{OH} \quad \text{OH} \]

where, n represents an integer of 1-4; X represents a hydrogen atom or the following group represented by formula [II]:

\[ \text{O} \quad \text{P} \quad \text{O} \quad \text{R}_1 \quad \text{OH} \quad \text{OH} \]

where, R and R, which may be identical or different from each other, represent a uracil group, thymine group, adenine group, hypoxanthine group, or guanine group.

[0011] In these compounds, the uracil group, thymine group, adenine group, hypoxanthine group, and guanine group are optionally substituted with the following groups: a halogen atom such as fluorine, chlorine, and bromine, methyl group, ethyl group, propyl group, hexyl group, and C₃₋₅ straight-chain or branched lower alkyl groups, methoxy group, ethoxy group, propoxy group, hexyloxy group, and other C₁₋₅ straight-chain or branched lower alkoxy groups, phenyl group, tolyl group, and other aryl groups, phenoxy group, and other aryleoxy groups, benzyl group, phenethyl group, and other aralkyl groups, hydroxy group, etc. Also, amino groups in the adenine group or guanidine group may be protected with generally used protecting groups. Examples of protecting groups include an acetyl group, pivaloyl group, and other C₂₋₅ lower alkanoyl groups, a benzoyl group, and other arylelloyl groups. Examples of preferable groups of R₁ and R₂ include the adenine group and uracil group.

[0012] There is no special limitation on the salts of these compounds, as long as they are tolerated pharmaceutically.
Examples of salts that may be used include salts of sodium, potassium, calcium, and other alkali metals or alkaline earth metals; salts of ammonia or diethylamine, triethanolamine, and other organic amines; salts of hydrochloric acid, sulfuric acid, phosphoric acid, and other inorganic acids; salts of lactic acid, maleic acid, fumaric acid, oxalic acid, methanesulfonic acid, para-toluenesulfonic acid, and other organic acids; etc.

Among these compounds, there are optical isomers and diastereoisomers. These isomers are also included in the present invention. Also, these compounds may be in the form of a hydrate or other solvates.

Examples of compounds having particularly excellent effects include uracil 5'-diphosphoric acid, adenosine 5'-diphosphoric acid, uridine 5'-triphosphoric acid, adenosine 5'-triphosphoric acid, and P', P'-di(uridine-5')tetraphosphoric acid represented by formula (III), or their salts.

Among these compounds, the sodium salt represented by formula (IV) in particular, displays an excellent corneal epithelial migration promoting effect.

As pointed out in the prior art section, repair of a cornea that was damaged for various reasons is closely related to corneal epithelial migration. As proved in the pharmacological tests to be described later, P2Y receptor agonists of the present invention display excellent corneal epithelial migration promoting effects. Consequently, they may be used in treating various corneal diseases. Examples of corneal diseases include corneal ulcers, exfoliation of corneal epithelium, keratitis, etc. Also, since no substantial difference has been observed between corneas and conjunctiva, P2Y receptor agonists can display repair effects not only for corneas, but also for diseases of conjunctiva. In summary, P2Y receptor agonists are useful in treating diseases of corneas and conjunctiva.

Among the several sub-types of P2Y receptors, P2Y$_1$ receptors are particularly good. Typical compounds of P2Y$_1$ receptor agonists are disclosed in U.S. Pat. No. 5,292,498, WO 97/29756, etc.

There is no special limitation on the method of administration of a P2Y receptor agonist in the present invention. However, it is preferred that a P2Y receptor agonist be administered by local administration, in particular, as eye drops.

The concentration of P2Y receptor agonist in eye drops is selected corresponding to symptoms, age, etc., and there is no special limitation on it. Usually, however, the concentration should be in the range of 0.0001% - 15%, or preferably in the range of 0.01% - 10%. The dose of eye drops is in the range of one drop—several drops for each round of administration, and one—several rounds a day. In addition to conventional eye drops, the form of preparation of the eye drops may also be such that the agonist is dissolved just before use. Also, the form of preparation may be an eye ointment.

When the formulation is prepared it is possible to add various additives, as needed, such as sodium chloride, potassium chloride, or other isotonic agents, sodium phosphate, sodium hydrogen phosphate, sodium dihydrogen phosphate, or other buffers, sodium edetate or other stabilizers, benzalconium chloride, sorbic acid, or other preservatives, sodium hydroxide, dilute hydrochloric acid, or other pH adjustors, white Vaseline, liquid paraffin, or other base agents for eye ointments. The formulation is prepared using a conventional method.

In the following, the present invention will be explained in detail with reference to application examples. However, these examples are only to help understand the present invention. They do not limit the range of the present invention.

**OPTIMUM EMBODIMENT OF THE PRESENT INVENTION**

**Pharmacological Tests**

Using cornea specimens collected from male Japanese white rabbits, the corneal epithelial migration length
was used as an index in studying the cornea tissue culturing system according to the method of Nishida, et al. (J. Cell Biol., 97, 1653-1657 (1983)).

Experimental method

Cornea blocks (6 specimens for each group) cut from rabbit cornea pieces were cultured in a culture solution (TCM-199) containing an invention compound at 37.5°C and 5% CO₂ for 24 h. After culturing, the cornea blocks were fixed in an ethanol/glacial acetic acid (95:5 by volume) mixture solution, followed by embedding with paraffin to form slices. After removal of the paraffin, the slices were subjected to hematoxylin-eosin staining, and the length of migration of the corneal epithelial layer was observed using a microscope. As a control, culturing was also performed using a culture solution not containing an invention compound.

Results

Table 1 lists the results of corneal epithelial migration rates under action of the following compounds, with a control set at 100%: P₁, P₁-di(uridine-5')tetraphosphate tetra-sodium [DUTP—Na], uridine 5'-diphosphate di-sodium [UDP—Na], adenosine 5'-diphosphate disodium [ADP—Na], uridine 5'-triphosphate trisodium [UTP—Na], and adenosine 5'-triphosphate trisodium [ATP—Na].

<table>
<thead>
<tr>
<th>Compound (Concentration)</th>
<th>Corneal Epithelial Migration Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DUTP—Na (100 μM)</td>
<td>118.9</td>
</tr>
<tr>
<td>UDP—Na (100 μM)</td>
<td>115.3</td>
</tr>
<tr>
<td>ADP—Na (10 μM)</td>
<td>116.1</td>
</tr>
<tr>
<td>UTP—Na (100 μM)</td>
<td>123.1</td>
</tr>
<tr>
<td>ATP—Na (10 μM)</td>
<td>119.3</td>
</tr>
<tr>
<td>Control</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Example Formulations

Typical formulations were prepared using P₁, P₁-di(uridine-5')tetraphosphate tetra-sodium [DUTP—Na], uridine 5'-triphosphate tri-sodium [UTP—Na], and uridine 5'-diphosphate di-sodium [UDP—Na]. Eye drops were prepared according to the following examples.

EXAMPLE 1

Sterilized purified water: appropriate amount

Sterilized refined water: appropriate amount

By changing the amount of P₁, P₁-di(uridine-5')tetraphosphate tetra-sodium [DUTP—Na], eye drop concentrations of 0.03% (w/v), 0.1% (w/v), 0.3% (w/v), 1.0% (w/v), and 3.0% (w/v), were obtained.

EXAMPLE 3

In 100 g:

DUTP—Na: 0.3 g

Fluidic paraffin: 10.0 g

White Vaseline: appropriate amount

By changing the amount of P₁, P₁-di(uridine-5')tetraphosphate tetra-sodium [DUTP—Na], eye ointment concentrations of 1% (w/v) and 3% (w/v) were obtained.

EXAMPLE 4

In 100 g:

UDP—Na: 0.3 g

Fluidic paraffin: 10.0 g

White Vaseline: appropriate amount

By changing the amount of uridine-5'-diphosphoric acid disodium [UDP—Na], eye ointment concentrations of 1% (w/v) and 5% (w/v) were obtained.

As can be seen in Table 1, in the present invention, P₁, P₁-di(uridine-5')tetraphosphate tetra-sodium [DUTP—Na], uridine 5'-diphosphate disodium [UDP—Na], adenosine 5'-diphosphate disodium [ADP—Na], uridine 5'-triphosphate trisodium [UTP—Na], and adenosine 5'-triphosphate acid trisodium [ATP—Na], all can display significant corneal epithelial migration promoting effects. From such results of pharmacological tests, it is found that formulations containing P₂Y receptor agonists in the present invention as the effective component can display an excellent corneal epithelial migration promoting effect, and can be used in treating diseases of corneas and conjunctiva.

INDUSTRIAL APPLICATION FIELD

P₂Y receptor agonists can display an excellent corneal epithelial migration promoting effect, and can be used in treating diseases of corneas and conjunctiva.

1. A method of promoting epithelial migration in the cornea or conjunctiva, said method comprising:

administering to the eye of a subject an epithelial migration promoter formulation comprising a P₂Y receptor agonist in an amount effective to promote epithelial migration in the cornea or conjunctiva, wherein said P₂Y receptor agonist is a compound of formula I or its pharmacologically tolerated salts:
wherein \( n \) is an integer of 1-4; \( X \) is hydrogen, and \( R_1 \) is a uracil group, thymine group, adenine group, hypoxanthine group, or guanine group.

2. The method according to claim 1, wherein said promoting epithelial migration is to repair epithelial defects.

3. The method according to claim 1, wherein said promoting epithelial migration to repair epithelial defects is such that the epithelium extends to cover a damaged portion of the cornea or conjunctiva.

4. The method according to claim 3, wherein said damaged portion of the cornea or conjunctiva results from corneal disease.

5. The method according to claim 4, wherein said corneal disease is selected from the group consisting of corneal ulcers, exfoliation of corneal epithelium, and keratitis.

6. The method according to claim 1, wherein said epithelial migration promoter formulation contains an effective amount of said P2Y receptor agonist or its pharmacologically tolerated salt together with a pharmacologically tolerated additive.

7. The method according to claim 6, wherein said uracil group, thymine group, adenine group, hypoxanthine group, or guanine group is a substituted uracil, thymine, adenine, hypoxanthine, or guanine group.

8. The method according to claim 7, wherein said substituted uracil, thymine, adenine, hypoxanthine, or guanine group is substituted with a group selected from the group consisting of: halogens, \( \text{C}_1-\text{C}_8 \) straight-chain or branched lower alkyl groups, \( \text{C}_1-\text{C}_8 \) straight-chain or branched lower alkoxy groups, ary groups, aryl groups, aryloxy groups, aralkyl groups, hydroxyl group, amino groups, and protecting groups.

9. The method according to claim 8, wherein said protecting groups are selected from the groups consisting of \( \text{C}_2-\text{C}_8 \) lower alkanoyl groups and arylcarbonyl groups.

10. The method according to claim 1, wherein said pharmacologically tolerated salts are selected from the group consisting of alkali metal salts, alkaline earth metal salts, ammonia salts, organic amine salts, hydrochloric acid salts, sulfuric acid salts, phosphoric acid salts, lactic acid salts, maleic acid salts, fumaric acid salts, oxalic acid salts, methanesulfonic acid salts, and para-toluensulfonic acid salts.

11. The method according to claim 10, wherein said pharmacologically tolerated salts are selected from the group consisting of sodium, potassium, calcium, ammonia, diethylamine, triethanolamine, hydrochloric acid, sulfuric acid, phosphoric acid, lactic acid, maleic acid, fumaric acid, oxalic acid, methanesulfonic acid, and para-toluensulfonic acid salts.

12. The method according to claim 1, wherein said amount of the P2Y receptor agonist effective to promote epithelial migration in the cornea or conjunctiva is in the range of 0.0001% to 15%.

13. The method according to claim 1, wherein said epithelial migration promoter formulation is in the form selected from the group consisting of eye drops and eye ointments.

14. The method according to claim 1, wherein said formulation comprises at least one additive selected from the group consisting of: sodium chloride, potassium chloride, sodium phosphate, sodium hydrogen phosphate, sodium diohydrogen phosphate, sodium edetate, benzalconium chloride, sorbic acid, sodium hydroxide, dilute hydrochloric acid, white Vaseline, and fluidic paraffin.

15. The method according to claim 14, wherein said additive is selected from the group consisting of: sodium chloride, potassium chloride, sodium phosphate, sodium hydrogen phosphate, sodium diohydrogen phosphate, sodium edetate, benzalconium chloride, sorbic acid, sodium hydroxide, dilute hydrochloric acid, white Vaseline, and fluidic paraffin.

16. The method according to claim 1, wherein said P2Y receptor agonist uridine 5'-diphosphoric acid, adenosine 5'-diphosphoric acid, uridine 5'-triphosphoric acid, adenosine 5'-triphosphoric acid, or the salts thereof.

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