DESCRIPTION

TITLE OF INVENTION

Corneal Epithelial Cell Death Inhibitor Containing Flavin Adenine Dinucleotide or Salt Thereof as Active Ingredient

TECHNICAL FIELD

The present invention relates to a corneal epithelial cell death inhibitor comprising flavin adenine dinucleotide (FAD) or a salt thereof (hereinafter, these are collectively called "FAD or the like") as an active ingredient, the corneal epithelial cell death being induced by ultraviolet radiation. The present invention also provides a method for inhibiting corneal epithelial cell death including administering a pharmaceutically effective amount of FAD or the like to a patient, the corneal epithelial cell death being induced by ultraviolet radiation. The present invention also relates to use of FAD or the like for inhibiting corneal epithelial cell death, the corneal epithelial cell death being induced by ultraviolet radiation. Further, the present invention also relates to use of FAD or the like for manufacturing a corneal epithelial cell death inhibitor, the corneal epithelial cell death being induced by ultraviolet radiation.

BACKGROUND ART

Cornea is a tissue that is in direct contact with the external world, and is usually exposed to various stresses. In ultraviolet light which is a sort of the external stresses, three kinds including ultraviolet A wave (wavelength: greater than or equal to 320 nm and less than 400 nm), ultraviolet B wave (wavelength: greater than or equal to 290 nm and less than 320 nm), and ultraviolet C wave (wavelength: greater than or equal to 200 nm and less than 290 nm) are known. Folia ophthalmologica Japonica, 57, 734-738 (2006) (Non-Patent Document 1) describes that ultraviolet B wave (hereinafter, also referred to as "UV-B") and ultraviolet C wave (hereinafter, also referred to as "UV-C") are absorbed in corneal epithelium, and that UV-B will be clinically problematic while UV-C will not be problematic because it is absorbed in the ozone layer.

Further, Exp. Eye Res., 90 (2), 216-22 (2010) (Non-Patent Document 2)

discloses that apoptosis (cell death) of corneal epithelial cells is induced by UV-B, and actually, Non-Patent Document 1 discloses that UV-B induces damage to corneal epithelium, corneal edema or opacity and so on.

On the other hand, FAD is coenzyme-form vitamin B_2 that is directly involved in redox of cells, and is known to promote enzymatic respiration metabolism of cornea. Further, the package insert of Flavitan (registered trade name) ophthalmic solution 0.05% (Non-Patent Document 3) describes that a disodium salt of FAD is useful for keratitis in which involvement of vitamin B_2 deficiency or metabolic abnormality is supposed.

However, the influence of FAD on corneal epithelial cell death induced by ultraviolet rays has not been clarified at all, and instead, International Patent Publication No. 99/43347 (Patent Document 1) exemplifies Flavitan (registered trade name) ophthalmic solution containing a disodium salt of FAD as an ophthalmic agent that induces a corneal epithelium disorder.

CITATION LIST

PATENT DOCUMENT

PTD 1: International Patent Publication No. 99/43347

NON PATENT DOCUMENT

NPD 1: Folia ophthalmologica Japonica, 57, 734-738 (2006)

NPD 2: Exp. Eye Res., 90 (2), 216-22 (2010)

NPD 3: Package insert of Flavitan (registered trade name) ophthalmic solution 0.05%

SUMMARY OF INVENTION

TECHNICAL PROBLEM

As described above, it is an interesting issue to search for drugs that inhibit corneal epithelial cell death induced by ultraviolet radiation.

SOLUTION TO PROBLEM

As a result of diligent studies for searching for drugs that inhibit corneal epithelial cell death induced by ultraviolet radiation, the present inventors found that flavin adenine dinucleotide disodium salt (hereinafter, also referred to as the "present

compound") has a significant cell death inhibitory effect, and accomplished the present invention.

That is, the present invention is a corneal epithelial cell death inhibitor comprising flavin adenine dinucleotide or a salt thereof (FAD or the like) as an active ingredient, the corneal epithelial cell death being induced by ultraviolet radiation (hereinafter, also referred to as the "present agent").

Another aspect of the present invention is a corneal epithelial cell death inhibitor comprising FAD or the like as an active ingredient, the corneal epithelial cell death being induced by ultraviolet B wave radiation.

Another aspect of the present invention is the present agent, wherein the concentration of the FAD or the like is 0.01 to 1% (w/v).

Another aspect of the present invention is the present agent, wherein the concentration of the FAD or the like is 0.05% (w/v).

Another aspect of the present invention is the present agent, wherein an administration route is ophthalmic instillation.

Another aspect of the present invention is the present agent, wherein a dosage form is an ophthalmic solution.

The present invention also provides a method for inhibiting corneal epithelial cell death comprising administering a pharmaceutically effective amount of FAD or the like to a patient, the corneal epithelial cell death being induced by ultraviolet radiation (hereinafter, also referred to as the "present method").

Another aspect of the present invention is a method for inhibiting corneal epithelial cell death comprising administering a pharmaceutically effective amount of FAD or the like to a patient, the corneal epithelial cell death being induced by ultraviolet B wave radiation.

Another aspect of the present invention is the present method, wherein the concentration of the FAD or the like is 0.01 to 1% (w/v).

Another aspect of the present invention is the present method, wherein the concentration of the FAD or the like is 0.05% (w/v).

Another aspect of the present invention is the present method, wherein an administration route is ophthalmic instillation.

Another aspect of the present invention is the present method, wherein a dosage form is an ophthalmic solution.

The present invention also provides use of FAD or the like for inhibiting corneal epithelial cell death, the corneal epithelial cell death being induced by ultraviolet radiation (hereinafter, also referred to as the "first present use").

Another aspect of the present invention is use of FAD or the like for inhibiting corneal epithelial cell death, the corneal epithelial cell death being induced by ultraviolet B wave radiation.

Another aspect of the present invention is the first present use, wherein the concentration of the FAD or the like is 0.01 to 1% (w/v).

Another aspect of the present invention is the first present use, wherein the concentration of the FAD or the like is 0.05% (w/v).

Another aspect of the present invention is the first present use, wherein an administration route is ophthalmic instillation.

Another aspect of the present invention is the first present use, wherein a dosage form is an ophthalmic solution.

The present invention also provides use of FAD or the like for manufacturing a corneal epithelial cell death inhibitor, the corneal epithelial cell death being induced by ultraviolet radiation (hereinafter, also referred to as the "second present use").

Another aspect of the present invention is use of FAD or the like for manufacturing a corneal epithelial cell death inhibitor, the corneal epithelial cell death being induced by ultraviolet B wave radiation.

Another aspect of the present invention is the second present use, wherein the concentration of the FAD or the like is 0.01 to 1% (w/v).

Another aspect of the present invention is the second present use, wherein the concentration of the FAD or the like is 0.05% (w/v).

Another aspect of the present invention is the second present use, wherein an

administration route is eye ophthalmic instillation.

Another aspect of the present invention is the second present use, wherein a dosage form is an ophthalmic formulation.

ADVANTAGEOUS EFFECTS OF INVENTION

As will be described later, FAD or the like including the present compound can be an inhibitor for corneal epithelial cell death induced by ultraviolet radiation because it significantly inhibits the decline of the viable cell count of corneal epithelial cells induced by ultraviolet radiation. The present invention also provides a method for inhibiting corneal epithelial cell death comprising administering a pharmaceutically effective amount of FAD or the like to a patient, the corneal epithelial cell death being induced by ultraviolet radiation. The present invention also provides use of FAD or the like for inhibiting corneal epithelial cell death, the corneal epithelial cell death being induced by ultraviolet radiation. The present invention also provides use of FAD or the like for manufacturing a corneal epithelial cell death inhibitor, the corneal epithelial cell death being induced by ultraviolet radiation.

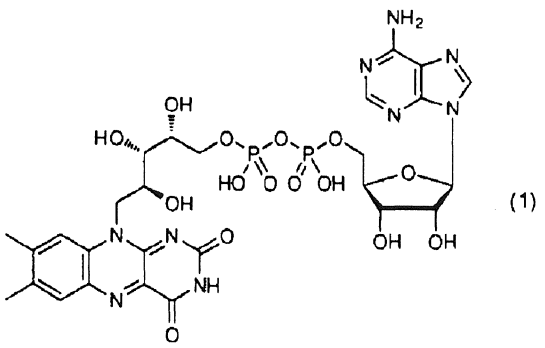
BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 is a graph showing the viability when corneal epithelial cells are treated with the present compound before UV-B radiation.

DESCRIPTION OF EMBODIMENTS

Flavin adenine dinucleotide is a compound represented by the following formula (1).

[Chemical formula 1]

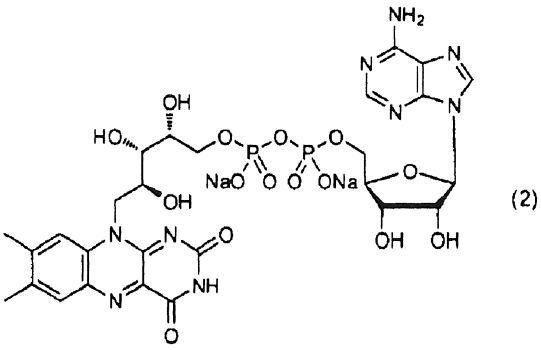


(1)

A salt of flavin adenine dinucleotide is not particularly limited as far as it is a pharmaceutically acceptable salt, and examples include salts with an inorganic acid such as hydrochloric acid, hydrobromic acid, hydroiodic acid, nitric acid, sulfuric acid, or phosphoric acid; salts with an organic acid such as acetic acid, fumaric acid, maleic acid, succinic acid, citric acid, tartaric acid, adipic acid, gluconic acid, glucoheptonic acid, glucuronic acid, terephthalic acid, methanesulfonic acid, lactic acid, hippuric acid, 1,2-ethanedisulfonic acid, isethionic acid, lactobionic acid, oleic acid, pamoic acid, polygalacturonic acid, stearic acid, tannic acid, trifluoromethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, lauryl sulfate ester, methyl sulfate, naphthalenesulfonic acid, or sulfosalicylic acid; quaternary ammonium salts with methyl bromide, methyl iodide or the like; salts with a halogen ion such as a bromine ion, a chlorine ion or an iodine ion; salts with an alkali metal such as lithium, sodium or potassium; salts with an alkaline earth metal such as calcium or magnesium; metal salts with iron, zinc or the like; salts with ammonia; and salts with an organic amine such as triethylenediamine, 2-aminoethanol, 2,2-iminobis(ethanol), 1-deoxy-1-(methylamino)-2-D-sorbitol, 2-amino-2-(hydroxymethyl)-1,3-propanediol, procaine, or N,N-bis(phenylmethyl)-1,2-ethanediamine.

As a salt of flavin adenine dinucleotide, a sodium salt is preferred, and flavin adenine dinucleotide disodium salt represented by the following formula (2) is particularly preferred.

[Chemical formula 2]



(2)

Flavin adenine dinucleotide or a salt thereof (FAD or the like) may be in the form of a hydrate or a solvate.

When flavin adenine dinucleotide has geometric isomers or optical isomers, these isomers and salts thereof are also embraced in the scope of the present invention. When flavin adenine dinucleotide has proton tautomers, the tautomers or salts thereof are also embraced in the scope of the present invention.

When flavin adenine dinucleotide or a salt thereof (including the case where it is in the form of a hydrate or a solvate) has polymorphs and a polymorphic group (polymorphic system), the polymorphs and the polymorphic group (polymorphic system) are also embraced in the scope of the present invention. Here, the polymorphic group (polymorphic system) means individual crystalline forms in each step and the entire process in the case where the crystalline form changes with the condition and the state of production, crystallization, storage and the like of the crystals (the state includes the formulated state).

Flavin adenine dinucleotide or a salt thereof may be produced according to an ordinary method in the field of organic synthetic chemistry. Also, a product commercially available from, for example, Sigma may be used.

In the present invention, "inhibition of corneal epithelial cell death induced by ultraviolet radiation" means not only inhibiting the decline of the viable cell count of corneal epithelial cells induced by ultraviolet radiation, but also means preventing or treating damage to corneal epithelium, corneal edema or opacity and so on associated with the decline of the viable cell count (namely, corneal epithelial cell death).

In the present invention, "ultraviolet rays" means the following three kinds: ultraviolet A wave (wavelength: greater than or equal to 320 nm and less than 400 nm), ultraviolet B wave (wavelength: greater than or equal to 290 nm and less than 320 nm), and ultraviolet C wave (wavelength: greater than or equal to 200 nm and less than 290 nm). As described in the section of background art, it is known that the "ultraviolet B wave (UV-B)" is clinically problematic as it induces corneal epithelial cell death.

The present agent may be formulated into a combination ophthalmic agent

using a generally used technique by mixing other active ingredients and a pharmaceutically acceptable additive with FAD or the like, or may be formulated into a single ophthalmic agent by using a generally used technique by adding a pharmaceutically acceptable additive to FAD or the like. In Japan, an ophthalmic solution such as "Flavitan (registered trade name) ophthalmic solution 0.05%" containing the present compound at a concentration of 0.05% (w/v) as an active ingredient is commercially available.

In the present invention, the present agent is locally administered to an eye. Examples of administration route of the present agent include ocular instillation (including instillation of an eye ointment), subconjunctival administration, intra-conjunctival sac administration, and subtenon administration, with the ocular instillation being particularly preferred.

The dosage form of the present agent is not particularly limited as far as the agent is usable for local administration to an eye, and examples include an ophthalmic solution, an ophthalmic ointment, an injection solution, a patch, a gel, and an intercalating agent, with the ophthalmic solution being preferred. These can be prepared by ordinary techniques that are generally used in the field of art. Besides these preparations, the present therapeutic agent may be formulated into a preparation for intraocular implant or a preparation designed to have DDS (drug delivery system) such as a microsphere.

An ophthalmic solution can be prepared by selectively using a tonicity agent such as sodium chloride, potassium chloride, or concentrated glycerin; a buffer such as sodium phosphate, sodium acetate, or epsilon-aminocaproic acid; a surfactant such as polyoxyethylene sorbitan monooleate, polyoxyl 40 stearate, or polyoxyethylene hydrogenated castor oil; a stabilizer such as sodium citrate or sodium edetate; and a preservative such as benzalkonium chloride or paraben as is necessary. The pH of an ophthalmic solution may be within the range that is pharmaceutically acceptable for an ophthalmic preparation, and is generally preferably within the range of 4 to 8.

An ophthalmic ointment can be prepared by using a generally used vehicle such

as white vaseline or liquid paraffin.

An injection solution can be prepared by selectively using a tonicity agent such as sodium chloride; a buffer such as sodium phosphate; a surfactant such as polyoxyethylene sorbitan monooleate; and a thickener such as methylcellulose as is necessary.

An intercalating agent can be prepared by grinding and mixing a biodegradable polymer, for example, a biodegradable polymer of hydroxypropyl cellulose, hydroxypropylmethyl cellulose, carboxyvinyl polymer, polyacrylic acid or the like with the present compound, and compression molding the resultant powder. An excipient, a binder, a stabilizer, a pH adjuster and the like may be used as is necessary.

A preparation for intraocular implant can be prepared by using a biodegradable polymer, for example, a biodegradable polymer of polylactic acid, polyglycolic acid, a lactic acid-glycolic acid copolymer, hydroxypropyl cellulose or the like.

A dose of the present agent can be varied appropriately depending on the dosage form, severity of symptom, age and body weight of the patient to which the agent is to be administered, decision by a physician and so on, and for example, when an ophthalmic solution is selected, one or two drops of the present agent comprising FAD or the like at a concentration of 0.005 to 5% (w/v), preferably 0.01 to 1% (w/v), more preferably 0.02 to 0.5% (w/v), and further preferably 0.05% (w/v) are instilled into an eye per one instillation.

The concentrations of the FAD or the like can be the concentration of flavin adenine dinucleotide (free form), and can be the concentration of a salt of flavin adenine dinucleotide.

Dosage regimen of the present agent can be varied appropriately depending on the dosage form, severity of symptom, age and body weight of the patient to which the agent is to be administered, decision by a physician and so on, and for example, when an ophthalmic solution is selected as a dosage form, it can be locally administered to an eye in 1 to 10 divided doses daily, preferably in 2 to 8 divided doses daily, and more preferably in 3 to 6 divided doses daily.

Further, the present invention provides a method for inhibiting corneal epithelial cell death comprising administering a pharmaceutically effective amount of FAD or the like to a patient, the corneal epithelial cell death being induced by ultraviolet radiation (present method), use of FAD or the like for inhibiting corneal epithelial cell death, the corneal epithelial cell death being induced by ultraviolet radiation (first present use), and use of FAD or the like for manufacturing a corneal epithelial cell death inhibitor, the corneal epithelial cell death being induced by ultraviolet radiation (second present use). Definitions of terms in these aspects of the present invention are also as described above, and preferred embodiments thereof are also the same as those described above.

In the following, results of pharmacological tests and formulation examples will be shown, which are given for better understanding of the present invention but not for limiting the scope of present invention.

EXAMPLES

[Pharmacological test]

Whether or not the decline of the viable cell count by ultraviolet radiation is inhibited when corneal epithelial cells are treated with FAD before ultraviolet (UV-B) radiation was evaluated.

(Test method)

SV40 immortalized human corneal epithelial cells (HCE-T: RIKEN Bio Resource Center, Japan, Cell No.: RCB2280) were inoculated in a 96-well plate (1×10^4 cells/well), and cultured in a 10% FBS-containing DMEM/F-12 culture medium for a day. On the next day, the 10% FBS-containing DMEM/F-12 culture medium was replaced by PBS containing 0.0125% (w/v) or 0.05% (w/v) flavin adenine dinucleotide disodium salt, PBS containing 0.5% (w/v) chondroitin sulfate sodium, or PBS, and then the cells were cultured at 37°C for 60 minutes (hereinafter, referred to as "0.0125% FAD group", "0.05% FAD group", "0.5% CS group" or "base group", respectively). Then, corneal epithelial cells were irradiated with UV-B (80 mJ/cm^2) for about 1 minute. After replacing the culture medium of each group again by a 10% FBS-

containing DMEM/F-12 culture medium, the cells were cultured at 37°C for 24 hours, and then the viable cell count was determined by using Cell Titer 96 (registered trade name) AQueous One Solution Cell Proliferation Assay (product of Promega, Catalogue No.: G3580), and the viability was calculated according to the following expression 1. The flavin adenine dinucleotide disodium salt and chondroitin sulfate sodium used in this test were purchased from KYOWA HAKKO BIO CO., LTD. and Maruha Nichiro Foods, Inc., respectively.

[Expression 1]

Viability (%) = (Viable cell count of each group/Viable cell count of UV-B non-irradiation group) × 100

(Results)

The test results are shown in Fig. 1. In Fig.1, each value represents an average value ± standard deviation (N = 3).

(Discussion)

As is apparent from Fig. 1, when corneal epithelial cells were pre-treated with the present compound before UV-B radiation, a concentration-dependent corneal epithelial cell death inhibitory action was observed. On the other hand, in the group treated with chondroitin sulfate sodium (see the package insert of Visine (registered trade name) UV) that is known to protect the corneal surface exposed to ultraviolet rays, the action was not observed. In other words, it was revealed that FAD inhibits corneal epithelial cell death induced by ultraviolet radiation.

[Formulation examples]

The drug of the present invention will be described more concretely by way of formulation examples, but it is to be noted that the present invention will not be limited to these formulation examples.

(Formulation example 1)

Ophthalmic solution (FAD concentration: 0.05% (w/v)), in 100 ml:

Flavin adenine dinucleotide disodium salt	0.05g
Sodium chloride	0.9g

Sodium hydrogen phosphate hydrate	quantum sufficit
Sterilized purified water	quantum sufficit

To sterilized purified water, flavin adenine dinucleotide disodium salt and other ingredients described above are added, and they are sufficiently mixed to prepare an ophthalmic solution. By varying the adding amount of flavin adenine dinucleotide disodium salt, it is possible to prepare an ophthalmic solution wherein the concentration of FAD is 0.005% (w/v), 0.01% (w/v) or 0.1% (w/v).

(Formulation example 2)

Ophthalmic ointment (FAD concentration: 0.05% (w/w)), in 100 g:

Flavin adenine dinucleotide disodium salt	0.05 g
Liquid paraffin	10 mg
White vaseline	quantum sufficit

To white vaseline and liquid paraffin that are uniformly melted, flavin adenine dinucleotide disodium salt is added, and these are sufficiently mixed, and then gradually cooled to prepare an ophthalmic ointment. By varying the adding amount of flavin adenine dinucleotide disodium salt, it is possible to prepare an ophthalmic ointment wherein the concentration of FAD is 0.005% (w/w), 0.01% (w/w) or 0.1% (w/w).

INDUSTRIAL APPLICABILITY

Flavin adenine dinucleotide or a salt thereof is expected to be an inhibitor for corneal epithelial cell death induced by ultraviolet radiation because it significantly inhibits the decline of the viable cell count of corneal epithelial cells induced by ultraviolet radiation.

CLAIMS

1. A corneal epithelial cell death inhibitor comprising flavin adenine dinucleotide or a salt thereof as an active ingredient, the corneal epithelial cell death being induced by ultraviolet radiation.

2. The inhibitor according to claim 1, wherein the ultraviolet is ultraviolet B wave.

3. The inhibitor according to claim 1 or 2, wherein the concentration of the flavin adenine dinucleotide or a salt thereof is 0.01 to 1% (w/v).

4. The inhibitor according to claim 1 or 2, wherein the concentration of the flavin adenine dinucleotide or a salt thereof is 0.05% (w/v).

5. The inhibitor according to claim 1 or 2, wherein an administration route is ocular instillation.

6. The inhibitor according to claim 5, wherein a dosage form is an ophthalmic solution.

7. A method for inhibiting corneal epithelial cell death comprising administering a pharmaceutically effective amount of flavin adenine dinucleotide or a salt thereof to a patient, the corneal epithelial cell death being induced by ultraviolet radiation.

8. The method according to claim 7, wherein the ultraviolet is ultraviolet B wave.

9. The method according to claim 7 or 8, wherein the concentration of the flavin adenine dinucleotide or a salt thereof is 0.01 to 1% (w/v).

10. The method according to claim 7 or 8, wherein the concentration of the flavin adenine dinucleotide or a salt thereof is 0.05% (w/v).

11. The method according to claim 7 or 8, wherein an administration route is ocular instillation.

12. The method according to claim 11, wherein a dosage form is an ophthalmic solution.

13. Use of flavin adenine dinucleotide or a salt thereof for inhibiting corneal epithelial cell death, the corneal epithelial cell death being induced by ultraviolet radiation.

14. The use according to claim 13, wherein the ultraviolet is ultraviolet B wave.

15. The use according to claim 13 or 14, wherein the concentration of the flavin adenine dinucleotide or a salt thereof is 0.01 to 1% (w/v).

16. The use according to claim 13 or 14, wherein the concentration of the flavin adenine dinucleotide or a salt thereof is 0.05% (w/v).

17. The use according to claim 13 or 14, wherein an administration route is ocular instillation.

18. The use according to claim 17, wherein a dosage form is an ophthalmic

solution.

19. Use of flavin adenine dinucleotide or a salt thereof for manufacturing a corneal epithelial cell death inhibitor, the corneal epithelial cell death being induced by ultraviolet radiation.

20. The use according to claim 19, wherein the ultraviolet is ultraviolet B wave.

21. The use according to claim 19 or 20, wherein the concentration of flavin adenine dinucleotide or a salt thereof is 0.01 to 1% (w/v).

22. The use according to claim 19 or 20, wherein the concentration of flavin adenine dinucleotide or a salt thereof is 0.05% (w/v).

23. The use according to claim 19 or 20, wherein an administration route is ocular instillation.

24. The use according to claim 23, wherein a dosage form is an ophthalmic solution.