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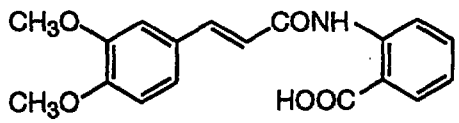
DESCRIPTION

AGENTS INHIBITING PROGRESS OF PTERYGIUM AND POSTOPERATIVE RECURRENCE OF THE SAME

Technical Field

The present invention relates to a pharmaceutical composition being useful as an agent inhibiting progress of pterygium and postoperative recurrence of the same.

More particularly, the present invention relates to an agent inhibiting progress of pterygium and postoperative recurrence of the same, which comprises as the active ingredient N-(3,4-dimethoxycinnamoyl)anthranilic acid (generic name: tranilast) represented by the formula:



or a pharmaceutically acceptable salt thereof.

Background Art

Pterygium is an ocular disease which is characterized by the encroachment of a fleshly and congestively triangular portion of the bulbar conjunctiva onto the cornea and caused by external stimuli such as ultraviolet light, dry atmosphere, heat, cold and dust. Especially, pterygium has been largely observed on the cornea at the nasal side along the palpebral fissure. Pterygium

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progresses gradually toward the top of the cornea from the conjunctiva and reaches pupillary area to cause visual loss. However, at the present time, there is no therapeutic agent inhibiting and being surely effective against progress of pterygium. Therefore, development of an effective therapeutic agent has been desired. Accordingly, in the field of the pterygium treatment, surgical operations such as erasion of pterygium tissues have been solely performed.

In the pterygium surgery, high incidence of recurrence of pterygium, about 30-50 %, has been pointed out as a serious problem. Therefore, in the field of the pterygium medication, earnest study has been actively promoted in order to find substances inhibiting recurrence of pterygium. Up to this time, for example, it was confirmed that anti-tumor agents such as mitomycin were effective. However, theses drugs have been concerned about serious side effects such as scleromalacia [Connective Tissue, Vol.26, pp135-137 (1994) etc.] and are not enough to use clinically. In addition, it has been reported that beta-ray radiation using strontium 90 was effective in inhibiting postoperative recurrence of pterygium. However, injuries such as cataract has been pointed out [Ganka, Vol.24, pp917-923 (1982) etc.].

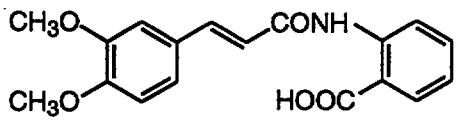
On the other hand, tranilast has been widely used as drugs for the treatment of allergic disorders such as bronchial asthma, allergic rhinitis, atopic dermatitis and allergic conjunctivitis, and cutaneous disorders such as keloid and hypertrophic scar. For example, it has been known that tranilast has inhibitory

activities on chemical mediator release caused by an allergic reaction, excessive collagen accumulation by fibroblast cells in cutaneous tissues and excessive proliferation of smooth muscle cells in coronary artery vessels.

However, it is disclosed in no way that tranilast has an inhibitory activity on progress of pterygium and postoperative recurrence of the same and it is not known at all that tranilast is useful as an agent inhibiting progress of pterygium and postoperative recurrence of the same.

Disclosure of Invention

The present invention relates to an agent inhibiting progress of pterygium and postoperative recurrence of the same, which comprises as the active ingredient N-(3,4-dimethoxycinnamoyl)anthranilic acid represented by the formula:



or a pharmaceutically acceptable salt thereof.

The present inventors have been earnestly studied to find compounds having an inhibitory activity on proliferation of pterygium tissues. As a result, it was found that tranilast has a marked inhibitory effect on proliferation of pterygium tissues and is extremely useful as an agent inhibiting progress of pterygium and postoperative recurrence of the same, thereby forming the basis of the present invention.

Accordingly, the present inventors confirmed that tranilast significantly suppressed proliferation of pterygium tissues in the *in vitro* test for inhibition of cell proliferation using rat pterygium tissue-derived cells.

As a consequence, tranilast has an excellent inhibitory effect on proliferation of pterygium tissue-derived cells and is an extremely useful compound as an agent inhibiting progress of pterygium and postoperative recurrence of the same.

Therefore, pharmaceutical compositions which are useful as agents inhibiting progress of pterygium and postoperative recurrence of the same can be prepared by comprising as the active ingredient tranilast or a pharmaceutically acceptable salt thereof.

Various methods for the preparation of tranilast and salts thereof which are active ingredients are known, and these compounds can be readily prepared according to methods described in literatures and the like (Japanese Patent Application Publication (*kokoku*) No.Sho.56-40710; ibid. No.Sho.57-36905; ibid. No.Sho. 58-17186; ibid. No.Sho.58-48545; ibid. No.Sho.58-55138; ibid. No.Sho.58-55139; ibid. No.Hei.01-28013; ibid. No.Hei.01-50219; ibid. No.Hei.03-37539 etc.).

As examples of pharmaceutically acceptable salts of tranilast, salts with inorganic bases such as a sodium salt and a potassium salt, salts formed with organic amines such as morpholine, piperazine, piperidine and pyrrolidine and salts formed with amino acids can be illustrated.

When the pharmaceutical compositions of the present invention are employed in the practical treatment, they may be administered orally, preferably, topically as eyedrops, eye ointments and the like.

For example, eyedrops can be formulated by dissolving tranilast or a pharmaceutically acceptable salt thereof together with a basic substance with heating in a proper quantity of sterilized water in which a surface active agent is dissolved, adding polyvinylpyrrolidone, and optionally adding appropriate pharmaceutical additives such as a preservative, a stabilizing agent, a buffer, an isotonicity, an antioxidant and a viscosity improver to dissolve completely.

Eye ointments can be appropriately formulated by using bases which are generally used in eye ointments. Eye ointments can be also used as reversible thermally gelling water-base pharmaceutical compositions.

When the pharmaceutical compositions of the present invention are employed in the practical treatment, the dosage of tranilast or a pharmaceutically acceptable salt thereof as the active ingredient is appropriately decided depending on the age, degree of symptoms of each patient to be treated, therapeutic value and the like. The dosage should be fixed at an appropriate concentration to be curable. For example, in case of eyedrops, preferably 0.001-2 weight % eyedrops are instillated 1 to several times per day and applied 1 to several droplets per time.

Brief Description of Drawings

Figure 1 is a graph illustrating an inhibitory activity on proliferation of human pterygium tissue-derived cells by tranilast. The axis of the ordinates shows number of human pterygium tissue-derived cells (x 10^4 cells), and the axis of the abscissas shows tranilast concentrations (μg/ml). The symbol * in the graph shows the significantly difference at $p<0.01$.

Best Mode for Carrying Out the Invention

The present invention is further illustrated in more detail by way of the following Example.

Example

Inhibitory activity on proliferation of pterygium tissue-derived cells

①Culture of pterygium tissue-derived cells

Human pterygium tissues were cut into narrow strips. The strips were adhered to a culture plate and subcultured in Dulbecco's modified Eagle's Medium (DMEM) containing 10 % fetal calf serum (FBS) at 37 °C in an atmosphere of 5 % CO_2 in air. At the point that cells were migrated and proliferated from the tissues, the medium was aspirated and cells were washed with phosphate-buffered saline (PBS(-)) gently. Then, the PBS(-) was aspirated, an aliquot of 0.25 % trypsin solution containing 0.02 % EDTA was added to the culture plate, and the morphology of cells was observed under a phase-contrast microscopy. When cells were

going to be round, an equal value of DMEM containing 10 % FBS was added to the trypsin solution to stop the action of trypsin. Attached cells were harvested from the plate by pipetting the medium using a slender pasteur pipette. Cell suspension was transferred into spit, then medium was added to the spit, and the cell suspension was mixed about 20 times vigorously by pipetting with a pasteur pipette and centrifuged at 100-110xg for 1 minute. After the supernatant was discarded, fresh medium was added to the precipitate, and pterygium-derived cell suspension was prepared by pipetting using a pasteur pipette. The suspension was further subcultured in DMEM containing 10 % FBS to use for experiment.

② Preparation of test drugs

Tranilast was added to 1 % aqueous sodium bicarbonate solution at a final concentration of 1.0 % and dissolved by warming at 70 °C. The solution was sterilized with millipore filter and diluted with DMEM containing 10 % FBS to a final prescribed concentration.

③ Experimental method

DMEM medium (2 ml) containing cells (2 x 10^4 cells) and 10 % FBS was added to each well using a culture plate (6-well), and cultured. After 1 day, the medium was aspirated, DMEM medium (2 ml) containing various concentration of tranilast and 10 % FBS was added to the plate, and cultured at 37 °C in an atmosphere

of 5 % CO_2 in air. After 2 days, the medium was aspirated, the cells were washed with PBS(-), and 1 ml of 0.25 % trypsin solution containing 0.02 % EDTA was added to the plate. After harvesting cells from the plate by pipetting using a pasteur pipette, number of viable cells was counted under a phase-contrast microscopy using a hemocytometer.

④ Assessment of effect

Mean and standard error value of each group were calculated. Statistical analysis of significance was performed by a one-way analysis of variance and statistical significance was confirmed. Thereafter, analysis of significance between groups was performed by Dunnett's multiple test.

⑤ Results

As shown in Figure 1, tranilast significantly suppressed the proliferation of pterygium tissue-derived cells in a concentration-dependent manner.

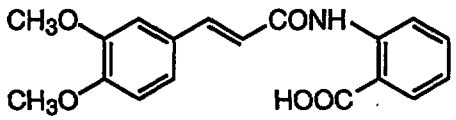
Industrial Applicability

A pharmaceutical composition of the present invention comprising as the active ingredient tranilast has a marked inhibitory activity on proliferation of pterygium tissues and is suitable as an agent inhibiting progress of pterygium and postoperative recurrence of the same.

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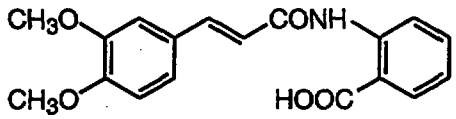
CLAIMS

1. An agent inhibiting progress of pterygium and postoperative recurrence of the same, which comprises as the active ingredient N-(3,4-dimethoxycinnamoyl)anthranilic acid represented by the formula:



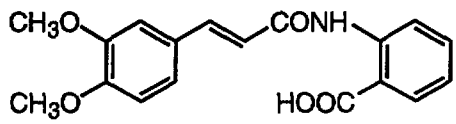
or a pharmaceutically acceptable salt thereof.

2. A use of N-(3,4-dimethoxycinnamoyl)anthranilic acid represented by the formula:



or a pharmaceutically acceptable salt thereof as an agent inhibiting progress of pterygium or postoperative recurrence of the same.

3. A use of N-(3,4-dimethoxycinnamoyl)anthranilic acid represented by the formula:

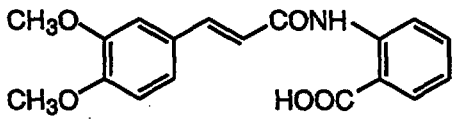


or a pharmaceutically acceptable salt thereof for the manufacture

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of a pharmaceutical composition for the prevention and treatment of pterygium and postoperative recurrence of the same.

4. A method for the prevention and treatment of pterygium or postoperative recurrence of the same, which comprises administering N-(3,4-dimethoxycinnamoyl)anthranilic acid represented by the formula:



or a pharmaceutically acceptable salt thereof.