and the supernatant was collected after 24 hours. The following western blot analysis and gelatin zymography analysis were used on the collected culture solution.

[0078]

(Western blot analysis)

After developing the supernatant of the collected culture solution in SDS-PAGE using 10% polyacrylamide gel, the separated proteins were transferred onto a nitrocellulose filter. Then, non-specific sites on the nitrocellulose filter were blocked and incubated for 24 hours at 4°C with anti-human MMP-1 antibodies (R&D Systems) and anti-rabbit MMP-3 antibodies (Daiichi Fine Chemical Co., Ltd). ECL® reagent (GE Healthcare) was used for detection.

[0079]

The results are shown in Figure 4A. The band for each of the non-active Pro-MMP-1, Pro-MMP-3 and active MMP-1, MMP-3 was diminished, where the extent thereof was dependent on the concentration of RARγ agonist A. Thus, it was revealed that RARγ agonist A concentration-dependently suppresses the expression and activation of MMP-1 and MMP-3.

[0080]

(Gelatin zymography analysis)

After developing the supernatant of the collected culture solution on SDS-PAGE using 10% polyacrylamide gel containing 0.1% of gelatin, it was incubated for one hour at room temperature with a TBS solution containing 2.5% Triton X-100. The gel after incubation was dyed with a solution of Coomassie Brilliant Blue (Wako Pure Chemical Industries, Ltd.) and decolored with a 5% methanol-7.5% acetic acid solution (Nacalai Tesque, Inc).

[0081]

The results are shown in Figure 4B. The band for each of the non-active Pro-MMP-2, Pro-MMP-9 and active MMP-2, MMP-9 was diminished, where the extent thereof was dependent on the concentration of RARγ agonist A. Thus, it was revealed that RARγ agonist A concentration-dependently suppresses the expression and activation of MMP-2 and MMP-9.

Example 5

[0082]

(Test Example) Test on suppression of corneal opacity and ulceration with RARγ agonist A

Systemic anesthesia was applied to male Japanese white rabbits (body weight 2.5-3.5 kg, 27 rabbits) by intramuscular administration of a solution of a mixture of ketamine and xylazine. Then, topical anesthesia was applied by a 0.4% oxybuprocaine hydrochloride instillation. Furthermore, 30 µl of 1% LPS (Sigma Aldrich) was injected into the corneal stroma of one eye, but not in the other eye.

[0083]

50 µl of 0.1% PBS/0.1% polysorbate 80 solution containing RARγ agonist A (R667; 0.1%, 24 mM) was instillatively administered to the rabbits injected with LPS twice after the injection on the day of LPS injection and four times daily thereafter until day 10 by using a micropipette . As a control, a 0.1% PBS/0.1% polysorbate 80 solution free of RARγ agonist A (Vehicle) was similarly administered. Figure 5 shows representative examples of a control and a case where RARγ agonist A was administered.

[0084]

In Figure 5, corneal opacity and ulceration were observed in the control (Vehicle) shown in the top row. However, in a case where RARγ agonist A was administered (0.1% R667) shown in the bottom row, corneal opacity and ulceration were not observed. Thus, it was revealed that RARγ agonist A suppresses corneal opacity and ulceration.

[Industrial Applicability]

[0085]

RARγ agonists, which are effective ingredients of the therapeutic agent for a keratoconjunctive disorder of the present invention, are useful in preventing or as a therapeutic agent for keratoconjunctive disorders, such as corneal ulcer, corneal epithelial abrasion, keratitis, dry eye, conjunctivitis, chronic superficial keratitis, corneal erosion, persistent

and the supernatant was collected after 24 hours. The following western blot analysis and gelatin zymography analysis were used on the collected culture solution.

[0078]

(Western blot analysis)

After developing the supernatant of the collected culture solution in SDS-PAGE using 10% polyacrylamide gel, the separated proteins were transferred onto a nitrocellulose filter. Then, non-specific sites on the nitrocellulose filter were blocked and incubated for 24 hours at 4°C with anti-human MMP-1 antibodies (R&D Systems) and anti-rabbit MMP-3 antibodies (Daiichi Fine Chemical Co., Ltd). ECL® reagent (GE Healthcare) was used for detection.

[0079]

The results are shown in Figure **5A**. The band for each of the non-active Pro-MMP-1, Pro-MMP-3 and active MMP-1, MMP-3 was diminished, where the extent thereof was dependent on the concentration of RARγ agonist A. Thus, it was revealed that RARγ agonist A concentration-dependently suppresses the expression and activation of MMP-1 and MMP-3.

[0080]

(Gelatin zymography analysis)

After developing the supernatant of the collected culture solution on SDS-PAGE using 10% polyacrylamide gel containing 0.1% of gelatin, it was incubated for one hour at room temperature with a TBS solution containing 2.5% Triton X-100. The gel after incubation was dyed with a solution of Coomassie Brilliant Blue (Wako Pure Chemical Industries, Ltd.) and decolored with a 5% methanol-7.5% acetic acid solution (Nacalai Tesque, Inc).

[0081]

The results are shown in Figure **5B**. The band for each of the non-active Pro-MMP-2, Pro-MMP-9 and active MMP-2, MMP-9 was diminished, where the extent thereof was dependent on the concentration of RARγ agonist A. Thus, it was revealed that RARγ agonist A concentration-dependently suppresses the expression and activation of MMP-2 and MMP-9.

Example 5

[0082]

(Test Example) Test on suppression of corneal opacity and ulceration with RARγ agonist A

Systemic anesthesia was applied to male Japanese white rabbits (body weight 2.5-3.5 kg, 27 rabbits) by intramuscular administration of a solution of a mixture of ketamine and xylazine. Then, topical anesthesia was applied by a 0.4% oxybuprocaine hydrochloride instillation. Furthermore, 30 µl of 1% LPS (Sigma Aldrich) was injected into the corneal stroma of one eye, but not in the other eye.

[0083]

50 µl of 0.1% PBS/0.1% polysorbate 80 solution containing RARγ agonist A (R667: 0.1%, 24 mM) was instillatively administered to the rabbits injected with LPS twice after the injection on the day of LPS injection and four times daily thereafter until day 10 by using a micropipette . As a control, a 0.1% PBS/0.1% polysorbate 80 solution free of RARγ agonist A (Vehicle) was similarly administered. Figure **6** shows representative examples of a control and a case where RARγ agonist A was administered.

[0084]

In Figure **6**, corneal opacity and ulceration were observed in the control (Vehicle) shown in the top row. However, in a case where RARγ agonist A was administered (0.1% R667) shown in the bottom row, corneal opacity and ulceration were not observed. Thus, it was revealed that RARγ agonist A suppresses corneal opacity and ulceration.

[Industrial Applicability]

[0085]

RARγ agonists, which are effective ingredients of the therapeutic agent for a keratoconjunctive disorder of the present invention, are useful in preventing or as a therapeutic agent for keratoconjunctive disorders, such as corneal ulcer, corneal epithelial abrasion, keratitis, dry eye, conjunctivitis, chronic superficial keratitis, corneal erosion, persistent