Description

COMPOSITION FOR PREVENTING OR TREATING DAMAGES OF THE MUCOSA IN THE GASTROINTESTINAL TRACTS

Technical Field

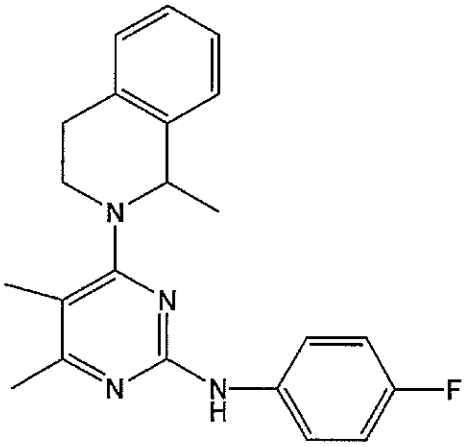
[1] The present invention relates to a composition for preventing or treating damages of the mucosa in the gastrointestinal tracts, comprising revaprazan or its salt.

Background Art

[2] Revaprazan, known by the chemical name, 5,6-dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl) pyrimidine, is a compound represented by Formula 1 below, and is available as an acid addition salt (e.g., revaprazan hydrochloride) [PCT Publication No. WO96/05177].

[3] <Formula 1>

[4]



[5] Revaprazan or its salt binds reversibly to a H^+/K^+ exchange site of proton pumps (H^+/K^+ ATPase) existing in gastric parietal cells to competitively inhibit the secretion of H^+ into the gastric lumen. Revaprazan or its salt binds to a specific site of the H^+/K^+ ATPase to block the transport of H^+ and acid secretion into the gastric lumen, thereby raising intragastric pH. Unlike irreversible proton pump inhibitors such as omeprazole, revaprazan or its salt is not affected by gastric acid activation of the drug or gastric acid secretion of the proton pumps. Based on the mechanism of revaprazan or its salt that is different from that of irreversible proton pump inhibitors such as omeprazole, revaprazan or its salt is classified as an Acid Pump Antagonist (APA).

[6] Meanwhile, gastrointestinal disorders are caused when offensive factors (e.g., gastric acid) are strengthened or defense factors are weakened. Both proton pump inhibitors (e.g., omeprazole) and acid pump antagonists (e.g., revaprazan) are compounds inhibiting the secretion of the offensive factor, i.e., the gastric acid, and cy-

toprotective agents (e.g., sucralfate, rebamipide) are compounds potentiating defensive factors.

Disclosure of Invention

Technical Problem

[7] The present inventors have conducted various clinical trials using an acid pump antagonist, i.e., revaprazan or its salt, and have found that revaprazan or its salt has a cytoprotective activity in the gastrointestinal tracts, beyond proton pump inhibitory activity. Such surprising findings suggest that revaprazan or its salt has not only the effect of inhibiting the secretion of the gastric acid (an offensive factor), but also the gastrointestinal cytoprotective effect of potentiating a defensive factor.

Technical Solution

[8] Therefore, the present invention provides a composition for preventing or treating damages of the mucosa in the gastrointestinal tracts, comprising revaprazan or its salt.

[9] The present invention provides a composition for preventing or treating damages of the mucosa in the gastrointestinal tracts, comprising revaprazan or its salt and a pharmaceutically acceptable carrier.

[10] In accordance with one aspect of the present invention, there is provided a method for preventing or treating damage to the gastric mucosa comprising administering to a human in need thereof a pharmaceutical composition comprising an effective amount for preventing or treating damage to gastric mucosa of revaprazan, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

[11] In accordance with another aspect of the present invention, there is provided a method for preventing or treating drug-induced damage to the gastric mucosa comprising administering to a human in need thereof a pharmaceutical composition comprising an effective amount for preventing or treating drug-induced damage to gastric mucosa of revaprazan, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

[12] In accordance with still another aspect of the present invention, there is provided a method for preventing or treating alcohol-induced damage to the gastric mucosa comprising administering to a human in need thereof a pharmaceutical composition comprising an effective amount for preventing alcohol-induced damage to gastric mucosa of revaprazan, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

[13] In accordance with still another aspect of the present invention, there is provided a method for providing cytoprotection of the gastric mucosa in a human receiving non-steroidal anti-inflammatory drugs (NSAIDs), comprising administering to the human prior to or concurrently with NSAIDs a pharmaceutical composition comprising a cy-

toprotectively effective amount of revaprazan, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier. The NSAIDs used in the present invention are not particularly restricted and include all NSAIDs widely used, such as Aspirin, Indomethacin, Diclofenac, Ibuprofen, Naproxen, Piroxicam, Mefenamic Acid, Flufenamic Acid, Floctafenine, Ethenzamide, Sodium salicylate, Diflunisal, Clofezone, Ketophenylbutazone, Phenylbutazone, Alclofenac, Alminoprofen, Ketoprofen, Flurbiprofen, Pranoprofen, Loxoprofen-Na, Tiaramide hydrochloride, Perisoxal citrate, Emorfazone, Acemetacin, Proglumetacin maleate, Bucolome and the like.

Brief Description of the Drawings

[14] The above and other features and advantages of the present invention will become more apparent by describing in detail exemplary embodiments thereof with reference to the attached drawings in which:

[15] FIG. 1 shows MTT assay results for the inhibitory effect of revaprazan on cell death due to *Helicobacter pylori* (*H. pylori*)-induced gastrointestinal mucosal damage;

[16] FIG. 2 shows MTT assay results for the inhibitory effect of revaprazan on cell death due to ethanol-induced gastrointestinal mucosal damage;

[17] FIGS. 3A and 3B are images showing the cytoprotective effect of revaprazan against *in vivo* gastrointestinal damage;

[18] FIG. 4 is a Western blot image showing an inhibitory effect of revaprazan on *H. pylori*-induced ERK (extracellular signal-regulated kinase) activation;

[19] FIG. 5 is Electrophoretic Mobility Shift Assay (EMSA) results showing an inhibitory effect of revaprazan on *H. pylori*-induced NF-kB activation;

[20] FIG. 6 is RT-PCR results showing an effect of revaprazan on the activity of pro-angiogenic growth factors;

[21] FIG. 7 is a Western blot image showing an effect of revaprazan on the activity of heat-shock proteins;

[22] FIG. 8 is a comparative image showing the preventive effects for gastric mucosal lesions with the treatment of rebamipide, omeprazole, and revaprazan prior to indomethacin administration; and

[23] FIG. 9 is a comparative image showing the treatment effect of revaprazan for gastric mucosal lesions after indomethacin administration.

Best Mode for Carrying Out the Invention

[24] Revaprazan may be prepared according to the methods disclosed in WO96/05177, WO97/42186, and/or WO98/18784. The salt of revaprazan may be an inorganic acid salt such as hydrochloride, sulfate, phosphate, and nitrate, or an organic acid salt such as tartrate, fumarate, citrate, mesylate, and acetate. Revaprazan hydrochloride is

preferred.

[25] The composition of the present invention may include additives such as lactose or corn starch, lubricants such as magnesium stearate, emulsifiers, suspending agents, stabilizers, and isotonic agents. If necessary, sweetening agents and/or flavoring agents may be added.

[26] The composition of the present invention may be administered orally or parenterally, including intravenous, intraperitoneal, subcutaneous, rectal and topical routes of administration. Therefore, the composition of the present invention may be formulated into various forms such as tablets, capsules, aqueous solutions or suspensions. In the case of tablets for oral use, carriers such as lactose, corn starch, and lubricating agents, e.g. magnesium stearate, are commonly added. In the case of capsules for oral administration, lactose and/or dried corn starch can be used as a diluent. When an aqueous suspension is required for oral use, the active ingredient may be combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring agents may be added. For intramuscular, intraperitoneal, subcutaneous and intravenous use, sterile solutions of the active ingredient are usually prepared, and the pH of the solutions should be suitably adjusted and buffered. For intravenous use, the total concentration of solutes should be controlled in order to render the preparation isotonic. The composition of the present invention may be in the form of an aqueous solution containing pharmaceutically acceptable carriers, e.g., saline, at a pH level of 7.4. The solutions may be introduced into a patient's intramuscular blood-stream by local bolus injection.

[27] Revaprazan or its salt can be administered to a patient who needs the prevention or treatment of gastrointestinal mucosal damage in an effective amount ranging from about 50 mg to 400 mg per day, preferably about from 100 mg to 300 mg per day, more preferably about from 150 mg to 250 mg per day, and most preferably about 200 mg per day. Of course, the dosage may be changed according to the patient's age, weight, susceptibility, or symptom.

Mode for the Invention

[28] Hereinafter, the present invention will be described more specifically by the following working examples. However, the following working examples are provided only for illustrations and thus the present invention is not limited to or by them.

[29] **Example 1: Measurement of incidence of *Helicobacter pylori* (*H. pylori*)-induced cell death (MTT assay-1)**

[30] In order to determine cytoprotective properties of revaprazan against *H. pylori* infection, gastric mucosal cells were pretreated with revaprazan and rebamipide prior to *H. pylori* infection, and the incidence of cell death was measured. Rebamipide,

known to have cytoprotective properties, was used as a control drug.

[31] The human gastric epithelial (AGS, KCLB 21739) cells were seeded onto 96-well plate at 5 X 10^4 cells/ml and cultured in RPMI 1640 (Gibco BRL, Grand Island, New York, U.S.A.), supplemented with 100 units/mL penicillin, 100 ug/mL streptomycin, and 10 % FBS (Fetal Bovine Serum). Revaprazan and rebamipide, dissolved in sterilized water, were added to AGS cells to reach a final concentration of 50 uM and the cells were incubated at 37 °C for 16 hours. To remove RPMI 1640 media, the plate was centrifuged at 24 °C, 3000 rpm for 5 minutes and washed three times with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4). For inoculation, *H. pylori* (ATCC 43504) cultures were resuspended in PBS and cocultured with AGS cells at a final concentration of 5 X 10^8 CFU/ml. After 48 hours of incubation at 37 °C, the culture was centrifuged at 24 °C, 3000 rpm for 5 minutes to remove the culture medium, i.e., RPMI 1640, and then total viable cell numbers were assessed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolim bromide) assay method. 1 mg/ml of an MTT solution prepared by dissolving MTT (Amresco, Ohio, U.S.A.) in PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4) was added to each well (50 □/well), followed by incubation at 37 °C for 4 hours. The supernatant was then removed by centrifugation at 24 °C, 3000 rpm for 5 minutes and the formazan grains formed by the viable cells were dissolved in 99.5% dimethyl sulfoxide (DMSO) (Kanto, Tokyo, Japan) (50 □/well), and the optical intensity was measured at 540 nm using an ELISA reader (TECAN, Maennedorf, Switzerland).

[32] The MTT assay results are shown in FIG. 1. Referring to FIG. 1, the 48-hour *H. pylori* infection caused significant gastric mucosal cytotoxicity. However, pretreatment of rebamipide and revaprazan was significantly reduced gastric mucosal cytotoxicity by *H. pylori* infection. These results show that revaprazan has cytoprotective effect equal to or greater than rebamipide against *H.pylori*-induced cytotoxicity.

[33]

[34] **Example 2: Measurement of incidence of ethanol-induced cell death (MTT assay-2)**

[35] The rat gastric mucosal (RGM-1, RIKEN cell bank, Japan) cells were seeded onto 96-well plate at 5 X 10^4 cells/ml and cultured in DMEM-F12 (Gibco BRL, Grand Island, New York, U.S.A.), supplemented with 100 units/mL penicillin, 100 ug/mL streptomycin, and 10 % FBS (Fetal Bovine Serum). Revaprazan and rebamipide, dissolved in sterilized water, were added to RGM-1 cells to reach a final concentration of 50 uM and the cells were incubated at 37 °C for 16 hours. To remove DMEM-F12 media, the plate was centrifuged at 24 °C, 3000 rpm for 5 minutes and washed three times with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4). Then, ethanol-containing media (200 mM ethanol-containing DMEM-F12, sup-

plemented with 100 units/mL penicillin, 100 ug/mL streptomycin, and 10% FBS) was added to the cells, and the cells were cultured at 37 °C for 16 hours. After the incubation, the culture was centrifuged at 24 °C, 3000 rpm for 5 minutes to remove the culture medium and then total viable cell numbers were assessed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolim bromide) assay method. 1 mg/ml of an MTT solution prepared by dissolving MTT (Amresco, Ohio, U.S.A.) in PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4) was added to each well (50 □/well), followed by incubation at 37 °C for 4 hours. The supernatant was then removed by centrifugation at 24 °C, 3000 rpm for 5 minutes and the formazan grains formed by the viable cells were dissolved in 99.5% dimethylsulfoxide (DMSO) (Kanto, Tokyo, Japan) (50 □/well), and the optical intensity was measured at 540 nm using an ELISA reader (TECAN, Maennedorf, Switzerland).

[36] The MTT assay results are shown in FIG. 2. Referring to FIG. 2, in the ethanol-only treatment group, significant gastric mucosal cytotoxicity was induced. On the other hand, in the rebamipide/revaprazan-ethanol treatment groups, gastric mucosal cytotoxicity was significantly reduced. These results show that revaprazan has cytoprotective effect equal to or greater than rebamipide.

[37]

[38] **Example 3: Evaluation of preventive effect of revaprazan on *in vivo* gastrointestinal damage**

[39] Six-week old specific-pathogen-free (SPF) Sprague-Dawley male rats (Charles River, Tokyo, Japan) were used for experiments. The rats were fed a sterilized commercial pellet diet (Biogenomics Co., Seoul, Korea), given sterile water ad libitum, and housed in air-conditioned biohazard room with a 12-h light:12-h dark cycle. The rats were divided into four groups; indomethacin-only treatment group, revaprazan-indomethacin treatment group, ethanol-only treatment group and revaprazan-ethanol treatment group. The rats were food-deprived 24 hours, and then administered with 10 mg/kg revaprazan suspended in 0.5% CMC (carboxymethylcellulose) via oro-gastric tube prior to exposure to either indomethacin (40 mg/kg for 12hrs) or absolute ethanol (6 ml/kg for 1hr).

[40] The stomachs of the rats belonging to the groups treated with indomethacin were excised and the degree of gastric mucosal lesions was observed (see FIG. 3A). Referring to FIG. 3A, in the indomethacin-only treatment group, increased hemorrhagic lesions in gastric mucosa were observed visually. However, in the revaprazan-indomethacin treatment group that had been treated with indomethacin after the revaprazan administration, indomethacin-induced gastric injury was completely inhibited. Also, the stomachs of the rats belonging to the ethanol treatment groups were excised and the degree of gastric mucosal lesions was observed (see FIG.

3B). Referring to FIG. 3B, in the ethanol-only treatment group, remarkably increased hemorrhagic lesions in gastric mucosa were also observed visually. However, in the revaprazan-ethanol treatment group that had been treated with ethanol after the revaprazan administration, ethanol-induced gastric injury was completely inhibited. These results show that pretreatment of revaprazan significantly reduced Non-Steroidal Anti-Inflammatory Drug (NSAID) (e.g., indomethacin)- or ethanol-induced gastric mucosal lesions, thereby achieving excellent cytoprotective effect of the gastrointestinal mucosa.

[41]

[42] **Example 4: Evaluation of activity of extracelluar signal-regulated kinase (ERK) inducing *H. pylori* -mediated cytotoxicity**

[43] *H. pylori*-induced cytotoxicity activates MAPK (Mitogen-Activated Protein Kinase), thereby resulting in apoptosis. Therefore, *H. pylori* infection leads to an increase in phosphorylation of ERK among three major subfamilies of MAPKs.

[44] The human gastric epithelial (AGS, KCLB 21739) cells were seeded in culture dishs at 2 X 10^6 cells/100mm^2 and cultured in RPMI 1640 (Gibco BRL, Grand Island, New York, U.S.A.), supplemented with 100 units/mL penicillin, 100 ug/mL streptomycin, and 10 % FBS (Fetal Bovine Serum). Revaprazan, dissolved in sterilized water, were added to AGS cells to reach final concentrations of 5 and 25 uM and the cells were incubated at 37 °C for 16 hours. RPMI 1640 media was removed and then the cells washed three times with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4). For inoculation, *H. pylori* (ATCC 43504) cultures were re-suspended in PBS and cocultured with AGS cells at a final concentration of 5 X 10^8 CFU/ml. After 30 minutes of incubation at 37 °C, the cells were resuspended in lysis buffer (20 mM Tris-Cl (pH 7.5), 150 mM NaCl, 1 % Triton X-100, 1 mM EDTA, and protease inhibitor cocktail (Roche, Mannheim, Germany)). The suspension was sonicated for approximately 10 seconds, four times using the BIORUPTOR (Cosmo Bio, Koto-ku, Tyoko), and centrifuged at 4 °C, 12000 rpm for 30 minutes. Supernatants were used as protein extracts, electrophoresed on 10% SDS-PAGE gels, and transferred to PVDF membranes (Millipore, Massachusetts, U.S.A.) using a semidry transfer system (Hoeffer Pharmacia Biotech, San Francisco, CA, U.S.A.). In order to prevent non-specific binding between the primary antibodies and the proteins, the PVDF membranes were blocked with blocking buffer (TBST: 10 mM Tris-Cl, pH 8.0, 150 mM NaCl, and 0.1% Tween 20 (v/v)) containing 5% skim milk (Difco, Livonia MI, U.S.A.) for one hour at 23 °C. The PVDF membranes were incubated at 4 °C for 15 hours with 1:1000 dilution (200ng/ml) of primary antibodies for p-ERK or ERK. The PVDF membranes were incubated with 1:2000 dilution (100 ng/ml) of the HRP (horseradish peroxidase)-conjugated secondary antibody (Santa Cruz Biotech,

California, U.S.A.) for one hour at 23 °C. The immunocomplex was visualized with an ECL (enhanced chemiluminescence) detection kit (Amersham-Pharmacia Biotec, Buckinghamshire, UK).

[45] The Western blot results for the p-ERK of the untreatment group and the revaprazan treatment groups of the *H. pylori*-infected cells are shown in FIG. 4. Referring to FIG. 4, revaprazan exhibits a significant, concentration-dependent inhibitory effect on ERK activation.

[46]

[47] **Example 5: Evaluation of activity of NF-kB transcription factor associated with *H. pylori* infection (Electrophoretic Mobility Shift Assay, EMSA)**

[48] The DNA binding activity of NF-kB (nuclear factor - kappa B), a redox-sensitive transcription factor known to be associated with *H. pylori* infection was evaluated using EMSA. Rebamipide (50 uM), known to have cytoprotective properties, was used as a control drug, and revaprazan (25 uM) was used as a test drug.

[49] The human gastric epithelial (AGS, KCLB 21739) cells were seeded in culture dishes at 2 X 10^6 cells/100 mm^2 and cultured in RPMI 1640 (Gibco BRL, Grand Island, New York, U.S.A.), supplemented with 100 units/mL penicillin, 100 ug/mL streptomycin, and 10 % FBS (Fetal Bovine Serum). Revaprazan and rebamipide, dissolved in sterilized water, were added to AGS cells to reach final concentrations of 25 uM and 50 uM, respectively, and the cells were incubated at 37 °C for 16 hours. The culture media were removed and the cells were twice washed with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4). For inoculation, *H.pylori* (ATCC 43504) cultures were resuspended in PBS and cocultured with AGS cells at a final concentration of 5 X 10^8 CFU/ml at 37 °C for one hour. The nuclear fractions for EMSA were prepared using the NE-PER Nuclear and the Cytoplasmic Extraction Kit (Pierce, Rockford, IL, U.S.A.). Sequences of double-stranded oligonucleotides used for EMSA of NF-kB were as follows; 5'-AGT TGA GGG GAC TTT CCC AGG C-3', and the oligonucleotides were labeled with a Biotin 3' End DNA Labeling Kit (Pierce, Rockford, IL, U.S.A.). The EMSA was performed with a Light Shift Chemiluminescent EMSA Kit (Pierce, Rockford, IL, U.S.A.).

[50] The evaluation results for the DNA binding activity of the NF-kB using EMSA are shown in FIG. 5. These results indicated that NF-kB activity was significantly inhibited by revaprazan pretreatment, thereby achieving cytoprotective effect equal to or greater than rebamipide.

[51]

[52] **Example 6: Measurement of expression levels of proteins participating in cytoprotection (RT-PCR)**

[53] In order to evaluate cytoprotective effect of revaprazan, 100 uM of sulindac

belonging to NSAIDs was added to the rat cells, and the expression levels of pro-angiogenic growth factors, VEGF (Vascular Endothelial Growth Factor), interlukin-8 (IL-8), and COX-2 (cyclooxygenase-2) were measured. Rebamipide (50 uM), known to have cytoprotective properties, was used as a control drug and revaprazan (25 uM) was used as a test drug.

[54] The rat gastric mucosal (RGM-1, RIKEN cell bank, Japan) cells were seeded in culture dishes at 2 X 10^6 cells/100 mm^2 and cultured in RPMI 1640 (Gibco BRL, Grand Island, New York, U.S.A.), supplemented with 100 units/mL penicillin, 100 ug/mL streptomycin, and 10% FBS (Fetal Bovine Serum). Revaprazan and rebamipide, dissolved in sterilized water, were added to RGM-1 cells to reach final concentrations of 25 uM and 50uM, respectively, and the cells were cultured at 37 ℃ for 16 hours. The culture media were removed and the cells were washed three times with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4). A solution of sulindac in DMSO was added to the cells to reach a final concentration of 100 uM and the cells were cultured at 37 ℃ for 8 hours. The cultured cells were collected by centrifugation at 23 ℃ at 3000 rpm for 3 minutes, and the total RNA was isolated from the cells using the TRIzol reagent (Life technologies, Milan, Italy). Sterilized RNase-free water was added to 2 □of the total RNA and 1 □ of 10 pmol/oligo dT to obtain reaction solutions with a volume of 34 □. The reaction solutions were incubated at 65 ℃ for 5 minutes, and 10 □ of a 5 X reverse-transcription buffer, 5 □ of 10 mM dNTPs, and 1 □ of M-MLV reverse transcriptase were added thereto. The resultant solutions were incubated at 37 ℃ for one hour to obtain cDNA (Promega, Madison, WI), and the cDNA were amplified by PCR. The PCR was performed using the Premix Ex *Taq* kit (Takara, Chiba, Japan) with specific primers as follows;
5'-TGCACCCACGACAGAAGGGGA-3' and
5'-TCACCGCCTTGGCTTGTCACAT-3' (for VEGF);
5'-GAAGATAGATTGCCCGA-3' and 5'-CATAGCCTCTCACACATTTC-3' (for IL-8); 5'-ATCTGTGTGGGTACAAATTTG-3' and
5'-GTCTCTCATCTGCAATAATGTG-3' (for COX-2);
5'-TGAAGGTCGGTGTCAACGGATTTGTC-3' and
5'-CATGTAGGCCATGAGGTCCACCAC-3' (for GAPDH). As described above, GAPDH was used as positive control. The PCR was performed as follows: 35 cycles (VEGF, IL-8, and COX-2) or 28 cycles (GAPDE) of 94 ℃ for one minute, 60 ℃ (VEGF), 45 ℃ (IL-8), 48 ℃ (COX-2), and 55 ℃ (GAPDH) for one minute (each), and 72 ℃ for one minute. The PCR product was resolved on 1% agarose gels, and stained with 10 mg/ml of an ethidium bromide .

[55] The expression levels of IL-8, VEGF, and COX-2 in the rebamipide-sulindac treatment group and the revaprazan-sulindac treatment group are shown in FIG. 6.

Referring to FIG. 6, the expression levels of IL-8, VEGF, and COX-2 in the revaprazan-sulindac treatment group were much higher than those in the rebamipide-sulindac treatment group. It is known that one of mechanisms contributing to NSAID-induced gastric mucosal damage is associated with a reduction in VEGF, IL-8, and COX-2 causing gastric mucosal regeneration and ischemia. In this regard, the results of FIG. 6 showing the increased expression levels of IL-8, VEGF, and COX-2 in the revaprazan-sulindac treatment group reveal that revaprazan has a good cytoprotective effect for the gastrointestinal mucosa.

[56]

[57] **Example 7: Evaluation of activities of heat-shock proteins (Western blotting)**

[58] To measure expression of heat-shock proteins, HO-1, HSP27, HSP70, cells were pretreated with concentrations of 5, 10, and 25 uM revaprazan, and then treated with indomethacin (a NSAID, 0.5 uM).

[59] The rat gastric mucosal (RGM-1, RIKEN cell bank, Japan) cells were seeded in culture dishes at 2 X 10^6 cells/100 mm^2 and cultured in DMEM-F12 (Gibco BRL, Grand Island, New York, U.S.A.), supplemented with 100 units/mL penicillin, 100 ug/mL streptomycin, and 10% FBS (Fetal Bovine Serum). RGM-1 cells were pretreated with revaprazan (5, 10, and 25uM) and cultured 37 °C for 16 hours. The culture media were removed and the cells were washed three times with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4). A solution of indomethacin in sterilized water was added to the cells to reach a final concentration of 0.5 uM, and the cells were incubated at 37 °C for 16 hours. After incubation, the cells were resuspended in a lysis buffer (20 mM Tris-Cl (pH 7.5), 150 mM NaCl, 1 % Triton X-100, 1 mM EDTA, and protease inhibitor cocktail (Roche, Mannheim, Germany)). The suspension was sonicated for approximately 10 seconds, four times using the BIORUPTOR (Cosmo Bio, Koto-ku, Tokyo), and centrifuged at 4 °C, 12000 rpm for 30 minutes. Supernatants were used as protein extracts, the extracted proteins were electrophoresed on 10% SDS-PAGE gels and transferred to PVDF membranes (Millipore, Massachusetts, U.S.A.) using a semidry transfer system (Hoeffer Pharmacia Biotech, San Francisco, CA, U.S.A.). In order to prevent non-specific binding between the primary antibodies and the proteins, the PVDF membranes were blocked with blocking buffer (TBST: 10 mM Tris-Cl, pH 8.0, 150 mM NaCl, and 0.1% Tween 20 (v/v)) containing 5% skim milk (Difco, Livonia MI, U.S.A.) for one hour at 23 °C. The PVDF membranes were incubated at 4 °C for 15 hours with 1:1000 dilution (200 ng/ml) of the primary antibodies for HO-1, HSP27, HSP70 or α-tubulin (TU-02). The PVDF membranes were incubated with 1:2000 dilution (100 ng/ml) of HRP (horseradish peroxidase)-conjugated secondary antibody (Santa Cruz Biotech, California, U.S.A.) for one hour at 23 °C. The immunocomplex was visualized with an ECL (enhanced

chemiluminescence) detection kit (Amersham-Pharmacia Biotec, Buckinghamshire, UK) (see FIG. 7).

[60] It can be seen from FIG. 7 that revaprazan exhibits the cytoprotective effect by stimulating expression of heat-shock proteins.

[61]

[62] **Example 8: Evaluation of the preventive effect of revaprazan on NSAID-induced gastropathy**

[63] Six-week old specific-pathogen-free (SPF) Sprague Dawley male rats (Charles River, Tokyo, Japan) were used for experiments. The rats were fed a sterilized commercial pellet diet (Biogenomics Co., Seoul, Korea), given sterile water ad libitum, and housed in air-conditioned biohazard room with a 12-h light:12-h dark cycle. The rats were food-deprived 24 hours, and then administered with 5, 10 mg/kg revaprazan, 30 mg/kg rebamipide and 5 mg/kg omeprazole suspended in 0.5% CMC (carboxymethylcellulose) via oro-gastric tube prior to exposure to indomethacin (40 mg/kg for 12hrs). Phosphate-buffered saline was perfused into the gastric lumen of the rats to swell the stomachs. Then, the stomachs were opened by an incision along the greater curvature, and the total area of the gastric mucosal lesions was calculated to obtain the average size of the lesions.

[64] Comparative results of the preventive effects for gastric mucosal lesions in the control group and the test groups are shown in FIG. 8. Referring to FIG. 8, hemorrhagic lesions are observed in the control group, the omeprazole pretreatment group, and the rebamipide pretreatment group, whereas little hemorrhagic lesions are observed in the revaprazan pretreatment group. These results show that revaprazan has an excellent preventive effect on gastric mucosal lesions.

[65]

[66] **Example 9: Evaluation of the treatment effect of revaprazan on NSAID-induced gastropathy**

[67] Six-week old specific-pathogen-free (SPF) Sprague Dawley male rats (Charles River, Tokyo, Japan) were used for experiments. The rats were fed a sterilized commercial pellet diet (Biogenomics Co., Seoul, Korea), given sterile water ad libitum, and housed in air-conditioned biohazard room with a 12-h light:12-h dark cycle. The rats were food-deprived 24 hours, and then treated with 1 ml of a solution of indomethacin (40 mg/kg) in 0.5% CMC (carboxymethylcellulose) via oro-gastric tube. 8 hours after the indomethacin treatment, the rats were treated with 1 ml of a solution of revaprazan (5 mg/kg and 10 mg/kg) in 0.5% CMC for one hour, and the degree of gastropathy was determined in the same manner as in Example 8.

[68] Comparative results of the treatment effects for gastric mucosal lesions in the indomethacin-only treatment group and the indomethacin-revaprazan treatment groups

are shown in FIG. 9. Referring to FIG. 9, in the indomethacin-revaprazan treatment groups, gastric mucosal hemorrhagic lesions were remarkably improved and the number of erosions and ulcers was also reduced. These results show that revaprazan has an excellent treatment effect for gastric mucosal lesions.

Industrial Applicability

[69] As described above, revaprazan or its salt has an excellent preventive or treatment effect for gastrointestinal mucosal damage by potentiating a defensive factor in the gastrointestinal mucosa, simultaneously with acting as an acid pump antagonist.

Claims

[1] A composition for preventing or treating damages of the mucosa in the gas-trointestinal tracts, comprising revaprazan or a salt thereof and a pharma-ceutically acceptable carrier.

[2] The composition of claims 1, wherein the salt of revaprazan is revaprazan hy-drochloride.

[3] Use of revaprazan or a pharmaceutically acceptable salt thereof for the preparation of a medicament for preventing or treating damage to the gastric mucosa.

[4] Use of revaprazan or a pharmaceutically acceptable salt thereof for the preparation of a medicament for preventing or treating drug-induced damage to the gastric mucosa.

[5] Use of revaprazan or a pharmaceutically acceptable salt thereof for the preparation of a medicament for preventing or treating alcohol-induced damage to the gastric mucosa.

[6] Use of revaprazan or a pharmaceutically acceptable salt thereof for the preparation of a medicament for providing cytoprotection of the gastric mucosa in a human receiving non-steroidal anti-inflammatory drugs (NSAIDs).

[7] The use according to any one of claims 3 to 6, characterized in that the pharmaceutically acceptable salt is revaprazan hydrochloride.

[8] The use according to any one of claims 3 to 6, characterized in that the effective amount of revaprazan or a pharmaceutically acceptable salt thereof is 100 mg to 300 mg per day.

[9] The use according to any one of claims 3 to 6, characterized in that the effective amount of revaprazan or a pharmaceutically acceptable salt thereof is 150 mg to 250 mg per day.

[10] The use according to any one of claims 3 to 6, characterized in that the effective amount of revaprazan or a pharmaceutically acceptable salt thereof is 200 mg per day.