Description

Title of Invention

COMPOSITION FOR ALLEVIATING OR TREATING PAIN

Technical Field

The present invention relates to a composition for alleviating or treating pain and to the use of the composition for alleviating or treating pain.

Background Art

Pain means an experience of actual or potential tissue damage or unpleasant sensations and feelings associated with such damage. Pain protects parts of the body that have been damaged during the healing of the damaged tissues from the damaged situation and provides motivation to avoid similar experiences in the future. Most pain is alleviated slowly when the causal stimulus is removed, but sometimes pain persists even though the tissues have been healed as the stimulus has disappeared and the damage has clearly healed, or pain occurs in a state without any irritation, damage or disease.

For the treatment of pain, mainly, narcotic analgesics such as morphine, which is an opioid alkaloid, or non-narcotic analgesics such as non-steroidal anti-inflammatory

Recently, new therapeutic agents for neuropathic pain have been developed, but still have side effects. For example, sodium channel blockers are mostly in the form of small molecules and show low selectivity for isoform proteins. In addition, they show side effects such as cardiac toxicity and movement disorder.

Therefore, there is an imperative need to develop a new analgesic for neuropathic pain which is excellent in analgesic efficacy while reducing side effects.

Disclosure of Invention

Aspects of the invention are defined in the independent claims. Some optional features are defined in the dependent claims.

The present inventors have endeavored to develop a new analgesic for neuropathic pain exhibiting an excellent analgesic efficacy even at a low dosage. As a result, the present inventors have found that when a combination of two or more of glutamate decarboxylase, an anti-inflammatory cytokine, in particular a gene encoding interleukin-10 (IL-10), and a glial cell-derived neurotrophic factor is used, pain can be significantly alleviated or treated as compared with an individual use.

Disclosed herein is a pharmaceutical composition for alleviating or treating pain comprising two or more selected from the group consisting of a gene encoding glutamate decarboxylase (GAD), a gene encoding interleukin-10 (IL-10), and a gene encoding a glial cell-derived neurotrophic factor (GDNF).

Advantageous Effects

A pharmaceutical composition as disclosed herein comprises two or more selected from the group consisting of genes encoding GAD, IL-10, and GDNF. Therefore, the pharmaceutical composition as disclosed herein exhibits an excellent analgesic efficacy at a dosage lower than that of individual administration since genes are co-administered, and thus conventional side effects and toxicity can be reduced. Therefore, the disclosed pharmaceutical composition can be useful in alleviating or treating pain.

Brief Description of Drawings

Fig. 1 shows the schematic diagram of the plasmids pAAV-GAD65 and pAAV-GAD65-modi used for the construction of the recombinant adeno-associated virus:

(a) shows the schematic diagram of pAAV-GAD65, and (b) shows the schematic diagram of pAAV-GAD65-modi.

Fig. 2 shows the schematic diagram of the plasmid pAAV-IL-10 used for the construction of the recombinant adeno-associated virus.

Fig. 3 shows the schematic diagram of the plasmid pAAV-GDNF used for the construction of the recombinant adeno-associated virus.

Fig. 4 is a schematic diagram showing the pAAV-GDNF-IL-10 plasmid.

Fig. 5 shows the expression of each introduced gene by the pAAV-GAD65, pAAV-IL-10, or pAAV-GDNF plasmid:

(a) shows the expression of GAD65 by pAAV-GAD65 plasmid; (b) shows the expression of IL-10 by pAAV-IL-10 plasmid; and (c) shows the expression of GDNF by the pAAV-GDNF plasmid.

Fig. 6 shows the expression of GDNF gene and IL-10 gene by the pAAV-GDNF-IL-10 plasmid:

(a) shows the expression of IL-10 by pAAV-GDNF-IL-10 plasmid; and (b) shows the expression of GDNF by pAAV-GDNF-IL-10 plasmid.

Fig. 7 shows the results of western blot showing expression of each protein after treatment of 293T or HeLa cells with each recombinant adeno-associated virus after construction of the recombinant adeno-associated viruses into which GAD65 gene, IL-10 gene and GDNF gene were introduced, respectively:

(a) shows the expression of GAD65 after treatment of 293T or HeLa cells with the recombinant adeno-associated virus AAV-GAD65; (b) shows the expression of GAD65 after treatment of 293T or HeLa cells with the recombinant adeno-associated virus AAV-GAD65-modi; (c) shows the expression of IL-10 after treatment of 293T or HeLa cells with the recombinant adeno-associated virus AAV-IL-10; and (d) shows the expression of GDNF after treatment of 293T or HeLa cells with the recombinant adeno-associated virus AAV-GDNF.

Fig. 8 shows the levels of GABA expression measured by ELISA after treatment of 293T or HeLa cells with the recombinant adeno-associated virus AAV-GAD65 or AAV-GAD65-modi:

(a) is a graph showing the level of GABA expression after 293T or HeLa cells were treated with the recombinant adeno-associated virus AAV-GAD65; and (b) is a graph showing the level of GABA expression after 293T or HeLa cells were treated with the recombinant adeno-associated virus AAV-GAD65-modi.

Fig. 9 is a graph showing the results of comparing the pain alleviating efficacies between individual administration of AAV-GAD65, AAV-IL-10, or AAV-GDNF virus and co-administration of AAV-GAD65 and AAV-GDNF viruses, or AAV-IL-10 and AAV-GDNF viruses.

Fig. 10 is a graph showing the results of comparing the pain alleviating efficacies between individual administration of AAV-GAD65, AAV-IL-10, or AAV-GDNF virus and co-administration of AAV-GAD65, AAV-IL-10 and AAV-GDNF viruses.

Fig. 11 is a graph showing the results of comparing the pain alleviating efficacies between co-administration of AAV-GAD65 and AAV-GDNF viruses, or AAV-IL-10 and AAV-GDNF viruses and co-administration of all of the AAV-GAD65, AAV-IL-10 and AAV-GDNF viruses.

Fig. 12 is a graph showing the results of comparing the pain alleviating efficacies between individual administration of pAAV-GAD65, pAAV-IL-10, or pAAV-GDNF plasmid and co-administration of pAAV-GAD65 and pAAV-GDNF plasmids, or pAAV-IL-10 and pAAV-GDNF plasmids.

Fig. 13 is a graph showing the results of comparing the pain alleviating efficacies between individual administration of pAAV-GAD65, pAAV-IL-10, or pAAV-GDNF

plasmid and co-administration of all of the pAAV-GAD65, pAAV-IL-10 and pAAV-GDNF plasmids.

Fig. 14 is a graph showing the results of comparing the pain alleviating efficacies between co-administration of pAAV-GAD65 and pAAV-GDNF plasmids, or pAAV-IL-10 and pAAV-GDNF plasmids and co-administration of all of the pAAV-GAD65, pAAV-IL-10 and pAAV-GDNF plasmids.

Fig. 15 is a graph showing the results of comparing the pain alleviating efficacies between co-administration of AAV-GAD65-modi and AAV-GDNF-IL-10 viruses and co-administration of all of the AAV-GAD65, AAV-IL-10 and AAV-GDNF viruses.

Detailed Description

The present disclosure provides a pharmaceutical composition for alleviating or treating pain comprising two or more selected from the group consisting of a gene encoding glutamate decarboxylase (GAD), a gene encoding interleukin-10 (IL-10), and a gene encoding a glial cell-derived neurotrophic factor (GDNF).

In one example, the combination of two or more may be GAD and IL-10, GAD and GDNF, IL-10 and GDNF, or GAD, IL-10 and GDNF.

Two or more genes selected from the group consisting of a gene encoding GAD, a gene encoding IL-10, and a gene encoding GDNF may be in a form of being contained in a carrier. Herein, the carrier may be a viral vector, or a non-viral vector such as a plasmid,

a liposome, etc. In addition, the genes may be in a form in which some of the genes are contained in a viral vector and the remaining genes are contained in a non-viral vector.

In one example, the genes may be in a form in which GAD is contained in a viral vector, and IL-10 is contained in a non-viral vector. In addition, the genes may be in a form in which GAD is contained in a viral vector, and GDNF is contained in a non-viral vector. Further, the genes may be in a form in which IL-10 is contained in a viral vector, and GDNF is contained in a non-viral vector. In addition, the genes may be in a form in which GAD is contained in a viral vector, and IL-10 and GDNF are contained in a non-viral vector. In addition, the genes may be in a form in which GAD and IL-10 are contained in a viral vector, and GDNF is contained in a non-viral vector. In addition, the genes may be in a form in which GAD and GDNF are contained in a viral vector, and IL-10 is contained a non-viral vector. In addition, the genes may be in a form in which IL-10 is contained in a viral vector, and GAD and GDNF are contained in a non-viral vector. In addition, the genes may be in a form in which IL-10 and GDNF are contained in a viral vector, and GAD is contained in a non-viral vector. Also, the genes may be in a form in which GDNF is contained in a viral vector, and GAD and IL-10 are contained in a non-viral vector.

In addition, the gene may be in a form of being operably contained in a vector. Specifically, the gene may be in a form of being operably contained in a viral vector or a non-viral vector.

The viral vector may be at least one selected from the group consisting of adenovirus, adeno-associated virus (AAV), herpes simplex virus, lentivirus, retrovirus,

cytomegalovirus, baculovirus, poxvirus, etc. Specifically, the viral vector may be adeno-associated virus.

In one example, the gene encoding GAD may be operably contained in a carrier 1 (e.g., a first vector), and the gene encoding IL-10 may be operably contained in a carrier 2 (e.g., a second vector), and the gene encoding GDNF may be operably contained in a carrier 3 (e.g., a third vector). In addition, one carrier may contain two or more genes.

The non-viral vector may be at least one selected from the group consisting of a plasmid, a liposome, a cationic polymer, a micelle, an emulsion, and solid lipid nanoparticles.

The term "plasmid" as used herein refers to a circular DNA fragment existing separately outside the chromosome of bacteria. Plasmids have no genes essential for

As used herein, the term "solid lipid nanoparticle" refers to a preparation of a form in which a drug is contained in a nano-sized microparticle made of a solid lipid instead of a liquid lipid.

A carrier 1 (e.g., a first vector) comprising any one gene selected from the group consisting of GAD, IL-10, and GDNF, and a carrier 2 (e.g., a second vector) comprising any one gene selected from the remaining gene group not included in the carrier 1 as disclosed herein may have a virus titer-based mixing ratio per unit volume of 1: 1 to 100 or 1 to 100: 1. Specifically, the virus titer-based mixing ratio per unit volume of the carrier 1 and the carrier 2 may be 1: 1 to 10 or 1 to 10: 1.

A carrier 1 (e.g., a first vector) comprising a gene encoding GAD, a carrier 2 (e.g., a second vector) comprising a gene encoding IL-10 and a carrier 3 (e.g., a third vector) comprising a gene encoding GDNF as disclosed herein may have a virus titer-based mixing ratio per unit volume of 1: 0.1 to 10: 0.1 to 10.

As used herein, the term "operably" means that an introduced gene is linked to a regulatory sequence in such a way that expression can take place in a host cell. The regulatory sequence is a DNA sequence that regulates the expression of the gene, and may include other regulatory elements such as promoters and enhancers or polyadenylation. In addition, the regulatory sequence provides a site for binding of a transcription factor that controls the expression of the introduced gene, and can influence the complex structure with the transcription factor to determine the function of the transcription factor.

drowsiness, etc., as well as the side effects such as increase in the heart rate or respiratory rate (Longo, Am Fam Physician, 2000).

IL-10 is known to be a cytokine which shows anti-inflammatory actions, but side effects such as flu symptoms and the like can occur (Friedrich, J Invest Dermatol, 2002).

Furthermore, it is known that the expression of GDNF exhibits analgesic efficacies on a variety of pains such as neuropathic pain and the like, but it has been reported in monkey experiments that administration in excess caused neuronal damage of brain (Hovland, Toxicol Pathol, 2007).

A pharmaceutical composition as disclosed herein can exhibit analgesic actions with a small amount of genes or carriers containing the same. The composition typically comprises, or consists of, a vector containing a gene encoding GAD, a vector containing a gene encoding an anti-inflammatory cytokine in nervous tissues, and/or a vector containing a gene encoding GDNF. And by co-administering substances having different analgesic mechanisms, it is possible to achieve the same or better pain alleviation or treatment effects at a dosage lower than that of individual administration.

Particularly, as disclosed herein, when two or more genes selected from the group consisting of genes encoding GAD65, IL-10, and GDNF are provided for use in the alleviation or treatment of pain, a synergistic pain-alleviating effect takes place. Therefore, the pharmaceutical composition can be useful for alleviating or treating pain.

According to one example, the first vector, the second vector, and/or the third vector may be an adeno-associated virus. The adeno-associated virus is not limited to a particular serotype, and preferably may be any one of AAV1 to AAV9.

The pain may be selected from the group consisting of nociceptive pain, psychogenic pain, inflammatory pain, pathological pain, neuropathic pain, cancer pain, postoperative pain, trigeminal neuralgia pain, idiopathic pain, diabetic neuropathic pain, or migraine. In a specific example, the pain may be lumbosacral radiculopathy (LSR).

The inflammatory pain refers to the pain associated with a tissue damage and infiltration of immune cells. In addition, the pathological pain means a disease state in which pain is caused by damage to a nerve tissue or its abnormal function. Also, the pathological pain may be dysfunctional pain, such as fibromyalgia, irritable bowel syndrome, or tension headache.

In addition, pain can include back pain which can be anatomically distinguished: neck pain, middle back pain, lower back pain, or tailbone pain. In addition, the pain may be at least one selected from the group consisting of neuropathic pain, cancer pain, postoperative pain, trigeminal neuralgia pain, idiopathic pain, diabetic neuropathic pain, migraine, and the like. In a specific example, the pain may be lumbosacral radiculopathy.

Neuropathic pain can be caused by a damage or disease that affects the somatosensory system. Neuropathic pain can be an abnormal sensation called allodynia and dysesthesia. In addition, the general characteristics of neuropathic pain include the sense of hot or cold, pins and needles, numbness, and itching. In contrast, nociceptive pain is often expressed as aching.

In addition, migraine is associated with a number of autonomic nervous system symptoms, and is a chronic disorder that causes headaches of normal to serious severities. Migraine is known to be associated with increased excitability of the cerebral cortex and

abnormal regulation of pain neurons in the trigeminal nucleus of the brainstem (Noseda, Pain, 2013).

Specifically, a pharmaceutical composition as disclosed herein can be used for alleviating or treating neuropathic pain and chronic cancer pain.

As used herein, the term "alleviating or treating" means any action that improves or alters pain symptom in a beneficial way by administering the composition as disclosed herein.

The pharmaceutical composition may further comprise a physiologically acceptable carrier. In addition, the pharmaceutical compositions may further comprise suitable excipients and diluents conventionally used in the preparation of pharmaceutical compositions. In addition, the compositions may be provided for use in the alleviation or treatment of pain by preparing them as oral formulations such as powders, granules, tablets, capsules, suspensions, emulsions, syrups, aerosols, etc., external formulations, suppositories, or injections by general methods. Specifically, the pharmaceutical composition may be in the form of an injection. As for the suitable formulations known in the art, those listed in Remington's Pharmaceutical Science (1985) may be used.

In addition, the pharmaceutical composition may comprise a salt (sodium chloride), lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starch, acacia gum, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methylcellulose, microcrystalline cellulose, polyvinylpyrrolidone, water, methyl hydroxybenzoate, propyl hydroxybenzoate, talc, magnesium stearate, mineral oil, etc., as carriers, excipients, and diluents. For the formulation of the pharmaceutical composition as disclosed herein,

generally used diluents or excipients such as fillers, extenders, binders, humectants, disintegrators, surfactants, etc. may be utilized.

Formulations for parenteral administration may include sterile solutions, non-aqueous solvents, suspensions, emulsions, freeze-dried formulations, and suppositories. Propylene glycol, polyethylene glycol, vegetable oil such as olive oil, and injectable ester such as ethylolate, etc., may be used for non-aqueous solvents and suspensions. Witepsol, macrogol, Tween 61, cacao oil, laurin oil, glycerogelatin, etc. may be used for suppository bases.

Also disclosed herein is a pharmaceutical composition for use in the alleviation or treatment of pain, the pharmaceutical composition comprising two or more selected from the group consisting of genes encoding GAD, IL-10, and GDNF.

The pain is as described above with regard to the pharmaceutical composition.

The subject may be a mammal including a human, or a cell and/or tissue isolated from a mammal including a human. The term "non-human animal" as used herein is intended to encompass all vertebrate animals, which include mammals and non-mammals such as primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc.

As for the administration route, dosage, and administration frequency, the pharmaceutical composition may be provided for use in the alleviation or treatment of pain in a subject in various ways and amounts depending on the condition of a patient and the presence or absence of side effects, and the range of optimal administration methods, dosages and administration frequencies may be appropriately selected by those having ordinary skill in the art. In addition, the pharmaceutical composition may be provided for

use in the alleviation or treatment of pain in combination with another drug or a physiologically active substance which is known to show a therapeutic efficacy on a disorder to be treated. Also, the pharmaceutical composition may be prepared in the form of a combination formulation.

Specifically, the pharmaceutical composition as disclosed herein may be provided in the form of an injection. For example, subcutaneous injection, intramuscular injection, intravenous injection, epidural injection, or intrathecal injection, and the like may be included. Specifically, the pharmaceutical composition may be provided for use in the alleviation or treatment of pain via epidural injection or intrathecal injection, and more specifically, it may be administered via transforaminal epidural injection or intrathecal injection.

As used herein, the term "transforaminal epidural injection" refers to a method of injecting a drug into the inside of an intervertebral foramen which is a space where nerves emerge from the spinal cord through the space between spinal bones, and into the space outside of the dura which surrounds the spinal cord and spinal nerves. In one example, if the pharmaceutical composition is made of viruses, the drug may be provided such that it can be administered to the inside of the intervertebral foramen of a subject by conducting epidural injection therapy.

As used herein, the term "intrathecal injection" refers to a method of administration by injecting a drug to a space inside dura in the spinal canal. In one embodiment, if the pharmaceutical composition as disclosed herein is made of plasmids, the drug may be

provided such that it can be administered to the inside of the spinal canal of a subject by conducting intrathecal injection therapy.

Specifically, if the pharmaceutical composition is made of viral vectors, it may be provided such that it can be administered in an amount of 1.0×10^6 to 1.0×10^{14} vg on an adult basis. In addition, when there are two types of viruses to be administered, each type of the viruses can be administered in an amount of 5.0×10^5 to 5.0×10^{13} vg. If there are three types of viruses to be administered, each type of the viruses may be provided such that it can be administered in an amount of 3.0×10^5 to 3.0×10^{13} vg.

In addition, if the pharmaceutical composition is made of non-viral vectors, it may be provided such that it can be administered in a concentration of 0.1 mg/ml to 10 mg/ml, on an adult basis. Also, if the pharmaceutical composition is made of plasmid vectors, the dosage may be 0.1 ml, 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, 8 ml, 9 ml, 10 ml or more, including all values and ranges between them.

As for the administration frequency of a viral vector, it may be provided for administration once or more, or 1 to 10 times. Also, it may be provided for administration at the interval of 1 day to 1 month, or 1 month to 1 year in the case of repeated administration. In addition, if the pharmaceutical composition is made of non-viral vectors, it may be provided for administration 1 or more, or 1 to 10 times. Also, it may be provided for administration at the interval of 12 to 24 hours or 1 to 14 days in the case of repeatedly administration.

The pharmaceutical composition as disclosed herein is provided for use in the alleviation or treatment of pain.

Also disclosed herein is the use of the pharmaceutical composition in the manufacture of a medicament for the alleviation or treatment pain.

Modes for Carrying Out the Invention

Hereinafter, the present disclosure will be described in more detail with reference to examples. However, the following examples are for illustrative purposes only and are not intended to limit the scope of the present disclosure.

Example 1. Preparation and property analysis of recombinant adeno-associated virus

The adeno-associated viruses required were constructed and produced on the basis of the AAV helper-free system (Agilent).

Example 1.1. Construction of pAAV-GAD65 plasmid

To construct the pAAV-GAD65 plasmid of Fig. 1, the CMV promoter region of pJDK-rGAD65 (Lee, Gene Ther, 2005) was amplified by PCR, and then the resultant was introduced into pGEM-T (Promega) to construct pGEM-T-CMV. The primer sequences used for CMV promoter amplification are as follows:

F-JDK (SEQ ID NO: 15): 5'-TTCGGCCGTCGAGGAGCTTGGCCCATTG-3'

R-JDK (SEQ ID NO: 16): 5'-GACGTCGACCTAGCTAGCGAATTCGGGGCCGCGGAG-3'.

As for the GAD65 gene, the gene represented by SEQ ID NO: 3 was designed by codon-optimization to be suitable for humans based on the human GAD65 (NCBI NM_000818.2) represented by the amino acid sequence of SEQ ID NO: 1, and referred

to Bioneer for gene synthesis. The hGAD65 gene introduced into pGEM-T was treated with NheI and SalI to prepare a 1.7 Kb DNA fragment. Thereafter, it was subjected to ligation with the 3.7 Kb DNA fragment obtained by treating pGEM-T-CMV with NheI and SalI, to complete pGEM-T-CMV-hGAD65 construction.

SV40pA was amplified by conducting PCR using pCI (Invitrogen) as a template, and then the resultant was treated with ClaI and SalI to prepare a 222 bp DNA fragment. The DNA fragment was subjected to ligation with the 5.4 Kb DNA fragment prepared by cutting pGEM-T-CMV-hGAD65 with ClaI and SalI, to finally prepare pGEM-T-CMV-hGAD65-SV40pA. The primer sequences used for SV40pA amplification are as follows:

To introduce the GAD65 expression cassette into pVAX1 (Invitrogen), the BamHI site was generated by mutagenesis downstream of the bGHpA. Then, the resultant was cut with MluI and NheI to prepare DNA fragments. The LITR and CMV promoter regions were amplified by PCR using pssAAV-GAD65 as a template and cloned into pGEM-T easy (Promega). Thereafter, the resultant was cut with AscI and NheI, and subjected to ligation with the pVAX1 vector previously prepared, to construct pVAX1-LITR-CMV. Primer sequences used for LITR and CMV promoter region amplification are as follows:

F-ITR (SEQ ID NO: 21): 5'-ATGGCGCGCCCCTGGCCTTTTGCTGGCC-3',

R-JDK (SEQ ID NO: 16): 5'-GACGTCGACCTAGCTAGCGAATTCGGGGCCGCGGAG-3'.

pVAX1-LITR-CMV was cut with NotI and NheI again to prepare DNA fragments. pssAAV-GAD65 was cut with EagI and NheI, and subjected to ligation with the DNA fragments previously prepared, to construct pVAX1-LITR-CMV-hGAD65-SV40pA.

The pVAX1-LITR-CMV-hGAD65-SV40pA was cut with HpaI and BamHI to prepare DNA fragments. In addition, pssAAV-SV40pA-RITR, which had been prepared by amplifying through PCR using pssAAV-GAD65 as a template and cloning into pGEM-T easy, was treated with HpaI and BamHI, to prepare DNA fragments. The two DNA fragments were ligated to complete pVAX1-LITR-CMV-hGAD65-SV40pA-RITR (hereinafter abbreviated as "pAAV-GAD65"). Primer sequences used for SV40pA and RITR region amplification are as follows:

The pAAV-GAD65, pAAV-IL-10, pAAV-GDNF, or pAAV-GDNF-IL-10 plasmids prepared in the Examples 1.1. to 1.4 were respectively transfected into human embryonic kidney cell line 293T cells using jetPRIME (Polyplus). The transfected cells were cultured in a 37°C incubator for 48 hours. Thereafter, the cell culture medium or cultured cells were harvested. The cells were dissolved with a solvent, and the prepared samples were treated with each of the antibodies to GAD65 (Merck Millipore), IL-10 (Santa Cruz), and GDNF (R&D systems), and subjected to western blotting.

Specifically, in the case of pAAV-GAD65, the human embryonic kidney cell line 293T cells were treated with 2 μg of pAAV-GAD65 plasmid and cultured for 48 hours. Thereafter, the cultured cells were dissolved and the expression of GAD65 in the cells was confirmed through western blotting.

In the case of pAAV-IL-10 and pAAV-GDNF plasmids, the human embryonic kidney cell line 293T cells were treated with 1 μg of pAAV-IL-10 or pAAV-GDNF plasmid, and cultured for 48 hours. Thereafter, the culture medium was harvested and the expression of IL-10 or GDNF in the medium was confirmed through western blotting.

In the case of the pAAV-GDNF-IL-10 plasmid, the human embryonic kidney cell line 293T cells were treated with 1 μg of pAAV-GDNF-IL-10 plasmid and cultured for 48 hours. Thereafter, the cultured cells were dissolved, and the expression of intracellular IL-10 and GDNF was confirmed through western blotting.

As a result, it was confirmed that the transfected pAAV-GAD65, pAAV-IL-10, pAAV-GDNF or pAAV-GDNF-IL-10 plasmid was expressed (Figs. 5 and 6).

Example 2. Preparation of recombinant adeno-associated virus

The AAV-IL-10 virus used in the experiment was produced and purified by UNC vector core. The production method is as follows. The pVAX-rIL-10, pHelper and pRC5 were transfected into human embryonic kidney cell line 293T cells. Thereafter, the resultant was subject to purification by column chromatography to secure AAV5-IL-10 virus. The titer of the produced virus was measured using qPCR.

The AAV-GAD65 and AAV-GDNF viruses were produced and purified by KRcrogen. The production method is as follows. The AAV-transgenes (pAAV-GAD65 and pAAV-GDNF plasmids) were respectively transfected into the human embryonic kidney cell line 293T cells using the calcium phosphate method along with pHelper and pRC. In the case of GAD65, pRC5 introduced with the capsid gene of serotype 5 was used. In the case of GDNF, pRC1 introduced with the capsid gene of AAV serotype 1 was used. The transfected cells were cultured in a 37°C incubator and the cells were harvested after 48 hours.

Thereafter, only the bands containing viruses were isolated and purified through the ultrahigh speed centrifugation method according to the cesium concentration gradient, to secure AAV5-GAD65, and AAV1-GDNF viruses. The titers of the produced viruses were measured using qPCR.

treated with the AAV-GAD65, AAV-GAD65-modi, AAV-IL-10 or AAV-GDNF virus obtained above, and protein expression was examined by western blotting. Specifically, 293T or HeLa cells were seeded at 5 x 10^5 cells/well in a 6-well plate. And on the next day, the cells were respectively treated with 3 types of viruses at 10,000 vg/well, and then cultured in a 37°C incubator. After 48 hours, the cells were harvested and were dissolved with a solvent and the culture medium was concentrated using the amicon (Merck Millipore). Then, the prepared samples were respectively treated with the antibodies to GAD65 (Cell signaling), IL-10 (Santa Cruz) and GDNF (R&D systems), and subjected to western blotting.

As a result, it was confirmed that each target protein was expressed in the cell lysate of human embryonic kidney cell line 293T or HeLa cell line treated with AAV-GAD65, AAV-GAD65-modi, AAV-IL-10 or AAV-GDNF virus (Fig. 7). Therefore, it was confirmed that there was no abnormality in the structures and properties of the recombinant adeno-associated viruses used in the experiment.

Also, in order to confirm that GABA is produced by AAV-GAD65 or AAV-GAD65-modi virus, the culture medium of the cells treated with the AAV-GAD65 or AAV-GAD65-modi virus was harvested and subjected to GABA ELISA (LDN) analysis. For each experimental group, two identical samples were prepared separately to conduct the analysis, and the bar graph shows the value for each sample.

As a result, it was confirmed that GABA was secreted to the culture medium by GAD65 introduced into cells by AAV-GAD65 or AAV-GAD65-modi virus (Fig. 8).

	vg/5 μl			
AAV-IL-10	-	9.0×10^8 vg/5 μl	-	-
AAV-GDNF	-	-	9.0×10^8 vg/5 μl	-
AAV-GAD65 + AAV-GDNF	4.5×10^8 vg/5 μl	-	4.5×10^8 vg/5 μl	-
AAV-IL-10 + AAV-GDNF	-	4.5×10^8 vg/5 μl	4.5×10^8 vg/5 μl	

Experimental Example 3.2. Construction of neuropathic pain-induced rats and administration of samples

150 to 200 g male SD-rats were subjected to inhalation anesthesia. And then the upper part of the calf was incised and both ends of the common peroneal nerve and tibial nerve were tied and knots were made at the interval of 0.5 to 1 cm by 7-0 suture. The regions between the knots of the two nerve bundles were cut with a scissor and the incision site was sutured. Thereafter, the rats were recovered to be awakened from the anesthesia and returned to the cage. Two weeks later, the *von Frey* filament test was

Claims:

1. A pharmaceutical composition for alleviating or treating pain comprising two or more selected from the group consisting of a gene encoding glutamate decarboxylase (GAD), a gene encoding interleukin-10 (IL-10), and a gene encoding a glial cell-derived neurotrophic factor (GDNF).

2. The pharmaceutical composition of claim 1, wherein the gene is in a form of being operably contained in a vector.

3. The pharmaceutical composition of claim 2, wherein the vector is at least one viral vector selected from the group consisting of adenovirus, adeno-associated virus, herpes simplex virus, lentivirus, retrovirus, and poxvirus.

4. The pharmaceutical composition of claim 2, wherein the vector is at least one non-viral vector selected from the group consisting of a plasmid, a liposome, a cationic polymer, a micelle, an emulsion, and solid lipid nanoparticles.

5. The pharmaceutical composition of any preceding claim, wherein the GAD is GAD65 or GAD67.

6. The pharmaceutical composition of any preceding claim, wherein the gene encoding GAD is the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 32, SEQ ID NO: 34 or SEQ ID NO: 36.

7. The pharmaceutical composition of any of claims 1 to 5, wherein the gene encoding GAD is the nucleotide sequence represented by SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 33, SEQ ID NO: 35 or SEQ ID NO: 37.

8. The pharmaceutical composition of any preceding claim, wherein the gene encoding IL-10 is the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 38, SEQ ID NO: 40 or SEQ ID NO: 42.

9. The pharmaceutical composition of any of claims 1 to 7, wherein the gene encoding IL-10 is the nucleotide sequence represented by SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 39, SEQ ID NO: 41 or SEQ ID NO: 43.

10. The pharmaceutical composition of any preceding claim, wherein the gene encoding GDNF is the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 11, SEQ ID NO: 44, SEQ ID NO: 46, or SEQ ID NO: 48.

11. The pharmaceutical composition of any of claims 1 to 9, wherein the gene encoding GDNF is the nucleotide sequence represented by SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 45, SEQ ID NO: 47 or SEQ ID NO: 49.

12. The pharmaceutical composition of any preceding claim, wherein the pain is nociceptive pain, psychogenic pain, inflammatory pain, pathological pain, neuropathic pain, cancer pain, postoperative pain, trigeminal neuralgia pain, idiopathic pain, diabetic neuropathic pain, or migraine.

13. The pharmaceutical composition of any preceding claim, wherein the pharmaceutical composition further comprises a physiologically acceptable carrier.

14. The pharmaceutical composition of any preceding claim, wherein the pharmaceutical composition is provided in the form of an injection.

15. A pharmaceutical composition as defined in any of claims 1 to 14 for use in the alleviation or treatment of pain.

16. The pharmaceutical composition of claim 15, wherein the use in the alleviation or treatment of pain is via epidural injection or intrathecal injection.

17. The pharmaceutical composition of claim 15 or claim 16, wherein the pain is nociceptive pain, psychogenic pain, inflammatory pain, pathological pain, neuropathic pain, cancer pain, postoperative pain, trigeminal neuralgia pain, idiopathic pain, diabetic neuropathic pain, or migraine.

18. Use of a pharmaceutical composition as defined in any of claims 1 to 14 in the manufacture of a medicament for the alleviation or treatment of pain.