

Description

Title of Invention

Composition for treating pain

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Technical Field

The present invention relates to a composition for relieving or treating pain, the composition comprising a gene encoding glutamate decarboxylase and a gene encoding an anti-inflammatory cytokine; and use of the composition for relieving or treating pain.

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Background Art

The term “pain” used by the International Association for the Study of Pain is defined as “actual or potential tissue damage or an unpleasant sensory and emotional experience associated therewith.” Pain protects parts of the damaged body during the healing of the damage from the damage occurrence and motivates a human being to avoid similar experiences in the future. Most pain is relieved gradually if the cause of stimulus is removed, but sometimes the pain persists even though the damaged site has clearly healed as the stimulus has disappeared, or the pain develops in a state where no irritation, damage or disease has occurred.

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Neuropathic pain is a type of non-malignant chronic pain that is induced by abnormality of a nerve, a spinal cord or a brain and is assumed that 1% or more of the population is suffering therefrom. The most common causes of neuropathic pain are trauma, metabolic and ischemic disorders, etc., which cause pathological nerve impulses to be transmitted to the brain through the spinal cord, resulting in pain occurrence. Neuropathic pain can be classified into peripheral neuropathic pain and central neuropathic pain depending on the location affected.

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If such neuropathic pain persists for a long period of time, it may lead to mental

pain as well as physical pain, and thus, the life quality of a patient is likely to decline. Therefore, active treatment for pain relief should be carried out. Currently, as the pain itself is recognized as a disease and interests in pain is spreading, demand for analgesics is expected to increase steadily in the future. Mainly, analgesics such as acetylsalicylic acid, ibuprofen, and acetaminophen are widely used in current pain treatments. If aspirin, whose main ingredient is acetylsalicylic acid, is used for analgesic purposes, a high dose of at least 500 mg should be administered. However, since aspirin is a non-steroidal anti-inflammatory analgesic (NSAIDs) and interferes with the production of gastric mucosa by blocking the enzyme (COX-1) that promotes the production of prostaglandin which plays a role in protecting gastric mucosa, the stomach can be easily damaged by gastric acid and gastrointestinal bleeding can easily occur. Further, since aspirin prevents thrombus formation, it can cause bleeding. Ibuprofen is also a non-steroidal anti-inflammatory analgesic ingredient, and therefore, it can cause gastric disturbances as well. In case of analgesics whose main ingredient is acetaminophen such as Tylenol, since acetaminophen is metabolized mostly in the liver, the liver may be damaged. Therefore, there are safety issues to lower its maximum dose, etc. In addition, long-term use of the above-mentioned analgesics often lead to resistance and loss of efficacies even if they are efficacious in the early stage, and specifically in case of neuropathic pain, there is a problem that the nonsteroidal anti-inflammatory agent is not effective even in the maximum dose for each patient, and thus high-dose administration for short term is prescribed. Therefore, the development of a new analgesic for neuropathic pain which exhibits excellent analgesic efficacy at low dose without side effects is urgently required.

Currently, several new substances are being developed as analgesics for neuropathic pain. Recently, sodium channel blockers have been developed, but they are mostly in the form of small molecules and have low selectivity to certain isoforms. Further, they show side effects such as cardio-toxicity, motor impairment, etc., and thus, more research in the future is necessary. As for a gene therapeutic agent, Periphagen

Holdings Inc. has developed a therapeutic agent in which a gene encoding enkephalin, an opioid peptide, was introduced into herpes simplex virus (HSV) gene carrier. However, in a clinical trial, the problem of declining in its analgesic efficacy has been found, and thus, its progression was halted at phase 2 study. HSV had the advantage of delivering genes to peripheral nerves even when subcutaneously administered, but, in fact, gene delivery efficiency is inferior and it seems to have difficulty in suppressing pain with only enkephalin, an opioid peptide.

Disclosure of Invention

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

An example as disclosed herein relates to a composition for relieving or treating pain, which comprises a gene encoding glutamate decarboxylase (GAD) and a gene encoding an anti-inflammatory cytokine, in particular a gene encoding interleukin-10 (IL-10).

An additional example is to a composition comprising a gene encoding GAD and a gene encoding an anti-inflammatory cytokine, in particular IL-10, for use in the relief or treatment of pain.

Also disclosed herein is a pharmaceutical composition for relieving or treating pain, which comprises a gene encoding glutamate decarboxylase (GAD) and a gene encoding an anti-inflammatory cytokine, in particular IL-10; and the pharmaceutical composition for use in the relief or treatment of pain.

Advantageous Effects

A pharmaceutical composition as disclosed herein can exhibit analgesic effect with only a small amount of gene delivery, and the pain-relieving efficacy can be observed even with a smaller amount of gene delivery as compared to single administration. GABA, a product of the GAD gene, has the efficacy of blocking pain

signal transduction, but excessive amount of it can cause symptoms such as itching, dizziness, drowsiness, etc., as well as side effects such as increased heartbeat rate or respiration rate. IL-10 is known to be a cytokine that exhibits anti-inflammatory effect, but side effects such as reducing RBC level, etc., were observed when high dose was used systemically. On the other hand, a pharmaceutical composition as disclosed herein can exhibit excellent analgesic efficacy even with a smaller dose by employing co-administration the combination of GAD and IL-10 as compared to single administration, and thus, it shows the synergistic efficacy of lowering conventional side effects and toxicity by low dose administration.

Brief Description of Drawings

Fig. 1 shows a vector map of the plasmid pAAV-hGAD65 used for the production of recombinant adeno-associated viruses.

Fig. 2 shows a vector map of the plasmid pAAV-rIL-10 used for the production of recombinant adeno-associated viruses.

Fig. 3 shows a vector map of the plasmid pAAV-rIL-4 used for the production of recombinant adeno-associated viruses.

Fig. 4 is a diagram confirming the expression level of each protein by Western blot, where adeno-associated viruses respectively introduced with GAD65, IL-10 and IL-4 genes were prepared, and then 293T cells, a human embryonic kidney cell line, were treated with the viruses, and cells or culture media were collected after 48 hours.

Fig. 5 illustrates the expression of GABA by a recombinant adeno-associated virus AAV-hGAD65, which is a diagram showing GABA levels in the media measured by ELISA, where 293T cells, a human embryonic kidney cell line, were treated with AAB-GAD65, and the culture media were collected after 48 hours. Two identical samples were prepared separately for each experiment group, and the bar represents the value for each sample.

Fig. 6 shows the results of comparing the efficacies of co-administration of AAV-

GAD65 and AAV-IL-10 with gabapentin administration, which is a graph confirming the difference in synergistic efficacies of co-administration of AAV-GAD65 and AAV-IL-10 as compared to gabapentin used as a neuropathic pain reliever in the market.

Fig. 7 illustrates efficacies of AAV-GAD65 and AAV-IL-10 depending on combination composition ratios thereof. Particularly, it is a graph showing the synergistic effects in animal behavior analysis with the combination composition ratios of AAV-IL-10 to AAV-GAD of 1: 1, 1: 5, 1: 30.

Fig. 8 is a graph showing the synergistic effects when AAV-GAD65 and AAV-IL-10 were co-administered at 1: 10 and 1: 30 by transforaminal epidural injection.

Fig. 9 shows the results of comparing the efficacies of AAV-GAD65 and AAV-IL-10 with AAV-GAD65 and AAV-IL-4, which illustrates the synergistic effects in animal behavior analysis when IL-10 and IL-4 were respectively combined with GAD65 for use.

Detailed Description

In one disclosed technique, a gene encoding GAD and a gene encoding an anti-inflammatory cytokine are provided in a form of being contained in a carrier, and the carrier includes viral vectors and non-viral vectors such as a plasmid, liposome, or the like.

The gene encoding GAD may be operably contained in a first vector, and the gene encoding an anti-inflammatory cytokine may be operably contained in a second vector.

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

A carrier containing a gene encoding GAD (e.g., a first vector) and a carrier containing a gene encoding an anti-inflammatory cytokine

(e.g., a second vector) may have a virus titer-based mixing ratio per unit volume of 1: 1 to 1: 100, 1: 1 to 1: 80, 1: 1 to 1: 60, 1: 1 to 1: 40, 1: 1 to 1: 20, 1: 1 to 1: 10, 1: 3 to 1: 100, 1: 3 to 1: 80, 1: 3 to 1: 60, 1: 3 to 1: 40, 1: 3 to 1: 20, or 1: 3 to 1: 10, more preferably 1: 1 to 1: 50, and most preferably 1: 5 to 1: 30.

5 A pharmaceutical composition as disclosed herein can exhibit analgesic effect with only a small amount of genes or carriers containing the same. The composition consists of a vector containing a gene encoding GAD and a vector containing an anti-inflammatory action gene in neural tissues, and the pain-relieving or treating efficacy can be achieved with a smaller amount as compared to single administration by co-
10 administering substances having different analgesic mechanisms. Accordingly, since a composition uses a small amount of genes or carriers containing the same, synergy of superior analgesic efficacies is shown while lowering toxicity.

 According to an example, the first vector and the second vector may be adeno-associated viruses. The adeno-associated virus is not limited to a specific serotype, and
15 preferably, it may be any one of AAV1 to AAV5.

 GAD may be an enzyme which decarboxylates glutamate to produce GABA (gamma-aminobutyric acid). The gene encoding GAD may be one of GAD65 and GAD67, which are two isoforms. The GAD65 may be a human or rat protein or a gene encoding the same, and specific example thereof may consist of the amino acid
20 sequence of SEQ ID NO: 1 of NCBI accession no. NM_000818, and also may consist of the base sequence of SEQ ID NO: 2 or SEQ ID NO: 3. The GAD67 may be a human or rat protein or a gene encoding the same, and specific example thereof may consist of the amino acid sequence of SEQ ID NO: 4 of NCBI accession no. NM_000817, and also may consist of the base sequence of SEQ ID NO: 5.

25 In a pharmaceutical composition as disclosed herein, the inflammatory cytokine may be interleukin-10 (IL-10), and synergistic pain-relieving

efficacy is exhibited by co-administration of IL-10 and GAD65.

The IL-10 is one of anti-inflammatory cytokines and is also known as a cytokine synthesis inhibitory factor (CSIF). IL-10 belongs to the class II cytokine and is a homodimer consisting of two subunits of 178 amino acids in length. IL-10 serves the function of inhibiting the activity of NK (natural killer) cells in an immune response, and is involved in signal transduction by forming a complex with the IL-10 receptor. IL-10 may be a human or rat protein or a gene encoding the same, and specific example thereof may consist of the amino acid sequence of SEQ ID NO: 6 of NCBI accession no. NM_012854 or SEQ ID NO: 9 of NCBI accession no. NM_000572, and also may consist of the base sequence of SEQ ID NO: 7, SEQ ID NO: 8 or SEQ ID NO: 10.

The base sequences of the gene encoding GAD and/or the gene encoding IL-10 of may include mutants thereof, which may be base sequences modified by codon optimization for rats or humans. Specifically, the base sequences having sequence homology of at least 60%, preferably 70% or more, more preferably 80% or more, even more preferably 90% or more, most preferably 95% or more are included, where the “sequence homology %” is identified by comparing two optimally arranged sequences and comparison regions, and a part of the sequences in the comparison region may include addition or deletion (i.e., gap) as compared to a reference sequence (not including addition or deletion) regarding the optimal arrangements of the two sequences.

The present techniques may also provide the pharmaceutical composition for use in the relief and/or treatment of pain. A therapeutically effective amount of the pharmaceutical composition may be administered to a patient in need of relieving and/or treating pain. Prior to administration, it is typically determined whether the patient is a patient in need of relieving and/or treating pain. The term “therapeutically effective amount” may depend on the amount of an active gradient to achieve a desired efficacy, pain relief and/or therapeutic efficacy.

The pain according may include nociceptive pain, psychogenic pain, inflammatory pain associated with tissue damage and immune cell infiltration, pathological pain which is a disease state induced by damage to the nervous system or its abnormal function (dysfunctional pain such as fibromyalgia, irritable bowel syndrome, tension headache), etc. Also, the pain may include back pain distinguished anatomically, such as: neck pain, middle back pain, lower back pain, or tailbone pain. In addition, the pain may include pain such as neuropathic pain, migraine, etc. Neuropathic pain can result from damage or diseases that affect the somatosensory system. Neuropathic pain may be associated with an abnormal sensation called
5 dysesthesia, and with allodynia in which pain sensation is evoked even with painless stimulation. Also, the neuropathic pain may also be a continuous and/or intermittent (seizure) factor. The latter is linked to electric shock in the figurative sense. General characteristics include being hot or cold, pins and needles, numbness, and itching.

In contrast, nociceptive pain is often expressed as “ache”. In addition, migraine is
15 a chronic disorder that is associated with a number of autonomic nervous system symptoms and causes headaches of ordinary to severe intensities. The precise mechanisms of these migraines have not been clarified yet. The basic theory is related to the increased excitability of the cerebral cortex and the abnormal regulation of pain nerve cells in the trigeminal nucleus of the brainstem.

As a specific example, pain may be at least one selected from the group consisting
20 of neuropathic pain, cancer pain, postoperative pain, trigeminal neuralgia, idiopathic pain, diabetic neuropathic pain, migraine, etc. As another specific example, the pain may not be a muscle spasm associated with lumbago.

Preferably, a composition may be used for relieving or treating neuropathic pain
25 or chronic cancer pain. The term “relieving or treating” refers to any action that alleviates or improves pain symptom by administering a composition as disclosed herein.

An additional example relates to a method for relieving or

treating pain comprising administering a gene encoding GAD and a gene encoding an anti-inflammatory cytokine to a subject in need thereof. The subject may be a mammal including a human, or a cell and/or tissue isolated from a mammal including a human. Also, the subject may be a non-human animal, and the term “non-human animal” includes vertebrates such as mammals and non-mammals, for example, primates except humans, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc.

The therapeutically effective amount and administration route of a pharmaceutical composition can be appropriately adjusted in consideration of a condition of the patient, a desired efficacy, and the like by those of ordinary skill in the art. For example, a composition may be provided in the form of an injection, and may include, for example, nerve injection, subcutaneous injection, intramuscular injection, or gene gun injection.

Mode for the Invention

Hereinafter, the present invention is explained in detail by Examples, but the following Examples are intended to further illustrate the present invention without limiting its scope.

<Example 1> Preparation and property analysis of recombinant adeno-associated viruses

The adeno-associated viruses required for the present techniques were prepared and produced based on the AAV helper-free system (Agilent, USA).

A. Preparation of pAAV-hGAD65

To prepare pAAV-hGAD65 of Fig. 1, the CMV promoter region of pJDK-rGAD65 [Lee B et al., Gene Ther, 12: 1215-1222 (2005)] was amplified by PCR and then introduced into pGEM-T (Promega, USA), to prepare pGEM-T-CMV. The primer

[Table 1]

Samples	Virus types and contents	
	AAV-GAD65	AAV-IL-10
Comparative Example 1 (GAD alone)	5.4×10^5 VG/ 2 μ L	0
Comparative Example 2 (IL-10 alone)	0	1.8×10^7 VG/ 2 μ L
Experimental Example 1 (1:1)	5.4×10^5 VG/ 1 μ L	5.4×10^5 VG/ 1 μ L
Experimental Example 2 (1:5)	5.4×10^5 VG/ 1 μ L	2.7×10^6 VG/ 1 μ L
Experimental Example 3 (1:30)	5.4×10^5 VG/ 1 μ L	1.8×10^7 VG/ 1 μ L

B. Observation of analgesic efficacy using *von Frey* filament test

Samples were administered to the pain animal models prepared by the same method as in Example 2, and pain was observed using a *von Frey* filament test, and the results are shown in Fig. 7.

Fig. 7 illustrates efficacies of AAV-GAD65 and AAV-IL-10 depending on composition ratios thereof. Particularly, compared to trace amounts of AAV-GAD65 and AAV-rIL-10 which showed no analgesic efficacy experiments with the mixing composition ratios of AAV-rIL-10 to AAV-GAD of 1:1 (Experimental Example 1), 1:5 (Experimental Example 2) and 1:30 (Experimental Example 3) exhibited synergistic efficacies in animal behavior analysis. As a result, the co-administration composition of AAV-GAD65 and AAV-rIL-10 showed an increasing pattern of the pain treatment efficacy as the mixing composition ratio of AAV-rIL-10 to AAV-GAD increased.

<Example 4> Analgesic efficacy tests of AAV-IL-10 and AAV-GAD65 using transforaminal epidural injection

Claims:

Claim 1. A pharmaceutical composition for relieving or treating pain, which comprises a gene encoding glutamate decarboxylase (GAD) and a gene encoding interleukin-10 (IL-10).

Claim 2. The composition of claim 1, wherein the composition comprises a first vector operably containing a gene encoding GAD and a second vector operably containing a gene encoding IL-10.

Claim 3. The composition of claim 2, wherein the composition comprises a first vector containing a gene encoding GAD and a second vector containing a gene encoding IL-10 in a virus titer-based mixing ratio per unit volume of 1: 1 to 1: 50.

Claim 4. The composition of claim 3, wherein the first vector and the second vector comprise at least one viral vector selected from the group consisting of adenovirus, adeno-associated virus, herpes simplex virus, lentivirus, retrovirus and poxvirus.

Claim 5. The composition according to any preceding claim, wherein the GAD is at least one selected from the group consisting of GAD65 and GAD67.

Claim 6. The composition of claim 5, wherein the GAD consists of the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 4.

Claim 7. The composition of claim 5, wherein the gene encoding GAD consists of the base sequence of SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 5.

Claim 8. The composition according to any preceding claim, wherein the IL-10

consists of the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 9.

Claim 9. The composition according to any of claims 1 to 7, wherein the gene encoding IL-10 consists of the base sequence of SEQ ID NO: 7, SEQ ID NO: 8 or SEQ ID NO: 10.

Claim 10. The composition according to any preceding claim, wherein the pain is nociceptive pain, psychogenic pain, inflammatory pain, or pathological pain.

Claim 11. The composition of claim 10, wherein the pain is neuropathic pain, cancer pain, postoperative pain, trigeminal neuralgia, idiopathic pain, diabetic neuropathic pain, or migraine.

Claim 12. The composition of claim 1, wherein the pharmaceutical composition further comprises a physiologically acceptable carrier.

Claim 13. The composition according to any preceding claim, wherein the pharmaceutical composition is provided in the form of an injection.

Claim 14. A pharmaceutical composition as defined in any one of claims 1 to 13 for use in the relief or treatment of pain.

Claim 15. The pharmaceutical composition of claim 14, wherein the pain is nociceptive pain, psychogenic pain, inflammatory pain, or pathological pain.

Claim 16. The pharmaceutical composition of claim 15, wherein the pain is neuropathic pain, cancer pain, postoperative pain, trigeminal neuralgia, idiopathic pain, diabetic neuropathic pain, or migraine.