

Description

ANTI-INFLAMMATORY AGENT

Technical Field

[0001]

The present invention relates to a highly safe anti-inflammatory agent.

Background Art

[0002]

With the super-aging society, the importance of efforts to prevent and treat lifestyle-related diseases such as metabolic diseases, e.g., diabetes and obesity, and cardiovascular diseases such as arteriosclerosis, which are associated with chronic inflammation, is increasing. In fact, for diabetes, inhibition of IL-1 β signaling and NF- κ B inhibitory action have been developed. In chronic inflammatory diseases represented by rheumatoid arthritis, large amounts of inflammatory cytokines such as TNF- α , IL-1 and IL-6 are abnormally produced and secreted, and inhibitors of these inflammatory cytokines are useful as excellent anti-inflammatory agents. For example, as TNF- α inhibitors, antibody drugs such as infliximab has been used as therapeutic drugs for rheumatoid arthritis (Non Patent Literature 1). In addition, as an IL-6 inhibitor, the antibody drug tocilizumab has been used (Non Patent

Literature 2). Furthermore, as IL-1 inhibitors, protein preparations and antibody drugs such as anakinra, rilonacept and canakinumab have been developed (Non Patent Literature 3).

Citation List

Non Patent Literature

[0003]

Non Patent Literature 1: TNF inhibitor guidelines for rheumatoid arthritis (RA) (Japan College of Rheumatology)

Non Patent Literature 2: Yakugaku Zasshi, 129(6) 667-674 (2009)

Non Patent Literature 3: The Journal of the Japanese Society of Internal Medicine, 100:10 p. 2985-2990 (October 10, 2011)

Summary of the Invention

Problems to be Solved by the Invention

[0004]

However, most of the conventional inflammatory cytokine inhibitors are biological preparations represented by antibody drugs, and are reported to have adverse effects such as abnormal liver functions, infection and anaphylaxis, thus usage guidelines such as Non Patent Literature 1 have been created.

Therefore, an object of the present invention is to provide an anti-inflammatory agent based on new

inflammatory cytokine inhibition that is highly safe and can be widely used.

Means for Solving the Problems

[0005]

Therefore, the present inventors have examined the actions of various components on the production of inflammatory cytokines, found that a liver hydrolysate, which has been widely used for the improvement of liver functions in chronic liver disease, the improvement of hangover and the like, has a strong IL-1 production suppressing action and therefore is useful as an anti-inflammatory agent by suppressing the production of inflammatory cytokines, and completed the present invention.

[0006]

That is, the present invention provides the following [1] to [15].

[0007]

[1] An anti-inflammatory agent, comprising a liver hydrolysate as an active ingredient.

[2] An agent for suppressing inflammatory cytokine production, comprising a liver hydrolysate as an active ingredient.

[3] An agent for suppressing IL-1 production, comprising a liver hydrolysate as an active ingredient.

[4] A food composition for improving inflammation, comprising a liver hydrolysate as an active ingredient.

[5] A food composition for suppressing inflammatory cytokine production, comprising a liver hydrolysate as an active ingredient.

[6] A food composition for suppressing IL-1 production, comprising a liver hydrolysate as an active ingredient.

[7] Use of a liver hydrolysate for producing an anti-inflammatory agent.

[8] Use of a liver hydrolysate for producing an agent for suppressing inflammatory cytokine production.

[9] Use of a liver hydrolysate for producing an agent for suppressing IL-1 production.

[10] A liver hydrolysate for use in treating an inflammatory disease.

[11] A liver hydrolysate for use in suppressing inflammatory cytokine production.

[12] A liver hydrolysate for use in suppressing IL-1 production.

[13] A method for treating an inflammatory disease, the method comprising administering an effective amount of a liver hydrolysate.

[14] A method for suppressing inflammatory cytokine production, the method comprising administering an effective amount of a liver hydrolysate.

[15] A method for suppressing IL-1 production, the method comprising administering an effective amount of a liver hydrolysate.

Effects of the Invention

[0008]

Since the liver hydrolysate has a strong IL-1 production suppressing action, it suppresses the production of inflammatory cytokines and is useful as an anti-inflammatory agent. In addition, the liver hydrolysate can also be used as a food composition since it can be orally administered, has very few adverse effects and is excellent in safety and usability.

Brief Description of the Drawing

[0009]

[Figure 1] Figure 1 shows the effect of liver hydrolysate on IL-1 β production.

Modes for Carrying out the Invention

[0010]

The active ingredient of the anti-inflammatory agent of the present invention is a liver hydrolysate. The liver hydrolysate, which is also called a hydrolyzed liver, a liver extract, a hydrolyzed liver extract, or a hepatic hydrolysate, is obtained by hydrolyzing liver with digestive enzymes and the like, and is used as an agent for improving liver functions. As the liver, which is the raw material, fresh livers of cows, pigs, bonitos, whales and the like are used. It is preferable to use the obtained hydrolysate after concentrating it. Preferably, the liver hydrolysate defined as the above medicine can be mentioned.

[0011]

The liver hydrolysate contains various amino acids, nucleotides, vitamins, minerals and the like, with low molecular weight peptides as the main component. More specifically, the liver hydrolysate preferably contains 19 to 78 mass% of amino acids, 17 to 73 mass% of peptides and proteins, 1.8 to 11 mass% of saccharides, 0.005 to 0.04 mass% of lipids, 0.7 to 2.5 mass% of nucleic acids, 1.6 to 5.4 mass% of inorganic substances, 0.03 to 0.2 mass% of vitamins and 0.8 mass% or less of glutathione. In addition, the liver hydrolysate more preferably contains 23 to 65 mass% of amino acids, 20 to 61 mass% of peptides and proteins, 2.2 to 8.6 mass% of saccharides, 0.006 to 0.035 mass% of lipids, 0.9 to 2.1 mass% of nucleic acids, 1.9 to 4.5 mass% of inorganic substances, 0.04 to 0.15 mass% of vitamins and 0.7 mass% or less of glutathione, and it even more preferably contains 29 to 52 mass% of amino acids, 25 to 49 mass% of peptides and proteins, 2.8 to 6.9 mass% of saccharides, 0.008 to 0.03 mass% of lipids, 1.1 to 1.7 mass% of nucleic acids, 2.4 to 3.6 mass% of inorganic substances, 0.05 to 0.12 mass% of vitamins and 0.6 mass% or less of glutathione.

[0012]

Among these components, the amino acid composition is preferably: 17 to 68 mg/g Ala, 0.6 to 4.4 mg/g Arg, 9 to 48 mg/g Asp, 5 mg/g or less of Cystine, 18 to 63 mg/g Glu, 10 to 39 mg/g Gly, 3 to 17 mg/g His, 14 to 56 mg/g Ile, 26 to 98 mg/g Leu, 15 to 65 mg/g Lys, 0.3 to 20 mg/g

Met, 13 to 46 mg/g Phe, 10 to 48 mg/g Pro, 12 to 49 mg/g Ser, 12 to 45 mg/g Thr, 3 to 13 mg/g Trp, 1.6 to 41 mg/g Tyr, and 18 to 71 mg/g Val. In addition, the amino acid composition is more preferably: 21 to 57 mg/g Ala, 0.8 to 3.6 mg/g Arg, 11 to 40 mg/g Asp, 4 mg/g or less of Cystine, 22 to 53 mg/g Glu, 13 to 32 mg/g Gly, 4 to 14 mg/g His, 17 to 47 mg/g Ile, 32 to 82 mg/g Leu, 18 to 54 mg/g Lys, 0.4 to 17 mg/g Met, 15 to 38 mg/g Phe, 12 to 40 mg/g Pro, 15 to 41 mg/g Ser, 14 to 38 mg/g Thr, 3.8 to 11 mg/g Trp, 1.9 to 34 mg/g Tyr and 21 to 59 mg/g Val, and even more preferably: 26 to 45 mg/g Ala, 1 to 2.9 mg/g Arg, 14 to 32 mg/g Asp, 3 mg/g or less of Cystine, 27 to 42 mg/g Glu, 16 to 26 mg/g Gly, 5 to 11 mg/g His, 21 to 40 mg/g Ile, 40 to 66 mg/g Leu, 22 to 43 mg/g Lys, 0.5 to 14 mg/g Met, 19 to 31 mg/g Phe, 15 to 32 mg/g Pro, 18 to 33 mg/g Ser, 18 to 30 mg/g Thr, 4.8 to 8.4 mg/g Trp, 2.4 to 27 mg/g Tyr, and 27 to 48 mg/g Val.

[0013]

Moreover, in the present invention, low molecular weight fractions of the liver hydrolysate can also be used. As the low molecular weight fraction of the liver hydrolysate, a fraction having a molecular weight of 3000 or less, for example, one obtained by collecting a fraction having a molecular weight of 3000 or less from the above liver hydrolysate using an ultrafiltration membrane or the like, can be used.

[0014]

As shown in the Examples described later, the liver hydrolysate and its low molecular weight fractions have an action of strongly suppressing the production of IL-1 β , which is a type of inflammatory cytokines, and an action of suppressing the mRNA expression of IL-1 β . Therefore, the liver hydrolysate suppresses the production of inflammatory cytokines represented by IL-1, and is useful as a therapeutic agent for inflammatory diseases involving inflammatory cytokines and as a food and beverage composition for improving inflammation. Here, diseases involving inflammatory cytokines such as IL-1 include rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, sepsis, acute and chronic myeloid leukemia, osteoporosis and lifestyle-related diseases.

[0015]

The anti-inflammatory agent of the present invention can be administered by oral administration, percutaneous administration, enteral administration, intravenous administration and the like, but oral administration is more preferable. Examples of preparations for oral administration include solutions, tablets, powders, fine granules, granules and capsules, but solutions and tablets are preferable, and solutions are more preferable.

[0016]

To prepare these oral administration preparations, excipients such as lactose, mannitol, corn starch and crystalline cellulose, binders such as cellulose

derivatives, gum arabic and gelatin, disintegrants such as calcium carboxymethylcellulose, lubricants such as talc and magnesium stearate, solubilizing agents such as nonionic surfactants, flavoring agents, sweetening agents, stabilizers, pH adjusters, water, ethanol, propylene glycol, glycerin and the like can be used. Moreover, coating agents such as hydroxymethylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, cellulose acetate phthalate and methacrylate copolymer may also be used.

[0017]

In addition, the anti-inflammatory agent of the present invention can be formulated with other active ingredients. Other active ingredients include vitamin B₁ compounds such as thiamine, thiamine nitrate, thiamine hydrochloride, fursultiamine, bisbentiamine, benfotiamine, thiamine disulfide, dicethiamine, thiamine propyl disulfide and derivatives thereof; vitamin B₂ compounds such as riboflavin and its derivatives and salts thereof; vitamin B₃ compounds such as niacin, nicotinic acid, nicotinamide and their derivatives and salts thereof; vitamin B₅ compounds such as panthenol, pantothenic acid and their derivatives and salts thereof; vitamin B₆ compounds such as pyridoxine and its derivatives and salts thereof; vitamin B₁₂ compounds such as cyanocobalamin and its derivatives and salts thereof; other vitamins such as vitamin A, vitamin C, vitamin E, vitamin K and vitamin P; diisopropylamine dichloroacetate,

taurine, chondroitin sulfate, royal jelly, caffeine, turmeric, milk thistle, Japanese dandelion, dandelion, burdock, garlic, chrysanthemum, yarrow, common gardenia, sesame, notoginseng, asparagus, onion, chicory, common sage, artichoke, Chinese desert-thorn, plants of the family Fabaceae or the family of Iridaceae, miyama-uzura (*Goodyera schlechtendaliana*), erva de passarinho, sete sangrias, Japanese Mallotus, black tea, resveratrol, catechins, berberine, rosemary, bean extract, metformin, bromelain, arginine, black cohosh, eyebright, propolis, shark cartilage, horehound, alfalfa, astragalus, belladonna, blessed thistle, marigold, chamomile, devil's clow, toki (*Angelica acutiloba*), echinacea, elder, eucalyptus oil, feverfew, ginger, ginseng, golden seal, Indian pennywort, hop, kava, lavender, licorice, saw palmetto, spirulina, St. John's wort, tea tree oil, valerian and wild yam.

[0018]

In addition, since the anti-inflammatory agent of the present invention is highly safe and orally administrable, it can be used not only as a medicine, but also as a quasi-drug, a food composition such as a functional food, e.g., a health functional food, a sport drink, a beverage for rehabilitation and a pet food, and the like.

[0019]

The content of the liver hydrolysate in the anti-inflammatory agent of the present invention varies

depending on the mode of administration, but usually, 0.001 to 10 mass% is preferable, and 0.001 to 5 mass% is more preferable, in dry weight. Moreover, the daily dose of the liver hydrolysate in the anti-inflammatory agent of the present invention is preferably 100 mg to 1076 mg, more preferably 383 mg to 623 mg and even more preferably 351 mg to 680 mg, in dry weight.

Examples

[0020]

Next, the present invention is described in detail by showing Examples, but the present invention is not in any way limited thereto.

[0021]

Production Example 1

The filter part of an ultrafiltration membrane (MICROCON (registered trademark) Centrifugal Devices YM-3) capable of fractionating with a molecular weight of 3000 is prewashed with phosphate buffer (PBS). After diluting 100 mg/mL of the liver hydrolysate (manufactured by Zeria Pharmaceutical Co., Ltd.) stock to 12 mg/mL with PBS, a portion of the sample was stored for addition to cells (pre-fractionation sample). 500 μ L of the diluted 12 mg/mL sample was dispensed to the ultrafiltration membrane, centrifuged at 12000 rpm, 4°C for 260 min, and the filtrate fraction (liver hydrolysate low molecular weight fraction: under Molecular Weight 3000: uMW 3 k) was recovered.

[0022]

Test Example 1

The number of RAW264.7 cells was adjusted to 1.5×10^6 cells/dish, seeded, and cultured overnight. The pre-fractionation sample and the filtrate fraction were diluted 20 times with EMEM culture medium (final concentration: about 0.6 mg/mL), respectively. For the control, PBS was diluted 20 times with EMEM culture medium instead of the sample. Each of the above samples diluted in culture medium (control, pre-fraction and filtrate fraction) was added to each dish group. After culturing for 24 hours, lipopolysaccharide (LPS) was added to a final concentration of 1.0 $\mu\text{g/mL}$. After leaving it to stand for 3 hours, RNA was recovered and cDNA synthesis was performed by reverse transcription. Next, the measurement of EEF1A1 and IL-1 β was performed by RT-PCR.

[0023]

The results are shown in Figure 1.

When the amount of mRNA expression of inflammation-related genes in the liver hydrolysate group was compared with the Cont group to which PBS had been added instead of the liver hydrolysate in RAW cells stimulated with LPS, IL-1 β was significantly decreased, suggesting the inflammation suppressing effect of liver hydrolysate (PLH: pre-fractionation). In addition, in the liver hydrolysate low molecular weight fraction (uMW3k) group,

a significant decrease in the amount of mRNA expression of IL-1 β was similarly observed.

The above results suggest that liver hydrolysate has an anti-inflammatory effect and that a physiologically active substance exhibiting an anti-inflammatory effect is present in the low molecular weight fraction having a molecular weight of 3000 or less in the liver hydrolysate.

Claims

[Claim 1]

An anti-inflammatory agent, comprising a liver hydrolysate as an active ingredient.

[Claim 2]

An agent for suppressing inflammatory cytokine production, comprising a liver hydrolysate as an active ingredient.

[Claim 3]

An agent for suppressing IL-1 production, comprising a liver hydrolysate as an active ingredient.

[Claim 4]

A food composition for improving inflammation, comprising a liver hydrolysate as an active ingredient.

[Claim 5]

A food composition for suppressing inflammatory cytokine production, comprising a liver hydrolysate as an active ingredient.

[Claim 6]

A food composition for suppressing IL-1 production, comprising a liver hydrolysate as an active ingredient.

[Claim 7]

Use of a liver hydrolysate for producing an anti-inflammatory agent.

[Claim 8]

Use of a liver hydrolysate for producing an agent for suppressing inflammatory cytokine production.

[Claim 9]

Use of a liver hydrolysate for producing an agent for suppressing IL-1 production.

[Claim 10]

A liver hydrolysate for use in treating an inflammatory disease.

[Claim 11]

A liver hydrolysate for use in suppressing inflammatory cytokine production.

[Claim 12]

A liver hydrolysate for use in suppressing IL-1 production.

[Claim 13]

A method for treating an inflammatory disease, the method comprising administering an effective amount of a liver hydrolysate.

[Claim 14]

A method for suppressing inflammatory cytokine production, the method comprising administering an effective amount of a liver hydrolysate.

[Claim 15]

A method for suppressing IL-1 production, the method comprising administering an effective amount of a liver hydrolysate.