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(54) Title: DIPEPTIDES FOR PREVENTION OF MUSCLE BREAKDOWN AND MICROBIAL INFECTION

(57) Abstract: The subject invention provides dipeptides useful in promoting healthy muscle tissues as well as effective immune responses. The dipeptides of the subject invention are particularly advantageous because they are stable, bioavailable, and can be formulated in an aqueous solution.



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DESCRIPTIONDIPEPTIDES FOR PREVENTION OF MUSCLE BREAKDOWN AND
MICROBIAL INFECTION

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Background of the Invention

Prevention or inhibition of muscle breakdown, and the facilitation of efficient muscle repair is of great interest to athletes, the elderly, and those with muscle-wasting conditions. A wide range of products and methods have been proposed for enhancing healthy muscle tissues and enhancing athletic performance. Existing compositions and methods which are available for these purposes suffer from a variety of shortcomings. These shortcomings range from potentially dangerous side affects, to a lack of bioavailability and difficulty in formulation and/or administration.

It is well known that proteins are converted to amino acids in the digestive system and that the resulting amino acids are used by the body for growth and development. In certain medical situations a patient may be unable to receive proteins. In these situations patients have been given free amino acids. Free amino acids, however, are sometimes not tolerated well by patients and may cause diarrhea and dehydration. Also, the free amino acids may be unstable and/or difficult to formulate.

It has been observed that the body can more effectively absorb certain small molecules called dipeptides or tripeptides. These molecules consist of, for example, two to three amino acids. It has been observed, for example, that peptides containing the amino acid residue glycine in the N-terminal position are readily assimilable. See for example, U.S. Patent No. 4,340,592.

European Patent Application No. 0,182,356 discloses a nutritional composition containing at least one oligopeptide consisting of a dipeptide or a tripeptide wherein the N-terminal amino acid residue is selected from the class consisting of alanine, lysine and arginine.

One group conducting research in this area concluded that glycine is generally superior to other amino acids as the N-terminal amino acid residue in a dipeptide. This superiority was attributed to a greater fraction of such an intravenously administered

dipeptide reaches the tissues. S. Adibi *et al.*, *Influence of Molecular Structure on Half-life and Hydrolysis of Dipeptides in Plasma: Importance of Glycine as N-Terminal Amino Acid Residue*, 35 Metabolism 850, 835 (1986).

Two European patents, 0,087,751 and 0,087,750 disclose water-soluble peptides. The '751 patent discloses a method to parenterally administer low water-soluble amino acids. Two amino acids, tyrosine and cystine, individually have low solubility in water. These amino acids, however, are clinically useful and, therefore, it was desirable to find an effective formulation. The '751 patent describes an infusion method which involves bonding these relatively insoluble amino acids to the amino acid lysine to produce a tripeptide.

The '750 patent discloses the infusion of glutamine as a derivative substituted by α -aminoacyl residues on the α amino group. That is, glutamine is in the "c-terminal" position, in that its alpha amino nitrogen becomes part of the peptide bond with the other amino acid. The preferred dipeptide preparation disclosed in the '750 patent is alanyl-glutamine. The aminoacylation of glutamine is reported to achieve a stabilization of the terminal amide group.

Experiments involving the use of total parenteral nutrition (TPN) containing glycyl-glutamine dipeptides, however, suggest potential adverse effects of the TPN formulation containing glycyl-glutamine (U.S. Patent no. 5,189,016).

Two commercially available dipeptides of glutamine are Dipeptiven, which is an alanyl-glutamine (Fresenius Laboratories, Germany) and Glamin (Pharmacia and Upjohn Laboratory, Sweden), which is an amino acid solution containing glycyl-glutamine dipeptide. To this date, there are no studies of the arginyl-L-glutamine dipeptide.

It is well known that nutrition can impact the functioning of the immune system. Proper nutrition can promote healthy immune responses. There are many aspects to the immune response of humans and animals. One component of the immune response is mucosal immunity. Mucosal immunity provides a first line of defense for the body against a broad range of pathogens.

There remains a great need in the art for compositions and methods which promote healthy muscle tissue, reduce muscle deterioration, and/or promote a healthy immune system.

Brief Summary of the Invention

In one embodiment, the subject invention provides materials and methods useful in promoting healthy muscle tissue. The promotion of healthy muscle tissue according to the subject invention is particularly advantageous for athletes and others engaging in rigorous physical exertion and/or training. The promotion of healthy muscle tissue according to the subject invention is also advantageous for the treatment and/or prevention of muscle wasting conditions. This is particularly advantageous for critically ill hospitalized patients including neonates.

The compositions of the subject invention are also useful in promoting mucosal immunity. Such immunity, which is often typified by an IgA response, can be a critical defense against a variety of human and animal pathogens. These pathogens may be, for example, bacteria, viruses, or parasites.

In a preferred embodiment of the subject invention, the amino acids arginine and glutamine are combined as the dipeptide arginyl-glutamine in order to provide beneficial effects in a safe, easily absorbable formulation. The dipeptide of the subject invention promotes healthy muscle tissue and an advantageous immune response. The dipeptide of the subject invention is also advantageous because it is safe for human or animal consumption and can be readily formulated in an aqueous solution for internal consumption.

The compositions of the subject invention can be used in a variety of situations where it is desired to promote healthy muscle tissue. For example, the dipeptides of the subject invention can be used in medical applications to aid critically ill patients. Also, the arginyl-glutamine dipeptide can be applied to enhance athletic performance.

The compositions can also be used to promote a healthy immune system in humans or animals. This is particularly advantageous for patients and hospital workers, or others who may be exposed to pathogens.

These peptides of the subject invention can be administered as one component of a nutrient composition. The use of these peptides, compared to the administration of equivalent amounts of the free amino acids, cause a decrease in osmolarity of the solution, facilitate the administration of amino acids having low water solubility, and stabilize heat unstable amino acids such as glutamine. The aqueous solution is suitable

for intravenous feeding or for intragastrintestinal administration. The aqueous solution itself may contain other nutrient additives such as fats, glucose, mono- or oligo-saccharides, minerals, trace elements and/or vitamins.

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Detailed Disclosure of the Invention

The present invention relates to nutrient compositions containing dipeptides and methods for administering the same. Advantageously, the subject invention provides a dipeptide having water solubility, stability to sterilization, long-term stability, and bioavailability for humans and animals. A preferred embodiment of the present invention provides a nutrient composition comprising an aqueous solution having at least one arginyl-glutamine dipeptide.

Among the advantages of the dipeptide of the subject invention over the existing alanyl-glutamine and glycyl-glutamine dipeptides is that the arginine moiety is particularly advantageous because it is a creatine phosphate precursor, a stimulator of immune function, a stimulator of growth hormone production and, in combination with glutamine, is particularly useful in strengthening mucosal immune defenses.

In one embodiment of the subject invention the arginyl-glutamine dipeptides described herein are useful for promoting healthy muscle tissue. Thus, these dipeptides can be used by athletes or others who are undergoing training or other rigorous physical exertion. The dipeptide compositions of the subject invention can also be used to promote muscle repair and maintenance in hospital or hospice patients, or other individuals subject to muscle deterioration.

In a further embodiment, the subject invention provides materials and methods for enhancing the immune system functioning of humans and animals. In this embodiment, compositions which comprise the arginyl-glutamine dipeptide are administered to people or animals who could benefit from an improved immune response. Specifically, the dipeptides of the subject invention can be used to enhance mucosal immunity. This enhancement of mucosal immunity reduces the risk of infection by a variety of pathogens including bacteria, viruses, and parasites.

In view of the muscle maintenance characteristics of the arginyl-glutamine dipeptide combined with its ability to stimulate an effective immune response, the

compositions of the subject invention are particularly attractive for use in hospitals. For example, compositions containing the dipeptide of the subject invention can be used to maintain muscle mass in inactive patients while having the added benefit of reducing susceptibility to hospital acquired infections in patients.

5 In accordance with the teachings provided herein, aqueous clinical nutrient compositions can be prepared which include at least one arginyl-glutamine dipeptide. The dipeptide can be added to enteral or parenteral formulations of either complete or incomplete nutritional content. Each dipeptide has an N-terminal amino acid which is arginine. The C-terminal amino acid is glutamine.

10 The concentration of the dipeptide in the aqueous solution can be, for example, from about 0.1 to about 25.0 percent by weight. In addition to dipeptides, the clinical nutritional solution can contain, for example, dextrose, liquid emulsions, vitamins, minerals and trace elements. The selection of the particular dipeptide formulation depends upon the particular use.

15 Dipeptide additives such as single or multiple entities, as well as a total nutritional formulation which contains dipeptides as one component among many are contemplated by this invention.

 The aqueous dipeptide formulation may be ingested orally along with other nutrients such as conventional foods or prepared vitamins, fats, glucose or other mono-
20 saccharides, oligosaccharides, minerals and trace elements. For parenteral administration, a supply of the dipeptide solution may be merged through a Y-connection with a supply of glucose solution or other parenteral solutions. The dipeptide solutions may be mixed with glucose solutions and/or other parenteral solutions to create a mixture which may be administered parenterally. The administration of dipeptides rather than
25 free amino acids allows administration of the same amount of amino acid residue in solutions which are less hypertonic and therefore can be introduced into peripheral veins.

 The dipeptides of the subject invention can be readily synthesized and/or formulated by a person skilled in the art having the benefit of the instant disclosure. Alternatively, the dipeptides can be purchased commercially from, for example, Bachem
30 Biosciences, Inc. which sells the H-Arg-Glu-OH salt.

In a preferred embodiment, the dipeptide is used up to a dose of 10-15% of the usual protein intake in these animals. It is able to "rescue" the animals from the detrimental effects of a low protein diet and the protein wasting effects of dexamethasone as determined by improved survival, improved intestinal morphology and an increased capability for protein synthesis.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

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Example 1 — Dose Response and Safety Study

Rats were fed a low protein diet through specially designed feeding tubes. The arginine-glutamine dipeptide was provided at doses of 0, 1, 2 and 5 grams/Kg/day. Outcome measures included survival, weight change, intestinal digestive enzyme measurements, intestinal inflammation, and a measurement of the ability of these animals to synthesize new protein (P70s6kinase assay). The results were that all doses appeared to be safe. Survival was highest in the animals fed the highest dose (5 gm/kg/d). There was a trend toward less weight loss in the 5-gm/Kg/d group. There was also an increase in the ability to synthesize new protein (P70s6kinase). None of the other outcomes differed between the groups.

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Example 2 — Reduction in Mortality Using Dipeptide

Studies were conducted to determine whether 5 gm/kg/d of an arginyl-glutamine dipeptide results in improved survival, growth, altered hematological indices, intestinal digestive enzymes, intestinal inflammation, intestinal microscopic morphology and ability to synthesize new protein (P70s6kinase). All animals were fed a low protein diet. The controls used in this experiment were 2 other dipeptides. The first, alanyl-glycine was used as an isonitrogenous control, in order to ensure that any beneficial effects incurred would be specific to the dipeptide and not to the amount of nitrogenous substances provided. The second control was alanyl-glutamine, a glutamine-containing dipeptide that does not contain arginine. This study demonstrated a trend toward lower

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mortality in the arginyl glutamine-treated rats. There were no detrimental hematologic consequences and the protein synthesis was significantly greater in the arginyl-glutamine treated rats than in any of the other groups.

5 Example 3 — Reduction in Weight Loss Using Dipeptide

Further studies were conducted to determine whether arginyl-glutamine rescues animals from a combination of protein restriction and the catabolic effects of dexamethasone, a drug used widely in neonates to hasten weaning from mechanical ventilation. Dexamethasone is also widely used in children and adults in the treatment of asthma and other autoimmune and allergic disorders. One of its major detrimental side effects is protein wasting. It was found that the arginyl-glutamine dipeptide resulted in a significant amelioration of weight loss caused by the dexamethasone, an improved intestinal villus morphology as determined by standard microscopic analyses and improved protein synthesis, as measured by P70s6kinase activity.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

Claims

I claim:

1 1. A peptide composition comprising an arginyl-glutamine dipeptide formulated
2 as a nutrient formulation, wherein the arginine residue is the amino terminus of said
3 dipeptide and the glutamine residue is the carboxy terminus of said dipeptide.

1 2. The peptide composition, according to claim 1, wherein said formulation is
2 suitable for enteral administration.

1 3. The peptide composition, according to claim 1, wherein said formulation is
2 suitable for parenteral administration.

1 4. The peptide composition, according to claim 1, wherein the concentration of
2 said dipeptide is from about 0.1% to about 25.0% by weight of said formulation.

1 5. The peptide composition, according to claim 1, wherein said nutrient
2 formulation comprises an additive selected from the group consisting of vitamins,
3 minerals, trace elements, fats, monosaccharides and oligosaccharides.

1 6. The peptide composition, according to claim 5, wherein said monosaccharide
2 is glucose.

1 7. A method for promoting healthy muscle tissue in a human or animal, said
2 method comprising administering to a human or animal in need of such treatment an
3 effective amount of a dipeptide composition comprising an arginyl-glutamine dipeptide
4 formulated as a nutrient formulation, wherein the arginine residue is the amino terminus
5 of said dipeptide and the glutamine residue is the carboxy terminus of said dipeptide.

1 8. The method, according to claim 7, wherein said human or animal has
2 undergone, is undergoing, or will undergo physical exertion or training.

1 9. The method, according to claim 7, wherein said human or animal is in need
2 of maintenance of muscle mass.

1 10. The method, according to claim 9, wherein said human or animal is
2 hospitalized.

1 11. The method, according to claim 10, wherein said hospitalized human or
2 animal is a neonate.

1 12. The method, according to claim 9, wherein said human or animal is subjected
2 to an environment of decreased gravity relative to gravity on earth.

1 13. A method for promoting increased immunity to pathogens in a human or
2 animal, said method comprising administering to a human or animal in need of such
3 treatment an effective amount of a dipeptide composition comprising an arginyl-
4 glutamine dipeptide formulated as a nutrient formulation, wherein the arginine residue
5 is the amino terminus of said dipeptide and the glutamine residue is the carboxy terminus
6 of said dipeptide.

1 14. The method, according to claim 13, wherein said human or animal is at risk
2 for infection by a pathogen.

1 15. The method, according to claim 14, wherein said pathogen is selected from
2 the group consisting of bacteria, viruses and parasites.

1 16. The method, according to claim 13, wherein said human or animal is an
2 employee, worker or patient in a hospital or medical facility.

1 17. The method, according to claim 13, wherein said immunity is mucosal
2 immunity.

- 1 18. The method, according to claim 17, wherein said mucosal immunity
2 comprises an IgA response to said pathogen.

INTERNATIONAL SEARCH REPORT

National Application No

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A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 A23L1/305 A61K38/05 C07K5/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23L A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, FSTA, PAJ, BIOSIS

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

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