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(54) Title: PHARMACEUTICAL COMPOSITION FOR THE TREATMENT OF PERIODONTAL DISEASES

(57) Abstract: A pharmaceutical composition for the treatment of periodontitis. A pharmaceutical composition according to the present invention is in the form of a gel with a selected viscosity. Its active ingredient is a base comprising a cysteine protease inhibitor from egg protein, urine, human or animal amniotic fluid, human or animal placentae as well as plants, with an activity of 1 - 50 inhibition units, suspended in 50 g of a polyhydroxyl alcohol and 50 g of a 0.05 molar phosphate buffer solution at a p H of 6.0 to 7.5, from 20 to 50 % by mass, whereas its carrier comprises gelling substances, from 7 to 20 % by mass, as well as water to 100% by mass. The gelling substances may be methylcellulose, hydroxypropylmethyl-cellulose, carboxymethylcellulose sodium salt, gelatin, apple-citrus pectin or a dextran. Preferentially, additives in the form of polyhydroxyl alcohols and Nipagin are used.

Pharmaceutical composition for the treatment of periodontal diseases

The subject of the present invention is a pharmaceutical composition for the treatment of periodontal disease during both walk-in and hospitalized dentistry, as well as in dental surgery.

Due to the common occurrence of periodontitis, it is viewed as a social disease. Missing teeth, ill gums and teeth often lead to the development of cardiac, arthroid and gastric illnesses.

Periodontitis does not only concern older persons, but also 20 and 30-year olds, and a number of forms are observed in children as well, and constitute the most common cause of tooth loss. Statistical analyses show that inflammatory changes of the gums constitute the most serious cause of tooth loss, and some 60% of the population of the European Union suffers from this problem. This is most often caused by improper oral hygiene and leads to plaque formation, which may be a cause of gingivitis. It has also been reported that the development of periodontitis is aided by some genetic factors, smoking, cavities, over or under-bites, poorly set fillings, hormonal disruptions, the activity of certain drugs or heavy metal poisoning. Anatonomically, teeth are supported by the gum, the periodontium or connective tissue between the tooth and the jaw, as well as the jaw bone. The periodontal apparatus, is susceptible to inflammation, chiefly due to improper oral hygiene.

It has been shown that periodontal disease, including gingivitis, leads to a disappearance of tissues around the teeth. In most cases, they disappear and leave the neck of the tooth exposed. The disease develops slowly, and symptoms may include bleeding during brushing or flossing, reddened gums, receding gums or plaque. When these problems are not observed sufficiently early, this leads to osteological changes in periodontium, which is evidenced by its increased mobility.

The cause of changes in the gums and the bone surrounding the tooth is inflammation. Bacteria accrue in the periodontal space, which cause inflammation, whence the teeth begin to move and fall out. Inflammation is caused by bacteria which settle on the surface of the root of the tooth, which develop underneath the gum, and the inflammation progresses deeper. The space between the gum and root deepens gradually, and it becomes impossible to clean the root through brushing. Bacterial accretions, plaque, overtime change into hard to clean sub-gingival mineral

deposits. The best method of treatment is to remove the bacteria settling on the root. Often, the gums are surgically exposed so as to reach the deeper portions of the root. However, the treatment is difficult and not always effective. (Spratt DA., Greenman J., Schaffer AG. (1995): Capnocytophaga gingivalis aminopeptidase: a potential virulence factor. Mikrobiol. 141.3087-3093).

Changes in the periodontal tissue are dependent not only on the microorganisms themselves, but also on the biological activity of the substances secreted by them or by degraded periodontal tissue. It was confirmed that increased numbers of pathogenic microorganisms develop locally and the range of their effects depends on local immune mechanisms and not the entire periodontium of a patient. (Grenco RJ. (1992): Host responses in periodontal diseases: Current Concept. J. Periodont. 63, 338 - 355).

At first, a membrane appears on plaque, whose main constituents are glycoproteins which are immune factors in saliva. Only further on, to we observe bacteria and other microorganisms on it, which lead to the development of various states of the gum and periodontium. (Komman KS., Loe H. (1993): The role of local factors in the etiology of periodontal diseases. Periodontology 2000 2, 83 - 97).

As a result of infection by bacteria settled on the plaque, fluid appears in the increased periodontal space, which aside from microorganisms and enzymes, contains proteinaceous growth factors. These stimulate bacterial growth and facilitate their settlement on the plaque. The increased numbers and variety of pathogenic microorganisms as well as of factor aiding their growth limits the host immune mechanisms as well as the effectiveness of antibiotic treatment. (Genco R., Loe H. (1993): The role of systemic factors in the etiology of periodontal diseases. Periodontology 2000 2, 98-116).

Research on the microflora causing periodontal disease, it was confirmed that the first to develop in the in the gingival crevice are facultative anaerobes, which create optimal conditions for the development of the anaerobic bacteria being the main cause of gingivitis. The bacteria which most often cause juvenile periodontitis and gingivitis are Actinomycetes, whereas in adults this is most often Porphyronomas gingiva lis, intermedia and Eubacterium. (Polanowska J. Polanowski A. (1997).: The role of Porphyromonas gingivalis proteases in periodontal pathogenesis. Post. Hig. Med. Dosw.51.149-169).

In gingival crevices, whose depth exceeds 2.0 mm, the changes in the periodontium are so advanced that basic oral hygiene treatments are insufficient to remove the

bacteria accrued at the sites of infection. These processes indicate a correlation between gingivitis and periodontitis.

It is thought that gingivitis jest the direct cause of inflammation, whereas periodontitis of the host immune response to an inflammation caused by bacteria on the dental plaque. (0' Leary TJ., Barrington EP., Gottsegen R. (1988): Periodontal therapy: a summary status report 1987-1988. J. Periodontol. 59, 306-310).

Antibodies formed in the salivary glands appear in persons with advanced periodontitis induced by bacterial infection in the fluid in the gingival crevices, in order to control the development of the microorganism. (Henskens YM., van der Weijden FA., van den Keijbus PA., Veennan EC., Timmerman MF., van der Velden U., Amerongen AV. (1996): Effect of periodontal treatment on the protein composition of whole and parotid saliva. J. Periodont. 67.205-212).

It was determined that inflammations in periodontal tissue cause only local changes, whereas no such changes are observed in healthy tissue. (Page RC. (1986): Gingivitis J. Clin Periodontol. 13, 345 - 355). It was shown that similar proteolytic enzymes play a role in the development of periodontal disease and in tumour take. These are connected with closely related autoactivation processes. It was determined that the gingivital crevice fluid with inflammation becomes host to increased levels of cathepsin D, elastases, collagenases and cysteine endopeptidases, including cathepsisns B and L. (Turk D., Guncar G., Podobnik M., Turk B. (1998): Reviewed definition of substrate binding sites of papain-like cysteine proteases. Biol. Chern. 379, 137147). It was confirmed that an important disease-limiting role is played by peptidase inhibitors produced in salivary glands, which inhibit the activity of enzymes catalysing the degradation of healthy tissue. (Kennett CN., Cox SW., Eley BM. (1997): Investigation into the cellular contribution to host tissue proteinases cervicular fluid. J. Clin. Periodont. 24, 424-431). Changes in periodontal tissues connected with their degradation appear locally at sites of developing inflammation, whereas this type of enzyme activity is not observed in healthy tissues. (Page RC. (1986): Gingivitis J. Clin. Periodontol. 13, 345-355). These changes are controlled in vivo by inhibitors of plasminogen activator, elastoproteases and cysteine peptidases. It is perceived that an imbalance between the activities of these enzymes and their inhibitors is the direct cause of pathological changes, and a key role is played by cysteine peptidases. (Bobek L.A., Levine M.J. (1992): Cystatins - inhibitors of cysteine proteinases. Crit. Rev. Oral Biol. Med.1992, 3, 307-332). Three genes encoding specific cysteine peptidases were isolated from an inflamed gingival crevice. These enzymes were called gingipains (R1,

R2 and K). A direct connection between them and periodontal tissue degradation was shown. (Madden TE., Clark VI., Kuramitsu HK. (1995): Revised sequence of the Porphyronomas gingival is cysteine proteinase/hemaglutinin gene homology with streptococal pyrogenic exotoxin B/streptococal proteinase. Infect. Immun. 63, 238-247). It was also shown that cysteine peptidases catalyse changes in the structure of specific inhibitors, including kininogen and cystatin (C. Skaleric U, Babnik J., Curin V., Lah T., Turk V.(1989): Immunochemical quantitation of cysteine proteinase inhibitor cystatin C in inflamed human gingiva. Arch. Oral. Biol. 34, 301 - 305).

Cystatin S in saliva inhibits the development of P. gingivalis as well as the activity of cysteine endopeptidases secreted by periodontal bacteria as well as egg protein inhibitors which inhibit the growth of P. gingivalis in vitro in gingival crevice fluid. (Blankenvoorde MFJ., Henskens Wim van 't Hot YMC., Amerongen AV N. (1996): Inhibition of the growth and cysteine proteinase activity of porphyromonas gingivalis by human salivary cystatin S and chicken cystatin. Biol. Chern. 377, 847-850).

Three cystatins have been isolated from human saliva so far, which are produced in the salivary glands. These have been assigned the symbols S, SN and SA. These inhibitors exhibit almost 90% mutual homology and 60% similarity to cystatin C, the most commonly seen cysteine peptidase inhibitor in the human. They have been determined in the gingival crevice fluid. They play a protective role against degradation of healthy tissue by cysteine peptidases. (Saitoh E., Isemura S., Sanada K. (1988): Cystatin superfamily. Evidence that family II cystatin genes are evolutionary related to family III cystatin genes. Biol. Chern. Hoppe-Seyler 369.191 - 197).

It is supposed that the inhibitors of these enzymes may play a protective function and be useful as potential drugs against gingivitis (Hugly TE. Protease inhibitortors: novel therapeutic application and development. TIBTECH 1996, 14.409-412).

An interesting method of determining the density of pathogenic bacteria in a patient's gingival crevice fluid is used in the "BANA" test, such as that from OraTec. These tests make use of a specific substrate to estimate the number of pathogenic bacteria and their accompanying active cysteine peptidases using benzoyl-arginyl-beta-naphthylamide - BANA. This confirms the suggestion that these enzymes play a key role in periodontitis. Their presence in connection with bacteria may be observed even under a light microscope with a special additional attachment. (Professional Catalog; OraTec Periodontal Home Care Dental Catalog Copyright 2005 Oratec

Corporation Manassas, Virginia) (OraTec 12181 Balls Ford Road Manassas, VA 20109-2449, USA) BANA and Phase Contrast Video Microscope Corporation).

During more advanced inflammation, a curettage is performed. This consists of scraping off the plaque. Bacteria and diseased tissues are thus removed, and the periodontium can heal and regenerate, thus form new tissue. If the disease has destroyed most of the periodontium, and the teeth are poorly held in the bone, they can be bridged, or stiffened. In this procedure, the teeth are connected to one another using various techniques. This may consist of a ligature, or special wires, or a bridging prosthesis, or splints of a composite material. Such a stiff structure of connected teeth stops them from swinging too much. When the wounds in the gums have healed and the periodontium has regenerated, these may be removed.

The goal of surgical treatment is to remove disease-altered tissues as well as their regeneration or reformation. A periodontologist has in his arsenal several treatment methods including: root-to-crown, controlled tissue regeneration using implantable barrier membranes, bone grafts of human or animal bone or natural or synthetic bioglass. As a result of the treatment process, new tissue is formed which is a copy of the natural one, both in terms of structure and functionality. Of paramount importance is the reconstruction of adhesive function, of the periodontal attachments. When the damage to the periodontium and periodontal attachments and the bone of the tooth socket is extensive, a novel preparation, emdogain, gives some hope. It contains enamel proteins, the so-called hydrophobic proteins, which stimulate the regeneration of tissues. It contains amelogenins, which play an important role in tooth formation and protection, but are only produced by the human organism during foetal life. This preparation stimulates all types of cells that are responsible for the regeneration of periodontal tissues and in the elimination of gingivial crevices more than 6 mm deep, which are accompanied by vertical bone loss. Its use has been deemed to be the simplest periodontal disease treatment for the patient and doctor.

American patents (Takada et al. 2003; Reynolds et al. 2003) reveal the use of cysteine inhibitors in the reconstruction of periodontal bone, as additives in nutraceuticals, most often as compositions with milk or milk products. Such preparations are also manufactured by the Kazain company. Most often, cysteine peptidase inhibitors are used in the form of preparations purified from various sources, including egg proteins in a composition with vitamins K and D, and the fundamental goal is to rebuild a degraded periodontium and bony periodontal

fragments, including the root degraded during periodontitis (Takada Y., Atsushi Serizawa A., Hidetoshi Ishikawa H., Tomoe Y.,_ Seiichiro Aoe S. (2003) P USA 420102. Bone resorption suppressing agent. Reynolds EC., Bhogal PS., Slakeski N. (2003): Diagnostics and treatments of periodontal disease. P USA 66330).

Polish patent No. 148352 reveals an agent for the treatment of advanced periodontitis in the form of a cellulose derivative at 3 - 7 % by mass, with a polyhydroxyl alcohol or a polymer thereof at 1 - 5% by mass, with an addition of 0.25 % by mass of Kunitz protease inhibitor and water to 100% by mass. This complex is a hydrophilic gel with thixotropic properties and a high flow threshold, due to which it may be considered a pseudoplastic substance, which makes it resistant to the activity of mechanical factors such as the activity of saliva, beverages and food.

Polish patent No. 164585 reveals a therapeutic agent for use in dental surgery, composed of 10-50% gelatin by mass, 2 - 50 % glycerol by mass, or 1.2-propylene glycol, or polyoxyethylene glycol with a molar mass of 200-15000 as well as 1 - 25% by mass of polyvinylpyrolidone with a molar mass of 160000-360000, or polyvinyl alcohol and water to 100% by mass. This substance is dosed into wounds, where it becomes hydrolysed by systemic enzymes, and the hydrolysis products comprising amino acids are resorbed. As the bone becomes replenished, the therapeutic agent diminishes in volume, so it requires no replacement. The use of gelatin as a carrier, a substance of animal origin, ensures resorption due to hydrolysis into amino-acids. Polyvinyl alcohol and polyvinylpyrolidone are also metabolised in the human. The release of therapeutically active substances into the tissues follows a zero-order kinetic process, which ensures a constant concentration of the drug in the tissue.

Polish patent No. 311322 releases a dental gel containing 70-90% by mass of a carrier, composed of water physiological saline and a moisturizer, preferentially hydroxyethylcellulose, a plant extract containing tannins and 0.1-5% by mass of sweeteners and aromas. This gel also contains a composition comprising antibiotics such as dethreomycine, colistine, clindamycine in a mass percentage ratio of 1:0.23:5, a steroid anti-inflammatory substance, particularly hydrocortisone at 0.02-1% by mass as well as a plant extract, preferentially of Bergenia, at 1-5 % by mass. The dental gel according to the application in question is useful in the treatment of periodontitis.

The present invention relaters to a pharmaceutical composition for the treatment of periodontitis containing at least gel-forming substances.

The nature of the invention is based on the fact that the active substance of the composition is a cysteine protease inhibitor from egg proteins, urine, human and

animal amniotic fluid, human and animal placentae, and from plants, which is suspended in 50 g of a polyhydroxyl alcohol, preferentially glycerol, and 50 g of a 0.05 M phosphate buffer solution , pH 6.0 to 7.5, which constitutes a base for the composition in an amount from 20 to 50% by mass, whereas the carrier comprises gelling substances at 7 to 20 % by mass as well as water to 100 % by mass.

Preferentially, the gelling substances comprise methylcellulose, hydroxypropylmethylcellulose, carboxymethylcellulose sodium salt, gelatin, apple-citrus pectin, dextran or a mixture thereof.

Also preferentially, the composition contains additives in the form of polyhydroxyl alcohols such as glycerol, polyvinylpirolidone K-30 or K-90, polyoxyethylene glycol-200 or glycol propylene-l,2 at 5 to 25 % by mass.

In accordance with storage requirements, a preservative is included in the composition, preferentially in the form of Nipagin.

The compositions in the example sets were used in tests of their ability to treat periodentosis. The observation showed that the activity of cysteine proteases was inhibited in the gingival crevices by specific inhibitors obtained from chicken eggs. The determinations were made on a 30-patient group at the Facial and Jaw clinic of the Medical Academy of Wrocław. Gingival crevice fluid was collected from two distant crevices from a patient, and then cysteine peptidase activity was quantified, and then inhibited with cystein protease inhibitors. The inhibition exceeded 90%. As is evident from this research, these inhibitors are suitable for treatment of periodontal inflammations by using appropriate compositions thereof administered directly into periodontal crevices and in mouthwashes for periodontitis patients.

Pharmaceutical compositions using cysteine peptidase inhibitors, particularly of cystatins from egg proteins, which are genetically similar to human protease inhibitors, are easily obtained and inexpensive. The research also indicates the possibility of using other sources of inhibitors in novel drugs, provided that they are not toxic to the human. The research was performed in vitro in fluid collected from the periodontal crevices of patients with diagnosed periodontitis.

The base, which is also an active substance of the composition, composed of the cysteine peptidase inhibitor, polyhydroxyl alcohol and phosphate buffer solution of an appropriate pH may be maintained at 4°C for up to 12 months. The use if a polyhydroxyl alcohol in the composition, mainly glycerol, as a solvent for the inhibitor stabilises its activity and makes it possible to maintain the drug at the site of treatment.

Enriching the base with a series of selected components makes it possible to produce a composition in liquid or semi-liquid form, particularly for the us in injections, for rinsing or washing or for direct administration into gingival crevice fluid during inflammation. Compositions according to the invention are suitable for both prophylaxis as well as in curettage or surgical treatment as a supplemental treatment.

The subject of the present invention is shown in example embodiments.

Example 1. Mass composition in g:

BASE = cysteine peptidase inhibitor, 1-50 activity units suspended in 50 g glycerol, and 50 g 0.05 M phosphate buffer solution

pH 6.0 to 7.5	30.0
Methylcellulose	7.0
Gelatin	9.0
Glycerol	5.0
Polyvinyl alcohol	0.5
Nipagin (Aseptins, Parabens)	0.1
Water	48.

The composition base is prepared by mixing the planned quantities of its components, meaning an appropriate number of units of the cysteine peptidase inhibitor with the polyhydroxyl alcohol and 0.05M phosphate buffer solution with a selected pH. in the range of 6.0 to 7.5. The base is apportioned into plastic or glass containers, and stored, preferentially at 4°C.

The measured quantity of base is supplemented with the given quantities of the remaining components and mixed until homogeneity.

The gel produced, with a viscosity of 337.53 mPa*s is apportioned into 20 or 50 ml containers. The example composition is meant for rinsing the jaw pocket.

Example 2. Mass composition in g:

BASE	30.0
Carboxymethylcellulose sodium salt	6.0
Hydroxypropylmethylcellulose	4.0
Gelatin	9.0
Glycol polyoxyethylene-200	5.0
Nipagin	0.1
Water	45.9

The example composition is produced as in Example 1, by first selecting the components, and then apportioning in the same way.

It has a viscosity of 624.43 mPa*s and is meant for use on the border between the hard and soft palate.

Example 3. Mass composition in g:

BAZA 20.0

Methylcellulose 10.0

Sodium alginate 1.0

Propylene glycol-l,2 20.0

Nipagin 0.1

Water 48.9

The example composition is produced as in Example 1, by first selecting the components, and then apportioning in the same way.

It has a viscosity of 506.30 mPa*s and is meant for use in the gingival crevices.

Example 4. Mass composition in g :

BASE 20.0

Gelatin 8.0

Glycerol 25.0

Apple-Citrus pectin 3.0

Nipagin 0.1

Water 43.9

The example composition is produced as in Example 1, by first selecting the components, and then apportioning in the same way.

It has a viscosity of 421.91 mPa*s and is meant for use in the sublingual space near the egress of the sublingual salivary glands.

Example 5. Mass composition in g :

BASE 30.0

Methylcellulose 10.0

Carboxymethylcellulose sodium salt 6.0

Calcium alginate 1.0

Glycol polyoxyethylene-200 10.0

Nipagin 0.1

Water 42.9

The example composition is produced as in Example 1, by first selecting the components, and then apportioning in the same way.

It has a viscosity of 607.56 mPa*s and is meant for use at the base of the tongue in the corner of the lower jaw.

Example 6. Mass composition in g :

BASE 30.0

Methylcellulose 10.0

Carboxymethylcellulose sodium salt 6.0

Dextran 1.0

Glycerol 15.0

Nipagin 0.1

Water 37.9

The example composition is produced as in Example 1, by first selecting the components, and then apportioning in the same way.

It has a viscosity of 708.81 mPa*s and is meant for use on the cheeks, at the bite line.

Table 1. Inhibition of cysteine peptidases in the gingival crevice fluid by egg protein cystatins using a colorimetric method.

Sample No.	Amount of added inhibitor (units/sample)	Enzymatic activity (units/mg protein)	Remaining activity (%)
1a.	0.0	0.43	100
	0.5	0.28	65
	1.0	0.14	32
	2.0	0.07	16
1b.	0.0	0.37	100
	0.5	0.21	56
	1.0	0.12	32
	2.0	0.05	13
2a	0.0	0.84	100
	0.5	0.32	38
	1.0	0.21	25
	2.0	0.07	8
2b	0.0	0.47	100
	0.5	0.31	66
	1.0	0.11	23
	2.0	0.03	6
3a	0.0	0.92	100
	0.5	0.47	51
	1.0	0.24	38
	2.0	0.07	4
3b	0.0	0.65	100
	0.5	0.27	41
	1.0	0.12	18
	2.0	0.09	14
4a	0.0	0.53	100
	0.5	0.21	40
	1.0	0.11	21
	2.0	0.06	11
4b	0.0	0.82	100
	0.5	0.34	41
	1.0	0.22	27
	2.0	0.11	13
5a	0.0	0.31	100
	0.5	0.24	77
	1.0	0.05	16
	2.0	0.04	13
5b	0.0	0.63	100
	0.5	0.35	56
	1.0	0.27	43
	2.0	0.09	14

Table 2: Inhibition of cysteine peptidases in gingival crevice fluid by egg protein cystatins (5 µg). Inhibitor activity (180 mEU/mg protein) using spectrofluorometry.

Protein	Food cathepsin L U/mg protein	Food cathepsins B and L U/ml	casein peptidase inhibitors mEU/ml	inhibition of food cathepsin B by egg protein cysteins /Umg protein	percent of inhibited enzymes
78	9	0	23	98	70
70	6	20	02	86	90
97	9	95	30	22	0
40	94	93	53	34	2
06	9	40	85	78	0
70	8	82	34	05	3
25	3	98	29	00	0
95	0	88	66	62	0
57	3	01	71	43	21
05	2	0	36	75	33
70	3	21	25	67	6
30	5	80	81	83	3
60	3	0	44	89	46
30	9	84	40	45	6
0	10	28	10	76	2
75	92	47	63	492	1
80	5	95	45	10	9
05	0	19	20	36	1
98	3	98	25	67	84
70	4	88	35	45	18
15	86	5	95	139	6
14	1	5	05	34	30
05	0	73	32	89	60
98	A	15	66	121	83
03	1	52	35	89	88
35	5	36	64	61	3
88	8	8	75	56	53
58	6	6	06	08	78
85	5	56	48	01	i7

Claims

1. A pharmaceutical composition for the treatment of periodontitis containing at least a gelling substance, characterised in that the active component is a cysteine peptidase inhibitor from egg protein, urine, human or animal amniotic fluid, human or animal placentae or plants, with an activity of 1-50 inhibition units, suspended in 50 g of a polyhydroxyl alcohol, preferentially glycerol, and 50 g of 0.05 molar phosphate buffer with a pH of 6.0 to 7.5, which constitutes the base of the composition at 20 to 50 % by mass, and its carriers are gelling substances at 7 to 20 % by mass and water to 100 % by mass.

2. A pharmaceutical composition according to Claim 1, characterised in that the gelling substance is methylcellulose, hydroxypropylmethylcellulose, carboxymethylcellulose sodium salt, gelatin, apple-citrus pectin or dextran or a mixture thereof.

3. A pharmaceutical composition according to Claim 1, characterised in that it contains additives in the form of glycerol, polyvinylpirolidone K-30 or K-90, polyoxyethylene glycol-200 or glycol propylene-l,2 or a mixture thereof at 5 to 25 % by mass.

4. A pharmaceutical composition according to Claim 1, characterised in that it contains an addition of a preservative, preferentially in the form of Nipagin