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(54) Title: A METHOD OF TREATING ALZHEIMER'S DISEASE

(57) Abstract: The present invention relates to a method of treating Alzheimer's disease by administration of activated-potentiated form of antibodies to brain-specific protein S-100 and activated-potentiated form of antibodies to endothelial NO synthase.

A Method Of Treating Alzheimer's Disease

FIELD

The present invention relates to the field of medicine and can be used for the treatment of Alzheimer's disease.

Alzheimer's disease (AD) is neurodegenerative disease and is characterized by impairment of cognitive functions, memory loss, mental confusion, deterioration of emotional control, and dementia (progressive decline in memory with inability to remember information that was known in the past or inability to learn new information). The principal cause of the development of AD is thought to be the accumulation of a beta amyloid, leading to formation of beta amyloid plaques and neurofibrillar balls in tissues of a brain. AD is also accompanied by deficiency of cholinergic system. Impairment of learning and memory can be induced chemically in experimental animals by scopolamine, a cholinergic antagonist known to interfere with acetylcholine transmission. The experimental animal model of scopolamine-induced amnesia has been extensively used to screen for compounds with potential therapeutic value for dementia.

Known in the art are neurotropic drug based on antiserum to brain specific protein S-100 (RU 2156621 C1, A61K39/395, 27.09.2000).

The therapeutic effect of an extremely diluted form (or ultra-low form) of antibodies potentized by homeopathic technology (activated potentiated form) has been discovered by the inventor of the present patent application, Dr. Oleg I. Epshtein. U.S. Patent No. 7,582,294 discloses a medicament for treating Benign Prostatic Hyperplasia or prostatitis by administration of a homeopathically activated form of antibodies to prostate specific antigen (PSA). U.S. Patent No. 7,700,096 discloses a homeopathically potentized form of antibodies to endothelial NO-synthase.

The S-100 protein is a cytoplasmic acidic calcium binding protein found predominantly in the gray matter of the brain, primarily in glia and Schwann cells. The protein exists in several homo-or heterodimeric isoforms consisting of two immunologically distinct subunits, alpha and beta. The S-100 protein has been suggested for use as an aid in the diagnosis and assessment of brain lesions and neurological damage due to brain injury, as in stroke. Yardan et al., *Usefulness of*

S100B Protein in Neurological Disorders, J Pak Med Assoc Vol. 61, No. 3, March 2011, which is incorporated herein by reference.

Ultra low doses of antibodies to S-100 protein have been shown to have anxiolytic, anti-asthenic, anti-aggressive, stress-protective, anti-hypoxic, anti-ischemic, neuroprotective and nootropic activity. *See* Castagne V. et al., *Antibodies to S100 proteins have anxiolytic-like activity at ultra-low doses in the adult rat*, J Pharm Pharmacol. 2008, 60(3):309-16; Epshtein O. I., *Antibodies to calcium-binding S100B protein block the conditioning of long-term sensitization in the terrestrial snail,* Pharmacol Biochem Behav., 2009, 94(1):37-42; Voronina T.A. et al., Chapter 8. *Antibodies to S-100 protein in anxiety-depressive disorders in experimental and clinical conditions. In "Animal models in biological psychiatry",* Ed. Kalueff A. V. N-Y, "Nova Science Publishers, Inc.", 2006, pp. 137-152, all of which are incorporated herein by reference.

Nitric oxide (NO) is a gaseous molecule that has been shown to acts in the signaling of different biological processes. Endothelium-derived NO is a key molecule in regulation of vascular tone and its association with vascular disease has long been recognized. NO inhibits many processes known to be involved in the formation of atherosclerotic plaque, including monocyte adhesion, platelet aggregation and vascular smooth muscle cell proliferation. Another important role of endothelial NO is the protection of the vascular wall from the oxidative stress induced by its own metabolic products and by the oxidation products of lipids and lipoproteins. Endothelial dysfunction occurs at very early stages of atherosclerosis. It is therefore possible that deficiency in local NO availability could be a final common pathway that accelerates atherogenesis in humans. In addition to its role in the vascular endothelium, NO availability has been shown to modulate metabolism of lipoproteins. Negative correlation has been reported between plasma concentrations of NO metabolic products and plasma total and Low Density Lipoprotein [LDL] cholesterol levels while High Density Lipoprotein [HDL] improves vascular function in hypercholesterolaemic subjects. The loss of NO has considerable effect on the development of the disease. Diabetes mellitus is associated with increased rates of morbidity and mortality caused primarily by the accelerated development of atherosclerotic disease. Moreover, reports show that diabetics have impaired lung functions. It has been proposed that insulin

resistance leads to airway inflammation. Habib et al., *Nitric Oxide Measurement From Blood To Lungs, Is There A Link?* Pak J Physiol 2007; 3(1).

Nitric oxide is synthesized by the endothelium from L-arginine by nitric oxide synthase (NO synthase). NO synthase occurs in different isoforms, including a constitutive form (cNOS) and an inducible form (iNOS). The constitutive form is present in normal endothelial cells, neurons and some other tissues.

There is a continuing need for new drug products with desired therapeutic efficacy for treatment of neurodegenerative diseases such as Alzheimer's disease.

SUMMARY

The invention provides a more effective remedy for treatment of Alzheimer's disease.

The present invention provides a method of treating Alzheimer's disease, the method comprising administering a pharmaceutical composition comprising activated-potentiated form of antibodies to brain-specific protein S-100 and activated-potentiated form of antibodies to endothelial NO synthase as an additional strengthening component.

In one variant, the present invention provides a combination pharmaceutical composition comprising activated-potentiated form of antibodies to brain-specific protein S-100 and activated-potentiated form of antibodies to endothelial NO synthase, wherein the antibody is to the entire protein S-100 or fragments thereof.

In one variant, the present invention provides a combination pharmaceutical composition comprising activated-potentiated form of antibodies to brain-specific protein S-100 and activated-potentiated form of antibodies to endothelial NO synthase, wherein the antibody is to the entire endothelial NO synthase or fragments thereof.

In one variant, the combination pharmaceutical composition of this aspect of the invention includes activated-potentiated form of an antibody to protein S-100 which is in the form of a mixture of (C12, C30, and C50) or (C12, C30 and C200) homeopathic dilutions impregnated onto a solid carrier. The activated-potentiated form of an antibody to NO synthase is in the form of mixture of (C12, C30, and C50)

or (C12, C30 and C200) homeopathic dilutions may be subsequently impregnated onto the solid carrier.

In one variant, the combination pharmaceutical composition of this aspect of the invention includes activated-potentiated form of an antibody to endothelial NO synthase which is in the form of a mixture of (C12, C30, and C50) or (C12, C30 and C200) homeopathic dilutions impregnated onto a solid carrier. The activated-potentiated form of an antibody to protein S-100 is in the form of mixture of (C12, C30, and C50) or (C12, C30 and C200) homeopathic dilutions may be subsequently impregnated onto the solid carrier.

Preferably, the activated-potentiated form of an antibody to protein S-100 is a monoclonal, polyclonal or natural antibody, more preferably, a polyclonal antibody. In one variant of this aspect of the invention, the activated-potentiated form of an antibody to a protein S-100 is prepared by successive centesimal dilutions coupled with shaking of every dilution. Vertical shaking is specifically contemplated

Preferably, the activated-potentiated form of an antibody to endothelial NO synthase is a monoclonal, polyclonal or natural antibody, more preferably, a polyclonal antibody. In one variant of this aspect of the invention, the activated-potentiated form of an antibody to NO synthase is prepared by successive centesimal dilutions coupled with shaking of every dilution. Vertical shaking is specifically contemplated

In one variant of the invention, there is provided administration of from one to two unit dosage forms of the activated-potentiated form of an antibody to protein S-100 and one to two unit dosage forms of the activated-potentiated form of an antibody to endothelial NO synthase, each of the dosage form being administered from once daily to six times daily. Preferably, the one to two unit dosage forms of each of the activated-potentiated forms of antibodies is administered twice daily.

DETAILED DESCRIPTON

The invention is defined with reference to the appended claims. With respect to the claims, the glossary that follows provides the relevant definitions.

The term "antibody" as used herein shall mean an immunoglobulin that specifically binds to, and is thereby defined as complementary with, a particular spatial and polar organization of another molecule. Antibodies as recited in the claims may include a complete immunoglobulin or fragment thereof, may be

natural, polyclonal or monoclonal, and may include various classes and isotypes, such as IgA, IgD, IgE, IgG1, IgG2a, IgG2b and IgG3, IgM, etc. Fragments thereof may include Fab, Fv and $F(ab')_2$, Fab', and the like. The singular "antibody" includes plural "antibodies".

The term "activated-potentiated form" or "potentiated form" respectively, with respect to antibodies recited herein is used to denote a product of homeopathic potentization of any initial solution of antibodies. "Homeopathic potentization" denotes the use of methods of homeopathy to impart homeopathic potency to an initial solution of relevant substance. Although not so limited, 'homeopathic potentization" may involve, for example, repeated consecutive dilutions combined with external treatment, particularly vertical (mechanical) shaking. In other words, an initial solution of antibody is subjected to consecutive repeated dilution and multiple vertical shaking of each obtained solution in accordance with homeopathic technology. The preferred concentration of the initial solution of antibody in the solvent, preferably water or a water-ethyl alcohol mixture, ranges from about 0.5 to about 5.0 mg/ml. The preferred procedure for preparing each component, i.e. antibody solution, is the use of the mixture of three aqueous or aqueous-alcohol dilutions of the primary matrix solution (mother tincture) of antibodies diluted 100^{12}, 100^{30} and 100^{200} times, respectively, which is equivalent to centesimal homeopathic dilutions (C12, C30, and C200) or the use of the mixture of three aqueous or aqueous-alcohol dilutions of the primary matrix solution of antibodies diluted 100^{12}, 100^{30} and 100^{50} times, respectively, which is equivalent to centesimal homeopathic dilutions (C12, C30 and C50). Examples of homeopathic potentization are described in U.S. Patent. Nos. 7,572,441 and 7,582,294, which are incorporated herein by reference in their entirety and for the purpose stated. While the term "activated-potentiated form" is used in the claims, the term "ultra-low doses" is used in the examples. The term "ultra-low doses" became a term of art in the field of art created by study and use of homeopathically diluted and potentized form of substance. The term "ultra-low dose" or "ultra-low doses" is meant as fully supportive and primarily synonymous with the term 'activated-potentiated" form used in the claims.

In other words, an antibody is in the "activated-potentiated" or "potentiated" form when three factors are present. First, the "activated-potentiated" form of the antibody is a product of a preparation process well accepted in the homeopathic

art. Second, the "activated-potentiated" form of antibody must have biological activity determined by methods well accepted in modern pharmacology. And third, the biological activity exhibited by the "activated potentiated" form of the antibody cannot be explained by the presence of the molecular form of the antibody in the final product of the homeopathic process.

For example, the activated potentiated form of antibodies may be prepared by subjecting an initial, isolated antibody in a molecular form to consecutive multiple dilutions coupled with an external impact, such as mechanical shaking. The external treatment in the course of concentration reduction may also be accomplished, for example, by exposure to ultrasonic, electromagnetic, or other physical factors. V. Schwabe "Homeopathic medicines", M., 1967, U.S. Patents Nos. 7,229,648 and 4,311,897, which are incorporated by reference in their entirety and for the purpose stated, describe such processes that are well accepted methods of homeopathic potentiation in the homeopathic art. This procedure gives rise to a uniform decrease in molecular concentration of the initial molecular form of the antibody. This procedure is repeated until the desired homeopathic potency is obtained. For the individual antibody, the required homeopathic potency can be determined by subjecting the intermediate dilutions to biological testing in the desired pharmacological model. Although not so limited, 'homeopathic potentization" may involve, for example, repeated consecutive dilutions combined with external treatment, particularly (mechanical) shaking. In other words, an initial solution of antibody is subjected to consecutive repeated dilution and multiple vertical shaking of each obtained solution in accordance with homeopathic technology. The preferred concentration of the initial solution of antibody in the solvent, preferably, water or a water-ethyl alcohol mixture, ranges from about 0.5 to about 5.0 mg/ml. The preferred procedure for preparing each component, i.e. antibody solution, is the use of the mixture of three aqueous or aqueous-alcohol dilutions of the primary matrix solution (mother tincture) of antibodies diluted 100^{12}, 100^{30} and 100^{200} times, respectively, which is equivalent to centesimal homeopathic dilutions C12, C30 and C200 or the mixture of three aqueous or aqueous-alcohol dilutions of the primary matrix solution (mother tincture) of antibodies diluted 100^{12}, 100^{30} and 100^{50} times, respectively, which is equivalent to centesimal homeopathic dilutions C12, C30 and C50. Examples of how to obtain the desired potency are also provided, for example, in U.S. Patent

Nos. 7,229,648 and 4,311,897, which are incorporated by reference for the purpose stated. The procedure applicable to the "activated potentiated" form of the antibodies described herein is described in more detail below.

There has been a considerable amount of controversy regarding homeopathic treatment of human subjects. While the present invention relies on accepted homeopathic processes to obtain the "activated-potentiated" form of antibodies, it does not rely solely on homeopathy in human subjects for evidence of activity. It has been surprisingly discovered by the inventor of the present application and amply demonstrated in the accepted pharmacological models that the solvent ultimately obtained from consecutive multiple dilution of a starting molecular form of an antibody has definitive activity unrelated to the presence of the traces of the molecular form of the antibody in the target dilution. The "activated-potentiated" form of the antibody provided herein are tested for biological activity in well accepted pharmacological models of activity, either in appropriate in vitro experiments, or *in vivo* in suitable animal models. The experiments provided further below provide evidence of biological activity in such models. Human clinical studies also provide evidence that the activity observed in the animal model is well translated to human therapy. Human studies have also provided evidence of availability of the "activated potentiated" forms described herein to treat specified human diseases or disorders well accepted as pathological conditions in the medical science.

Also, the claimed "activated-potentiated" form of antibody encompasses only solutions or solid preparations the biological activity of which cannot be explained by the presence of the molecular form of the antibody remaining from the initial, starting solution. In other words, while it is contemplated that the "activated-potentiated" form of the antibody may contain traces of the initial molecular form of the antibody, one skilled in the art could not attribute the observed biological activity in the accepted pharmacological models to the remaining molecular form of the antibody with any degree of plausibility due to the extremely low concentrations of the molecular form of the antibody remaining after the consecutive dilutions. While the invention is not limited by any specific theory, the biological activity of the "activated-potentiated' form of the antibodies of the present invention is not attributable to the initial molecular form of the antibody. Preferred is the "activated-potentiated" form of antibody in liquid or solid form in

which the concentration of the initial molecular form of the antibody is below the limit of detection of the accepted analytical techniques, such as capillary electrophoresis and High Performance Liquid Chromatography. Particularly preferred is the "activated-potentiated" form of antibody in liquid or solid form in which the concentration of the initial molecular form of the antibody is below the Avogadro number. In the pharmacology of molecular forms of therapeutic substances, it is common practice to create a dose-response curve in which the level of pharmacological response is plotted against the concentration of the active drug administered to the subject or tested in vitro. The minimal level of the drug which produces any detectable response is known as a threshold dose. It is specifically contemplated and preferred that the "activated-potentiated" form of the antibodies contains molecular antibody, if any, at a concentration below the threshold dose for the molecular form of the antibody in the given biological model.

In one aspect, the present invention provides a combination pharmaceutical composition comprising a) an activated-potentiated form of an antibody to endothelial NO synthase and b) an activated-potentiated form of an antibody to brain-specific protein S-100. As set forth herein above, each of the individual components of the combination is generally known for its won individual medical uses. However, the inventors of the present application surprisingly discovered that administration of the combination remarkably is useful for the treatment of Alzheimer's disease.

In another aspect, the invention provides the method of treatment of Alzheimer's disease by means of insertion in an organism of activated-potentiated form of antibodies to brain-specific protein S-100 simultaneously with activated-potentiated form of antibodies to endothelial NO synthase in ultra-low doses of affinity purified antibodies.

Preferably, for the purpose of treatment, the combination pharmaceutical composition is administered from once daily to four times daily, each administration including one or two combination unit dosage forms.

The pharmaceutical composition of the present application for the purpose of treatment of Alzheimer's disease contains active components in volume primarily in 1:1 ratio.

For the purpose of treatment of Alzheimer's disease the components of the pharmaceutical composition may be administered separately. However, the simultaneous administration of the combined components in one form of solutions and/or solid dosage form (tablet), which contains activated-potentiated form of antibodies to brain-specific protein S-100 and, accordingly, activated-potentiated form of antibodies to endothelial NO synthase is preferred.

In addition, during treatment of Alzheimer's disease, separate and simultaneous application (intake to organism) of the declared pharmaceutical composition in the form of two separately prepared medications both in the form of solutions and solid dosage forms (tablets) each of which contains activated-potentiated form of antibodies to endothelial NO-synthase or to S-100 protein is possible.

The medical product is prepared mainly as follows.

The combination pharmaceutical composition in accordance with the present invention may be in the liquid form or in solid form. Each of the activated potentiated forms of the antibodies included in the pharmaceutical composition is prepared from an initial molecular form of the antibody via a process accepted in homeopathic art. The starting antibodies may be monoclonal, or polyclonal antibodies prepared in accordance with known processes, for example, as described in *Immunotechniques*, G. Frimel, M., "Meditsyna", 1987, p. 9-33; "*Hum. Antibodies. Monoclonal and recombinant antibodies, 30 years after*" by Laffly E., Sodoyer R. – 2005 – Vol. 14. – N 1-2. P.33-55, both incorporated herein by reference.

Monoclonal antibodies may be obtained, e.g., by means of hybridoma technology. The initial stage of the process includes immunization based on the principles already developed in course of polyclonal antisera preparation. Further stages of work involve production of hybrid cells generating clones of antibodies with identical specificity. Their separate isolation is performed using the same methods as in case of polyclonal antisera preparation.

Polyclonal antibodies may be obtained via active immunization of animals. For this purpose, for example, suitable animals (e.g. rabbits) receive a series of injections of the appropriate antigen: brain-specific protein S-100 and endothelial NO synthase. The animals' immune system generates corresponding antibodies,

which are collected from the animals in a known manner. This procedure enables preparation of a monospecific antibody-rich serum.

If desired, the serum containing antibodies may be purified, e.g., using affine chromatography, fractionation by salt precipitation, or ion-exchange chromatography. The resulting purified, antibody-enriched serum may be used as a starting material for preparation of the activated-potentiated form of the antibodies. The preferred concentration of the resulting initial solution of antibody in the solvent, preferably, water or water-ethyl alcohol mixture, ranges from about 0.5 to about 5.0 mg/ml.

The preferred procedure for preparing each component is the use of the mixture of three aqueous-alcohol dilutions of the primary matrix solution of antibodies diluted 100^{12}, 100^{30} and 100^{200} times, respectively, which is equivalent to centesimal homeopathic dilutions C12, C30 and C200. To prepare a solid dosage form, a solid carrier is treated with the desired dilution obtained via the homeopathic process. To obtain a solid unit dosage form of the combination of the invention, the carrier mass is impregnated with each of the dilutions. Both orders of impregnation are suitable to prepare the desired combination dosage form.

In a preferred embodiment, the starting material for the preparation of the activated potentiated form that comprise the combination of the invention is polyclonal antibodies to brain-specific protein S-100 and endothelial NO synthase an initial (matrix) solution with concentration of 0.5 to 5.0 mg/ml is used for the subsequent preparation of activated-potentiated forms.

To prepare the pharmaceutical composition preferably polyclonal antibodies to brain-specific protein S-100 and endothelial NO synthase are used.

Polyclonal antibodies to endothelial NO synthase are obtained using adjuvant as immunogen (antigen) for immunization of rabbits and whole molecule of bovine endothelial NO synthase of the following sequence:

SEQ.ID. NO. 1

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Met Gly Asn Leu Lys Ser Val Gly Gln Glu Pro Gly Pro Pro Cys
 1               5                  10                  15
Gly Leu Gly Leu Gly Leu Gly Leu Gly Leu Cys Gly Lys Gln Gly
 16              20                  25                  30
Pro Ala Ser Pro Ala Pro Glu Pro Ser Arg Ala Pro Ala Pro Ala
 31              35                  40                  45
Thr Pro His Ala Pro Asp His Ser Pro Ala Pro Asn Ser Pro Thr
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 46                  50                  55                  60
Leu Thr Arg Pro Pro Glu Gly Pro Lys Phe Pro Arg Val Lys Asn
 61                  65                  70                  75
Trp Glu Leu GLys er Ile Thr Tyr Asp Thr Leu Cys Ala Gln Ser
 76                  80                  85                  90
Gln Gln Asp Gly Pro Cys Thr Pro Arg Cys Cys Leu GLys er Leu
 91                  95                 100                 105
Val Leu Pro Arg Lys Leu Gln Thr Arg Pro Ser Pro Gly Pro Pro
106                 110                 115                 120
Pro Ala Glu Gln Leu Leu Ser Gln Ala Arg Asp Phe Ile Asn Gln
121                 125                 130                 135
Tyr Tyr Ser Ser Ile Lys Arg Ser GLys er Gln Ala His Glu Glu
136                 140                 145                 150
Arg Leu Gln Glu Val Glu Ala Glu Val Ala Ser Thr Gly Thr Tyr
151                 155                 160                 165
His Leu Arg Glu Ser Glu Leu Val Phe Gly Ala Lys Gln Ala Trp
166                 170                 175                 180
Arg Asn Ala Pro Arg Cys Val Gly Arg Ile Gln Trp Gly Lys Leu
181                 185                 190                 195
Gln Val Phe Asp Ala Arg Asp Cys Ser Ser Ala Gln Glu Met Phe
196                 200                 205                 210
Thr Tyr Ile Cys Asn His Ile Lys Tyr Ala Thr Asn Arg Gly Asn
211                 215                 220                 225
Leu Arg Ser Ala Ile Thr Val Phe Pro Gln Arg Ala Pro Gly Arg
226                 230                 235                 240
Gly Asp Phe Arg Ile Trp Asn Ser Gln Leu Val Arg Tyr Ala Gly
241                 245                 250                 255
Tyr Arg Gln Gln Asp GLys er Val Arg Gly Asp Pro Ala Asn Val
256                 260                 265                 270
Glu Ile Thr Glu Leu Cys Ile Gln His Gly Trp Thr Pro Gly Asn
271                 275                 280                 285
Gly Arg Phe Asp Val Leu Pro Leu Leu Leu Gln Ala Pro Asp Glu
286                 290                 295                 300
Ala Pro Glu Leu Phe Val Leu Pro Pro Glu Leu Val Leu Glu Val
301                 305                 310                 315
Pro Leu Glu His Pro Thr Leu Glu Trp Phe Ala Ala Leu Gly Leu
316                 320                 325                 330
Arg Trp Tyr Ala Leu Pro Ala Val Ser Asn Met Leu Leu Glu Ile
331                 335                 340                 345
Gly Gly Leu Glu Phe Ser Ala Ala Pro Phe Ser Gly Trp Tyr Met
346                 350                 355                 360
Ser Thr Glu Ile Gly Thr Arg Asn Leu Cys Asp Pro His Arg Tyr
361                 365                 370                 375
Asn Ile Leu Glu Asp Val Ala Val Cys Met Asp Leu Asp Thr Arg
376                 380                 385                 390
Thr Thr Ser Ser Leu Trp Lys Asp Lys Ala Ala Val Glu Ile Asn
391                 395                 400                 405
Leu Ala Val Leu His Ser Phe Gln Leu Ala Lys Val Thr Ile Val
406                 410                 415                 420
Asp His His Ala Ala Thr Val Ser Phe Met Lys His Leu Asp Asn
421                 425                 430                 435
Glu Gln Lys Ala Arg Gly Gly Cys Pro Ala Asp Trp Ala Trp Ile
436                 440                 445                 450
Val Pro Pro Ile Ser GLys er Leu Thr Pro Val Phe His Gln Glu
451                 455                 460                 465
Met Val Asn Tyr Ile Leu Ser Pro Ala Phe Arg Tyr Gln Pro Asp
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466 470 475 480
Pro Trp Lys GLy Ser Ala Thr Lys Gly Ala Gly Ile Thr Arg Lys
481 485 490 495
Lys Thr Phe Lys Glu Val Ala Asn Ala Val Lys Ile Ser Ala Ser
496 500 505 510
Leu Met Gly Thr Leu Met Ala Lys Arg Val Lys Ala Thr Ile Leu
511 515 510 525
Tyr Ala Ser Glu Thr Gly Arg Ala Gln Ser Tyr Ala Gln Gln Leu
526 530 535 540
Gly Arg Leu Phe Arg Lys Ala Phe Asp Pro Arg Val Leu Cys Met
541 545 550 555
Asp Glu Tyr Asp Val Val Ser Leu Glu His Glu Ala Leu Val Leu
556 560 565 570
Val Val Thr Ser Thr Phe Gly Asn Gly Asp Pro Pro Glu Asn Gly
571 575 580 585
Glu Ser Phe Ala Ala Ala Leu Met Glu Met Ser Gly Pro Tyr Asn
586 590 595 600
Ser Ser Pro Arg Pro Glu Gln His Lys Ser Tyr Lys Ile Arg Phe
601 605 610 615
Asn Ser Val Ser Cys Ser Asp Pro Leu Val Ser Ser Trp Arg Arg
616 620 625 630
Lys Arg Lys Glu Ser Ser Asn Thr Asp Ser Ala Gly Ala Leu Gly
631 635 640 645
Thr Leu Arg Phe Cys Val Phe Gly Leu GLy Ser Arg Ala Tyr Pro
646 650 655 660
His Phe Cys Ala Phe Ala Arg Ala Val Asp Thr Arg Leu Glu Glu
661 665 670 675
Leu Gly Gly Glu Arg Leu Leu Gln Leu Gly Gln Gly Asp Glu Leu
676 680 685 690
Cys Gly Gln Glu Glu Ala Phe Arg Gly Trp Ala Lys Ala Ala Phe
691 695 700 705
Gln Ala Ser Cys Glu Thr Phe Cys Val Gly Glu Glu Ala Lys Ala
706 710 715 720
Ala Ala Gln Asp Ile Phe Ser Pro Lys Arg Ser Trp Lys Arg Gln
721 725 730 735
Arg Tyr Arg Leu Ser Thr Gln Ala Glu Gly Leu Gln Leu Leu Pro
736 740 745 750
Gly Leu Ile His Val His Arg Arg Lys Met Phe Gln Ala Thr Val
751 755 760 765
Leu Ser Val Glu Asn Leu Gln Ser Ser Lys Ser Thr Arg Ala Thr
766 770 775 780
Ile Leu Val Arg Leu Asp Thr Ala Gly Gln Glu Gly Leu Gln Tyr
781 785 790 795
Gln Pro Gly Asp His Ile Gly Ile Cys Pro Pro Asn Arg Pro Gly
796 800 805 810
Leu Val Glu Ala Leu Leu Ser Arg Val Glu Asp Pro Pro Pro Pro
811 815 820 825
Thr Glu Ser Val Ala Val Glu Gln Leu Glu Lys GLys er Pro Gly
826 830 835 840
Gly Pro Pro Pro Ser Trp Val Arg Asp Pro Arg Leu Pro Pro Cys
841 845 850 855
Thr Leu Arg Gln Ala Leu Thr Phe Phe Leu Asp Ile Thr Ser Pro
856 860 865 870
Pro Ser Pro Arg Leu Leu Arg Leu Leu Ser Thr Leu Ala Glu Glu
871 875 880 885
Pro Ser Glu Gln Gln Glu Leu Glu Thr Leu Ser Gln Asp Pro Arg

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886                 890                 895                 900
Arg Tyr Glu Glu Trp Lys Trp Phe Arg Cys Pro Thr Leu Leu Glu
901                 905                 910                 915
Val Leu Glu Gln Phe Pro Ser Val Ala Leu Pro Ala Pro Leu Leu
916                 920                 925                 930
Leu Thr Gln Leu Pro Leu Leu Gln Pro Arg Tyr Tyr Ser Val Ser
931                 935                 940                 945
Ser Ala Pro Asn Ala His Pro Gly Glu Val His Leu Thr Val Ala
946                 950                 955                 960
Val Leu Ala Tyr Arg Thr Gln Asp Gly Leu Gly Pro Leu His Tyr
961                 965                 970                 975
Gly Val Cys Ser Thr Trp Leu Ser Gln Leu Lys Thr Gly Asp Pro
976                 980                 985                 990
Val Pro Cys Phe Ile Arg Gly Ala Pro Ser Phe Arg Leu Pro Pro
991                 995                1000                1005
Asp Pro Tyr Val Pro Cys Ile Leu Val Gly Pro Gly Thr Gly Ile
1006               1010                1015                1020
Ala Pro Phe Arg Gly Phe Trp Gln Glu Arg Leu His Asp Ile Glu
1021               1025                1030                1035
Ser Lys Gly Leu Gln Pro Ala Pro Met Thr Leu Val Phe Gly Cys
1036               1140                1145                1050
Arg Cys Ser Gln Leu Asp His Leu Tyr Arg Asp Glu Val Gln Asp
1051               1155                1160                1065
Ala Gln Glu Arg Gly Val Phe Gly Arg Val Leu Thr Ala Phe Ser
1066               1170                1175                1080
Arg Glu Pro Asp Ser Pro Lys Thr Tyr Val Gln Asp Ile Leu Arg
1081               1185                1190                1095
Thr Glu Leu Ala Ala Glu Val His Arg Val Leu Cys Leu Glu Arg
1096               1100                1105                1110
Gly His Met Phe Val Cys Gly Asp Val Thr Met Ala Thr Ser Val
1111               1115                1120                1125
Leu Gln Thr Val Gln Arg Ile Leu Ala Thr Glu Gly Asp Met Glu
1126               1130                1135                1140
Leu Asp Glu Ala Gly Asp Val Ile Gly Val Leu Arg Asp Gln Gln
1141               1145                1150                1155
Arg Tyr His Glu Asp Ile Phe Gly Leu Thr Leu Arg Thr Gln Glu
1156               1160                1165                1170
Val Thr Ser Arg Ile Arg Thr Gln Ser Phe Ser Leu Gln Glu Arg
1171               1175                1180                1185
His Leu Arg Gly Ala Val Pro Trp Ala Phe Asp Pro Pro Gly Pro
1186               1190                1195                1200
Asp Thr Pro Gly Pro
1201               1205
```

Polyclonal antibodies to endothelial NO synthase may be obtained using the whole molecule of human endothelial NO synthase of the following sequence:

SEQ ID NO:2

```
Met Gly Asn Leu Lys Ser Val Ala Gln Glu Pro Gly Pro Pro Cys
 1                5                  10                  15
Gly Leu Gly Leu Gly Leu Gly Leu Gly Leu Cys Gly Lys Gln Gly
 16               20                  25                  30
Pro Ala Thr Pro Ala Pro Glu Pro Ser Arg Ala Pro Ala Ser Leu
```

```
 31                35                40                45
Leu Pro Pro Ala Pro Glu His Ser Pro Pro Ser Ser Pro Leu Thr
 46                50                55                60
Gln Pro Pro Glu Gly Pro Lys Phe Pro Arg Val Lys Asn Trp Glu
 61                65                70                75
Val GLys er Ile Thr Tyr Asp Thr Leu Ser Ala Gln Ala Gln Gln
 76                80                85                90
Asp Gly Pro Cys Thr Pro Arg Arg Cys Leu GLys er Leu Val Phe
 91                95               100               105
Pro Arg Lys Leu Gln Gly Arg Pro Ser Pro Gly Pro Pro Ala Pro
106               110               115               120
Glu Gln Leu Leu Ser Gln Ala Arg Asp Phe Ile Asn Gln Tyr Tyr
121               125               130               135
Ser Ser Ile Lys Arg Ser GLys er Gln Ala His Glu Gln Arg Leu
136               140               145               150
Gln Glu Val Glu Ala Glu Val Ala Ala Thr Gly Thr Tyr Gln Leu
151               155               160               165
Arg Glu Ser Glu Leu Val Phe Gly Ala Lys Gln Ala Trp Arg Asn
166               170               175               180
Ala Pro Arg Cys Val Gly Arg Ile Gln Trp Gly Lys Leu Gln Val
181               185               190               195
Phe Asp Ala Arg Asp Cys Arg Ser Ala Gln Glu Met Phe Thr Tyr
196               200               205               210
Ile Cys Asn His Ile Lys Tyr Ala Thr Asn Arg Gly Asn Leu Arg
211               215               220               225
Ser Ala Ile Thr Val Phe Pro Gln Arg Cys Pro Gly Arg Gly Asp
226               230               235               240
Phe Arg Ile Trp Asn Ser Gln Leu Val Arg Tyr Ala Gly Tyr Arg
241               245               250               255
Gln Gln Asp GLy Ser Val Arg Gly Asp Pro Ala Asn Val Glu Ile
256               260               265               270
Thr Glu Leu Cys Ile Gln His Gly Trp Thr Pro Gly Asn Gly Arg
271               275               280               285
Phe Asp Val Leu Pro Leu Leu Leu Gln Ala Pro Asp Glu Pro Pro
286               290               295               300
Glu Leu Phe Leu Leu Pro Pro Glu Leu Val Leu Glu Val Pro Leu
301               305               310               315
Glu His Pro Thr Leu Glu Trp Phe Ala Ala Leu Gly Leu Arg Trp
316               320               325               330
Tyr Ala Leu Pro Ala Val Ser Asn Met Leu Leu Glu Ile Gly Gly
331               335               340               345
Leu Glu Phe Pro Ala Ala Pro Phe Ser Gly Trp Tyr Met Ser Thr
346               350               355               360
Glu Ile Gly Thr Arg Asn Leu Cys Asp Pro His Arg Tyr Asn Ile
361               365               370               375
Leu Glu Asp Val Ala Val Cys Met Asp Leu Asp Thr Arg Thr Thr
376               380               385               390
Ser Ser Leu Trp Lys Asp Lys Ala Ala Val Glu Ile Asn Val Ala
391               395               400               405
Val Leu His Ser Tyr Gln Leu Ala Lys Val Thr Ile Val Asp His
406               410               415               420
His Ala Ala Thr Ala Ser Phe Met Lys His Leu Glu Asn Glu Gln
421               425               430               435
Lys Ala Arg Gly Gly Cys Pro Ala Asp Trp Ala Trp Ile Val Pro
436               440               445               450
Pro Ile Ser GLys er Leu Thr Pro Val Phe His Gln Glu Met Val
```

451 455 460 465
Asn Tyr Phe Leu Ser Pro Ala Phe Arg Tyr Gln Pro Asp Pro Trp
466 470 475 480
Lys Gly Ser Ala Ala Lys Gly Thr Gly Ile Thr Arg Lys Lys Thr
481 485 490 495
Phe Lys Glu Val Ala Asn Ala Val Lys Ile Ser Ala Ser Leu Met
496 500 505 510
Gly Thr Val Met Ala Lys Arg Val Lys Ala Thr Ile Leu Tyr Gly
511 515 510 525
Ser Glu Thr Gly Arg Ala Gln Ser Tyr Ala Gln Gln Leu Gly Arg
526 530 535 540
Leu Phe Arg Lys Ala Phe Asp Pro Arg Val Leu Cys Met Asp Glu
541 545 550 555
Tyr Asp Val Val Ser Leu Glu His Glu Thr Leu Val Leu Val Val
556 560 565 570
Thr Ser Thr Phe Gly Asn Gly Asp Pro Pro Glu Asn Gly Glu Ser
571 575 580 585
Phe Ala Ala Ala Leu Met Glu Met Ser Gly Pro Tyr Asn Ser Ser
586 590 595 600
Pro Arg Pro Glu Gln His Lys Ser Tyr Lys Ile Arg Phe Asn Ser
601 605 610 615
Ile Ser Cys Ser Asp Pro Leu Val Ser Ser Trp Arg Arg Lys Arg
616 620 625 630
Lys Glu Ser Ser Asn Thr Asp Ser Ala Gly Ala Leu Gly Thr Leu
631 635 640 645
Arg Phe Cys Val Phe Gly Leu GLys er Arg Ala Tyr Pro His Phe
646 650 655 660
Cys Ala Phe Ala Arg Ala Val Asp Thr Arg Leu Glu Glu Leu Gly
661 665 670 675
Gly Glu Arg Leu Leu Gln Leu Gly Gln Gly Asp Glu Leu Cys Gly
676 680 685 690
Gln Glu Glu Ala Phe Arg Gly Trp Ala Gln Ala Ala Phe Gln Ala
691 695 700 705
Ala Cys Glu Thr Phe Cys Val Gly Glu Asp Ala Lys Ala Ala Ala
706 710 715 720
Arg Asp Ile Phe Ser Pro Lys Arg Ser Trp Lys Arg Gln Arg Tyr
721 725 730 735
Arg Leu Ser Ala Gln Ala Glu Gly Leu Gln Leu Leu Pro Gly Leu
736 740 745 750
Ile His Val His Arg Arg Lys Met Phe Gln Ala Thr Ile Arg Ser
751 755 760 765
Val Glu Asn Leu Gln Ser Ser Lys Ser Thr Arg Ala Thr Ile Leu
766 770 775 780
Val Arg Leu Asp Thr Gly Gly Gln Glu Gly Leu Gln Tyr Gln Pro
781 785 790 795
Gly Asp His Ile Gly Val Cys Pro Pro Asn Arg Pro Gly Leu Val
796 800 805 810
Glu Ala Leu Leu Ser Arg Val Glu Asp Pro Pro Ala Pro Thr Glu
811 815 820 825
Pro Val Ala Val Glu Gln Leu Glu Lys Gly Ser Pro Gly Gly Pro
826 830 835 840
Pro Pro Gly Trp Val Arg Asp Pro Arg Leu Pro Pro Cys Thr Leu
841 845 850 855
Arg Gln Ala Leu Thr Phe Phe Leu Asp Ile Thr Ser Pro Pro Ser
856 860 865 870
Pro Gln Leu Leu Arg Leu Leu Ser Thr Leu Ala Glu Glu Pro Arg

```
871                 875                 880                 885
Glu Gln Gln Glu Leu Glu Ala Leu Ser Gln Asp Pro Arg Arg Tyr
886                 890                 895                 900
Glu Glu Trp Lys Trp Phe Arg Cys Pro Thr Leu Leu Glu Val Leu
901                 905                 910                 915
Glu Gln Phe Pro Ser Val Ala Leu Pro Ala Pro Leu Leu Leu Thr
916                 920                 925                 930
Gln Leu Pro Leu Leu Gln Pro Arg Tyr Tyr Ser Val Ser Ser Ala
931                 935                 940                 945
Pro Ser Thr His Pro Gly Glu Ile His Leu Thr Val Ala Val Leu
946                 950                 955                 960
Ala Tyr Arg Thr Gln Asp Gly Leu Gly Pro Leu His Tyr Gly Val
961                 965                 970                 975
Cys Ser Thr Trp Leu Ser Gln Leu Lys Pro Gly Asp Pro Val Pro
976                 980                 985                 990
Cys Phe Ile Arg Gly Ala Pro Ser Phe Arg Leu Pro Pro Asp Pro
991                 995                1000                1005
Ser Leu Pro Cys Ile Leu Val Gly Pro Gly Thr Gly Ile Ala Pro
1006                1010                1015                1020
Phe Arg Gly Phe Trp Gln Glu Arg Leu His Asp Ile Glu Ser Lys
1021                1025                1030                1035
Gly Leu Gln Pro Thr Pro Met Thr Leu Val Phe Gly Cys Arg Cys
1036                1040                1045                1050
Ser Gln Leu Asp His Leu Tyr Arg Asp Glu Val Gln Asn Ala Gln
1051                1055                1060                1065
Gln Arg Gly Val Phe Gly Arg Val Leu Thr Ala Phe Ser Arg Glu
1066                1070                1075                1080
Pro Asp Asn Pro Lys Thr Tyr Val Gln Asp Ile Leu Arg Thr Glu
1081                1085                1090                1095
Leu Ala Ala Glu Val His Arg Val Leu Cys Leu Glu Arg Gly His
1096                1100                1105                1110
Met Phe Val Cys Gly Asp Val Thr Met Ala Thr Asn Val Leu Gln
1111                1115                1120                1125
Thr Val Gln Arg Ile Leu Ala Thr Glu Gly Asp Met Glu Leu Asp
1126                1130                1135                1140
Glu Ala Gly Asp Val Ile Gly Val Leu Arg Asp Gln Gln Arg Tyr
1141                1145                1150                1155
His Glu Asp Ile Phe Gly Leu Thr Leu Arg Thr Gln Glu Val Thr
1156                1160                1165                1170
Ser Arg Ile Arg Thr Gln Ser Phe Ser Leu Gln Glu Arg Gln Leu
1171                1175                1180                1185
Arg Gly Ala Val Pro Trp Ala Phe Asp Pro Pro Gly Ser Asp Thr
1186                1190                1195                1200
Asn Ser Pro
1201    1203
```

To obtain polyclonal antibodies to NO synthase, it is also possible to use a fragment of endothelial NO synthase, selected, for example, from the following sequences:

SEQ ID NO:3

```
Pro Trp Ala Phe
1192        1195
```

SEQ ID NO:4
Gly Ala Val Pro
1189 1192

SEQ ID NO:5
Arg
1185
His Leu Arg Gly Ala Val Pro Trp Ala Phe Asp Pro Pro Gly Pro
1186 1190 1195 1200
Asp Thr Pro Gly Pro
1201 1205

SEQ ID NO:6
Ala Phe Asp Pro Pro Gly Pro
11941195 1200
Asp Thr Pro Gly Pro
1201 1205

SEQ ID NO:7
His Leu Arg Gly Ala Val Pro Trp Ala Phe Asp
1186 1190 11951196

SEQ ID NO:8
His Leu Arg Gly Ala Val Pro Trp Ala Phe Asp Pro Pro Gly Pro
1186 1190 1195 1200
Asp Thr Pro Gly Pro
1201 1205

The exemplary procedure for preparation of starting polyclonal antibodies to NO synthase may be described as follows: 7-9 days before blood sampling 1-3 intravenous injections are made to the rabbits to increase the level of polyclonal antibodies in the rabbit blood stream. Upon immunization, blood samples are taken to test the antibody level. Typically, the maximum level of the immune reaction of the soluble antigen is reached in 40-60 days after the first injection. After the termination of the first immunization cycle, rabbits have a 30-day rehabilitation period, after which re-immunization is performed with another 1-3 intravenous injections.

To obtain antiserum containing the desired antibodies, the immunized rabbits' blood is collected from rabbits and placed in a 50ml centrifuge tube Product clots formed on the tube sides are removed with a wooden spatula, and a rod is placed into the clot in the tube center. The blood is then placed in a refrigerator for one night at the temperature of about 4°C. On the following day,

the clot on the spatula is removed, and the remaining liquid is centrifuged for 10 min at 13,000 rotations per minute. Supernatant fluid is the target antiserum. The obtained antiserum is typically yellow. 20% of NaN_3 (weight concentration) is added in the antiserum to a final concentration of 0.02% and stored before use in frozen state at the temperature of -20°C (or without addition NaN_3 – at temperature -70^{0}C). To separate the target antibodies to endothelial NO synthase from the antiserum, the following solid phase absorption sequence is suitable:

(a) 10 ml of antiserum of rabbit is diluted twofold with 0.15 M NaCl, after which 6.26 g Na_2SO_4, is added, mixed and incubated for about 12-16 hours at 4°C;

(b) the sediment is removed by centrifugation, dissolved in 10 ml of phosphate buffer and dialyzed against the same buffer within one night at room temperature;

(c) after the sediment is removed by centrifugation, the solution is put on the column with DEAE-cellulose, counterbalanced by phosphate buffer;

(d) the antibody fraction is determined by measuring the optical density of eluate at 280 nanometers.

The isolated crude antibodies are purified using affine chromatography method by attaching the obtained antibodies to endothelial NO synthase located on the insoluble matrix of the chromatography media, with subsequent elution by concentrated aqueous salt solutions.

The resulting buffer solution is used as the initial solution for the homeopathic dilution process used to prepare the activated potentiated form of the antibodies. The preferred concentration of the initial matrix solution of the antigen-purified polyclonal rabbit antibodies to endothelial NO synthase is 0.5 to 5.0 mg/ml, preferably, 2.0 to 3.0 mg/ml.

The brain-specific S100 protein, expressed by neurons and glial cells (astrocytes and oligodendrocytes), directly or through interactions with other proteins executes in the CNS a number of functions directed at maintaining normal brain functioning, including affecting learning and memory processes, growth and viability of neurons, regulation of metabolic processes in neuronal tissues and others. To obtain polyclonal antibodies to brain-specific protein S-100, brain-specific protein S-100 is used, which physical and chemical properties are described in the article of M. V. Starostin, S. M. Sviridov, Neurospecific

Protein S-100, *Progress of Modern Biology*, 1977, Vol. 5, P. 170-178; found in the book M. B. Shtark, *Brain-Specific Protein Antigenes and Functions of Neuron*, "Medicine", 1985; P. 12-14. Brain-specific protein S-100 is allocated from brain tissue of the bull by the following technique:

- the bull brain tissue frozen in liquid nitrogen is converted into powder using a specialized mill;
- proteins are extracted in the ratio of 1:3 (weight/volume) using an extracting buffer with homogenization;
- the homogenate is heated for 10 min at 60°C and then cooled to 4°C in an ice bath;
- thermolabile proteins are removed by centrifugation;
- ammonium sulfate fractionation is carried out in stages, with subsequent removal of precipitated proteins;
- the fraction containing S-100 protein is precipitated using 100% saturated ammonium sulfate accomplished by pH drop to 4.0; the desired fraction is collected by centrifugation;
- the precipitate is dissolved in a minimum buffer volume containing EDTA and mercaptoethanol, the precipitate is dialyzed with deionized water and lyophilized;
- fractionation of acidic proteins is followed by chromatography in ion-exchanging media, DEAE-cellulose DE-52 and then DEAE-sephadex A-50;
- the collected and dialyzed fractions, which contain S-100 protein, are divided according to molecular weight by gel filtration on sephadex G-100;
- purified S-100 protein is dialyzed and lyophilized.

The molecular weight of the purified brain-specific protein S-100 is 21000 D.

Owing to the high concentration of asparaginic and glutaminic acids brain-specific protein S-100 is highly acidic and occupies extreme anode position during electroendosmosis in a discontinuous buffer system of polyacrylamide gel which facilitates its identification.

The polyclonal antibodies to S-100 protein may also be obtained by a similar methodology to the methodology described for endothelial NO synthase antibodies using an adjuvant. The entire molecule of S-100 protein may be used as immunogen (antigen) for rabbits' immunization:

Bovine S100B (SEQ ID NO:9)

```
Met Ser Glu Leu Glu Lys Ala Val Val Ala Leu Ile Asp Val Phe
 1               5                  10                  15
His Gln Tyr Ser Gly Arg Glu Gly Asp Lys His Lys Leu Lys Lys
 16              20                  25                  30
Ser Glu Leu Lys Glu Leu Ile Asn Asn Glu Leu Ser His Phe Leu
 31              35                  40                  45
Glu Glu Ile Lys Glu Gln Glu Val Val Asp Lys Val Met Glu Thr
 46              50                  55                  60
Leu Asp Ser Asp Gly Asp Gly Glu Cys Asp Phe Gln Glu Phe Met
 61              65                  70                  75
Ala Phe Val Ala Met Ile Thr Thr Ala Cys His Glu Phe Phe Glu
 76              80                  85                  90
His Glu
 91  92
```

Human S100B (SEQ ID NO:10)

```
Met Ser Glu Leu Glu Lys Ala Met Val Ala Leu Ile Asp Val Phe
 1               5                  10                  15
His Gln Tyr Ser Gly Arg Glu Gly Asp Lys His Lys Leu Lys Lys
 16              20                  25                  30
Ser Glu Leu Lys Glu Leu Ile Asn Asn Glu Leu Ser His Phe Leu
 31              35                  40                  45
Glu Glu Ile Lys Glu Gln Glu Val Val Asp Lys Val Met Glu Thr
 46              50                  55                  60
Leu Asp Asn Asp Gly Asp Gly Glu Cys Asp Phe Gln Glu Phe Met
 61              65                  70                  75
Ala Phe Val Ala Met Val Thr Thr Ala Cys His Glu Phe Phe Glu
 76              80                  85                  90
His Glu
91  92
```

Human S100A1 (SEQ ID NO:11)

```
Met Gly Ser Glu Leu Glu Thr Ala Met Glu Thr Leu Ile Asn Val
 1               5                  10                  15
Phe His Ala His Ser Gly Lys Glu Gly Asp Lys Tyr Lys Leu Ser
 16              20                  25                  30
Lys Lys Glu Leu Lys Glu Leu Leu Gln Thr Glu Leu Ser Gly Phe
 31              35                  40                  45
Leu Asp Ala Gln Lys Asp Val Asp Ala Val Asp Lys Val Met Lys
 46              50                  55                  60
Glu Leu Asp Glu Asn Gly Asp Gly Glu Val Asp Phe Gln Glu Tyr
 61              65                  70                  75
Val Val Leu Val Ala Ala Leu Thr Val Ala Cys Asn Asn Phe Phe
 76              80                  85                  90
Trp Glu Asn Ser
 91          94
```

Bovine S100A1 (SEQ ID NO:12)

```
Met Gly Ser Glu Leu Glu Thr Ala Met Glu Thr Leu Ile Asn Val
 1               5                  10                  15
Phe His Ala His Ser Gly Lys Glu Gly Asp Lys Tyr Lys Leu Ser
```

```
 16              20                  25                  30
Lys Lys Glu Leu Lys Glu Leu Leu Gln Thr Glu Leu Ser Gly Phe
 31              35                  40                  45
Leu Asp Ala Gln Lys Asp Ala Asp Ala Val Asp Lys Val Met Lys
 46              50                  55                  60
Glu Leu Asp Glu Asn Gly Asp Gly Glu Val Asp Phe Gln Glu Tyr
 61              65                  70                  75
Val Val Leu Val Ala Ala Leu Thr Val Ala Cys Asn Asn Phe Phe
 76              80                  85                  90
Trp Glu Asn Ser
 91          94
```

To obtain antiserum, brain-specific S-100 protein or the mixture of S-100 protein s (antigens) in complex with methylated bull seralbumin as the carrying agent with full Freund's adjuvant is prepared and added to allocated brain-specific protein S-100 which is injected subdermally to a laboratory animal – a rabbit into area of back in quantity of 1-2 ml. On 8th, 15th day repeated immunization is made. Blood sampling is made (for example, from a vein in the ear) on the 26th and the 28th day.

The obtained antiserum titre is 1:500 - 1:1000, forms single precipitin band with an extract of nervous tissue but does not react with extracts of heterological bodies and forms single precipitin peak both with pure protein S-100 and with the extract of nervous tissue indicating that the antiserum obtained is monospecific.

The activated potentiated form of each component of the combination may be prepared from an initial solution by homeopathic potentization, preferably using the method of proportional concentration decrease by serial dilution of 1 part of each preceding solution (beginning with the initial solution) in 9 parts (for decimal dilution), or in 99 parts (for centesimal dilution), or in 999 parts (for millesimal dilution – attenuation M) of a neutral solvent, starting with a concentration of the initial solution of antibody in the solvent, preferably, water or a water-ethyl alcohol mixture, in the range from about 0.5 to about 5.0 mg/ml, coupled with external impact. Preferably, the external impact involves multiple vertical shaking (dynamization) of each dilution. Preferably, separate containers are used for each subsequent dilution up to the required potency level, or the dilution factor. This method is well-accepted in the homeopathic art. See, e.g. V. Schwabe "*Homeopathic medicines*", M., 1967, p. 14-29, incorporated herein by reference for the purpose stated.

For example, to prepare a 12-centesimal dilution (denoted C12), one part of the initial matrix solution of antibodies to brain-specific protein S-100 (or to endothelial NO - synthase) with the concentration of 2.5 mg/ml is diluted in 99 parts of neutral aqueous or aqueous-alcohol solvent (preferably, 15%-ethyl alcohol) and then vertically shaken many times (10 and more) to create the 1st centesimal dilution (denoted as C1). The 2nd centesimal dilution (C2) is prepared from the 1st centesimal dilution C1. This procedure is repeated 11 times to prepare the 12th centesimal dilution C12. Thus, the 12th centesimal dilution C12 represents a solution obtained by 12 serial dilutions of one part of the initial matrix solution of antibodies to brain-specific protein S-100 with the concentration of 2.5 mg/ml in 99 parts of a neutral solvent in different containers, which is equivalent to the centesimal homeopathic dilution C12. Similar procedures with the relevant dilution factor are performed to obtain dilutions C30, C50 and C 200. The intermediate dilutions may be tested in a desired biological model to check activity. The preferred activated potentiated forms for both antibodies comprising the combination of the invention are a mixture of C12, C30, and C200 dilutions or C12, C30 and C50 dilutions. When using the mixture of various homeopathic dilutions (primarily centesimal) of the active substance as biologically active liquid component, each component of the composition (e.g., C12, C30, C50, C200) is prepared separately according to the above-described procedure until the next-to-last dilution is obtained (e.g., until C11, C29, C49 and C199 respectively), and then one part of each component is added in one container according to the mixture composition and mixed with the required quantity of the solvent (e.g. with 97 parts for centesimal dilution).

Thus, activated-potentiated form of antibodies to brain-specific protein S-100 in ultra low dose is obtained by extra attenuation of matrix solution, accordingly in 100^{12}, 100^{30} and 100^{200} times, equal to centesimal C12, C30 and C200 solutions or 100^{12}, 100^{30} and 100^{50} times, equal to centesimal C12, C30 and C50 solutions prepared on homoeopathic technology.

Use of active substance in the form of mixture of other various solutions on homoeopathic technology, for example, decimal and/or centesimal, (C12, C30, C100; C12, C30, C50; D20, C30, C100 or D10, C30, M100 etc.) is possible. The efficiency is defined experimentally.

External processing in the course of potentiation and concentration reduction can also be carried out by means of ultrasound, of electromagnetic or any other physical influence accepted in the homeopathic art.

Preferably, the combination pharmaceutical composition of the invention may be in the form of a liquid or in the solid unit dosage form. The preferred liquid form of the pharmaceutical composition is a mixture, preferably, at a 1:1 ratio of the activated potentiated form of antibodies to endothelial NO synthase and the activated potentiated form of antibodies to protein S-100. The preferred liquid carrier is water or water-ethyl alcohol mixture.

The solid unit dosage form of the pharmaceutical composition of the invention may be prepared by using impregnating a solid, pharmaceutically acceptable carrier with the mixture of the activated potentiated form aqueous or aqueous-alcohol solutions of active components that are mixed, primarily in 1:1 ratio and used in liquid dosage form. Alternatively, the carrier may be impregnated consecutively with each requisite dilution. Both orders of impregnation are acceptable.

Preferably, the pharmaceutical composition in the solid unit dosage form is prepared from granules of the pharmaceutically acceptable carrier which was previously saturated with the aqueous or aqueous-alcoholic dilutions of the activated potentiated form of antibodies. The solid dosage form may be in any form known in the pharmaceutical art, including a tablet, a capsule, a lozenge, and others. As an inactive pharmaceutical ingredients one can use glucose, sucrose, maltose, amylum, isomaltose, isomalt and other mono- olygo- and polysaccharides used in manufacturing of pharmaceuticals as well as technological mixtures of the above mentioned inactive pharmaceutical ingredients with other pharmaceutically acceptable excipients, for example isomalt, crospovidone, sodium cyclamate, sodium saccharine, anhydrous citric acid etc), including lubricants, disintegrants, binders and coloring agents. The preferred carriers are lactose and isomalt. The pharmaceutical dosage form may further include standard pharmaceutical excipients, for example, microcrystalline cellulose, magnesium stearate and citric acid.

The example of preparation of the solid unit dosage form is set forth below. To prepare the solid oral form, 100-300 μm granules of lactose are impregnated with aqueous or aqueous-alcoholic solutions of the activated-potentiated form of

antibodies to endothelial NO synthase and the activated potentiated form of antibodies to protein S-100 in the ratio of 1 kg of antibody solution to 5 or 10 kg of lactose (1:5 to 1:10). To effect impregnation, the lactose granules are exposed to saturation irrigation in the fluidized boiling bed in a boiling bed plant (e.g. "Hüttlin Pilotlab" by Hüttlin GmbH) with subsequent drying via heated air flow at a temperature below 40°C. The estimated quantity of the dried granules (10 to 34 weight parts) saturated with the activated potentiated form of antibodies is placed in the mixer, and mixed with 25 to 45 weight parts of "non-saturated" pure lactose (used for the purposes of cost reduction and simplification and acceleration of the technological process without decreasing the treatment efficiency), together with 0.1 to 1 weight parts of magnesium stearate, and 3 to 10 weight parts of microcrystalline cellulose. The obtained tablet mass is uniformly mixed, and tableted by direct dry pressing (e.g., in a Korsch – XL 400 tablet press) to form 150 to 500 mg round pills, preferably, 300 mg. After tableting, 300 mg pills are obtained that are saturated with aqueous-alcohol solution (3.0-6.0 mg/pill) of the combination of the activated-potentiated form of antibodies. Each component of the combination used to impregnate the carrier is in the form of a mixture of centesimal homeopathic dilutions, preferably, C12, C30 and C200.

Preferably, 1-2 tablets of the claimed pharmaceutical composition are administered 2-4 times a day.

Moreover the declared drug broadens assortment of medications designed for the treatment of Alzheimer's disease.

In addition, the combination pharmaceutical composition of the present invention may be used for the treatment of Alzheimer's disease. For the treatment of said disorder the combination pharmaceutical composition may contain active components in volume ratio 1:1, thus, each component is used as the mixture of three matrix solutions (mother tincture) of antibodies diluted 100^{12}, 100^{30} and 100^{200} times, respectively, which is equivalent to centesimal homeopathic dilutions (C12, C30, and C200) or mixture of three matrix solutions of antibodies diluted 100^{12}, 100^{30} and 100^{50} times, respectively, which is equivalent to centesimal homeopathic dilutions (C12, C30 and C50). The claimed pharmaceutical composition is recommended to be taken, preferably in 1-2 tablets 2-6 times (preferably 2-4 times) a day.

The claimed pharmaceutical composition as well as its components does not possess sedative and myorelaxant effect, does not cause addiction and habituation.

EXAMPLES

Example 1.

Study of effect of a complex preparation containing ultra-low doses (ULD) of polyclonal affinity purified rabbit antibodies to brain-specific protein S-100 (anti-S100) and endothelial NO-synthase (anti-eNOS), obtained by super-dilution of initial matrix solution (concentration: 2,5 mg/ml) (100^{12}, 100^{30}, 100^{200} times), equivalent to a blend of centesimal homeopathic dilutions C12, C30, C200 (ratio: 1:1) (ULD anti-S100+anti-eNOS), as well as its components – ultra-low doses (ULD) of polyclonal affinity purified rabbit antibodies to of brain-specific protein S-100 (anti-S100), purified on antigen, obtained by super-dilution of initial matrix solution (100^{12}, 100^{30}, 100^{200} times, equivalent to a blend of centesimal homeopathic dilution C12, C30, C200, and ultra-low doses of polyclonal rabbit antibodies to endothelial NO-synthase (ULD anti-eNOS), obtained by super-dilution of initial matrix solution (100^{12}, 100^{30}, 100^{200} times), equivalent to a blend of centesimal homeopathic dilution C12, C30, C200 *in vitro* on binding of standard ligand [^{3}H]pentazocine to human recombinant σ1 receptor was evaluated using radioligand method. Potentiated distilled water (blend of homeopathic dilutions C12+C30+C200) was used as test preparations control.

Sigma-1 (σ1) receptor - an intracellular one which is localized in the cells of central nervous system, the cells of the most of peripheral tissues and immune component cells. Receptors exhibit a unique ability to be translocated which is caused by many psychotropic medications. The dynamics of sigma-1 receptors is directly linked to various influences which are performed by preparations acting to the sigma-1 receptors. These effects include the regulation of activity channels, ecocytosis, signal transfering, remodeling of the plasma membrane (formation of rafts) and lipid transportation / metabolism. All this can contribute to the plasticity of neurons in a brain. There is evidence that the sigma-1 receptors have a modulating effect on all the major neuromediator systems: noradrenergic, serotonergic, dopaminergic, cholinergic systems and NMDA- adjustable glutamate effects. Sigma-1 receptor plays an important role in the pathophysiology of

neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson), psychiatric and affective disorders, stroke and takes part in the processes of learning and memory. In this regard, the ability of drugs to influence the efficiency of interaction of ligands with sigma-1 receptor indicates on the presence of neuroprotective, anti-ischemic, anxiolytic, antidepressant and anti astenic components in the spectrum of its pharmacological activity that allows to consider these drugs as effective preparations particularly for the treatment of cerebrovascular diseases.

During the test (to measure total binding) 20 µl of complex preparation of ULD anti-S100+anti-eNOS or 10 µl of ULD AB to S100 or 10 µl of ULD AB to NOS were transferred in the incubation medium. Thus, the quantity of ULD anti-S100+anti-eNOS, transferred into the test well when testing the complex preparation was identical to that of ULD AB to S100 and ULD AB to NOS tested as monopreparations, which allow comparing the efficiency of the preparation to its separate components. 20 µl and 10 µl of potentiated water were transferred in the incubation medium.

Further, 160 µl (about 200µg of protein) of Jurkat cell line membranes homogenate (human leukemic T-lymphocyte line), and finally, 20 µl of tritium-labeled radioligand [^{3}H]pentazocine (15 nm) were transferred.

In order to measure non-specific binding, 20 µl of non-labeled ligand-haloperidol (10 µM) were transferred in the incubation medium instead of the preparations or potentiated water.

Radioactivity was measured using a scintillometer (Topcount, Packard) and scintillation blend (Microscint 0, Packard) following the incubation within 120 minutes at 22^{0}C in 50 mM Tris-HCl buffer (pH = 7,4) and filtration using fiberglass filters (GF/B, Packard). Specific binding (during the test or control) was calculated as a difference between total (during the test or control) and non-specific binding.

Results are represented as percentage of specific binding inhibition in control (distilled water was used as control) (Table 1).

Table 1.

Test group	Quantity per test well	% of radioligand specific binding in control			% of radioligand binding inhibition in
		1st test	2nd test	Average	

					control
ULD anti-S100+anti-eNOS	20 µl	48.4	35.5	42.0	58.0
ULD anti-S100	10 µl	67.3	63.1	65.2	34.8
ULD anti-eNOS	10 µl	147.5	161.1	154.3	-54.3
Potentiated water	20 µl	98.1	75.8	86.9	13.1
Potentiated water	10 µl	140.1	106.2	123.2	-23.2

Effect of the preparations and potentiated water on binding of standard ligand [^{3}H]pentazocine to human recombinant σ 1 receptor

Note:

% of specific binding in control = (specific binding during the test/ specific binding in control)* 100%;

% of specific binding inhibition in control = 100% - (specific binding during the test/ specific binding in control) * 100%).

The outcomes reflecting inhibition above 50% represent significant effects of the tested compounds; inhibition from 25% to 50% confirm mild to moderate effects; inhibition less than 25% is considered to be insignificant effect of the tested compound and is within background level.

Therefore, the conditions of this test model showed that the complex preparation of ULD anti-S100+anti-eNOS is more efficient than its separate components (ULD anti-S100 and ULD anti-eNOS) in inhibiting the binding of standard radioligand [^{3}H]pentazocine to human recombinant σ1 receptor; ULD anti-S100, transferred into the test well, namely 10 µl, inhibit the binding of standard radioligand [3H]pentazocine to human recombinant σ1 receptor, but the effect intensity is inferior to that of the complex preparation of ULD anti-S100+anti-eNOS; ULD anti-eNOS, transferred into the test well, namely 10 µl, had no effect on the binding of standard radioligand [3H]pentazocine to human recombinant σ1 receptor; potentiated water, transferred into the test well, namely 10 µl or 20 µl,

had no effect on the binding of standard radioligand [3H]pentazocine to human recombinant σ1 receptor.

Example 2.

To study the properties of the combination pharmaceutical composition of the present application for the treatment of Alzheimer's disease, tablets with weight of 300 mg were used. The tablets were impregnated with pharmaceutical composition containing water-alcohol solutions (6 mg/tablet.) of activated-potentiated forms of polyclonal affinity purified rabbit brain-specific proteins antibodies S-100 (anti-S100) and to endothelial NO-synthase (anti-eNOS) in ultra low doses (ULD) obtained by super dilution of initial solution (with concentration of 2.5 mg/ml) in 100^{12}, 100^{30}, 100^{200} times, of equivalent mixture of centesimal homeopathic dilutions C12, C30, C200 (ratio: 1:1) ("ULD anti-S100 + anti-eNOS").

The control group patients received 300 mg tablets impregnated with pharmaceutical composition containing water-alcohol solutions (3 mg/tablet) of activated-potentiated forms of polyclonal affinity purified rabbit brain-specific proteins antibodies S-100 (anti-S100) in ultra low doses (ULD) obtained by super dilution of initial solution (with concentration of 2.5 mg/ml) in 100^{12}, 100^{30}, 100^{200} times, of equivalent mixture of centesimal homeopathic dilutions C12, C30, C200

The study included patients diagnosed with Alzheimer's disease. Alzheimer's disease is characterized by dementia (acquired dementia, stable impairment of cognitive activity with certain loss of previously acquired knowledge and practical skills, difficulties or impossibility to gain new knowledge).

The study was an open-label randomized comparative clinical trial of efficiency and safety of the therapy in two parallel groups (preparations of ULD anti-S100 and ULD anti-S100+anti-eNOS) in the treatment of patients with mild to moderate Alzheimer's disease.

The study included 6 patients aged 55 – 64 years old (mean age 59.0 ± 3.58) diagnosed with mild to moderate Alzheimer's disease.

Compliance of patients to following inclusion and exclusion criteria was checked:

Inclusion criteria are as follows:

1. Patients with mild to moderate Alzheimer's disease, confirmed by medical history, neurological examinations and medical records.
2. Patient without change in concomitant therapy within at least one month prior to Visit 1.
3. No need for change in concomitant therapy for the whole observation period.
4. No need for immunomodulatory drugs prescription for the next 6 months.
5. Patients with a level of education sufficient to adequately communicate with the researcher and study coordinator.
6. Patients assessed by the researcher as reliable and ready to perform all scheduled clinical visits, tests and procedures stipulated in the protocol.
7. Patients having a valid home address.

Exclusion criteria are as follows:

1. Any brain surgery in medical history.
2. Acute myocardial infarction.
3. Hemorrhagic stroke.
4. The diagnosis of psychosis, bipolar disorder or schizoaffective disorder in medical history.
5. Major depressive disorder according to criteria of depression module of international neuropsychiatric mini-interview (MINI).
6. Factors/conditions of medical or another character which in the opinion of the researcher may affect to the test results for patients in the study.
7. Answers "2A", "2B", "2C" or "3" in the section "I" of Beck Depression questionnaire (active suicidal ideation with some intent to act, without a specific plan, or active suicidal ideation with a specific plan and intent).
8. Autoimmune disease in medical history.
9. Acute damage of liver or severe cirrhosis (class C by Child-Pugh).
10. Non-corrected disorder of thyroid gland function.
11. Decompensated arterial hypertension in medical history.
12. Serious or decompensated cardiovascular disease, liver disease, kidney disease, metabolic, respiratory or hematological disease, symptomatic peripheral vascular disease or another medical or psychiatric condition which in the opinion of the researcher, may affect the patient's participation

in the study or could lead to prolonged hospitalization or re-hospitalization during the study.

13. Diseases and conditions which in the opinion of researcher may prevent patient from the participation in the study.
14. The intake of the drug containing ULD anti-eNOS or the drug containig ULD anti-S100 before inclusion in the study.
15. The intake of antidepressants of any group including plant and homeopathic preparations.
16. The intake of anxiolytics of any group including plant and homeopathic preparations.
17. The intake of immunomodulators including plant and homeopathic preparations.
18. The treatment with systemic steroids within 1 month before Visit 0.
19. The participation in the study of the drug containing ULD anti-eNOS or the drug containing ULD anti-S100 if patients took at least one doze of preparation.
20. Participation in other clinical studies within 1 month before within 1 month before being enrolled in this study.
21. Pregnancy, breast feeding, impossibility to use an adequate contraception during the study period and within 1 month after the last intake of the studied drug.
22. The presence of allergy/intolerance of any component of drugs including lactose intolerance.
23. Patients taking narcotic drugs and neuroleptics, alcoholic dependence, psychiatric diseases in patients.
24. Patients are the staff of the center which directly related to the conducted study and/or are family members of the research center staff's which directly associated with the ongoing study. The "family members" are a husband (wife), parents, children, brothers (sisters).
25. Participation in the trial or presumable receiving of compensation or participation in the judicial process in the opinion of a researcher.

After the determination of patient conformity to inclusion and exclusion criteria the patients were randomized into two study groups: a group of patients

receiving ULD anti-S100 (3 patients, women - 100%, men - 0%, mean age – 59.0 ± 3.6 years old), a group of patients receiving ULD anti-S100 + anti-eNOS (3 patients, women – 66.66 % men – 33.33 %, mean age – 59.0 ± 4.36 years old).

During this study the five visits were carried out. Treatment phase lasted from Visit 1 to Visit 4 for 84 ± 5 days on average. Visit 4 (Day 84 ± 5) was the first endpoint of the study followed by a follow-up observation. Follow-up phase continued from Visit 4 to Visit 5 (Day 168 ± 5 on average).

In the safety analysis the data of all patients participating in the study (n = 6) was included. During the study good tolerance of the drug was recorded. No adverse events were registered. All patients of studied groups have completed the treatment according to the protocol; no early dropouts.

The effect of ULD anti-S100 + anti-eNOS preparation on the main clinical signs and symptoms of Alzheimer's disease (NPI neuropsychiatric inventory, Intensity section), on the intensity of concomitant distress of the person attending to the patient (NPI Neuropsychiatric Inventory, Distress section) as well as the on patient's cognitive functions (The Mini Mental State Examination, MMSE) were assessed. An improvement was found in the key symptoms of Alzheimer's disease such as statistically significant reduction of the intensity section of NPI neuropsychiatric inventory (from 24.33± 4.73 to 12.0±3.46, $p<0.05$) at Visit 4 (Table 2).

A tendency for reduction of distress of the person attending to the patient was also found as well as for the reduction in activity of the patient's everyday life at the end of therapy (however, without any statistically significant difference, possibly due to the small number of patients included in the study).

Besides, a tendency for improvement of cognitive functions was found, manifested by increase of MMSE score from 23.66±3.21 to 26.66±1.53 points, however, the difference also failed to reach statistically significant values at the end of therapy, which may also be related to the small sample size.

The same endpoints in the group of patients receiving ULD anti-S100, showed no trend for improvement, except a statistically insignificant improvement of MMSE score from 22.66±0.58 to 23.33±0.58 points.

At that, a difference between the groups of patients in the total MMSE score at the end of therapy was statistically significant at $p<0.05$.

Table 2.

	NPI (intensity)	NPI (distress)	ADCS-ADL	MMSE
ULD anti-S100+anti-eNOS before treatment	24.33±4.73	9.66±1.53	71.0±6.56	23.66±3.21
ULD anti-S100+anti-eNOS after treatment	12.0±3.46 *	5.0±3.61	74.33±2.51	26.66±1.53#
ULD anti-S100 before treatment	35.66±5.50	22.33±5.50	61.66±5.13	22.66±0.58
ULD anti-S100 after treatment	38.33±8.5	23.0±5.0	61.33±5.86	23.33±0.58

* - p from baseline <0.05; # - p from control <0.05

Thus, in the conducted clinical study a positive effect of combined pharmaceutical composition ULD anti-S100 + anti-eNOS on the main clinical signs and symptoms of Alzheimer's disease and tendency to effect cognitive functions with Alzheimer's disease. In addition, good drug tolerability was confirmed. No drug-related adverse events were registered.

Example 3.

The efficacy of preparations in rats with scopolamine amnesia (model of Alzheimer's disease).

Alzheimer's disease (AD) is a neurodegenerative disease characterized by loss of cognitive functions, memory deterioration, confused consciousness, emotional liability. At present the main cause of this disease is thought to be the accumulation of beta amyloid in the brain which leads to the formation of beta-amyloid plaques and neurofibrillary tangles in brain tissues; AD is also accompanied by a deficiency of cholinergic system. This is the basis of a most common way of modeling of AD in animals with the help of scopolamine , an antagonist of cholinergic system. An injection of scopolamine to experimental animals (usually rodents, rats or mice) blocks the ability to learn and leads to deterioration of memory.

To assess cognitive functions of rats and mice various methods and tests including Morris water maze can be used. The essence of this test is that animals being released in a container with cloudy water from different points are forced to look for a hidden fixed platform. The advantage of this method is that it allows as to monitor the process of animal training (the formation of the idea about the

spatial location of the platform no matter in what place it was put into the water), so as to assess the memory strength (for this the testis being conducted when removing platform).

In the following Example 3 the effectiveness in rats with Scopolamine amnesia of claimed medical preparation in the form of composition containing activated-potentiated forms of polyclonal affinity purified on antigen of rabbit brain-specific proteins S-100 (anti-S100) and to endothelial NO-synthase (anti-eNOS) in ultra low doses (ULD) obtained by super dilution of storage stock solution (with concentration of 2.5 mg/ml) in в 100^{12}, 100^{30}, 100^{200} times, of equivalent mixture of centesimal homeopathic dilutions C12, C30, C200 (ULD anti-S100 + anti-eNOS).

In a study of the effectiveness of the drug ULD anti-S100 + anti-eNOS at scopolamine amnesia in rats (a model of Alzheimer's disease) 48 male rats of the Rj: Wistar (Han) line (weight 180-280g) were used. During 4 days the rats were subdermally injecting with normal saline (n = 12, intact) or scopolamine in doze of 0.5 mg / kg (n = 36) (scopolamine-induced amnesia). Rats with scopolamine-induced amnesia were divided into three groups and administered with, respectively, distilled water (7.5 ml / kg, n = 12, control group 1), ULD anti-S100 (7.5 ml / kg, n = 12, group 2) and ULD anti-S100 + anti-eNOS (7.5 ml / kg, n = 12, group 3) intragastrically for 9 days (4 days prior to the injection of scopolamine, 4 days against the background of scopolamine and 1 day after the last scopolamine injection).

Within 4 days of scopolamine injection through 60 minutes after administration of tested drugs and 30 minutes after administration of scopolamine the training session in the Morris water maze was conducted (4 sequential tests at interval of 60 seconds). Morris' maze is a round reservoir (diameter - 150 cm, height - 45 cm) at 30 cm filled with water (26-28 ° C). At 18 cm from the edge of the container there is hidden platform (diameter - 15 cm) buried on 1.5 cm below the water level. Cloudy water made by adding to it non-toxic dye (e.g., milk powder) which makes the platform invisible. For each test the animal was placed in a maze in one of the initial points that are equidistant from the hidden platform and allowed them find it. If the animal could not find the platform within 120 seconds it was being stood on the platform for 60 seconds and then started a new test. During the four tests in random order the animals began to swim through the

maze twice from each starting point. The tests were recorded on videotape and then analyzed for distance covered searching the platform in each trial and the latent period of searching for the platform. On day 5 the test was performed: the platform was removed from the maze and rats were given free float for 60 seconds. The time spent in the place where the platform used to be was recorded.

The administration of scopolamine significantly worsened the ability of animals to learn: in the control group 1 the time spent by animals for searching platforms and the distance that the animals swam searching the platform significantly increased (Table 3, 4). The test showed that the memory of animals in control group 1 is much worsened too: in a place where the platform used to be located they were spending less time than intact rats (Table 5). The administration of ULD anti-S100 in the group 2 did not lead to improvement of the studied parameters (Tables 3, 4, 5). The administration of ULD anti-S100 + anti-eNOS in group 3 led to some improvement in learning which was reflected in a shortening of the latent time of the platform search time (Table 3) and covered distance (Table 4) within 4 days of training and to improvement of memory as reflected in increase of the time spent in a place where the platform was (Table 5).

Table 3.

Latent period of the platform search, sec

Group	Training			
	1st day	2nd day	3rd day	4th day
Intact, n=12	54.7±6.2	30.8±2.8	26.9±5.1	20.5±3.6
Control, n=12	100.1±6.8***	92.4±9.3***	81.4±10.7***	77.7±9.4***
ULD anti-S100. n=12	106.8±7.0	99.3±7.8	95.6±9.0	80.4±11.1
ULD anti-S100 + anti-eNOS, n=12	94.4±7.2	90.7±8.2	78.3±8.6	60.1±10.2

*** - difference from intact is significant, $p<0.05$

Table 4.

Distance overcome to search the platform, cm

Group	Training

	1st day	2nd day	3rd day	4th day
Intact, n=12	1055.7±94.6	659.5±62.2	564.8±119.3	406.1±61.2
Control, n=12	2587.1±217.2 ***	2559.6±250.5 ***	2397.9±312.6	2366.1±293.8 ***
ULD anti-S100, n=12	2797.2±208.9	2865.2±255.1	2857.0±300.8	2457.4±344.4
ULD anti-S100 + anti-eNOS, n=12	2434.3±222.8	2529.9±282.7	2344.2±283.0	1905.1±343.7

*** - difference from intact is significant, p<0.05

Table 5.

Time spent in a place where the platform used to be located, sec.

Group	Test		
	0-30 sec.	30-60 sec.	0-60 sec.
Intact, n=12	40.8±4.1	36.8±3.6	38.5±2.6
Control, n=12	18.4±2.8***	18.8±1.9***	18.8±1.7***
ULD anti-S100, n=12	13.3±2.1	21.5±2.6	17.6±1.3
ULD anti-S100 + anti-eNOS, n=12	19.1±4.8	23.8±2.2	21.2±2.5

*** - difference from intact is significant, p<0.05

Thus, in the model of Alzheimer's disease the use of complex pharmaceutical composion of ULD anti-S100 + anti-eNOS was more effective in comparison with administration of ULD anti-S100 alone.

CLAIMS:

1. A method of treating Alzheimer's disease, said method comprising administering a combination pharmaceutical composition comprising a) an activated-potentiated form of an antibody to brain-specific protein S-100 and b) activated-potentiated form of antibodies to endothelial NO synthase.

2. The method of claim 1, wherein the activated-potentiated form of an antibody to brain-specific protein S-100 is to the entire bovine brain-specific protein S-100.

3. The method of claim 1, wherein the activated-potentiated form of an antibody to brain-specific protein S-100 is to brain-specific protein S-100 having SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12 .

4. The method of claim 1, wherein the activated-potentiated form of an antibody to endothelial NO synthase is to the entire bovine NO synthase.

5. The method of claim 1, wherein the activated-potentiated form of an antibody to endothelial NO synthase is to the entire human NO synthase.

6. The method of claim 1, wherein the activated-potentiated form of an antibody to brain-specific protein S-100 is in the form of a mixture of C12, C30, and C50 homeopathic dilutions impregnated onto a solid carrier and the activated-potentiated form of an antibody to endothelial NO synthase is in the form of mixture of C12, C30, and C50 homeopathic dilutions impregnated onto the solid carrier.

7. The method of claim 1, wherein the activated-potentiated form of an antibody to brain-specific protein S-100 is in the form of a mixture of C12, C30, and C200 homeopathic dilutions impregnated onto a solid carrier and the activated-potentiated form of an antibody to endothelial NO synthase is in the form of mixture of C12, C30, and C200 homeopathic dilutions impregnated onto the solid carrier.

8. The method of claim 1, wherein the activated-potentiated form of an antibody to endothelial NO synthase is in the form of mixture of C12, C30, and C50

homeopathic dilutions impregnated onto a solid carrier and the activated-potentiated form of an antibody to brain-specific protein S-100 is in the form of mixture of C12, C30, and C50 homeopathic dilutions impregnated onto the solid carrier.

9. The method of claim 1, wherein the activated-potentiated form of an antibody to endothelial NO synthase is in the form of mixture of C12, C30, and C200 homeopathic dilutions impregnated onto a solid carrier and the activated-potentiated form of an antibody to brain-specific protein S-100 is in the form of mixture of C12, C30, and C200 homeopathic dilutions impregnated onto the solid carrier.

10. The method of claim 1, wherein the activated-potentiated form of an antibody to brain-specific protein S-100 is a monoclonal, polyclonal or natural antibody.

11. The method of claim 10, wherein the activated-potentiated form of an antibody to brain-specific protein S-100 is a polyclonal antibody.

12. The method of claim 1, wherein the activated-potentiated form of an antibody to brain-specific protein S-100 is prepared by successive centesimal dilutions coupled with shaking of every dilution.

13. The method claim 1, wherein the activated-potentiated form of an antibody to endothelial NO synthase is a monoclonal, polyclonal or natural antibody.

14. The method of claim 13, wherein the activated-potentiated form of an antibody to endothelial NO synthase is a polyclonal antibody.

15. The method of claim 1, wherein the activated-potentiated form of an antibody to endothelial NO synthase is prepared by successive centesimal dilutions coupled with shaking of every dilution.

16. The method of claims 1, wherein the combination pharmaceutical composition is administered in one to two unit dosage forms, each of the dosage form being administered from once daily to six times daily.

17. The method of claim 16, wherein the combination pharmaceutical composition is administered in one to two unit dosage forms, each of the dosage form being administered twice daily.

18. A method of improvement of cognitive functions as manifested by increase of MMSE score by administration of the combination pharmaceutical composition of claim 1.

19. A pharmaceutical composition for use in treating a patient suffering from Alzheimer's disease, said composition having been obtained by providing a) an activated-potentiated form of an antibody to brain-specific protein S-100 and b) activated-potentiated form of antibodies to endothelial NO synthase, each prepared by consecutive repeated dilution and multiple shaking of each obtained solution in accordance with homeopathic technology, and then either combining the potentiated solutions by mixing them, or, alternatively, impregnating a carrier mass with said combined solution or with the solutions separately.

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Thr Pro Ala Pro Glu Pro Ser Arg Ala Pro Ala Ser Leu Leu Pro Pro
        35                  40                  45
Ala Pro Glu His Ser Pro Pro Ser Ser Pro Leu Thr Gln Pro Pro Glu
    50                  55                  60
Gly Pro Lys Phe Pro Arg Val Lys Asn Trp Glu Val Gly Ser Ile Thr
65                  70                  75                  80
Tyr Asp Thr Leu Ser Ala Gln Ala Gln Gln Asp Gly Pro Cys Thr Pro
                85                  90                  95
Arg Arg Cys Leu Gly Ser Leu Val Phe Pro Arg Lys Leu Gln Gly Arg
            100                 105                 110
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Pro Ser Pro Gly Pro Pro Ala Pro Glu Gln Leu Leu Ser Gln Ala Arg
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Asp Phe Ile Asn Gln Tyr Tyr Ser Ser Ile Lys Arg Ser Gly Ser Gln
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Ala His Glu Gln Arg Leu Gln Glu Val Glu Ala Glu Val Ala Ala Thr
145                 150                 155                 160
Gly Thr Tyr Gln Leu Arg Glu Ser Glu Leu Val Phe Gly Ala Lys Gln
                165                 170                 175
Ala Trp Arg Asn Ala Pro Arg Cys Val Gly Arg Ile Gln Trp Gly Lys
            180                 185                 190
Leu Gln Val Phe Asp Ala Arg Asp Cys Arg Ser Ala Gln Glu Met Phe
        195                 200                 205
Thr Tyr Ile Cys Asn His Ile Lys Tyr Ala Thr Asn Arg Gly Asn Leu
    210                 215                 220
Arg Ser Ala Ile Thr Val Phe Pro Gln Arg Cys Pro Gly Arg Gly Asp
225                 230                 235                 240
Phe Arg Ile Trp Asn Ser Gln Leu Val Arg Tyr Ala Gly Tyr Arg Gln
                245                 250                 255
Gln Asp Gly Ser Val Arg Gly Asp Pro Ala Asn Val Glu Ile Thr Glu
            260                 265                 270
Leu Cys Ile Gln His Gly Trp Thr Pro Gly Asn Gly Arg Phe Asp Val
        275                 280                 285
Leu Pro Leu Leu Leu Gln Ala Pro Asp Glu Pro Pro Glu Leu Phe Leu
    290                 295                 300
Leu Pro Pro Glu Leu Val Leu Glu Val Pro Leu Glu His Pro Thr Leu
305                 310                 315                 320
Glu Trp Phe Ala Ala Leu Gly Leu Arg Trp Tyr Ala Leu Pro Ala Val
                325                 330                 335
Ser Asn Met Leu Leu Glu Ile Gly Gly Leu Glu Phe Pro Ala Ala Pro
            340                 345                 350
Phe Ser Gly Trp Tyr Met Ser Thr Glu Ile Gly Thr Arg Asn Leu Cys
        355                 360                 365
Asp Pro His Arg Tyr Asn Ile Leu Glu Asp Val Ala Val Cys Met Asp
    370                 375                 380
Leu Asp Thr Arg Thr Thr Ser Ser Leu Trp Lys Asp Lys Ala Ala Val
385                 390                 395                 400
Glu Ile Asn Val Ala Val Leu His Ser Tyr Gln Leu Ala Lys Val Thr
                405                 410                 415
Ile Val Asp His His Ala Ala Thr Ala Ser Phe Met Lys His Leu Glu
            420                 425                 430
Asn Glu Gln Lys Ala Arg Gly Gly Cys Pro Ala Asp Trp Ala Trp Ile
        435                 440                 445
Val Pro Pro Ile Ser Gly Ser Leu Thr Pro Val Phe His Gln Glu Met
    450                 455                 460
Val Asn Tyr Phe Leu Ser Pro Ala Phe Arg Tyr Gln Pro Asp Pro Trp
465                 470                 475                 480
Lys Gly Ser Ala Ala Lys Gly Thr Gly Ile Thr Arg Lys Lys Thr Phe
                485                 490                 495
Lys Glu Val Ala Asn Ala Val Lys Ile Ser Ala Ser Leu Met Gly Thr
            500                 505                 510
Val Met Ala Lys Arg Val Lys Ala Thr Ile Leu Tyr Gly Ser Glu Thr
        515                 520                 525
Gly Arg Ala Gln Ser Tyr Ala Gln Gln Leu Gly Arg Leu Phe Arg Lys
    530                 535                 540
Ala Phe Asp Pro Arg Val Leu Cys Met Asp Glu Tyr Asp Val Val Ser
545                 550                 555                 560
Leu Glu His Glu Thr Leu Val Leu Val Val Thr Ser Thr Phe Gly Asn
                565                 570                 575
Gly Asp Pro Pro Glu Asn Gly Glu Ser Phe Ala Ala Ala Leu Met Glu
            580                 585                 590
Met Ser Gly Pro Tyr Asn Ser Ser Pro Arg Pro Glu Gln His Lys Ser
        595                 600                 605
Tyr Lys Ile Arg Phe Asn Ser Ile Ser Cys Ser Asp Pro Leu Val Ser
    610                 615                 620
Ser Trp Arg Arg Lys Arg Lys Glu Ser Ser Asn Thr Asp Ser Ala Gly
625                 630                 635                 640
Ala Leu Gly Thr Leu Arg Phe Cys Val Phe Gly Leu Gly Ser Arg Ala
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645 650 655
Tyr Pro His Phe Cys Ala Phe Ala Arg Ala Val Asp Thr Arg Leu Glu
660 665 670
Glu Leu Gly Gly Glu Arg Leu Leu Gln Leu Gly Gln Gly Asp Glu Leu
675 680 685
Cys Gly Gln Glu Glu Ala Phe Arg Gly Trp Ala Gln Ala Ala Phe Gln
690 695 700
Ala Ala Cys Glu Thr Phe Cys Val Gly Glu Asp Ala Lys Ala Ala Ala
705 710 715 720
Arg Asp Ile Phe Ser Pro Lys Arg Ser Trp Lys Arg Gln Arg Tyr Arg
725 730 735
Leu Ser Ala Gln Ala Glu Gly Leu Gln Leu Leu Pro Gly Leu Ile His
740 745 750
Val His Arg Arg Lys Met Phe Gln Ala Thr Ile Arg Ser Val Glu Asn
755 760 765
Leu Gln Ser Ser Lys Ser Thr Arg Ala Thr Ile Leu Val Arg Leu Asp
770 775 780
Thr Gly Gly Gln Glu Gly Leu Gln Tyr Gln Pro Gly Asp His Ile Gly
785 790 795 800
Val Cys Pro Pro Asn Arg Pro Gly Leu Val Glu Ala Leu Leu Ser Arg
805 810 815
Val Glu Asp Pro Pro Ala Pro Thr Glu Pro Val Ala Val Glu Gln Leu
820 825 830
Glu Lys Gly Ser Pro Gly Gly Pro Pro Pro Gly Trp Val Arg Asp Pro
835 840 845
Arg Leu Pro Pro Cys Thr Leu Arg Gln Ala Leu Thr Phe Phe Leu Asp
850 855 860
Ile Thr Ser Pro Pro Ser Pro Gln Leu Leu Arg Leu Leu Ser Thr Leu
865 870 875 880
Ala Glu Glu Pro Arg Glu Gln Gln Glu Leu Glu Ala Leu Ser Gln Asp
885 890 895
Pro Arg Arg Tyr Glu Glu Trp Lys Trp Phe Arg Cys Pro Thr Leu Leu
900 905 910
Glu Val Leu Glu Gln Phe Pro Ser Val Ala Leu Pro Ala Pro Leu Leu
915 920 925
Leu Thr Gln Leu Pro Leu Leu Gln Pro Arg Tyr Tyr Ser Val Ser Ser
930 935 940
Ala Pro Ser Thr His Pro Gly Glu Ile His Leu Thr Val Ala Val Leu
945 950 955 960
Ala Tyr Arg Thr Gln Asp Gly Leu Gly Pro Leu His Tyr Gly Val Cys
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Ser Thr Trp Leu Ser Gln Leu Lys Pro Gly Asp Pro Val Pro Cys Phe
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Ile Arg Gly Ala Pro Ser Phe Arg Leu Pro Pro Asp Pro Ser Leu Pro
995 1000 1005
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Trp Gln Glu Arg Leu His Asp Ile Glu Ser Lys Gly Leu Gln Pro Thr
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Pro Met Thr Leu Val Phe Gly Cys Arg Cys Ser Gln Leu Asp His Leu
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Val Leu Thr Ala Phe Ser Arg Glu Pro Asp Asn Pro Lys Thr Tyr Val
1075 1080 1085
Gln Asp Ile Leu Arg Thr Glu Leu Ala Ala Glu Val His Arg Val Leu
1090 1095 1100
Cys Leu Glu Arg Gly His Met Phe Val Cys Gly Asp Val Thr Met Ala
1105 1110 1115 1120
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1140 1145 1150
Gln Arg Tyr His Glu Asp Ile Phe Gly Leu Thr Leu Arg Thr Gln Glu
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35 40 45
Lys Glu Gln Glu Val Val Asp Lys Val Met Glu Thr Leu Asp Ser Asp
50 55 60
Gly Asp Gly Glu Cys Asp Phe Gln Glu Phe Met Ala Phe Val Ala Met
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20 25 30
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35 40 45
Lys Glu Gln Glu Val Val Asp Lys Val Met Glu Thr Leu Asp Asn Asp
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35 40 45
Gln Lys Asp Val Asp Ala Val Asp Lys Val Met Lys Glu Leu Asp Glu
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35 40 45
Gln Lys Asp Ala Asp Ala Val Asp Lys Val Met Lys Glu Leu Asp Glu
50 55 60
Asn Gly Asp Gly Glu Val Asp Phe Gln Glu Tyr Val Val Leu Val Ala
65 70 75 80
Ala Leu Thr Val Ala Cys Asn Asn Phe Phe Trp Glu Asn Ser
85 90