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(21) International Application Number: PCT/EP97/02050 (22) International Filing Date: 22 April 1997 (22.04.97) (30) Priority Data: 9608657.4 26 April 1996 (26.04.96) GB (71) Applicant (for all designated States except US): SMITHKLINE BEECHAM PLC [GB/GB]; New Horizons Court, Brentford, Middlesex TW8 9EP (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): KARRAN, Eric, H. [GB/GB]; SmithKline Beecham Pharmaceuticals, New Frontiers Science Park South, Harlow, Essex CM19 5AW (GB). GRAY, Carol [IE/GB]; SmithKline Beecham Pharmaceuticals, New Frontiers Science Park South, Harlow, Essex CM19 5AW (GB). DAVIS, John, B. [GB/GB]; SmithKline Beecham Pharmaceuticals, New Frontiers Science Park South, Harlow, Essex CM19 5AW (GB). WARD, Robin, V. [GB/GB]; SmithKline Beecham Pharmaceuticals, New Frontiers Science Park South, Harlow, Essex CM19 5AW (GB).		(74) Agent: VALENTINE, Jill, Barbara; SmithKline Beecham, Corporate Intellectual Property, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB). (81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: NOVEL TREATMENT (57) Abstract A method of screening compounds to identify those compounds which inhibit or otherwise modulate the cleavage of presenilin-1 (PS-1) comprising measuring the extent of cleavage of PS-1 into 18kDa and 28kDa species as compared with the extent of cleavage in the absence of test compound, compounds identified thereby and their use in therapy.		

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NOVEL TREATMENT

The present invention relates to an assay used in identifying compounds which are potentially useful in therapy. The present invention also relates to the use of modulators of polypeptide cleavage in therapy and to the use of the polypeptide cleavage products in prognosing Alzheimer's disease.

Alzheimer's disease (AD) is a progressive degenerative disease of the central nervous system characterized clinically by dementia and neuropathologically by the presence of numerous senile plaques and neurofibrillary tangles. AD is typically a late onset disease of the elderly. However, a small number of pedigrees have been described wherein an early onset form of the disease is inherited as an autosomal dominant with age dependent penetrance. Most commonly, the age of onset of the disease is below 60 years old. Genetic factors have been implicated in both early and late onset AD.

Mutations in at least four different genetic loci are associated with an inherited susceptibility to AD. The e4 allele of the apolipoprotein E (ApoE) gene on chromosome 19 is associated with late onset AD (Strittmatter et al. Proc. Natl. Acad. Sci. USA 1993, 90:1977-1981; Saunders et al. Neurology 1993, 43:1467-1472); Corder et al. Science 1993, 261:921-923). Mutations in the β -amyloid precursor protein (BAPP) gene on chromosome 21 have been found in a small number of families with early onset AD (Goate et al. Nature 1991, 349:704-706; Chartier-Harlin et al. Nature 1991, 353:844-846; Murrell et al. Science 1991, 254:97-99; Karlinsky et al. Neurology 1992, 42:1445-1453). Most recently, a novel AD locus in a gene referred to as STM2 was identified on chromosome 1 (1q31-41) from genetic linkage analysis of "Volga German" kindred (Ephrat Levy-Lahad et al. Science 1995, 269:970-973; *ibid.* 973-977). The STM2 gene bears a remarkable similarity to the AD-associated gene, S182.

The fourth locus (AD3) has been mapped by genetic linkage studies to chromosome 14q24.3 and may account for up to 70% of early-onset autosomal dominant AD. Schellenberg et al. Science 1992, 258:668-670; George-Hyslop et al. Nature Genet. 1992, 2:330-334; Van Broeckhoven et al. Nature Genet. 1992, 2:335-339). The AD3 locus is associated with the most aggressive form of this disease (onset between 30 and 60 years of age) and it has been suggested that mutations at this locus put into effect a biologically fundamental process leading to AD.

Recently, a novel gene with five missense mutations in seven pedigrees segregating early-onset autosomal dominant AD at the AD3 locus, the S182 gene, was cloned and described by Sherrington et al. Nature 1995, 375:754-760. Analysis of the nucleotide sequence of the S182 transcript revealed heterozygous nucleotide substitutions

in the reverse transcriptase-polymerase chain reaction products from affected members of six large pedigrees. The putative open reading frame (ORF) of S182 encodes a protein predicted to be a seven-transmembrane protein and the pedigree-associated nucleotide substitutions change the encoded amino acids in transmembrane (TM) helices II(L146M), VI(E246A), and VII(Y410C) and in loops between TMII-TMIII(R163H) and TMVI-TMVII(V286L). In addition, a polymorphism in intron 9 of the gene that does not affect the coding region of the S182 gene, hereafter referred to as the presenilin-1 gene, was demonstrated to be a risk factor for late-onset sporadic Alzheimer's disease (M. Wragg et al, The Lancet, Volume 347, 509-512, (1996).

10 The present invention is based on the finding that presenilin-1 is proteolytically cleaved within cells predominantly between Methionine 298 and Alanine 299 (D. Selkoe, Neurobiology of Aging Supplement Volume 17, number 4S Abstract 146, pS37 1996) by an enzyme resulting in:

15 an N-terminal fragment of 28kDa that can be immunodetected by antibodies raised to the N-terminal region of presenilin-1 but not detected by antibodies raised to the C-terminus; and

a C-terminal fragment of 18kDa that can be immunodetected with antibodies raised to the C-terminal region of presenilin-1 but not detected with antibodies raised to the N-terminus.

20 Because mutations in presenilin 1 cause early-onset AD, and because an intronic polymorphism is associated with an increased risk of developing late-onset sporadic Alzheimer's disease, it follows that the cleavage of presenilin-1 is an important event in its role in the pathology of Alzheimer's disease.

25 The present invention therefore provides a method of screening compounds to identify those compounds which inhibit or otherwise modulate the cleavage of presenilin-1 (PS-1) comprising measuring the extent of cleavage of PS-1 into 18kDa and 28kDa species as compared with the extent of cleavage in the absence of test compound. The invention also relates to compounds identified thereby.

30 In a preferred embodiment the method employs a human host cell transfected with an expression vector containing DNA encoding PS-1. The presence of the cleavage products may be detected by assaying the protein content of the host cell by using polyclonal or monoclonal antibodies raised against fragments of PS-1, such as the cleavage products, and specific to the 18kDa species over the 28kDa species and vice versa. Any suitable configuration of immunoassay may be employed, for example a
35 Western blot assay or an ELISA.

Monoclonal and polyclonal antibodies raised against fragments of PS-1 and specific to the 18kDa species over the 28kDa species and vice versa are themselves novel and form a further aspect of the invention. In particular the invention provides antibodies raised to the polypeptides CRSLGHPEPLSNGRPQGNSR and CRDSHLGPHRSTPESR.

5 The invention also provides the novel 18kDa and 28kDa cleavage products in isolated form, specific antibodies raised thereto and polynucleotides (DNA or RNA) encoding the polypeptide cleavage products. The cleavage site may be determined by routine N-terminal sequencing the C-terminal 18kDa species and has been found to exist predominantly between Methionine 298 and Alanine 299 (D. Selkoe, Neurobiology of
10 Aging Supplement Volume 17, number 4S Abstract 146, pS37 1996). Knowledge of the cleavage site allows design of short polypeptides (for example 3 to 10 amino acids long) spanning the cleavage site which can be used in the method of the invention in place of full length PS-1 and to characterize and measure the activity of the enzymes that are involved in the cleavage of PS-1. Knowledge of the cleavage site also allows design of
15 short polypeptides (3 to 20 amino acids long) corresponding to the C-terminus of the 28kDa species and the N-terminus of the 18kDa species of PS-1, which polypeptides can be used to raise specific antibodies in accordance with the invention and useful in the method of the invention.

20 The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

25 The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment. The polypeptide is preferably in purified form. By purified form is meant at least 80%, more preferably 90%, still more preferably 95% and most preferably 99%
30 pure with respect to other protein contaminants.

 The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

35 In accordance with yet a further aspect of the present invention, there is therefore provided a process for producing polypeptides of the invention by recombinant

techniques by expressing a polynucleotide encoding said polypeptide in a host and recovering the expressed product. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Host cells are genetically engineered (transduced or transformed or transfected) with vectors which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a cosmid, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Suitable expression vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the *E. coli. lac* or *trp*, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli*.

The gene can be placed under the control of a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator (collectively referred to herein as "control" elements), so that the DNA sequence encoding the desired protein is transcribed

into RNA in the host cell transformed by a vector containing this expression construction. The coding sequence may or may not contain a signal peptide or leader sequence. The protein sequences of the present invention can be expressed using, for example, the *E. coli* tac promoter or the protein A gene (spa) promoter and signal sequence. Leader sequences can be removed by the bacterial host in post-translational processing. Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are PKK232-8 and PCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In addition to control sequences, it may be desirable to add regulatory sequences which allow for regulation of the expression of the protein sequences relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

An expression vector is constructed so that the particular coding sequence is located in the vector with the appropriate regulatory sequences, the positioning and orientation of the coding sequence with respect to the control sequences being such that the coding sequence is transcribed under the "control" of the control sequences (i.e., RNA polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence). Modification of the coding sequences may be desirable to achieve this end. For example, in some cases it may be necessary to modify the sequence so that it may be attached to the control sequences with the appropriate orientation; i.e., to maintain the reading frame. The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector, such as the cloning vectors described above. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site. Modification of the coding sequences may also be performed to alter codon usage to suit the chosen host cell, for enhanced expression.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin

resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

Examples of recombinant DNA vectors for cloning and host cells which they can transform include the bacteriophage λ (*E. coli*), pBR322 (*E. coli*), pACYC177 (*E. coli*), pKT230 (gram-negative bacteria), pGV1106 (gram-negative bacteria), pLAFR1 (gram-negative bacteria), pME290 (non-*E. coli* gram-negative bacteria), pHV14 (*E. coli* and *Bacillus subtilis*), pBD9 (*Bacillus*), pIJ61 (*Streptomyces*), pUC6 (*Streptomyces*), YIp5 (*Saccharomyces*), a baculovirus insect cell system, , YCp19 (*Saccharomyces*). See, generally, "DNA Cloning": Vols. I & II, Glover *et al.* ed. IRL Press Oxford (1985) (1987) and; T. Maniatis *et al.* ("Molecular Cloning" Cold Spring Harbor Laboratory (1982).

In some cases, it may be desirable to add sequences which cause the secretion of the polypeptide from the host organism, with subsequent cleavage of the secretory signal.

Yeast expression vectors are also known in the art. See, e.g., U.S. Patent Nos. 4,446,235; 4,443,539; 4,430,428; see also European Patent Applications 103,409; 100,561; 96,491. pSV2neo (as described in J. Mol. Appl. Genet. 1:327-341) which uses the SV40 late promoter to drive expression in mammalian cells or pCDNA1neo, a vector derived from pCDNA1(Mol. Cell Biol. 7:4125-29) which uses the CMV promoter to drive expression. Both these latter two vectors can be employed for transient or stable(using G418 resistance) expression in mammalian cells. Insect cell expression systems, e.g., *Drosophila*, are also useful, see for example, PCT applications WO 90/06358 and WO 92/06212 as well as EP 290,261-B1.

Transcription of DNA by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus

early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Host cell containing the above vectors can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. As representative examples of appropriate hosts, there may be mentioned: prokaryotes for example bacterial cells, such as *E. coli*, *Streptomyces*, *Salmonella typhimurium* and eukaryotes for example fungal cells, such as yeast, insect cells such as *Drosophila* and *Spodoptera frugiperda*, mammalian cells such as CHO, COS or Bowes melanoma, plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation. (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well known to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

Transgenic non-human animals may also be used as hosts.

The method of recovery of expressed polypeptide depends on the expression system and host selected. If the expression system secretes the polypeptide into growth media, the polypeptide can be purified directly from the media. If the polypeptide is not

secreted, it is isolated from cell lysates or recovered from the cell membrane fraction.

Where the polypeptide is localized to the cell surface, whole cells or isolated membranes can be used as an assayable source of the desired gene product. Polypeptide expressed in bacterial hosts such as *E. coli* may require isolation from inclusion bodies and refolding.

- 5 The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

The polypeptide can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic
10 interaction chromatography, affinity chromatography hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

- 15 Depending upon the host employed in a recombinant production procedure, the polypeptides may be glycosylated or may be non-glycosylated. Polypeptides may also include an initial methionine amino acid residue.

"Recombinant" polypeptides refer to polypeptides produced by recombinant DNA techniques; i.e., produced from cells transformed by an exogenous DNA construct encoding the desired polypeptide. "Synthetic" polypeptides are those prepared by
20 chemical synthesis.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; i.e., capable of replication under its own control.

- 25 A "vector" is a replicon, such as a plasmid, phage, or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "double-stranded DNA molecule" refers to the polymeric form of deoxyribonucleotides (bases adenine, guanine, thymine, or cytosine) in a double-stranded helix, both relaxed and supercoiled. This term refers only to the primary and secondary
30 structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction
35 along the sense strand of DNA.

A DNA "coding sequence of" or a "nucleotide sequence encoding" a particular protein, is a DNA sequence which is transcribed and translated into a polypeptide when placed under the control of appropriate regulatory sequences.

5 A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes.

10 DNA "control sequences" refers collectively to promoter sequences, ribosome binding sites, polyadenylation signals, transcription termination sequences, upstream regulatory domains, enhancers, and the like, which collectively provide for the expression (i.e., the transcription and translation) of a coding sequence in a host cell.

15 A control sequence "directs the expression" of a coding sequence in a cell when RNA polymerase will bind the promoter sequence and transcribe the coding sequence into mRNA, which is then translated into the polypeptide encoded by the coding sequence.

A "host cell" is a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous DNA sequence.

20 A cell has been "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element, such as a plasmid. With respect to eukaryotic cells, a stably
25 transformed or transfected cell is one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cell containing the exogenous DNA.

30 A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

35 Two DNA or polypeptide sequences are "substantially homologous" or "substantially the same" when at least about 85% (preferably at least about 90%, and most preferably at least about 95%) of the nucleotides or amino acids match over a

defined length of the molecule and includes allelic variations. As used herein, substantially homologous also refers to sequences showing identity to the specified DNA or polypeptide sequence. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent
5 conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., "Current Protocols in Mol. Biol." Vol. I & II, Wiley Interscience. Ausbel *et al.* (ed.) (1992). Protein sequences that are substantially the same can be identified by proteolytic digestion, gel electrophoresis and microsequencing.

10 The term "functionally equivalent" intends that the amino acid sequence of the subject protein is one that will exhibit activity of the same kind as that of the native protein.

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the
15 other molecule in nature.

The polypeptides or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression
20 library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against polypeptides can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself.
25 In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, *Nature*, 256:495-497), the trioma
30 technique, the human B-cell hybridoma technique (Kozbor *et al.*, 1983, *Immunology Today* 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, *et al.*, 1985, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptides.

5 The present invention is also directed to compounds which are modulators of the cleavage of PS-1 into 18kDa and 28kDa species, and their use in modulating the function of PS-1.

The invention further provides the use of a compound according to the invention in the preparation of a medicament for modulating the function of PS-1.

10 The invention further provides a method of treatment or prophylaxis of Alzheimer's disease, which method comprises administering to a patient an effective amount of a compound of the invention, in particular a compound which is a modulator of the cleavage of PS-1 into 18kDa and 28kDa species

When used in therapy, the compounds of the invention are formulated in accordance with standard pharmaceutical practice.

15 The present invention therefore also provides a pharmaceutical composition comprising a compound of the invention and a pharmaceutically acceptable carrier.

The compounds which are active when given orally can be formulated as liquids, for example syrups, suspensions or emulsions, tablets, capsules and, lozenges.

20 A liquid formulation will generally consist of a suspension or solution of the compound or pharmaceutically acceptable salt in a suitable liquid carrier(s) for example, ethanol, glycerine, non-aqueous solvent, for example polyethylene glycol, oils, or water with a suspending agent, preservative, flavouring or colouring agent.

25 A composition in the form of a tablet can be prepared using any suitable pharmaceutical carrier(s) routinely used for preparing solid formulations. Examples of such carriers include magnesium stearate, starch, lactose, sucrose and cellulose.

30 A composition in the form of a capsule can be prepared using routine encapsulation procedures. For example, pellets containing the active ingredient can be prepared using standard carriers and then filled into a hard gelatin capsule; alternatively, a dispersion or suspension can be prepared using any suitable pharmaceutical carrier(s), for example aqueous gums, celluloses, silicates or oils and the dispersion or suspension then filled into a soft gelatin capsule.

Typical parenteral compositions consist of a solution or suspension of the compound or pharmaceutically acceptable salt in a sterile aqueous carrier or parenterally acceptable oil, for example polyethylene glycol, polyvinyl pyrrolidone, lecithin, arachis

oil or sesame oil. Alternatively, the solution can be lyophilised and then reconstituted with a suitable solvent just prior to administration.

A typical suppository formulation comprises a compound of formula (I) or a pharmaceutically acceptable salt thereof which is active when administered in this way, with a binding and/or lubricating agent such as polymeric glycols, gelatins or cocoa butter or other low melting vegetable or synthetic waxes or fats.

Preferably the composition is in unit dose form such as a tablet or capsule.

Each dosage unit for oral administration contains preferably from 1 to 250 mg (and for parenteral administration contains preferably from 0.1 to 25 mg) of an inhibitor of the invention.

The daily dosage regimen for an adult patient may be, for example, an oral dose of between 1 mg and 500 mg, preferably between 1 mg and 250 mg, or an intravenous, subcutaneous, or intramuscular dose of between 0.1 mg and 100 mg, preferably between 0.1 mg and 25 mg, of the compound of the formula (I) or a pharmaceutically acceptable salt thereof calculated as the free base, the compound being administered 1 to 4 times per day. Suitably the compounds will be administered for a period of continuous therapy.

The present invention provides in a still further aspect a diagnostic method for prognosing Alzheimer's disease in a patient comprising isolating a sample of tissue from the patient, and assaying said sample for cleavage of PS-1 into 18kDa and 28kDa species.

The presence of the cleavage products may be detected by assaying protein content of the sample by using polyclonal or monoclonal antibodies prepared against the cleavage products as described above for the method of screening compounds.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

EXAMPLES

Example 1

30 Demonstration of cleavage of presenilin-1 in cells

Rabbit polyclonal antibodies were raised to the peptide CRSLGHPEPLSNGRPQGNSR, which contains amino acids 42 - 60 at the N-terminal region of PS-1, and CRDSHLGPHRSTPESR which contains amino acids 344-358 at the C-terminal region of PS-1 (R. Sherrington et al, Nature Vol. 375 754-760, 1995). These antibodies have been characterized by:

Western immunoblot analysis of cell lysates from cells transfected with a cDNA for PS-1, and demonstrating greater immunoreactivity of protein bands in transfected cells compared to vector control cells;

demonstrating that immunoreactivity to protein bands can be competed against with the peptide used to raise the antibody;

demonstrating that serum taken from rabbits prior to inoculation with the peptides is unable to detect protein bands that are detected using serum after inoculation with peptides.

The neuroblastoma cell line SHSY5Y was transfected with expression vectors containing cDNA for the PS-1 gene, and established stably transfected cell lines. In transfected cells, and also in vector control cells, PS-1 protein appears as a 43-46 kDa protein that can be detected with antibodies raised to both N- and C-terminal regions of the protein. The full-length PS-1 protein can be more readily detected in PS-1 transfected cells than in vector control cells. PS-1 is proteolytically cleaved within both transfected and untransfected cells resulting in: an N-terminal fragment of 28kDa that can be immunodetected by antibodies raised to the N-terminal region of presenilin-1 but not detected by antibodies raised to the C-terminus; and a C-terminal fragment of 18kDa that can be immunodetected with antibodies raised to the C-terminal region of presenilin-1 but not detected with antibodies raised to the N-terminus.

Example 2

Assay for Modulators of cleavage of presenilin-1

The antibodies described above can be used to measure the cleavage of PS-1 in cells, and also the effects of external agents that may affect the extent of cleavage of PS-1. This would include agents that inhibit or otherwise modulate the activity of the enzyme that cleaves PS-1 protein, such agents having utility in therapies for Alzheimer's disease.

The ability of inhibitors of the cleavage of presenilin-1 in human neuroblastoma cells transfected with expression vectors containing cDNA for the presenilin-1 (PS-1) gene is investigated by use of the following procedure:

Adherent SHSY5Y human neuroblastoma cells which have been transfected with PS-1 cDNA in either pcDNA3 or pCEP4 expression vectors are grown in microtitre plates. Cells are grown to 80% confluency in the following growth medium (pH 7.4) consisting of 88 % (v/v) DMEM/ NUT MIX F-12, 10% (v/v) foetal calf serum, 50microg/ml penicillin/streptomycin, G-418 Geneticin 400microg/ml, and 2mM

glutamine. Test compounds are dissolved in dimethyl sulphoxide at a stock concentration of 2mM, then diluted 1 in 200 in growth medium. After removal of the growth medium, 200microlitres of the diluted compounds are added to the adherent cells in triplicate wells. Appropriate control cultures containing diluted solvent are set up in triplicate. The plates are incubated for 24 hours at 37°C, 95% air/5% CO₂ in a humidified incubator. The cells are washed twice with phosphate buffered saline pH 7.4.

The cells are lysed in RIPA buffer consisting of 150mM sodium chloride, 1% v/v NP40, 0.5% w/v sodium deoxycholic acid, 0.1% w/v sodium dodecyl sulphate in 50mM Tris HCl pH 8.0 and assayed in a 96-well immunoassay format which utilizes specific antibodies in a sandwich assay. One anti-presenilin-1 antibody serves as the capture reagent for the C-terminal fragment within cells, the second anti-presenilin-1 specific antibody (which recognises the N-terminal neoepitope generated by cleavage) serves as a component of the complex used for detection. A rabbit IgG specific third antibody which has been conjugated with dextran and biotinylated is detected by time-resolved fluorescence following further incubations with a streptavidin-europium complex (Delfia; EG&G Berthold and Wallac) followed by enhancer solution. A positive result is one in which there is change in fluorescence relative to the control group, indicating a modulation of the cleavage of as a result of the effects of the test compound. The results are expressed as the percentage inhibition of the signal obtained in wells conditioned with medium which contained solvent alone.

Example 3

Diagnostic method

The antibodies described above can be used diagnostically to assay the extent of PS-1 cleavage into 18kDa and 28kDa species in patients tissue e.g. blood as a means of prognosing Alzheimer's disease, and also as a means of assessing the efficacy of agents that have been administered with a view to modulating the cleavage of the Presenilins as a therapy for Alzheimer's disease.

The presence of the cleavage products may be detected by assaying the immunoreactivity of the sample by using polyclonal or monoclonal antibodies prepared against the cleavage products as described above for the method of screening compounds.

Claims

1. A method of screening compounds to identify those compounds which inhibit or otherwise modulate the cleavage of presenilin-1 (PS-1) comprising measuring the extent of cleavage of PS-1 into 18kDa and 28kDa species as compared with the extent of cleavage in the absence of test compound.
2. A method according to claim 1 which employs a human host cell transfected with an expression vector containing DNA encoding PS-1.
3. A method according to claim 1 or 2 wherein the presence of the cleavage products is detected by assaying the protein content of the host cell by using polyclonal or monoclonal antibodies raised against fragments of PS-1 and specific to the 18kDa species over the 28kDa species and vice versa.
4. A method according to claim 3 which is a Western blot assay or an ELISA.
5. PS-1 18kDa or 28kDa cleavage product in isolated form
6. A polypeptide 3 to 20 amino acids long corresponding to the C-terminus of the 28kDa species or the N-terminus of the 18kDa species of PS-1
7. An antibody raised against a fragment of PS-1 and specific to the 18kDa species over the 28kDa species or vice versa
8. An antibody according to claim 7 raised against the polypeptide of claim 5 or 6.
9. An antibody according to claim 7 raised to the polypeptide
CRSLGHPPEPLSNGRPQGNSR or CRDSHLGPHRSTPESR.
10. A method according to claim 3 or 4 wherein the antibodies are as defined in claims 7 to 9.
11. A polypeptide of 3 to 10 amino acids spanning the cleavage site of PS-1.
12. A method according to claim 1 which employs a polypeptide as defined in claim 11.
13. A polynucleotide (DNA or RNA) encoding the polypeptide of claim 5, 6 or 11.
14. A vector comprising a polynucleotide of claim 13.
15. A host cell transformed with a vector of claim 14.
16. A process for producing a polypeptide of claim 5, 6 or 11 by recombinant techniques by expressing a polynucleotide encoding said polypeptide in a host and recovering the expressed product.
17. SHSY5Y human neuroblastoma cells transfected with PS-1 cDNA in either pcDNA3 or pCEP4 expression vectors.
18. A compound identified by the method of any of claims 1 to 4.

19. A compound which is a modulator of the cleavage of PS-1 into 18kDa and 28kDa species.
20. A pharmaceutical composition comprising a compound according to claim 18 or 19 and a pharmaceutically acceptable carrier.
- 5 21. Use of a compound according to claim 18 or 19 in the preparation of a medicament for modulating the function of PS-1.
22. A method of treatment or prophylaxis of Alzheimer's disease, which method comprises administering to a patient an effective amount of a compound according to claim 18 or 19.
- 10 22. A diagnostic method for prognosing Alzheimer's disease in a patient comprising isolating a sample of tissue from the patient, and assaying said sample for cleavage of PS-1 into 18kDa and 28kDa species.