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(71) Applicants *(for all designated States except US)*: **RAMOT AT TEL-AVIV UNIVERSITY LTD.** [IL/IL]; P.O. Box 392, 61392 Tel Aviv (IL). **ALLON THERAPEUTICS, INC.** [CA/CA]; 315 - 1681 Chestnut Street, Vancouver, British Columbia V6J 4M6 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GOZES, Illana** [IL/IL]; 14 Ha'amal St., 47445 Ramat- Hasharon (IL). **MILLER, James** [CA/CA]; 17 - 5650 Hampton Place, Vancouver, British Columbia V6T 2G5 (CA).

(74) Agent: **FETHERSTONHAUGH & CO.**; Suite 2200, 650 West Georgia Street, Box 11560, Vancouver Centre, Vancouver, British Columbia V6B 4N8 (CA).

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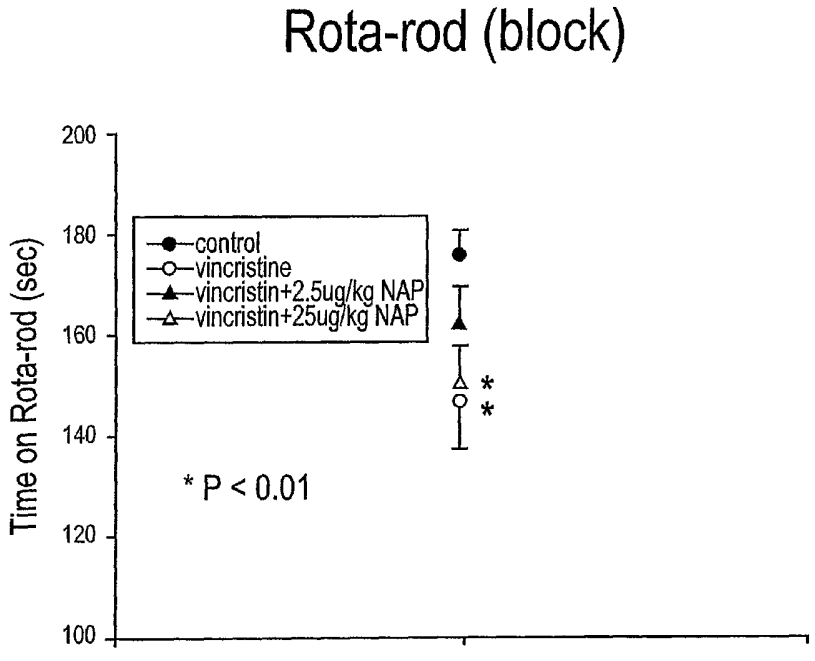
(54) Title: USE OF ADNF POLYPEPTIDES FOR TREATING PERIPHERAL NEUROTOXICITY



(57) Abstract: This invention relates to the use of ADNF polypeptides in the treatment of neurotoxicity induced by chemical agents or by disease processes. The ADNF polypeptides include ADNF I and ADNF III (also referred to as ADNP) polypeptides, analogs, subsequences such as NAP and SAL, and D-amino acid versions (either wholly D-amino acid peptides or mixed D- and L-amino acid peptides), and combinations thereof which contain their respective active core sites.

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USE OF ADNF POLYPEPTIDES FOR TREATING PERIPHERAL NEUROTOXICITY

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/664908, filed March 23, 2005; which is herein incorporated by reference for all purposes.

FIELD OF INVENTION

[0002] This invention relates to the use of ADNF polypeptides in the treatment of neurotoxicity. The present invention also relates to the manufacture of medicaments, methods of formulation and uses thereof. The ADNF polypeptides include ADNF I and ADNF III (also referred to as ADNP) polypeptides, analogs, subsequences such as NAP and SAL (defined below), and D-amino acid versions (either wholly D-amino acid peptides or mixed D- and L-amino acid peptides), and combinations thereof which contain their respective active core sites.

BACKGROUND OF THE INVENTION

[0003] NAP, an 8-amino acid peptide (NAPVSIPQ = Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln), is derived from a novel protein, activity-dependent neuroprotective protein, ADNP (US Patent 6613740, Bassan *et al., J. Neurochem.* 72: 1283-1293 (1999); Zamostiano, *et al., J. Biol. Chem.* 276:708-714 (2001)). The NAP sequence within the ADNP gene is identical in rodents and humans (US Patent 6613740, Zamostiano, *et al., J. Biol. Chem.* 276:708-714 (2001)).

[0004] In cell cultures, NAP has been shown to have neuroprotective activity on cells of the central nervous system (CNS) at femtomolar concentrations (Bassan *et al.*, 1999; Offen *et al., Brain Res.* 854:257-262 (2000)). Several animal models have also demonstrated NAP activity on diseases of the CNS. In animal models simulating parts of the Alzheimer's disease pathology, NAP was protective (Bassan *et al.*, 1999; Gozes *et al., J. Pharmacol. Exp. Ther.* 293:1091-1098 (2000); see also US Patent 6613740). In normal aging rats, intranasal administration of NAP improved performance in the Morris water maze. (Gozes *et al., J. Mol. Neurosci.* 19:175-178 (2002). NAP reduced infarct volume and motor function deficits after ischemic injury, by decreasing apoptosis (Leker *et al., Stroke* 33:1085-1092 (2002)) and reducing damage caused by closed head injury in mice by

decreasing inflammation (Beni Adani *et al., J. Pharmacol. Exp. Ther.* 296:57-63 (2001); Romano *et al., J. Mol. Neurosci.* 18:37-45 (2002); Zaltzman *et al., NeuroReport* 14:481-484 (2003)). NAP has been shown to provide protective intervention in a model of fetal alcohol syndrome, reducing fetal demise and growth restrictions. (Spong *et. al., J Pharmacol Exp Ther.* 297:774-9 (2001)). Additionally, long term nasal NAP application in mice resulted in decreased anxiety (Alcalay *et al., Neurosci Lett.* 361(1-3):128-31 (2004)).

[0005] SAL, a 9-amino acid peptide (SALLRSIPA = Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala), also known as ADNF-9, was identified as the shortest active form of ADNF (see US Pat. No. 6174862). SAL has been shown in in-vitro assays and in vivo disease models to keep neurons of the central nervous system alive in response to various insults (e.g. Gozes *et al.*, 2000, infra; Brenneman *et al.*, 1998. *J. Pharmacol. Exp. Ther.* 285, 619-627). D-SAL is an all D-amino acid derivative of SAL that is stable and orally available (Brenneman, *et al., J Pharmacol Exp Ther.* 309:1190-7 (2004)) and surprisingly exhibits similar biological activity (potency and efficacy) to SAL in the systems tested.

[0006] ADNF polypeptides, including NAP and SAL, and uses thereof in neuroprotection against disorders of the central nervous system, are the subject of patents and patent applications including PCT WO 1/92333;U.S.S.N. 07/871,973 filed April 22, 1992, now U.S. Patent No. 5,767,240; U.S.S.N. 08/342, 297, filed October 17, 1994 (published as WO96/11948), now U.S. Patent 6,174,862; U.S.S.N. 60/037,404, filed February 7, 1997 (published as WO98/35042); U.S.S.N. 09/187,330, filed November 11, 1998 (published as WO00/27875); U.S.S.N. 09/267,511, filed March 12, 1999 (published as WO00/53217); US6613740, U.S.S.N. 60/149,956, filed August 18, 1999 (published as WO01/12654); U.S.S.N. 60/208,944, filed May 31, 2000; and U.S.S.N. 60/267,805, filed February 8, 2001; PCT/IL2004/000232, filed March 11, 2004 (published as WO 2004/080957) herein each incorporated by reference in their entirety.

[0007] This disclosure provides new and surprising uses for ADNF polypeptides, including, *e.g.*, NAP, SAL, D-NAP and D-SAL, in the treatment of neurotoxicity in the peripheral nervous system.

BRIEF SUMMARY OF THE INVENTION

[0008] In one aspect, the present invention provides a method for treating peripheral neurotoxicity in a subject, the method comprising administering a therapeutically effective amount of an ADNF polypeptide to a subject in need thereof.

[0009] In one embodiment, the ADNF polypeptide is a member selected from the group consisting of:

(a) an ADNF I polypeptide comprising an active core site having the following amino acid sequence: Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:1), or an analogue thereof;

(b) an ADNF III polypeptide comprising an active core site having the following amino acid sequence: Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln (SEQ ID NO:2), or an analogue thereof, and

(c) a mixture of the ADNF I polypeptide of part (a) and the ADNF III polypeptide of part (b), or their respective analogues.

[0010] In another embodiment, the ADNF polypeptide is a member selected from the group consisting of a full length ADNF I polypeptide, a full length ADNF III polypeptide (ADNP), and a mixture of a full length ADNF I polypeptide and a full length ADNF III polypeptide.

[0011] In one embodiment, the ADNF polypeptide is prepared by recombinant DNA methodology. In another embodiment, the active core site of the ADNF polypeptide comprises at least one D-amino acid. In another embodiment, the active core site of the ADNF polypeptide comprises all D-amino acids.

[0012] In one embodiment, the ADNF I polypeptide has the formula (R1)x-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala-(R2)y (SEQ ID NO:27), or an analogue thereof, in which

R1 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs;

R2 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; and

x and y are independently selected and are equal to zero or one.

[0013] In one embodiment, the ADNF I polypeptide is selected from the group consisting of:

Val-Leu-Gly-Gly-Gly- Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:3);

Val-Glu-Glu-Gly-Ile-Val-Leu-Gly-Gly-Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:4);

Leu-Gly-Gly-Gly- Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:5);

Gly-Gly-Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:6);

Gly-Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:7);

Gly- Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:8);

Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:1); and

SALLRSIPAPAGASRLLLLTGEIDLP (SEQ ID NO:28).

[0014] In one embodiment, the ADNF I polypeptide comprises up to about 20 or 40 amino acids at either or both of the N-terminus and the C-terminus of the active core site.

[0015] In another embodiment, the ADNF III polypeptide has the formula (R1)x-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-(R2)y (SEQ ID NO:13), or an analogue thereof, in which

R1 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs;

R2 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; and

x and y are independently selected and are equal to zero or one.

[0016] In another embodiment, the ADNF III polypeptide is a member selected from the group consisting of:

Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln (SEQ ID NO:9);

Leu-Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-Gln-Ser (SEQ ID NO:10);

Leu-Gly-Leu-Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-Gln-Ser (SEQ ID NO:11);

Ser-Val-Arg-Leu-Gly-Leu-Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-Gln-Ser (SEQ ID NO:12); and

Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln (SEQ ID NO:2).

[0017] In another embodiment, the ADNF III polypeptide comprises up to about 20 amino acids at at least one of the N-terminus and the C-terminus of the active core site.

[0018] In one embodiment, an ADNF I polypeptide of part (a) and an ADNF III polypeptide of part (b) are administered to the subject.

[0019] In one embodiment, the ADNF polypeptide is administered intranasally. In another embodiment, the ADNF polypeptide is administered orally. In another embodiment, the ADNF polypeptide is administered intravenously or subcutaneously.

[0020] In one aspect the invention provides the use of an ADNF polypeptide in the manufacture of a medicament for the treatment of peripheral neurotoxicity.

[0021] In one embodiment, the symptoms of said peripheral neurotoxicity are measured by motor dysfunction, muscle wasting, or a change selected from among a change in sense of smell, vision or hearing, deep tendon reflexes, vibratory sense, cutaneous sensation, gait and balance, muscle strength, orthostatic blood pressure, and chronic or intermittent pain.

[0022] In another embodiment, the peripheral neurotoxicity is a consequence of treatment with one or more chemical agents. In another embodiment, the peripheral neurotoxicity is a consequence of treatment with a chemical agent selected from among chemical agents for cancer, multiple sclerosis, gout, arthritis, Bechet's disease, psychiatric disorder, immunosuppression and infectious disease.

[0023] In another embodiment, one or more chemical agents is selected from among the vinca alkaloids (*e.g.*, vincristine, vindesine, vinorelbine and vinblastine), platinum drugs (*e.g.*, cisplatinum, carboplatinum), L-asparaginase and the taxanes (*e.g.*, taxol, taxotere). In addition to anti-cancer agents, neurotoxicity may be caused by thalidomide, methotrexate, colchicine and anti-infective agents (including but not limited to nucleoside analogs such as lamivudine, zalcitabine, didanosine and stavudine).

[0024] In another embodiment, peripheral neurotoxicity is a consequence of a disease process. In another embodiment, the disease process selected from among diabetes, leprosy, Charcot-Marie-Tooth Disease, hereditary sensory and autonomic neuropathies (HSAN), Guillain-Barré syndrome, viral illnesses, (*e.g.*, cytomegalovirus, Epstein-Barr virus, varicella-zoster virus, and human immunodeficiency virus (HIV)), bacterial infection (including *Campylobacter jejuni* and Lyme disease), chronic alcoholism, botulism, poliomyelitis, uremia, chronic kidney failure, and atherosclerosis.

[0025] In another aspect, the present invention provides, the treatment of cancer or neoplasia comprising

a) administering an anti-cancer agent; and

b) administering, contemporaneously or sequentially with the anti-cancer agent of step a), an ADNF polypeptide in a pharmaceutically acceptable carrier.

[0026] In another aspect, the present invention provides a method of testing for response to a therapeutic agent for a neurodegenerative disease or peripheral neurotoxicity comprising the following steps,

a) measuring olfaction capacity in a subject having a neurodegenerative disease or potential peripheral neurotoxicity;

b) administering a therapeutic agent to the subject;

c) measuring olfaction capacity in the subject subsequent to step b);

d) comparing olfaction capacity from step a) and step c).

[0027] In another embodiment, the therapeutic agent is an ADNF polypeptide. In another embodiment, the neurodegenerative disease is Alzheimer's disease. In another embodiment, the subject has potential peripheral neurotoxicity associated with treatment by a chemotherapeutic agent.

[0028] In another aspect, the present invention provides a method of treatment of tauopathy in a subject comprising administering to a subject having or suspected of having a tauopathy, a therapeutically effective amount of an ADNF polypeptide.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] Figure 1: Rota-rod tests were performed on rats receiving 0.175 mg/kg vincristine, and similar rats receiving vincristine plus subcutaneous NAP. Rota-rod test shows vincristine and NAP treated animals (n=10) perform better than vincristine treated alone (n=10). (**p< 0.01).

[0030] Figure 2: Motor evaluations: The ability to exit a circle 30 cm in diameter within 20 seconds. Vincristine-treated rats (n=10) are significantly worse as compared to control animals (n=10) (P<0.001). Treatment with NAP (25 microgram/kg) (n=10) significantly improved the performance similar to control values (P<0.01).

[0031] Figures 3, 4 and 5: Time spent with new odors: olfaction capacity. The time spent with each odor over the three consecutive tests was recorded. Figure 3, for control rats, Figure 4 for vincristine treated rats and Figure 5 for vincristine and 25 micogram/kg NAP-treated rats. While the vincristine-treated rats did not show any initial interest in the new smell, a trend toward increased interest toward a new odor was observed in the control and the vincristine-NAP-treated rats.

[0032] Figure 6: Comparison between time periods spent with a certain odor after exchanging for a former scent – odor discrimination test: The figure depicts 4 points, point 1 = water (ddw) trial 3; point 2 = odor #1, trial #1; point 3 = odor #1, trial 3; point 4 = odor #2, trial 1. Results showed a significant difference in the time taken to sniff the third odor when comparing control to vincristine-treated rats or vincristine-treated rats with vincristine+25micogram/kg NAP ($P<0.05$).

[0033] Figure 7: Plantar tests were performed on rats receiving a total dose of 5.6 mg/kg taxol, and similar rats receiving taxol plus 2.5 μg/kg/day NAP. The plantar test shows taxol and 2.5 μg/kg/day NAP treated animals (n=10) perform better than taxol treated alone (n=10). (*$p< 0.01$).

[0034] Figure 8: Plantar tests were performed on rats receiving a total dose of 9 mg/kg taxol, and similar rats receiving taxol plus 2.5 μg/kg/day NAP. The plantar test shows taxol and 2.5 μg/kg/day NAP treated animals (n=10) perform better than taxol treated alone (n=5). (*$p< 0.04$).

[0035] Figure 9: Rotarod tests were performed on rats receiving a total dose of 9 mg/kg taxol, and similar rats receiving taxol plus 2.5 μg/kg/day NAP. The rotarod test shows taxol and 2.5 μg/kg/day NAP treated animals (n=5) perform better than taxol treated alone (n=10). (*$p< 0.04$).

DEFINITIONS

[0036] The phrase "ADNF polypeptide" refers to one or more activity dependent neurotrophic factors (ADNF) that have an active core site comprising the amino acid sequence of SALLRSIPA (referred to as "SAL") or NAPVSIPQ (referred to as "NAP"), or conservatively modified variants thereof that have neurotrophic/neuroprotective activity as measured with in vitro cortical neuron culture assays described by, e.g., Hill *et al., Brain Res.* 603:222-233 (1993); Brenneman & Gozes, *J. Clin. Invest.* 97:2299-2307 (1996), Gozes

et al., Proc. Natl. Acad. Sci. USA 93, 427-432 (1996). An ADNF polypeptide can be an ADNF I polypeptide, an ADNF III polypeptide, their alleles, polymorphic variants, analogs, interspecies homolog, any subsequences thereof (e.g., SALLRSIPA or NAPVSIPQ) or lipophilic variants that exhibit neuroprotective/neurotrophic action on, e.g., neurons originating in the central nervous system either *in vitro* or *in vivo*. An "ADNF polypeptide" can also refer to a mixture of an ADNF I polypeptide and an ADNF III polypeptide.

[0037] The term "ADNF I" refers to an activity dependent neurotrophic factor polypeptide having a molecular weight of about 14,000 Daltons with a pI of 8.3 ± 0.25. As described above, ADNF I polypeptides have an active site comprising an amino acid sequence of Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (also referred to as "SALLRSIPA" or "SAL" or "ADNF-9"). *See* Brenneman & Gozes, *J. Clin. Invest.* 97:2299-2307 (1996), Glazner *et al., Anat. Embryol.* ((Berl). 200:65-71 (1999), Brenneman *et al., J. Pharm. Exp. Ther.*, 285:619-27 (1998), Gozes & Brenneman, *J. Mol. Neurosci.* 7:235-244 (1996), and Gozes *et al., Dev. Brain Res.* 99:167-175 (1997). Unless indicated as otherwise, "SAL" refers to a peptide having an amino acid sequence of Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala, not a peptide having an amino acid sequence of Ser-Ala-Leu. A full length amino acid sequence of ADNF I can be found in WO 96/11948.

[0038] The phrase "ADNF III polypeptide" or "ADNF III" also called activity-dependent neuroprotective protein (ADNP) refers to one or more activity dependent neurotrophic factors (ADNF) that have an active core site comprising the amino acid sequence of NAPVSIPQ (referred to as "NAP"), or conservatively modified variants thereof that have neurotrophic/neuroprotective activity as measured with in vitro cortical neuron culture assays described by, e.g., Hill *et al., Brain Res.* 603, 222-233 (1993); Gozes *et al., Proc. Natl. Acad. Sci. USA* 93, 427-432 (1996). An ADNF polypeptide can be an ADNF III polypeptide, allelelic or polymorphic variant, analog, interspecies homolog, or any subsequences thereof (e.g., NAPVSIPQ) that exhibit neuroprotective/neurotrophic action on, e.g., neurons originating in the central nervous system either in vitro or in vivo. ADNF III polypeptides can range from about eight amino acids and can have, e.g., between 8-20, 8-50, 10-100 or about 1000 or more amino acids.

[0039] Full length human ADNF III has a predicted molecular weight of 123,562.8 Da (>1000 amino acid residues) and a pI of about 6.97. As described above, ADNF III polypeptides have an active site comprising an amino acid sequence of Asn-Ala-Pro-Val-

Ser-Ile-Pro-Gln (also referred to as "NAPVSIPQ" or "NAP"). *See* Zamostiano *et al., J. Biol. Chem.* 276:708-714 (2001) and Bassan *et al., J. Neurochem.* 72:1283-1293 (1999). Unless indicated as otherwise, "NAP" refers to a peptide having an amino acid sequence of Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln, not a peptide having an amino acid sequence of Asn-Ala-Pro. Full-length amino acid and nucleic acid sequences of ADNF III can be found in WO 98/35042, WO 00/27875, US Patent 6613740. The Accession number for the human sequence is NP_852107, *see also* Zamostiano *et al.,* infra.

[0040] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. For the purposes of this application, amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an a carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. For the purposes of this application, amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0041] Amino acids may include those having non-naturally occurring D-chirality, as disclosed in WO 01/12654, incorporated herein by reference, which may improve oral availability and other drug like characteristics of the compound. In such embodiments, one or more, and potentially all of the amino acids of NAP or the ADNF polypeptide will have D-chirality. The therapeutic use of peptides can be enhanced by using D-amino acids to provide longer half life and duration of action. However, many receptors exhibit a strong preference for L-amino acids, but examples of D-peptides have been reported that have equivalent activity to the naturally occurring L-peptides, for example, pore-forming antibiotic peptides, beta amyloid peptide (no change in toxicity), and endogenous ligands for the CXCR4 receptor. In this regard, NAP and ADNF polypeptides also retain activity in

the D-amino acid form (Brenneman *et al., J. Pharmacol. Exp. Ther.* 309(3):1190-7 (2004), infra).

[0042] Amino acids may be referred to by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0043] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al., Nucleic Acid Res.* 19:5081 (1991); Ohtsuka *et al., J. Biol. Chem.* 260:2605-2608 (1985); Rossolini *et al., Mol. Cell. Probes* 8:91-98 (1994)). Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

[0044] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known

in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0045] The following groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);
2) Serine (S), Threonine (T);
3) Aspartic acid (D), Glutamic acid (E);
4) Asparagine (N), Glutamine (Q);
5) Cysteine (C), Methionine (M);
6) Arginine (R), Lysine (K), Histidine (H);
7) Isoleucine (1), Leucine (L), Valine (V); and
8) Phenylalanine (F), Tyrosine (Y), Tryptophan (W). (*see, e.g.,* Creighton, *Proteins* (1984)).

[0046] One of skill in the art will appreciate that many conservative variations of the nucleic acid and polypeptide sequences provided herein yield functionally identical products. For example, due to the degeneracy of the genetic code, "silent substitutions" (i.e., substitutions of a nucleic acid sequence that do not result in an alteration in an encoded polypeptide) are an implied feature of every nucleic acid sequence that encodes an amino acid. Similarly, "conservative amino acid substitutions," in one or a few amino acids in an amino acid sequence are substituted with different amino acids with highly similar properties (see the definitions section, supra), are also readily identified as being highly similar to a disclosed amino acid sequence, or to a disclosed nucleic acid sequence that encodes an amino acid. Such conservatively substituted variations of each explicitly listed nucleic acid and amino acid sequences are a feature of the present invention.

[0047] The term "subject" refers to any mammal, in particular human, at any stage of life.

[0048] The term "contacting" is used herein interchangeably with the following: combined with, added to, mixed with, passed over, incubated with, flowed over, etc. Moreover, the ADNF III polypeptides or nucleic acids encoding them of the present invention can be "administered" by any conventional method such as, for example, parenteral, oral, topical, and inhalation routes. In some embodiments, parenteral and nasal inhalation routes are employed.

[0049] "Neurotoxicity" as used herein is defined as adverse effects on the structure or functioning of the cells of the nervous system that result from exposure to chemical substances or to disease processes. Among other things, neurotoxicants can cause morphological changes that lead to generalized damage to nerve cells (neuronopathy), injury to axons (axonopathy), or destruction of the myelin sheath (myelinopathy). It is well established that exposure to certain chemotherapeutic agents, agricultural and industrial chemicals can damage the nervous system, resulting in neurological and behavioral dysfunction. Symptoms of neurotoxicity include muscle weakness, loss of sensation and motor control, tremors, alterations in cognition, and impaired functioning of the autonomic nervous system. Neurotoxicological assessments use a battery of functional and observational tests. Neurotoxicity in humans is most commonly measured by neurological tests that assess cognitive, sensory, and motor function.

[0050] "Peripheral neurotoxicity" refers to neurotoxicity of the peripheral nervous system (PNS). The PNS includes all the nerves not in the brain or spinal cord, and includes the dorsal root ganglia (DRG). These nerves carry sensory information and motor impulses. Damage to the nerve fibers of the PNS can disrupt communication between the CNS and the rest of the body. Peripheral neurotoxicity is also sometimes referred to in the literature as peripheral neuropathy, and can include hundreds of identifiable conditions, as further described below. "Peripheral neuropathy" encompasses a wide range of conditions in which the nerves outside of the brain and spinal cord have been damaged, and may include crush injury and section.

[0051] "Central nervous system" or "CNS" means the brain and spinal cord.

[0052] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. Generally, a peptide refers to a short polypeptide. The terms apply to amino acid polymers in which one or more amino acid residue is an analog or mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

[0053] As used herein 'treatment' includes preventative treatment or prophylaxis, such as treatment for prevention of disease progression or onset of further symptoms, or for avoidance or reduction of side-effects or symptoms of a disease.

[0054] As used herein, 'disease' includes an incipient condition or disorder or symptoms of a disease, incipient condition or disorder.

[0055] The terms "isolated," "purified" or "biologically pure" refer to material that is substantially or essentially free from components that normally accompany it as found in its native state.

[0056] "An amount sufficient" or "an effective amount" or a "therapeutically effective amount" is that amount of an ADNF polypeptide that exhibits the activity of interest or which provides either a subjective relief of a symptom(s) or an objectively identifiable improvement as noted by the clinician or other qualified observer. In therapeutic applications, the ADNF polypeptides of the invention are administered to a patient in an amount sufficient to reduce or eliminate symptoms of the disease. An amount adequate to accomplish this is defined as the "therapeutically effective dose." The dosing range varies with the ADNF polypeptide used, the route of administration and the potency of the particular ADNF polypeptide, as further set out below, and as described in patents CA Patent 2202496, US Patent 6174862 and US Patent 6613740.

[0057] "Inhibitors," "activators," and "modulators" of expression or of activity are used to refer to inhibitory, activating, or modulating molecules, respectively, identified using in vitro and in vivo assays for expression or activity, e.g., ligands, agonists, antagonists, and their homologs and mimetics. The term "modulator" includes inhibitors and activators. Inhibitors are agents that, e.g., inhibit expression of a polypeptide or polynucleotide of the invention or bind to, partially or totally block stimulation or enzymatic activity, decrease, prevent, delay activation, inactivate, desensitize, or down regulate the activity of a polypeptide or polynucleotide of the invention, e.g., antagonists. Activators are agents that, e.g., induce or activate the expression of a polypeptide or polynucleotide of the invention or bind to, stimulate, increase, open, activate, facilitate, enhance activation or enzymatic activity, sensitize or up regulate the activity of a polypeptide or polynucleotide of the invention, e.g., agonists. Modulators include naturally occurring and synthetic ligands, antagonists, agonists, small chemical molecules and the like. Assays to identify inhibitors and activators include, e.g., applying putative modulator compounds to cells, in the presence or absence of a polypeptide or polynucleotide of the invention and then determining the functional effects on a polypeptide or polynucleotide of the invention activity. Samples or assays comprising a polypeptide or polynucleotide of the invention that are treated with a

potential activator, inhibitor, or modulator are compared to control samples without the inhibitor, activator, or modulator to examine the extent of effect. Control samples (untreated with modulators) are assigned a relative activity value of 100%. Inhibition is achieved when the activity value of a polypeptide or polynucleotide of the invention relative to the control is about 80%, optionally 50% or 25-1%. Activation is achieved when the activity value of a polypeptide or polynucleotide of the invention relative to the control is 110%, optionally 150%, optionally 200-500%, or 1000-3000% higher.

DETAILED DESCRIPTION OF THE INVENTION

[0058] This invention discloses the surprising finding that an ADNF polypeptide that was shown previously to be neuroprotective of the CNS and to provide cognitive enhancement can alternatively be used in the treatment of peripheral neurotoxicity induced by chemical agents or disease processes. The invention is supported by the findings set out in the Examples that *in vivo* administration of NAP peptide significantly reduces peripheral neurotoxicity induced by chemical agents.

ADNF polypeptides: composition and synthesis

[0059] In one embodiment, the ADNF polypeptides of the present invention comprise the following amino acid sequence:$(R^1)_x$-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-$(R^2)_y$ (SEQ ID NO:13) and conservatively modified variations thereof. In this designation, R^1 denotes the orientation of the amino terminal (NH_2 or N-terminal) end and R^2 represents the orientation of the carboxyl terminal (COOH or C-terminal) end.

[0060] In the above formula, R^1 is an amino acid sequence comprising from 1 to about 40 amino acids, wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs. The term "independently selected" is used herein to indicate that the amino acids making up the amino acid sequence R^1 may be identical or different (e.g., all of the amino acids in the amino acid sequence may be threonine, etc.). Moreover, as previously explained, the amino acids making up the amino acid sequence R^1 may be either naturally occurring amino acids, or known analogues of natural amino acids that functions in a manner similar to the naturally occurring amino acids (i.e., amino acid mimetics and analogs). Suitable amino acids that can be used to form the amino acid sequence R^1 include, but are not limited to, those listed in Table I, infra. The indexes "x" and "y" are independently selected and can be equal to one or zero.

[0061] As with R^1, R^2, in the above formula, is an amino acid sequence comprising from 1 to about 40 amino acids, wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs. Moreover, as with R^1, the amino acids making up the amino acid sequence R^2 may be identical or different, and may be either naturally occurring amino acids, or known analogues of natural amino acids that functions in a manner similar to the naturally occurring amino acids (i.e., amino acid mimetics and analogs). Suitable amino acids that can be used to form R^2 include, but are not limited to, those listed in Table I, infra.

[0062] As used herein, "NAP" or "NAP peptide" refers to the formula above where x and y both equal 0. "NAP related peptide" refers to any of the other variants of NAP which are described the formula.

[0063] R^1 and R^2 are independently selected. If R^1 R^2 are the same, they are identical in terms of both chain length and amino acid composition. For example, both R^1 and R^2 may be Val-Leu-Gly-Gly-Gly (SEQ ID NO:14). If R^1 and R^2 are different, they can differ from one another in terms of chain length and/or amino acid composition and/or order of amino acids in the amino acids sequences. For example, R^1 may be Val-Leu-Gly-Gly-Gly (SEQ ID NO:15), whereas R^2 may be Val-Leu-Gly-Gly (SEQ ID NO:16). Alternatively, R^1 may be Val-Leu-Gly-Gly-Gly (SEQ ID NO:17), whereas R^2 may be Val-Leu-Gly-Gly-Val (SEQ ID NO:18). Alternatives, R^1 may be Val-Leu-Gly-Gly-Gly (SEQ ID NO:19), whereas R^2 may be Gly-Val-Leu-Gly-Gly (SEQ ID NO:20).

[0064] Within the scope of the above formula, certain NAP and NAP related polypeptides are preferred, namely those in which x and y are both zero (i.e. NAP). Equally preferred are NAP and NAP related polypeptides in which x is one; R^1 Gly-Gly; and y is zero (SEQ ID NO:21). Also equally preferred are NAP and NAP related polypeptides in which is one; R^1 is Leu-Gly-Gly; y is one; and R^2 is -Gln-Ser (SEQ ID NO:22). Also equally preferred are NAP and NAP related polypeptides in which x is one; R^1 is Leu-Gly-Leu-Gly-Gly- (SEQ ID NO:23); y is one; and R^2 is -Gln-Ser (SEQ ID NO:24). Also equally preferred are NAP and NAP related polypeptides in which x is one; R^1 is Ser-Val-Arg-Leu-Gly-Leu-Gly-Gly- (SEQ ID NO:25); y is one; and R^2 is -Gln-Ser (SEQ ID NO:26). Additional amino acids can be added to both the N-terminus and the C-terminus of the active peptide without loss of biological activity.

[0065] In another aspect, the present invention provides pharmaceutical compositions comprising one of the previously described NAP and NAP related polypeptides in an amount sufficient to exhibit desired therapeutic activity, in a pharmaceutically acceptable diluent, carrier or excipient. In one embodiment, the NAP or NAP related peptide has an amino acid sequence selected from the group consisting of SEQ ID NO:2, and 9-12, and conservatively modified variations thereof.

[0066] In another embodiment, the ADNF polypeptide comprises the following amino acid sequence: $(R^1)_x$-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala-$(R^2)_y$ (SEQ ID NO:27) and conservatively modified variations thereof. In this designation, R^1 denotes the orientation of the amino terminal (NH_2 or N-terminal) end and R^2 represents the orientation of the carboxyl terminal (COOH or C-terminal) end.

[0067] In the above formula, R^1 is an amino acid sequence comprising from 1 to about 40 amino acids, wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs. The term "independently selected" is used herein to indicate that the amino acids making up the amino acid sequence R^1 may be identical or different (e.g., all of the amino acids in the amino acid sequence may be threonine, etc.). Moreover, as previously explained, the amino acids making up the amino acid sequence R^1 may be either naturally occurring amino acids, or known analogues of natural amino acids that functions in a manner similar to the naturally occurring amino acids (i.e., amino acid mimetics and analogs). Suitable amino acids that can be used to form the amino acid sequence R^1 include, but are not limited to, those listed in Table I, infra. The indexes "x" and "y" are independently selected and can be equal to one or zero.

[0068] As with R^1, R^2, in the above formula, is an amino acid sequence comprising from 1 to about 40 amino acids, wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs. Moreover, as with R^1, the amino acids making up the amino acid sequence R^2 may be identical or different, and may be either naturally occurring amino acids, or known analogues of natural amino acids that functions in a manner similar to the naturally occurring amino acids (i.e., amino acid mimetics and analogs). Suitable amino acids that can be used to form R^2 include, but are not limited to, those listed in Table I, infra.

[0069] As used herein, "SAL" or "SAL peptide" refers to the formula above where x and y both equal 0. "SAL related peptide" refers to any of the other variants of SAL which are described the formula.

[0070] R^1 and R^2 are independently selected. If R^1 R^2 are the same, they are identical in terms of both chain length and amino acid composition. Additional amino acids can be added to both the N-terminus and the C-terminus of the active peptide without loss of biological activity.

[0071] In another aspect, the present invention provides pharmaceutical compositions comprising one of the previously described SAL and SAL-related polypeptides in an amount sufficient to desired therapeutic activity, in a pharmaceutically acceptable diluent, carrier or excipient. In one embodiment, the SAL or SAL related peptide has an amino acid sequence selected from the group consisting of SEQ ID NO:1 and 3-8, and conservatively modified variations thereof. In a further embodiment, the SAL related peptide comprises SALLRSIPAPAGASRLLLLTGEIDLP (SEQ ID NO:28). The sequence SALLRSIPAPAGASRLLLLTGEIDLP (SEQ ID NO:28) is also known as Colivelin and is a combination of the SAL active site and a derivative of the Humanin protein named AGA-(C8R)HNG17. Colivelin is described in Chiba *et al.*, *J. Neurosci.* 25:10252-10261 (2005), which is herein incorporated by reference for all purposes.

[0072] It will be readily apparent to those of ordinary skill in the art that preferred ADNF polypeptides can readily be selected for peripheral neuroprotective activity by employing suitable assays and animal models known to those skilled in the art, some of which are disclosed herein.

[0073] In addition, one of skill in the art will recognize that a variety of chemical modifications can be made to the peptides without diminishing their biological activity. In addition to replacement of specific amino acids with other amino acids, there may also be a wide range of modifications to specific amino acids, and conjugates with a wide variety of polymers, proteins, carbohydrates or other organic moieties.

[0074] The peptides of the invention may be prepared via a wide variety of well-known techniques. Peptides of relatively short size are typically synthesized on a solid support or in solution in accordance with conventional techniques (*see, e.g.*, Merrifield, *Am. Chem. Soc.* 85:2149-2154 (1963)). Various automatic synthesizers and sequencers are commercially available and can be used in accordance with known protocols (*see, e.g.*, Stewart & Young,

Solid Phase Peptide Synthesis (2nd ed. 1984)). Solid phase synthesis in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the remaining amino acids in the sequence is the preferred method for the chemical synthesis of the peptides of this invention. Techniques for solid phase synthesis are described by Barany & Merrifield, *Solid-Phase Peptide Synthesis*; pp. 3-284 in *The Peptides: Analysis, Synthesis, Biology*. Vol. 2: *Special Methods in Peptide Synthesis,* Part A.; Merrifield et al 1963; Stewart *et al.* 1984). NAP and related peptides are synthesized using standard Fmoc protocols (Wellings & Atherton, *Methods Enzymol.* 289:44-67 (1997)).

[0075] Other synthetic methods for peptides include liquid phase synthesis (e.g. Fischer and Zheleva *J Pept Sci.* 8(9):529-42 (2002).

[0076] In addition to the foregoing techniques, the ADNF peptides, in particular the full length proteins ADNF I and ADNF III for use in the invention may be prepared by recombinant DNA methodology. Generally, this involves creating a nucleic acid sequence that encodes the protein, placing the nucleic acid in an expression cassette under the control of a particular promoter, and expressing the protein in a host cell. Recombinantly engineered cells known to those of skill in the art include, but are not limited to, bacteria, yeast, plant, filamentous fungi, insect (especially employing baculoviral vectors) and mammalian cells.

Use of ADNF polypeptides for treating peripheral neurotoxicity

[0077] Peripheral neurotoxicity may be identified and diagnosed in a subject by a variety of techniques. Typically it may be measured by motor dysfunction, muscle wasting, or a change in sense of smell, vision or hearing, or changes in deep tendon reflexes, vibratory sense, cutaneous sensation, gait and balance, muscle strength, orthostatic blood pressure, and chronic or intermittent pain. In humans these symptoms are also sometimes demonstrative of toxic effects in both the PNS and the CNS. Ultimately, there are hundreds of possible peripheral neuropathies that may result from neurotoxicity. Reflecting the scope of PNS activity, symptoms may involve sensory, motor, or autonomic functions. They can be classified according to the type of affected nerves and how long symptoms have been developing.

[0078] Peripheral neurotoxicity can be induced by chemotherapeutic agents (anti-cancer, anti-microbial and the like) and by disease processes. These two different areas are discussed separately below.

[0079] Regarding chemotherapeutic agents, it is well known that patients exposed to agents such as *Vinca* alkaloids, suramin, taxanes, and cisplatin can develop peripheral neurotoxicity. Neurological observations published in recent years indicate that administration of taxanes and cisplatin in patients affected by neoplasm induces nerve deficits in a dose- and time-dependent manner. (Bedikian A. Y., *et al.* 1995. *J. Clin. Oncol.*, 13: 2895-2899). Moreover, when platinum compounds and taxanes are used in combination, the patients develop more severe peripheral neuropathies. The pathophysiology of chemotherapeutic agent-induced neuropathy is still not clear, although a variety of studies have shown that taxanes interfere with axonal transport, causing axonal distal sensory-motor lesions, whereas platinum compounds induce sensory neuropathy acting mainly on the neuronal cell bodies of the spinal ganglion. Pathological and electrophysiological studies have also indicated that neurons of the dorsal root ganglion are selectively damaged after cisplatin treatment. It has been reported that the development of this peripheral neurotoxicity can induce clinicians to interrupt therapy to prevent more severe neurological deficits (Amato A. A., Collins M. P. *Semin. Neurol.*, 18: 125-142, 1998.). Because of the neurotoxic effects, much effort has been devoted to the identification of potential neuroprotective agents. It is reasonable, therefore, to hypothesize that ADNF polypeptides, which can prevent neurotoxicity and/or promote peripheral innervation after chemotherapy, will be clinically useful. Those skilled in the art are familiar with chemotherapeutic agents that may cause peripheral neurotoxicities. In general such chemotherapeutic agents are used in the treatment of cancer, multiple sclerosis, gout, arthritis, Bechet's disease, psychiatric disorders, familial Mediterranean fever, amyloidosis, immunosuppression and infectious disease. A representative list includes vinca alkaloids (vincristine, vindesine, vinorelbine and vinblastine), platinum drugs (cisplatinum, carboplatinum), L-asparaginase and the taxanes (taxol, taxotere). In addition to anti-cancer agents, neurotoxicity may be cause by thalidomide, methotrexate, colchicine and anti-infective agents (including but not limited to nucleoside analogs such as lamivudine, zalcitabine, didanosine and stavudine).

[0080] The method of the invention recognizes that administration of a therapeutically effective amount of an ADNF polypeptide is useful to treat or prevent peripheral neurotoxicity in a subject receiving a chemotherapeutic agent, such as those described above. Relative to the administration of the chemotherapeutic agent, the administration of the ADNF polypeptide can occur before, at the same time, subsequent to or on an irregular basis. Those skilled in the art are able to identify a suitable temporal relationship between the agents which is designed to establish peripheral neuroprotection before the consequences of the neurotoxicity develop. Treatment may continue until chemotherapeutic agent is discontinued, or until the neurotoxicity resulting from the agent is resolved and not expected to worsen.

[0081] When administered non-contemporaneously (e.g. sequentially) with the chemotherapeutic agent, the ADNF polypeptide will typically be formulated separately from the agent. When administered contemporaneously, it may be advantageous to provide the ADNF polypeptide in a dosage form in combination with the agent. Thus the invention recognizes a formulation of a chemotherapeutic agent and an ADNF polypeptide, wherein the dose of the ADNF polypeptide is effective to reduce or eliminate the peripheral neurotoxicity associated with the chemotherapeutic agent. Those skilled in the art are able to select a proper dose of ADNF polypeptide based this disclosure and on the anticipated neurotoxic effects of the selected chemotherapeutic agent.

[0082] As mentioned previously, certain disease processes can also result in peripheral neurotoxicity. For example, the diabetes/peripheral neuropathy link has been well established. A typical pattern of diabetes-associated neuropathic symptoms includes sensory effects that first begin in the feet. The associated pain or pins-and-needles, burning, crawling, or prickling sensations form a typical "stocking" distribution in the feet and lower legs.

[0083] Other diseases that may result in peripheral neurotoxicity include inherited or acquired disorders, including infectious diseases. Such diseases include leprosy, Charcot-Marie-Tooth Disease, Inherited neurological disorders such as the hereditary sensory and autonomic neuropathies (HSAN), Guillain-Barré syndrome which may arise from complications associated with viral illnesses, such as cytomegalovirus, Epstein-Barr virus, and human immunodeficiency virus (HIV), or bacterial infection, including *Campylobacter jejuni* and Lyme disease. Other well-known causes of peripheral neuropathies include

chronic alcoholism, infection varicella-zoster virus, botulism, and poliomyelitis. Peripheral neuropathy may develop as a primary symptom, or it may be less significant. Uremia, or chronic kidney failure, carries a 10-90% risk of eventually developing neuropathy, and there may be an association between liver failure and peripheral neuropathy. Accumulation of lipids inside blood vessels (atherosclerosis) can choke-off blood supply to certain peripheral nerves.

[0084] As recognized in the case of chemotherapeutic agents, use of ADNF polypeptides to treat or prevent neurotoxicity from disease processes requires administration of a therapeutically effective amount of an ADNF polypeptide sufficient to treat or prevent peripheral neurotoxicity in a subject suffering from such disease. In this case, relative to the onset of the peripheral neurotoxicity, the administration of the ADNF polypeptide can occur before, at the same time, subsequent to or on an irregular basis. Those skilled in the art are able to identify a suitable temporal relationship between the agents which is designed to establish peripheral neuroprotection before the consequences of the disease induced neurotoxicity develop. Treatment may continue until the underlying disease resolves, or until the neurotoxicity resulting from the disease is resolved and not expected to worsen. In some cases, administration of ADNF polypeptides may be chronic.

[0085] Because the disease processes of concern to this invention are often treated with other therapeutic agents, the invention recognizes that it may be advantageous to provide the ADNF polypeptide in a dosage form in combination with such an agent. Thus the invention recognizes a formulation of a therapeutic agent and an ADNF polypeptide, wherein the dose of the ADNF polypeptide is effective to reduce or eliminate the peripheral neurotoxicity associated with the chemotherapeutic agent. Those skilled in the art are able to select a proper dose of ADNF polypeptide based this disclosure and on the anticipated neurotoxic effects of the selected therapeutic agent.

Use of ADNF Polypeptides to Treat Tauopathy and Related Diseases

[0086] Tauopathy means the accumulation of microtubule-associated protein tau in the neuronal and glial cytoplasm. This terminology is relatively new, but it relates to neurodegenerative diseases evidencing widespread accumulation of tau epitopes both in neurons and glia, sometimes without deposition of amyloid beta protein. Tauopathy is now considered to be one of the primary causes of neuronal degeneration, with about one third of the very elderly presenting with deposition of abnormally phosphorylated tau proteins with

relative paucity of amyloid beta protein (Abeta). In the course of neurofibrillary tangle formation (including tau aggregates), the major proteinaceous components of these lesions undergo post-translational modifications. In the case of tau, these include phosphorylation of mainly serine and threonine, but also tyrosine residues. In addition, tau is subject to ubiquitination, nitration, truncation, prolyl isomerization, association with heparan sulfate proteoglycan, glycosylation, glycation and modification by advanced glycation end-products (AGEs). Human tauopathies include Alzheimer's disease and frontotemporal dementia with parkinsonism linked to chromosome 17 (Chen *et al. Curr Drug Targets.* 5(6):503-15 (2004)). Furthermore, recent studies have shown that as a consequence of chemotherapy there was an increase in cerebrospinal fluid tau, which is a marker of neurodegeneration (Van Gool *et al. Leukemia.* 14:2076-84 (2000); Lee *et al., Biochem. Biophys Acta.* 1739: 251-9 (2005))

[0087] The instant invention relates to a method of treatment of tauopathy in a subject comprising administering to the subject a therapeutically effective amount of an ADNF polypeptide. Treatment of tauopathy with the NAP peptide is a specific embodiment of this invention.

[0088] The inventors have recognized, based on the instant disclosure, that ADNF polypeptides such as NAP effectively prevent neurotoxic damage by the vinca alkaloid vincristine (see Examples), and without wishing to be bound to any particular theory or mechanism of action, that this effect of NAP can be combined with the teachings of PCT publication WO 2004/080957 (Gozes *et al.*) and Divinski *et al. J Biol Chem.* 279(27):28531-8. (2004) that demonstrate that NAP interacts with tubulin to enhance microtubule formation and stabilize microtubular structure in glial and neural cells, to establish for the first time that NAP is useful for the treatment of tauopathy. Other peptides of the ADNF family including ADNF-9 (or SAL) and all D-amino acids SAL (termed D-SAL, Brenneman *et al.* (2004), infra) as well as full length ADNP (ADNFIII) interact with tubulin. (Furman *et al., Neuron Glia Biology* 1:193-9 (2004).

[0089] It is well recognized that tau performs an important function of stabilizing and maintaining the microtubular network, that in turn is important for axonal transport in neurons. The formation of the pathological neurofibrillary tangles which results from the hyperphosphorylation of tau, leads to microtubule breakdown and impaired axonal transport (Ishihara *et al. Neuron* 24:751-62 (1999); Lee *et al., Annu Rev Neurosci.* 24:1121-59

(2001); Morfini *et al. Neuromolecular Med.* 2:89-99 (2002); Gozes. *J Mol Neurosci.* 19(3):337-8 (2002)). Divinski *et al. J Biol Chem.* 279(27):28531-8. (2004) have demonstrated that exposure to zinc toxicity resulted in microtubule breakdown in astrocytes and neurons and that NAP protects these cells from this toxicity by promoting the reorganization of the microtubular network. In the same experiments, tubulin was identified as a NAP binding molecule. Furthermore, in the presence of NAP, there is an increase in the ratio of non-phosphorylated tau to phosphorylated tau (Gozes & Divinski, *Journal of Alzheimer's Disease* 6(6 Suppl.):S37-41 (2004)) and increased neurite outgrowth, a process that is dependent on slow axoplasmic transport (Lagreze *et al.*, *Invest Opthalmol Vis Sci.* 46:933-8 (2005); Gozes. *Neurochem Int.*4:101-20 (1982); Smith-Swintosky *et al. J Mol Neurosci.* 25:225-38 (2005). Therefore, it is possible that NAP functions to promote the assembly and stability of the microtubular network either directly by binding to tubulin or indirectly through changes in the levels of the different forms of tau. The promotion of proper microtubule assembly is also important in the case of vicristine treatment, as vincristine and related compounds facilitate the tubulin spiral filaments and aggregated spiral formation (Verdier-Pinard *et al. Biochem Pharmacol.* 58(6):959-71 (1999)) Any other tubulin binding and modifying agents including, but not limited to vinca alkaloids (vincristine, vindesine, vinorelbine and vinblastine), the taxanes (taxol, taxotere), nocodazole and colchicines will affect axoplasmic transport which can in turn be protected by the specific neuroprotective effect of NAP treatment (Gozes *et al. J Mol Neurosci.* 20(3):315-22, (2003)).

Use of olfaction testing to measure effectiveness of neurological therapeutics, such as ADNF polypeptides.

[0090] Olfaction disabilities, including hyposmia (reduction in ability to taste and smell) or anosmia (total loss of ability to taste and smell), are associated with neurodegenerative disease (such as Alzheimer's disease, multiple sclerosis, Huntington disease, amyotrophic lateral sclerosis, Parkinson's disease and others) and peripheral neurotoxicity induced by chemotherapeutic agents and by disease processes. In all these cases, olfaction disabilities are generally progressive.

[0091] The present invention provides a method to identify whether a subject having a neurodegenerative disease or peripheral neurotoxicity is responding to therapeutic agents administered to treat the disease by measuring olfaction in the subject. A response to

therapy is indicated either by an improvement in olfaction capacity or quality of the subject after treatment with a therapeutic agent, or at least a reduction, after such treatment, in the degree of hyposmia or the progress of hyposmia to anosmia that would be expected in subjects with untreated disease.

[0092] While the method can be used to test response to any therapeutic agent for the treatment of the neurodegenerative disease or the peripheral neurotoxicity, in particular, this invention provides a method to identify a response to therapy with ADNF polypeptide.

[0093] The method involves testing for response to a therapeutic agent for a neurodegenerative disease comprising the following steps: a) measuring olfaction capacity in a subject having a neurodegenerative disease or potential peripheral neurotoxicity; b) administering a therapeutic agent to the subject; c) measuring olfaction capacity in the subject subsequent to step b); and d) comparing olfaction capacity from step a) and step c).

[0094] Based on the results of the comparison of step d), the subject and care-giver can determine whether there is either an improvement in olfaction capacity or quality of the subject after treatment with a therapeutic agent, or at least a reduction, after such treatment, in the degree of hyposmia or the progress of hyposmia to anosmia that would be expected in subjects with untreated disease, thus indicating a response to the therapeutic agent, or not. Patients and care-givers can then go on to decide whether treatment with the therapeutic agent should continue or be halted.

[0095] This method provides many advantages for assessing a response to a therapeutic agent, in particular because olfaction is one of the first senses to be lost or diminished as a result of a neurodegenerative disease or the onset of peripheral neurotoxicity.

Pharmaceutical administration

[0096] ADNF polypeptides of the invention are generally administered in a pharmaceutical formulation. Suitable formulations for use in the present invention are found in Remington's *Pharmaceutical Sciences* (17th ed. 1985). In addition, for a brief review of methods for drug delivery, *see* Langer, *Science* 249:1527-1533 (1990).

[0097] As such, the present invention provides for therapeutic compositions or medicaments comprising one or more of the ADNF polypeptides described herein in combination with a pharmaceutically acceptable excipient, wherein the amount of the ADNF polypeptide is sufficient to provide a therapeutic effect.

[0098] The ADNF polypeptides of the present invention are embodied in pharmaceutical compositions intended for administration by any effective means, including parenteral, topical, nasal, oral, pulmonary (e.g. by inhalation) or local administration. Preferably, the pharmaceutical compositions are administered parenterally, e.g., intravenously, subcutaneously, intradermally, intramuscularly, or intranasally.

[0099] Thus, the invention provides compositions for parenteral administration that comprise a solution of ADNF polypeptide, as described above, dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used including, for example, water, buffered water, 0.4% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques or, they may be sterile filtered. The resulting aqueous solutions may be packaged for use as is or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions including pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, such as, for example, sodium acetate, sodium lactate, sodium chloride potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

[0100] For solid compositions, conventional nontoxic solid carriers may be used that include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient and more preferably at a concentration of 25%-75%.

[0101] For aerosol administration, the ADNF polypeptides are preferably supplied in finely divided form along with a surfactant and propellant. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. A carrier can also be included, as desired, as with, e.g., lecithin for intranasal delivery. An example includes a solution in which each milliliter

included 7.5 mg NaCl, 1. 7 mg citric acid monohydrate, 3 mg disodium phosphate dihydrate and 0.2 mg benzalkonium chloride solution (50%) (Gozes *et al., J Mol Neurosci.* 19(1-2):167-70 (2002)). The ADNF polypeptides of the invention can therefore be used in the manufacture of a medicament for the treatment or prevention of peripheral neurotoxicity. The medicament can comprise any of the pharmaceutical formulations contemplated herein, with any amount of active ingredient (e.g. ADNF polypeptide) contemplated herein.

[0102] In therapeutic applications, the ADNF polypeptides of the invention are administered to a patient in an amount sufficient to reduce or eliminate symptoms of peripheral neurotoxicity. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, for example, the particular NAP or ADNF polypeptide employed, the type of disease or disorder to be prevented, the manner of administration, the weight and general state of health of the patient, and the judgment of the prescribing physician.

[0103] For example, an amount of polypeptide falling within the range of a 100 ng to 10 mg dose given intranasally once a day (e.g., in the evening) would be a therapeutically effective amount. Alternatively, dosages may be outside of this range, or on a different schedule. For example, dosages may range from 0.0001 mg/kg to 1000 mg/kg, and will preferably be about 0.001 mg/kg, 0.1 mg/kg, 1 mg/kg, 5 mg/kg, 50 mg/kg or 500 mg/kg per dose. Doses may be administered hourly, every 4, 6 or 12 hours, with meals, daily, every 2, 3, 4, 5, 6, or 7 days, weekly, every 2, 3, 4 weeks, monthly or every 2, 3 or 4 months, or any combination thereof. The duration of dosing may be single (acute) dosing, or over the course of days, weeks, months, or years, depending on the condition to be treated. Those skilled in the art can determine the suitable dosage, and may rely on preliminary data reported in Gozes *et al.*, 2000, Gozes *et al.*, 2002), Bassan *et al.* 1999; Zemlyak *et al., Regul. Pept.* 96:39-43 (2000); Brenneman *et al., Biochem. Soc. Trans.* 28: 452-455 (2000); *Erratum Biochem Soc. Trans.* 28:983; Wilkemeyer *et al. Proc. Natl. Acad. Sci. USA* 100:8543-8548 (2003)). Suitable dose ranges are described in the examples provided herein, as well as in WO 9611948.

[0104] It must be noted that as used herein and in the appended claims, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and equivalents thereof known to those skilled in the art, and so forth.

[0105] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed. Citations are incorporated herein by reference.

EXAMPLES

Example 1: Intranasal administration of NAP decreases peripheral neurotoxicity induced by vinca alkaloids in rats

[0106] The present study was designed to evaluate if NAP treatment successfully reduced peripheral neurotoxicity induced by the vinca alkaloid vincristine in rats.

Methods

[0107] Rats (200-300g), divided into 4 groups of 10, were injected with vincristine sulfate dissolved in saline. Stock concentration was 2 mg/ml, pH 4.5 - 5.2. Aliquots of the drug were diluted daily in saline to concentrations of 0.175 mg/ml and were administered i.p. at a dose of 0.175 mg/kg. NAP was prepared at 0.1 mg/1.3 ml in saline (0.9% NaCl) and about 0.1 ml was injected subcutaneously to a ~300g rat to achieve a dose of 25 microgram/kg (exact calculations were made based on the daily body weights). For 2.5 microgram/kg, the injection solution was diluted by 10 and again 0.1 ml was injected per rat. Treatments took place daily (5 days a week) for ~3 weeks (20 days) with the dosage calculated on daily body weight. NAP was administered at the same time as the vincristine, in the dosage and the amount where indicated the following schedule.

[0108] Four groups were evaluated: 1) Control, saline (n=10), 2) Vincristine (i.p.) 0.175 mg/kg (n=10), 3) Vincristine (i.p.) 0.175 mg/kg + NAP (s.c.) 2.5 microgram/kg (n=10), and 4) Vincristine (i.p.) 0.175 mg/kg + NAP (s.c.) 25 microgram/kg (n=10).

[0109] After a week cessation of treatment, a final boost of vincristine was given i.p. daily for three days and NAP at 25 microgram/kg was given intranasally to group number 4 only. The intranasal formulation followed previous experiment (7.5 mg Sodium Chloride, 1.7mg Citric Acid Monohydrate, 3.0mg Disodium Phosphate Dihydrate, 0.2mg Benzalkonium Chloride Solution (50%) per 1 mL Sterile Water (U.S.P.) Alcalay *et al.*, infra). For behavioral testing baseline toxin effects were evaluated 2-23 days following the first injection, using the testing procedures below:

1) Rota-rod

[0110] Vincristine treated animals show impaired performance on the rota-rod test which evaluates muscle innervation and strength (Boyle *et al., J Pharmacol Exp Ther* 279: 410-415 (1996)) Here, the rota-rod test was performed on days 3, 8, 15 and 23 after the initiation of the vincristine injections.

[0111] In the Rota-rod test a rodent is placed on a rotating rod. The speed of rotation is gradually increased and the rodent's ability to remain on the rotating rod is recorded. Here, the speed was gradually increased from 3-30rpm every 30 seconds with 3rpm increments up to 200 seconds. The time spent on the rota-rod without falling was recorded. Impaired animals fall earlier from the rota-rod. The time spent by each animal in a single treatment group was summed for all treatment and test days as seen on Figure 1. Statistical analysis showed a significant difference between the vincristine treated animals and the control animals indicating impairment in the vincristine animals ($P<0.01$). Group 3 treatment with NAP (2.5 microgram/kg) significantly improved the performance similar to control values Animals treated with NAP (25 micrograms/kg) in group 4 showed no significant difference from animals treated with vincristine treated alone and were significantly different from controls.

2) Motor Evaluation

[0112] Motor examination was performed on days 6, 9, 13 and 24 after the initiation of the vincristine injections. Rats were examined after vincristine injection, with the use of a motor disability scale. (Bederson JB, *et al., Stroke.* 1986; 17: 472–476; Leker RR, *et al., J Neurol Sci.* 1999; 162: 114–119).

[0113] Animals were assessed based on their failure to walk out of a circle 30 cm in diameter within 20 seconds.

[0114] Figure 2 shows the results of motor evaluations. The ability to exit a 30 cm in diameter within 20 seconds was significantly reduced for vincristine-treated rats as compared to control animals ($P<0.001$). Additional treatment with NAP (25 microgram/kg) in group 4 significantly improved the performance similar to control values ($P<0.01$). Animals treated with NAP (2.5 micrograms/kg) in group 3 showed no significant difference from animals treated with vincristine alone.

3) Olfactory Discrimination Test

[0115] Treatments took place daily (5 days a week) for ~3 weeks (20 days) with the dosage calculated on daily body weight. NAP was administered subcutaneously at the same time as the vincristine was administered intraperitoneally, in the dosage and the amount indicated in the schedule above. After a week cessation of treatment a final boost of vincristine was given i.p. daily for three days and NAP at 25 microgram/kg was given intranasally to group number 4 only. The intranasal formulation followed the previous experiment (Alcalay *et al.*, infra). An olfactory discrimination test was performed on each of the three days of NAP treatment during the final boost period. (Macknin *et al.*, *Brain Res.* 1000, 174-78 (2004)) The odors tested were 1) Deionized water (ddw) and 2) Scented extracts, *i.e.*, vanilla/almond.

[0116] Overall the experiment included 3 odors (water and two scented extracts) each encompassing 3 trials of 2 minutes. New dipped tip was introduced at the beginning of each trial for the different scents.

[0117] Thirty minutes before the testing the subjects were housed in separate cages and a cotton tip dipped in water was placed hanging into the cage. During these 30 minutes the animal was acclimatized to the tip and the cage. Thereafter, the cotton tip was dipped in the desired scent and placed hanging from the top of the cage.

[0118] The duration (seconds) of tip sniffing with count starting when the nose of the animal is approximately 1 cm. from the tip and stopping when the animal places its paws on the tip or tries to bite it reflects olfactory responses.

[0119] If the animal can discriminate odors, then each trial of the same scent it will be less and less interested – thus the sniffing time will go down. But when the odor is replaced by a new one – the sniffing time will increase.

[0120] T-test was used to make comparisons of individual test groups with control group or between unpaired groups.

[0121] Figures 3-5 show the time spent with new odors, *i.e.*, olfaction capacity. The time spent with each odor over the three consecutive tests was recorded. Figure 3, for control rats (Group 1), Figure 4 for vincristine treated rats (Group 2) and Figure 5 for vincristine and 25 micogram/kg NAP treated rats (Group 4). While the Group 2 vincristine-treated rats did not show any initial interest in the new smell, a trend toward increased interest toward a

new odor was observed in the control and the vincristine-NAP treated rats. These results were further evaluated in Figure 6.

[0122] Figure 6 shows a comparison between time periods spent with a certain odor after exchanging for a new scent – odor discrimination test: The figure depicts 4 points, point 1 = water (ddw) trial 3; point 2 = odor #1, trial #1; point 3 = odor #1, trial 3; point 4 = odor #2, trial 1. Results showed a significant difference in the time taken to sniff the third odor when comparing vincristine-treated rats (Group 2) to either control rats (Group 1) or to vincristine + 25micogram/kg NAP rats (Group 4) ($P<0.05$).

Results

[0123] The result of these three independent tests evaluating peripheral neurotoxicity, specifically muscle innervation or strength (e.g. rota-rod test); Motor abilities (movement and circle exit); and behavioral/olfaction abilities, indicates that treatment of animals with NAP significantly reduced impairment and evidence of peripheral neurotoxicity that results from vincristine treatment.

[0124] The results of the olfaction experiment in particular demonstrates that intranasal NAP provides a benefit to animals that lose olfaction capacity as a result of chemotherapy. This supports the finding that measurement of olfaction capacity can be used to determine whether subjects are responding to ADNF polypeptide treatment, either for neurodegenerative disease or peripheral neurotoxicity, by measuring whether the progress of hyposmia or anosmia has been halted, reduced or reversed in patients receiving treatment with ADNF polypeptides.

Example 2: Administration of NAP reduces neurotoxicity induced by chemotherapeutic agents.

[0125] Aim of the study: The aim of the present randomized blind study is to investigate the efficacy of NAP in reducing neurotoxicity induced by the chemotherapeutic agent vincristine in men and women with advanced carcinoma.

Methods:

[0126] Initially 21 patients with advanced carcinoma are randomized between groups A and B. In group A (11 patients) NAP is administered at a dose of 15mg/45-70 kg before vincristine infusion (1.4 mg/m^2.). In group B (10 patients) the same chemotherapeutic protocol is followed without administration of NAP. Before beginning of chemotherapy

and after 6 chemotherapeutic cycles all patients will undergo clinical neurologic examination and nerve conduction study by a neurologist who is blind to the randomization.

Results:

[0127] Clinical neurologic examination is assessed neurotoxicity indicators such as tendon reflexes, superficial sensory perception and muscle strength, as well as neuropathic symptoms. Nerve conduction study assesses nerve conduction velocity and action potential amplitude in 7 peripheral nerves. Deterioration of nerve conduction parameters, tendon reflexes, muscle strength, superficial sensory perception, but also of patient's symptoms is significantly more severe in group B.

Conclusion:

[0128] Concurrent NAP administration will be found to significantly reduce neurotoxicity induced by the chemotherapeutic agent vincristine in men and women with advanced carcinoma.

Example 3: Randomized trial with or without NAP to reduce neurotoxicity side effects under chemotherapy with oxaliplatin (L-OHP), FA/5-FU.

[0129] Aim of the study: Chemotherapy with L-OHP, FA, 5-FU has a high activity in advanced colorectal cancer (ACRC). The main dose-limiting toxicity of chemotherapy with L-OHP is a peripheral sensory neuropathy. In this study the patients (pts) will receive a chemotherapy with L-OHP, FA and 5-FU with or without NAP. The question is whether a reduction of side effects of neurotoxicity is seen after application of NAP.

Materials and Methods

[0130] We include 27 patients with ACRC. In arm A chemotherapy is applied with L-OHP 85 mg/m2 d1, FA 500 mg/m2 d1+d2 and 5-FU 4000 mg/m2 over 48 h continuous infusion as biweekly schedule. In arm B, 15mg/45-70 kg NAP is given over 10 min i.v. before application of the same schedule of chemotherapy. Investigation of toxicity, neurological examination and a blood count is performed before every cycle. For a daily documentation of the side effects every patient receives a questionnaire. The NAP group shows a significant reduction of peripheral neurotoxicity. In the NAP group grade II/III leucopenia occurs at a lower frequency than in the control group.

Conclusion

[0131] Side effects such as peripheral neurotoxicity under chemotherapy including L-OHP, FA/5-FU will be reduced under supportive care with NAP.

Example 4: Taxol Neurotoxicity and Protection by NAP

[0132] The present study was designed to evaluate whether NAP treatment successfully reduced peripheral neurotoxicity induced by the taxane taxol in rats.

Methods

Experiment 1

[0133] Forty Sprague-Dawley rats (eight weeks old) were divided into four groups that received the following treatments: a) 10% Cremophor EL in Saline; b) taxol for a cumulative dose of 5.6mg/kg; c) taxol for a cumulative dose of 5.6mg/kg + NAP 2.5μg/kg/Day; or d) taxol for a cumulative dose of 5.6mg/kg + NAP 25μg/kg/Day. The taxol was reconstituted in a vehicle of 10% Cremophor EL in Saline.

[0134] Taxol treatments were administered four times intraperitoneally (i.p.) on nonconsecutive days in a volume of 250μl each time. NAP was administered daily on 8 consecutive days from the first day of taxol injection (with a 2 day brake on the weekend).

[0135] Rats were tested in the rota-rod and plantar test. The rota-rod test is described above and assesses muscle strength. The plantar test evaluates thermal hyperalgesia. For the plantar test, each rat was placed in a clear plastic chamber with a plastic floor and allowed a short period to acclimatize to the new environment (approximately 2-5 min). The animals were then challenged with a radiant Infrared (IR) heat source directed at the plantar surface of the hind paw from below (7371 Plantar Test, Ugo Basile). The withdrawal latency of both the ipsilateral and contralateral hind paw was evaluated. The infrared intensity was set at IR55 and the maximum length of exposure to the IR source was eighteen seconds. Statistical differences were tested by ANOVA and by t-test.

Results:

[0136] A statistically significant difference between groups was observed only in the plantar test and results are shown in Figure 7. After the last taxol injection on day three, the taxol-treated rats exhibited thermal hyperalgesia, which was ameliorated by 2.5μg/kg/day NAP injections. 25ug/kg NAP did not affect the plantar test. The hyperalgesia induced by

the taxol treatment diminished after 4 days. Rota-rod experiments did not show statistically significant differences between groups in this experiment.

Experiment 2

[0137] Fifteen Sprague-Dawley Rats (seven weeks old) were randomly divided into three groups that received the following treatments: a) 10% Cremophor EL in Saline; b) taxol for a cumulative dose of 9mg/kg; or c) taxol for a cumulative dose of 9mg/kg + 2.5µg/kg/Day NAP. The taxol was reconstituted in a vehicle of 10% Cremophor EL in Saline.

[0138] Taxol was administered twice on nonconsecutive days; each rat received 0.4-0.5ml Taxol solution (i.p.). NAP was administered in 0.1ml volume for five consecutive days starting with first injection of Taxol.

[0139] The rats were tested a day after the last Taxol injection on rota-rod and plantar tests as described above. Statistical significance was only observed using non-paired, one tail t-test.

Results

[0140] Results are shown in Figure 8 (Plantar test) and Figure 9 (Rota-rod test). Taxol-treated rats exhibited a significant thermal hyperalgesia eight days after the last Taxol injection. The hyperalgesia was ameliorated by NAP treatment. At this higher dose of Taxol, a significant effect on neuromuscular function was measured using the rota-rod test five days after the first taxol injection. The neuromuscular effect was ameliorated by NAP treatment.

Conclusion:

[0141] NAP protects against Taxol-induced neuropathy in vivo.

[0142] The examples set out above are intended to be exemplary of the effects of the invention, and are not intended to limit the embodiments or scope of the invention contemplated by the claims set out below. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, databases, Genbank sequences, GO terms, patents, and patent applications cited herein are hereby incorporated by reference.

WHAT IS CLAIMED IS:

1. A method for treating peripheral neurotoxicity in a subject, the method comprising administering a therapeutically effective amount of an ADNF polypeptide to a subject in need thereof, wherein the ADNF polypeptide is a member selected from the group consisting of:

(a) an ADNF I polypeptide comprising an active core site having the following amino acid sequence:

Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:1), or an analogue thereof;

(b) an ADNF III polypeptide comprising an active core site having the following amino acid sequence:

Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln (SEQ ID NO:2), or an analogue thereof, and

(c) a mixture of the ADNF I polypeptide of part (a) and the ADNF III polypeptide of part (b), or their respective analogues.

2. The method of claim 1, wherein the ADNF polypeptide is a member selected from the group consisting of a full length ADNF I polypeptide, a full length ADNF III polypeptide (ADNP), and a mixture of a full length ADNF I polypeptide and a full length ADNF III polypeptide.

3. The method of claim 1, wherein the ADNF polypeptide is an ADNF I polypeptide.

4. The method of claim 1, wherein the active core site of the ADNF polypeptide comprises at least one D-amino acid.

5. The method of claim 1, wherein the ADNF I polypeptide has the formula $(R^1)_x$-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala-$(R^2)_y$ (SEQ ID NO:27), or an analogue thereof, in which

R^1 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs;

R^2 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; and

x and y are independently selected and are equal to zero or one.

6. The method of claim 5, wherein the ADNF I polypeptide is Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:1).

7. The method of claim 5, wherein the ADNF I polypeptide is selected from the group consisting of:

Val-Leu-Gly-Gly-Gly- Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:3);

Val-Glu-Glu-Gly-Ile-Val-Leu-Gly-Gly-Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:4);

Leu-Gly-Gly-Gly- Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:5);

Gly-Gly-Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:6);

Gly-Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:7);

Gly- Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:8); and

Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:1).

8. The method of claim 5, wherein the ADNF I polypeptide comprises up to about 20 amino acids at either or both of the N-terminus and the C-terminus of the active core site.

9. The method of claim 1, wherein the ADNF polypeptide is an ADNF III polypeptide.

10. The method of claim 9, wherein the ADNF III polypeptide has the formula $(R^1)_x$-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-$(R^2)_y$ (SEQ ID NO:13), or an analogue thereof, in which

R^1 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs;

R^2 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; and

x and y are independently selected and are equal to zero or one.

11. The method of claim 9, wherein the ADNF III polypeptide is Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln (SEQ ID NO:2).

12. The method of claim9, wherein the active core site of the ADNF III polypeptide comprises at least one D-amino acid.

13. The method of claim9, wherein the ADNF III polypeptide is a member selected from the group consisting of:

Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln (SEQ ID NO:9);

Leu-Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-Gln-Ser (SEQ ID NO:10);

Leu-Gly-Leu-Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-Gln-Ser (SEQ ID NO:11);

Ser-Val-Arg-Leu-Gly-Leu-Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-Gln-Ser (SEQ ID NO:12); and

Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln (SEQ ID NO:2).

14. The method of claim9, wherein the ADNF III polypeptide comprises up to about 20 amino acids at either or both of the N-terminus and the C-terminus of the active core site.

15. The method of claim 1, wherein a mixture of the ADNF I polypeptide of part (a) and the ADNF III polypeptide of part (b), or their respective analogues are administered to the subject.

16. The method of claim 15, wherein either or both active core sites of the ADNF I polypeptide and the ADNF III polypeptide comprise at least one D-amino acid.

17. The method of claim 15, wherein the ADNF I polypeptide is Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:1), and wherein the ADNF III polypeptide is Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln (SEQ ID NO:2).

18. The method of claim 15, wherein the ADNF I polypeptide is a member selected from the group consisting of:

Val-Leu-Gly-Gly-Gly- Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:3);

Val-Glu-Glu-Gly-Ile-Val-Leu-Gly-Gly-Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:4);

Leu-Gly-Gly-Gly- Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:5);

Gly-Gly-Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:6);

Gly-Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:7);

Gly- Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:8); and

Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala (SEQ ID NO:1); and

wherein the ADNF III polypeptide is selected from the group consisting of:

Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln (SEQ ID NO:9);

Leu-Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-Gln-Ser (SEQ ID NO:10);

Leu-Gly-Leu-Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-Gln-Ser (SEQ ID NO:11);

Ser-Val-Arg-Leu-Gly-Leu-Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-Gln-Ser (SEQ ID NO:12); and

Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln (SEQ ID NO:2).

19. The method of claim 15, wherein the ADNF I polypeptide comprises up to about 20 amino acids at either or both of the N-terminus and the C-terminus of the active core site of the ADNF I polypeptide, and wherein the ADNF III polypeptide comprises up to about 20 amino acids at either or both of the N-terminus and the C-terminus of the active core site of the ADNF III polypeptide.

20. The method of claim 1, wherein the ADNF polypeptide is administered intranasally, orally, intravenously or subcutaneously.

21. Use of an ADNF polypeptide in the manufacture of a medicament for the treatment of peripheral neurotoxicity.

22. The method of claim 1 or 21, wherein said peripheral neurotoxicity is a consequence of treatment with one or more chemical agents or a consequence of a disease process.

23. The method of claim 22, wherein said peripheral neurotoxicity is a consequence of treatment with a chemical agent selected from among chemical agents for cancer, multiple sclerosis, gout, arthritis, Behcet's disease, psychiatric disorder, immunosuppression and infectious disease.

24. The method of claim 22, wherein said one or more chemical agents is selected from the group consisting of vinca alkaloids, platinum drugs, L-asparaginase, taxanes, thalidomide, methotrexate, colchicines, and anti-infective agents.

25. The method of claim 22, wherein said peripheral neurotoxicity is a consequence of a disease process selected from among diabetes, leprosy, Charcot-Marie-Tooth Disease, hereditary sensory and autonomic neuropathies (HSAN), Guillain-Barré syndrome, viral illnesses, bacterial infection, chronic alcoholism, botulism, poliomyelitis, uremia, chronic kidney failure, and atherosclerosis.

26. A method for the treatment of cancer or neoplasia comprising

a) administering an anti-cancer agent; and

b) administering, contemporaneously or sequentially with the anti-cancer agent of step a), an ADNF polypeptide in a pharmaceutically acceptable carrier.

27. The method of claim 26, wherein said anti-cancer agent is a vinca alkaloid.

28. The method of claim 26, wherein said ADNF polypeptide is NAP.

29. A method of testing for response to a therapeutic agent for a neurodegenerative disease or peripheral neurotoxicity, wherein the therapeutic agent is an ADNF polypeptide, the method comprising the following steps,

a) measuring olfaction capacity in a subject having a neurodegenerative disease or potential peripheral neurotoxicity;

b) administering a therapeutic agent to the subject;

c) measuring olfaction capacity in the subject subsequent to step b);

d) comparing olfaction capacity from step a) and step c).

30. The method of claim 29, wherein the neurodegenerative disease is Alzheimer's disease.

31. The method of claim 29, wherein the subject has potential peripheral neurotoxicity associated with treatment by a chemotherapeutic agent.

32. A method of treatment of tauopathy in a subject comprising administering to a subject having or suspected of having a tauopathy, a therapeutically effective amount of an ADNF polypeptide.

33. The method of claim 32 wherein the ADNF polypeptide is NAP.

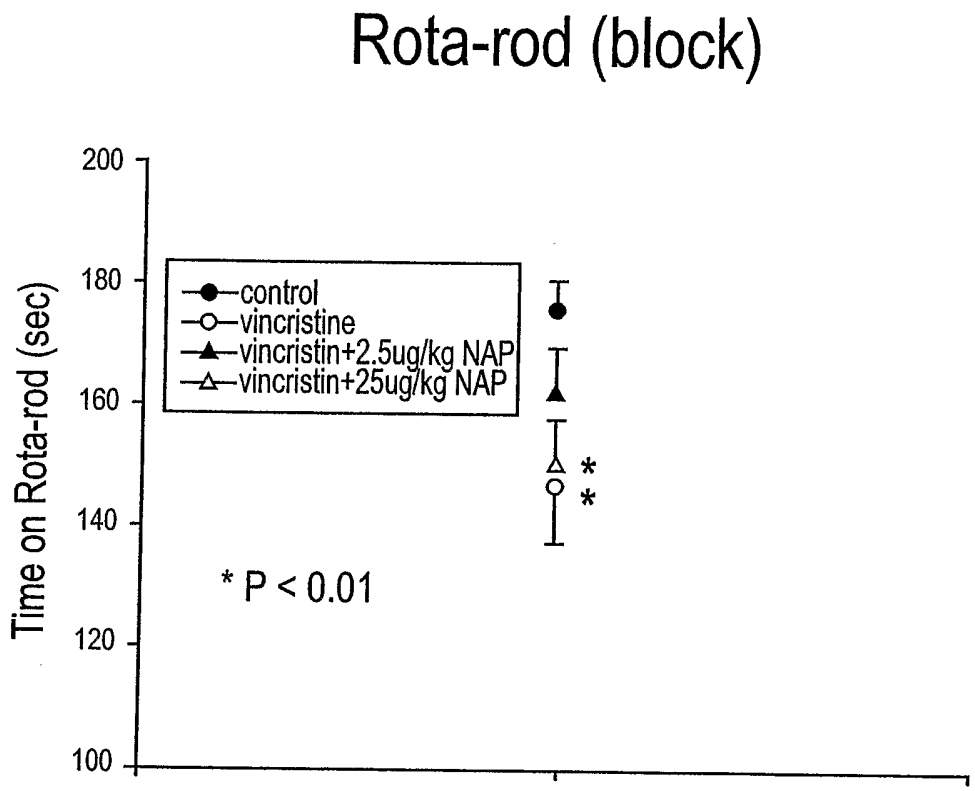
Rota-rod (block)
Time on Rota-rod (sec)
200
180
160
140
120
100
control
vincristine
vincristin+2.5ug/kg NAP
vincristin+25ug/kg NAP
* P < 0.01
*
*

FIG. 1

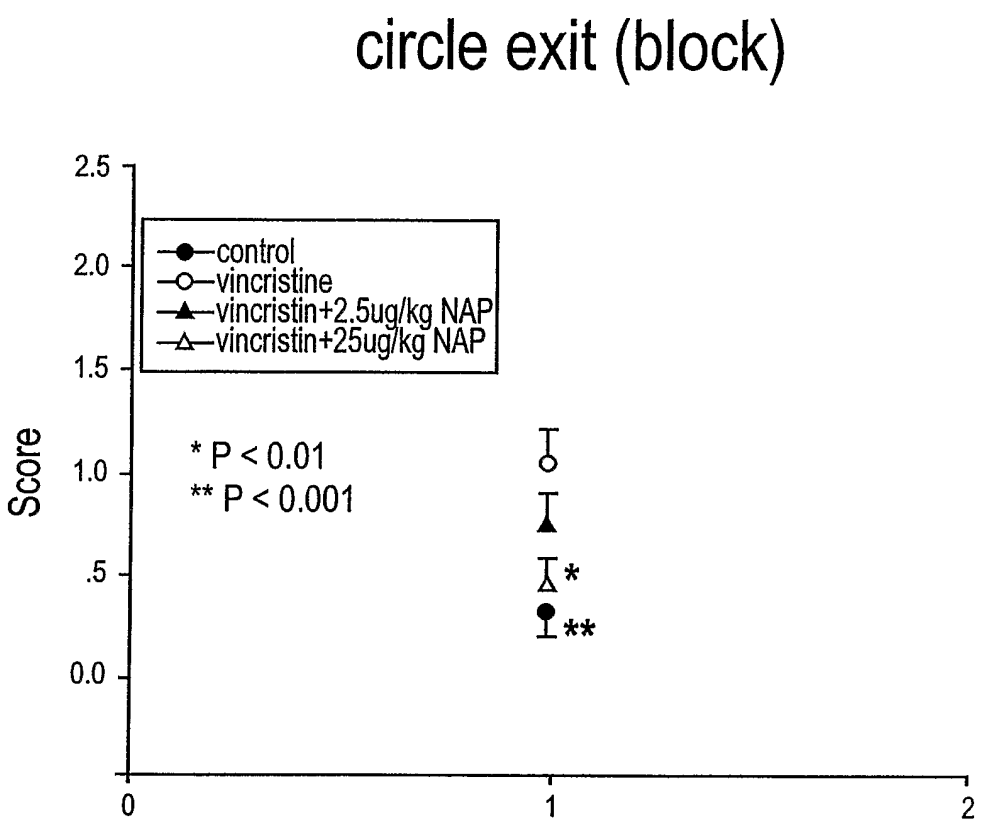
circle exit (block)
Score
2.5
2.0
1.5
1.0
.5
0.0
0
1
2
control
vincristine
vincristin+2.5ug/kg NAP
vincristin+25ug/kg NAP
* P < 0.01
** P < 0.001
*
**

FIG. 2

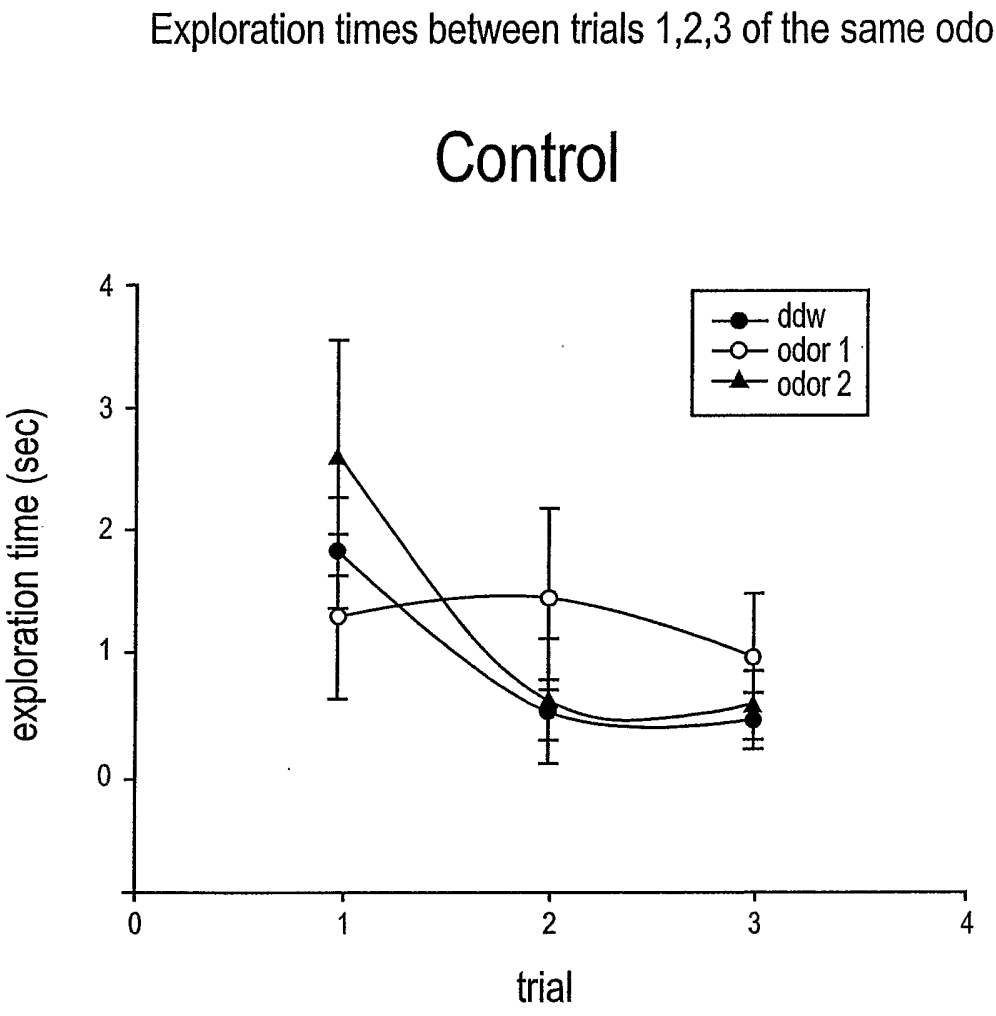
Exploration times between trials 1,2,3 of the same odor
Control
exploration time (sec)
trial
ddw
odor 1
odor 2
0
1
2
3
4

FIG. 3

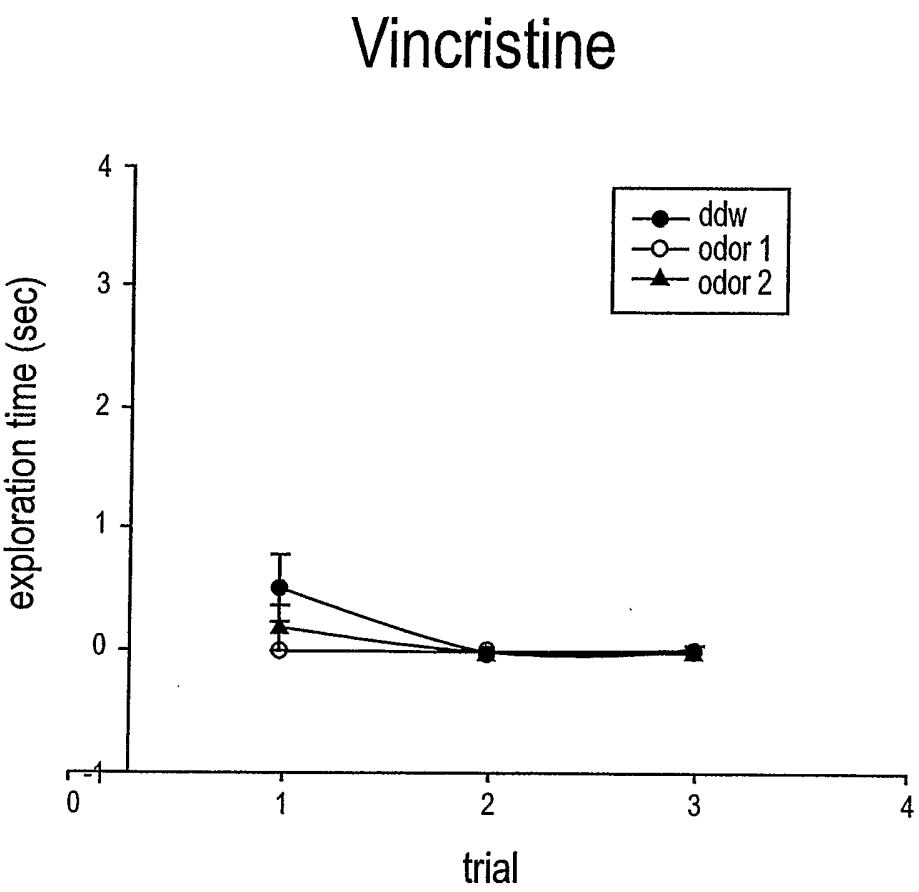
Vincristine
ddw
odor 1
odor 2
exploration time (sec)
4
3
2
1
0
-1
0
1
2
3
4
trial

FIG. 4

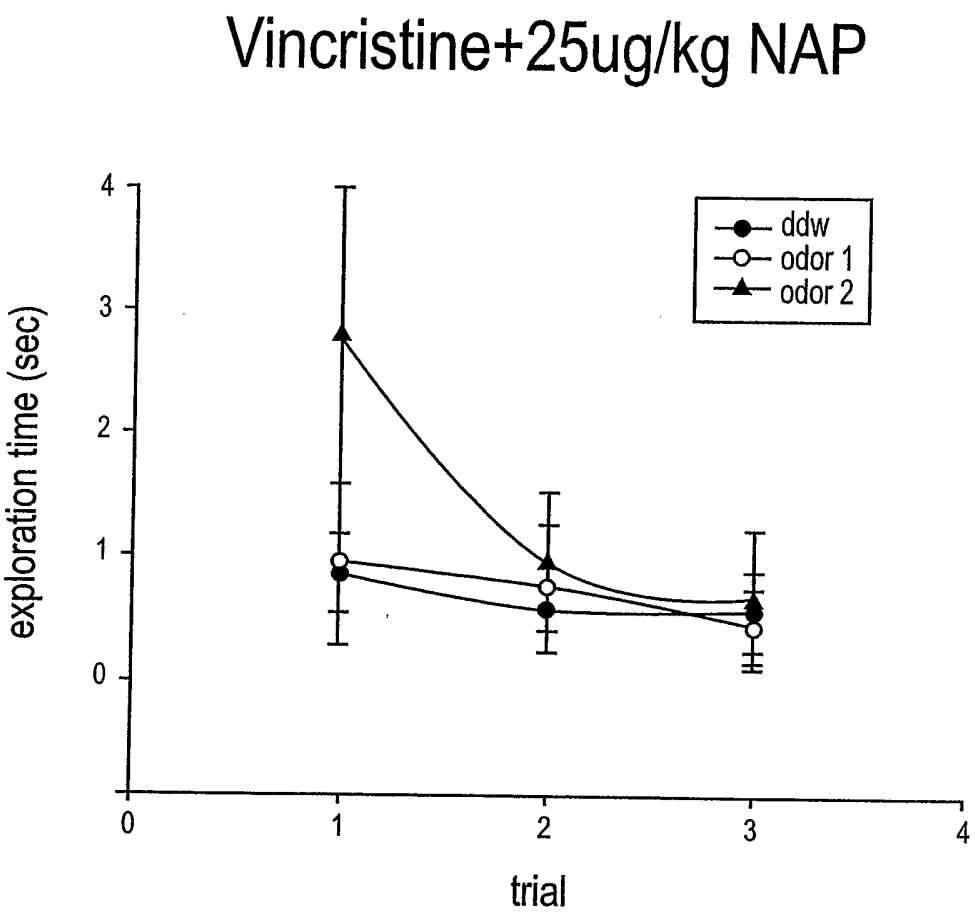
Vincristine+25ug/kg NAP
exploration time (sec)
trial
ddw
odor 1
odor 2
0
1
2
3
4

FIG. 5

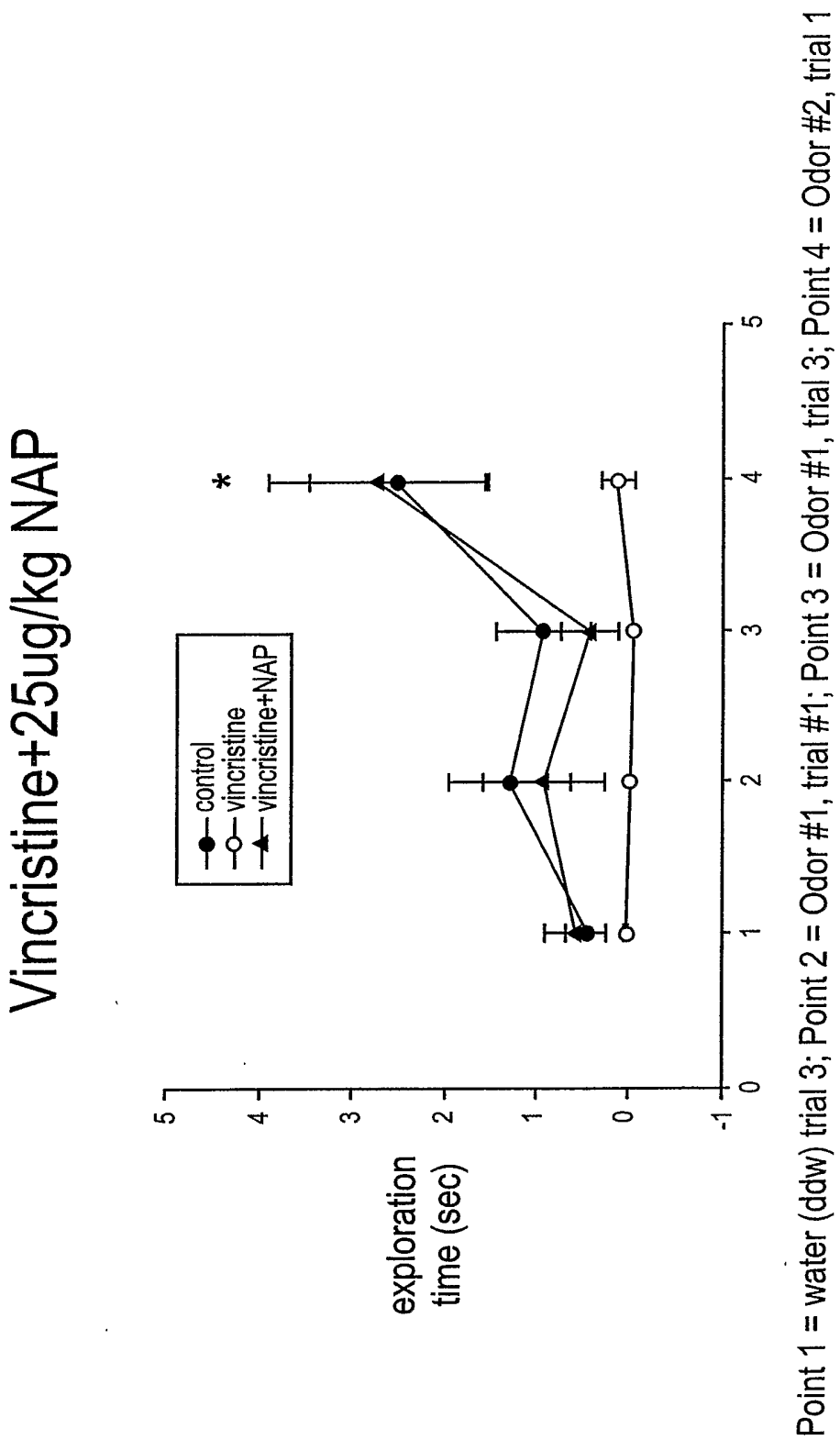
Vincristine+25ug/kg NAP
control
vincristine
vincristine+NAP
*
exploration time (sec)
5
4
3
2
1
0
-1
0
1
2
3
4
5
Point 1 = water (ddw) trial 3; Point 2 = Odor #1, trial #1; Point 3 = Odor #1, trial 3; Point 4 = Odor #2, trial 1
* P<0.05, control and vincristine + NAP as compared to vincristine alone

FIG. 6

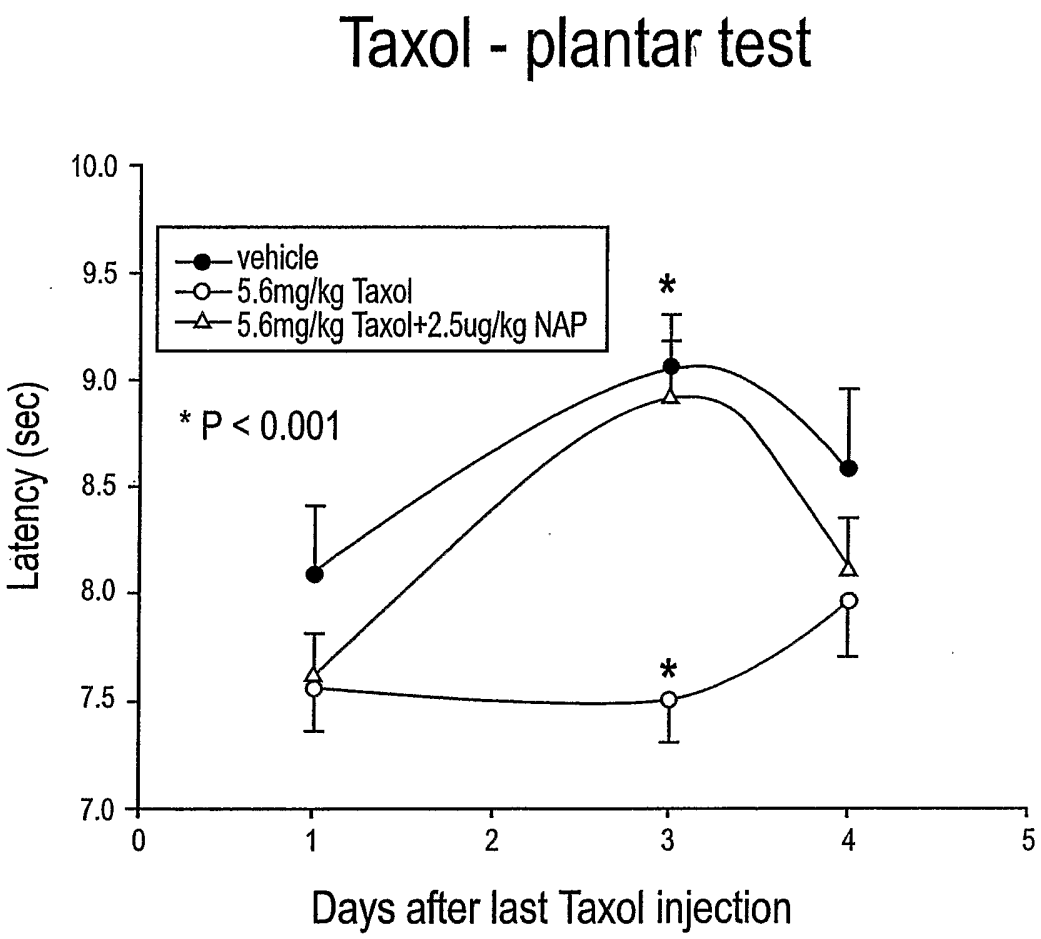
Taxol - plantar test
vehicle
5.6mg/kg Taxol
5.6mg/kg Taxol+2.5ug/kg NAP
* P < 0.001
Latency (sec)
10.0
9.5
9.0
8.5
8.0
7.5
7.0
0
1
2
3
4
5
Days after last Taxol injection

FIG. 7

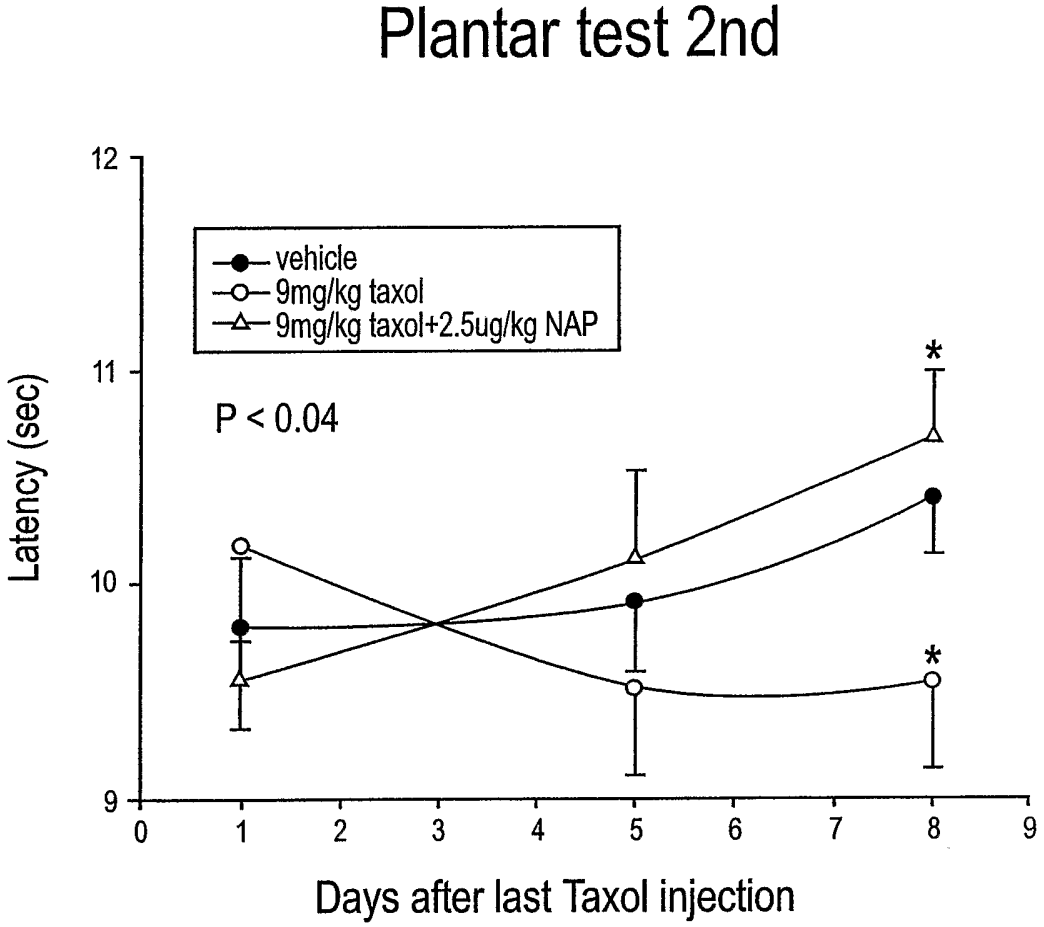
Plantar test 2nd
vehicle
9mg/kg taxol
9mg/kg taxol+2.5ug/kg NAP
P < 0.04
Latency (sec)
Days after last Taxol injection
12
11
10
9
0
1
2
3
4
5
6
7
8
9
*
*

FIG. 8

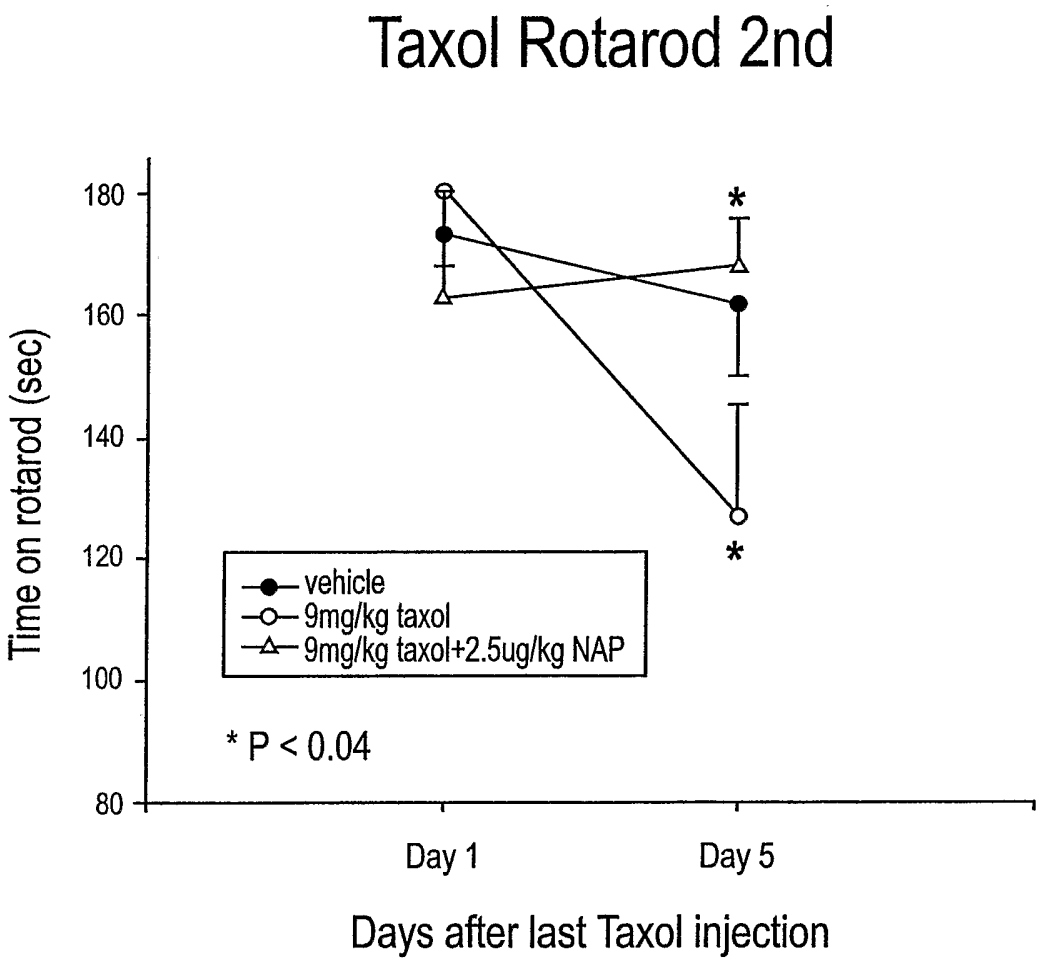
Taxol Rotarod 2nd
Time on rotarod (sec)
180
160
140
120
100
80
vehicle
9mg/kg taxol
9mg/kg taxol+2.5ug/kg NAP
* P < 0.04
*
*
Day 1
Day 5
Days after last Taxol injection

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2006/000438

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 38/18*** (2006.01) , ***A61P 35/00*** (2006.01) , ***A61P 25/28*** (2006.01) , ***A61K 45/08*** (2006.01) , ***C12Q 1/00*** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: ***A61K 38/18*** (2006.01) , ***A61P 35/00*** (2006.01) , ***A61P 25/28*** (2006.01) , ***A61K 45/08*** (2006.01) , ***C12Q 1/00*** (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Databases: MEDLINE, CAPLUS, BIOSIS, DELPHION, CANADIAN PATENT DATABASE
Keywords: activity-dependent neurotrophic factor (ADNF), activity-dependent neuroprotective protein (ADNP), SAL, NAP, peripheral neurotoxicity, chemotherapeutic agent, vinca, vincristine, taxol, neuroprotection

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 0112654 A2 RAMOT UNIVERSITY [IL/IL] and GOVERNMENT OF THE UNITED STATES [US/US] 22 February 2001 Abstract pages 3, 4, 21, 24-26, 29-32, 37-40 and 46-48 Figures 1-3 claims 42-69	1-22 and 25
X	WO 0027875 A2 GOVERNMENT OF THE UNITED STATES [US/US] and RAMOT UNIVERSITY [IL/IL] 18 May 2000 Abstract pages 3, 5, 7, 8, 35, 36 and 57-66 Figures 6 and 7	1, 2, 9-14, 20, 21, 22 and 25

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 July 2006 (11-07-2006)	12 July 2006 (12-07-2006)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476	Raffaele Salvino (819) 997-3031

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 20030166544 A1 ALCON RESEARCH LTD. 4 September 2003 whole document, especially paragraphs 15, 16, 18, 19, 23 and claims 1-5	1-22
X	LAGREZE WA et al. "The peptides ADNF-9 and NAP increase survival and neurite outgrowth of rat retinal ganglion cells in vitro'. Invest. Ophthamol. Vis. Sci., March 2005, Vol. 46, No. 3, pp. 933-938 ISSN 0146-0404 Abstract page 933, right column, last paragraph to page 934, left column, first full paragraph page 936, right column, second full paragraph Figures 2, 5 and 6	1-22
X	CHIBA T et al., " Neuroprotective effect of activity-dependent neurotrophic factor against toxicity from familial amyotrophic lateral sclerosis-linked mutant SOD1 *in vitro* and *in vivo*". J. Neurosci. Res., November 2004, Vol. 78, No. 4, pp. 542-552, ISSN 0360-4012 pages 544-545 and 548-550 Figures 1, 2 and 6	1-8, 21 and 22

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2006/000438

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO0112654 A2	22-02-2001	AT266044T T	15-05-2004
		AU761597 B2	05-06-2003
		AU6920100 A	13-03-2001
		CA2381981 A1	22-02-2001
		DE60010478D D1	09-06-2004
		DE60010478T T2	09-06-2005
		EP1206489 A2	22-05-2002
		EP1206489 B1	06-05-2004
		ES2220522T T3	16-12-2004
		WO0112654 A3	24-01-2002
WO0027875 A2	18-05-2000	AU737406 B2	16-08-2001
		AU776833 B2	23-09-2004
		AU1469800 A	29-05-2000
		AU6322298 A	26-08-1998
		CA2279231 A1	13-08-1998
		CA2349159 A1	18-05-2000
		EP0966533 A1	29-12-1999
		EP1124960 A2	22-08-2001
		JP2001522228T T	13-11-2001
		US6613740 B1	02-09-2003
		US2004053313 A1	18-03-2004
		WO0027875 A3	27-07-2000
		WO9835042 A2	13-08-1998
US2003166544 A1	04-09-2003	none	

Continuation of Box No. II

The scope of products defined by the "ADNF" polypeptide of claim 1 as evidenced by the description of the present application and in particular, claims dependent on claim 1 (e.g. claims 5 and 10) encompass an infinite number of products. The application provides support within the meaning of Article 6 and disclosure within the meaning of Article 5 for only a limited number of such products. In the present case, the claims so lack clarity and support and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been established for the parts of the application which appear to be clear and supported, namely full length ADNF I and ADNF III and the subfragments therewith comprising the ADNF core sequences set forth in SEQ ID Nos: 1 and 2 respectively which represent old and known peptides designated SAL and NAP in the art. It should be made clear that the specific peptide embodiments defined by SEQ ID Nos: 3-12 (i.e. claims 7 and 13) were not specifically searched. These peptide embodiments merely represent minor variations of the SAL and NAP peptides (i.e. addition of a few amino acids at the N- and/or C-terminal ends of the core sequences) which were not tested in the present application.

Further, the present application lacks a clear and unambiguous meaning for the term "analogue", found in claims 1, 5, 10 and 15, in contravention of Article 6 PCT. Thus, the scope of subject matter encompassed by said term is unclear and not searchable.

Continuation of Box No. III

This International Searching Authority found multiple inventions (groups) in this international application as follows:

Group 1. claim 3 (fully); claims 1, 2, 4-8 and 10-25 (partially)

A method for treating peripheral neurotoxicity in a subject comprising administering an ADNF I polypeptide.

Group 2. claim 9 (fully); claims 1, 2, 4-8 and 10-25 (partially)

A method for treating peripheral neurotoxicity in a subject comprising administering an ADNF III polypeptide.

Group 3. claims 1, 2, 4-8 and 10-25 (partially)

A method for treating peripheral neurotoxicity in a subject comprising administering a mixture of an ADNF I polypeptide and an ADNF III polypeptide.

Group 4. claims 26-28

A method for the treatment of cancer or neoplasia comprising administering an anti-cancer agent and an ADNF polypeptide contemporaneously or sequentially in a pharmaceutically acceptable carrier.

Group 5. claims 29-31

A method of testing for response to an ADNF polypeptide for a neurodegenerative disorder or peripheral neurotoxicity comprising comparing the olfaction capacity in a subject before and after administration of the ADNF polypeptide.

Group 6. claims 32-33

A method of treating of tauopathy in a subject comprising administration of an ADNF polypeptide.

(continued on next page)

Continuation of Box No. III (from previous page)

The requirements of unity of invention are not fulfilled in that there is no technical relationship among the inventions as they do not involve one or more of the same or corresponding technical features. The expression "special technical features" means those features which define a contribution which each of the claimed inventions considered as a whole makes over the prior art.

ADNF I and ADNF III are *a priori*, structurally distinct old and known polypeptides. SAL and NAP (defined by SEQ ID Nos: 1 and 2 respectively), peptides derived from ADNF I and ADNF III and representing preferred embodiments of the present application, are also old and known. Utilities for ADNF I and ADNF III (and said derived peptides) are also old and known (see page 1 line 16 to page 2 line 25 of the present application), including uses involving neuroprotection against disorders of the central nervous system such as Alzheimers disease, a tauopathy. As well, SAL and/or NAP have been shown to provide neuroprotective activity on cells of the peripheral nervous system (see LAGREZE W.A. et al., ARVO Annual Meeting of the Association for Research in Vision and Ophthalmology. Fort Lauderdale, Fl., USA. May 4, 2003, Abstract No. 5229 or CLARK A.F. et al., US2003166544, September 4, 2003). As such, because of a priori and a posteriori considerations, the inventions defined by the subject matter of Groups 1 to 6 cannot be viewed as being unified by a single inventive concept.

This Authority would add the following:

The above groupings and the claims assigned therewith are those that were sent to the applicant by way of a PCT/ISA/206 form. Upon subsequent review of the contents of said form, this Authority has found errors in the claims assigned to Groups 1, 2 and 3. The correct claim assignment should be claims 3 and 5-8 (fully) for Group 1, claims 9-14 (fully) for Group 2 and claims 15-19 (fully) for Group 3. Further, claims 1, 2, 4 and 20-25 are all partially encompassed in Groups 1, 2 and 3 respectively. To avoid confusion and out of courtesy to the Applicant, this Authority has done a search directed at the subject matter of Groups 1, 2 and 3 as originally put forth in the PCT/ISA/206 form, specifically a method for treating peripheral neurotoxity in a subject comprising an ADNF 1 polypeptide, an ADNF III polypeptide or a mixture thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2006/000438

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 1-20 and 22-25
because they relate to subject matter not required to be searched by this Authority, namely :

Claims 1-20 and 22-25, directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search under Rule 39.1(iv) of the PCT. Regardless, this Authority has carried out a search based on the alleged effects of the product defined in claims 1-20 and 22-25.

2. [X] Claim Nos. : 1-25
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

See extra sheet (Continuation of Box No. II)

3. [ ] Claim Nos. :
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

See extra sheet (Continuation of Box No. III)

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

claims 1-25; see explanation on extra sheet (Continuation of Box No. III)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.