

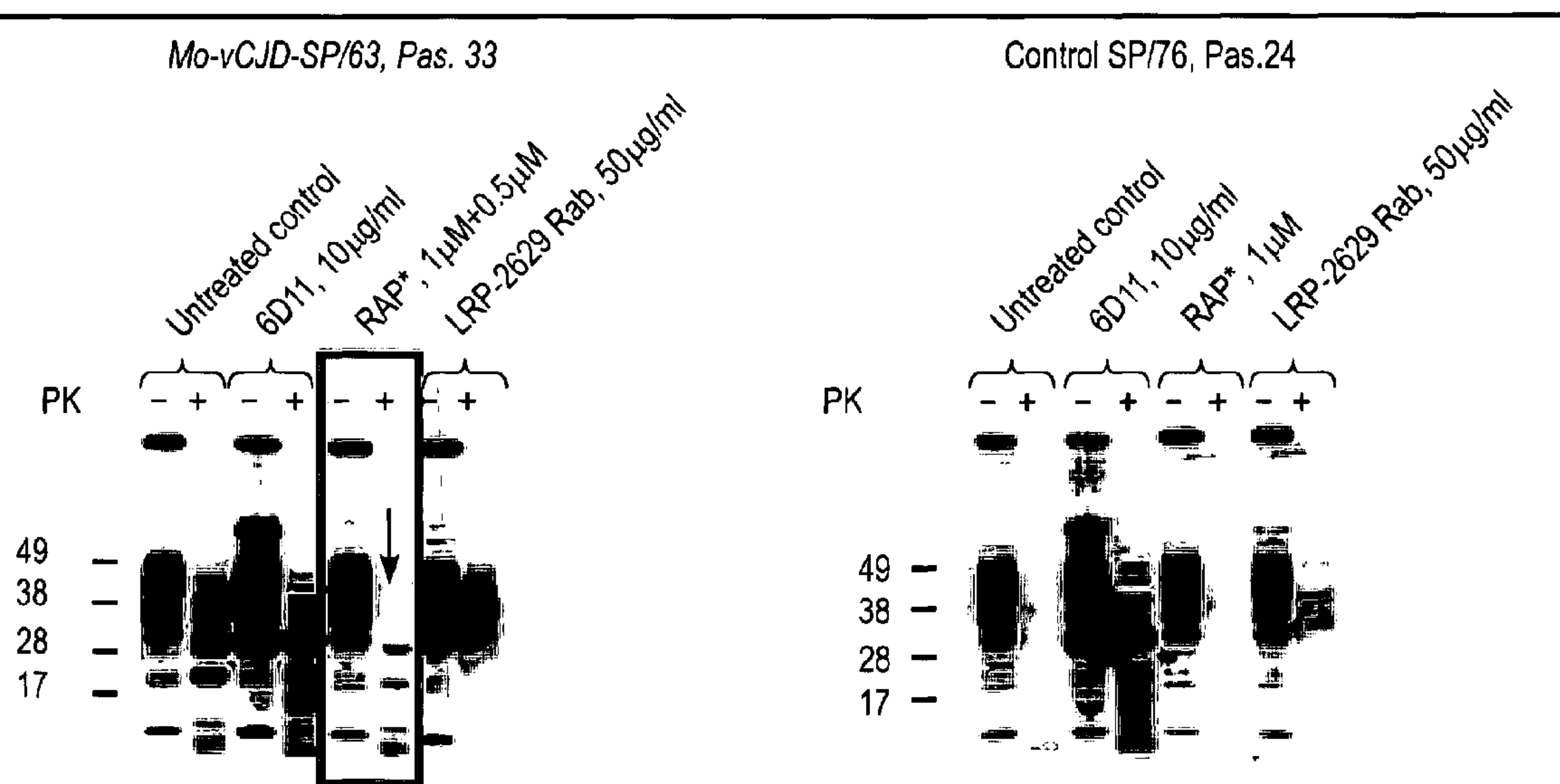


(86) Date de dépôt PCT/PCT Filing Date: 2010/05/17
(87) Date publication PCT/PCT Publication Date: 2010/11/25
(85) Entrée phase nationale/National Entry: 2011/09/19
(86) N° demande PCT/PCT Application No.: US 2010/001455
(87) N° publication PCT/PCT Publication No.: 2010/134970
(30) Priorités/Priorities: 2009/05/21 (US61/180,406);
2009/06/19 (US61/218,752)

(51) Cl.Int./Int.Cl. *A61K 38/00* (2006.01),
C07K 14/00 (2006.01)
(71) Demandeur/Applicant:
AMERICAN NATIONAL RED CROSS, US
(72) Inventeurs/Inventors:
CERVENAKOVA, LARISA, US;
YAKOVLEVA, OKSANA, US
(74) Agent: TORYS LLP

(54) Titre : INHIBITION DE PROPAGATION DE PROTEINES PRION PAR LA PROTEINE ASSOCIEE AU RECEPTEUR,
SES DERIVES, MIMETIQUES ET PEPTIDES SYNTHETIQUES
(54) Title: INHIBITION OF PRION PROTEIN PROPAGATION BY RECEPTOR ASSOCIATED PROTEIN (RAP), ITS
DERIVATIVES, MIMETICS AND SYNTHETIC PEPTIDES

FIG. 1 Effect of RAP on PrPres formation in spleen-derived mo-vCJD-
SP/63 cell line: 24 hours after the treatment



- Control SP/76 and mo-vCJD-SP/63 cells were treated with RAP twice initially with 1µM and in 8 hours with 0.5 µM.
- Proteins were concentrated by Methanol precipitation.
- (-) and (+) Proteinase K (PK)- treated samples represent total PrP and pathologic PrPres, respectively.
- Anti-PrP monoclonal antibody 6D11 diluted 1:10,000.
- Inhibition of PrPres formation by RAP is shown by arrows.

(57) **Abrégé/Abstract:**

A pharmaceutical formulation and method of treatment of prion disease include a RAP agent with a pharmaceutically acceptable carrier and/or excipient, and the administration of same to a subject suffering from or at risk of a prion disease. The RAP agent is an effective means for the prevention and/or treatment of various prion diseases regardless whether the disease is acquired by infection or by genetic mutation.



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
25 November 2010 (25.11.2010)(10) International Publication Number
WO 2010/134970 A1

(51) International Patent Classification:

A61K 38/00 (2006.01) **C07K 14/00** (2006.01)

(21) International Application Number:

PCT/US2010/001455

(22) International Filing Date:

17 May 2010 (17.05.2010)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/180,406	21 May 2009 (21.05.2009)	US
61/218,752	19 June 2009 (19.06.2009)	US

(71) Applicant (for all designated States except US): **AMERICAN NATIONAL RED CROSS** [US/US]; 2025 E. Street, NW, Washington, DC 20006 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **CERVENAKOVA, Larisa** [US/US]; 310 Fallsgrave Drive, Rockville, MD 20850 (US). **YAKOVLEVA, Oksana** [UA/US]; 15933 Yukon Lane, Derwood, MD 20855 (US).(74) Agent: **O'SHAUGHNESSY, Brian, P.**; Buchanan, Ingersoll & Rooney PC, Post Office Box 1404, Alexandria, VA 22313-1404 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

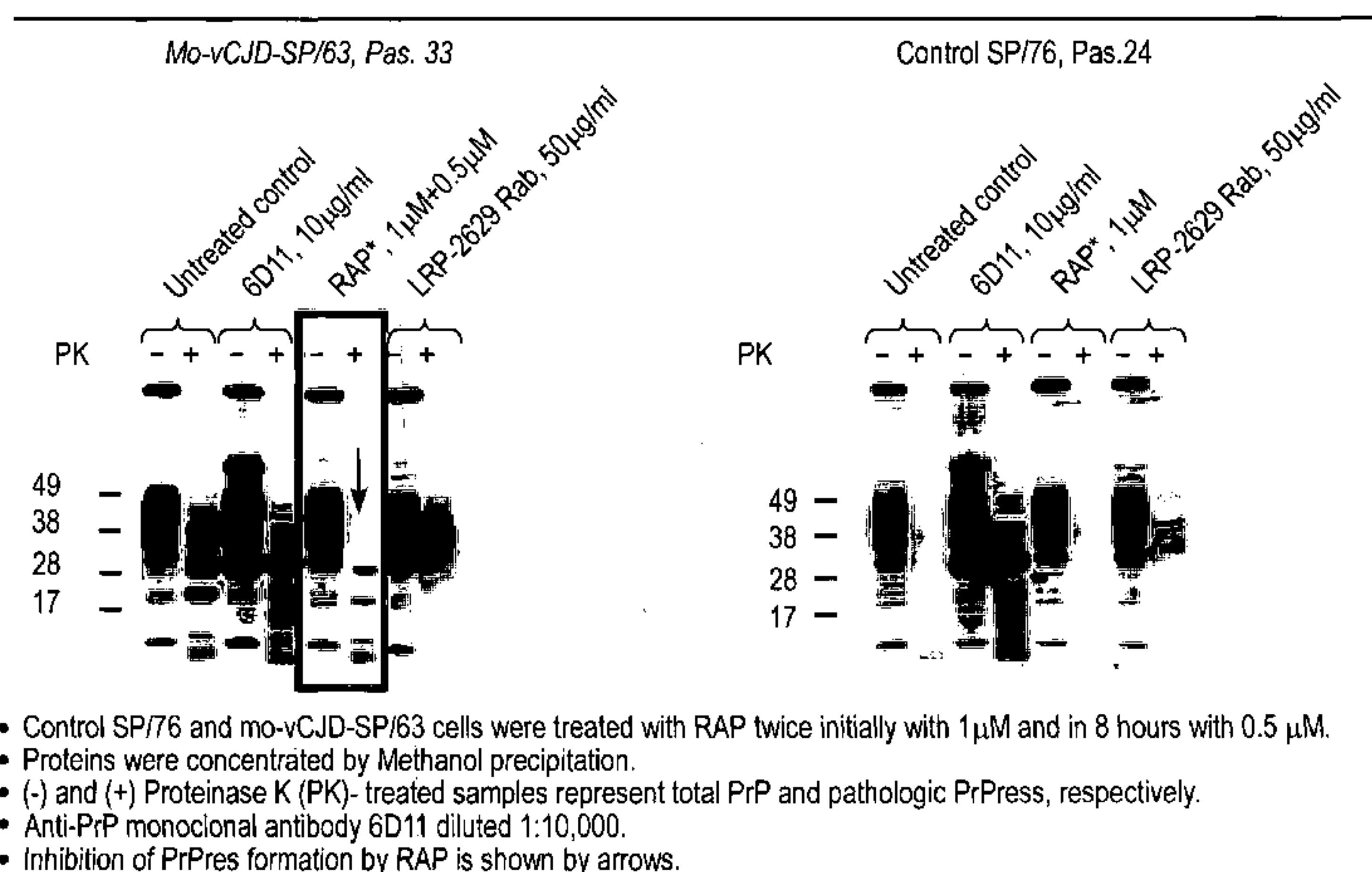
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: INHIBITION OF PRION PROTEIN PROPAGATION BY RECEPTOR ASSOCIATED PROTEIN (RAP). ITS DERIVATIVES, MIMETICS AND SYNTETIC PEPTIDES

FIG. 1 Effect of RAP on PrPres formation in spleen-derived mo-vCJD-SP/63 cell line: 24 hours after the treatment



(57) Abstract: A pharmaceutical formulation and method of treatment of prion disease include a RAP agent with a pharmaceutically acceptable carrier and/or excipient, and the administration of same to a subject suffering from or at risk of a prion disease. The RAP agent is an effective means for the prevention and/or treatment of various prion diseases regardless whether the disease is acquired by infection or by genetic mutation.

INHIBITION OF PRION PROTEIN PROPAGATION BY RECEPTOR ASSOCIATED PROTEIN (RAP), ITS DERIVATIVES, MIMETICS AND SYNTHETIC PEPTIDES

BACKGROUND

[0001] Prion diseases, otherwise known as Transmissible Spongiform Encephalopathies (TSEs), are a family of progressive neurodegenerative disorders that affect both humans and animals. They are distinguished by long incubation periods, characteristic spongiform changes associated with neuronal loss, and a failure to induce classic immune response. The causative agent of TSEs is believed to be a prion. A prion is a transmissible agent mostly consisting of a conformationally changed prion protein that is able to induce abnormal folding of normal cellular prion protein. Prion replication in the brain leads to brain damage and the characteristic signs and symptoms of the disease. Human prion diseases are rare, usually rapidly progressive and fatal; no preclinical diagnostic test or treatment is currently available.

[0002] The normal cellular prion protein is found in various organs and tissues throughout the body, including the brain, in healthy people and animals. However, prion protein found in the brains of disease-affected people or animals has a different "mis-folded" structure and is partially resistant to proteases. The normal cellular form of the prion protein is generally called PrP^C (the "c" refers to "cellular"). The infectious form is variously called PrP^{Sc} (the "Sc" is from "scrapie"); PrP^{Sc, TSE, CJD, GSS, FFI, BSE, CWD, etc} (the Sc, TSE, CJD, GSS, FFI, and CWD refer to the abnormal protein of a TSE disease, and more specifically to scrapie, various forms of Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease, fatal familial insomnia, bovine spongiform encephalopathy, chronic wasting disease, etc.); and more generally PrP^d (the "d" refers to "disease-associated" prion protein).

[0003] Human PrP^C is a 253-amino acid protein produced from a single-copy gene located on chromosome 20 (LIAO et al. "Human prion protein cDNA: molecular cloning, chromosomal mapping, and biological implications," *Science*, 1986, Vol. 233, pp. 364-367) which undergoes various posttranslational modifications that include formation of a disulphide bond (STAHL et al., "Prions and prion proteins," *FASEB J.*, 1991, Vol. 5, pp. 2799-2807), glycosylation (RUDD et al., "Prion glycoprotein: structure, dynamics, and roles for the sugars," *Biochemistry*, 2001, Vol. 40, pp. 3759-66), removal of 22 amino acids from C-terminus and addition of glycoposphatidylinositol(GPI) moiety (STAHL et al., "; *Cell*,

1987, Vol. 51, pp. 229-40). The normal cellular protein is attached to the plasma membrane through a GPI anchor and has a predominantly alpha helical structure. The fully glycosylated protein has a molecular weight of 38 kDa, is monomeric in structure and sensitive to proteolysis (CAUGHEY et al., "Prions and their partners in crime," *Nature*, 2006, Vol. 443, pp. 803-10, Review). PrP^C is known to interact with various proteins, including heat-shock proteins (EDENHOFER et al., "Prion protein PrP^C interacts with molecular chaperones of the Hsp60 family," *J Virol.*, 1996, Vol. 70, pp. 4724-8), a 37 kDa/67 kDa laminin receptor (RIEGER et al., "The human 37-kDa laminin receptor precursor interacts with the prion protein in eukaryotic cells," *Nat Med.*, 1997, Vol. 3, pp. 1383-8; GAUCZYNSKI et al., "The 37-kDa/67-kDa laminin receptor acts as the cell-surface receptor for the cellular prion protein," *EMBO J.*, 2001, Vol. 20, pp. 5863-75), stress-inducible protein-1 of 66 kDa predominantly present in cytoplasm (ZANATA et al., "Stress-inducible protein 1 is a cell surface ligand for cellular prion that triggers neuroprotection," *EMBO J.*, 2002, Vol. 21, pp. 3307-16), plasminogen (ELLIS et al., "Plasminogen activation is stimulated by prion protein and regulated in a copper-dependent manner," *Biochemistry*, 2002, Vol. 41, pp. 6891-6; PRAUS et al., "Stimulation of plasminogen activation by recombinant cellular prion protein is conserved in the NH2-terminal fragment PrP23-110," *Thromb Haemost.*, 2003, Vol. 89, pp. 812-9; KORNBLATT et al., "The fate of the prion protein in the prion/plasminogen complex," *Biochem Biophys Res Commun.*, 2003, Vol. 305, pp. 518-22), a neuronal cell adhesion molecule (NCAM) (SANTUCCIONE et al., "Prion protein recruits its neuronal receptor NCAM to lipid rafts to activate p59fyn and to enhance neurite outgrowth," *J Cell Biol.*, 2005, Vol. 169, pp. 341-54), heparan sulphate proteoglycans (HPSG) (HORONCHIK et al., "Heparan sulfate is a cellular receptor for purified infectious prions," *J Biol Chem.*, 2005, Vol. 280, p.17062, Epub., 2005 Jan 24), the low-density lipoprotein receptor-related protein (LRP1) (TAYLOR et al., "Role of lipid rafts in the processing of the pathogenic prion and Alzheimer's amyloid-beta proteins," *Semin Cell Dev Biol.*, 2007, Vol. 18, pp. 638-48, Epub., 2007 Jul 24, Review; PARKYN et al., "LRP1 controls biosynthetic and endocytic trafficking of neuronal prion protein," *J Cell Sci.*, 2008, Vol. 121(Pt 6), pp. 773-83, Epub., 2008 Feb 19; Cervenakova et al., unpublished data, 2004), other LDL receptor superfamily members, megalin receptor and VLDLR (Cervenakova et al., unpublished data, 2004). It has been suggested that PrP^C could function as a part of the LRP1 scavenger complex, because its N-terminal domain has multiple binding motifs (CAUGHEY et al., "Prions and transmissible spongiform encephalopathy (TSE) chemotherapeutics: A common mechanism for anti-TSE

compounds?" *Acc Chem Res.*, 2006, Vol. 39, pp. 646-53) and its hydrophobic sequence (amino acids 112-130) exposed to an aqueous environment could bind to denatured proteins as PrP^C rapidly traffics across the neuronal surface. Recently, it has been shown that LRP1 binds to and is involved in both the biosynthetic and the endocytic trafficking of neuronal PrP^C (PARKYN et al., "LRP1 controls biosynthetic and endocytic trafficking of neuronal prion protein," *J Cell Sci.*, 2008, Vol. 121(Pt 6), pp. 773-83, Epub., 2008 Feb 19). The function of the normal PrP^C is not known, but there is evidence that it may function as a copper-dependent antioxidant, a signaling molecule, an anti- and pro-apoptotic molecule, as a proteinsupporting neuronal morphology and adhesion, and it may play a role in maintenance of long-term memory (ZOMOSA-SIGNORET et al., Physiological role of the cellular prion protein, *Vet Res.* 2008, Vol. 39:9. Epub., 2007 Nov 27. Review). It has been recently proposed that PrP^C is a marker of long-term bone marrow hematopoietic stem cells and supports their self-renewal (ZHANG et al., "Prion protein is expressed on long-term repopulating hematopoietic stem cells and is important for their self-renewal," *Proc Natl Acad Sci, U S A*, 2006, Vol. 103, pp. 2184-9, Epub., 2006 Feb 7).

[0004] No differences in the primary structure (i.e. amino acid sequence) of PrP^C and PrP^d have been detected, nor have any differences been found between PrP genes or mRNAs from normal and infected brains with respect to structure or copy number. The physical differences (such as three-dimensional configuration) between the two proteins are therefore attributed to post-translational chemical modification. However, familial prion disease can occur in families with a mutation in the PrP gene, and mice with PrP mutations develop prion disease despite controlled conditions where transmission is prevented (HSIAO et al., "Spontaneous neurodegeneration in transgenic mice with prion protein codon 101 proline----leucine substitution," *Ann N Y Acad Sci.*, 1991, Vol. 640, pp. 166-70). Many different mutations have been identified and it is hypothesized that the mutations somehow make PrP^C more likely to change spontaneously into the abnormal PrP^d form.

[0005] PrP^d is able to convert normal PrP^C proteins into the infectious isoform by changing their conformation, or shape; this, in turn, alters the way the proteins interconnect. Data from animal transmission studies has pointed to the existence of an unidentified factor, termed "protein X," which may control the conversion process (TELLING et al., "Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein," *Cell*, 1995, Vol. 83, pp. 79-90). Although the exact 3D structure of PrP^d is not known, during the refolding of PrP^C into PrP^d, some of the normal α -

helical protein structure is partially converted into β -sheet. Aggregations of these abnormal isoforms form highly structured amyloid fibers, which accumulate to form plaques consisting of tightly packed β -sheets. Unlike PrP^C, this altered structure is extremely stable and accumulates in infected tissue. This stability means that prions are largely resistant to denaturation by chemical and physical agents, making disposal and containment of the particles difficult. The term "PrP^{res}" (the "res" is from "resistant") is generally used to refer to the resistant proteolytic cleavage product of PrP^d after treatment with Proteinase K.

[0006] Prions cause neurodegenerative disease by damaging neurons within the central nervous system and disrupting the normal tissue structure. While the incubation period for prion diseases is generally quite long, once symptoms appear the disease progresses rapidly, leading to brain damage and death. All known prion diseases are currently untreatable and fatal. Many different mammalian species can be affected by prion diseases. Due to the minor differences in PrP between different species, it is not unusual for a prion disease to be transmitted from one species to another. However, species to species transmission can only occur under certain conditions, and mechanisms of the transmission are not fully understood. The most recent example of such transmission is variant Creutzfeldt-Jakob disease (vCJD) affecting humans, which is believed to be caused by a prion which typically infects cattle, causing bovine spongiform encephalopathy (BSE), that was transmitted through contaminated bovine-derived food products (WILL et al., "A new variant of Creutzfeldt-Jakob disease in the UK," *Lancet*, 1996, Vol. 347, pp. 921-5).

[0007] The primary route of natural TSE infection, for example scrapie in sheep and goats, BSE in cattle and sheep, CWD in deer and elk, and vCJD in humans, is thought to be through ingestion of contaminated sources. Prions may be deposited in the environment through the remains of dead animals and via urine, saliva, and other body fluids (e.g. in the case of CWD) (HALEY et al., "Detection of CWD prions in urine and saliva of deer by transgenic mouse bioassay," *PLoS ONE*, 2009, Vol. 4, p. 4848, Epub., 2009 Mar 18). They may then linger in the soil by binding to clay and other minerals (SAUNDERS et al., Prions in the environment: occurrence, fate and mitigation. *Prion*. 2008 Vol. 2:162-9. Epub 2008 Oct 26. Review.). Other methods of infection are also known.

[0008] Low density lipoprotein receptor-related protein associated protein 1, also known as LRPAP1 or Receptor-Associated Protein (RAP), is encoded in humans by the *LRPAP1* gene (STRICKLAND et al., Primary structure of alpha 2-macroglobulin receptor-associated protein. Human homologue of a Heymann nephritis antigen". *J. Biol. Chem.*, 1991

Vol. 266, pp. 13364–9. KORENBERG et al., "Chromosomal localization of human genes for the LDL receptor family member glycoprotein 330 (LRP2) and its associated protein RAP (LRPAP1)". *Genomics* 1994, Vol. 22, pp. 88–93. The protein was first isolated from mice as a 44-kD heparin-binding protein and was initially termed HBP-44 (FURUKAWA et al., "A heparin binding protein whose expression increases during differentiation of embryonal carcinoma cells to parietal endoderm cells: cDNA cloning and sequence analysis," *J. Biochem.*, 1990, Vol. 108, No. 2, pp. 297–302). In humans a 39-kD associated protein was purified as a part of the alpha-2-macroglobulin receptor complex (ASHCOM et al., "The human alpha 2-macroglobulin receptor: identification of a 420-kD cell surface glycoprotein specific for the activated conformation of alpha 2-macroglobulin," *J. Cell. Biol.*, 1990, Vol. 110, pp. 1041-8; STRICKLAND et al., "Primary structure of alpha 2-macroglobulin receptor-associated protein. Human homologue of a Heymann nephritis antigen," *J Biol Chem.*, 1991, Vol. 266, pp. 13364–9. The primary structure of the 39-kD polypeptide, termed alpha-2-macroglobulin receptor-associated protein (α_2 MRAP), was determined by cDNA cloning (STRICKLAND et al., "Primary structure of alpha 2-macroglobulin receptor-associated protein. Human homologue of a Heymann nephritis antigen," *J Biol Chem.*, 1991, Vol. 266, pp. 13364–9). Functional studies revealed that RAP blocked ligand binding by LRP1 (HERZ et al., "39-kDa protein modulates binding of ligands to low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor," *J. Biol. Chem.*, 1991, Vol. 266, pp. 21232-8; WILLIAMS et al., "A novel mechanism for controlling the activity of alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein. Multiple regulatory sites for 39-kDa receptor-associated protein," *Biol Chem.*, 1992, Vol. 267, pp. 9035-40). The deduced amino acid sequence of human RAP contains a putative signal sequence that precedes the 323-residue mature protein. The sequence showed 73% identity with a rat protein and 77% identity to a 44-kD mouse HBP-44. There are also similarities between RAP and apolipoprotein E. RAP is localized in the rough endoplasmic reticulum where it binds to LDL-receptor related proteins functioning as a specialized chaperone assisting in the folding and intracellular transport of members of the LDL receptor family. RAP is expressed in various organs and tissues throughout the body, including the brain. Experimental evidence suggests that RAP acts as a receptor antagonist and prevents association of newly synthesized LDL-receptor related proteins with their ligands during transport to the cell surface (WILLNOW, "Receptor-associated protein (RAP): a specialized chaperone for endocytic receptors," *Biol Chem.*, 1998, Vol. 379, pp. 1025-31). RAP is efficiently transferred across

the blood-brain barrier and may provide a means of protein-based drug delivery to the brain (PAN et al., "Efficient transfer of receptor-associated protein (RAP) across the blood-brain barrier," J Cell Sci., 2004, Vol. 117(Pt 21), pp. 5071-8, Epub., 2004 Sep 21). Recently, the importance of RAP has been shown in amyloid depositions in a mouse model of Alzheimer's disease (XU et al. "Receptor-associated protein (RAP) plays a central role in modulating Abeta deposition in APP/PS 1 transgenic mice," PLoS ONE, 2008, Vol. 3, p. 3159). RAP also inhibited beta-amyloid protein (Abeta) oligomerization, neurotoxic effects of Abeta in cell cultures and blocked an Abeta-induced inhibition of long-term memory consolidation in 1-day-old chicks (KERR et al., "Inhibition of Abeta aggregation and neurotoxicity by the 39-kDa receptor-associated protein", J Neurochem. 2010 Vol. 112:1199-209. Epub 2009 Dec 10).

SUMMARY

[0009] A method for prophylaxis or treatment of a prion disease in a subject comprises administering to the subject a therapeutically effective amount of an agent selected from the group consisting of an RAP polypeptide and a derivative, variant, fragment, and mimetic thereof. The subject may be one who is at risk of developing, or is infected with, or is otherwise suffering from a prion disease.

[0010] In an exemplary embodiment, the prion disease is selected from the group consisting of various forms of Creutzfeldt-Jakob disease (CJD) such as iatrogenic Creutzfeldt-Jakob disease (iCJD), variant Creutzfeldt-Jakob disease (vCJD), familial Creutzfeldt-Jakob disease (fCJD), sporadic Creutzfeldt-Jakob disease (sCJD)); Gerstmann-Sträussler-Scheinker syndrome (GSS); Fatal insomnia familial (FFI) and sporadic; Kuru, Scrapie, Bovine spongiform encephalopathy (BSE); Transmissible mink encephalopathy (TME); Chronic wasting disease (CWD); Feline spongiform encephalopathy; and Exotic ungulate encephalopathy (EUE).

[0011] The subject can be a mammal, and the mammal can be selected from the group consisting of humans, sheep, goats, cows, mink, white-tailed deer, elk, mule deer, moose, cats, nyala, gemsbok, oryx, eland, kudu, ankole, and bison. In an exemplary embodiment, the mammal is a human.

[0012] Examples of suitable agents include polypeptide sequences comprising the following RAP sequences: amino acids 35-357 of SEQ ID NO: 1, amino acid sequences having at least 70% to 100% sequence identity to amino acids 35-357 of SEQ ID NO: 1, and

any one of SEQ ID NOS: 1-7. In an exemplary embodiment, the agent comprises amino acids 35-357 of SEQ ID NO: 1.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Fig. 1 shows the effect of RAP on PrP^{res} formation in spleen-derived mo-vCJD-SP/63 and control SP/76 cell lines 24 hours after treatment.

[0014] Fig. 2 shows the effect of 1 μ M RAP on PrP^{res} formation in spleen-derived mo-vCJD-SP/63 cell line (time-course).

[0015] Fig. 3 shows the effect of RAP on PrP^{res} formation in spleen-derived mo-vCJD-SP/63 cell line (dose-dependent inhibition).

[0016] Fig. 4 shows the effect of 500 nM RAP on PrP^{res} formation in spleen-derived mo-vCJD-SP/63 and Fu-SP/58 cell lines persistently infected with either mo-vCJD or Fukuoka.

[0017] Fig. 5 shows the effect of 500 nM RAP on PrP^{res} formation in spleen-derived mo-vCJD-SP/63 cell line (time-course).

[0018] Fig. 6 shows the inhibitory effect of 500 nM RAP on PrP^{res} formation in bone-marrow-derived cell line 336-2BMS-Fu2 persistently infected with Fukuoka.

[0019] Fig. 7 shows the inhibitory effect of multiple treatments with 250 nM RAP on PrP^{res} formation in bone marrow-derived 336-2-BM-Fu2 cell line persistently infected with Fukuoka.

[0020] Fig. 8 shows the human RAP polypeptide sequence, including the signal peptide (SEQ ID NO: 1).

[0021] Fig. 9 shows sequence alignments for RAP polypeptides from various species. In the figure, the sequences identified as 1-7 are from human (amino acids 35-357 of SEQ ID NO: 1), African clawed frog (SEQ ID NO: 2), zebrafish (SEQ ID NO: 3), orangutan (SEQ ID NO: 4), mouse (SEQ ID NO: 5), rat (SEQ ID NO: 6), and chicken (SEQ ID NO: 7), respectively.

DETAILED DESCRIPTION

[0022] We have discovered that Receptor-Associated Protein (RAP), also known as low density lipoprotein receptor-related protein associated protein 1 or LRPAP1 is a potent inhibitor of replication of various TSE agents, and particularly replication of mis-folded prion protein or PrP^d.

[0023] The phenomenon of mis-folded prion proteins is associated with a wide variety of prion diseases, including, but not limited to, those generally referred to as TSEs.

[0024] RAP and its variants, derivatives, fragments, and/or mimetics (also referred to herein as "RAP agents") are useful in the prophylaxis and/or treatment of prion diseases in various organisms, particularly mammals. Notably, RAP agents can be used to prevent, treat, or delay the onset or progression of prion disease, and can do so regardless whether the prion disease is caused by infection from another organism or by genetic mutation.

[0025] According to various embodiments, RAP and its variants, derivatives, fragments, and/or mimetics may be administered to a diseased or at risk animal by varied means and in various forms. In one embodiment, a RAP agent is administered via intranasal delivery or parenterally. The RAP agent may be compounded as a pharmaceutical formulation in aqueous media.

[0026] In one embodiment, the RAP agent is formulated for injection as an aqueous mixture that is about 2 μ M RAP. Such formulations can be administered to the subject in volumes of about 30 ml to about 3 μ l. In another embodiment, the formulation is administered to the subject at about 3 ml to about 3 μ l of a 2 μ M RAP solution, or an equivalent dose. As discussed elsewhere, the administration of such doses can be made intranasally or by parenteral injection.

[0027] As used herein, the terms "cellular prion protein," "normal prion protein" or "PrP^C" mean prion proteins in their normal (or wild-type) state and include the naturally occurring prion protein and its variants.

[0028] The terms "disease-associated prion protein," "mis-folded prion protein," and "PrP^d" mean the infectious isoform, and refer to a prion protein which has undergone a three-dimensional structural change resulting in increased β -sheet structure, decreased solubility, and/or increased proteolytic resistance when compared with its normal state (i.e., that not associated with disease). The term "PrP^{res}" indicates the proteolytic cleavage product or digestion product of PrP^d.

[0029] By "prion diseases" or "prion disorders" is meant those disorders associated

with or caused by the conversion of PrP^C into PrP^d and/or the consequent aggregation of prion proteins. The term "prion diseases" is used herein interchangeably with "TSE" (transmissible spongiform encephalopathies) or spongiform encephalopathies. Prion diseases affect humans and other mammals, including livestock.

[0030] In humans, prion diseases include Creutzfeldt-Jakob disease (CJD) and its varieties (e.g. iatrogenic Creutzfeldt-Jakob disease (iCJD), variant Creutzfeldt-Jakob disease (vCJD), familial Creutzfeldt-Jakob disease (fCJD), and sporadic Creutzfeldt-Jakob disease (sCJD)); Gerstmann-Sträussler-Scheinker syndrome (GSS); Fatal familial insomnia (FFI); sporadic fatal insomnia; and Kuru.

[0031] In animals, the diseases include Scrapie (sheep and goats), Bovine spongiform encephalopathy (BSE, known as "mad cow disease") (cattle); Transmissible mink encephalopathy (TME) (mink); Chronic wasting disease (CWD) (white-tailed deer, elk, mule deer and moose); Feline spongiform encephalopathy (cats, e.g. domestic cat, puma, cheetah, ocelot, tiger); Exotic ungulate encephalopathy (EUE) or spongiform encephalopathy of exotic ruminants (nyala, gemsbok, oryx (e.g. Arabian oryx and scimitar-horned oryx), eland, kudu (e.g. greater kudu), ankole, and bison); and (possibly) Spongiform encephalopathy of the ostrich (ostrich).

[0032] RAP refers to Receptor-Associated Protein, also known as low density lipoprotein receptor-related protein associated protein 1 or LRPAP1, regardless of the species of origin. In an exemplary embodiment, RAP is the full-length human RAP polypeptide sequence, minus the signal sequence (amino acids 35-357 of SEQ ID NO: 1; see Fig. 8 and STRICKLAND et al., "Primary structure of alpha 2-macroglobulin receptor-associated protein. Human homologue of a Heymann nephritis antigen," J Biol Chem., 1991, Vol. 266, pp. 13364-9), as used in the present examples.

[0033] As used herein, the term "RAP" includes any naturally-occurring RAP polypeptide sequence, such as a mammalian or a non-mammalian RAP polypeptide sequence. The term "RAP polypeptide" as used herein refers to full-length RAP sequences of natural origin as well as truncated forms, e.g., amino acids 35-357 of SEQ ID NO: 1, and those having at least 90% homology to a RAP sequence of natural origin.

[0034] Amino acid sequences of RAP from various mammalian and non-mammalian species have been identified (see, e.g., the sequences for human (amino acids 35-357 of SEQ ID NO: 1); African clawed frog (SEQ ID NO: 2); zebrafish (SEQ ID NO: 3); orangutan (SEQ ID NO: 4); mouse (SEQ ID NO: 5); rat (SEQ ID NO: 6); and chicken (SEQ

ID NO: 7); identified as sequences 1-7, respectively, in Fig. 9); as well as Common Chimpanzee, *Macaca mulatta*- Rhesus macaque, *Bos Taurus*- Cow, *Capra hircus* – Goat, *Ovis aries*- Sheep, *Sus scrofa*- Pig, *Canis lupus familiaris*-Dog. RAP sequences can also be found in the literature, e.g., GenBank, according to the following accession numbers: Human [GenBank Acc: NM_002337]; *Pan troglodytes*- Common Chimpanzee [GenBank Acc: XM_517082]; *Pongo abeli*-Sumatran Orangutan [GenBank Acc: NM_001131664.1]; *Macaca mulatta*- Rhesus macaque [GenBank Acc: XM_001085674]; *Bos Taurus*- Cow [GenBank Acc: NM_001080225]; *Capra hircus* – Goat [GenBank Acc: EV438413]; *Ovis aries*- Sheep [Assembled from the 4 EST clones, GenBank Acc. numbers: GO756662.1; GO772827.1; 114717509; 88624253]; *Sus scrofa*- Pig [GenBank Acc: NM_001113436]; *Canis lupus familiaris*-Dog [GenBank Acc: XM_536218]; *Mus musculus*- Mouse [GenBank Acc: NM_013587]; *Rattus norvegicus*- Rat [GenBank Acc: NM_001169113]; *Gallus gallus*- Chicken [GenBank Acc: NM_205062]; *Danio rerio*- Zebrafish [GenBank Acc: NM_201306]; *Xenopus laevis*- African clawed frog [GenBank Acc: BC054293]. The following also demonstrate homology to the foregoing RAP sequences: *Drosophila melanogaster*-Fruit fly; Gene symbol: CG8507; GenBank Acc: NP_649950.1; *Anopheles gambiae*- Mosquito; Gene symbol: AgaP_AGAP003521; GenBank Acc: XP_313261.4; *Caenorhabditis elegans* –Worm; hypothetical protein with gene symbol C15C8.4, GenBank Acc: NP_506187.2.

[0035] The term "RAP agents" includes RAP as well as derivatives, variants, fragments, or mimetics of RAP that inhibit PrP^d formation and/or promote the transformation of PrP^d to non-infectious form(s).

[0036] RAP agents include polypeptides with significant sequence homology to amino acids 35-357 of SEQ ID NO: 1, such as from about 70% to about 100% sequence identity to amino acids 35-357 of SEQ ID NO: 1 (e.g. at least 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with amino acids 35-357 of SEQ ID NO: 1).

[0037] In one embodiment, there is a method for prophylaxis and/or treatment of a mammal at risk of or suffering from a prion disease, comprising administering to the mammal a therapeutically effective amount of an agent selected from the group consisting of: mammalian RAP polypeptide; and a derivative, variant, fragment, and mimetic thereof; and combinations thereof. The prion disease may be selected from the group of various forms of Creutzfeldt-Jakob disease (CJD), iatrogenic Creutzfeldt-Jakob disease (iCJD), variant

Creutzfeldt-Jakob disease (vCJD), familial Creutzfeldt-Jakob disease (fCJD), sporadic Creutzfeldt-Jakob disease (sCJD)); Gerstmann-Sträussler-Scheinker syndrome (GSS); Fatal insomnia, familia (FFI) or sporadic; Kuru, Scrapie, Bovine spongiform encephalopathy (BSE); Transmissible mink encephalopathy (TME); Chronic wasting disease (CWD); Feline spongiform encephalopathy; and Exotic ungulate encephalopathy (EUE). The mammal may be selected from the group of human, sheep, goat, cow, mink, white-tailed deer, elk, mule deer, moose, cat, nyala, gemsbok, oryx, eland, kudu, ankole, and bison.

[0038] The therapeutically effective agent may be selected from the group of:
 a) a polypeptide comprising amino acids 35-357 of SEQ ID NO: 1; b) a polypeptide comprising an amino acid sequence having 70% to 100% sequence identity to amino acids 35-357 of SEQ ID NO: 1; and c) a polypeptide comprising an amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7; as well as the RAP sequences (also disclosed herein) of: Common Chimpanzee, *Macaca mulatta*- Rhesus macaque, *Bos Taurus*- Cow, *Capra hircus* – Goat, *Ovis aries*- Sheep, *Sus scrofa*- Pig, *Canis lupus familiaris*-Dog.

[0039] In another embodiment, the method of prophylaxis and/or treatment further includes intranasal administration of a therapeutically effective amount of a RAP agent in an aqueous vehicle. In one embodiment, the RAP agent is a polypeptide comprising amino acids 35-357 of SEQ ID NO: 1. The administration of the RAP agent can be repeated until a diminution or eradication of symptoms is achieved, or until the subject is no longer exposed or at risk of contracting a prion disease.

[0040] The terms "treatment" or "treating" and the like are used herein to generally mean obtaining a desired pharmacological and/or physiological effect, and refer to complete elimination as well as to any clinically or quantitatively measurable reduction in the symptoms of the prion disease for which the subject is being treated. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof, and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effects attributable to the disease. The effect may also relate to reversing or delaying progression of a prion disease or symptom thereof. Thus, for example, treatment may indicate that any symptoms of a prion disease are reduced or alleviated; the conversion of PrP^C into PrP^d is inhibited; and/or the processing of PrP^d into non-infectious fragments is promoted by administration of the agent (i.e. RAP and derivatives, variants, fragments, and mimetics thereof) described herein.

[0041] A “therapeutically effective amount” or “pharmaceutically effective amount” means the amount of an agent that, when administered to a subject in need thereof, is sufficient to effect such treatment. Thus a “therapeutically effective amount” is an amount indicated for treatment while not exceeding an amount which may cause significant adverse effects (commensurate with a reasonable risk/benefit ratio). The “therapeutically effective amount” will vary depending on the agent, and will also be determined by physical and physiological factors such as the age, body weight, and/or clinical history of the subject to be treated. Methods for evaluating the effectiveness of therapeutic treatments are known to those of skill in the art.

[0042] A “subject in need thereof” refers to any subject or individual who could benefit from the method of treatment described herein, and includes those that are infected, suffering from, or at risk of developing a prion disease. In some embodiments, a subject in need thereof is a subject predisposed for the development of a prion disease; a subject exposed to infection with a prion disease; a subject having one or more prion diseases but not exhibiting any clinical symptoms; and/or a subject exhibiting one or more symptoms of one or more prion diseases.

[0043] The “subject in need thereof” is generally a vertebrate, such as a mammal. Mammals include, but are not limited to, humans, other primates, farm animals, exotic animals, sport animals and pets. Examples include sheep, goats, cows, mink, white-tailed deer, elk, mule deer, moose, cats (e.g. domestic cat, puma, cheetah, ocelot, tiger), nyala, gemsbok, oryx (e.g. Arabian oryx and scimitar-horned oryx), eland, kudu (e.g. greater kudu), ankole, and bison. In an exemplary embodiment, the subject is a human. In other embodiments, the methods find use in experimental animals, in veterinary application, and/or in the development of animal models for disease.

[0044] As used herein, the term “administering” or “introducing” an agent to a subject means providing the agent to a subject in a therapeutically effective manner. Methods of administering RAP agents to a subject includes a number of known means including, but not limited to, systemic administration (e.g. parenteral administration (intravenous, subcutaneous, or intramuscular), intraperitoneal administration, inhalation, transdermal delivery, oral delivery, nasal delivery, rectal delivery, etc.) and/or local administration (e.g. direct injection into a target tissue, delivery into a tissue via cannula, delivery into a target tissue by implantation of a time-release material, or delivery through the skin via a topical composition such as a cream, lotion, or the like), delivery into a tissue by a

pump, etc., intraosseously, in the cerebrospinal fluid, or the like. The terms "orally" and "oral delivery" refer to administration by mouth and include ingestion of the formulation as well as oral gavage. Additional modes of administration include ocular (e.g. via eye drops), buccal, sublingual, vaginal, subcutaneous, or intradermal administration. In an exemplary embodiment, the target tissue is the brain.

[0045] Modes of administration include delivery via a sustained release and/or controlled release drug delivery formulation and/or device. "Sustained release" refers to release of a drug or an active metabolite thereof into the systemic circulation over a prolonged period of time relative to that achieved by oral administration of a conventional formulation of the drug. "Controlled release" is a zero order release; that is, the drug releases over time irrespective of concentration. Single, multiple, continuous or intermittent administration can be effected.

[0046] One skilled in the art will appreciate that the RAP agent can be formulated in a variety of styles, and using a wide variety of carriers, solvents, diluents, and other excipients. For example, the RAP agent can be formulated in an aqueous mixture or solution. The mixture may further include excipients such as buffers, preservatives, antioxidants, and the like all in accordance with commonly accepted principles of pharmaceutical formulation. Additionally, the RAP agent can be formulated as a cream, an oil, a solid release form, or in a patch, e.g., for transdermal delivery.

[0047] Doses to be administered are variable according to the treatment period, frequency of administration, the host, and the nature and severity of the disorder. The dose can be determined by one of skill in the art without an undue amount of experimentation. The agents are administered in dosage concentrations sufficient to ensure the release of a sufficient dosage unit into the patient to provide the desired treatment of the prion disease. The active ingredients may be administered to achieve therapeutic or prophylactic blood concentrations, such as in vivo plasma concentrations of the agents of from about 0.01 to about 10,000 ng/cc, such as from about 0.01 to about 1,000 ng/cc. "Therapeutic or prophylactic blood concentrations" refers to systemic exposure to a sufficient concentration of a drug or an active metabolite thereof over a sufficient period of time to effect disease therapy or to prevent the onset or reduce the severity of a disease in the treated animal.

[0048] For example, the methods described herein may use compositions to provide from about 0.01 to about 100 mg/kg body weight/day of the agents, from about 0.01 to about 10 mg/kg body weight/day of the agents, or about 30 mg/kg body weight/day of the agents.

It will be understood, however, that dosage levels that deviate from the ranges provided may also be suitable in the treatment of a given disorder.

[0049] The agents may be in any form suitable for administration. Such administrable forms include tablets, buffered tablets, pills, capsules, enteric-coated capsules, dragees, cachets, powders, granules, aerosols, liposomes, suppositories, creams, lotions, ointments, skin patches, parenterals, lozenges, oral liquids such as suspensions, solutions and emulsions (oil-in-water or water-in-oil), ophthalmic liquids and injectable liquids, or sustained- and/or controlled release forms thereof. The desired dose may be provided in several increments at regular intervals throughout the day, by continuous infusion, or by sustained and/or controlled release formulations, or may be presented as a bolus, electuary or paste.

[0050] "Practical dosage regimen" refers to a schedule of drug administration that is practical for a patient to comply with. For human patients, a practical dosage regimen for an orally administered drug is likely to be an aggregate dose of less than 10 g/day.

[0051] In one embodiment, a pharmaceutical composition or formulation comprising the agents is prepared by admixture with one or more pharmaceutically acceptable carriers. Other products may be added, if desired, to maximize agent preservation, or to optimize a particular method of delivery. In addition, the present methods include use of combination compositions comprising the agents as described herein in combination with other agents suitable for the treatment of prion diseases.

[0052] "Pharmaceutically acceptable carrier" or "diluent" means a carrier that is useful in preparing a pharmaceutical composition that is generally safe, neither biologically nor otherwise undesirable, not toxic or otherwise unacceptable commensurate with a reasonable risk/benefit ratio, compatible with other ingredients of the formulation, and includes a carrier that is acceptable for veterinary use as well as human pharmaceutical use. "A pharmaceutically acceptable carrier" as used in the specification and claims includes both one and more than one such carrier.

[0053] A "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and anti-fungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration of a composition comprising agents. Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions and dextrose solution. The volume of the pharmaceutical composition is based on the intended mode of administration and the safe

volume for the individual patient, as determined by a medical professional.

[0054] The present disclosure relates to use of any one or more of the agents described herein for the treatment of a prion disease. The present disclosure also relates to the use of any one or more of the agents described herein for manufacture of a medicament, particularly the manufacture of a medicament for treating a prion disease.

[0055] RAP agents, including RAP and its various mimetics, can also be used as a surface active agent for TSE disinfection. RAP inhibits or prevents or destroys PrP^d replication by acting, at least in part, at the cell surface. By placing a RAP agent in contact with any of the various forms of PrP^d (or TSE, e.g., BSE, CWD, CJD, scrapie, etc.), the RAP agent inhibits or destroys replication, and thus significantly reduces or eliminates or prevents PrP^d infection. The RAP agent can be used as a TSE disinfectant on a host of surfaces including man-made surfaces as are found in a hospital or dwelling (e.g., metal or plastic devices, ceramics), those used in association with livestock including barns or rendering plants, as well as on natural surfaces such as grass, soil, etc.

[0056] The surface active agent can be applied as a surface coating, or it can be embedded in the surface material to be treated. For example, the RAP agent can be embedded in a polymer, ceramic, or composite material forming the structural elements of the surface to be disinfected; or it can be applied to a surface in a matrix as in a paint, varnish, polymer, gel, paste, oil, ointment, or other coating. The RAP agent disinfectant can also be formulated in a variety of embodiments, including a solution for liquid, aerosol, or spray administration, or as a slurry, powder, or paste.

[0057] The various matrixes or vehicles in which the RAP agent is compounded may be further formulated to include agents such as buffers, preservatives, excipients, or other known agents useful for protection of e.g., polypeptides, from light, heat, oxidation, free-radicals, and the like. The manner of formulation, the vehicle, and the ultimate disposition or means of application is not thought to be particularly significant, and are subject to development and manipulation according to known principles and methods.

[0058] RAP agents can be used in a method of TSE disinfection by administering a RAP agent to a material or surface likely to be contaminated with or exposed to a TSE. The RAP agent disinfectant can be used to treat areas in and around those used to house animals. The material or surface to be disinfected can be those likely to contact or harbor TSEs, and particularly those associated with, or exposed to food, including livestock feed, and may include the food itself, as well as materials used to store, transport, or process the food. In

this manner, the methods include both a prophylactic effect, and therapeutic effect as the treatment of food can be used to disinfect the food, and can be a means of administration to the animal ingesting the food. As used herein, the term "animal" includes human.

[0059] Additionally, the TSE disinfectant described herein can be readily and inexpensively compounded for wide scale administration to animals in the wild, and so can be used to fight the spread of CWD. In such an embodiment, the RAP agent can be compounded, e.g., as an aqueous mixture, alone or with other excipients and/or additives, and sprayed over areas where TSE-infected animals, or animals at risk of TSE infection, are living and/or feeding.

[0060] As used herein, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an RAP" includes a plurality of RAP molecules and reference to "the dosage" includes reference to one or more dosages and equivalents thereof known to those skilled in the art, and so forth.

[0061] 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 disclosure is not entitled to antedate such publication by virtue of prior disclosure. Further, the dates of publication provided may be different from the actual publication dates, which may need to be independently confirmed. All publications, patents, patent applications and other references cited herein are hereby incorporated by reference.

[0062] While the disclosure has been described in detail with reference to certain embodiments thereof, it will be apparent to one skilled in the art that various changes can be made, and equivalents employed, without departing from the scope of the disclosure. In addition, the following examples are illustrative of the methods described herein and should not be considered as limiting the foregoing disclosure in any way.

EXAMPLES

[0063] The experiments set forth herein demonstrate that RAP inhibits PrP^d formation in prion infected murine cell cultures of bone marrow and spleen origin. The inhibition of PrP^d formation was observed over a time course of exposure to various concentrations of RAP. PrP^d, unlike PrP^C, is partially resistant to proteolysis by proteinase K. Thus, the formation of PrP^d was determined by testing for the presence of PrP^{res}, the digestion product of PrP^d. PrP^{res} is a recognized marker of prion infection.

[0064] The results show that a suitable treatment time for the cell cultures is

between 12 and 24 hours, with an inhibitory concentration of between 50 and 250 nM. Multiple treatments inhibited PrP^d formation at least through 20 passages (~70 days after the treatment was stopped). A similar approach has been used to treat other TSE infected cells with various substances including monoclonal anti-PrP-specific antibodies, and in *in vivo* experiments showing the delay in development of the disease in mice treated with such substances (CAUGHEY et al., "Prions and transmissible spongiform encephalopathy (TSE) chemotherapeutics: A common mechanism for anti-TSE compounds?" *Acc Chem Res.*, 2006, Vol. 39, pp. 646-53; PANKIEWICZ et al., "Clearance and prevention of prion infection in cell culture by anti-PrP antibodies," *Eur J Neurosci.*, 2006, Vol. 23, pp. 2635-47; and TELLING et al., "Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein," *Cell*, 1995, Vol. 83, pp. 79-90).

[0065] The murine spleen-derived stromal cell (SP) lines (Holland Laboratory) used in the experiments were developed to persistently propagate mouse-adapted human prion agents, Fukuoka-1 (Fu) (cell line Fu-SP/58) and mouse-adapted variant Creutzfeldt-Jakob disease (cell line mo-vCJD-SP/63) (AKIMOV et al., "Persistent propagation of variant Creutzfeldt-Jakob disease agent in murine spleen stromal cell culture with features of mesenchymal stem cells," *J Virol.*, 2008, Vol. 82, pp. 10959-62, Epub., 2008 Aug 20). The murine bone marrow (BM) stromal cell line (Holland Laboratory) also used in experiments was developed to persistently propagate Fu (cell line 336-2BMSFu2) (AKIMOV et al., "Murine bone marrow stromal cell culture with features of mesenchymal stem cells susceptible to mouse-adapted human TSE agent, Fukuoka-1," *Folia Neuropathol.* 2009, Vol. 47, pp. 205-14).

Experiment 1

[0066] The initial experiment was performed to determine if treatment of mo-vCJD-SP/63 cell cultures with low density lipoprotein receptor-related protein (LRP) specific polyclonal antibodies 2629, PrP specific monoclonal antibodies 6D11, or RAP have any effect on levels of total PrP and PrP^{res}.

[0067] Cell line mo-vCJD-SP/63 propagating PrP^{res} through multiple passages, and control uninfected cell line SP/76, expressing normal PrP^C, were plated at a density of 1:3 of confluent monolayer. On the next day the growth medium was exchanged for fresh medium containing either 1 μ M RAP (gift of Dr. Strickland), or 10 μ g/ml 6D11 monoclonal antibody

(Signet) or 50 µg/ml 2629 rabbit polyclonal antibody (gift of Dr. Strickland) or 3 µl control anti-alpha-tubulin rabbit polyclonal antibodies (Cell Signaling Tech). After 8 hours of incubation, an additional 0.5 µM RAP was added only to the cells which were already exposed to RAP. During each experiment, cell cultures were maintained at 37°C in the presence of 5% CO₂. Cells were harvested using lysis buffer after 24 hours following initial treatment. A portion of the sample was treated with proteinase K (10 µg/ml) for digestion of normal PK-sensitive PrP^C and another portion was left untreated. The proteins in PK-treated and untreated samples were precipitated by methanol at -80°C. After centrifugation the pellets were solubilized in a denaturing buffer containing 2 % SDS, 62.5 mM Tris-HCl, 25% glycerol and 0.1 % bromophenol blue, resolved on NuPAGE, 4-12% Bis-Tris gradient gel, followed by transfer to nitrocellulose membrane and probing with anti-PrP specific monoclonal antibodies 6D11 diluted 1:10,000. Data of this experiment are shown in Figure 1. RAP significantly inhibited the generation of PrP^{res} in mo-vCJD-SP/63 cells but had no effect on total PrP in either mo-vCJD-SP/63 or control SP/76 cells. Treatment of both cell lines with anti-LRP 2629 antibodies had no visible effect on amount of total PrP or PrP^{res} in mo-vCJD-SP/63 cells and PrP^C in control SP/76 cells. On further propagation of up to three passages, recovered generation of PrP^{res} in mo-vCJD-SP/63 cells treated with RAP to the levels similar to cells which were treated with antibodies or left untreated was observed.

Experiment 2

[0068] Next, a "time-course" experiment was performed to examine when the inhibition of PrP^{res} generation occurred after the treatment of cells with 1 µM RAP.

[0069] Cells of mo-vCJD-SP/63 cell line were plated into 6 cm Petri dishes ~ 3 hrs prior experiment at ~ 50% confluent density and were incubated at 37°C in presence of 5% CO₂ to allow cell adhesion. Next, 1 µM RAP was added and cell cultures were maintained at 37°C in presence of 5% CO₂. Cells were collected after 15 min, 30 min, 1, 2, 8, 12 and 24 hrs and processed for western blotting using the procedures described in Experiment 1. Data from this experiment are shown in Figure 2.

[0070] The western blot shows significant inhibition of PrP^{res} generation in cells collected 24 hours following exposure to RAP.

Experiment 3

[0071] In our previous experiments, a 1 µM concentration of RAP was used. In this

experiment, the effect of lower and higher concentrations of RAP on PrP^{res} formation and the minimal inhibiting concentration of RAP were determined.

[0072] Cells of mo-vCJD-SP/63 cell line were plated into 6 cm Petri dishes ~ 3 hrs prior experiment at ~ 50% confluent density and were maintained at 37°C in presence of 5% CO₂ to allow cell adhesion. Next, RAP at various concentrations, 50 nM, 250 nM, 500 nM, 750 nM, 1 µM, 1.5 µM and 2 µM, was added to the plates and cell cultures were maintained at 37°C in presence of 5% CO₂. Cells were collected after 24 hrs and processed using the procedures described in Experiment 1. Data from this experiment are shown in Figure 3.

[0073] RAP profoundly inhibited the generation of PrP^{res} when used at 250 nM concentration. Increasing concentrations up to 2 µM produced no further inhibiting effect. No inhibitory effect was observed when RAP was used at concentrations of 50 nM and below.

Experiment 4

[0074] In this experiment the inhibitory effect of 500 nM RAP was tested in two cell culture persistently infected either with mo-vCJD (mo-vCJD-SP/63) or Fukuoka-1 agent (Fu-SP/58).

[0075] Cells of either mo-vCID-SP/63 or Fu-SP/58 cell lines were plated into 6 cm Petri dishes ~ 1 hrs prior experiment at ~ 50% confluent density for each cell line and were maintained at 37°C in presence of 5% CO₂ to allow cell adhesion. One hour later the medium was exchanged for either BM or BLGM medium and RAP was added to each cell culture to reach final concentration of 500 nM. Cell cultures were maintained at 37°C in presence of 5% CO₂. Cells were collected after 24 hrs and processed using the procedures described in Experiment 1. Data from this experiment are shown in Figure 4.

[0076] RAP significantly inhibited the generation of PrP^{res} under tested conditions in mo-vCJD-SP/63 and in Fu-SP/58 cell cultures.

Experiment 5

[0077] A "time-course" experiment was repeated to examine when the inhibition of PrP^{res} generation occurred after the treatment with 500 nM of RAP. Cells of mo-vCJD-SP/63 cell line were plated into 6 cm Petri dishes ~ 1 hrs prior experiment at ~ 50% confluent density and were incubated at 37°C in presence of 5% CO₂ to allow cell adhesion. Next, either 500 nM RAP was added to the cell cultures or cell cultures were left

untreated. Cell cultures were maintained at 37°C in presence of 5% CO₂. Cells were collected after 4, 8, 12 and 24 hrs and processed for western blotting using the procedures described in Experiment 1. Data from this experiment are shown in Figure 5.

[0078] Once again, RAP inhibited PrP^{res} formation in a timely manner with significant inhibition observed 12 hours following treatment. Cells that were left untreated with RAP generated PrP^{res} during the 24 hours with slightly increased amounts after 12 hours.

Experiment 6

[0079] The inhibitory effect of 500 nM RAP was tested in bone marrow-derived cell culture 336-2BMS-Fu2 persistently propagating Fu agent.

[0080] Cells of 336-2BMSFu2 cell line were plated into 25 cm² flasks ~ 1 hrs prior experiment at ~ 25% confluent density for each cell line and were maintained at 37°C in presence of 5% CO₂ to allow cell adhesion. After one hour the medium was exchanged for a fresh one and 500 nM RAP (final concentration) was added to the cell culture or cell cultures was left untreated (control). Cell cultures were maintained at 37°C in presence of 5% CO₂. Cells were collected after 24 hrs and were processed using the procedures described in Experiment 1. Data from this experiment are shown in Figure 6.

[0081] RAP significantly inhibited PrP^{res} formation in 336-2BMS-Fu2 cell culture (lane 4) as compared to untreated control (lane 2) but had no effect on the level of total PrP (compare lane 1 and 3).

Experiment 7

[0082] In this experiment the effect of multiple treatments with 250 nM RAP on 336-2BMS-Fu2 cells was investigated. In addition the cell culture was further propagated to establish whether the treatment of cells caused transient inhibition of PrP^{res} formation or lead to stable inhibition or cured cells completely.

[0083] Cells of the 336-2BMS-Fu2 cell line were plated into 25 cm² flasks ~ 1 hrs prior experiment at ~ 10 % confluent density for each cell line and were maintained at 37°C in presence of 5% CO₂ to allow cell adhesion. After one hour the medium was exchanged for a fresh one and 250 nM RAP (final concentration) was added to the cell culture or cell cultures was left untreated (control). The treatment of cells continued and 250 nM RAP was added after 8, 12, 12, 12, 12, 12 hours (total six treatments). Control cells were treated with medium only. During the experiment cell cultures were maintained at 37°C in presence of

5% CO₂. Cells were collected after 12 hrs following the last treatment and portion of cells was used for further propagation and portion was processed for detection of PrP^{res} by Western blotting using the procedures described in Experiment 1. Cells were propagated through 20 passages with splitting of cells every 3-4 days and at each passage portion of cells was collected and processed for detection of PrP^{res} by Western blotting. Data from this experiment are shown in Figure 7.

[0084] Multiple treatments of cells with 250 nM RAP inhibited formation of PrP^{res} as shown by the absence of the signal in lysates collected at 68 hours following initial treatment. During further propagation through 20 passages the untreated cell culture continue to propagate PrP^{res} while RAP-treated bone-marrow derived 336-2-BM-Fu2 cell culture infected with Fukuoka agent produced significantly lower, if any, PrP^{res}. The amount of total PrP was comparable in both RAP-treated and untreated cell cultures during the experiment.

[0085] The foregoing experiments demonstrate that RAP has a significant inhibitory effect *in vitro* on the generation of PrP^d in murine spleen- and bone-marrow-derived stromal cell cultures persistently infected with either mouse-adapted vCJD or Fukuoka-1 agents. Data from experiment 7 show that continuous treatment with RAP has a long-term (up to 70 days) inhibitory effect on PrP^{res} propagation in infected cells. This shows that RAP, either directly or through interaction with other protein(s), is involved in inhibiting the formation of, and/or promoting the clearance of, PrP^{res}.

Experiment 8

[0086] To evaluate effect of RAP on TSE agent propagation *in vivo* we performed the following experiment.

[0087] Brain tissue from a sporadic CJD case was homogenized and serially diluted 10-fold from 10⁻³ to 10⁻⁶ suspension in PBS. Part of the volume of the samples was left untreated (Sample 1) and part was treated with 2 μM RAP (Sample 2). Groups of homozygous transgenic mice carrying human prion protein gene with methionine at position 129 were created: Mice in Groups 1 and 3, 4 and 6, 7 and 9, 10 received single intracranial injections of serially diluted (10⁻³, 10⁻⁴, 10⁻⁵ and 10⁻⁶, respectively) Sample 1 in volume of 30 μl. Mice in Groups 2, 5, 8 and 11 received single intracranial injections of serially diluted (10⁻³, 10⁻⁴, 10⁻⁵ and 10⁻⁶, respectively) Sample 2 in volume of 30 μl. To evaluate the therapeutic effect of RAP on development of the disease, mice from Groups 3, 6 and 9 received intracranial injections of 2 μM RAP in volume of 30 μl at 100 days following the

injection of the infectious agent (Sample 1).

[0088] Summary of the experiment is provided in Table 1. At high concentration of the brain inoculum (10^{-3}) for infecting mice, RAP showed no effect on their survival when added to the brain homogenate immediately before the injection to prevent the infection (Group 2) or when used as a single dose treatment at 100 days following the injection of the infectious agent (Group 3). However, all mice in Groups 1, injected with brain homogenate, and in Group 2; injected with brain homogenate that was treated with RAP immediately before inoculation, died as result of infection as confirmed by the presence of PrP^{res} in their brains while only four animals out of five from Group 3, that were treated intracranially with RAP at 100 days, died as a result of infection (one animal was tested negative for PrP^{res} by western blotting). At lower dilution of brain homogenate (10^{-4}) for infecting mice we again observed differences in survival of mice untreated and treated with RAP. While all five mice died from infection in untreated by RAP Group 4 only four animals died as a result of infection in Group 5 and Group 6. One dead animal from Group 5 was tested negative for PrP^{res} by Western blotting and one animal from Group 6 is still alive for more than 300 days following inoculation. The same trend was observed for infection while using even lower (10^{-5}) concentration of brain homogenate: as a result of infection three mice out of five died in Group 7, two mice out of five died in Group 8 and all three mice died in Group 9. We also observed statistically significant prolongation in the incubation period between Groups 4 and 6 ($p=0.004$) and 5 and 6 ($p=0.001$) while using t-test (SigmaPlot 8). However it seems that addition of RAP to the brain homogenate immediately before injecting the sample into mice shortened the incubation period (comparison between Groups 4 and 5, $p=0.036$). Differences in incubation periods were not statistically significant between Groups 7, 8 and 9 due to high variation which is generally observed for higher dilutions of the infectious agent. This phenomenon may obfuscate the significance of the RAP effect while tested on small size groups of mice. To address this, the larger groups of animals should be used.

[0089] RAP showed therapeutic effect on progression of TSE when administered to mice that received lower concentrations of the TSE agent.

Table 1. Effect of RAP on development of TSE in transgenic mice injected intracranially with sporadic CJD agent

Groups	Dilution of the brain	Number of dead/alive/inoculated animals	Number of animals tested (pos/neg) by WB	Incubation period (average ± SD)
1	10 ⁻³ (Sample 1)	5/0/5	5/0	173.2±5.8
2	10 ⁻³ +RAP (Sample 2)	5/0/5	5/0	164.6±9.8
3	10 ⁻³ (Sample 1)+RAP 100day	5/0/5	4/1	179.5±18.5
4	10 ⁻⁴ (Sample 1)	5/0/5	5/0	193±5.6
5	10 ⁻⁴ +RAP (Sample 2)	5/0/5	5/1	185±1.4
6	10 ⁻⁴ (Sample 1)+RAP 100day	4/1/5	4/0	215.3±11.4
7	10 ⁻⁵ (Sample 1)	4/1/5	4/0	246±47
8	10 ⁻⁵ +RAP (Sample 2)	2/3/5	2/0	245±58
9	10 ⁻⁵ (Sample 1)+RAP 100day	3/0/3	3/0	240.3±20.5
10	10 ⁻⁶	0/5/5	n.a.	345 days
11	10 ⁻⁶ +RAP	0/5/5	n.a.	345 days

Statistical analysis was performed using t-test (SigmaPlot 8). Statistically significant differences were found when comparing Groups 4 and 5 (p=0.03), 4 and 6 (p=0.006) and 5 and 6 (p=0.002)

[0090] The findings disclosed herein suggest that RAP will be effective in the treatment, and prophylaxis, of other protein misfolding diseases. Exemplary of such diseases are those of Table 2, below.

TABLE 2
Other Protein Misfolding Diseases In Which RAP Is Likely To Have Therapeutic Effect

Protein	Disease
Amylin	Type II Diabetes
α _{1A} -voltage-dependent calcium channel subunit	Spinocerebral ataxia type 6
ABri	Familial British dementia
α-Galactosidase A	Fabry's disease
Androgen receptor	Spinobulbular muscular atrophy

Protein	Disease
α -Synuclein	Parkinson's disease
Ataxins	Ataxia
ATP7B	Wilson disease
Atrial natriuretic factor	Atrial amyloidosis of heart
Atrophin	Dentatorubral pallidoluysian atrophy
β_2 -Microglobulin	Haemodialysis-associated amyloidosis
β -Amyloid	Alzheimer's disease
β -Amyloid	Hereditary cerebral amyloid angiopathy
β -Glucocerebrosidase	Gaucher's disease
Calcitonin	Medullary carcinoma of thyroid
Cystatin C	Hereditary cerebral amyloid angiopathy
Cystic fibrosis transmembrane regulator protein	Cystic fibrosis
Fibrillin	Marfan syndrome
Fragile X mental retardation-1 protein	Fragile X syndrome
Fragile X mental retardation-2 protein	Fragile XE syndrome
Gelsolin	Finnish type familial amyloidosis
Glial fibrillary acidic protein (GFAP)	Alexander disease
Gonadotropin-release hormone receptor	Huntington's disease
Huntingtin	Huntington's disease
Ig V _L domain	Light chain amyloidosis
Lysozyme	Fatal systemic amyloidosis
Machado-Joseph disease protein 1	Machado-Joseph disease
Medin	Hereditary systemic amyloidosis
Myotonic dystrophy protein kinase	Myotonic dystrophy
Nephrogenic diabetes isipidus	Aquaporin-Vasopressin-1
p53	Cancer
Prion proteins	Prion diseases
Rhodopsin	Retinitis pigmentosa protein
Serum amyloid A (SAA)	Secondary systemic amyloidosis
Superoxide dismutase	Amyotrophic lateral sclerosis
Tau	Alzheimer's disease
Tau	Frontotemporal dementia
Transthyretin	Familial amyloid polyneuropathy
von Hippel Lindau protein	Cancer

What is claimed is:

1. A method of treating a subject suffering from a prion disease, comprising administering to the subject a therapeutically effective amount of a RAP polypeptide.
2. The method of claim 1, wherein the prion disease is selected from the group consisting of various forms of Creutzfeldt-Jakob disease (CJD), iatrogenic Creutzfeldt-Jakob disease (iCJD), variant Creutzfeldt-Jakob disease (vCJD), familial Creutzfeldt-Jakob disease (fCJD), sporadic Creutzfeldt-Jakob disease (sCJD)); Gerstmann-Sträussler-Scheinker syndrome (GSS); Fatal insomnia, familial (FFI) or sporadic Kuru; Scrapie; Bovine spongiform encephalopathy (BSE); Transmissible mink encephalopathy (TME); Chronic wasting disease (CWD); Feline spongiform encephalopathy; and Exotic ungulate encephalopathy (EUE).
3. The method of claim 1, wherein the subject is a mammal selected from the group consisting of human, sheep, goat, cow, mink, white-tailed deer, elk, mule deer, moose, cat, nyala, gemsbok, oryx, eland, kudu, ankole, and bison.
4. The method of claim 1, wherein the subject is a human.
5. The method of claim 1, wherein the RAP polypeptide is selected from the group consisting of:

- a) a polypeptide comprising amino acids 35-357 of SEQ ID NO: 1;
- b) a polypeptide comprising an amino acid sequence having 90% to 100% sequence identity to amino acids 35-357 of SEQ ID NO: 1;
- c) a polypeptide comprising an amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7; and
- d) combinations thereof.

6. The method of claim 5, wherein the RAP polypeptide comprises amino acids 35-357 of SEQ ID NO: 1.

7. The method of claim 1, wherein the mode of administration is intranasal.

8. The method of claim 7, wherein the agent is compounded with an aqueous vehicle prior to administration to the subject.

9. The method of claim 1, wherein the RAP agent is administered to achieve *in vivo* plasma concentration of the agent of about 0.01 ng/cc to about 10,000 ng/cc.

10. The method of claim 1, wherein the RAP agent is administered to achieve *in vivo* plasma concentration of the agent of about 0.01 ng/cc to about 1,000 ng/cc.

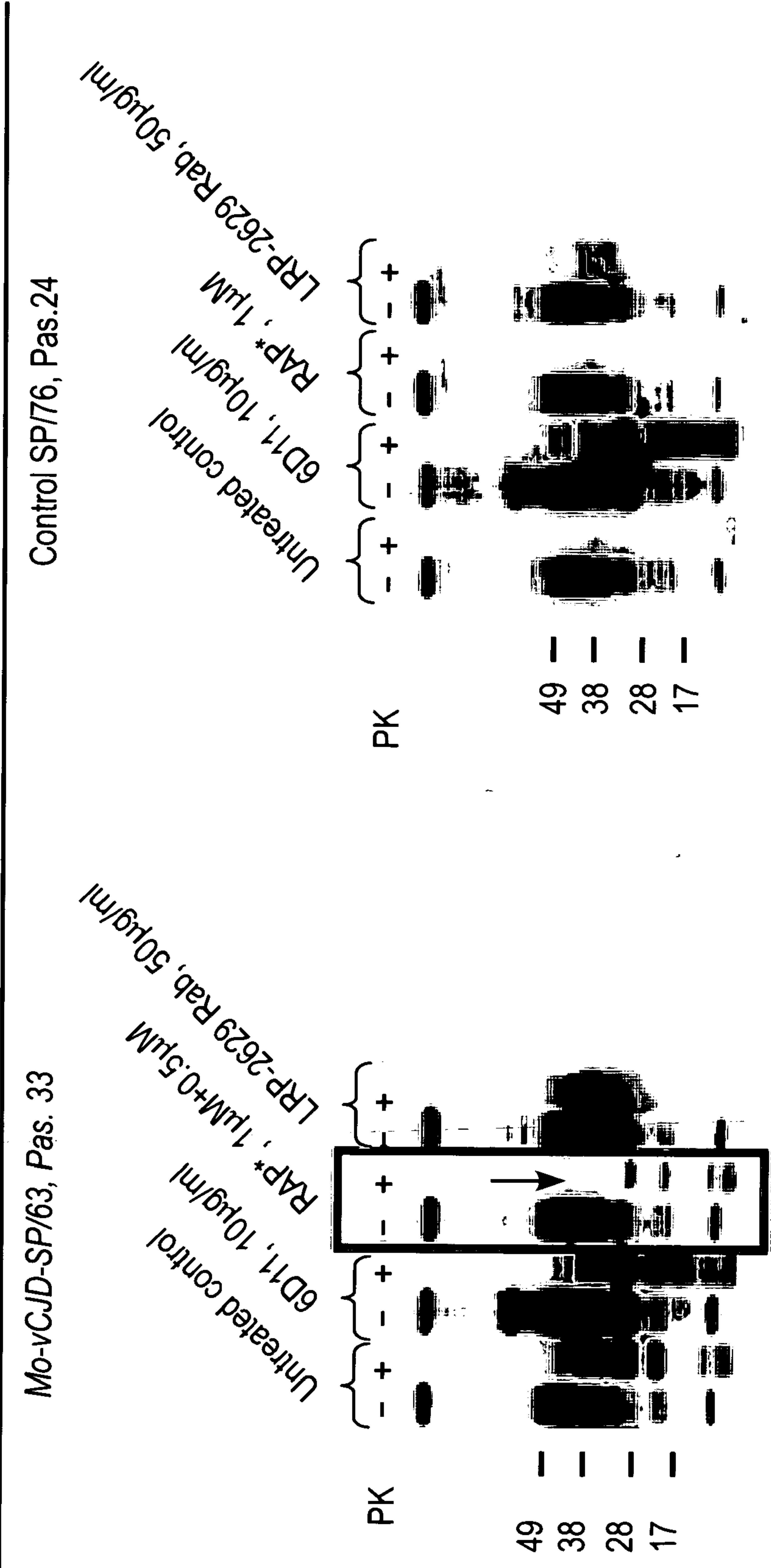
11. The method of claim 1, wherein the RAP polypeptide is administered to the subject at a dose of about 0.01 to about 100 mg/kg body weight/day.

12. The method of claim 1, wherein the RAP polypeptide is administered to the subject at a dose of about 30 mg/kg body weight/day.
13. A pharmaceutical formulation comprising a therapeutically effective amount of RAP polypeptide in combination with a carrier and a pharmaceutically acceptable excipient.
14. The formulation of claim 13, wherein the carrier is a liquid aqueous carrier.
15. The formulation of claim 14, wherein the RAP polypeptide is selected from the group consisting of:
- (a) a polypeptide comprising amino acids 35-357 of SEQ ID NO: 1;
 - (b) a polypeptide comprising an amino acid sequence having 70% to 100% sequence identity to amino acids 35-357 of SEQ ID NO: 1;
 - (c) a polypeptide comprising an amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7; and
 - (d) combinations thereof.

16. The formulation of claim 13, wherein the RAP polypeptide comprises amino acids 35-357 of SEQ ID NO: 1.

17. The formulation of claim 13, wherein the RAP polypeptide consists of amino acids 35-357 of SEQ ID NO: 1.

FIG. 1 Effect of RAP on PrPress formation in spleen-derived mo-vCJD-
SP/63 cell line: 24 hours after the treatment



- Control SP/76 and mo-vCJD-SP/63 cells were treated with RAP twice initially with 1µM and in 8 hours with 0.5 µM.
- Proteins were concentrated by Methanol precipitation.
- (-) and (+) Proteinase K (PK)- treated samples represent total PrP and pathologic PrPress, respectively.
- Anti-PrP monoclonal antibody 6D11 diluted 1:10,000.
- Inhibition of PrPres formation by RAP is shown by arrows.

FIG. 2 Effect of 1 mM RAP on PrPres formation in mo-vCJD-SP/63 cell line infected with mouse-vCJD: Time-course

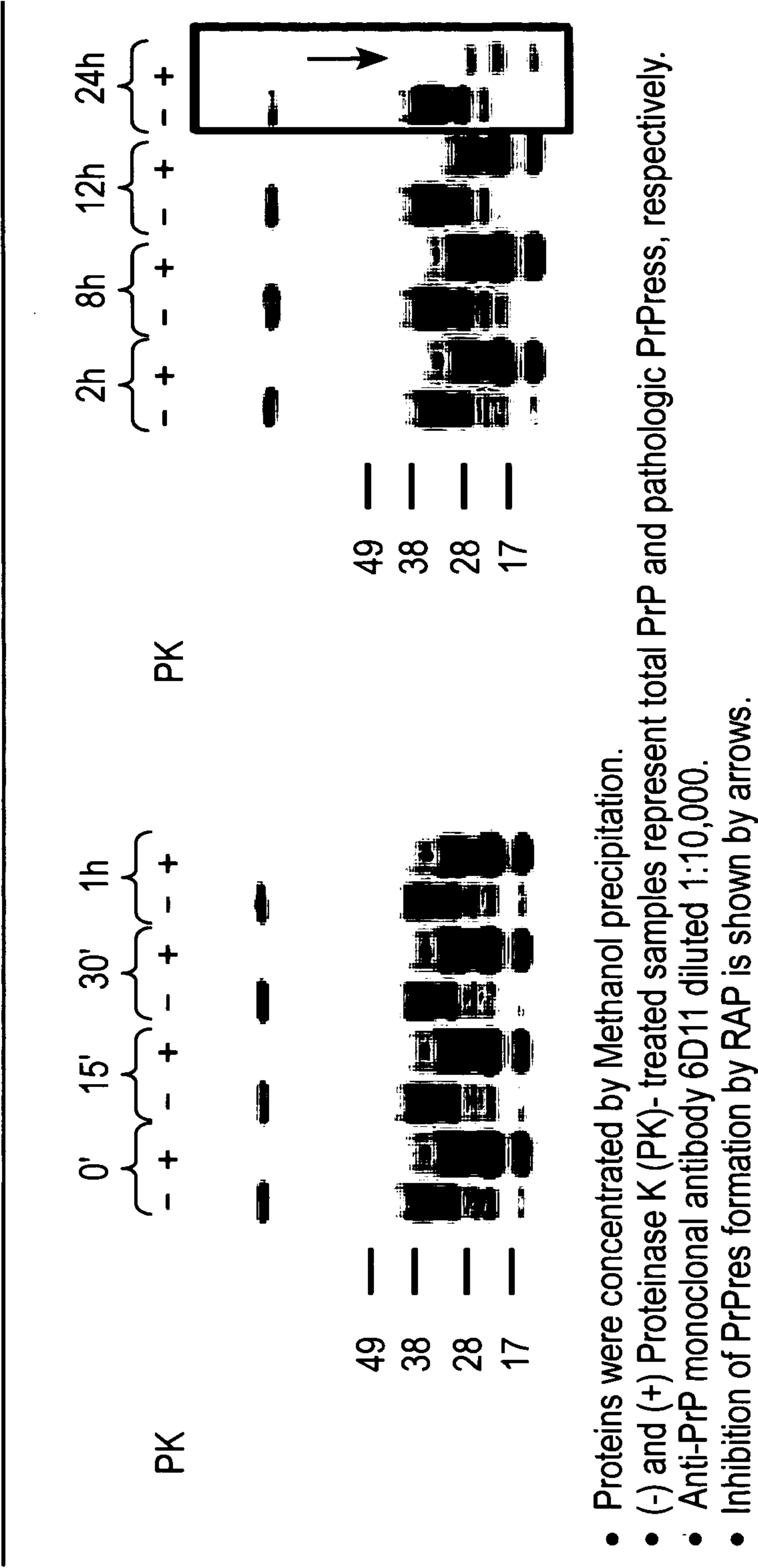
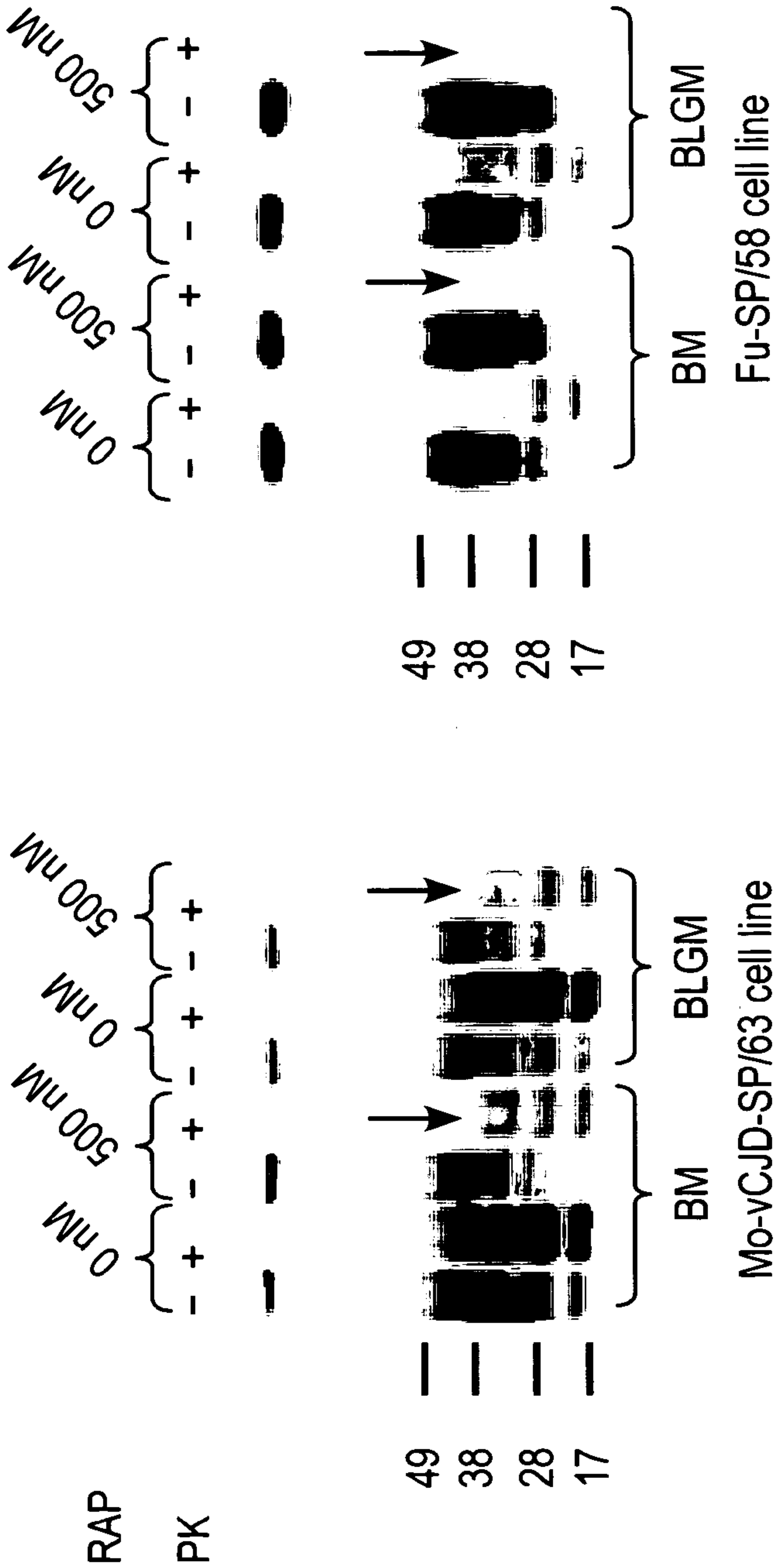


FIG. 3 Effect of RAP on PrPres formation in mo-vCJD-SP/63 cell line
infected with mouse-vCJD: Dose-dependent inhibition



- Proteins were concentrated by Methanol precipitation.
- (-) and (+) Proteinase K (PK)- treated samples represent total PrP and pathologic PrPres, respectively.
- Anti-PrP monoclonal antibody 6D11 diluted 1:10,000.
- Inhibition of PrPres formation by RAP is shown by arrows.

FIG. 4 Effect of 500 nM RAP on PrPres formation in spleen-derived cell lines persistently infected with either mo-vCJD or Fukuoka



- Cells were grown wither in BM or in BLGM medium for 24 hours following the treatment with RAP.
- Proteins were concentrated by Methanol precipitation.
- (-) and (+) Proteinase K (PK)- treated samples represent total PrP and pathologic PrPres, respectively.
- Anti-PrP monoclonal antibody 6D11 diluted 1:10,000.
- Inhibition of PrPres formation by RAP is shown by arrows.

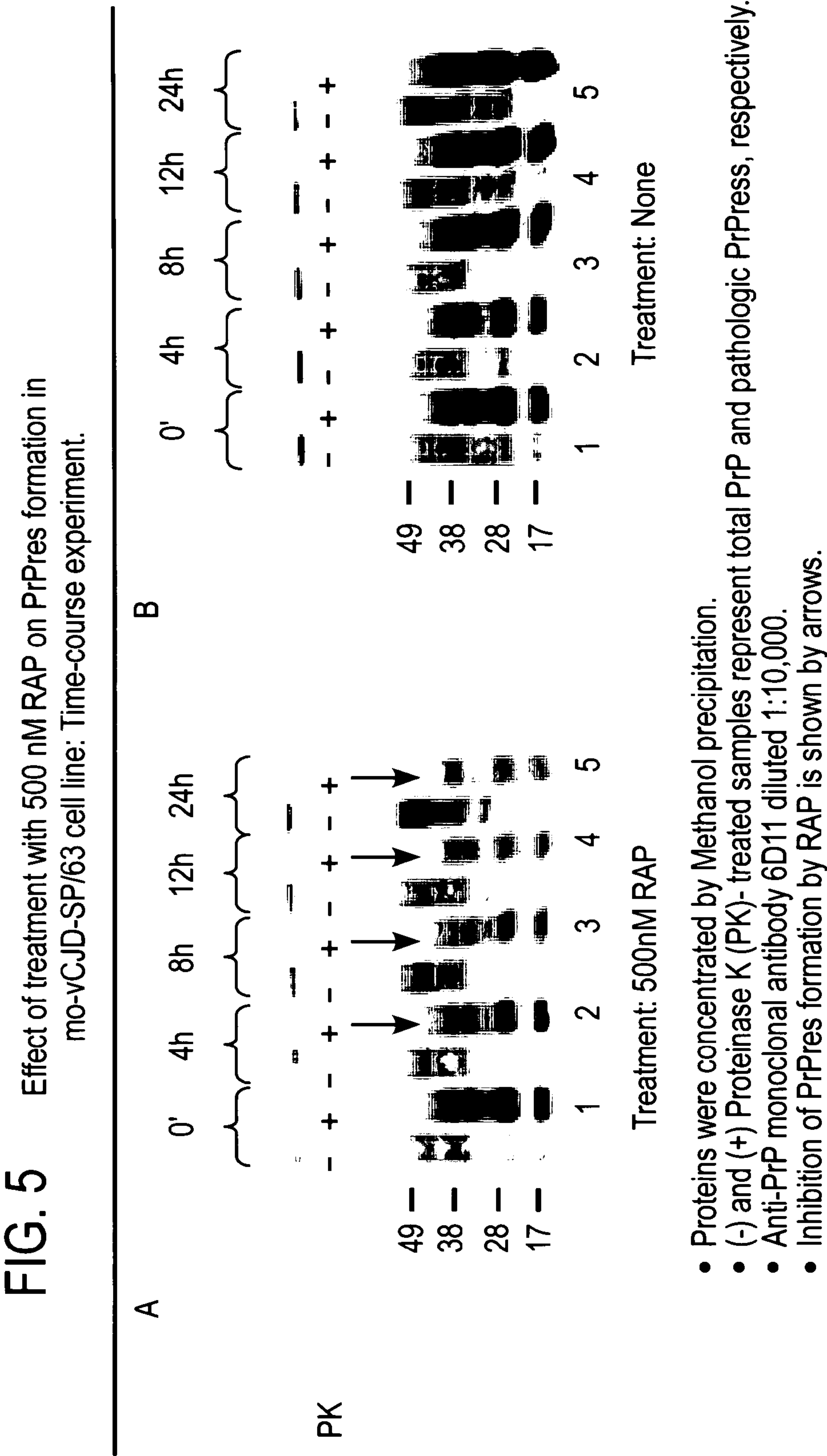
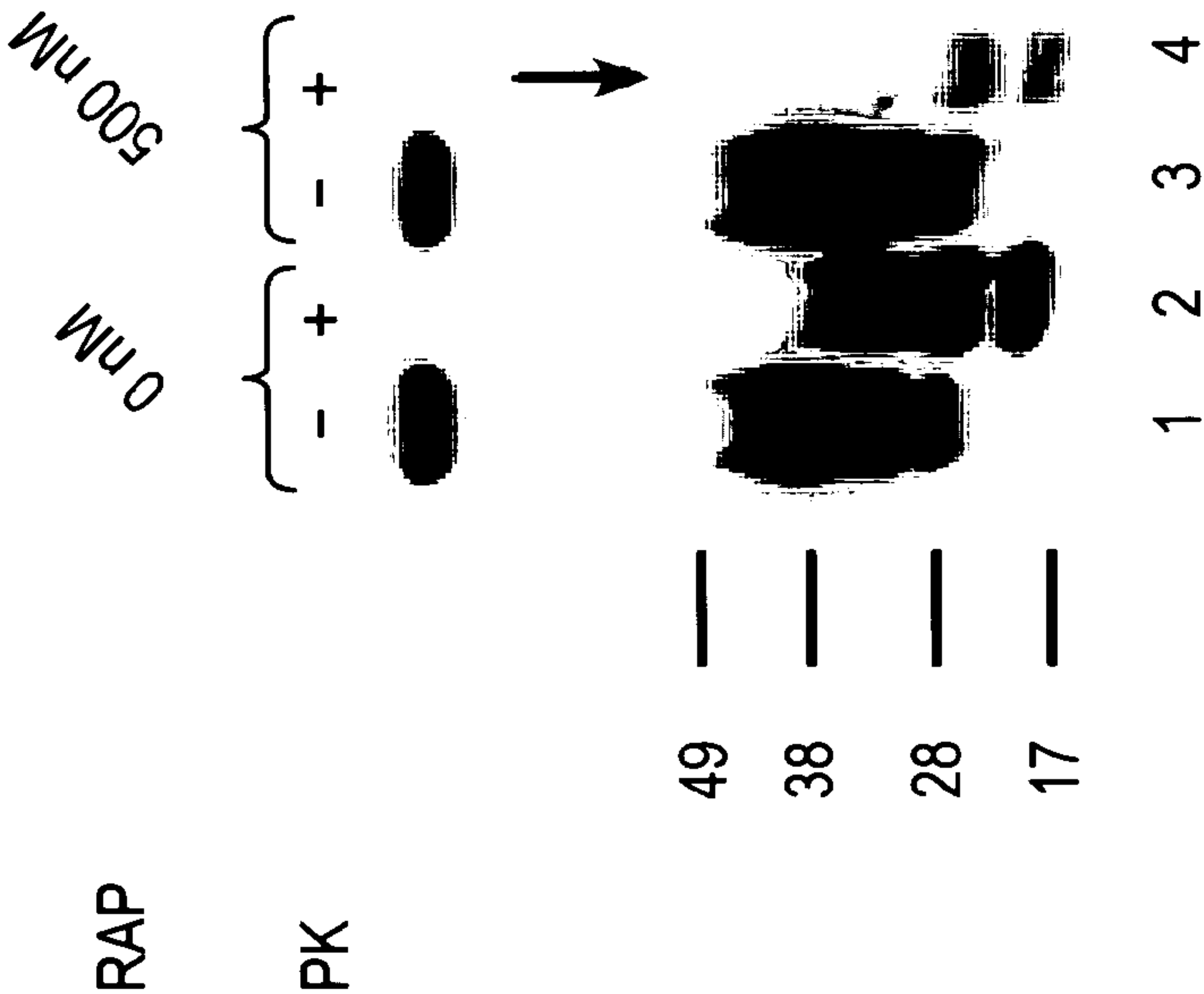
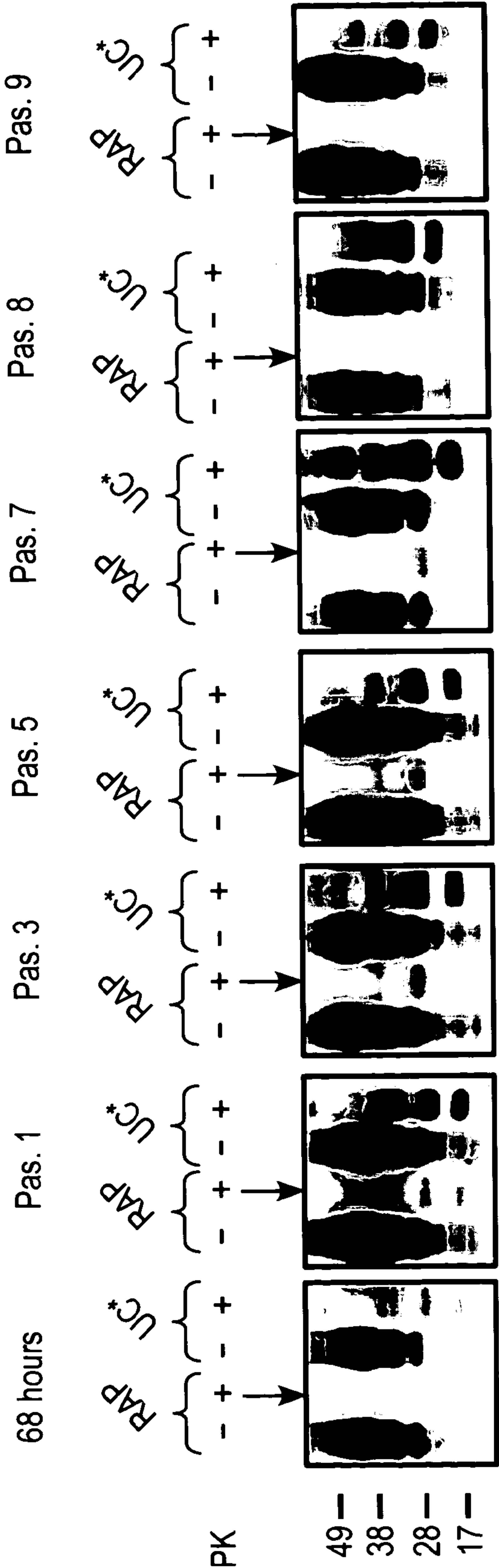


FIG. 6 Effect of 500 nM RAP on PrPres formation in bone-marrow derived 336-2-BM-Fu2 cell line persistently infected with Fukuoka



- Cells were grown for 24 hours following the treatment with RAP.
- Proteins were concentrated by Methanol precipitation.
- (-) and (+) Proteinase K (PK)- treated samples represent total PrP and pathologic PrPres, respectively.
- Anti-PrP monoclonal antibody 6D11 diluted 1:10,000.
- Inhibition of PrPres formation by RAP is shown by arrows.

FIG. 7 Inhibitory effect of multiple treatments with 250 nM RAP on PrPress formation in bone-marrow derived 336-2-BM-Fu2 cell line persistently infected with Fukuoka



UC* - untreated control

- Proteins were concentrated by Methanol precipitation.
- (-) and (+) Proteinase K (PK)- treated samples represent total PrP and pathologic PrPress, respectively.
- Anti-PrP monoclonal antibody 6D11 diluted 1:10,000.
- Inhibition of PrPress formation by RAP is shown by arrows.

FIG. 8

Human RAP polypeptide sequence (SEQ ID NO: 1); signal peptide is shown in bold.

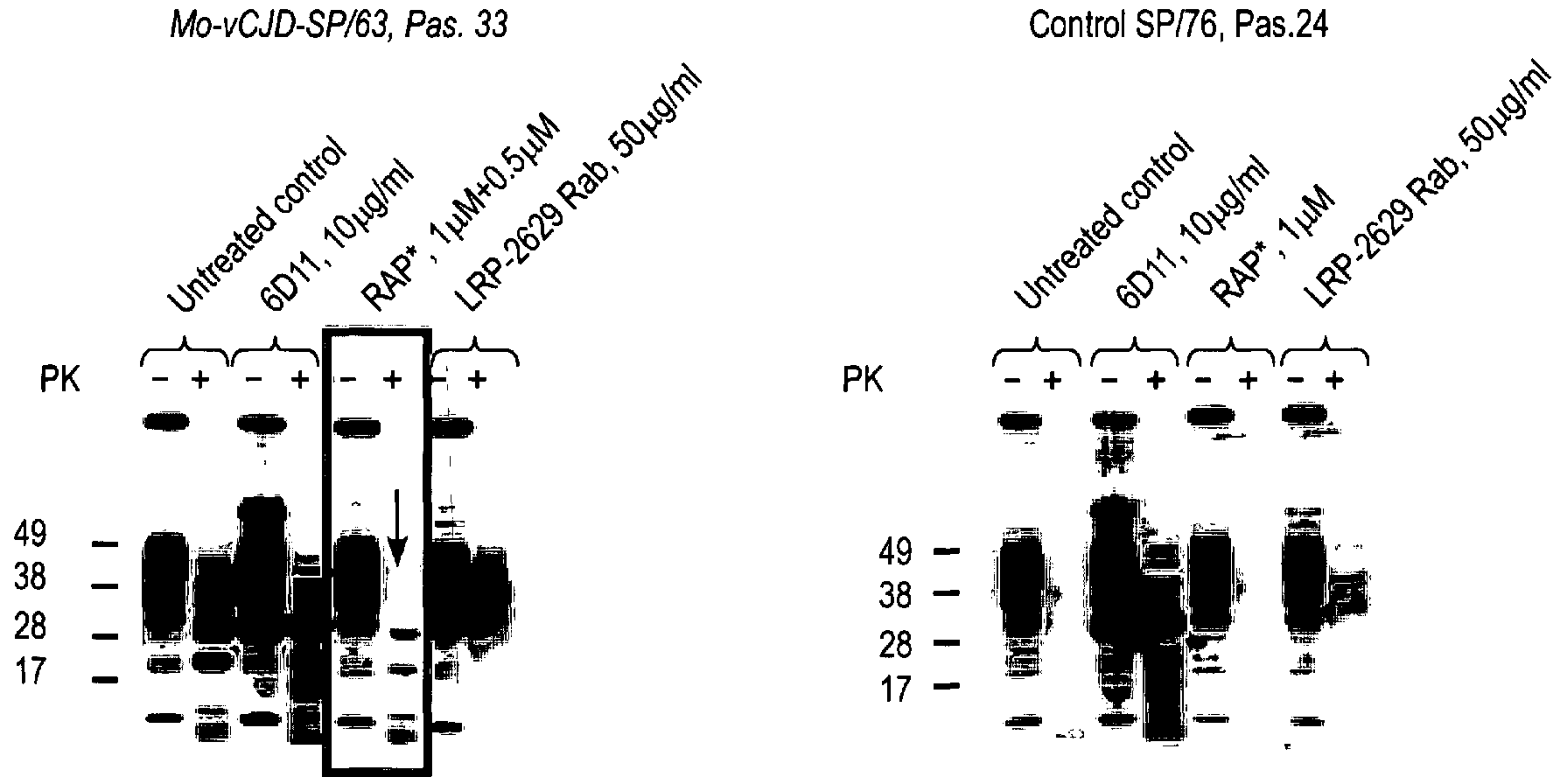
<u>10</u>	<u>20</u>	<u>30</u>	<u>40</u>	<u>50</u>	<u>60</u>
MAPRRRSFL	RGLPALLLL	LFLGPWPAAS	HGGKYSREKN	QPKPSPKRES	GEEFRMEKLN
<u>70</u>	<u>80</u>	<u>90</u>	<u>100</u>	<u>110</u>	<u>120</u>
QLWEKAQRLH	LPPVRRLAELH	ADLKIQRDE	LAWKKLKL DG	LDEdgeKEAR	LIRNLNVILA
<u>130</u>	<u>140</u>	<u>150</u>	<u>160</u>	<u>170</u>	<u>180</u>
KYGLDGKKDA	RQVTSNSLSG	TQEDGLDDPR	LEKLWHKAKT	SGKFSGEELD	KLWREFLHHK
<u>190</u>	<u>200</u>	<u>210</u>	<u>220</u>	<u>230</u>	<u>240</u>
EKVHEYNVLL	ETLSRTEEIH	ENVISPSDLS	DIKGSVLHSR	HTELKEKLRS	INQGLDRLRR
<u>250</u>	<u>260</u>	<u>270</u>	<u>280</u>	<u>290</u>	<u>300</u>
VSHQGYSTEA	EFEEPRVIDL	WDLAQSANLT	DKELEAFREE	LKHFEAKIEK	HNHYQKQLEI
<u>310</u>	<u>320</u>	<u>330</u>	<u>340</u>	<u>350</u>	
AHEKLRHAES	VGDGERVRS	REKHALLEGR	TKELGYTVKK	HLQDLSGRIS	RARHNEL

9/9

620

FIG. 1

Effect of RAP on PrP^{Sc} formation in spleen-derived mo-vCJD-SP/63 cell line: 24 hours after the treatment



- Control SP/76 and mo-vCJD-SP/63 cells were treated with RAP twice initially with 1 µM and in 8 hours with 0.5 µM.
- Proteins were concentrated by Methanol precipitation.
- (-) and (+) Proteinase K (PK)- treated samples represent total PrP and pathologic PrP^{Sc}, respectively.
- Anti-PrP monoclonal antibody 6D11 diluted 1:10,000.
- Inhibition of PrP^{Sc} formation by RAP is shown by arrows.