[0011] US Patent No. 8,088,359 and US Patent Application Publication No. 20120252868 relate to methods of treating hearing loss and phantom hearing.

[0012] US Patent Application Publication No. 20110142917 discloses non-invasive methods of delivering dsRNA molecules to the ear.

[0013] US Patent Application Publication No. 20110034534 relates to, *inter alia*, dsRNA molecules to target CAPNS1.

[0014] US Patent Application Publication No. 20110229557 relates to dsRNA molecules to various gene targets, including CASP2, useful in treating eye diseases.

[0015] PCT Patent Publication No. WO2011/163436 discloses dsRNA to target RHOA.

[0016] Tinnitus and Ménière's disease affects many individuals worldwide and current therapies have not been successful at preventing progression of neuronal degeneration and the attendant hearing loss. A therapeutic treatment, which would protect the inner ear neurons, including hair cells and spiral and vestibular ganglion cells, from damage and cell death (e.g. apoptosis), and thereby attenuate or prevent hearing loss in, for example, Ménière's patients would be highly desirable. It is, accordingly, an aspect to provide methods for neuroprotection of neurons in a subject's ear, including human subjects suffering from tinnitus or Ménière's disease or having similar symtoms, using dsRNA compounds not previously known to have such activity.

SUMMARY ~~OF THE INVENTION~~

[0017] This disclosure is directed to methods and kits for providing neuroprotection to neurons in subject's ear, for example to spiral ganglion cells and vestibular ganglion cells.

[0017a] In one aspect, there is provided use of a double-stranded RNA (dsRNA) compound which down regulates expression of a CASP2 gene encoding an mRNA having a sequence set forth in any one of SEQ ID NO:1-3, for the preparation of a medicament for providing neuroprotection of a neuron in the ear of a subject in need thereof.

[0017b] In one ~~aspect~~embodiment, disclosed herein is a double-stranded RNA (dsRNA) compound which down regulates expression of a CASP2 gene, a NOX3 gene, a CAPNS 1 gene or a RHOA gene encoding an mRNA having a sequence set forth in any one of SEQ ID

NO: 1-3, SEQ ID NO:4, SEQ ID NO:5-6 or SEQ ID NO:7 for use in neuroprotection of a neuron in the ear of a subject in need thereof. In various ~~aspects~~ embodiments provided is a method providing neuroprotection to neurons in the ear, the method comprising administering to the subject's ear a therapeutically effective amount of a double- stranded RNA (dsRNA) molecule compound which down

noted that the dsRNA compound can be administered as the compound per se or as pharmaceutically acceptable salt and can be administered alone or as an active ingredient in combination with pharmaceutically acceptable carriers, solvents, diluents, excipients, adjuvants and vehicles.

[0028] The dsRNA compounds are preferably administered transtympanically, including topically or via injection. Liquid forms may be prepared for administration. The liquid compositions include aqueous solutions, with and without organic co-solvents, aqueous or oil suspensions, emulsions with edible oils, as well as similar pharmaceutical vehicles. In one embodiment the administration comprises topical administration, in particular topical administration to the ear canal, topical administration to the tympanic membrane, or a combination thereof, thereby allowing the dsRNA compounds to pass the tympanic membrane. In some embodiments the compounds of the present application are applied to the tympanic membrane as an eardrop. In some preferred embodiments the dsRNA compounds are administered by transtympanic injection or by eardrops.

[0029] In various embodiments, particularly embodiments in which the pharmaceutical compositions of the ~~invention~~ disclosure are administered topically, the pharmaceutical compositions further comprise a permeability enhancer, also known as penetration enhancer.

[0030] In various embodiments the penetration enhancer is selected from any compound or any combination of two ore more compounds that enhance the penetration of a therapeutic oligonucleotide through the tympanic membrane in the ear of a subject suffering from Ménière's disease. In certain embodiments the permeability enhancer is a polyol. In some embodiments the oligonucleotide is in admixture with a polyol. In some embodiments the polyol is selected from the group consisting of glycerol, propylene glycol, polyethylene glycol, sorbitol, xylitol, maltitol and combinations thereof.

[0031] According to one embodiment the polyol is glycerol. In various embodiments glycerol is present at a final concentration of about 0.1% to about 35%; about 1% to about 30%; about 5% to about 25%, preferably about 10% to about 20% by volume of the pharmaceutical composition.

[0032] In some embodiments, the pharmaceutical composition is applied to the ear canal when the subject's head is tilted to one side and the treated ear is facing upward. In some

embodiments, the pharmaceutical composition is applied to the ear using a receptacle for liquid, for example using a dropper of for example, 10-100 microliter per drop, or a wick.

An additional embodiment of the present ~~invention~~ disclosure provides for the use of any of the above compositions in the preparation of a medicament for the treatment of a subject suffering from Meniere's disease. Further provided is compound that inhibits expression of CASP2 for providing neuroprotection to a neuron in an ear of a subject in need thereof.

[0033] The methods, materials, and examples that will now be described are illustrative only and are not intended to be limiting; materials and methods similar or equivalent to those described herein can be used in practice or testing of the invention. Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

[0034] This disclosure is intended to cover any and all adaptations or variations of combination of features that are disclosed in the various embodiments herein. Although specific embodiments have been illustrated and described herein, it should be appreciated that the invention encompasses any arrangement of the features of these embodiments to achieve the same purpose. Combinations of the above features, to form embodiments not specifically described herein, will be apparent to those of skill in the art upon reviewing the instant description.

BRIEF DESCRIPTION OF THE FIGURES

[0035] Figure 1 shows histological sections of wild type (left panels) and $Phex^{hyp\text{-}Duk}$ mutant (right panels) inner ear tissue. Arrows show Endolymphatic hydrop (ELH). In left bottom panel, G refers to spiral ganglion neurons, and in right bottom panel M refers to mutant spiral ganglion neurons, of which there are fewer (compare G to M) in the $Phex^{hyp\text{-}Duk}$ mutant mice. $Phex^{hyp\text{-}Duk}$ mutant mice spontaneously develop endolymphatic hydrop and are thus presented with balance problems and hearing loss phenotype. One of the primary defects is the loss of neurons in the inner ear ganglia (vestibular and spiral) by cell death, or apoptosis.

[0036] Figure 2 shows experimental design of safety assessment of siRNA by eardrops delivery. "Px" refers to x days postpartum.

CLAIMS

1. Use of ~~A~~ a double-stranded RNA (dsRNA) compound which down regulates expression of a CASP2 gene encoding an mRNA having a sequence set forth in any one of SEQ ID NO:1-3, for ~~use in~~ the preparation of a medicament for providing neuroprotection of a neuron in the ear of a subject in need thereof.

~~2. A method of providing neuroprotection to a neuron in the ear of a subject in need thereof, comprising administering to the subject's ear a therapeutically effective amount of a double-stranded RNA (dsRNA) compound which down regulates expression of a CASP2 gene, wherein the CASP2 gene encodes a mRNA having a sequence set forth in any one of SEQ ID NO:1-3, so as to thereby provide neuroprotection to the neuron in the subject's ear.~~

~~3.~~2. The use ~~or method~~ of claim~~s~~ 1 ~~or 2~~, wherein the dsRNA compound comprises a sense strand with a nucleotide sequence set forth in SEQ ID NO:8 and an antisense strand with a nucleotide sequence set forth in SEQ ID NO:9.

~~4.~~3. The use ~~or method~~ of any one of claims 1 ~~to 3~~or 2, wherein the neuron ~~is, or~~ is comprised within~~,~~ a ganglion.

~~5.~~4. The use ~~or method~~ of claim ~~4~~3, wherein the ganglion is a spiral ganglion or a vestibular ganglion.

~~6.~~5. The use ~~or method~~ of any one of claims 1 to ~~5~~4, wherein the neuroprotection comprises protecting the neuron from cell death.

~~7.~~6. The use ~~or method~~ of claim ~~6~~5, wherein cell death of the neuron comprises apoptotic cell death.

~~8.~~7. The use ~~or method~~ of claims ~~6~~ 5 or ~~7~~6, wherein cell death of the neuron is associated with one or more of: a disease or disorder, ischemia, physical/mechanical trauma, a chemical agent, an infectious agent, an immunologic reaction and a nutritional imbalance.

~~9.~~8. The use ~~or method~~ of claim ~~8~~7, wherein cell death of the neuron is associated with a disease or disorder.

~~10.~~9. The use ~~or method~~ of claim~~s~~ 1 ~~or 2~~, wherein the subject has or is at risk of developing one or more of a disease or condition associated with pathological abnormalities of an auditory organ or of a vestibular organ.

~~11.~~10. The use ~~or method~~ of claim ~~10~~9, wherein the disease or condition is associated with a symptom selected from the group consisting of: episodic vertigo, hearing loss, tinnitus and aural fullness.

~~12.~~11. The use ~~or method~~ of claim ~~10~~9, wherein the subject is afflicted with or susceptible to Ménière's disease.

~~13.~~12. The use ~~or method~~ of claim~~s~~ 1 ~~or 2~~, wherein the neuroprotection comprises restoration of cochlear function and/or of vestibular function.

~~14.~~13. The use ~~or method~~ of any of claims 1 to ~~13~~12, wherein the dsRNA compound down regulates CASP2 expression in a cell in the inner ear of the subject.

~~15.~~14. The use ~~or method~~ of any of claims 1 to ~~14~~13, wherein the dsRNA compound has the structure:

5′ z″-GCCAGAAUGUGGAACUCCU-Z′3′ (sense strand, SEQ ID NO:24)

3′ Z -CGGUCUUACACCUUGAGGA 5′(antisense strand, SEQ ID NO:25)

wherein each A, C, U, and G is an unmodified or modified ribonucleotide or an unconventional moiety and each consecutive ribonucleotide or unconventional moiety is joined to the next ribonucleotide or unconventional moiety by a phosphodiester bond; wherein the sense strand comprises, counting from the 5' terminus, an unmodified ribonucleotide at positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 19, a L-deoxycytidine at position 18 (set forth in SEQ ID NO:24); wherein the antisense strand comprises at least five alternating unmodified and 2'-O-methyl sugar modified ribonucleotides; and wherein z", Z and Z' are optional.

~~16.~~15. The use ~~or method~~ of claim ~~15~~14, wherein ~~the covalent bond is a phosphodiester bond; wherein~~ each Z and Z' is absent~~, wherein the sense strand comprises, counting from the 5' terminus, an unmodified ribonucleotide at positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 19, a L-deoxycytidine at position 18 (set forth in SEQ ID NO:24)~~; and wherein the antisense strand comprises, counting from the 5' terminus, a 2'O-

Methyl sugar modified ribonucleotide at each of positions 2, 4, 6, 8, 11, 13, 15, 17 and 19 and an unmodified ribonucleotide at each of positions 1, 3, 5, 7, 9, 10, 12, 14, 16 and 18 (set forth in SEQ ID NO:25).

~~17.~~16. The use ~~or method~~ of claim ~~16~~15, wherein z" is covalently attached to the 5' terminus of the sense strand (SEQ ID NO:26) and comprises an inverted deoxyabasic moiety.

~~18.~~17. The use ~~or method~~ of any of claims 1 to ~~17~~16, wherein the dsRNA molecule is formulated for transtympanic administration.

~~19.~~18. The use ~~or method~~ of any of claims 1 to 16~~claim 18~~, wherein the dsRNA molecule is formulated as eardrops.

~~20.~~19. The use ~~or method~~ of any of claims 1 to ~~19~~18, wherein the subject is a mammal.

~~21.~~20. The use ~~or method~~ of claim ~~20~~19, wherein the subject is a human.

~~22.A double stranded RNA (dsRNA) compound which down regulates expression of a CAPNS1 gene encoding an mRNA having a sequence set forth in any one of SEQ ID NO:5-6, for use in neuroprotection of a neuron in the ear of a subject in need thereof.~~

~~23.A method of providing neuroprotection to a neuron in the ear of a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a double-stranded RNA (dsRNA) molecule which down regulates expression of a CAPNS1 gene, wherein the CAPNS1 gene encodes a mRNA having a sequence set forth in any one of SEQ ID NO:5-6, so as to thereby provide neuroprotection to the neuron in the subject's ear.~~

~~24.The use or method of claims 22 or 23, wherein the neuron is, or is comprised within, a ganglion.~~

~~25.The use or method of claim 24, wherein the ganglion is a spiral ganglion or a vestibular ganglion.~~

~~26.The use or method of claims 22 or 23, wherein the neuroprotection comprises protecting the neuron from cell death.~~

~~27.The use or method of claim 26, wherein cell death of the neuron comprises apoptotic cell death.~~

28. The use or method of claims 26 or 27, wherein cell death of the neuron is associated with one or more of a disease or disorder, ischemia, physical/mechanical trauma, a chemical agent, an infectious agent, an immunologic reaction and a nutritional imbalance.

29. The use or method of claim 28, wherein cell death of the neuron is associated with a disease or disorder.

30. The use or method of claims 22 or 23, wherein the subject has or is at risk of developing one or more of a disease or condition associated with pathological abnormalities of an auditory organ or of a vestibular organ.

31. The use or method of claim 30, wherein the disease or condition is associated with a symptom selected from the group consisting of episodic vertigo, hearing loss, tinnitus and aural fullness.

32. The use or method of claim 31, wherein the subject is afflicted with or susceptible to Ménière's disease.

33. The use or method of claims 22 or 23, wherein the neuroprotection comprises partial or full restoration of cochlear function and/or of vestibular function.

34. The use or method of any of claims 22 to 33, wherein the dsRNA compound down regulates CAPNS1 expression in a cell in the inner ear of the subject.

35. A double-stranded RNA (dsRNA) compound which down regulates expression of a NOX3 gene encoding an mRNA having a sequence set forth in SEQ ID NO: 4, for use in neuroprotection of a neuron in the ear of a subject in need thereof.

36. A method of providing neuroprotection to a neuron in the ear of a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a double-stranded RNA (dsRNA) molecule which down regulates expression of a NOX3 gene, wherein the NOX3 gene encodes a mRNA having a sequence set forth in SEQ ID NO:4, so as to thereby provide neuroprotection to the neuron in the subject's ear.

37. The use or method of claims 35 or 36, wherein the neuron is, or is comprised within, a ganglion.

38. The use or method of claim 37, wherein the ganglion is a spiral ganglion or a vestibular ganglion.

39. The use or method of any one of claims 35 to 38, wherein the neuroprotection comprises protecting the neuron from cell death.

40. The use or method of claim 39, wherein cell death of the neuron comprises apoptotic cell death.

41. The use or method of claims 39 or 40, wherein cell death of the neuron is associated with one or more of a disease or disorder, ischemia, physical/mechanical trauma, a chemical agent, an infectious agent, an immunologic reaction and a nutritional imbalance.

42. The use or method of claim 41, wherein cell death of the neuron is associated with a disease or disorder.

43. The use or method of claims 35 or 36, wherein the subject has or is at risk of developing one or more of a disease or condition associated with pathological abnormalities of an auditory organ or of a vestibular organ.

44. The use or method of claim 42 wherein the disease or condition is associated with a symptom selected from the group consisting of episodic vertigo, hearing loss, tinnitus and aural fullness.

45. The use or method of claim 43, wherein the subject is afflicted with or susceptible to Ménière's disease.

46. The use or method of claim 35 or 36, wherein the neuroprotection comprises partial or full restoration of cochlear function and/or of vestibular function.

47. The use or method of any of claims 35 to 46, wherein the dsRNA compound down regulates NOX3 expression in a cell in the inner ear of the subject.

48. A double-stranded RNA (dsRNA) compound which down regulates expression of a RHOA gene encoding an mRNA having a sequence set forth SEQ ID NO:7, for use in neuroprotection of a neuron in the ear of a subject in need thereof.

49. A method of providing neuroprotection to a neuron in the ear of a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a double-stranded RNA (dsRNA) molecule which down regulates expression of a RHOA gene, wherein the RHOA gene encodes a mRNA having a sequence set forth in SEQ ID NO:7, so as to thereby provide neuroprotection to the neuron in the subject's ear.

50. The use or method of claims 48 or 49, wherein the neuron is, or is comprised within, a ganglion.

51. The use or method of claim 50, wherein the ganglion is a spiral ganglion or a vestibular ganglion.

52. The use or method of any one of claims 48 to 51, wherein the neuroprotection comprises protecting the neuron from cell death.

53. The use or method of claim 52, wherein cell death of the neuron comprises apoptotic cell death.

54. The use or method of claims 52 or 53, wherein cell death of the neuron is associated with one or more of a disease or disorder, ischemia, physical/mechanical trauma, a chemical agent, an infectious agent, an immunologic reaction and a nutritional imbalance.

55. The use or method of claim 54, wherein cell death of the neuron is associated with a disease or disorder.

56. The use or method of claims 48 or 49, wherein the subject has or is at risk of developing one or more of a disease or condition associated with pathological abnormalities of an auditory organ or of a vestibular organ.

57. The use or method of claim 56, wherein the disease or condition is associated with a symptom selected from the group consisting of episodic vertigo, hearing loss, tinnitus and aural fullness.

58. The use or method of claim 56, wherein the subject is afflicted with or susceptible to Ménière's disease.

59. The use or method of claims 48 or 49, wherein the neuroprotection comprises partial or full restoration of cochlear function and/or of vestibular function.

60. The use or method of any of claims 48 to 59, wherein the dsRNA compound down regulates RHOA expression in a cell in the inner ear of the subject.

61. A kit for providing neuroprotection to a neuron in an ear of a subject in need thereof comprising a dsRNA compound which down regulates expression of CASP2, NOX3, CAPNS1 or RHOA in the subject.

62. The kit of claim 61 wherein the dsRNA compound down regulates expression of CASP2.

63. The kit of claim 61 wherein the dsRNA compound down regulates expression of NOX3.

64. The kit of claim 61 wherein the dsRNA compound down regulates expression of CAPNS1.

65. The kit of claim 61 wherein the dsRNA compound down regulates expression of RHOA.

66. The kit of anyone of claims 61-65, wherein the neuron is, or is comprised within, a ganglion.

67. The kit of claim 66, wherein the ganglion is a spiral ganglion or a vestibular ganglion.

68. The kit of any one of claims 61, 62, 66 or 67, wherein the dsRNA compound has the double-stranded structure:

5′ z″-GCCAGAAUGUGGAACUCCU-Z′3' (sense strand, SEQ ID NO:8)

3' Z -CGGUCUUACACCUUGAGGA 5'(antisense strand, SEQ ID NO:9)

wherein each A, C, U, and G is an unmodified ribonucleotide, a modified ribonucleotide or an unconventional moiety and each consecutive ribonucleotide or unconventional moiety is joined to the next ribonucleotide or unconventional moiety by a covalent bond;

wherein each of Z and Z' is independently present or absent, but if present independently includes 1-5 consecutive nucleotides or non-nucleotide moieties or a

combination thereof covalently attached at the 3' terminus of the strand in which it is present; and

wherein z" may be present or absent, but if present is a capping moiety covalently attached at the 5' terminus of the sense strand.

69. The kit of claim 68, wherein the covalent bond is a phosphodiester bond; wherein each Z and Z' is absent, wherein the sense strand comprises, counting from the 5' terminus, an unmodified ribonucleotide at positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 19, a L-deoxycytidine at position 18, an inverted deoxyabasic moiety covalently attached to the 5' terminus (set forth in SEQ ID NO:26); and wherein the antisense strand comprises, counting from the 5' terminus, a 2'O-Methyl sugar modified ribonucleotide at each of positions 2, 4, 6, 8, 11, 13, 15, 17 and 19 and an unmodified ribonucleotide at each of positions 1, 3, 5, 7, 9, 10, 12, 14, 16 and 18 (set forth in SEQ ID NO:27).

70. The kit of claim 69, wherein z" is present and comprises an inverted deoxyabasic moiety.

71. The kit of any of claims 61 to 70, further comprising a device for transtympanic administration of the dsRNA compound to the subject.