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(54) Title: VEGF ANTAGONIST FORMULATIONS SUITABLE FOR INTRAVITREAL ADMINISTRATION

(57) Abstract: Ophthalmic formulations of a vascular endothelial growth factor (VEGF)-specific fusion protein antagonist are provided suitable for intravitreal administration to the eye. The ophthalmic formulations include a stable liquid formulation and a lyophilizable formulation. Preferably, the protein antagonist has the amino acid sequence shown in SEQ ID NO:4.

## VEGF ANTAGONIST FORMULATIONS SUITABLE FOR INTRAVITREAL ADMINISTRATION

### BACKGROUND OF INVENTION

#### Field of the Invention

[0001] The present invention is directed to pharmaceutical formulations suitable for intravitreal administration comprising agents capable of inhibiting vascular endothelial growth factor (VEGF), and to methods for making and using such formulations. The invention includes liquid pharmaceutical formulations having increased stability, as well as formulations that may be lyophilize and reconstituted for intravitreal administration.

#### Statement of Related Art

[0002] Vascular endothelial growth factor (VEGF) expression is nearly ubiquitous in human cancer, consistent with its role as a key mediator of tumor neoangiogenesis. Blockade of VEGF function, by binding to the molecule or its VEGFR-2 receptor, inhibits growth of implanted tumor cells in multiple different xenograft models (see, for example, Gerber et al. (2000) Cancer Res. 60:6253-6258). A soluble VEGF-specific fusion protein antagonist, termed a "VEGF trap" has been described (Kim et al. (2002) Proc. Natl. Acad. Sci. USA 99:11399-404; Holash et al. (2002) Proc. Natl. Acad. Sci. USA 99:11393-8).

[0003] Ophthalmic formulations are known, see for example, U.S. 7,033,604 and 6,777,429. An ophthalmic formulation of a VEGF antibody is described in US 6,676,941.

[0004] Lyophilization (freeze drying under controlled conditions) is commonly used for long-term storage of proteins. The lyophilized protein is substantially resistant to degradation, aggregation, oxidation, and other degenerative processes while in the freeze-dried state (see, for example, U.S. 6,436,897).

### BRIEF SUMMARY OF THE INVENTION

[0005] Stable formulations of a VEGF-specific fusion protein antagonist are provided. Pharmaceutically acceptable formulations are provided that comprise a VEGF "trap" antagonist with a pharmaceutically acceptable carrier. In specific embodiments, liquid and lyophilized formulations are provided.

[0006] In a first aspect, a stable liquid ophthalmic formulation of a VEGF-specific fusion protein antagonist is provided, comprising a fusion protein that comprises a receptor component consisting essentially of an immunoglobulin-like (Ig) domain 2 of a first VEGF receptor and Ig domain 3 of a second VEGF receptor, and a multimerizing component (also termed a "VEGF trap"). In a specific embodiment of the VEGF-specific fusion protein antagonist, the first VEGF receptor is Flt1 and the second VEGF receptor is Flk1 or Flt4. In a more specific embodiment the fusion protein has the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4. Preferably,

the VEGF antagonist is a dimer comprising two fusion proteins of SEQ ID NO:4.

**[0007]** In one aspect, a stable liquid ophthalmic formulation is provided that comprises 1-100 mg/ml VEGF-specific fusion protein antagonist, 0.01-5% of one or more organic co-solvent(s), 30-150 mM of one or more tonicity agent(s), 5-40 mM of a buffering agent, and optionally, 1.0-7.5% of a stabilizing agent, pH between about 5.8-7.0.

**[0008]** In one or more specific embodiments, the organic co-solvent may be polysorbate, for example, polysorbate 20 or polysorbate 80, polyethylene glycol (PEG), for example, PEG 3350, or propylene glycol, or a combination thereof; the tonicity agent may be, for example, sodium chloride or potassium chloride; the stabilizing agent may be sucrose, sorbitol, glycerol, trehalose, or mannitol; and the buffering agent may be, for example, phosphate buffer. In a specific embodiment, the phosphate buffer is a sodium phosphate buffer.

**[0009]** In various embodiments, the organic co-solvent is polysorbate and/or PEG, the stabilizing agent is sucrose, the buffering agent is phosphate buffer, and the tonicity agent is sodium chloride.

**[0010]** More specifically, the stable liquid ophthalmic formulation comprises about 40-50 mg/ml of the VEGF antagonist (SEQ ID NO:4), about 10 mM phosphate buffer, 0.01-3% polysorbate and/or PEG, 40-135 mM sodium chloride, and optionally 5.0% sucrose, pH about 6.2-6.3.

**[0011]** In a specific preferred embodiment, the stable liquid ophthalmic formulation comprises about 50 mg/ml of the VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 50 mM sodium chloride, 0.1% polysorbate, and 5% sucrose, pH about 6.2-6.3.

**[0012]** In a specific preferred embodiment, the stable liquid ophthalmic formulation comprises about 50 mg/ml of the VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 50 mM sodium chloride, 3% PEG, and 5% sucrose, pH about 6.2-6.3.

**[0013]** In a specific preferred embodiment, the stable liquid ophthalmic formulation comprises about 40 mg/ml of the VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 40 mM sodium chloride, 0.03% polysorbate, and 5% sucrose, pH about 6.2-6.3.

**[0014]** In a specific preferred embodiment, the stable liquid ophthalmic formulation comprises about 40 mg/ml of the VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 135 mM sodium chloride, and 0.03% polysorbate, pH about 6.2-6.3.

**[0015]** In another aspect, a stable liquid ophthalmic formulation is provided that comprises 1-100 mg/ml VEGF-specific fusion protein antagonist; 0.01-5% of one or more organic co-solvent(s); 5-40 mM of a buffering agent; and optionally 30-150 mM of one or more tonicity agent(s) and/or 1.0-7.5% of a stabilizing agent; having a pH between about 5.8-7.0.

**[0016]** In various embodiments, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 10 to about 80 mg/ml. In various embodiments, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 10, about 20, about 30, about 40, about 50, about 60, about 70, or about 80 mg/ml. In a preferred embodiment, the VEGF antagonist

(SEQ ID NO:4) is present at a concentration of about 40 mg/ml.

**[0017]** In another embodiment, the stabilizing agent is selected from one or more of sucrose, sorbitol, glycerol, trehalose, and mannitol.

**[0018]** In another embodiment, the organic co-solvent is selected from one or more of polysorbate, for example, polysorbate 20 or polysorbate 80, polyethylene glycol (PEG), for example, PEG 3350, and propylene glycol.

**[0019]** In another embodiment, the buffer is a phosphate buffer, for example, sodium phosphate.

**[0020]** In another embodiment, the tonicity agent is a salt, for example, sodium chloride.

**[0021]** In one embodiment, the stable liquid ophthalmic formulation comprises 10 mM sodium phosphate buffer, about 0.03 to about 0.1% polysorbate and/or about 3% PEG or propylene glycol, about 40 mM sodium chloride, and about 5% sucrose. In a specific embodiment, the stable liquid ophthalmic formulation comprises 10 mM sodium phosphate buffer, about 0.03% polysorbate, about 40 mM sodium chloride, and about 5% sucrose. In another specific embodiment, the pH of the formulation is about 6.2 to about 6.3. In another specific embodiment, the pH is achieved by mixing mono- and dibasic sodium phosphate to the desired pH without acid/base titration.

**[0022]** In a specific embodiment, the stable liquid ophthalmic formulation consists essentially of a VEGF antagonist (SEQ ID NO:4) at 40 mg/ml, 10 mM sodium phosphate buffer, polysorbate at 0.03%, sodium chloride at 40 mM, and sucrose at 5%, pH 6.2-6.3.

**[0023]** In another aspect, a stable liquid ophthalmic formulation is provided that comprises about 10 to about 80 mg/ml VEGF antagonist, about 10 mM sodium phosphate buffer, about 0.03% polysorbate, and about 135 mM sodium chloride, pH of 6.2 to 6.3.

**[0024]** In various embodiments, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 10 to about 80 mg/ml. In various embodiments, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 10, about 20, about 30, about 40, about 50, about 60, about 70, or about 80 mg/ml. In a specific embodiment, the VEGF antagonist (SEQ ID NO:4) is present at a concentration of about 40 mg/ml.

**[0025]** In one embodiment, the stable liquid ophthalmic formulation comprises 40 mg/ml of VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 0.03% polysorbate, and 135 mM sodium chloride at pH 6.2-6.3. In a specific embodiment, the stable liquid ophthalmic formulation consists essentially of 40 mg/ml of VEGF antagonist (SEQ ID NO:4), 10 mM sodium phosphate buffer, 0.03% polysorbate, and 135 mM sodium chloride at pH 6.2-6.3.

**[0026]** In another aspect, a lyophilizable formulation of a VEGF antagonist is provided, wherein upon lyophilization followed by reconstitution, a stable liquid ophthalmic formulation as described herein is obtained.

**[0027]** In another aspect, a lyophilizable formulation of a vascular endothelial growth factor

(VEGF)-specific fusion protein antagonist is provided, comprising 5-50 mg/ml of the VEGF antagonist, 5-25 mM buffer, such as phosphate buffer, 0.01 to 0.15% of one or more of an organic co-solvent, such as polysorbate, propylene glycol and/or PEG, and optionally 1-10% of a stabilizing agent such as sucrose, sorbitol, trehalose, glycerol, or mannitol, pH about 5.8-7.0. In various embodiments, the VEGF antagonist (SEQ ID NO:4) is present at about 5, about 10, about 20, about 30, or about 40 mg/ml. In a specific embodiment, the lyophilizable ophthalmic formulation of the invention comprises 20 mg/ml of the VEGF antagonist, 10 mM sodium phosphate buffer, 0.03% polysorbate, 0.1% PEG, and 2.5% sucrose, pH about 6.2-6.3. In further embodiments, the lyophilizable formulation further comprises sodium chloride. In a specific embodiment, the sodium chloride is present at a concentration of about 20 mM. In another specific embodiment, the sodium chloride is present at a concentration of about 67.5 mM.

**[0028]** In another specific embodiment, the lyophilizable ophthalmic formulation of the invention comprises 20 mg/ml of the VEGF antagonist, 5 mM sodium phosphate buffer, 0.015% polysorbate, 20 mM sodium chloride, and 2.5% sucrose, pH about 6.2-6.3.

**[0029]** In another embodiment, the lyophilizable ophthalmic formulation comprises 5 mg/ml, 10 mg/ml, or 40 mg/ml VEGF antagonist, 5 mM sodium phosphate buffer, 0.015% polysorbate, 20 mM sodium chloride, and 2.5% sucrose, at pH 6.2-6.3. In a specific embodiment, the lyophilizable ophthalmic formulation consists essentially of 5 mg/ml, 10 mg/ml, or 40 mg/ml VEGF antagonist (SEQ ID NO:4), 5 mM sodium phosphate buffer, 0.015% polysorbate, 20 mM sodium chloride, and 2.5% sucrose, at pH 6.2-6.3.

**[0030]** In another specific embodiment, the lyophilizable ophthalmic formulation comprises 20 mg/ml of the VEGF antagonist, 5 mM sodium phosphate buffer, 0.015% polysorbate, and 67.5 mM sodium chloride, pH about 6.2-6.3. In a more specific embodiment, the lyophilizable ophthalmic formulation consists essentially of 20 mg/ml of the VEGF antagonist (SEQ ID NO:4), 5 mM sodium phosphate buffer, 0.015% polysorbate, and 67.5 mM sodium chloride, pH 6.2-6.3.

**[0031]** In another specific embodiment, the lyophilizable ophthalmic formulation comprises 5 mg/ml, 10 mg/ml, or 40 mg/ml VEGF antagonist, 5 mM sodium phosphate buffer, 0.015% polysorbate, and 67.5 mM sodium chloride, pH about 6.2-6.3. In a more specific embodiment, the lyophilizable ophthalmic formulation consists essentially of 5 mg/ml, 10 mg/ml, or 40 mg/ml VEGF antagonist (SEQ ID NO:4), 5 mM sodium phosphate buffer, 0.015% polysorbate, and 67.5 mM sodium chloride, pH about 6.2-6.3.

**[0032]** Generally, the reconstituted formulation is about 2 times the concentration of the pre-lyophilized formulation, e.g., a 20 mg fusion protein/ml pre-lyophilized formulation is reconstituted to a final formulation of 40 mg fusion protein/ml.

**[0033]** Generally, the lyophilized formulation is reconstituted with sterile water suitable for injection. In one embodiment, the reconstitution liquid is bacteriostatic water.

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