

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
13 November 2003 (13.11.2003)

PCT

(10) International Publication Number
WO 03/093446 A2

- (51) International Patent Classification⁷: C12N (74) Agents: FARBER, Mark et al.; Acorda Therapeutics, Inc., 15 Skyline Drive, Hawthorne, NY 10532 (US).
- (21) International Application Number: PCT/US03/14157 (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (22) International Filing Date: 5 May 2003 (05.05.2003) (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 60/377,675 4 May 2002 (04.05.2002) US
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- Published:
— without international search report and to be republished upon receipt of that report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 03/093446 A2

(54) Title: ANTIBODIES FOR ENHANCING NEURITE OUTGROWTH

(57) Abstract: Neural outgrowth in the central nervous system is achieved by administering a neutralizing antibody, or fragments thereof, or combination of antibodies to overcome inhibitory molecules that inhibit or contribute to the inhibition of nervous tissue regeneration.

ANTIBODIES FOR ENHANCING NEURITE OUTGROWTH

CROSS-REFERENCE TO RELATED APPLICATION

Priority to U.S. Provisional Application Serial No. 60/377,675 filed May 4, 2002 is hereby claimed.

BACKGROUND

5 TECHNICAL FIELD

Antibodies which are reactive to inhibitory molecules, can enhance neurite outgrowth and generation in injured central nervous system sites.

DESCRIPTION OF RELATED ART

10 The ability of neurons to extend neurites is of primary importance in establishing nerve connections during development. A neurite is a process growing from a neuron, or nerve cell, in culture and generically applies to both dendrites and axons. The extension of neurons is also required to regenerate or re-establish neuronal connections which may have been lost as a result of injury or disease.

15 A number of molecules have been identified that inhibit the regeneration of injured neurites in the adult mammalian central nervous system. These molecules are expressed in adult mammalian central nervous system ("CNS"), for the most part, on oligodendrocytes and myelin, and on the glial scars of injured CNS tissue. Injury sites on the CNS develop glial scarring by an increase

in the deposition of extracellular matrix molecules by astrocytes and oligodendrocytes at the site of injury. The extracellular matrix molecules of the scar tissue also include chondroitin sulfate proteoglycans (CSPGs). CSPGs inhibit nerve tissue growth in vitro, and nerve tissue regeneration at CSPGs rich regions in vivo.

Proteoglycans contain long carbohydrate side-chains of glycosaminoglycans, which are covalently attached to a core protein by a glycosidic linkage. The glycosaminoglycans consist of repeating disaccharide units, all bearing negatively charged groups, usually sulfate or carbohydrate groups. The proteoglycan core protein is typically biologically inactive and the glycosaminoglycan chains account for inhibitory activity. (Nieto-Sampedro, M., Neurite outgrowth inhibitors in gliotic tissue. *Adv. Exp. Med. Biol.* 468: 207-24 (1999). The glycosaminoglycans (GAGs), most notably chondroitin sulfate (CS) and dermatan sulfate (DS), are important components of CSPG. They are inhibitory molecules that contribute to the lack of regeneration of the CNS in adult mammals, by hindering axonal and neuritic growth. (However, CSPGs are important in neuronal guidance and patterning during development, rather than inhibition).

The chondroitin sulfate family includes seven sub-types designated unsulfated chondroitin sulfate, oversulfated chondroitin sulfate, and chondroitin sulfates A-E, which vary in the number and position of their sulfate functional groups. Dermatan sulfate is also referred to as chondroitin sulfate B, and it differs in that iduronic acid is the predominant residue in the alternative hexuronic

acid position. Glycosaminoglycans are unbranched polysaccharides consisting of alternating hexosamine and hexuronic residues which carry sulfate groups in different positions. The GAGs are typically divided into three families according to the composition of the disaccharide backbone. These are: heparin/heparan sulfate [HexA-GlcNAc(SO.sub.4)]; chondroitin sulfate [HexA-GalNAc]; and keratan sulfate [Gal-GlcNAc].

Immunological studies using monoclonal antibodies (mAbs) have shown that the sulfation profile of chondroitin sulfate chains changes with concomitant specific spatio-temporal patterns in various tissues, suggesting that chondroitin sulfate ("CS") isoforms differing in sulfation position and degree perform distinct functions in development. (Mark, M.P. et al., Int. J. Dev. Biol. 34: 191-204(1990). Changes in the CS conformation of the CSPGs are believed to prohibit binding to glycosaminoglycan epitope, which recognize particular CS structure.

CNS- reactive antibodies have been identified that can neutralize and overcome these inhibitory molecules. By reacting with CNS tissues, the antibodies enhance neurite outgrowth and overcome substrates inhibitory to neurite growth.

Summary

This disclosure relates to a method of treating an injury to the central nervous system. Such a treatment involves administering antibodies that neutralize the inhibitory molecules expressed in a CNS injury, typically a glial scarring area.

Detailed Description

This disclosure relates to a method of administering antibodies to neutralize the inhibitory molecules often associated with diseased or injured CNS tissues. The neutralizing antibodies enhance the outgrowth of neurites in the CNS by providing a positive growth environment. The outgrowth of neurites will allow the regeneration of the injured or diseased neuronal tissues, thereby avoiding or reversing the motor function disabilities associated with such insults to CNS tissues.

When a nerve is severed, the distal neurites become separated from the nerve cell body and degenerate. Neurite death and degeneration leaves only the empty nerve sheath, which will also eventually degenerate. In addition, some degeneration of the proximal stump occurs. Where degeneration does not result in the death of the nerve cell body, neurons can regenerate by re-extension of the severed axons. This regeneration is more likely if it occurs at a sufficient distance from the nerve cell body. The newly regenerating neurites, or "nerve sprouts", grow distally toward the sheath of the distal portion of the severed nerve. If the neurites successfully enter the sheath, they will often grow down its length toward their synaptic target cells, and function may be restored.

Regeneration and regrowth of the neurites is impeded or prevented by scar formation, which can be stimulated by trauma or disease. The scar tissue is composed of extra cellular molecules, primarily chondroitin sulfate proteoglycans ("CSPGs").

The extent and orientation of nerve growth is regulated and guided by a variety of extra cellular matrix molecules, including CSPGs, which are synthesized by neurons as well as non-neuronal cells. These regulator molecules can be secreted or immobilized on the surface of the cell which produces them. The binding of the regulator molecule to a receptor on the neuronal cell surface causes a signal which regulates the intracellular molecules. Many types of molecules which regulate neuronal outgrowth are known. Some stimulate neurite growth (e.g., neurotrophic molecules, neurotransmitters, extracellular matrix molecules and cell adhesion molecules) while others function as inhibitors or negative regulators.

Antibodies have been isolated and identified which are suitable for overcoming neurite-inhibitory substrates. The antibodies are selected on the basis of their reactivity with CNS tissues. The antibodies are screened in a series of in vitro assays to determine their ability to neutralize inhibitory substrates, including CSPGs and white matter areas of brain sections, thereby promoting neurite outgrowth. Certain antibodies have been shown to promote and improve neurite outgrowths over inhibitory CSPGs and white matter tracts on unfixed brain sections. Identified antibodies have also been shown to enhance neurite outgrowth of the hippocampal and cortical neurons, when used as a substrate. In a preferred embodiment, the antibodies are monoclonal. In a more preferred embodiment, the antibodies are human and monoclonal. Suitable antibodies can be directed to CSPGs, cell adhesion molecules, and antibodies obtained from a spinal cord homogenate- immunized mammal. Suitable

antibodies include those described in U.S. Patent 5,591,629 and U.S. pending patent applications 08/692,084 filed August 6, 1996; 08/779,784 filed January 7, 1997; 09/322,862 filed May 28, 1999; 09/580,787 filed May 30, 2000; and 10/010,729 filed November 13, 2001. The disclosures of each application and
5 the patent are incorporated herein in their entirety.

The antibody, or fragments, or combination of antibodies, herein may be administered to a patient with a CNS injury or disease. The antibody, or fragments thereof, or combination of antibodies can be administered to the patient on a daily, weekly, bi-weekly or monthly basis, as medically determined
10 by the condition of the patient and the severity of the injury.

The antibodies or fragments can be typically administered in a composition comprising a pharmaceutical carrier. A pharmaceutical carrier can be any compatible, non-toxic substance suitable for delivery of the monoclonal antibodies to the patient, sterile water, alcohol, fats, waxes, and inert solids may
15 be included in the carrier. Pharmaceutically acceptable adjuvants (buffering agents, dispersing agent) may also be incorporated into the pharmaceutical composition.

The antibody/ pharmaceutical compositions may be administered parenterally, e.g., subcutaneously, intramuscularly or intravenously. Thus,
20 compositions for parental administration may include a solution of the antibody, antibody fragment or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions

are sterile and generally free of particulate matter. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody or antibody fragment in these formulations can vary widely, e.g., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Actual methods for preparing parenterally administrable compositions and adjustments necessary for administration to subjects will be known or apparent to those skilled in the art and are described in more detail in, for example, *Remington's Pharmaceutical Science*, 17th Ed., Mack Publishing Company, Easton, Pa (1985), which is incorporated herein by reference.

The regeneration of nerve cells in the affected CNS area allows the return of motor function and sensory function. Clinically relevant improvement will range from a detectable improvement to complete restoration of an impaired or lost nervous function, varying with the individual patient and injuries.

Although preferred and other embodiments of the invention have been described herein, further embodiments may be perceived by those skilled in the art without departing from the scope of the invention as defined by the following claims.

CLAIMS

What is claimed is:

1. A method of treating a subject having an injury to the central nervous system, comprising administering an effective amount of an antibody, or
5 fragments thereof, or combination of antibodies, that neutralizes inhibitory molecules expressed around the site of a mammalian central nervous system injury.
2. A method as in claim 1, wherein the neutralizing antibody, or fragments thereof, or combination of antibodies, are reactive with central nervous
10 system tissue.
3. A method as in claim 1, wherein the neutralizing antibody, or fragments thereof, or combination of antibodies, are reactive with inhibitory molecules expressed by the central nervous system.
4. A method as in claim 3, wherein the neutralizing antibody, or fragments
15 thereof, or combination of antibodies, are reactive with chondroitin sulfate proteoglycans.
5. A method as in claim 1, wherein a neutralizing antibody, or fragments thereof, or combination of antibodies, allow outgrowth of neurites from the site of injury.
- 20 6. A method as in claim 1, wherein the neutralizing antibody, or fragments thereof, or a combination of antibodies, are administered locally to the site of injury.

7. A method as in claim 6, wherein the neutralizing antibody, or fragments thereof, or combination of antibodies, is administered as a composition comprising a pharmaceutically acceptable carrier.
8. A method as in claim 7, wherein local administration is to the site of injury
5 is achieved using a mode selected from a catheter, a syringe, and direct application to the injury.
9. A method as in claim 8, wherein the effective amount is administered in multiple doses.
10. A method as in claim 9, wherein the central nervous system injury is
10 trauma to the spinal cord.
11. A method as in claim 1, wherein said mammal is a human.
12. A method of promoting neurite outgrowth comprising contacting neural cells with a composition comprising one or more antibodies, or fragments thereof, which are reactive with central nervous system tissue.
- 15 13. A method as in claim 12, wherein the composition is reactive to central nervous system inhibitory molecules.
14. A method as in claim 13, wherein the composition is reactive to chondroitin sulfate proteoglycans.
- 20 15. A method as in claim 13, wherein the composition is reactive to cellular adhesion molecules.
16. A method as in claim 13, wherein the composition is reactive to spinal cord homogenate.

17. A method as in claim 12, wherein the composition further comprises a pharmaceutically acceptable carrier.
18. A method as in claim 17, wherein the composition further comprises a stabilizer.
- 5 19. A method as in claim 12, wherein the step of contacting comprises administering the composition directly to a site of central nervous system injury.