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(54) **METHODS OF TREATING TYPE I DIABETES BY BLOCKING VEGF-MEDIATED ACTIVITY**

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This patent is subject to a terminal disclaimer.

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C07K 14/71 (2006.01)
C12N 15/62 (2006.01)

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(58) **Field of Classification Search** ..... None
See application file for complete search history.

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(57) **ABSTRACT**

Methods of treating diabetes in mammals, particularly humans, by blocking or inhibiting VEGF-mediated activity. A preferred inhibitor of VEGF-mediated activity is a VEGF antagonist such as the VEGF fusion protein trap of SEQ ID NO:2 capable of binding and blocking VEGF. The method of the invention may be combined with other therapies, such as with insulin therapy.

5 Claims, 2 Drawing Sheets

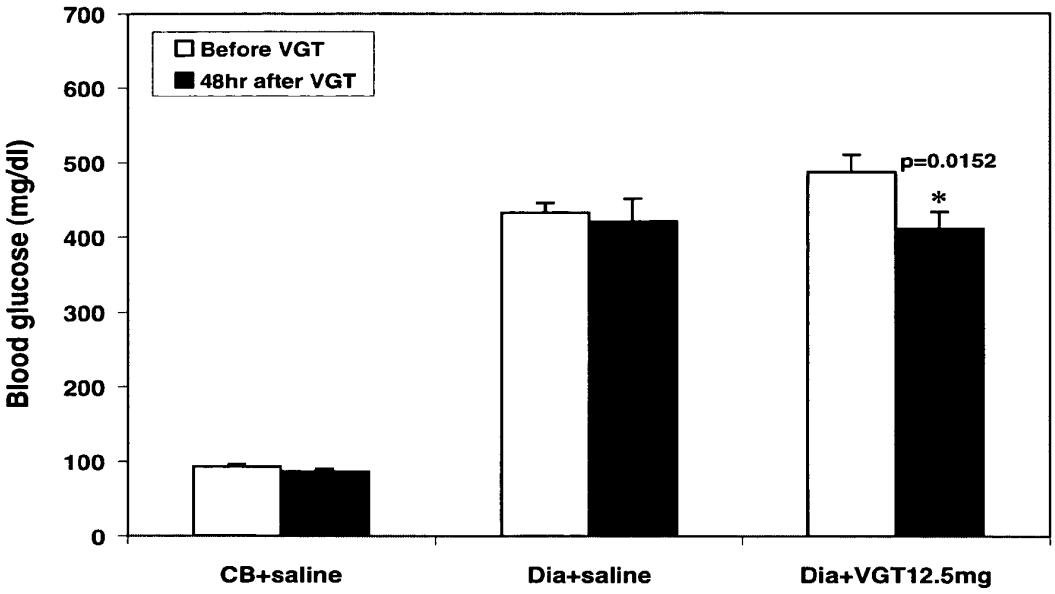

Fig. 1

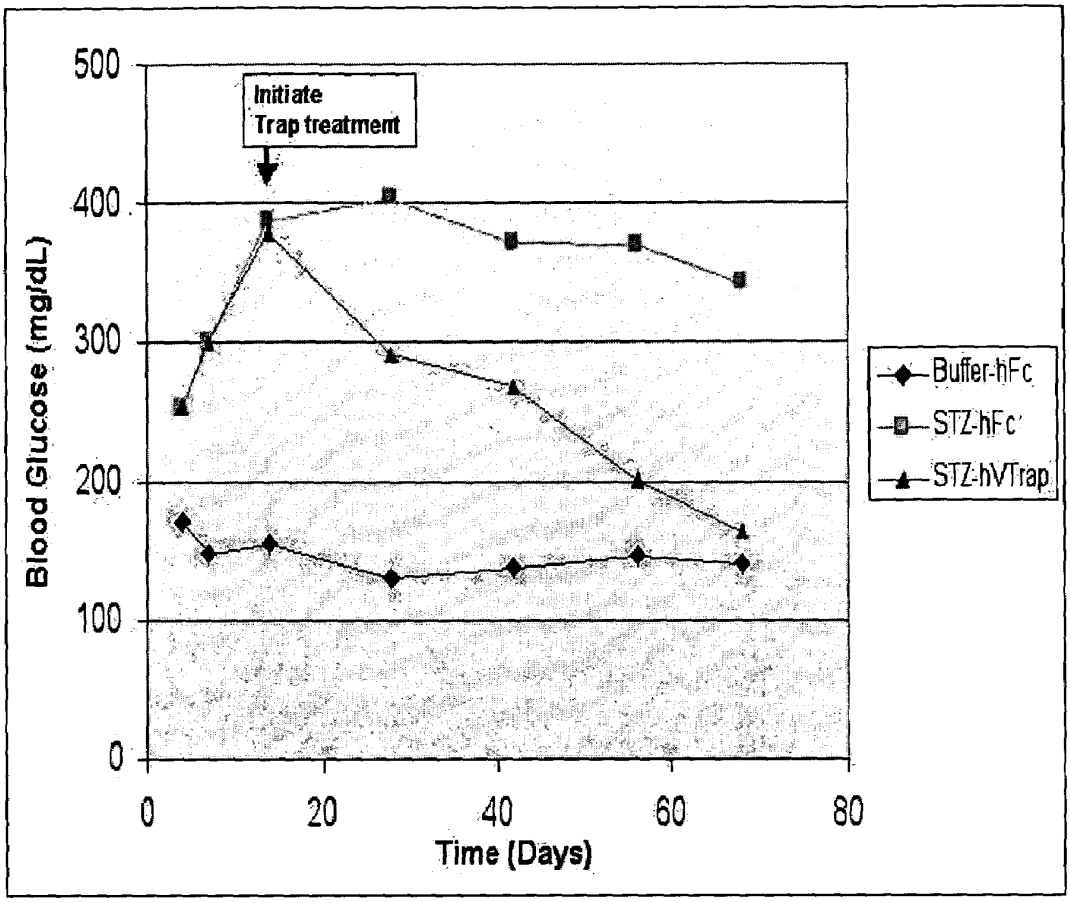

Fig. 2

METHODS OF TREATING TYPE I DIABETES BY BLOCKING VEGF-MEDIATED ACTIVITY

RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. 119(e) to U.S. Ser. No. 60/592,628 filed 30 Jul. 2004, which application is incorporated by reference in its entirety.

BACKGROUND

1. Field of the Invention

The field of the invention is generally related to methods of treating diabetes by administering agents capable of decreasing serum glucose levels. In particular, the field of the invention is methods of treating type I diabetes by administering agents capable of blocking, inhibiting, or ameliorating VEGF-mediated activity.

2. Description of Related Art

Streptozotocin (STZ)-induced diabetes is widely accepted as an animal model for human type I diabetes (see, for example, Susztak et al. (2004) Diabetes 53:784-794).

Vascular endothelial growth factor (VEGF) has been recognized as a primary stimulus of angiogenesis in pathological conditions. Approaches to methods of blocking VEGF are described, for example, in PCT WO/0075319 which describes a VEGF-specific fusion protein which binds and inhibits VEGF.

BRIEF SUMMARY OF THE INVENTION

The present invention is based, in part, on the observation that administration of a vascular endothelial growth factor (VEGF) antagonist is able to return blood glucose levels to normal in an animal model of human type I diabetes.

Accordingly, in a first aspect, the invention features a method of treating type I diabetes in a subject, comprising administering to the subject an agent capable of blocking, inhibiting, or ameliorating VEGF-mediated activity. In specific embodiments, the method of treatment of the invention results in decreased serum glucose levels, improved glucose tolerance, and/or improved glycemic control.

The agent capable of blocking, inhibiting, or ameliorating VEGF-mediated activity in specific embodiments is a VEGF antagonist. More specifically, the VEGF antagonist is a VEGF trap antagonist is a fusion protein selected from the group consisting of acetylated Flt-1(1-3)-Fc, Flt-1(1-3$_{R\rightarrow N}$) Flt-1(1-3$_{\Delta B}$)-Fc, Flt-1(2-3$_{\Delta B}$)-Fc, Flt-1(2-3)-Fc, Flt-1D2-VEGFR3D3-FcΔC1(a), Flt-1D2-Flk-1D3-FcΔC1(a), and VEGFR1R2-FcΔC1(a) (SEQ ID NOs:1-2). In a preferred embodiment, the VEGF antagonist is VEGFR1R2-FcΔC1 (a). In other embodiments, the VEGF antagonist is a VEGF-specific antibody, a nucleic acid such as an inhibitory ri0bozyme or antisense molecule, a small molecule, an aptamer, a carbohydrate, peptidomimetic, or hapten.

Administration of the agent may be by any method known in the art, including subcutaneous, intramuscular, intradermal, intraperitoneal, intravenous, intranasal, or oral routes of administration. Preferred methods of administered a VEGF trap antagonist is by subcutaneous or intravenous administration.

The subject treated is preferably a human suffering from type I diabetes.

Other objects and advantages will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1**. A single injection of VEGF trap antagonist significantly reduces blood glucose levels in diabetic rats. Normal control rats (CB+saline, n=4); Diabetic rats treated with saline (STZ+saline, n=6); Diabetic rats treated with VEGF trap (VGT) (12.5 mg/kg) (Dia+VGT 12.5 mg, n=6).

FIG. **2**. Changes in blood glucose levels produced by repeated administration of VEGF trap in diabetic mice. Normal control mice (CB) treated with a control protein (hFc, n=7, —◆—); Diabetic mice (STZ-induced) treated with VEGF trap (hVTrap, n=9, —▲—); Diabetic mice (STZ-induced) treated with a control protein (hFc, n=9, —■—).

DETAILED DESCRIPTION

Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only the appended claims.

As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus for example, a reference to "a method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

General Description

The invention is based in part on the finding that administration of an agent capable of blocking or inhibiting VEGF-mediated activity is capable of decreasing serum glucose and improving glucose disposal in an animal model of human type I diabetes. These findings represent the first time an agent capable of blocking or inhibiting VEGF-mediated activity has been shown to ameliorate type I diabetes. Thus, the invention provides for methods of treating diabetes in a mammal by administering a VEGF antagonist. More specifically, the method of the invention may be practiced with a VEGF antagonist such as a dimeric protein composed of two fusion polypeptides ("VEGF trap") (SEQ ID NO:2), as shown below, or a VEGF-specific antibody.

Definitions

By the term "therapeutically effective dose" is meant a dose that produces the desired effect for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

By the term "blocker", "inhibitor", or "antagonist" is meant a substance that retards or prevents a chemical or physiological reaction or response. Common blockers or

inhibitors include but are not limited to antisense molecules, antibodies, antagonists and their derivatives. More specifically, an example of a VEGF blocker or inhibitor is a VEGF receptor-based antagonist including, for example, an anti-VEGF antibody, or a VEGF trap such as VEGFR1R2-FcΔC1(a) (SEQ ID NOs:1-2). For a complete description of VEGF-receptor based antagonists including VEGFR1R2-FcΔC1(a), see PCT publication WO/00/75319, the contents of which is incorporated in its entirety herein by reference.

A "small molecule" is defined herein to have a molecular weight below about 500 Daltons, and may include chemical as well as peptide molecules.

Nucleic Acid Constructs

Individual components of the VEGF-specific fusion proteins of the invention may be constructed by molecular biological methods known to the art with the instructions provided by the instant specification. These components are selected from a first cellular receptor protein, such as, for example, VEGFR1; a second cellular receptor protein, such as, for example, VEGFR2; a multimerizing component, such as an Fc.

Specific embodiments of the VEGF-specific fusion proteins useful in the methods of the invention comprise a multimerizing component which allows the fusion proteins to associate, e.g., as multimers, preferably dimers. Preferably, the multimerizing component comprises an immunoglobulin derived domain. Suitable multimerizing components are sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al. 1982 Cell 29:671-679); immunoglobulin gene sequences, and portions thereof.

The nucleic acid constructs encoding the fusion proteins useful in the methods of the invention are inserted into an expression vector by methods known to the art, wherein the nucleic acid molecule is operatively linked to an expression control sequence. Host-vector systems for the production of proteins comprising an expression vector introduced into a host cell suitable for expression of the protein are known in the art. The suitable host cell may be a bacterial cell such as *E. coli*, a yeast cell, such as *Pichia pastoris*, an insect cell, such as *Spodoptera frugiperda*, or a mammalian cell, such as a COS, CHO, 293, BHK or NS0 cell.

Antisense Nucleic Acids

In one aspect of the invention, VEGF-mediated activity is blocked or inhibited by the use of VEGF antisense nucleic acids. The present invention provides the therapeutic or prophylactic use of nucleic acids comprising at least six nucleotides that are antisense to a gene or cDNA encoding VEGF or a portion thereof. As used herein, a VEGF "antisense" nucleic acid refers to a nucleic acid capable of hybridizing by virtue of some sequence complementarity to a portion of an RNA (preferably mRNA) encoding VEGF. The antisense nucleic acid may be complementary to a coding and/or noncoding region of an mRNA encoding VEGF. Such antisense nucleic acids have utility as compounds that prevent VEGF expression, and can be used in the treatment of diabetes. The antisense nucleic acids of the invention are double-stranded or single-stranded oligonucleotides, RNA or DNA or a modification or derivative thereof, and can be directly administered to a cell or produced intracellularly by transcription of exogenous, introduced sequences.

The VEGF antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides ranging from 6 to about 50 oligonucleotides. In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof and can be single-stranded or double-stranded. In addition, the antisense molecules may be polymers that are nucleic acid mimics, such as PNA, morpholino oligos, and LNA. Other types of antisence molecules include short double-stranded RNAs, known as siRNAs, and short hairpin RNAs, and long dsRNA (>50 bp but usually ≧500 bp).

Inhibitory Ribozymes

In another aspect of the invention, diabetes may be treated in a subject suffering from such disease by decreasing the level of VEGF activity by using ribozyme molecules designed to catalytically cleave gene mRNA transcripts encoding VEGF, preventing translation of target gene mRNA and, therefore, expression of the gene product.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event. The composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, e.g., U.S. Pat. No. 5,093,246. While ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy mRNAs encoding VEGF, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA has the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art. The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one that occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA). The Cech-type ribozymes have an eight base pair active site that hybridizes to a target RNA sequence where the cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes that target eight base-pair active site sequences that are present in the gene encoding VEGF.

Generation of Antibodies to VEGF Proteins

In another aspect of the invention, the invention may be practiced with an anti-VEGF antibody or antibody fragment capable of binding and blocking VEGF activity. Anti-VEGF antibodies are disclosed, for example, in U.S. Pat. No. 6,121,230, herein specifically incorporated by reference. The term "antibody" as used herein refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda , alpha, gamma, delta, epsilon, and mu constant regions, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD, and IgE, respectively. Within each IgG class, there are different isotypes (eg. IgG_1, IgG_2, etc.). Typically, the antigen-binding region of an antibody will be the most critical in determining specificity and affinity of binding.

Antibodies exist as intact immunoglobulins, or as a number of well-characterized fragments produced by digestion with various peptidases. For example, pepsin digests an antibody below the disulfide linkages in the hinge region to

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