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(54) PHARMACEUTICAL COMPOSITIONS FOR ANGIOGENIC THERAPY

PHARMAZEUTISCHE ZUBEREITUNGEN ZUR ANGIOGENESE-THERAPIE

COMPOSITIONS PHARMACEUTIQUES DE THERAPIE ANGIOGENIQUE

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Description

Technical Field

[0001] The present invention relates to novel pharmaceutical compositions for angiogenic therapy. More specifically, the present invention relates to hovel pharmaceutical compositions for angiogenic therapy that contain, as the active ingredients a gene encoding angiogenesis factor and a prostacyclin synthase gene for angiogenic therapy, etc.

Background Art

[0002] Development of new blood vessels and angiogenesis are initiated along with activation of endothelial cells of parental blood vessels. Growth factors that have been shown, in addition to the stimulation of such angiogenesis in vivo, to function mitogenically toward endothelial cells *in vitro* are termed "angiogenesis factor (angiogenesis growth factor)".

[0003] The first therapeutic application of angiogenesis factor was reported by Folkman et al (N. Engl. J. Med. 285, 1182-1186 (1971)). According to later studies, the use of recombinant angiogenesis factors, such as the fibroblast growth factor (FGF) family (Science 257, 1401-1403 (1992); Nature 362, 844-846 (1993)), endothelial growth factor (EGF) (J. Surg. Res.. 54, 575-583 (1993)), and vascular endothelial growth factor (VEGF), has been confirmed to promote and/or accelerate development of collateral circulatory tract in animal models of myocardial and hind limb ischemia (Circulation 90, 11-228-11-234 (1994)). Furthermore, the present inventors discovered that hepatocyte growth factor (HGF), like VEGF, functions as an endothelium-specific growth factor (J. Hypertens. 14, 1067-1072 (1996)).

[0004] The strategy wherein angiogenesis factors are used for treating angiopathy (as mentioned above) is referred to as "angiogenic therapy." Recently, extremely active research on angiogenic therapy is in progress for ischemic diseases and arterial diseases using genes of above-mentioned angiogenesis factors.

[0005] For example, the present inventors have elucidated the effectiveness of HGF genes against arteriosclerosis obliterans (ASO) (Circulation 100, No.18, No. 1672 (1999) ; Japanese Circulation Journal 64 (Suppl. I), 478, No.P079 (2000)). Furthermore, it has been revealed that the HGF gene effectively functions against ischemic-reperfusion injury in myocardial infarction (Circulation 96, No.8, No.3459 (1997); Ann. Thorac. Surg. 67, 1726-1731 (1999); Gene Therapy, 7, 417-427 (2000)).

[0006] Furthermore, the effectiveness of the VEGF gene on swine myocardial ischemia model (Human Gene Therapy 10, 2953 (1999)) and rabbit hind limb ischemia model (Circulation 96 (suppl II) : II-382-388 (1997)) has been established. In addition, the effect of VEGF on ASO patients (Circulation 97, 1114-1123 (1998)) and angina pectoris patients (Ann. Thorac. Surg. 68,830-837 (1999)) has also been reported. Currently, in the U.S., clinical studies of VEGF gene therapy for ASO patients and angina pectoris patients are being carried out by groups such as Isner et al.

[0007] Regarding the bFGF gene, it has been reported that the number of blood vessels increase due to intramuscular introduction of the bFGF gene into a mdx mouse, a model for muscular dystrophy (Gene Therapy 6(7), 1210-1221 (1999)).

[0008] Prostacyclin (prostaglandin I_2; PGI_2), a kind of prostaglandin, is an unstable lipid mediator having a half-life of 5 to 10 minutes (Arch. Gynecol. Obstet. 243, 187-190 (1988)). It elucidates a strong vasodilating effect and platelet aggregation inhibitory effect through an increase of the cAMP levels mediated via G protein-coupled receptor (N. Engl. J. Med. 17, 1142-1147 (1979)). Currently, vasodilators, such as the PGI_2, PGE_1 (prostaglandin E_1), and derivatives thereof (analogues), are widely used for the therapy of various types of angiopathy. Specifically, expecting functions, such as vasodilatation and platelet aggregation inhibition, intra-arterial injection and intravenous injection of the PGE_1 are performed against peripheral hematogenic disorders (e.g., ASO and TAO (thromboangiitis obliterans)). Such injections have become is an established therapeutic method. Furthermore, since the PGI_2 has a strong effect and its inactivation occurs rapidly, various derivatives (iloprost, beraprost sodium, etc.) have been developed. These derivatives are used for the therapy of peripheral vascular occlusive disease and chronic arterial occlusion (Prostaglandins, Leukotrienes and Essential Fatty Acids. 54, 327-333 (1996) ; Yakugaku Zasshi, 117, 509-521 (1997)). Furthermore, PGE_1 and PGI_2 are used against peripheral circulatory dysfunction due to collagen disease, Raynaud's phenomenon, maintenance of extracorporeal circulation (Minerva Med. 89, 405-409 (1998)), heart failure (Am. Heart J. 1.34, 44-54 (1997)), and so on.

[0009] As mentioned above, substances, such as PGI_2, that have vasodilating effect and platelet aggregation inhibitory effect are known to be effective against various types of angiopathies. However, these substances have never been used in combination in the aforementioned angiogenic therapy with the HGF gene, and it has not been determined as to what kind of effects can be expected by such combination.

[0010] Furthermore, angiogenesis factors, such as HGF, VEGF, bFGF, and EGF, are known to enhance the expression of ets-1 (erythroblastosis virus oncogene homolog 1), a transcription regulatory factor, and activate various types of factors involved in angiogenesis via the ets-1 (J. Cell. Physiol., 169, 522-531 (1996); "HGF no Bunshi Igaku (Molecular Medicine of HGF)", Medical Review, 179-185 (1998)). However, the ets-1 gene has never been used for angiogenic therapy and its effect completely unknown.

Disclosure of the Invention

[0011] The objective of the present invention is to provide novel pharmaceutical compositions for angiogenic therapy. The object of the present invention is to provide novel pharmaceutical compositions for angiogenic therapy that contain, as the active ingredient, a gene encoding an angiogenesis factor and a prostacyclin synthase gene for angiogenic therapy, etc.

[0012] The present inventors examined the effect of the combined use of a gene of the PGI_2-synthesizing enzyme (PGI_2 synthase, hereinafter referred to as "PGIS") in angiogenic therapy along with the HGF gene. No drug indicating a satisfying effect by the combination in general angiogenic therapy using a gene of an angiogenesis factor has been found so far. Furthermore, effects of combined application with other genes have not been elucidated so far.

[0013] As a result of examination using a mouse hind limb ischemia ASO model, it has been revealed that the combined application of HGF gene or VEGF gene with PGIS gene show an unexpectedly remarkable improvement in hind limb blood flow, compared to the use of each of these genes alone. Furthermore, for the first time, the PGIS gene was found to reinforce the angiogenic effect of the HGF gene or VEGF gene, and to express an angiogenic effect even used alone.

[0014] According to the above-mentioned result, it was revealed that combined application of substances, such as PGI_2, or substances producing them (such as the PGIS gene), having vasodilating effect or platelet aggregation inhibitory effect is extremely effective in angiogenic therapy wherein a gene of an angiogenesis factor is used.

[0015] The present invention was accomplished based on the above-mentioned findings.

[0016] More specifically, the subjects of the present invention are:

(1) a pharmaceutical composition for angiogenic therapy, which is characterized by the combined use of a gene encoding an angiogenesis factor with at least one substance selected from the group of: substances having vasodilating effect and platelet aggregation inhibitory effect, and substances producing them.;
wherein the angiogenesis factor is HGF and/or VEGF;
wherein the substance producing a substance having vasodilating effect and/or platelet aggregation inhibitory effect is prostacyclin synthase gene;

(2) a pharmaceutical composition for angiogenic therapy which contains HGF gene and prostacyclin synthase gene as the active ingredients;

(3) a pharmaceutical composition for angiogenic therapy which is
characterized by the combined use of HGF gene and prostacyclin synthase gene;

(4) a pharmaceutical composition for angiogenic therapy which contains VEGF gene and prostacylin synthase gene as the active ingredients;

(5) a pharmaceutical composition for angiogenic therapy, which is characterized by the combined use of VEGF gene and prostacyclin synthase gene;

(6) the pharmaceutical composition for angiogenic therapy of any one of (1) to (5) ; wherein the composition is used for treating or preventing ischemic disease or arterial disease;

(7) the pharmaceutical composition for angiogenic therapy of (6), wherein the ischemic disease or arterial disease is selected from the group of arteriosclerosis obliterans, myocardial infarction; angina pectoris, cardiomyopathy, and cerebrovascular disease;

(8) the pharmaceutical composition for angiogenic therapy of any one of (1) to (7), wherein the gene is introduced in the form of naked DNA;

(9) an agent for potentiating the angiogenic effect due to a gene encoding an angiogenesis factor, which contains, as the active ingredient, at least one substance selected from the group of: substances having vasodilating effect and platelet aggregation inhibitory effect, and substances producing them;
wherein the angiogenesis factor is HGF and/or VEGF; wherein the agent for potentiating the angiogenic effect is prostacyclin synthase gene.

(10) an agent for potentiating the angiogenic effect due to HGF gene which contains prostacyclin synthase gene as the active ingredient;

(11) the agent for potentiating the angiogenic effect of any one of (9) to (10), wherein the agent is used for treating or preventing ischemic disease or arterial disease;

[0017] The phrase "gene encoding an angiogenesis factor," as employed herein for angiogenic therapy refers to a gene that encodes a protein or polypeptide that can induce the formation of new blood vessels or parts thereof. The genes are HGF gene and VEGF gene; the HGF gene being more preferable. The genetic sequences of these genes are registered in public.databases and by utilizing these databases, one skilled in the art can readily clone the above-mentioned genes.

[0018] Hereinafter, the invention is explained using HGF gene and VEGF gene as the example.

[0019] In the present invention, the term "HGF gene," as employed herein refers to a gene that encodes HGF (HGF protein). In addition, an HGF gene incorporated into an expression plasmid to be expressed may also be simply referred to as "HGF gene." Specifically, the gene includes cDNAs of HGF, such as those described in Nature, 342, 440 (1989), Examined Published Japanese Patent Application No. 2777678, Biochem. Biophys. Res. Commun., 163, 967 (1989), incorporated into an appropriate expression vector (non-virus vector, virus vector), such as those mentioned below. The nucleotide sequence of the cDNA encoding HGF is described in the aforementioned literature. In addition, it is also registered in databases such as Genbank. Thus, the cDNA of HGF can be cloned by performing a RT-PCR reaction, for example, on mRNAs derived from liver or leukocytes using appropriate DNA segments as PCR primers based on the sequence information. The cloning can be performed readily by one skilled in the art by referring to references, such as Molecular Cloning 2nd edition, Cold Spring Harbor Laboratory Press (1989).

[0020] The HGF gene of the present invention is not limited to those mentioned above. So long as the protein expressed from the gene substantially has the same angiogenic effect as HGF, the gene can be used as the HGF gene of the present invention. More specifically, the HGF gene of the present invention encompasses: 1) DNAs that hybridize under stringent conditions to the aforementioned cDNA; 2) DNAs encoding a protein consisting of the amino acid sequence of the protein encoded by the aforementioned cDNA, wherein one or more (preferably several) amino acids are substituted, deleted, and/or added; and such, so long as they encode a protein with angiogenic effect. The above DNAs of 1) and 2) can be readily obtained, for example, by site-directed mutagenesis method, PCR method, conventional hybridization methods, etc. Specifically, these methods can be performed by referring to the aforementioned reference, such as Molecular Cloning 2nd edition, Cold Spring Harbor Laboratory Press (1989).

[0021] The term "VEGF gene," as employed herein refers to a gene encoding VEGF protein. A VEGF gene incorporated into an expression plasmid to be expressed may also be simply referred to as the "VEGF gene." Specifically, such a gene is exemplified by a cDNA of a VEGF incorporated into an appropriate expression vector (non-virus vector, virus vector) such as those mentioned below. Regarding the VEGF genes in humans, the existence of four kinds of subtypes (VEGF121, VEGF165, VEGF189, and VEGF206) due to selective splicing during transcription have been reported (Science, 219, 983 (1983); J. Clin. Invest., 84, 1470 (1989); Biochem. Biophys. Res. Commun., 161, 851 (1989)). Any of these VEGF genes can be used in the present invention. However, VEGF165 gene is more preferable due to its strongest biological activity among the VEGF genes. Furthermore, like in the case of the aforementioned HGF, a gene of these VEGF, which is modified, is also included in the category of the VEGF gene of the present invention so long as the gene encodes.a protein having an angiogenic effect.

[0022] Similar to the HGF gene, the VEGF gene can also be readily cloned by one skilled in the art based on the sequence described in the literature (for example, Science, 246, 1306 (1989)) and the sequence information registered in database; and modifications thereof can also be easily carried out.

[0023] Whether the above-mentioned HGF gene, VEGF gene, or genes encoding the modified forms of them possess angiogenic effect can be investigated, for example, via in vitro measuring the proliferative effect on vascular endothelial cells that is described in WO 97/07824. Alternatively, the angiogenic effect of the genes can be investigated via in vivo measuring the blood flow improving effect in a mouse hind limb ischemia model described in the Example, infra.

[0024] The above-mentioned genes encoding angiogenesis factors can be used alone or in combination in the angiogenic therapy of the present invention.

[0025] According to the Example mentioned below, for the first time it was revealed that the combined use of prostacyclin synthase gene (PGIS gene) in angiogenic therapy with HGF gene yields an unexpectedly remarkable effect. More specifically, it was demonstrated for the first time that a synergistic effect exceeding the sum of the effect of HGF gene alone and PGIS gene alone is achieved by the combination.

[0026] Herein, PGI_2 synthesized by PGIS has vasodilating effect, vascular permeability enhancing effect, and platelet aggregation inhibitory effect as mentioned above. Therefore, the reason for the aforementioned synergistic effect may be that the combined use of the HGF gene and PGIS gene provided an environment wherein the HGF can readily function at the ischemic site; i.e., an environment wherein angiogenesis by the HGF occurs easily through the effects, such as vasodilating effect and platelet aggregation inhibitory effect, possessed by PGI_2. As a result, this caused the aforementioned effect beyond expectation.

[0027] The phrase "substances having vasodilating effect," as employed herein includes all of the known substances having vasodilating effect (commercially available vasodilating agents, etc.), and may be any substance including, such as genes, proteins, and low molecular weight compounds. Specifically, the following substances can be presented as the examples.

[0028] The above term "substances that produce substances having vasodilating effect and/or platelet aggregation inhibitory effect" refers to substances that synthesize, produce, or induce the aforementioned substances having vasodilating effect and/or platelet aggregation inhibitory effect. Specifically, they indicate substances that synthesize, produce, or induce the aforementioned substances that increase prostaglandin. These substances are the PGIS gene.

[0029] The genetic sequences of all of these genes are registered in public databases, and those skilled in the art can readily clone the genes using these registered genes.

[0030] An example wherein the PGIS gene is used is explained below.

[0031] Herein, the term "PGIS gene" refers to a gene that encodes a PGIS protein. The PGIS gene incorporated into an expression plasmid so as to be expressed may also be simply referred to as the "PGIS gene". Specifically, a cDNA of PGIS described in BBRC 200(3), 1728-1734 (1994), and WO 95/30013 incorporated into an appropriate expression vector (non-virus vector, virus vector), such as those mentioned below, can be exemplified. Furthermore, similar to the aforementioned HGF gene and VEGF gene, a PGIS gene which is modified is also included in the category of the PGIS gene of the present invention so long as the gene encodes a protein having the effect as the PGIS.

[0032] As with the HGF gene and VEGF gene, the PGIS gene can also be cloned readily by one skilled in the art based on the sequence described in the aforementioned literature, or on the sequence information registered in the database. The PGIS gene can also be modified easily. Whether a protein encoded by the gene has the desired PGIS activity can be measured, for example, by enzyme immunoassay using 6-keto Prostaglandin F1 a enzyme immunoassay kit (Cayman, catalogue No. #515211), or by a method for detecting metabolites of the prostacyclin synthase by thin layer chromatography (TLC). Alternatively, the effect of enhancement of angiogenic effect due to an angiogenesis factor can be measured by measuring the effect of its combined use with an angiogenesis factor on a mouse hind limb ischemia model described below in the Example.

[0033] Below appear the method for introduction, form of introduction, and amount of introduction of the pharmaceutical compositions for angiogenic therapy according to the present invention.

<u>1) Use of a substance having vasolidating effect and/or platelet aggregation inhibitory effect or a substance (gene) producing it, and a gene encoding an angiogenesis factor</u>

[0034] To use a gene encoding an angiogenesis factor and a gene, such as the aforementioned PGIS gene, in combination, i.e., a combined application of two or more genes, both genes need to take the form of an agent for gene therapy. Representative combinations include the combination of the HGF gene and PGIS gene, or the VEGF gene and PGIS gene.

[0035] The form of administration to administer the agent for gene therapy to a patient can be classified into two groups, one using a non-virus vector, and the other using a virus vector. The method of preparation and administration thereof are described in detail in experiment manuals (Jikken Igaku (Experimental Medicine) Supplementary Volume, "Idenshichiryo no Kisogijyutsu (Fundamental Techniques for Gene Therapy)", Yodosha, 1996; Jikken Igaku (Experimental Medicine) Supplementary Volume, "Idenshidonyu & Hatsugenkaiseki Jikkenho (Experimental Methods for Gene Transfer & Expression Analysis)", Yodosha, 1997;. "Idenshi-chiryo Kaihatsu Kenkyu Handbook (Handbook of Gene Therapy Research and Development)", Nihon Idenshichiryo Gakkai (The Japan Society of Gene Therapy) Edition, NTS, 1999). Detailed explanations are given below.

A. Use of non-virus vector

[0036] Using a recombinant expression vector, a conventional gene expression vector introduced with an object gene, the object gene can be introduced into cells and tissues by the following method.

[0037] Examples of methods of gene transfection into cells include: calcium phosphate co-precipitation method, method of direct infusion of DNA using a glass capillary tube, etc.

[0038] Examples of gene transfection into tissues include: the method of gene transfection by internal type liposome, method of gene transfection by electrostatic type liposome, HVJ (hemagglutinating virus of Japan)-liposome method, improved type HVJ-liposome method (HVJ-AVE liposome method), receptor-mediated method of gene transfection, method of importing a carrier (metal particles) along with a DNA molecule into cells by particle gun, method of direct introduction of naked-DNA, method for introduction by a positively charged polymer, and so on. The recombinant expression vector can be introduced into a cell using any of these methods. Among these methods, the method of direct introduction of naked-DNA is most convenient, and thus is a preferred method for introduction from that perspective. Alternatively, due to its extremely high fusion activity with the cell membrane compared to conventional liposome methods, the HVJ-liposome method is a preferred form for transfection. Although the Z strain. (obtained from ATCC) is preferred as the HVJ, fundamentally, other HVJ strains (for example ATCC VR-907 , ATCC VR-105, and such) can be also used.

[0039] Any expression vector can be used in the present invention so long as it can express the desired gene *in vivo,* and includes, for example, pCAGGS (Gene, 108, 193-200 (1991)), pBK-CMV, pcDNA3.1, pZeoSV (Invitrogen, Stratagene).

[0040] The two or more genes mentioned above can be transfected into the body simultaneously as a mixture of two or more recombinant expression vectors, which were prepared by incorporating the genes into discrete expression vectors, or separately with a time interval. Alternatively, a single expression vector wherein the two or more genes are incorporated into one expression vector, can be also introduced. Furthermore, with the aforementioned liposomal preparations, transfection can be carried out by enclosing two or more recombinant expression vectors into one liposome, or by enclosing each recombinant expression vector into separate liposomes.

B. Use of virus vector

[0041] Examples of virus vectors include recombinant adenovirus, retrovirus, etc. More specifically, a gene is introduced into a cell by introducing a desired gene into a DNA virus or RNA virus, such as avirulent retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, poxvirus, poliovirus, Sindbis virus, Sendai virus, SV40, and immunodeficiency virus (HIV); the recombinant virus is infected into the cell.

[0042] Among the aforementioned virus vectors, the infection efficiency of adenoviruses is known to be much higher than other virus vectors. Thus, from this perspective, the use of the adenovirus vector system is preferred.

[0043] Similar to the above-mentioned non-virus vector, these adenovirus vectors mentioned above can be introduced simultaneously as a mixture, or separately with a time interval by preparing recombinant expression vectors introduced with respective two or more genes. Alternatively, a single recombinant expression vector wherein two or more genes are incorporated into one expression vector can be introduced.

[0044] Furthermore, two or more genes can be introduced into the living body using both of the aforementioned techniques using non-virus vector and virus vector.

[0045] Methods for introducing the agent of the present invention for gene therapy include: (i) the in vivo method that introduces the agent for gene therapy directly into the body; and (ii) the *ex vivo* method that harvests a certain type of cell from the body, introduces the agent for gene therapy into the cell outside the body, and then returns the modified cell to the body (Nikkei Science, April 1994, 20-45; Gekkann Yakuji 36 (1), 23-48, 1994; Jikken Igaku (Experimental Medicine) Supplementary Volume, 12 (15), 1994; "Idenshi-chiryo Kaihatsu Kenkyu Handbook (Handbook of Gene Therapy Research and Development) ", Nihon Idenshichiryo Gakkai (The Japan Society of Gene Therapy) Edition, NTS, 1999) . The in vivo method is preferred in the present invention.

[0046] When administering by the in vivo method, administration is carried out via an appropriate administration route depending on the disease to be treated, target organ, and so on. For example, the administration can be intravenous, intra-arterial, subcutaneous, intradermal, intramuscular, etc., or via direct local administration to the lesion itself.

[0047] Various formulations (for example, liquid preparations, etc.) suitable for each of the above-mentioned forms of administration may be adopted as the form of the preparation. For example, to prepare an injection containing a gene as the active ingredient, the injection can be prepared by conventional methods, for example, by dissolving in an appropriate solvent (buffer solution, such as PBS, physiological saline, sterilized water, etc.); sterilizing by filtration through a filter as necessary, and then loading into a sterile container. A conventional carrier and such may be added as required to the injection. Alternatively, a liposome, such as HVJ-liposome, can be in the form of liposome preparations, such as suspension, frozen agent, or centrifugally concentrated frozen agent.

[0048] Furthermore, to facilitate the presence of the genes around the diseased site, a controlled release preparation (miniature pellet preparation, etc.) can be prepared and implanted near the affected area. Alternatively, continuous and gradual administration to the affected area using an osmotic pump is available.

[0049] The aforementioned two or more recombinant expression vectors may take different formulations, or it may be a formulation of a mixed combined agent.

[0050] The amount of the genes contained in the preparation can be adjusted appropriately depending on the disease to be treated, age and weight of the patient, etc.; however, generally it is preferred that 0.0001 to 100 mg, or preferably 0.001 to 10 mg of each gene is administered once every few days or every few months.

<u>2) Use of substances having vasodilating effect and/or platelet aggregation inhibitory effect, and substances (low molecular weight compounds, proteins, etc.) producing them; and a gene encoding an angiogenesis factor</u>

[0051] When a gene encoding an angiogenesis factor and a low molecular weight compound, protein, peptide, etc. are used in combination, the gene encoding the angiogenesis factor should be in the form of the aforementioned agent for gene therapy. On the other hand, low molecular weight compounds and such are administered orally or parenterally in a conventional form of a pharmaceutical composition. Representative combinations include the combination of the HGF gene and PGI_2 derivative, the VEGF gene and PGI_2 derivative, and so on.

[0052] Explanation on pharmaceutical compositions containing the aforementioned low molecular weight compound, protein, and so on as the active ingredient are described in the following.

[0053] The administration method, dose, etc. of the aforementioned low molecular weight compounds or proteins that are already commercially available as vasodilating agents or platelet aggregation inhibitory agents (antiplatelet agents) can be set according to the statement of virtues. However, in general, examples of the form of administration and method of administration are the following.

[0054] For oral administration, it can be administered in an administration form conventionally used in the art. For parenteral administration, it can be administered in administration forms such as local administration agent (transdermal agent, etc.), rectal administration agent, injection, and nasal agent.

[0055] Examples of oral agents or rectal administration agents include capsules, tablets, pills, powders, drops, suppositories, liquid preparations, etc. Examples of injections include sterile solutions, suspensions, emulsions, and such; and specifically, water, water-propylene glycol solution, buffering solution, 0.4% physiological saline, and such can be presented as examples. Local administration agents include, for example, cream, ointment, lotion, transdermal agents, and such.

[0056] The above-mentioned dosage forms are formulated with pharmaceutically acceptable fillers and additives by methods conventionally performed in the art.

Pharmaceutically acceptable fillers and additives include carriers, binders, perfume, buffers, thickeners, coloring agents, stabilizers, emulsifiers, dispersants, suspending agents, preservatives, pH regulating agents, tonicity regulating agents, lubricants, and such. Pharmaceutically acceptable carriers include, for example, magnesium carbonate, lactose, pectin, starch, methyl cellulose, and such.

[0057] Such pharmaceutical compositions can be administered via an appropriate administration route depending on the disease to be treated, target organ, and such. For example, the administration can be intravenous, intra-arterial, subcutaneous, intradermal, intramuscular, etc., or direct local administration to the lesion itself. Furthermore, oral administration and administration as a suppository are also possible.

[0058] The dose and frequency of administrations vary depending on the symptom, age, weight of the patient, administration form, and such; but it is normally within the range of approximately 0.0001 to approximately 500 mg, preferably within the range of approximately 0.001 to approximately 100 mg for adults per day, which is administered at a time or divided for several administrations.

[0059] Pharmaceutical compositions that contain the above-mentioned low molecular weight compounds and proteins as the active ingredients can be administered simultaneously with the agent for gene therapy containing a gene encoding an angiogenesis factor, or they can be administered separately with a time interval.

[0060] The pharmaceutical compositions for angiogenic therapy of the present invention that have been described so far can be applied to all diseases that require angiogenic therapy. Specifically, ischemic disease or arterial disease can be exemplified as such diseases. More specifically, examples of heart diseases include ischemic heart disease, myocardial infarction, acute myocardial infarction, myocardosis, angina pectoris, unstable angina, coronary arteriosclerosis, heart failure, and such; and examples of ischemic diseases of the extremities include arteriosclerosis obliterans (ASO), Berger's disease, vascular injury, arterial embolism, arterial thrombosis, arterial occlusion of the organ, aneurysm, and such. Other examples are cerebrovascular diseases. Specifically, examples of cerebrovascular diseases include cerebrovascular occlusion, cerebral infarction, cerebral thrombosis, cerebral embolism, stroke, cerebral hemorrhage, moyamoya disease, cerebrovascular dementia, Alzheimer type dementia, sequela of cerebral hemorrhage, and sequela of cerebral infarction. Among these diseases, the pharmaceutical compositions of the present invention are effectively used particularly against arteriosclerosis obliterans.

[0061] Furthermore, the present invention also provides an agent for potentiating the angiogenic effect due to a gene encoding an angiogenesis factor which contains, as the active ingredient, at least one substance selected from the group consisting of substances having vasodilating effect and/or platelet aggregation inhibitory effect, and substances producing them. As mentioned above, the aforementioned substance that is the active ingredient of the pharmaceutical composition for angiogenic therapy of the present invention has the effect of enhancing the angiogenic effect due to a gene encoding an angiogenesis factor. Therefore, as mentioned above, it can be used as one of the components of a pharmaceutical composition for angiogenic therapy, or alternatively, it can be used alone as a potentiating agent to increase the angiogenic effect due to a gene encoding an angiogenesis factor. The potentiating agent of the present invention is used effectively in cases where the effect of the gene encoding the angiogenesis factor is insufficient. The potentiating agent of the present invention may comprise only one component (substance), or pulural components (substances) in combination.

[0062] Specifically, the active ingredient of the potentiating agents of the present invention is the aforementioned PGIS gene. The angiogenesis factor is HGF or VEGF as mentioned above.

[0063] The administration method, administration form, accommodated disease, and such of the potentiating agent of the present invention are the same as those of the aforementioned pharmaceutical compositions for angiogenic therapy.

<u>Brief Description of the Drawings</u>

[0064] Fig. 1 is a graph showing the result of examination wherein the changes in the left-right ratios with time were investigated by measuring the hind limb blood flow using Laser Doppler Imager after the administration of the respective genes (control, HGF gene, PGIS gene, HGF gene + PGIS gene) to a mouse hind limb ischemia ASO model.

[0065] Fig. 2 is a graph showing the result of examination wherein the proportion of increase with time of the left-right ratio compared to that before the administration of the genes was investigated by measuring the hind limb blood flow using Laser Doppler Imager after the administration of the respective genes (control, HGF gene, PGIS gene, HGF gene + PGIS gene) to a mouse hind limb ischemia ASO model.

[0066] Fig. 3 is a graph showing the result of examination wherein the number of capillaries in the ischemic limb muscle were investigated after the administration of the respective genes (control, HGF gene, PGIS gene, HGF gene + PGIS gene) to a mouse hind limb ischemia ASO model.

[0067] Fig. 4 is a graph showing the result of examination wherein the proportion of increase of the right-left hind limb blood flow ratio was investigated by measuring the hind limb blood flow using Laser Doppler Imager after the administration of the respective genes (control , HGF gene, ets-1 gene, HGF gene + ets-1 gene) to a rat hind limb ischemia ASO model.

[0068] Fig. 5 is a graph showing the result of examination wherein the capillary density in the ischemic limb muscle was measured after the administration of the respective genes (control, HGF gene, ets-1 gene, HGF gene + ets-1 gene) to a rat hind limb ischemia ASO model.

[0069] Fig. 6 is a graph showing the result of examination wherein the rat HGF concentration in the ischemic limb muscle was investigated after the administration of the respective genes (control, HGF gene, ets-1 gene, HGF gene + ets-1 gene) to a rat hind limb ischemia ASO model.

[0070] Fig. 7 is a graph showing the result of examination wherein the rat HGF concentration in the ischemic limb muscle was investigated after the administration of the ets-1 gene to a rat hind limb ischemia ASO model.

[0071] Fig. 8 is a graph showing the result of examination wherein the human VEGF concentration in the ischemic limb muscle was investigated after the administration of the PGIS gene, VEGF gene, or VEGF gene and PGIS gene to a mouse hind limb ischemia ASO model.

[0072] Fig. 9 is a graph showing the blood flow ratio of untreated right hind limb (normal), and left hind limb (ASO), determined by LDI, 10 days after surgery for producing a mouse hind limb ischemia ASO model.

[0073] Fig. 10 is a graph showing the result of examination wherein the proportion of increase of the amount of blood flow in the ischemic hind limb muscle was investigated by LDI, 2 weeks after the administration of the PGIS gene, VEGF gene, or VEGF gene and PGIS gene to a mouse hind limb ischemia ASO model.

[0074] Fig. 11 is a graph showing the result of examination wherein the proportion of increase of the amount of blood flow in the ischemic hind limb muscle was investigated by LDI, 4 weeks after the administration of the PGIS gene, VEGF gene, or VEGF gene and PGIS gene to a mouse hind limb ischemia ASO model.

[0075] Fig. 12 is a photograph of the frozen sections of the ischemic hind limb muscle stained by alkaline phosphatase staining, 4 weeks after the administration of the PGIS gene and HGF gene, VEGF gene, or VEGF gene and PGIS gene to a mouse hind limb ischemia ASO model.

[0076] Fig. 13 is a graph showing the result of examination wherein the capillary density was investigated 4 weeks after the administration of the PGIS gene, VEGF gene, or VEGF gene and PGIS gene to a mouse hind limb ischemia ASO model.

<u>Best Mode for Carrying out the Invention</u>

[0077] Herein below, the present invention will be described using Examples, however, it is not to be construed as being limited thereto.

[Example 1]

<u>Effect of administration of HGF gene, or PGIS gene to mouse hind limb ischemia ASO model</u>

(1) Materials

[0078] The cDNA of human HGF (described in Unexamined Published Japanese Patent Application No. (JP-A) Hei 5-111383) was cloned by a conventional method, and was inserted into an expression plasmid pcDNA3. 1 (+) (Invitrogen) containing a cytomegalovirus (CMV) promoter to be used as human HGF gene.

[0079] The cDNA of human PGIS (B.B.R.C., Vol. 200, No. 3, p1728-1734 (1994)) was cloned by a conventional method, and was inserted into an expression plasmid pCAGGS (Gene 108, 193-200 (1991)) containing a CMV enhancer and a β-actin promoter to be used as human PGIS gene.

(2) Methods

[0080] C57BL/6J mouse (8 weeks old, male) was used. The mouse was anesthetized by intraperitoneal injection of 200 μl of 10-fold diluted Nembutal, and further by ether inhalation when supplementation was necessary. Then, the arteriovenous of the left hind limb was tied up to produce a mouse hind limb ischemia ASO model. Ten days later, the blood flow in both hind limbs was evaluated using Laser Doppler Imager (LDI, Moor Instruments Ltd., MLDI5070), and the left-right ratio was calculated. After the evaluation, 500 μg each of the genes of above (1) was administered into the left hind limb muscle in the form of naked plasmids. Four groups were set up: i. e. , a control group without administration; a group given the HGF gene alone; a group given the PGIS gene alone; and a group given a combined application of the HGF gene and PGIS gene. 2 weeks and 4 weeks after the administration of the genes, the blood flow was evaluated using LDI, and the ratio was calculated. Furthermore, 4 weeks later, the left hind limb muscle was extirpated, and after preparing a frozen section, the capillary density in the muscle was measured by alkaline phosphatase staining. Significant difference test was performed by the Fisher's PLSD method.

(3) Result

[0081] The changes in the ratio of the left-right hind limb blood flow with time was measured by LDI, and is shown in Fig. 1. Furthermore, the proportion of increase compared to the LDI ratio before the administration of the genes is indicated in Fig. 2. The blood flow was improved 2 weeks after the administration of the PGIS gene, but 4 weeks after the administration, it was nearly the same as that of the control group. By administering the HGF gene, the blood flow was improved both at 2 and at 4 weeks after administration. Furthermore, unexpectedly, the combined application of the PGIS gene and HGF gene remarkably improved the blood flow compared to independent administration of the genes (2 weeks later: control: 100%, HGF gene administration: 132%, PGIS gene administration: 125%, HGF gene + PGIS gene administration: 177%, P< 0.01; 4 weeks later: control: 100%, HGF gene administration: 150%, PGIS gene administration: 104%, HGF gene + PGIS gene administration: 166%, P< 0.01).

[0082] The capillary densities in the muscles 4 weeks after the gene administration are shown in Fig. 3. The capillary density increased due to the administration of the PGIS gene or HGF gene. Furthermore, by the combined application of the PGIS gene and HGF gene, the capillary density increased remarkably compared to independent administration.

Reference Example

Effect of administration of HGF gene, and ets-1 gene to rat hind limb ischemia ASO model

(1) Materials

[0083] An expression plasmid carrying the human HGF gene, which is the same as that of Example 1, was used. The cDNA of human ets-1 (GenBank Acc. No. J04101, Proc. Natl. Acad. Sci. U.S.A., 85 (21), 7862-7866 (1988)) was cloned by a conventional method, and was inserted into a commercially available expression vector to be used as human ets-1 gene.

(2) Methods

[0084] Sprauge Dawley rats (12 weeks old, male) were used. The femoral artery from one side was extirpated to produce a rat hind limb ischemia ASO model. One week later, 100 μg each of the genes was administered into the left hind limb muscle using the HVJ-liposome method. Four groups were set up: a control group wherein the vector was administered alone; a group given the HGF gene alone; a group given the ets-1 gene alone; and a group wherein the HGF gene and ets-1 gene were used in combination. Using Laser Doppler Imager (LDI) before gene administration and 4 weeks after gene administration, the blood flow in both hind limbs was evaluated, and the proportion of increase in the left-right blood flow ratio was calculated. Furthermore, the left hind limb muscle was extirpated, and after preparing a frozen section, the capillary density in the muscle was measured by alkaline phosphatase staining. To investigate the influence of the gene administration on the expression of endogenous HGF, intramuscular rat HGF concentration in the ischemic limb was measured using ELISA kit (Institute of Immunology).

(3) Result

[0085] By the independent administration of the ets-1 gene, the ets-1 binding activity in the muscular tissue increased. Furthermore, by the administration of the ets-1 gene, the proportion of increase of hind limb blood flow ratio measured using LDI rose (Fig. 4), and the capillary density in the muscle increased (Fig. 5), which results indicate the effect of angiogenesis, and effectiveness towards the ASO model due to the independent administration of the ets-1 gene. Furthermore, the intramuscular HGF concentration in the ischemic limb increased in the group that were given the ets-1 gene alone (Fig. 6 and Fig. 7), and this was considered to be one of the mechanisms of the effect of the ets-1 gene administration.

[0086] In the group wherein the ets-1 gene and HGF gene was administered in combination, the proportion of increase of LDI blood flow ratio rose remarkably compared to the groups given ets-1 gene alone or HGF gene alone (Fig. 4). The intramuscular capillary density also increased significantly by the combined administration (Fig. 5). Therefore, gene transfection of both genes in combination was revealed to enhance angiogenesis more than when genes are used separately. Thus, the combination of the genes was more effective against ASO compared to the independent gene transfection.

[0087] According to the measurement of the intramuscular endogenous HGF concentrations in rat ischemic limbs, the rat HGF concentration was higher in the group wherein the HGF gene and ets-1 gene was used in combination compared to the group given HGF gene alone (Fig. 6). The HGF was suggested to have an auto-loop type regulatory mechanism through the activation of ets-1 because the expression of the internal HGF was enhanced much more with the combined administration of the ets-1 gene than the administration of the HGF gene alone.

[Example 3]

Effect of administration of VEGF gene, and PGIS gene to mouse hind limb ischemia ASO model

(1) Materials

[0088] The cDNA of human VEGF165 (gift from Prof. Yonemitsu at Kyushu University Department of Surgery II) was cloned by a conventional method, and was inserted into the EcoRI site of expression plasmid pCAGGS (Gene 108, 193-200 (1991)) having a CMV enhancer and a β-actin promoter to be used as human VEGF gene.

[0089] The cDNA of human PGIS (B.B.R.C., Vol. 200, No. 3, p1728-1734 (1994)) was cloned by a conventional method, and was inserted into an expression plasmid pCAGGS (Gene 108, 193-200 (1991)) having a CMV enhancer and a β-actin promoter to be used as human PGIS gene.

(2) Method

[0090]

1. C57BL/6J mouse (8 weeks old, male) was used. The mouse was anesthetized by intraperitoneal injection of 200 μl of 10-fold diluted Nembutal, and further by ether inhalation when supplementation was necessary. Then, the arteriovenous of the left hind limb was tied up to produce a mouse hind limb ischemia ASO model. After evaluation, 1 mg each of the above-mentioned genes of (1) was administered into the left hind limb muscle in the form of naked plasmids. Four groups were set up: a control group without administration; a group given the VEGF gene alone; a group given the PGIS gene alone; and a group given a combined application of the VEGF gene and PGIS gene. Four animals were included in each group. On the 5th day after the administration of each plasmid to the left tibialis muscle, the intramuscular concentration of human VEGF protein in the ischemic hind limb muscle was measured using AN'ALYZA Immunoassay System human VEGF kit (GENZYME) (Fig. 8).

2. Mouse hind limb ischemia ASO model was produced by a similar method as above. Ten days later, the blood flow in both hind limbs was evaluated using Laser Doppler Imager (LDI, Moor Instruments Ltd, MLDI5070), and the left-right ratio was calculated (Fig. 9; right leg (normal), left hind limb (ASO)). As a result, taking the normal blood flow as 100%, the amount of blood flow in the left hind limb was confirmed to have been decreased to approximately 30% thereof. After the evaluation, 500 μg each of the above-mentioned genes of (1) was administered into the left hind limb muscle in the form of naked plasmids. Four groups were set up:, a control group without administration; a group given the VEGF gene alone; a group given the PGIS gene alone; and a group given a combined application of the VEGF gene and PGIS gene. 2 weeks and 4 weeks after the gene administration, the blood flow was evaluated using LDI, and the proportion of increase was calculated. Then, 4 weeks later, the left hind limb muscle was extirpated, and after preparing a frozen section, the intramuscular capillary density was measured by alkaline phosphatase staining (Fig. 12). Significant difference test was performed by the Fisher's PLSD method.

(3) Result

[0091]

1. As indicated in Fig. 8, no intramuscular concentration of human VEGF protein in the ischemic hind limb was detected in the control and the PGIS gene administered groups, and the concentration was detected to be higher in groups to which the VEGF gene and PGIS gene were administered in combination than the group wherein the VEGF gene was administered alone.

2. The proportion of increase of blood flow in the left hind limb measured by LDI, 2 weeks later is shown in Fig. 10, and those 4 weeks later is shown in Fig. 11. The blood flow 2 weeks later was not improved, by either the administration of the VEGF gene alone, nor the VEGF gene and PGIS gene in combination. However, 4 weeks later, the blood flow was improved by the administration of the VEGF gene alone, and the VEGF gene and PGIS gene in combination compared to the control group. Unexpectedly, by the combined use of the PGIS gene and VEGF gene, the blood flow was remarkably improved compared to the independent administrations of the genes (2 weeks later: control: 100%, PGIS gene administration: 105%, VEGF gene administration: 117%, VEGF gene + PGIS gene administration: 115%; 4 weeks later: control: 100%, PGIS gene administration: 103%, VEGF gene administration: 130%, VEGF gene + PGIS gene administration: 169%, $P< 0.01$).

[0092] The intramuscular capillary density 4 weeks after the gene administration is shown in Fig. 13. The capillary density increased due to the VEGF gene administration. Furthermore, the combined use of the PGIS gene and VEGF gene remarkably increased the capillary density compared to the independent administration of the genes. (control: 100%, PGIS gene administration: 175%, VEGF gene administration: 221%, VEGF gene + PGIS gene administration: 338%, $P< 0.0001$).

Industrial Applicability

[0093] The present invention provides a novel and highly effective pharmaceutical composition for angiogenic therapy which contains as the active ingredients at least one substance selected from the group consisting of substances having vasodilating effect and/or platelet aggregation inhibitory effect, and substances producing them; and a gene encoding an angiogenesis factor. Furthermore, due to the present invention it was newly discovered that genes, such as prostacyclin synthase gene which were not known to be useable for angiogenic therapy can be applied to angiogenic therapy. Finally, pharmaceutical compositions for angiogenic therapy containing these genes as the active ingredients were provided.

Claims

1. A pharmaceutical composition for angiogenic therapy which contains, as the active ingredients, a prostacylin synthase gene and a gene encoding HGF and/or a gene encoding VEGF.

2. The pharmaceutical composition for angiogenic therapy of claim 1, wherein each of the genes take different formulations.

3. The pharmaceutical composition for angiogenic therapy of claim 1 or 2, which contains an HGF gene and a prostacyclin synthase gene as the active ingredients.

4. The pharmaceutical composition for angiogenic therapy of claim 1 or 2, which contains a VEGF gene and a prostacylin synthase gene as the active ingredients.

5. The pharmaceutical composition for angiogenic therapy of any one of claims 1 to 4, wherein the composition is used for treating or preventing ischemic disease or arterial disease.

6. The pharmaceutical composition for angiogenic therapy of claim 5, wherein the ischemic disease or arterial disease is selected from the group of arteriosclerosis obliterans, myocardial infarction, angina pectoris, cardiomyopathy, and cerebrovascular disease.

7. The pharmaceutical composition for angiogenic therapy of any one of claims 1 to 6, wherein the genes are in the form of naked DNA.

8. Use of a prostacyclin synthase gene and a gene encoding an angiogenic factor for the preparation of a pharmaceutical composition for angiogenic therapy, wherein the gene encoding an angiogenic factor is an HGF gene and/or a VEGF gene.

9. The use of claim 8, wherein the gene encoding an angiogenic factor is an HGF gene.

10. The use of claim 8, wherein the gene encoding an angiogenic factor is a VEGF gene.

11. The use of any one of claims 8 to 10, wherein each of the genes take different formulations.

12. The use of any one of claims 8 to 11, wherein the genes are in the form of naked DNA.

13. The use of any one of claims 8 to 12, wherein the pharmaceutical composition for angiogenic therapy is used for treating or preventing ischemic disease or arterial disease.

14. The use of claim 13, wherein the ischemic disease or arterial disease is selected from the group of arteriosclerosis obliterans, myocardial infarction, angina pectoris, cardiomyopathy, and cerebrovascular disease.

15. Use of a prostacyclin synthase gene for the preparation of a pharmaceutical composition for angiogenic therapy by potentiating the angiogenic effect due to a pharmaceutical composition comprising a gene encoding an angiogenic factor, wherein the gene encoding an angiogenic factor is an HGF gene and/or a VEGF gene.

16. The use of claim 15, wherein the gene encoding an angiogenic factor is an HGF gene.

17. The use of claim 15, wherein the gene encoding an angiogenic factor is a VEGF gene.

18. The use of any one of claims 15 to 17, wherein the prostacyclin synthase gene is in the form of naked DNA.

19. The use of any one of claims 15 to 18, wherein the pharmaceutical composition is used for treating or preventing ischemic disease or arterial disease.

20. The use of claim 19, wherein the ischemic disease or arterial disease is selected from the group of arteriosclerosis obliterans, myocardial infarction, angina pectoris, cardiomyopathy, and cerebrovascular disease.

Patentansprüche

1. Pharmazeutische Zusammensetzung zur Angiogenese-Therapie, die als Wirkstoffe ein Prostazyklinsynthasegen und ein für HGF codierendes Gen und/ oder ein für VEGF codierendes Gen enthält.

2. Pharmazeutische Zusammensetzung zur Angiogenese-Therapie nach Anspruch 1, wobei jedes der Gene in unterschiedlichen Formulierungen vorliegt.

3. Pharmazeutische Zusammensetzung zur Angiogenese-Therapie nach Anspruch 1 oder 2, die ein HGF-Gen und ein Prostazyklinsynthasegen als Wirkstoffe enthält.

4. Pharmazeutische Zusammensetzung zur Angiogenese-Therapie nach Anspruch 1 oder 2, die ein VEGF-Gen und ein Prostazyklinsynthasegen als Wirkstoffe enthält.

5. Pharmazeutische Zusammensetzung zur Angiogenese-Therapie nach einem der Ansprüche 1 bis 4, wobei die Zusammensetzung zum Behandeln oder Verhindern von einer ischämischen Krankheit oder einer arteriellen Krankheit verwendet wird.

6. Pharmazeutische Zusammensetzung zur Angiogenese-Therapie nach Anspruch 5, wobei die ischämische Krankheit oder die arterielle Krankheit ausgewählt ist aus der Gruppe von obliterierender Arteriosklerose, Myokardinfarkt, Angina pectoris, Kardiomyopathie und zerebrovaskulärer Erkrankung.

7. Pharmazeutische Zusammensetzung zur Angiogenese-Therapie nach einem der Ansprüche 1 bis 6, wobei die Gene in Form nackter DNA vorliegen.

8. Verwendung eines Prostazyklinsynthasegens und eines für einen angiogenen Faktor codierenden Gens zur Herstellung einer pharmazeutischen Zusammensetzung zur Angiogenese-Therapie, wobei das für einen angiogenen Faktor codierende Gen ein HGF-Gen und/oder ein VEGF-Gen ist.

9. Verwendung nach Anspruch 8, wobei das für einen angiogenen Faktor codierende Gen ein HGF-Gen ist.

10. Verwendung nach Anspruch 8, wobei das für einen angiogenen Faktor codierende Gen ein VEGF-Gen ist.

11. Verwendung nach einem der Ansprüche 8 bis 10, wobei jedes der Gene in unterschiedlichen Formulierungen vorliegt.

12. Verwendung nach einem der Ansprüche 8 bis 11, wobei die Gene in Form von nackter DNA vorliegen.

13. Verwendung nach einem der Ansprüche 8 bis 12, wobei die pharmazeutische Zusammensetzung zur Angiogenese-Therapie zum Behandeln oder Verhindern von einer ischämischen Krankheit oder einer arteriellen Krankheit verwendet wird.

14. Verwendung nach Anspruch 13, wobei die ischämische Krankheit oder die arterielle Krankheit ausgewählt ist aus der Gruppe von obliterierender Arteriosklerose, Myokardinfarkt, Angina pectoris, Kardiomyopathie und zerebrovaskulärer Erkrankung.

15. Verwendung eines Prostazyklinsynthasegens zur Herstellung einer pharmazeutischen Zusammensetzung zur Angiogenese-Therapie durch Verstärken der angiogenen Wirkung aufgrund einer pharmazeutischen Zusammensetzung, die ein für einen angiogenen Faktor codierendes Gen umfasst, wobei das für einen angiogenen Faktor codierende Gen ein HGF-Gen und/oder ein VEGF-Gen ist.

16. Verwendung nach Anspruch 15, wobei das für einen angiogenen Faktor codierende Gen ein HGF-Gen ist.

17. Verwendung nach Anspruch 15, wobei das für einen angiogenen Faktor codierende Gen ein VEGF-Gen ist.

18. Verwendung nach einem der Ansprüche 15 bis 17, wobei das Prostazyklinsynthasegen in Form von nackter DNA vorliegt.

19. Verwendung nach einem der Ansprüche 15 bis 18, wobei die pharmazeutische Zusammensetzung zum Behandeln oder Verhindern von einer ischämischen Krankheit oder einer arteriellen Krankheit verwendet wird.

20. Verwendung nach Anspruch 19, wobei die ischämische Krankheit oder die arterielle Krankheit ausgewählt ist aus der Gruppe von obliterierender Arteriosklerose, Myokardinfarkt, Angina pectoris, Kardiomyopathie und zerebrovaskulärer Erkrankung.

Revendications

1. Composition pharmaceutique pour thérapie angiogénique qui contient, en tant qu'ingrédients actifs, un gène de prostacycline synthase et un gène codant HGF et/ou un gène codant VEGF.

2. Composition pharmaceutique pour thérapie angiogénique selon la revendication 1, dans laquelle chacun des gènes prend des formulations différentes.

3. Composition pharmaceutique pour thérapie angiogénique selon la revendication 1 ou 2, qui contient un gène HGF et un gène de prostacycline synthase en tant qu'ingrédients actifs.

4. Composition pharmaceutique pour thérapie angiogénique selon la revendication 1 ou 2, qui contient un gène VEGF et un gène de prostacycline synthase en tant qu'ingrédients actifs.

5. Composition pharmaceutique pour thérapie angiogénique selon l'une quelconque des revendications 1 à 4, dans laquelle la composition est utilisée pour traiter ou prévenir une maladie ischémique ou une maladie artérielle.

6. Composition pharmaceutique pour thérapie angiogénique selon la revendication 5, dans laquelle la maladie ischémique ou la maladie artérielle est choisie dans le groupe de l'artériosclérose oblitérante, l'infarctus du myocarde, l'angine de poitrine, la cardiomyopathie et la maladie cérébrovasculaire.

7. Composition pharmaceutique pour thérapie angiogénique selon l'une quelconque des revendications 1 à 6, dans laquelle les gènes sont sous la forme d'ADN nu.

8. Utilisation d'un gène de prostacycline synthase et d'un gène codant un facteur angiogénique pour la préparation d'une composition pharmaceutique

pour thérapie angiogénique, dans laquelle le gène codant un facteur angiogénique est un gène HGF et/ou un gène VEGF.

9. Utilisation selon la revendication 8, dans laquelle le gène codant un facteur angiogénique est un gène HGF.

10. Utilisation selon la revendication 8, dans laquelle le gène codant un facteur angiogénique est un gène VEGF.

11. Utilisation selon l'une quelconque des revendications 8 à 10, dans laquelle chacun des gènes prend différentes formulations.

12. Utilisation selon l'une quelconque des revendications 8 à 11, dans laquelle les gènes sont sous la forme d'ADN nu.

13. Utilisation selon l'une quelconque des revendications 8 à 12, dans laquelle la composition pharmaceutique pour thérapie angiogénique est utilisée pour traiter ou prévenir une maladie ischémique ou une maladie artérielle.

14. Utilisation selon la revendication 13, dans laquelle la maladie ischémique ou la maladie artérielle est choisie dans le groupe de l'artériosclérose oblitérante, l'infarctus du myocarde, l'angine de poitrine, la cardiomyopathie et la maladie cérébrovasculaire.

15. Utilisation d'un gène de prostacycline synthase pour la préparation d'une composition pharmaceutique pour thérapie angiogénique en potentialisant l'effet angiogénique dû à une composition pharmaceutique comprenant un gène codant un facteur angiogénique, dans laquelle le gène codant un facteur angiogénique est un gène HGF et/ou un gène VEGF.

16. Utilisation selon la revendication 15, dans laquelle le gène codant un facteur angiogénique est un gène HGF.

17. Utilisation selon la revendication 15, dans laquelle le gène codant un facteur angiogénique est un gène VEGF.

18. Utilisation selon l'une quelconque des revendications 15 à 17, dans laquelle le gène de prostacycline synthase est sous la forme d'ADN nu.

19. Utilisation selon l'une quelconque des revendications 15 à 18, dans laquelle la composition pharmaceutique est utilisée pour traiter ou prévenir une maladie ischémique ou une maladie artérielle.

20. Utilisation selon la revendication 19, dans laquelle la maladie ischémique ou la maladie artérielle est choisie dans le groupe de l'artériosclérose oblitérante, l'infarctus du myocarde, l'angine de poitrine, la cardiomyopathie et la maladie cérébrovasculaire.

Fig. 1

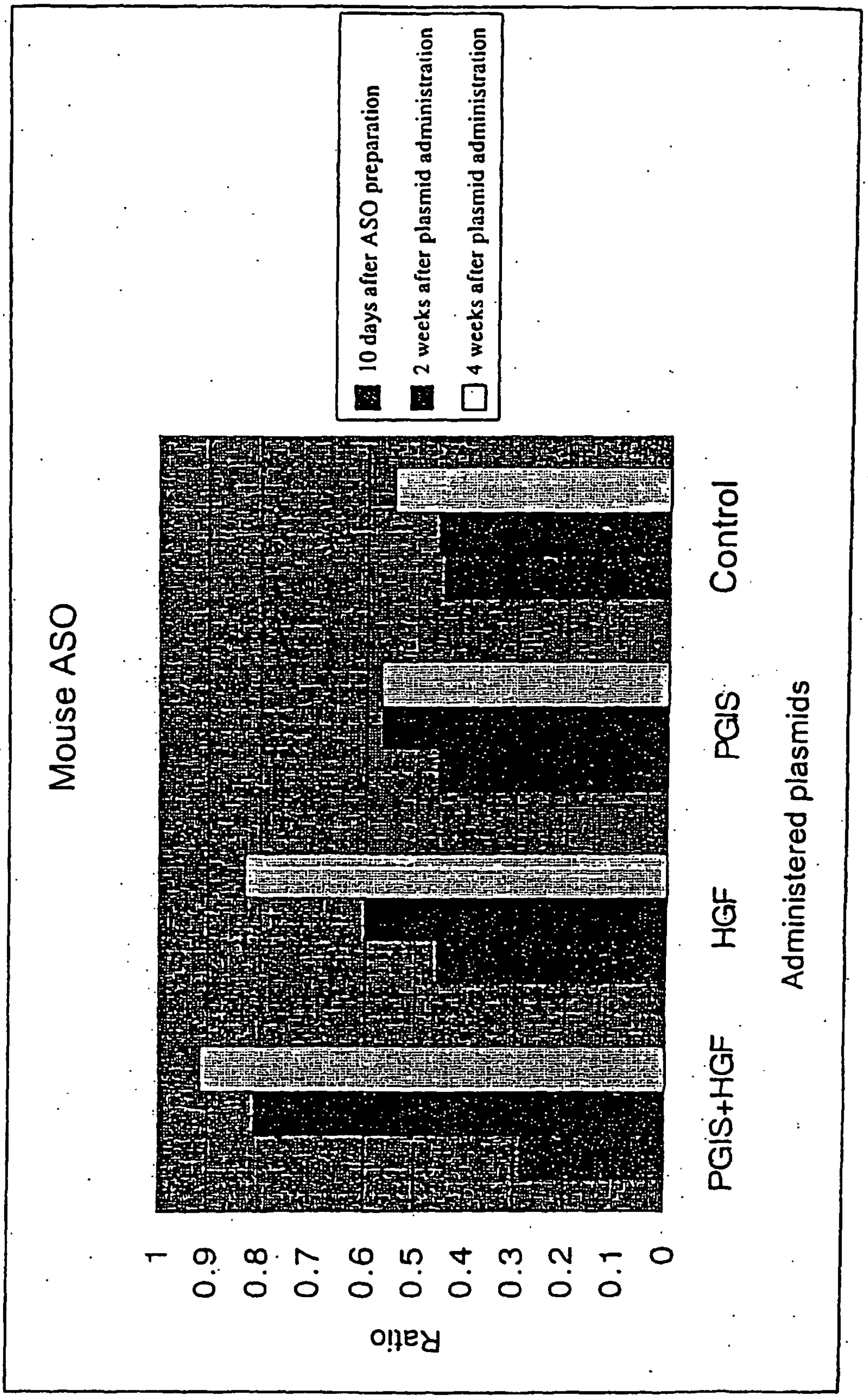

Mouse ASO
10 days after ASO preparation
2 weeks after plasmid administration
4 weeks after plasmid administration
Ratio
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
PGIS+HGF
HGF
PGIS
Control
Administered plasmids

Fig. 2

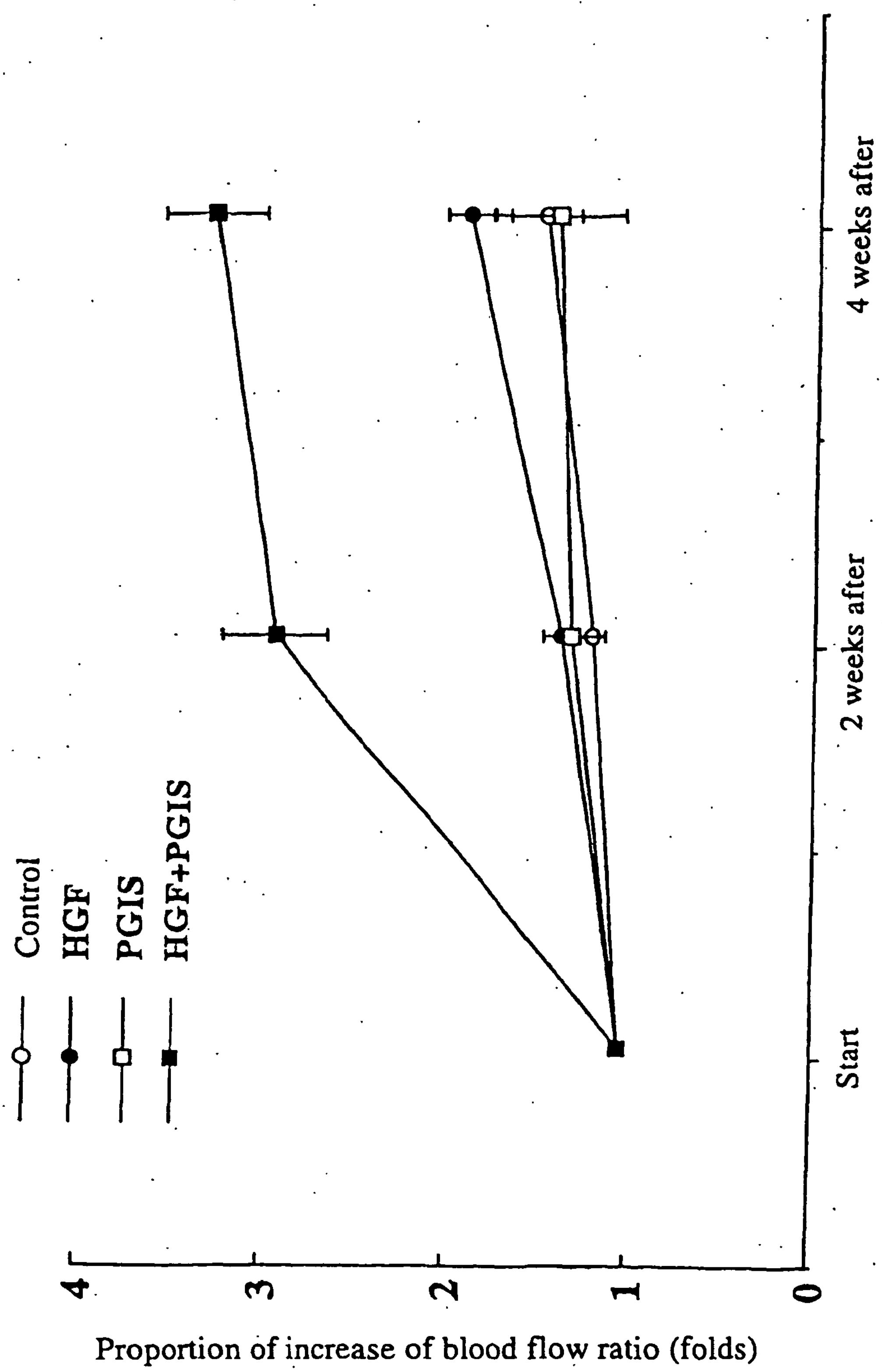

Control
HGF
PGIS
HGF+PGIS
0
1
2
3
4
Proportion of increase of blood flow ratio (folds)
Start
2 weeks after
4 weeks after

Fig. 3

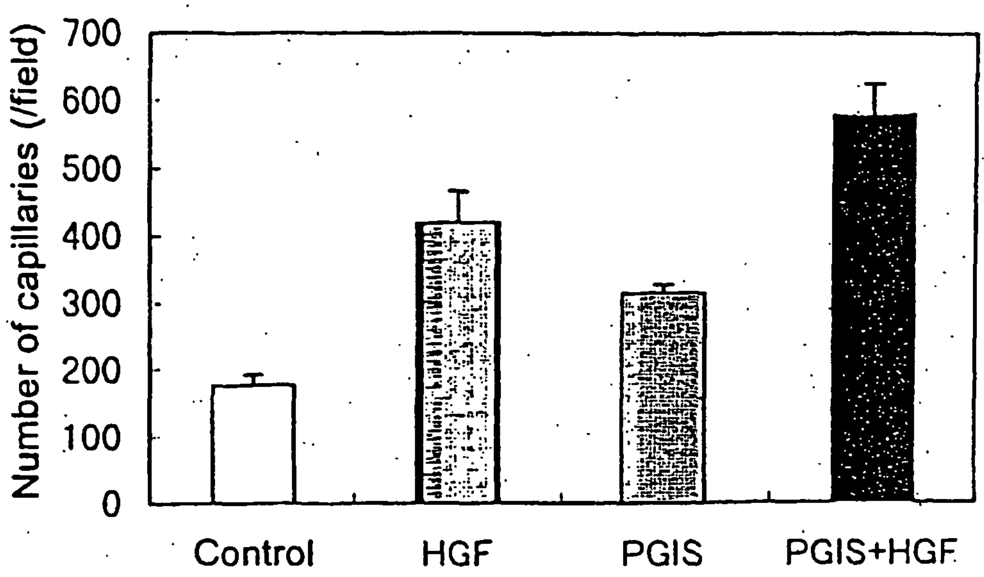
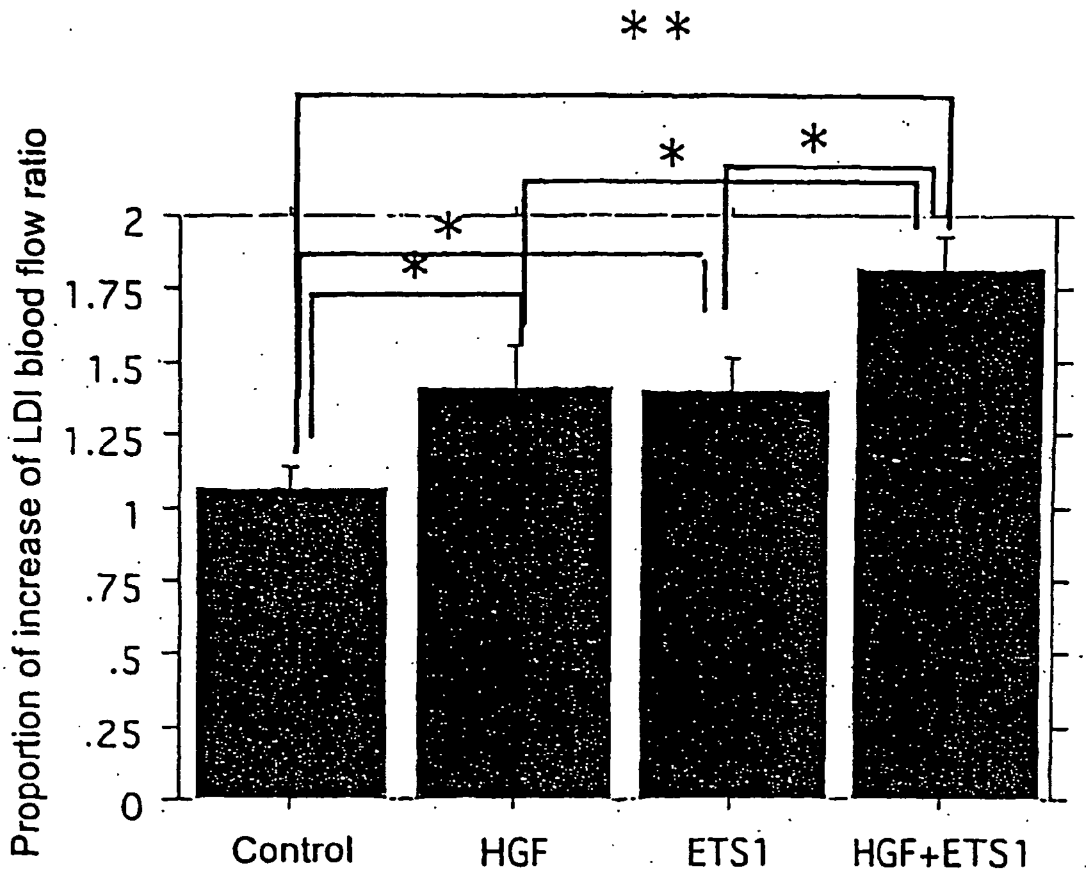

Number of capillaries (/field)
700
600
500
400
300
200
100
0
Control
HGF
PGIS
PGIS+HGF

Fig. 4



**
*
*
*
*
Proportion of increase of LDI blood flow ratio
2
1.75
1.5
1.25
1
.75
.5
.25
0
Control
HGF
ETS1
HGF+ETS1

Fig. 5

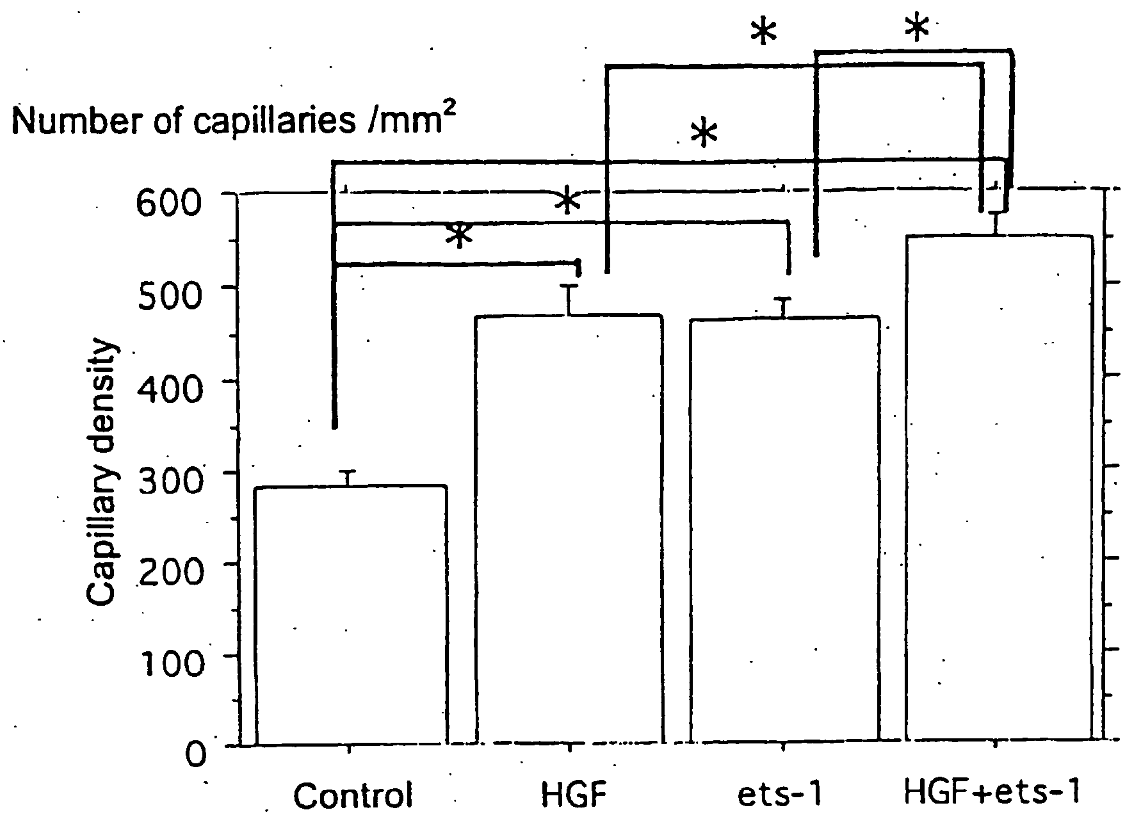

*
*
Number of capillaries /mm²
*
*
*
600
500
400
300
200
100
0
Capillary density
Control
HGF
ets-1
HGF+ets-1

Fig. 6

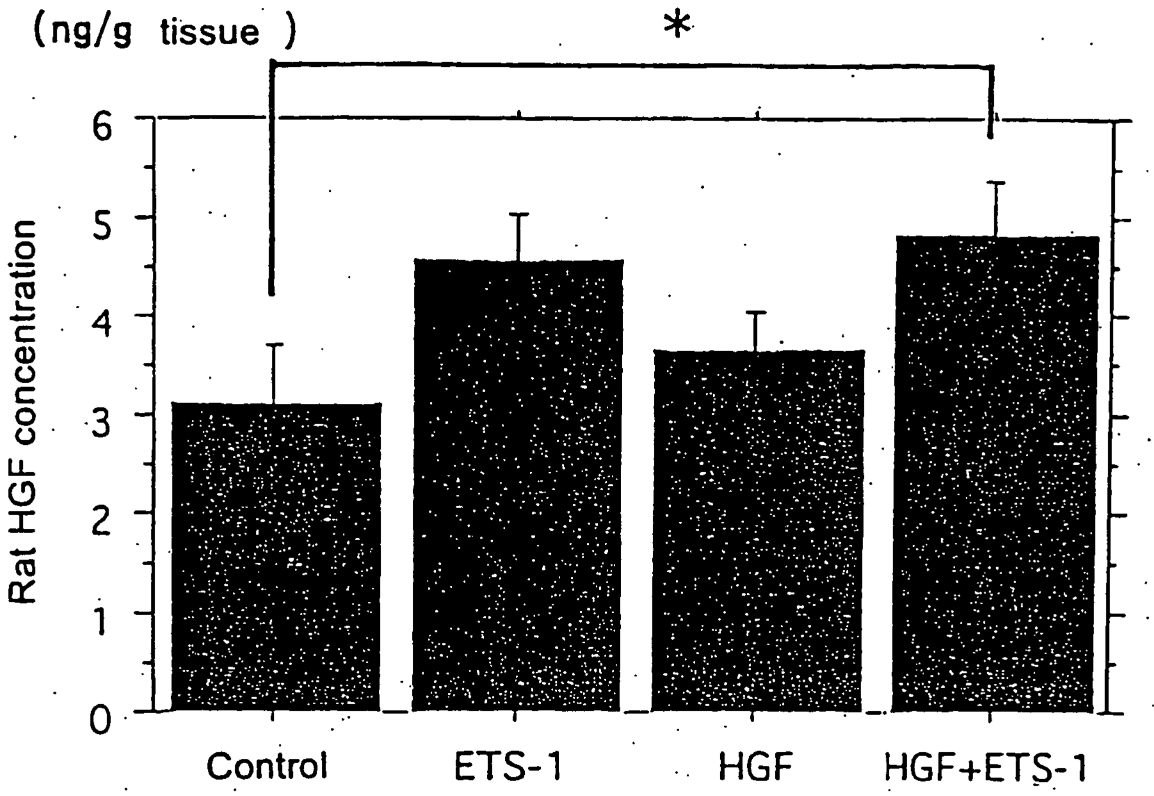

(ng/g tissue)
*
Rat HGF concentration
6
5
4
3
2
1
0
Control
ETS-1
HGF
HGF+ETS-1

Fig. 7

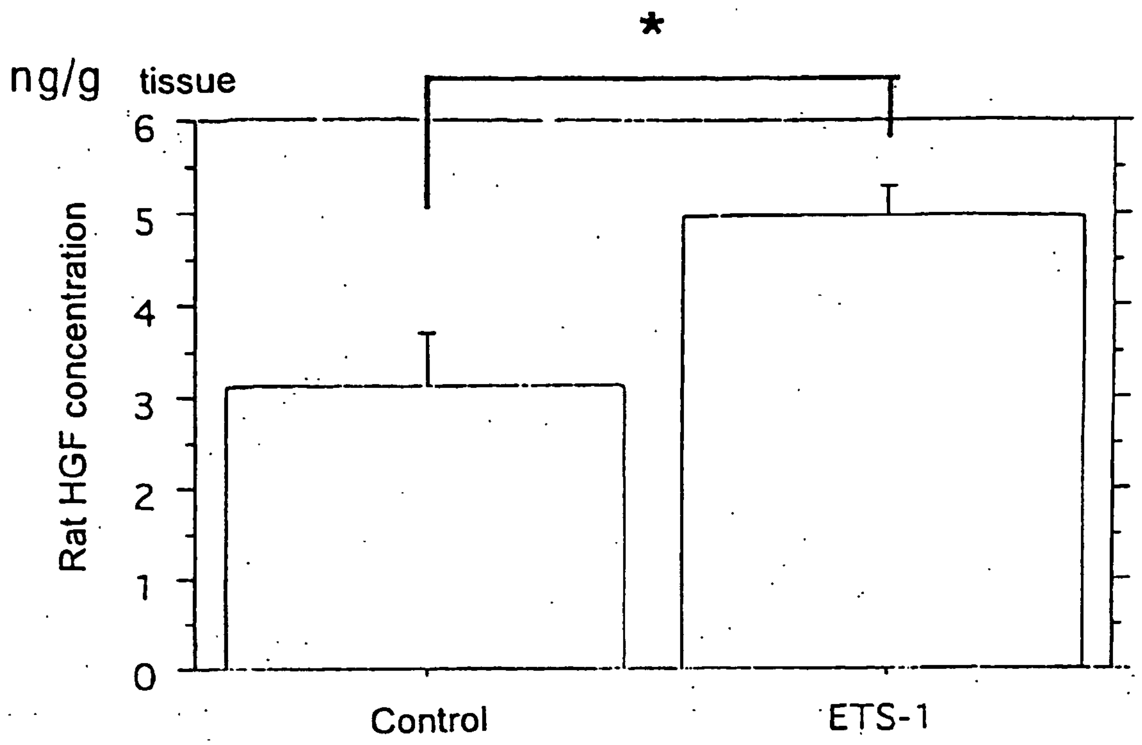


Fig. 8

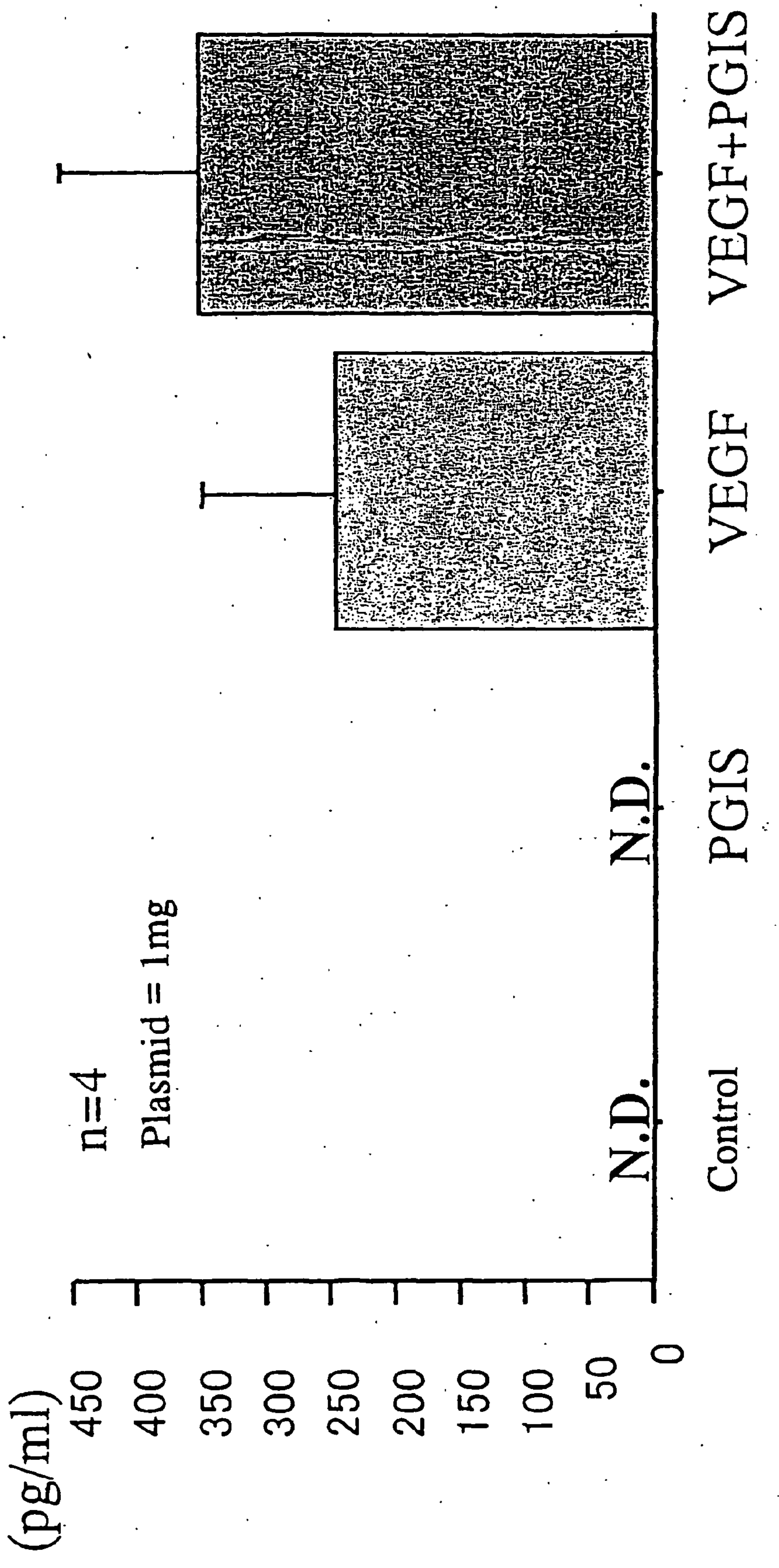

(pg/ml)
450
400
350
300
250
200
150
100
50
0
n=4
Plasmid = 1mg
N.D.
N.D.
Control
PGIS
VEGF
VEGF+PGIS

Fig. 9

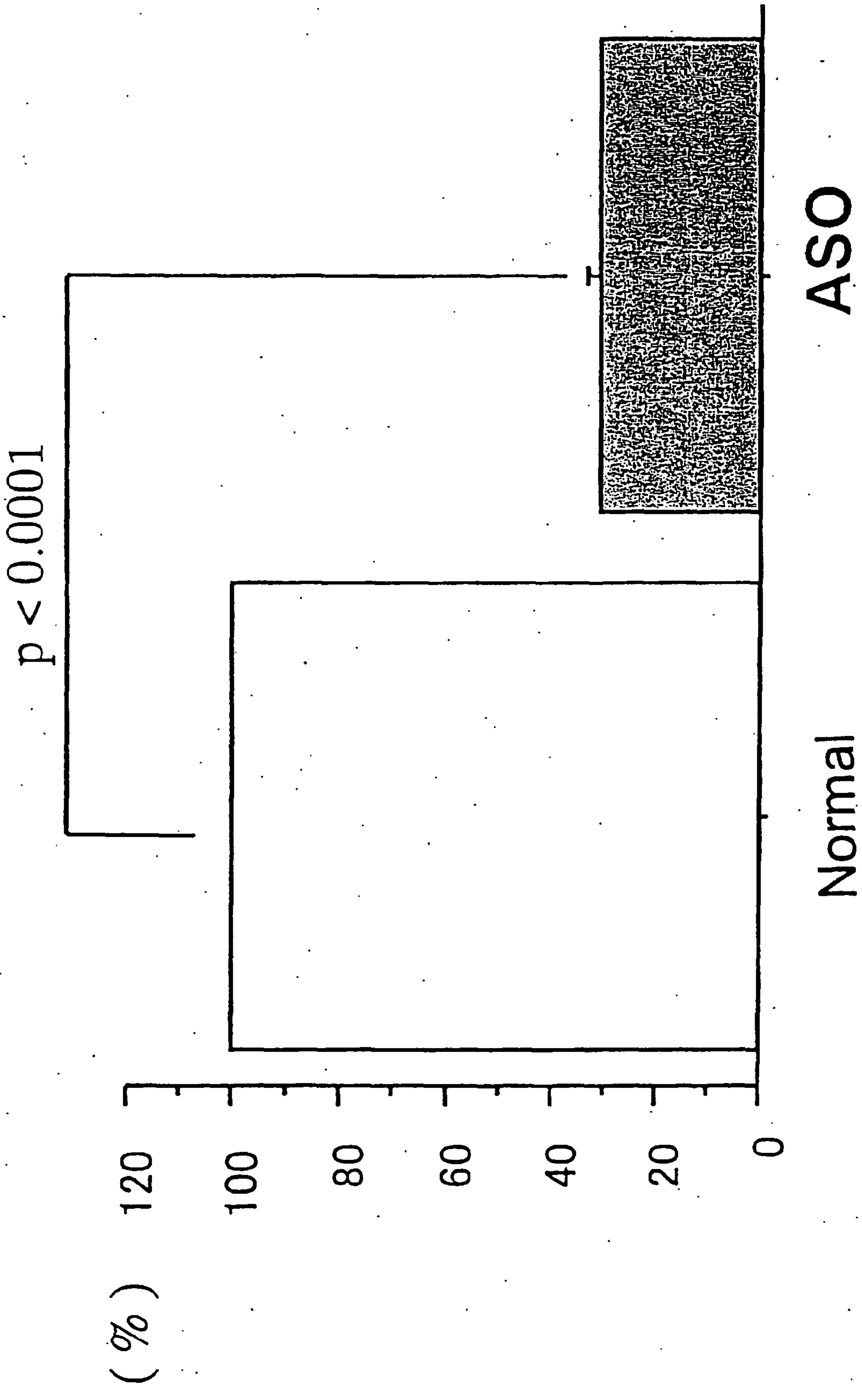


Fig. 10

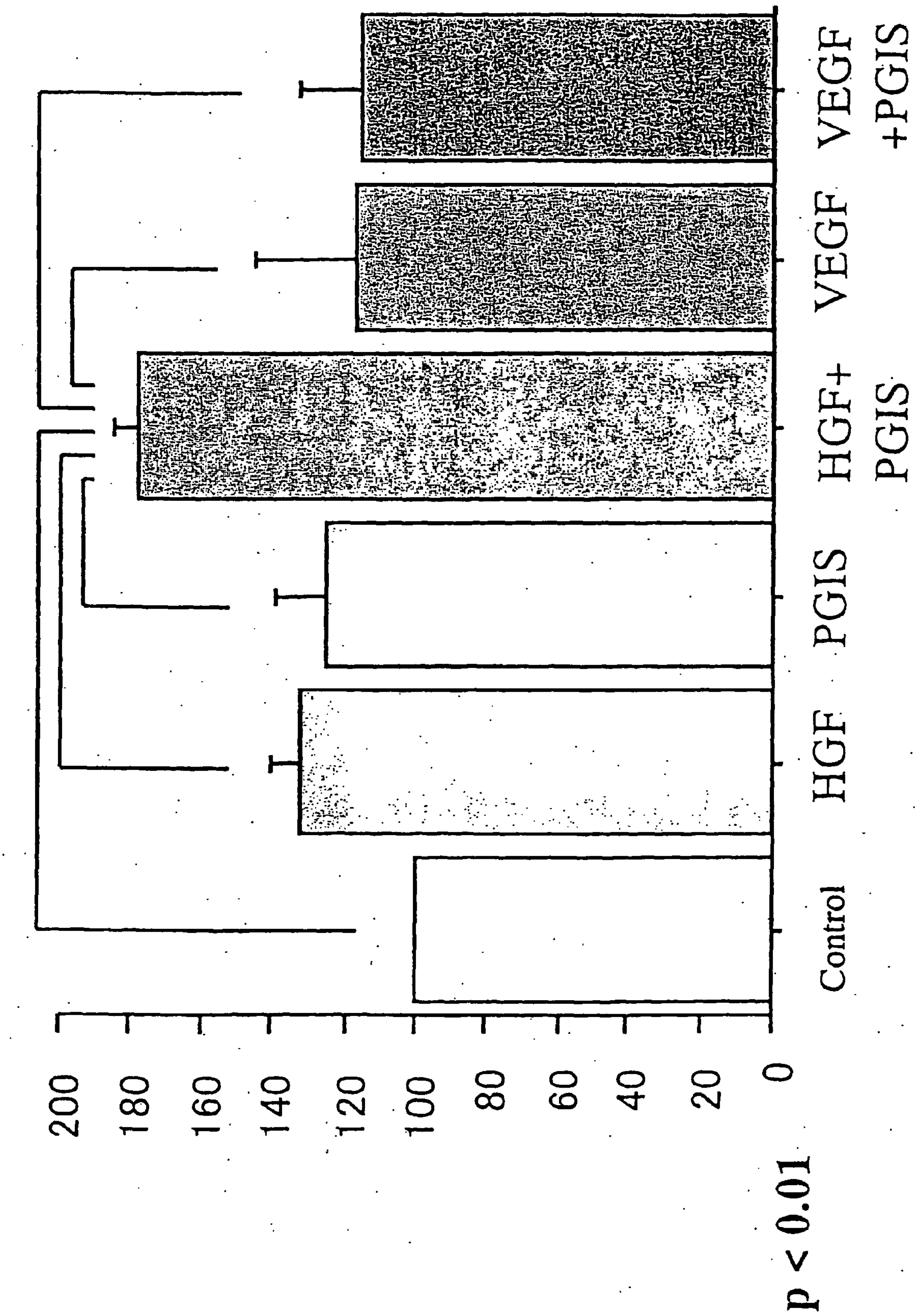

200
180
160
140
120
100
80
60
40
20
0
Control
HGF
PGIS
HGF+
PGIS
VEGF
VEGF
+PGIS
p < 0.01

Fig. 11

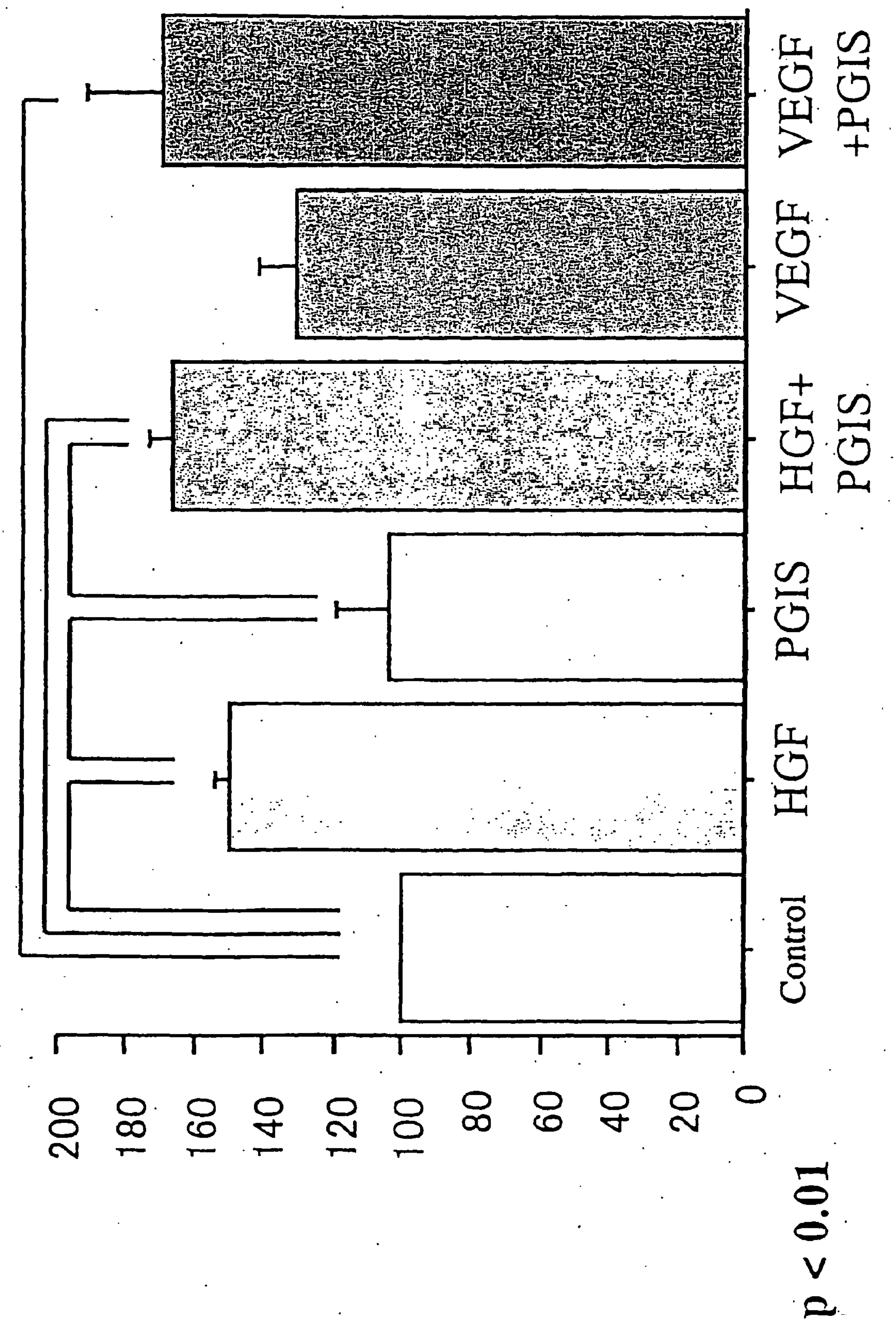

Control
HGF
PGIS
HGF+
PGIS
VEGF
VEGF
+PGIS
0
20
40
60
80
100
120
140
160
180
200
p < 0.01

Fig. 12

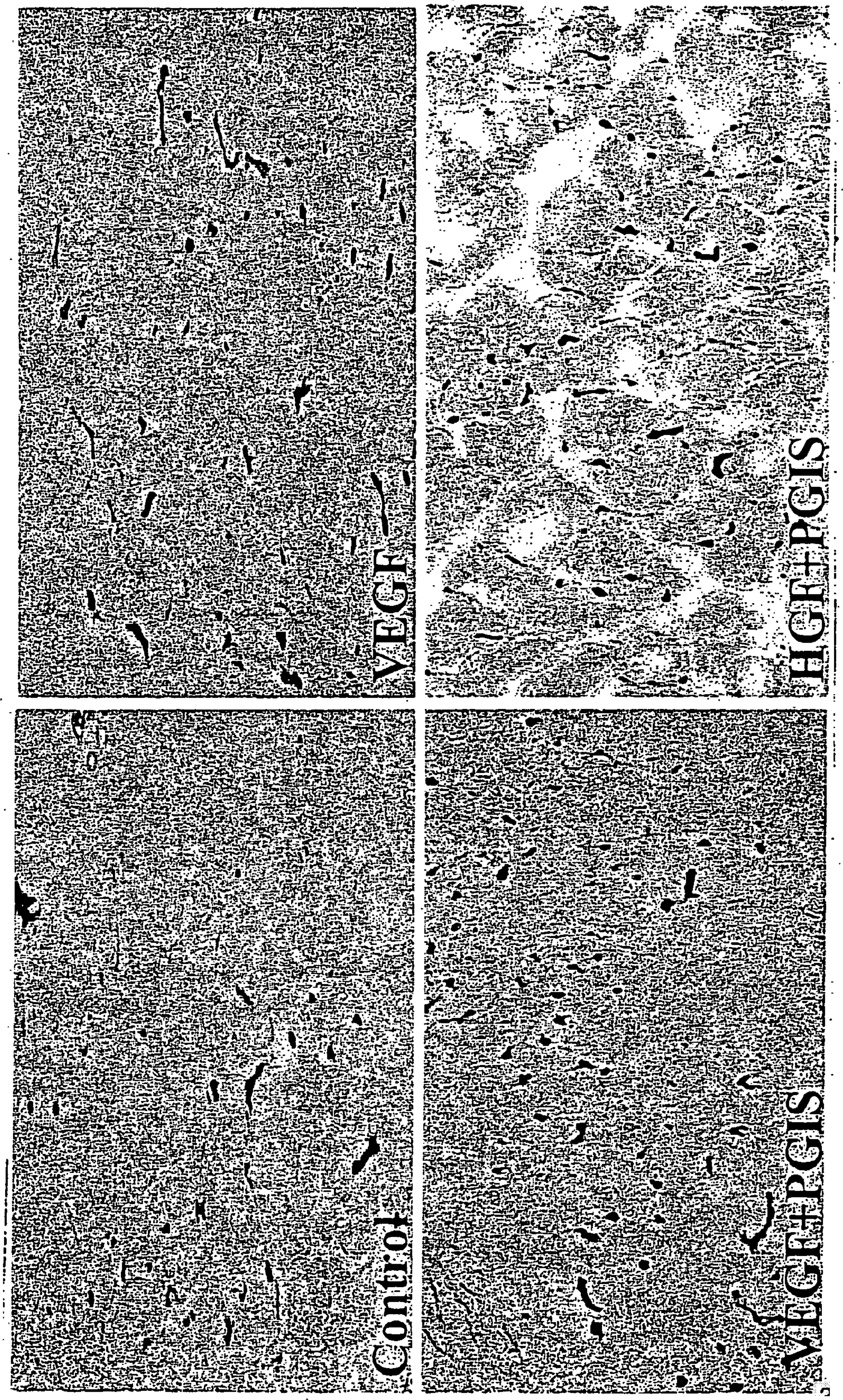
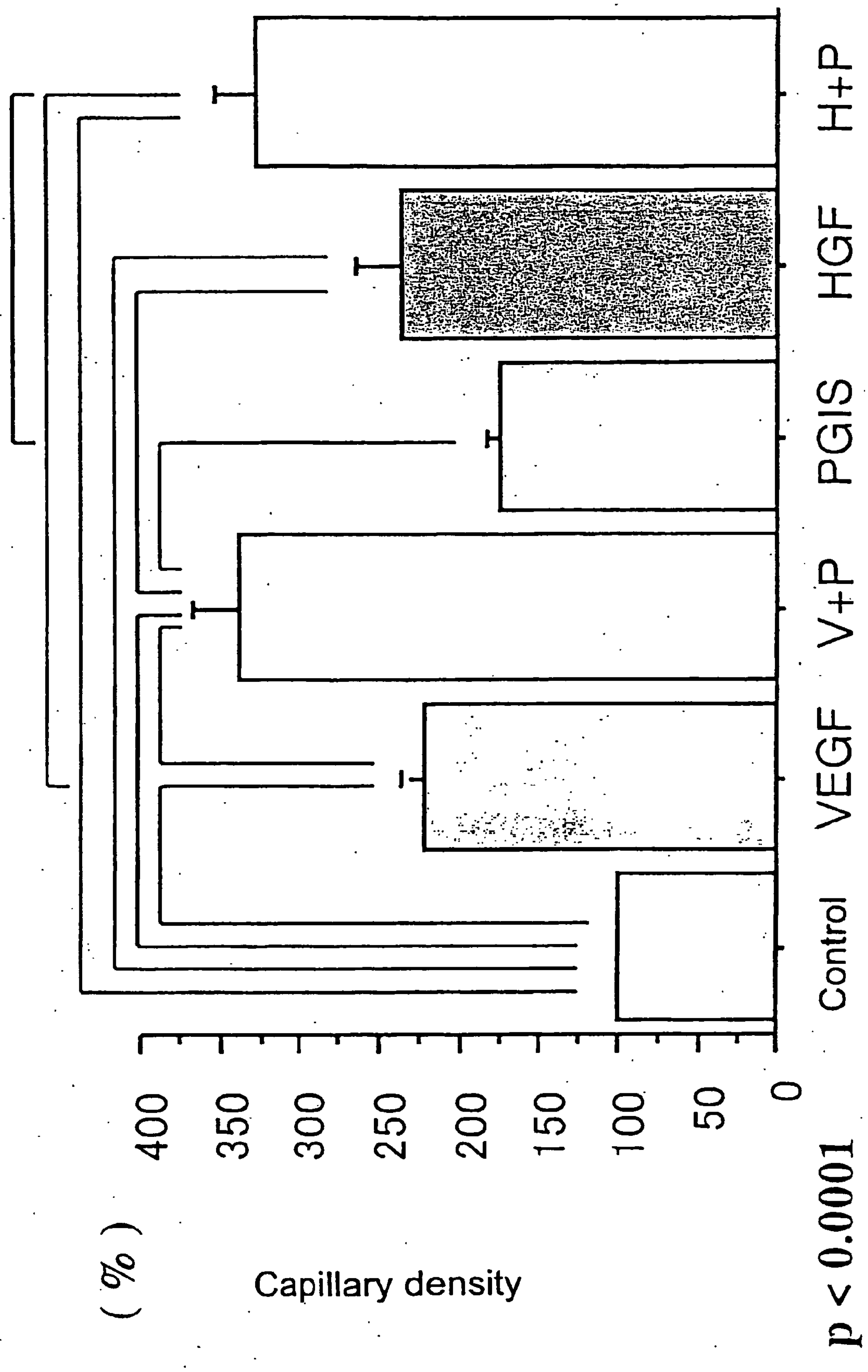


Fig. 13


Control
VEGF
V+P
PGIS
HGF
H+P
400
350
300
250
200
150
100
50
0
Capillary density
(%)
p < 0.0001

REFERENCES CITED IN THE DESCRIPTION

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