Title: INHIBITOR OF VASCULAR SUPEROXIDE ANION

Abstract: There is provided an inhibitor of superoxide anion. Also provided is a method of treating hypertension, atherosclerosis, diabetes and cancer by administering an effective amount of the production of superoxide anion. A viral vector expressing a superoxide anion is also provided. There is provided a composition for treating a patient comprising an inhibitor of superoxide anion.
INHIBITOR OF VASCULAR SUPEROXIDE ANION

BACKGROUND OF THE INVENTION

1. TECHNICAL FIELD

The present invention relates to inhibitors of vascular superoxide anion. More specifically, the present invention relates to inhibitors which block the superoxide anion for use in treating cardiovascular disease, cancer and other neoplastic effects.

2. DESCRIPTION OF RELATED ART

In acute myocardial infarction, cardiac tissue is damaged by two sequential events, hypoxia in the ischemic phase and oxidative damage in the reperfusion phase. Damaged cardiac tissue in the ischemic phase can be salvaged by restoring blood flow to the ischemic region through reperfusion. However, restoration of blood containing oxygen can result in injury due to the production of reactive oxygen species [Biochim. Biophys. Acta, 890:82-88 (1987)]. Two of these reactive oxygen species, hydrogen peroxide and superoxide radical, are thought to be of particular importance in causing injury to myocardial cells exposed to ischemia and reperfusion [Free Radical Res. Commun. 9:223-232 (1990); Basic Res. Cardiol. 84:191-196 (1989)]. Injury from hydrogen peroxide and superoxide radicals occurs when in the
of iron there is generation of highly toxic hydroxyl radicals [Am. J. Physiol. (1994) 266:H121-H127]. Hydroxyl radicals can also be produced from the simultaneous generation of superoxide radical and nitric oxide, and this reaction could also cause tissue injury ([Biochemical J. 281:419-424 (1992)].

If hydrogen peroxide, superoxide radical, or other reactive oxygen species accumulate during the reperfusion phase, various toxic reactions can occur which result in myocardial cell injury or death.

Similar injury to heart tissue can occur during heart surgery when bypass procedures or other manipulations result in an ischemic phase followed by a reperfusion phase. Similar injury to other organs such as the brain, kidney or intestine can also occur due to ischemia and reperfusion and production of reactive oxygen species [J. Appl. Physiol. 71:1185-1195 (1991); Kidney Int. 40:1041-1049 (1991)]. Injury due to generation of reactive oxygen species, probably resulting from exposure to ischemia and reperfusion, also occurs during transplantation of organs such as hearts, kidneys, livers or lungs [J. Thorax. Cardiovasc. Surgery (1992) 103:945-951; Clinical Transplantation (1995) 9:171-175]. In addition, injury due to reactive oxygen species to the coronary arteries or other blood vessels can occur either due to exposure to ischemia and reperfusion [Am. J. Physiol. 260H42-H49 (1990)] or under other conditions when they may contribute to atherosclerosis [Proc. Natl. Acad. Sci. U.S.A. 84:2995-2998 (1987)]. Although ischemia followed by reperfusion is the usual cause of production of reactive oxygen species in the
myocardium and blood vessels, there may be accumulation of reactive oxygen species in these organs from other mechanisms. For example, the accumulation of reactive oxygen species has been implicated in heart failure [Free Radical Biology Med. 14:643-647 (1993)]. Production of superoxide radical or other reactive oxygen species in vascular tissue can cause tolerance to certain drugs used for treatment of heart disease, such as nitroglycerin and related nitrates [J. Clin. Invest. 95:187-194 (1995)].

A major enzymatic source of superoxide anion which is the precursor to all major biological oxidants has been characterized [Pagano et al., 1995, 1997, 1998]. A number of groups have now identified the major source of vascular superoxide as NAD(P)H oxidases, and superoxide derived from this source has been implicated in a variety of disease processes including hypertension [Laursen et al., 1997; Rajagopalan et al., 1996; Pagano et al., 1998], atherosclerosis [White et al., 1994, Chatterjee, 1998], and diabetes [Rosen, Du, & Tschope, 1998; Ellis et al., 1998]. In particular, this enzyme has been shown to be induced in hypertension, i.e., the superoxide derived from it is involved in elevation of blood pressure and development of vascular hypertrophy [Griendling et al., 1994; Ushio-Fukai et al., 1996]. Superoxide has been shown to induce proliferation [Murrel et al., 1989, 1990], while NADPH oxidase-derived superoxide is a likely candidate for mediation of injury-induced vascular proliferation and is a basic underlying mechanism in carcinogenesis [Cerutti, 1985]. The primary cause of NADPH oxidase-derived
superoxide's deleterious vascular effects appears to be its ability to inactivate nitric oxide (NO) [Beckman et al., Gryglewski et al.], though a number of other superoxide-mediated mechanisms have also been proposed [Wu & de Champlain, 1999; Brzezinska et al., 2000].

There is a need for methods of treatment of hypertension, diabetes, and other vascular disease which avoid the problems in prior art methods. More specifically, there is a need for improved methods of treatment in which the effects of reactive oxygen species are neutralized.

SUMMARY OF THE INVENTION

According to the present invention, there is provided an inhibitor of production of superoxide anion. Also provided is a method of treating hypertension, atherosclerosis, diabetes and cancer by administering an effective amount of an inhibitor of superoxide anion. A viral vector expressing a this inhibitor is also provided. There is provided a composition for treating a patient comprising an inhibitor of superoxide anion.

DESCRIPTION OF THE DRAWINGS
Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

Figure 1 is a graph showing that AngII markedly increase superoxide levels in the murine aorta;

Figure 2 is a graph showing that gp91ds-tat attenuates AngII-induced elevations in blood pressure for up to seven days compared to a scrambled peptide control (10 mg/kg/d);

Figure 3 is a graph showing that gp91ds-tat-treated mice exhibited completely reduced superoxide levels compared with mice treated with AngII alone;

Figure 4 is a graph showing that Ang II increase monocyte infiltration approximately three-fold compared to sham; and

Figure 5 is a graph showing that gp91ds-tat at concentrations of up to 100 μM were capable of reducing superoxide-induced chemoluminescence (RCU) of phorbol myristate acetate by approximately 25%.
DETAILED DESCRIPTION OF THE INVENTION

Generally, the present invention relates to the use of inhibitors of superoxide anions and the use of such inhibitors for the treatment of vascular disease and hypertension. This general field of study has been impeded by the lack of specific inhibitors of NAD(P)H oxidases. The inhibitor used most often is diphenylene iodonium, a flavoprotein inhibitor, which is known to inhibit a variety of other important enzymes including nitric oxide synthases. This treatment can result in various side effects based upon the lack of specificity of the inhibition. These side effects can limit or counteract the effects of the treatment. These problems are overcome by the present invention, wherein a specific inhibitor is utilized which acts upon a specific region of interaction within the enzyme multimer. Specifically, the chimera gp91ds-tat specifically inhibits NAD(P)H oxidases based on unique regions of interaction within the enzyme multimer. Thus the inhibitor of the present invention has more specific interactions with the target site, thus enabling a more specific inhibition, thereby creating a more specific response to treatment.

Superoxide anion and NADPH oxidase are implicated in a variety of vascular diseases, as they have been shown to interfere with normal endothelium-dependent relaxation of blood vessels. gp91ds-tat can be used to enhance the patency of blood vessels and prevent their long-term
degeneration by blocking the production of superoxide and preserving NO. In fact, low levels of Ang II which can increase reactive oxygen species appear to underlie a number of disease states including essential hypertension and atherosclerosis. Even in the absence of blood pressure elevation, superoxide production in the vascular wall is likely to be involved in atherosclerosis and arteriosclerosis. As superoxide underlies the pro-proliferative effects of these vascular diseases, inhibition of this crucial mediator has broad anti-neoplastic effects.

Assembly of cytosolic and membrane-bound components is essential for activation of NADPH oxidase and production of superoxide [DeLeo and Quinn, 1996; DeLeo et al., 1995]. Based on the findings that (a) all four major components of phagocyte NADPH oxidase are present in vascular cells [Pagano et al., 1997]; (b) protein levels of components are upregulated during hypertension [Cifuentes et al., in press]; and (c) from cloning, it was determined that these components are highly homologous to phagocyte components [Pagano et al., 1998; Gauss, Pagano, & Quinn, unpublished data, 2000; Ushio-Fukai et al., 1996], specific inhibitors have been developed based on amino acid sequences from gp91<sup>phox</sup>, known to block interaction of gp91<sup>phox</sup> with p47<sup>phox</sup>, and linked it with a portion of HIV coat protein called tat, which is recognized by all cells and internalized [Fawell et al., 1994; Kim et al., 1997]. This first chimeric sequence is called gp91ds-tat. Other sequences are also provided which prevent the interaction of gp91<sup>phox</sup> and p47<sup>phox</sup>.
Generally, the patent also provides other peptide sequences or their chemical derivatives which inhibit any of the components of this enzyme class including other vascular cell types. This includes organic and inorganic chemical compounds which are designed to be peptidomimetics and/or which are expressly designed to inhibit the interaction of the components of the enzymes including gp91<sub>phox</sub>, p47<sub>phox</sub>, p22<sub>phox</sub>, p67<sub>phox</sub>, and p21rac. Additionally, the present invention also contemplates the use of viral vectors expressing these inhibitors for in vivo and in vitro expression, and agents which would interfere with the components’ involvement in restenosis, atherosclerosis, hypertension, diabetes, and other vascular diseases.

The sequence of the chimera (gp91ds-tat):

1: [H]-R-K-K-R-Q-R-R-R-C-S-T-R-I-R-R-Q-L-[NH<sub>2</sub>]

Some alternative sequences:


3: [H]-R-K-K-R-Q-R-R-R-G-V-H-F-I-F-[NH<sub>2</sub>]

Alternative chimeric sequence containing tat plus a region of p47<sub>phox</sub>:
4: [H]- R-K-R-R-Q-R-R-Q-R-R-Q-A-R-P-G-P-Q-S-P-G-[NH₂]

The above list provides some examples of sequences encoding for inhibitors of superoxide anions, but this not meant to be an exhaustive list of all alternative sequences. All possible amino acid sequences at the interface between the various components of NADPH oxidase are considered to be within the scope of the present invention.

The data show that angiotensin II (Ang II) directly induces vascular superoxide, consistent with increases in NADPH oxidase protein. Using the chimeric peptide sequence described above, Ang II-induced rises in O₂⁻ production in isolated aortas was prevented.

Mouse thoracic aortic rings were cleaned of adventitial adipose tissue at 4°C and placed in cold physiologic buffer. They were then placed in buffer at 37°C for three hours in the presence or absence of Ang II (10 pM) with or without the chimera. Ang II markedly increased superoxide levels in the murine aorta (Figure 1). Co-incubation with the chimera gp91ds-tat (25 FM) significantly blocked this increase, whereas co-incubation with tat alone (25-50 FM) had no effect on superoxide levels. These data show that Ang II induces O₂⁻ production in vascular tissue via a translational increase in components of phagocyte-like NADPH oxidase, and that interaction of these
components is necessary for this superoxide induction.

One of the primary reasons for these ongoing studies is the detrimental effect of superoxide on endothelium-derived relaxing factor NO and its suggested modulatory role in the development of blood pressure. The effect of this chimera on systolic blood pressure was tested. C57BL/6 mice were anesthetized and osmotic minipumps implanted in the peritoneal cavity to deliver Ang II (750 mg/kg/day) for seven days with or without the chimera (10 mg/kg/day). It was found that gp91ds-tat could attenuate AngII-induced elevations in blood pressure for up to seven days compared to a scrambled peptide control (10 mg/kg/d) (Figure 2), while at the same time ex vivo aorta from gp91ds-tat-treated mice exhibited completely reduced superoxide levels compared with mice treated with Ang II alone (Figure 3) whereas scramb-tat-treated mice did not. This shows that gp91ds-tat can reduce the degenerative effects of superoxide in end-organ disease independent of its effect on blood pressure.

It was also determined that in rats a 10-fold lower dose of gp91ds-tat (1 mg/kg/d) which did not lower blood pressure was capable of significantly lowering vascular monocyte infiltration in response to Ang II. Ang II (750 g/kg/d) increased monocyte infiltration approximately three-fold compared to sham (Figure 4). Monocytes and macrophages were detected immunohistochemically using antibodies to a cell-surface antigen (ED-1) and
counted in five fields. Co-infusion of gp91ds-tat markedly reduced this infiltration whereas scramb-tat did not, directly implicating vascular NAD(P)H oxidase superoxide involvement in this chemoattraction. Since macrophages participate in the early stages of vascular injury and atherosclerosis, these results show a broader utility of gp91ds-tat and related compounds in preventing these processes.

There is lower efficacy of gp91ds-tat in isolated neutrophils. Upon testing the effect of gp91ds-tat on whole activated neutrophils (PMNs) to produce superoxide, a much lower inhibitory capability was found. gp91ds-tat at concentrations up to 100 FM were only capable of reducing superoxide-induced chemiluminescence (RCU) of phorbol myristate acetate by approximately 25% (Figure 5, lower panel), in marked contrast to the ability of gp91ds-tat to lower superoxide levels by 80% in activated vascular rings (Figure 1). This difference can be explained by the subcellular location of NAD(P)H oxidase components in lysosomes or by the high concentration of proteases on neutrophils which degrades gp91ds-tat [Shipp et al., 1991].

There was an extension of treatment to treat atherosclerosis and angioplasty-induced injury. Recently the adventitia has been shown to play an important role in vascular remodeling following injury under normotensive conditions. It has been postulated that a fibroblast proliferation response is followed by modulation to myofibroblasts, which then migrate to the neointima
Since fibroblast proliferation is superoxide-dependent [Irani et al., 1997; Murrel et al., 1989] agents such as gp91ds-tat aimed at targeting superoxide production in adventitial fibroblasts and endothelial cells, which possess a gp91phox-containing NAD(P)H oxidase [G'rlach et al., 2000; Pagano, 2000] are useful in the treatment of injury and atherosclerosis. In the models of porcine coronary artery balloon injury used by Shi et al. [Shi et al., 1996] when dissection was produced by damaging the media, myofibroblasts were found to migrate along medial fissures to the media. However, medial dissection is not necessary, as direct adventitial injury can cause neointimal lesions even in the absence of endothelial denudation [Webster et al., 1974; Booth et al., 1989; Beesley et al., 1992; Barker et al., 1993]. In fact, moderate balloon injury mimicking the effects of angioplasty has recently been shown to induce migration of adventitial fibroblasts to the neointima, leading to enhanced proliferation [Li et al., 2000].

Furthermore, Shi et al. [1997] recently showed that in carotid artery-vein grafts, neointimal proliferation was preceded by proliferation of adventitial fibroblasts, modulation to myofibroblasts, and migration to the neointima.

It has been shown that Ang II can promote atherosclerotic lesions and aneurysms in apo E -/- mice, which are known to exhibit adventitial activation. Thus, gp91ds-tat can be used to prevent neointimal proliferation and aneurysms in these as well as balloon-injured mice.
Animal models of hypertension, diabetes, and atherosclerosis have been used to test the biological activity of this and other related compounds, as well as to verify the *in vivo* mechanism of action and specificity. Toxicology and pharmacokinetic studies can also be performed at Core Facilities at Henry Ford Cancer Center.


Introduction of nucleic acids by infection offers several advantages over the other listed methods. Higher efficiency can be obtained due to their
infectious nature. Moreover, viruses are very specialized and typically infect and propagate in specific cell types. Thus, their natural specificity can be used to target the vectors to specific cell types *in vivo* or within a tissue or mixed culture of cells. Viral vectors can also be modified with specific receptors or ligands to alter target specificity through receptor mediated events.

A specific example of DNA viral vector for introducing and expressing recombinant sequences is the adenovirus derived vector Adenop53TK. This vector expresses a herpes virus thymidine kinase (TK) gene for either positive or negative selection and an expression cassette for desired recombinant sequences. This vector can be used to infect cells that have an adenovirus receptor which includes most cancers of epithelial origin as well as others. This vector as well as others that exhibit similar desired functions can be used to treat a mixed population of cells and can include, for example, an *in vitro* or *ex vivo* culture of cells, a tissue or a human subject.

Additional features can be added to the vector to ensure its safety and/or enhance its therapeutic efficacy. Such features include, for example, markers that can be used to negatively select against cells infected with the recombinant virus. An example of such a negative selection marker is the TK gene described above that confers sensitivity to the antibiotic gancyclovir. Negative selection is therefore a means by which infection can be controlled.
because it provides inducible suicide through the addition of antibiotic. Such protection ensures that if, for example, mutations arise that produce altered forms of the viral vector or recombinant sequence, cellular transformation will not occur.

Features that limit expression to particular cell types can also be included. Such features include, for example, promoter and regulatory elements that are specific for the desired cell type.

In addition, recombinant viral vectors are useful for in vivo expression of a desired nucleic acid because they offer advantages such as lateral infection and targeting specificity. Lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. The result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. This is in contrast to vertical-type of infection in which the infectious agent spreads only through daughter progeny. Viral vectors can also be produced that are unable to spread laterally. This characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

As described above, viruses are very specialized infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically,
viruses infect and propagate in specific cell types. The targeting specificity of
viral vectors utilizes its natural specificity to specifically target predetermined
cell types and thereby introduce a recombinant gene into the infected cell.
The vector to be used in the methods of the invention will depend on desired
cell type to be targeted and will be known to those skilled in the art. For
example, if breast cancer is to be treated then a vector specific for such
epithelial cells would be used. Likewise, if diseases or pathological conditions
of the hematopoietic system are to be treated, then a viral vector that is
specific for blood cells and their precursors, preferably for the specific type of
hematopoietic cell, would be used.

Retroviral vectors can be constructed to function either as infectious
particles or to undergo only a single initial round of infection. In the former
case, the genome of the virus is modified so that it maintains all the
necessary genes, regulatory sequences and packaging signals to synthesize
new viral proteins and RNA. Once these molecules are synthesized, the host
cell packages the RNA into new viral particles which are capable of
undergoing further rounds of infection. The vector's genome is also
gineered to encode and express the desired recombinant gene. In the
case of non-infectious viral vectors, the vector genome is usually mutated to
destroy the viral packaging signal that is required to encapsulate the RNA into
viral particles. Without such a signal, any particles that are formed will not
contain a genome and therefore cannot proceed through subsequent rounds
of infection. The specific type of vector will depend upon the intended application. The actual vectors are also known and readily available within the art or can be constructed by one skilled in the art using well-known methodology.

The recombinant vector can be administered in several ways. If viral vectors are used, for example, the procedure can take advantage of their target specificity and consequently, do not have to be administered locally at the diseased site. However, local administration can provide a quicker and more effective treatment, administration can also be performed by, for example, intravenous or subcutaneous injection into the subject. Injection of the viral vectors into a spinal fluid can also be used as a mode of administration, especially in the case of neuro-degenerative diseases. Following injection, the viral vectors will circulate until they recognize host cells with the appropriate target specificity for infection.

An alternate mode of administration can be by direct inoculation locally at the site of the disease or pathological condition or by inoculation into the vascular system supplying the site with nutrients or into the spinal fluid. Local administration is advantageous because there is no dilution effect and, therefore, a smaller dose is required to achieve expression in a majority of the targeted cells. Additionally, local inoculation can alleviate the targeting requirement required with other forms of administration since a vector can be
used that infects all cells in the inoculated area. If expression is desired in only a specific subset of cells within the inoculated area, then promoter and regulatory elements that are specific for the desired subset can be used to accomplish this goal. Such non-targeting vectors can be, for example, viral vectors, viral genome, plasmids, phagemids and the like. Transfection vehicles such as liposomes can also be used to introduce the non-viral vectors described above into recipient cells within the inoculated area. Such transfection vehicles are known by one skilled within the art.

The compound of the present invention is administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, patient age, sex, body weight and other factors known to medical practitioners. The pharmaceutically "effective amount" for purposes herein is thus determined by such considerations as are known in the art. The amount must be effective to achieve improvement including but not limited to improved survival rate or more rapid recovery, or improvement or elimination of symptoms and other indicators as are selected as appropriate measures by those skilled in the art.

In the method of the present invention, the compound of the present invention can be administered in various ways. It should be noted that it can be administered as the compound or as pharmaceutically acceptable salt and
can be administered alone or as an active ingredient in combination with pharmaceutically acceptable carriers, diluents, adjuvants and vehicles. The compounds can be administered orally, subcutaneously or parenterally including intravenous, intraarterial, intramuscular, intraperitoneally, and intranasal administration as well as intrathecal and infusion techniques. Implants of the compounds are also useful. The patient being treated is a warm-blooded animal and, in particular, mammals including man. The pharmaceutically acceptable carriers, diluents, adjuvants and vehicles as well as implant carriers generally refer to inert, non-toxic solid or liquid fillers, diluents or encapsulating material not reacting with the active ingredients of the invention.

It is noted that humans are treated generally longer than the mice or other experimental animals exemplified herein which treatment has a length proportional to the length of the disease process and drug effectiveness. The doses may be single doses or multiple doses over a period of several days, but single doses are preferred.

The doses may be single doses or multiple doses over a period of several days. The treatment generally has a length proportional to the length of the disease process and drug effectiveness and the patient species being treated.
When administering the compound of the present invention parenterally, it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion). The pharmaceutical formulations suitable for injection include sterile aqueous solutions or dispersions and sterile powders for reconstitution into sterile injectable solutions or dispersions. The carrier can be a solvent or dispersing medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils.

Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Nonaqueous vehicles such as cottonseed oil, sesame oil, olive oil, soybean oil, corn oil, sunflower oil, or peanut oil and esters, such as isopropyl myristate, may also be used as solvent systems for compound compositions. Additionally, various additives which enhance the stability, sterility, and isotonicity of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. In many cases, it will be desirable to include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for
example, aluminum monostearate and gelatin. According to the present invention, however, any vehicle, diluent, or additive used would have to be compatible with the compounds.

Sterile injectable solutions can be prepared by incorporating the compounds utilized in practicing the present invention in the required amount of the appropriate solvent with various of the other ingredients, as desired.

A pharmacological formulation of the present invention can be administered to the patient in an injectable formulation containing any compatible carrier, such as various vehicle, adjuvants, additives, and diluents; or the compounds utilized in the present invention can be administered parenterally to the patient in the form of slow-release subcutaneous implants or targeted delivery systems such as monoclonal antibodies, vectored delivery, iontophoretic, polymer matrices, liposomes, and microspheres. Examples of delivery systems useful in the present invention include: 5,225,182; 5,169,383; 5,167,616; 4,959,217; 4,925,678; 4,487,603; 4,486,194; 4,447,233; 4,447,224; 4,439,196; and 4,475,196. Many other such implants, delivery systems, and modules are well known to those skilled in the art.

A pharmacological formulation of the compound utilized in the present invention can be administered orally to the patient. Conventional methods
such as administering the compounds in tablets, suspensions, solutions, emulsions, capsules, powders, syrups and the like are usable. Known techniques which deliver it orally or intravenously and retain the biological activity are preferred.

In one embodiment, the compound of the present invention can be administered initially by intravenous injection to bring blood levels to a suitable level. The patient's levels are then maintained by an oral dosage form, although other forms of administration, dependent upon the patient's condition and as indicated above, can be used. The quantity to be administered will vary for the patient being treated and will vary from about 100 ng/kg of body weight to 100 mg/kg of body weight per day and preferably will be from 10 mg/kg to 10 mg/kg per day.

The above discussion provides a factual basis for the use of an inhibitor of superoxide anions in treatment of patients. The methods used with and the utility of the present invention can be shown by the accompanying figures.

Throughout this application, various publications, including United States patents, are referenced by author and year and patents by number. Full citations for the publications are listed below. The disclosures of these publications and patents in their entireties are hereby incorporated by
reference into this application in order to more fully describe the state of the art to which this invention pertains.

The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation.

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.
REFERENCES


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25


p22phox is a critical component of the superoxide-generating NADH/NADPH 
oxidase system and regulates angiotensin II-induced hypertrophy in vascular 


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91:1044-1048.

Wu, L., and de Champlain, J. 1999. Effects of superoxide on signaling 

CLAIMS

What is claimed is:

1. An specific inhibitors of the production of superoxide anion.

2. The inhibitor according to claim 1, wherein said inhibitor is selected from the group consisting essentially of gp91phox, p47phox, p22phox, p67phox, p21rac and homologues thereof.

3. A method of treating hypertension by administering an effective amount of a specific inhibitors of superoxide anion production.

4. A method of treating atherosclerosis by administering an effective amount of a specific inhibitors of superoxide anion production.

5. A method of treating diabetes by administering an effective amount of a specific inhibitors of superoxide anion production.

6. A viral vector expressing a specific inhibitors of superoxide anion production.

7. The viral vector according to claim 6, wherein said superoxide anion which inhibits interaction of components of enzymes.

8. The viral vector according to claim 7, wherein said enzymes are selected from the group consisting essentially of gp91phox, p47phox, p22phox, p67phox, p21rac, and homologues thereof.

10. The composition according to claim 9, wherein said inhibitor inhibits vascular superoxide anion.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/70
US CL. : 514/43

According to International Patent Classification (IPC) or to both national classification and IPC.

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/43

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>A</td>
<td>US 6,011,019 A (THOMAS et al.) 04 January 2000 (04.01.00) see entire document.</td>
<td>1-10</td>
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<tr>
<td>A</td>
<td>US 5,891,459 A (COOKE et al.) 06 April 1999 (06.04.99) see entire document.</td>
<td>1-10</td>
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☐ Further documents are listed in the continuation of Box C.  ☐ See patent family annex.

* Special categories of cited documents:

- T: later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- &: document member of the same patent family

Date of the actual completion of the international search: 10 OCTOBER 2000

Date of mailing of the international search report: 17 NOV 2000

Name and mailing address of the ISA/US:

Box PCT

Authorized officer:

Telephone No.: (703) 305-3487

*Date of the international search report mailing (May 1998)
### B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):