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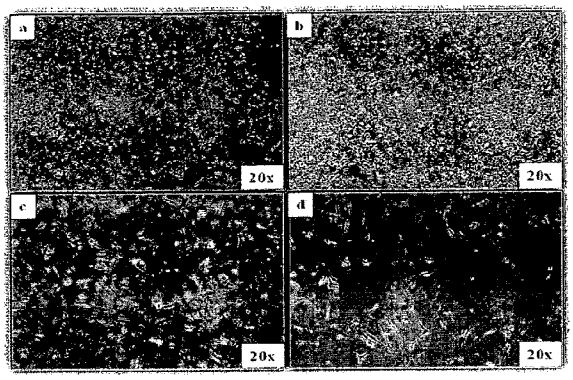
(54) **Title**: COMPOSITIOINS AND PREPARATION METHODS OF COMPOSITIONS FOR PREVENTION AND TREATMENT OF HYPERTENSION

FIG. 1



(57) **Abstract**: Disclosed herein are compounds, extracts, and active fractions of the plant Geumjaponicum and methods for preventing or treating hypertension. The compounds provided herein can be formulated into pharmaceutical compositions and medicaments that are useful in the disclosed methods. Also provided are the use of the compounds and extracts in preparing pharmaceutical formulations and medicaments.

WO 2010/143059 A1

COMPOSITIOINS AND PREPARATION METHODS OF COMPOSITIONS FOR PREVENTION AND TREATMENT OF HYPERTENSION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 61/186,709, filed June 12, 2009, and U.S. Provisional Application No. 61/187,905, filed June 17, 2009, the entire contents of which are hereby incorporated by reference in their entirety.

BACKGROUND

[0002] The following description is provided to assist the understanding of the reader. None of the information provided or references cited is admitted to be prior art to the present invention.

[0003] The American Heart Association estimates high blood pressure affects approximately one in three adults in the United States - 73 million people. According to the report of Journal of the American Medical Association, high blood pressure is also estimated to affect about two million American teens and children. Hypertension is clearly a major public health problem. Uncomplicated high blood pressure usually occurs without any symptoms and so hypertension has been labeled "the top silent killer." Hypertension can progress silently to finally develop any one or more of the several potentially fatal complications, such as heart attack or stroke, which are the leading causes of death among Western countries and now many Asian countries. Hypertension is already a highly prevalent cardiovascular risk factor worldwide because of increasing longevity and prevalence of contributing factors, such as obesity.

[0004] Despite the rapid advances of therapeutic technologies today, the currently available drugs and treatment modalities, such as vessel dilators, ß-blockers, diuretics, etc., for hypertension can only provide modest control of blood pressure under continuous medication and symptomatic improvement, and none of the drugs or therapeutic strategies is considered to have disease-modifying effects that can provide a curative effect on hypertension. They can only relieve the symptoms or slow down the pace of getting worse. Hypertension remains incurable and inadequately managed everywhere.

SUMMARY

[0005] In one aspect, the present invention provides a method for treating or preventing hypertension in mammalian subjects, the method comprising administering to a subject in need thereof an effective amount of an organic extract of *Geum japonicum* (OEGJ). In one embodiment, the peripheral resistance of small arteries is systemically decreased in a subject administered the OEGJ compared to a subject not administered the OEGJ. In one embodiment, the peripheral resistance of small arteries is decreased by collateral vessel formation in organs or tissues. In one embodiment, OEGJ stimulates collateral vessel formation in organs or tissues with increased resistance of small arteries so that the peripheral resistance will be decreased. In one embodiment, the subject's blood pressure is reduced compared to a subject not administered with the OEGJ. In one embodiment, the mammalian subject is a human.

[0006] In one embodiment, OEGJ is administered in an amount ranging from about 0.01 mg to about 10 g of the extract per kilogram of body weight per day. In one embodiment, OEGJ is administered in a dosage unit form. In one embodiment, OEGJ is administered in a dosage unit form comprising a pharmaceutically acceptable carrier. In one embodiment, OEGJ is administered orally. In one embodiment, OEGJ is administered by subcutaneous injection, intramuscular injection, or intravenous infusion.

[0007] In one embodiment, the OEGJ is a lower alkyl alcohol solvent extract of *Geum japonicum*. In one embodiment, the lower alkyl alcohol has 1-6 carbons atoms. In one embodiment, the lower alkyl alcohol is ethanol. In one embodiment, the lower alkyl alcohol is methanol.

[0008] In another aspect, the present invention provides a pharmaceutical composition for treating or preventing hypertension in mammalian subjects, comprising an effective amount of an organic extract of *Geum japonicum* (OEGJ) and a pharmaceutically acceptable carrier.

[0009] In another aspect, the present invention provides a kit comprising an effective amount of an organic extract of *Geum japonicum* (OEGJ) and a pharmaceutically acceptable carrier, a container and instructions indicating that the pharmaceutical composition is beneficial to a human suffering from hypertension or high blood pressure.

[0009a] In one form there is provided a method for treating hypertension in mammalian subjects, the hypertension being caused by peripheral resistance of small arteries, the method comprising administering to a subject in need thereof an effective amount of an organic extract of Geum japonicum (OEGJ), wherein the OEGJ is a lower alkyl alcohol solvent extract of Geum japonicum, wherein treating or preventing hypertension comprises stimulating collateral vessel formation, and wherein the peripheral resistance of small arteries is systemically decreased in a subject administered the OEGJ compared to a subject not administered the OEGJ.

[0009b] In another form there is provided a pharmaceutical composition for treating or preventing hypertension in a mammalian subject, wherein treating or preventing hypertension comprises stimulating collateral vessel formation, comprising an effective amount of an organic extract of Geum japonicum (OEGJ) and a pharmaceutically acceptable excipient.

BRIEF DESCRIPTION OF THE FIGURES

[0010] FIG. 1 is micrograph showing OEGJ-induced differentiation of vessel endothelial cells (HUVEC). Panel (a): The vehicle treated cells show proliferation and no sign of differentiation. Panel (b): OEGJ (30 μg/ml) treatment enhanced proliferation of the cells (30-50% more cells according to MTT assay). Panel (c): OEGJ (60 μg/ml) treated cells show elongated and refractive cells, which are characteristic of differentiation of vessel endothelial cells. Panel (d): OEGJ (180 μg/ml) treated cells display thin and elongated phenotype, forming tube-like structures.

[0011] FIG. 2 presents data showing that OEGJ treatment decreased the blood pressure in a 2VO rat model. Panel (a): The blood pressure was elevated (154.25±3.95 mmHg) after 2VO ligation measured 6 weeks post-2VO ligation in vehicle treated rats. Panel (b): By contrast, the elevated blood pressure due to 2VO was reduced (135±2.14 mmHg) measured 6 weeks post-ligation in OEGJ-treated rats.

[0012] FIG. 3 shows ultrasound Doppler evaluation of the reduced peripheral resistance of blood vessels in 2VO brain 2 weeks after termination of OEGJ treatment. The blood flow volume was evaluated by measuring basal artery of the experimental animals. Frequency is plotted vertically and time horizontally. Each signal corresponds to one cardiac cycle. A, Real time two dimensional image showing the basal artery in longitudinal section (upper panel) and Doppler shift signals recorded from basal artery (lower panel) in vehicle treated animals. B, Real time two dimensional image showing the basal artery in longitudinal section (upper panel) and Doppler shift signals recorded from basal artery (lower panel) in OEGJ treated animals. Although OEGJ treated animals had lower blood pressure (128mmHg) compared with the significantly elevated blood pressure (148mmHg) in vehicle treated, the cerebral blood flow volume was significantly higher (21.1ml/min) in OEGJ treated group than that (14.6ml/min) in vehicle treated. Based on the formula blood pressure (BP)/blood flow volume (BF) = blood vessel peripheral resistance, almost no difference of the peripheral resistance was observed between normal and control group, however, the resistance of OEGJ treated group is 43% lower, an indication of growth of new collateral arteries that increased the sectional area of peripheral arteries in brain by 37.4% obtained from vessel counting and brought about 30.8% more blood supply to the brain compared to the vehicle-treated 2VO animals.

[0013] FIG. 4 is a representative image of the cortex of the frontal lobe in OEGJ-treated 2VO rats showing significantly more vessels (~61.7±20.3/HPF) compared with that (38.5±12.6/HPF) of vehicle treated control 2VO rats (C).

[0014] FIG. 5 is a graph showing blood pressure and cerebral blood flow in a 2VO animal model. BP, denotes blood pressure. Basal CBFV, denotes the cerebral blood flow volume through basal artery. Nor, Normal control rats. Mod, Vehicle treated 2VO rats. OEGJ, OEGJ treated 2VO rats.

[0015] FIG. 6 is a graph showing blood pressure and cerebral blood flow in partial ligation (60%) of the right carotid artery in SD rats. (BP) blood pressure; (CBFV) the total cerebral blood flow volume; (Ctr) vehicle treated model rats; (Treated) OEGJ treated model rats. OEGJ treatment significantly increased cerebral blood flow through basal artery while the normal blood pressure was maintained. In comparison, the cerebral blood flow through basal artery in vehicle treated rats is significantly lower than that in OEGJ treated, while the blood pressure was elevated.

[0016] FIG. 7 is a graph showing blood pressure in APP mice. (OEGJ) OEGJ treated APP mice; (Ctr) vehicle treated APP mice. OEGJ treatment decreased the blood pressure by about 10% to a normal level in OEGJ treated APP mice compared with that in vehicle treated APP control mice.

DETAILED DESCRIPTION

[0017] In various aspects, the present invention provides compounds, extracts, and methods for preventing or treating hypertension. The compounds provided herein can be formulated into pharmaceutical compositions and medicaments that are useful in the disclosed methods. Also provided are the use of the compounds and extracts in preparing pharmaceutical formulations and medicaments.

[0018] It is to be appreciated that certain aspects, modes, embodiments, variations and features of the invention are described below in various levels of detail in order to provide a substantial understanding of the present invention. The following terms are used throughout as described below, unless context clearly indicates otherwise.

[0019] As used herein, the "administration" of an agent or drug to a subject or subject includes any route of introducing or delivering to a subject a compound to perform its

intended function. Administration can be carried out by any suitable route, including orally, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, or topically. Administration includes self-administration and the administration by another.

[0020] As used herein, the term "effective amount" or "pharmaceutically effective amount" or "therapeutically effective amount" of a composition, is a quantity sufficient to achieve a desired therapeutic and/or prophylactic effect, *e.g.*, an amount which results in the prevention of, or a decrease in, the symptoms associated with a disease that is being treated. The amount of a composition administered to the subject will depend on the type and severity of the disease and on the characteristics of the individual, such as general health, age, sex, body weight and tolerance to drugs. It will also depend on the degree, severity and type of disease. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. The compositions of the present invention can also be administered in combination with one or more additional therapeutic compounds.

[0021] The abbreviation "OEGJ" used in the invention, without specific indication, means an extract of the plant *Geum japonicum* Thunb. var. by an organic solvent described below.

[0022] As used herein, the term "disease" or "medical condition" are used interchangeably and includes, but is not limited to, any condition or disease manifested as one or more physical and/or psychological symptoms for which treatment and/or prevention is desirable, and includes previously and newly identified diseases and other disorders. For example, a medical condition may be hypertension.

[0023] As used herein, the term "subject" includes any mammalian subject, such as a human, but can also be an animal, *e.g.*, domestic animals (*e.g.*, dogs, cats and the like), farm animals (*e.g.*, cows, sheep, pigs, horses and the like) and laboratory animals (*e.g.*, monkey, rats, mice, rabbits, guinea pigs and the like).

[0024] As used herein, the terms "treating" or "treatment" or "alleviation" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. A subject is successfully "treated" for a disorder if, after receiving a therapeutic agent according to the methods of the present invention, the subject shows observable and/or measurable reduction in or absence of one or more signs and symptoms of a particular disease or condition.

[0025] As used herein, "prevention" or "preventing" of a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample.

Compositions of the Invention

[0026] The present disclosure provides methods of treating or preventing hypertension with agents and/or extracts and compounds, and derivatives of such compounds from a variety of plants including *Geum japonicum.* In some embodiments, the agent is an extract, *e.g.,* an organic extract, of *Geum japonicum.* In a particular embodiment, the agent is a methanol or ethanol extract of *Geum japonicum* or an active fraction thereof. An agent of the invention may be part of a pharmaceutical composition containing one or more excipients, carriers, or fillers. In one embodiment, the pharmaceutical composition is packaged in unit dosage form. The unit dosage form is effective in improving various diseases or medical conditions when administered to a subject in need thereof.

[0027] A method for preparing an organic extract from *Geum japonicum* is provided. This method comprises the step of (a) extracting the plant of *Geum japonicum* with alcohol selected from the group consisting of C1-C4 alcohols. This step maybe repeated 3-6 times, typically 5 times, at room temperature. Before performing step (a), the plant material may be powdered or cut into small pieces. The C1-C4 alcohols include methanol, ethanol, n-propanol, iso-propanol, n-butanol, iso-butanol, and ter-butanol. Typically, alcohol is added in 1-10 times by weight of the amount of the *Geum japonicum* to be extracted.

[0028] The methods may further comprise the step of (b) drying the extract obtained from the step of (a) into a dried powder; and (c) successively extracting the powder obtained from the step of (b) with C6 alkane, EtOAc and an alcohol selected from the group consisting of C1-C4 alcohols. The C6 alkane includes cyclic and non-cyclic alkane having 6 carbon atoms, including, for example, cyclohexane, n-hexane, and neo-hexane, etc. The C1-C4 alcohols include methanol, ethanol, n-propanol, iso-propanol, n-butanol, iso-butanol, and ter-butanol. The amount of organic solvent to be used is typically 1-10 times by weight of the amount of the powders to be further extracted.

[0029] The method as recited above may also include filtering the extract to remove any insoluble powders therein. A drying step may be completed under reduced pressure at a temperature higher than room temperature, for example, at 50°C.

[0030] To purify the OEGJ, the method may further comprise the steps of applying the powder to a chromatographic column; and eluting the column with an aqueous solution with increasing concentration of an alcohol selected from the group consisting of C1-C4 alcohols. For example, a Sephadex or reverse phase column may be used. The alcohol used may be any one selected from the group consisting of methanol, ethanol, n-propanol, iso-propanol, n-butanol, iso-butanol, and ter-butanol.

[0031] By NMR analysis, it is found that the OEGJ typically contains mainly tannins including Gemin A, B, C, D, E and F and triterpenes including 2-hydroxyoleanolic acid, 2-hydroxylursolic acid, 2,19-dihydroxy-ursolic acid, 2-α,19-α-dihydroxy-3-oxo-12-ursen-28-oic acid, ursolic acid, epimolic acid, maslinic acid, euscaphic acid, tormentic acid, 28-β-D-glucoside of tormentic acid.

[0032] In one embodiment, the extracts, fractions, and compounds of the invention are obtained by extraction, using water and/or of an organic solvent, from crude plant material comprises the following stages:

1. Extraction by addition to the plant material, of water and/or of organic solvent(s), by subjecting the whole to a treatment such as maceration/lixiviation, ultrasonics or microwaves;
2. Delipidation before or after the extraction stage using a solvent of petroleum ether, hexane or chloroform type;
3. Optionally, additional extraction of the extract recovered by an organic solvent of ethyl acetate or ethyl ether type,
4. Optionally, concentration of the crude extract obtained, and, if desired, its lyophilization.

[0033] According one aspect, considering the enrichment that it allows to be attained, the crude extract may be subjected to a purification stage by chromatography. In one embodiment, centrifugal partition chromatography (CPC) is used. This technique is in particular described by A.P. FOUCAULT, Ed., Centrifugal Partition Chromatography, Chromatographic Science Series, Marcel Dekker Inc., 1995, 68, or W.D. CONWAY, Ed., Countercurrent Chromatography apparatus theory and applications, VCH Publishers Inc.,

1990. CPC is based on the partition of the solutes between two non-miscible liquid phases prepared by the mixture of two or more solvents or solutions. One of the two phases is kept stationary by a centrifugal force. The solvents, their proportions and the flow rate chosen closely depend both on the stability of the stationary phase within the CPC column and the actual pressure.

[0034] A person skilled in the art will therefore choose the most appropriate solvent or solvents depending on the nature of the purified extract desired. These different extracts, namely crude or enriched also fall within the scope of the invention. The implementation of additional separation stages allows isolation of these extracts enriched with one or more compounds. These separations can be carried out on fractions enriched from a crude extract or on the crude extract itself by using mixtures of appropriate solvents according to the proportions which are suitable for the sought separation.

Methods for the Prevention and Treatment of Hypertension

[0035] Systolic blood pressure is the maximum pressure in the arteries when the heart contracts and pushes blood out into the body. The diastolic blood pressure is the minimum pressure in the arteries between beats when the heart relaxes to fill with blood. Hypertension is defined as an average systolic blood pressure above 140-150 mm Hg, a diastolic blood pressure above 90-95 mm Hg, or both. An elevation of the systolic and/or diastolic blood pressure increases the risk of developing heart disease, kidney disease, hardening of the arteries (atherosclerosis or arteriosclerosis), eye damage, and stroke (brain damage). These complications of hypertension are often referred to as end-organ damage because damage to these organs is the end result of chronic high blood pressure. For this reason, the diagnosis and early treatment of high blood pressure is important to normalize blood pressure and prevent complications.

[0036] Although current advanced treatment strategies can slow down the progress of the disease, it can at the same time develop severe complications, for example, ACE inhibitors stop the production of a hormone called angiotensin II that makes the blood vessels narrow; Angiotensin-II receptor antagonists work in a similar way as ACE inhibitors; Beta-blockers block the effect of the hormone adrenaline and the sympathetic nervous system on the body relaxing the heart; Alpha-blockers cause the blood vessels to relax and widen; and calcium-channel blockers reduce muscle tension in the arteries.

[0037] The nature of blood pressure is the force of blood as it is pumped through the arteries. The more blood the heart pumps and the narrower the arteries are, the higher the blood pressure. To maintain sufficient amount of blood supply to all organs and tissues to support the necessary requirement of normal activities of the body, the heart is required to pump certain volume of blood around the body through the arteries. Therefore, the best solution to reduce blood pressure without negatively affecting normal activities of the body is to permanently increase the cross-sectional area of the total arteries of the whole body.

[0038] The present inventors have discovered an organic extract of *Geum japonicum* that can significantly decrease the elevated blood pressure of subjects after about a two-week treatment with the extract. In some embodiments, treatment for four to eight weeks with the extract can permanently increase the cross-sectional area of the arteries in subjects, which substantially reduces the resistance of the peripheral arteries. As a result, the blood pressure decreases without compromising functional performance of the whole body.

[0039] The present invention is related to the use of an organic extract of *Geum japonicum* (OEGJ) and a method of treating hypertension in humans or animals and diseases associated with hypertension. Particularly, it relates to a pharmaceutical composition and method for reducing systemic blood pressure. Without wishing to be limited by theory, the OEGJ may act by stimulating the growth of new collateral capillaries, arterioles and micro-vessels systemically in the subject with increased peripheral resistance that substantially improves blood perfusion to important organs and tissues and increases cross-sectional area of the small blood vessels at different levels. As a result, the increased peripheral resistance of the small arterioles in hypertension is rectified due to the compensation of the newly grown collateral vessels to the narrowed arteries, thereby leading to a decrease in blood pressure. Therefore, the methods provide a substantial treatment modality that addresses the underlying pathological cause of hypertension.

[0040] In accordance with one aspect, the invention provides methods of treating or preventing hypertension in a subject in need thereof, which comprises administering to the subject an effective amount of a compound, composition, fraction, or extract described herein. In one aspect, the methods for the prevention or treatment of hypertension include administering to a mammal in need thereof fractions and/or extracts from a variety of plants including *Geum japonicum*. In some embodiments, the extract is an organic extract obtained from the plant *Geum japonicum*.

[0041] In another aspect, an agent for the treatment or prevention of hypertension is part of a pharmaceutical composition containing one or more excipients, carriers, or fillers. In one embodiment, the pharmaceutical composition is packaged in unit dosage form. The unit dosage form is effective in improving (*i.e.*, lowering) blood pressure in the subject.

[0042] In various embodiments of the invention, suitable *in vitro* or *in vivo* assays are performed to determine the effect of an agent (extracts, fractions, and compounds) of the invention and whether its administration is indicated for the treatment or prevention of hypertension in a subject. In some embodiments, *in vivo* models of hypertension are used to assess the effects of an agent on a subject. The effects of the agent in mediating the hypertension in the animal subject are investigated and compared to suitable controls.

[0043] In another embodiment, plants, extracts, active fractions, and/or compounds of the invention may be administered as part of a combination therapeutic with another cardiovascular agent. Examples of cardiovascular agents include vasodilators, for example, hydralazine; angiotensin converting enzyme inhibitors, for example, captopril; anti-anginal agents, for example, isosorbide nitrate, glyceryl trinitrate and pentaerythritol tetranitrate; anti-arrhythmic agents, for example, quinidine, procainaltide and lignocaine; cardioglycosides, for example, digoxin and digitoxin; calcium antagonists, for example, verapamil and nifedipine; diuretics, such as thiazides and related compounds, for example, bendrofluazide, chlorothiazide, chlorothalidone, hydrochlorothiazide and other diuretics, for example, fursemide and triamterene, and sedatives, for example, nitrazepam, flurazepam and diazepam.

[0044] Other exemplary cardiovascular agents include, for example, a cyclooxygenase inhibitor such as aspirin or indomethacin, a platelet aggregation inhibitor such as clopidogrel, ticlopidene or aspirin, fibrinogen antagonists or a diuretic such as chlorothiazide, hydrochlorothiazide, flumethiazide, hydroflumethiazide, bendroflumethiazide, methylchlorthiazide, trichloromethiazide, polythiazide or benzthiazide as well as ethacrynic acid ticrynafen, chlorthalidone, furosemide, muzolimine, bumetanide, triamterene, amiloride and spironolactone and salts of such compounds, angiotensin converting enzyme inhibitors such as captopril, zofenopril, fosinopril, enalapril, ceranopril, cilazapril, delapril, pentopril, quinapril, ramipril, lisinopril, and salts of such compounds, angiotensin II antagonists such as losartan, irbesartan or valsartan, thrombolytic agents such as tissue plasminogen activator (tPA), recombinant tPA, streptokinase, urokinase, prourokinase, and anisoylated plasminogen

streptokinase activator complex (APSAC, Eminase, Beecham Laboratories), or animal salivary gland plasminogen activators, calcium channel blocking agents such as verapamil, nifedipine or diltiazem, thromboxane receptor antagonists such as ifetroban, prostacyclin mimetics, or phosphodiesterase inhibitors. Such combination products if formulated as a fixed dose employ the compounds of this invention within the dose range described above and the other pharmaceutically active agent within its approved dose range.

[0045] Yet other exemplary cardiovascular agents include, for example, vasodilators, *e.g.*, bencyclane, cinnarizine, citicoline, cyclandelate, cyclonicate, ebumamonine, phenoxezyl, flunarizine, ibudilast, ifenprodil, lomcrizine, naphlole, nikamate, nosergoline, nimodipine, papaverine, pentifylline, nofedoline, vincamin, vinpocetine, vichizyl, pentoxifylline, prostacyclin derivatives (such as prostaglandin E1 and prostaglandin I2), an endothelin receptor blocking drug (such as bosentan), diltiazem, nicorandil, and nitroglycerin. Examples of the cerebral protecting drug include radical scavengers (such as edaravone, vitamin E, and vitamin C), glutamate antagonists, AMPA antagonists, kainate antagonists, NMDA antagonists, GABA agonists, growth factors, opioid antagonists, phosphatidylcholine precursors, serotonin agonists, Na^+/Ca^{2+} channel inhibitory drugs, and K^+ channel opening drugs. Examples of the brain metabolic stimulants include amantadine, tiapride, and gamma-aminobutyric acid. Examples of the anticoagulant include heparins (such as heparin sodium, heparin potassium, dalteparin sodium, dalteparin calcium, heparin calcium, pamaparin sodium, reviparin sodium, and danaparoid sodium), warfarin, enoxaparin, argatroban, batroxobin, and sodium citrate. Examples of the antiplatelet drug include ticlopidine hydrochloride, dipyridamole, cilostazol, ethyl icosapentate, sarpogrelate hydrochloride, dilazep hydrochloride, trapidil, a nonsteroidal antiinflammatory agent (such as aspirin), beraprostsodium, iloprost, and indobufene. Examples of the thrombolytic drug include urokinase, tissue-type plasminogen activators (such as alteplase, tisokinase, nateplase, pamiteplase, monteplase, and rateplase), and nasaruplase. Examples of the antihypertensive drug include angiotensin converting enzyme inhibitors (such as captopril, alacepril, lisinopril, imidapril, quinapril, temocapril, delapril, benazepril, cilazapril, trandolapril, enalapril, ceronapril, fosinopril, imadapril, mobertpril, perindopril, ramipril, spirapril, and randolapril), angiotensin II antagonists (such as losartan, candesartan, valsartan, eprosartan, and irbesartan), calcium channel blocking drugs (such as aranidipine, efonidipine, nicardipine, bamidipine, benidipine, manidipine, cilnidipine, nisoldipine, nitrendipine, nifedipine, nilvadipine, felodipine, amlodipine, diltiazem, bepridil, clentiazem, phendilin, galopamil,

mibefradil, prenylamine, semotiadil, terodiline, verapamil, cilnidipine, elgodipine, isradipine, lacidipine, lercanidipine, nimodipine, cinnarizine, flunarizine, lidoflazine, lomerizine, bencyclane, etafenone, and perhexiline), β-adrenaline receptor blocking drugs (propranolol, pindolol, indenolol, carteolol, bunitrolol, atenolol, acebutolol, metoprolol, timolol, nipradilol, penbutolol, nadolol, tilisolol, carvedilol, bisoprolol, betaxolol, celiprolol, bopindolol, bevantolol, labetalol, alprenolol, amosulalol, arotinolol, befunolol, bucumolol, bufetolol, buferalol, buprandolol, butylidine, butofilolol, carazolol, cetamolol, cloranolol, dilevalol, epanolol, levobunolol, mepindolol, metipranolol, moprolol, nadoxolol, nevibolol, oxprenolol, practol, pronetalol, sotalol, sufinalol, talindolol, tertalol, toliprolol, xybenolol, and esmolol), α-receptor blocking drugs (such as amosulalol, prazosin, terazosin, doxazosin, bunazosin, urapidil, phentolamine, arotinolol, dapiprazole, fenspiride, indoramin, labetalol, naftopidil, nicergoline, tamsulosin, tolazoline, trimazosin, and yohimbine), sympathetic nerve inhibitors (such as clonidine, guanfacine, guanabenz, methyldopa, and reserpine), hydralazine, todralazine, budralazine, and cadralazine. Examples of the anti anginal drug include nitrate drugs (such as amyl nitrite, nitroglycerin, and isosorbide), β-adrenaline receptor blocking drugs (such as propranolol, pindolol, indenolol, carteolol, bunitrolol, atenolol, acebutolol, metoprolol, timolol, nipradilol, penbutolol, nadolol, tilisolol, carvedilol, bisoprolol, betaxolol, celiprolol, bopindolol, bevantolol, labetalol, alprenolol, amosulalol, arotinolol, befunolol, bucumolol, bufetolol, buferalol, buprandolol, butylidine, butofilolol, carazolol, cetamolol, cloranolol, dilevalol, epanolol, levobunolol, mepindolol, metipranolol, moprolol, nadoxolol, nevibolol, oxprenolol, practol, pronetalol, sotalol, sufinalol, talindolol, tertalol, toliprolol, andxybenolol), calcium channel blocking drugs (such as aranidipine, efonidipine, nicardipine, bamidipine, benidipine, manidipine, cilnidipine, nisoldipine, nitrendipine, nifedipine, nilvadipine, felodipine, amlodipine, diltiazem, bepridil, clentiazem, phendiline, galopamil, mibefradil, prenylamine, semotiadil, terodiline, verapamil, cilnidipine, elgodipine, isradipine, lacidipine, lercanidipine, nimodipine, cinnarizine, flunarizine, lidoflazine, lomerizine, bencyclane, etafenone, and perhexiline) trimetazidine, dipyridamole, etafenone, dilazep, trapidil, nicorandil, enoxaparin, and aspirin. Examples of the diuretic include thiazide diuretics (such as hydrochlorothiazide, methyclothiazide, trichlormethiazide, benzylhydrochlorothiazide, and penflutizide), loop diuretics (such as furosemide, etacrynic acid, bumetanide, piretanide, azosemide, and torasemide), K^+ sparing diuretics (spironolactone, triamterene, and potassium can renoate), osmotic diuretics (such as isosorbide, D-mannitol, and glycerin), nonthiazide diuretics (such as meticrane, tripamide, chlorthalidone, and mefruside), and acetazolamide. Examples of the cardiotonic include

digitalis formulations (such as digitoxin, digoxin, methyldigoxin, deslanoside, vesnarinone, lanatoside C, and proscillaridin), xanthine formulations (such as aminophylline, choline theophylline, diprophylline, and proxyphylline), catecholamine formulations (such as dopamine, dobutamine, and docarpamine), PDE III inhibitors (such as amrinone, olprinone, and milrinone), denopamine, ubidecarenone, pimobendan, levosimendan, aminoethylsulfonic acid, vesnarinone, carperitide, and colforsin daropate. Examples of the antiarrhythmic drug include ajmaline, pirmenol, procainamide, cibenzoline, disopyramide, quinidine, aprindine, mexiletine, lidocaine, phenyloin, pilsicainide, propafenone, flecainide, atenolol, acebutolol, sotalol, propranolol, metoprolol, pindolol, amiodarone, nifekalant, diltiazem, bepridil, and verapamil. Examples of the antihyperlipidemic drug include atorvastatin, simvastatin, pravastatin sodium, fluvastatin sodium, clinofibrate, clofibrate, simfibrate, fenofibrate, bezafibrate, colestimide, and colestyramine.

Formulations and Dosages of Pharmaceutical Compositions.

[0046] Typically, an effective amount of the compositions of the present invention, sufficient for achieving a therapeutic or prophylactic effect, range from about 0.000001 mg per kilogram body weight per day to about 10,000 mg per kilogram body weight per day. Suitably, the dosage ranges are from about 0.0001 mg per kilogram body weight per day to about 10000 mg per kilogram body weight per day. For administration of an agent, the dosage ranges may be from about 0.0001 to 10000 mg/kg, and more usually 0.1 to 10000 mg/kg every week, every two weeks or every three weeks, of the host body weight. An exemplary treatment regime entails administration once per every two weeks or once a month or once every 3 to 6 months. The agent usually administered on multiple occasions. Intervals between single dosages can be daily, weekly, monthly or yearly. Alternatively, the agents can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the agent in the subject. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some subjects continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the subject shows partial or

complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

[0047] *Toxicity*. Suitably, an effective amount (*e.g.*, dose) of an agent described herein will provide therapeutic benefit without causing substantial toxicity to the subject. Toxicity of the agent described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, by determining the LD_{50} (the dose lethal to 50% of the population) or the LD_{100} (the dose lethal to 100% of the population). The dose ratio between toxic and therapeutic effect is the therapeutic index. The data obtained from these cell culture assays and animal studies can be used in formulating a dosage range that is not toxic for use in human. The dosage of the agent described herein lies preferably within a range of circulating concentrations that include the effective dose with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the subject's condition. See, *e.g.*, Fingl *et al.*, In: *The Pharmacological Basis of Therapeutics*, Ch. 1 (1975).

[0048] According to one embodiment, the agents can be incorporated into pharmaceutical compositions suitable for administration. In some embodiments, the pharmaceutical compositions may comprise purified or substantially purified extracts of *Geum japonicum* and a pharmaceutically-acceptable carrier in a form suitable for administration to a subject. In other embodiments, the pharmaceutical compositions may comprise Pharmaceutically-acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions for administering the compositions (see, *e.g.*, *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA 18th ed., 1990). The pharmaceutical compositions are generally formulated as sterile, substantially isotonic and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

[0049] The terms "pharmaceutically-acceptable," "physiologically-tolerable," and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a subject without the production of undesirable physiological effects to a degree that would prohibit administration of the composition. For example, "pharmaceutically-

acceptable excipient" means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients can be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous. "Pharmaceutically-acceptable salts and esters" means salts and esters that are pharmaceutically-acceptable and have the desired pharmacological properties. Such salts include salts that can be formed where acidic protons present in the agent are capable of reacting with inorganic or organic bases. Suitable inorganic salts include those formed with the alkali metals, *e.g.,* sodium and potassium, magnesium, calcium, and aluminum. Suitable organic salts include those formed with organic bases such as the amine bases, *e.g.,* ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like. Such salts also include acid addition salts formed with inorganic acids (*e.g.,* hydrochloric and hydrobromic acids) and organic acids (*e.g.,* acetic acid, citric acid, maleic acid, and the alkane- and arene-sulfonic acids such as methanesulfonic acid and benzenesulfonic acid). Pharmaceutically-acceptable esters include esters formed from carboxy, sulfonyloxy, and phosphonoxy groups present in the agent, *e.g.,* C_{1-6} alkyl esters. When there are two acidic groups present, a pharmaceutically-acceptable salt or ester can be a mono-acid-mono-salt or ester or a di-salt or ester; and similarly where there are more than two acidic groups present, some or all of such groups can be salified or esterified. The agent named in this invention can be present in unsalified or unesterified form, or in salified and/or esterified form, and the naming of such agent is intended to include both the original (unsalified and unesterified) compound and its pharmaceutically-acceptable salts and esters. Also, certain agents named in this invention can be present in more than one stereoisomeric form, and the naming of such agent is intended to include all single stereoisomers and all mixtures (whether racemic or otherwise) of such stereoisomers. A person of ordinary skill in the art, would have no difficulty determining the appropriate timing, sequence and dosages of administration for particular drugs and compositions of the present invention.

[0050] Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and compounds for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or compound is incompatible with the agent, use thereof in the

compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[0051] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. The compositions of the present invention can be administered by parenteral, topical, intravenous, oral, subcutaneous, intraarterial, intradermal, transdermal, rectal, intracranial, intraperitoneal, intranasal; intramuscular route or as inhalants. The agent can optionally be administered in combination with other agents that are at least partly effective in treating various diseases.

[0052] Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial compounds such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating compounds such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and compounds for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0053] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, *e.g.*, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, *e.g.*, 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. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal compounds, *e.g.*, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like.

In many cases, it will be preferable to include isotonic compounds, *e.g.*, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition a compound which delays absorption, *e.g.*, aluminum monostearate and gelatin.

[0054] Sterile injectable solutions can be prepared by incorporating the agents in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the binding agent into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The agents of this invention can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained or pulsatile release of the active ingredient.

[0055] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the binding agent can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding compounds, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating compound such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening compound such as sucrose or saccharin; or a flavoring compound such as peppermint, methyl salicylate, or orange flavoring.

[0056] In one embodiment, the agents are prepared with carriers that will protect the agent against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems, or protect the drug from degraded by the acid of the stomach. Biodegradable, biocompatible polymers can be used, such as ethylene

vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically-acceptable carriers. These can be prepared according to methods known to those skilled in the art, *e.g.*, as described in U.S. Pat. No. 4,522,811.

EXAMPLES

[0057] The present technology is further illustrated by the following examples, which should not be construed as limiting in any way.

Example 1 – Preparation of an organic extract of *Geum japonicum*

[0058] A bio-assay guided strategy was used for screening plant constituents to identify a composition of compounds showing the action on reducing peripheral resistance of small arteries through stimulating the growth of arterioles and micro-vessels. Briefly, 10 kg dried *Geum japonicum* collected from Anhui Province was cut into small pieces, which was percolated with 75% ethanol (10 x volume) at 40 °C for 3 days. The extract was electro-sprayed to yield a brown powder.

Example 2- OEGJ-enhanced proliferation and differentiation of vessel endothelial cells

[0059] Vessel endothelial cell proliferation analysis was conducted using an MTT assay following the modified ATCC protocol. Human umbilical vein endothelial cells (HUVECs, 2×10^3/well) were seeded onto a 96-well culture plate with growth medium (F12K medium with 15% FBS, 6 U/ml heparin and 30 μg/ml endothelial cell growth supplement). After cell attachment, the medium was changed to Ham's F12K medium with 2% FBS for 12 hours. Cells were then treated with OEGJ (prepared as described in Example 1) of gradient concentrations 50, 100 and 200 μg/ml, respectively, for 48 hours. The optical density (OD) was measured by Tecan Sunrise plate reader (GmbH, Australia). For evaluation of the phenotype and differentiation of the cultured endothelial cells, all wells with different treatments were examined under an inverted microscope before MTT measurement.

[0060] It was found that OEGJ not only promoted the proliferation of the HUVECs at low concentration of OEGJ, but also enhanced the differentiation of HUVECs forming thin,

elongated and connected tube-like structures in culture, indicating its potential in promoting angiogenesis (FIG. 1).

Example 3 – Therapeutic effects of OEGJ in a rat models of hypertension.

[0061] Our preliminary studies suggested that permanent ligation of bilateral carotid arteries (2VO) will cause mild hypertension due to the increased peripheral resistance of small arteries in the brain. After the 2VO, the cerebral blood supply should maximally rely on bilateral vertebral arteries, which supplies about 40% of the total blood volume to the brain normally. To compensate for the reduced 60% of blood supply, which was normally supplied by the bilateral carotid arteries, the heart needs to build up more pressure, which has been confirmed by blood pressure measurements during the experiment, to overcome the resistance of the small arteries in the brain for delivery of more volume of blood to the brain through the vertebral arteries.

[0062] To this end, male Sprague-Dawley (SD) rats, weighing 250-300 g were used. The study was conducted in accordance with the National Regulations of Experimental Animal Administration, and all animal experiments were approved by the Committee of Experimental Animal Administration of Zhangjiang High-tech Park. For 2VO (n=12), both of the common carotid arteries were exposed through a midline cervical incision under anesthesia and then they were ligated by 6-0 nylon suture, and cut by microscissors. The wound was thereafter closed with a suture. The rats (n=6) subjected to the operation of midline cervical incision but without common carotid artery ligation were taken as sham-operated controls. After recovering from anesthesia, the experimental animals were allowed free access to food and water.

[0063] The rats (n=6) of 2VO in the OEGJ treatment group were intragastricly administered daily with an OEGJ suspension (480mg/kg/day in water) for 4 weeks. The animals of 2VO in vehicle treatment group (n=6) were administered with an equal volume of water daily. Three of the rats in sham treated group were intragastricly administered daily with an OEGJ suspension (480mg/kg/day in water) for 4 weeks and the remaining other 3 animals were administered with an equal volume of water daily. The blood pressure and the blood flow to the brain were analyzed 4 weeks after the treatment.

[0064] *Measurement of blood pressure.* Systolic blood pressure (SBP) and mean blood pressure (MBP) of the experimental animals were measured after four weeks by a

computerized, automated system with a tail-cuff (BP-98A, Softron) after a warming period in unanesthetized rats. The room temperature was maintained constant at 27°C. It was found that the blood pressure was elevated (154.25±3.95mmHg) in vehicle treated 2VO rats 4 weeks after treatment (FIG. 2), which is significantly higher than that (around 125mmHg) in sham operated animals. By contrast, the blood pressure in OEGJ treated 2VO rats was 135.25 ±2.14 mmHg (FIG. 2), which is significantly lower than that in vehicle treated control rats, but slightly higher than the level in sham-operated rats indicating brain collateral vessel formation that reduced the peripheral resistance of brain arteries or vessel dilation due to OEGJ treatment.

[0065] To rule out the possibility of vessel dilation effect of OEGJ, we measured the brain blood flow volume and blood pressure again in the 2VO experimental animals two weeks after OEGJ treatment. It was found that two weeks after termination of the OEGJ treatment, the blood flow volume remained similar to the value of last measurement in OEGJ-treated rats with blood pressure approximately 128 mmHg. While in vehicle treated rats, both the blood flow volume and blood pressure were about the same to the values of last measurements two weeks ago. In conclusion, the significantly decreased blood supply to the brain due to 2VO was restored by OEGJ treatment. The effect is probably due to the enhanced collateral vessel formation in the ischemic brain that reduced the resistance of the arterioles of the brain, which was confirmed by histological studies (blood vessel counting) of the same brain samples.

[0066] *Doppler ultrasound evaluation of brain blood flow.* If OEGJ treatment stimulated the growth of collateral vessels in the brain that would reduce the peripheral resistance of small arteries in the brain, then the total cerebral blood flow volume would be increased under a certain blood pressure. To demonstrate this hypothesis, we measured the blood pressure as stated above and further evaluated the cerebral blood flow volume of the experimental animals using a Toshiba Aplio XG ultrasound with PLT-1202S linear array transducer by measuring extracranial basal artery of the experimental animals. After permanent 2VO, the blood supply to the rat brain depends dominantly on the bilateral common vertebral arteries-basal artery, which normally supplies approximately 40% of the total blood volume to the brain. Our results showed that although the blood flow volume of the basal artery in sham operated rats is around 12.4 ± 3.5 ml/min, it reached 16.04 ± 6.4 ml/min in vehicle-treated control due to the compensation mechanism to the 2VO (FIG. 3),

which accounts for 52% of the normal total blood volume to the brain. Interestingly, the blood flow volume of the basal artery in OEGJ-treated animals increased up to 25.9 ± 11.80 ml/min (FIG. 3), which is approximately 83.5% of the normal level of the total cerebral blood flow volume.

[0067] To determine whether the OEGJ-mediated reduction effect of peripheral blood vessel resistance were resulted from vessel dilation or growth of new collateral vessels in brain, we measured the blood flow volume again in these experimental animals two weeks after OEGJ treatment. It was found that two weeks after termination of the OEGJ treatment, the blood flow volume remained similar (21.1±6.3 ml/min) to the value of last measurement in OEGJ treated rats with blood pressure approximately 128 mmHg similar level to the normal blood pressure. While in vehicle treated rats, both the blood flow volume (14.6 ml/min) and blood pressure (148 mmHg) were about similar to the values of last measurements two weeks ago.

[0068] Based on the formula: *Blood pressure (BP)/blood flow volume (BF) = peripheral vessel resistance (PR),* for the normal rats: at the time of first measurement, Rn0 = 125/12.4 = 10.08, and 2 weeks later, Rn2 = 121/12 = 10.1; for the vehicle treated control rats: Rc0 = 154.25/16.04 = 9.62, 2 weeks post termination of vehicle treatment, Rc2 = 148/14.6 = 10.14; for the OEGJ treated rats: Ro0 = 135.25/25.9 = 5.22, 2 weeks post termination of OEGJ treatment, Ro2 = 128/21.1 = 6.06 (FIG. 3). It was derived from above calculations that the peripheral vessel resistance between normal and vehicle treated control rats showed almost no difference (~10). However, the peripheral vessel resistance of OEGJ treated group is about 43% lower, an indication of growth of new collateral arteries that increased the sectional area of peripheral arteries in brain, which was further confirmed by vessel counting in histological analysis.

[0069] *Confirmation of collateral neovascularization in the brain.* It was found that the average weight of the brain in OEGJ treated rats is 9.1±2.3% heavier than that in vehicle treated control rats ($P < 0.01$). Brains from the experimental animals sacrificed after cerebral blood flow measurement were removed, fixed in formalin and embedded in paraffin. Thin sections (5 μm) were cut from each slide and stained with H&E staining. The vascular densities were determined on the thin sections by counting the numbers of vessels within the cortex of frontal lobe and around hippocampus regions using a light microscope under a high power field (HPF) (40x). Six random and non-overlapping HPFs within the frontal lobe or

hippocampus were inspected for counting all the vessels in each section. The number of vessels in each HPF was averaged and expressed as the number of vessels per HPF. Vascular counts were performed by two investigators in a blind fashion.

[0070] It was found that the numbers of vessels are about 61.7±20.3/HPF in the regions of cortex in frontal lobe (FIG. 4) and 56.4±12.3/HPF around the regions of hippocampus in OEGJ treated rats. By contrast, the numbers of vessels are about 38.5±12.6/HPF in regions of cortex in frontal lobe (FIG. 4) and 30.7±10.5/HPF around the regions of hippocampus in vehicle treated rats. In summary, OEGJ treatment induced 37.4% more collateral vessels formed in ischemic brains that resulted in 30.8% more blood supply to the ischemic brain and 14.5% reduction of the blood pressure in the OEGJ treated 2VO induced hypertension animals (FIG. 5).

Example 4 – Therapeutic effects of OEGJ in mild hypertension animal models.

[0071] The therapeutic effects of OEGJ in several mild hypertension animal models were examined. All studies were conducted in accordance with the National Regulations of Experimental Animal Administration, and all animal experiments were approved by the Committee of Experimental Animal Administration of Zhangjiang High-tech Park. The animal models included a partial ligation of uniilateral carotid arteries in rat, APP mice, stroke rat, and senescence accelerated mouse induced mild hypertensionanimal models, which are described below.

[0072] *Induction of a milder high blood pressure rat model.* To this end, a partial ligation (60%) of right carotid artery (PLRCA) in SD rats (n=12) was performed. The right common carotid artery was exposed through a right midline cervical incision under anesthesia. The exposed right carotid artery was then partially (60%) ligated by a 6-0 nylon suture, and cut by microscissors. The wound was thereafter closed with a suture. Six rats subjected to the operation of right midline cervical incision but without common carotid artery ligation were taken as sham-operation control. After recovering from anesthesia, the experimental animals were allowed free access to food and water. The rats were randomly divided into the OEGJ treatment group (n=6) and vehicle treatment group (n=6).

[0073] APP mice develop fibrillar amyloid plaques. As the APP mice age, they exhibit impairments in spatial learning and memory with a mildly elevated blood pressure. SAMP10 mice, developed by Takeda and his colleagues (Takeda et al., 1991), show characteristics of

rapid aging. They develop early abnormalities in learning and memory with mild high blood pressure when they age. In this study, both APP (n=30) and SAMP10 mice (n=30) were used. Both the APP mice and SAMP10 mice were randomly divided into the OEGJ treatment group (n=15) and vehicle treatment group (n=15) respectively.

[0074] *Rat models of ischemic stroke induced high blood pressure.* Ischemic stroke was induced in SD rats by surgery according to the methods used previously (Mayzel-Oreg *et al.*, 2004). Briefly, the common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) around the carotid bifurcation were exposed through a midline incision in the right side of the neck. CCA was ligated proximal to the carotid bifurcation. Saline solution (0.5 ml) containing ~1000 microspheres (80–150 μM) was injected by a syringe inserted into the ECA pointing toward the carotid bifurcation. After ligation of the ECA distal to the injection site and removal of CCA ligation, the injected microspheres entered the ICA resulted in multi-infarct ischemic stroke in the brain. Rats (n=28) were divided into OEGJ treatment group (n=12) and vehicle treatment group (n=16) according to their neurological gradings so that rats in each group had an overall similar grade.

[0075] The rats and mice in OEGJ treatment groups of all above animal models were intragastricly treated with an OEGJ suspension (480mg/kg/day in water) for 4 weeks respectively. The rats and mice in the vehicle-treated groups were intragastricly administered with an equal volume of water daily for the same period.

[0076] The results showed that the average blood pressure of the vehicle treated rats (PLRCA) was increased to approximately 142 mmHg and the total blood flow volume was around 37ml/min (FIG. 6). By contrast, the elevated blood pressure due to the PLRCA was reduced to a normal level (121mmHg) in OEGJ treated rats, while the total cerebral blood flow volume significantly increased up to 58ml/min to maintain the blood supply to the ischemic brain (FIG. 6). In APP mice (6-7 months old), the average blood pressure of mice in vehicle treated group was ~124mmHg (FIG. 7). By contrast, the average blood pressure of mice in OEGJ treatment group was ~114mmHg, which is about 10% lower than that in the vehicle treated control mice (FIG. 7). It was also found that the average weight of the brains in OEGJ treatment group is about 15% heavier than that in vehicle treatment group. The histological studies also demonstrated that significantly more newly grown vessels were found in the cortex and hippocampus of brains in OEGJ treated APP mice. In conclusion, one month OEGJ treatment not only induced growth of new collateral vessels in brain and other

tissues, but also decreased the systemic blood pressure (~114mmHg) in APP mice. In contrast, the blood pressure in vehicle treated APP mice was about 10% higher (124mmHg) than that in OEGJ treated (FIG. 7). Furthermore, it was also demonstrated that, after one month OEGJ (480mg/kg) treatment, the mildly increased blood pressures(130-150mmHg) in rat stroke model and SAMP10 mouse model reduced by approximately 10%, similar to the normal blood pressure level in these animals.

Example 5 – The therapeutic effects of the OEGJ on hypertension in human subjects.

[0077] Based on above rather promising results from the OEGJ-enhanced proliferation and differentiation of vessel endothelial cells in cell cuture systems and more importantly, OEGJmediated substantial treatments on 2VO, stroke, PLRCA and APP induced hypertension in animal models, the potential as to whether the OEGJ-induced substantial treatment effects observed in hypertension animal model can be similarly translated in a clinical setting was tested in hypertension patients on the basis of mercy treatment. Our preliminary clinical mercy treatment was performed on 10 patients with primary or secondary hypertension.

[0078] All patients (n=10) with primary or secondary hypertension enrolled in the test reported having a long history of hypertension. Their hypertension could not be satisfactorily treated with currently commercial available medications in recent years. Their high blood pressure did not respond well to the traditional therapeutic strategy or combinations. At the time of examination, their blood pressures were around 162±20/106±10 mmHg. Furthermore, apart from high blood pressure, they also complained that they had been bothered with frequent headache, dizziness, chest suppression, shortness of breath and blurred vision.

[0079] They were treated on request and written consent with OEGJ (2-3 grams/day, oral administration) for 4-8 weeks. After 1-2 weeks oral administration of the OEGJ, all of these patients experienced a smooth decrease of blood pressure. After four to eight weeks treatment, the blood pressure of all these patients were back to within normal range (119±10/79±6 mmHg), and they reported relief from the accompaning symptoms of hypertension as mentioned above (Table 1).

Table 1 Relief of hypertension related symptoms after treatment

Hypertension Related Symptoms	Before Treatment	After Treatment
Headache	+++ ~ ++++	-
Dizziness	++ ~ +++	-
Chest Suppression	+ ~ ++	-
Shortness of Breath	++ ~ +++	-
Blurred Vision	+ ~ ++	-

Example 6 – Treatment of Hypertension using MEGJ

[0080] In a preliminary clinical test with the methanol extract of *Geum japonicum* Thunb. variant (MEGJ) (the composition of MEGJ is similar to OEGJ) for its therapeutic effects on hypertension, it was found that two weeks MEGJ treatment (orally, 3-4 grams/day) could smoothly decrease by 10-20% the blood pressure of patients who did not respond well to the conventional anti-high-blood pressure treatment. Furthermore, the reduced blood pressure can be maintained for several months without any further treatment, indicating its significant potential for treating hypertension, and especially for intractable hypertension. The mechanism underlying the remarkable therapeutic effect of MEGJ on hypertension is postulated to increase the total cross-sectional area of the arteriole bed in the treated subjects.

[0081] One week of MEGJ treatment in 5 patients with borderline hypertension (140-150/90-95 mm Hg) lowered blood pressure to 115-125/70-75 mm Hg for more than 3 months. Another 6 patients with refractory hypertension (140-180/95-140) received MEGJ treatment for one month after failing to respond to conventional anti-hypertension treatment. In the first week of treatment with MEGJ, blood pressure was not significantly lowered. However, after two weeks of treatment with MEGJ, the blood pressures of these patients were significantly and smoothly lowered to 135-145/85-100 mmHg and the symptoms of headache, dizziness, tinnitus, confusion, papilloedema were significantly improved or disappeared (Table 2). After four weeks of treatment with MEGJ, the blood pressures of all 6 patients were lowered and could be maintained for several months without any further medication.

[0082] In conclusion, two to four week oral administration of MEGJ can gradually and significantly lower the elevated blood pressures of patients, who have borderline or refractory hypertension, to a normal level, and the symptoms derived from high blood pressure were also significantly improved or eliminated. The MEGJ-induced blood pressure lowering effect seemed different from current clinically available anti-high blood pressure drugs. Increasing

the total cross-sectional area of the arteriole bed in treated subjects is considered to be one of the major mechanisms for smoothly lowering blood pressure as an effective and possible cure for hypertension.

Table 2. MEGJ treatment of hypertension.

Symptoms	Before treatment	After treatment
Blood pressure	140-180/95-140	120-140/75-95
Headache	++ --- +++	–
Tinnitus	+ --- ++	– --- +
Confusion	– --- ++	–
Papilloedema	+ --- +++	– --- +
Dizziness	++ --- +++	– --- +

* * * *

[0083] While certain embodiments have been illustrated and described, it should be understood that changes and modifications can be made therein in accordance with ordinary skill in the art without departing from the technology in its broader aspects as defined in the following claims.

[0084] The present disclosure is not to be limited in terms of the particular embodiments described in this application. Many modifications and variations can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatuses within the scope of the disclosure, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such modifications and variations are intended to fall within the scope of the appended claims. The present disclosure is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled. It is to be understood that this disclosure is not limited to particular methods, reagents, compounds compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0085] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0086] As will be understood by one skilled in the art, for any and all purposes, particularly in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 units refers to groups having 1, 2, or 3 units. Similarly, a group having 1-5 units refers to groups having 1, 2, 3, 4, or 5 units, and so forth.

[0087] While various aspects and embodiments have been disclosed herein, other aspects and embodiments will be apparent to those skilled in the art. The various aspects and embodiments disclosed herein are for purposes of illustration and are not intended to be limiting, with the true scope and spirit being indicated by the following claims.

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2010258352 18 Mar 2016

CLAIMS

1. A method for treating hypertension in mammalian subjects, the hypertension being caused by peripheral resistance of small arteries, the method comprising administering to a subject in need thereof an effective amount of an organic extract of Geum japonicum (OEGJ), wherein the OEGJ is a lower alkyl alcohol solvent extract of Geum japonicum, wherein treating or preventing hypertension comprises stimulating collateral vessel formation, and wherein the peripheral resistance of small arteries is systemically decreased in a subject administered the OEGJ compared to a subject not administered the OEGJ.

2. The method of claim 1, wherein OEGJ is administered in an amount ranging from about 0.01 mg to about 10,000 mg of the extract per kilogram of body weight per day.

3. The method of claim 1 or claim 2, wherein OEGJ is administered in a dosage unit form.

4. The method of any one of claims 1 to 3, wherein OEGJ is administered in a dosage unit form comprising a pharmaceutically acceptable carrier.

5. The method of any one of claims 1 to 4, wherein the subject's blood pressure is reduced compared to a subject not administered with the OEGJ.

6. The method of any one of claims 1 to 5, wherein OEGJ is administered orally.

7. The method of any one of claims 1 to 6, wherein OEGJ is administered by subcutaneous injection, intramuscular injection, or intravenous infusion.

8. The method of any one of claims 1 to 7, wherein lower alkyl alcohol has 1 to 6 carbons atoms.

9. The method of any one of claims 1 to 8, wherein the solvent is ethanol.

10. The method of any one of claims 1 to 8, wherein the solvent is methanol.

11. The method of any one of claims 1 to 10, wherein said mammalian subject is a human.

2010258352 18 Mar 2016

12. A pharmaceutical composition when used for treating hypertension in a mammalian subject, the hypertension being caused by peripheral resistance of small arteries, wherein treating hypertension comprises stimulating collateral vessel formation, said composition comprising an effective amount of a lower alkyl alcohol solvent extract of Geum japonicum (OEGJ) and a pharmaceutically acceptable excipient.

13. A method according to claim 1 and substantially as hereinbefore described with reference to any one of the Examples.

14. A composition according to claim 12 and substantially as hereinbefore described with reference to any one of the Examples.

Generex Pharmaceuticals, Inc.
Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

FIG. 1

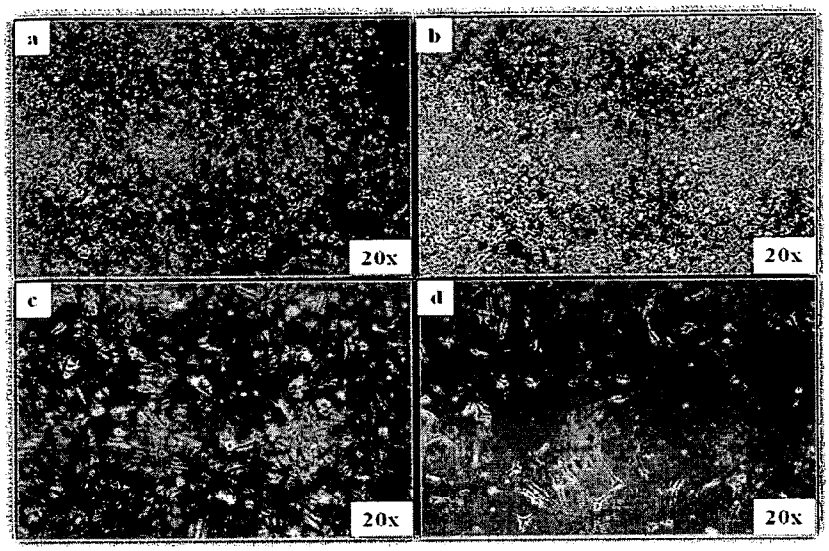


FIG. 2

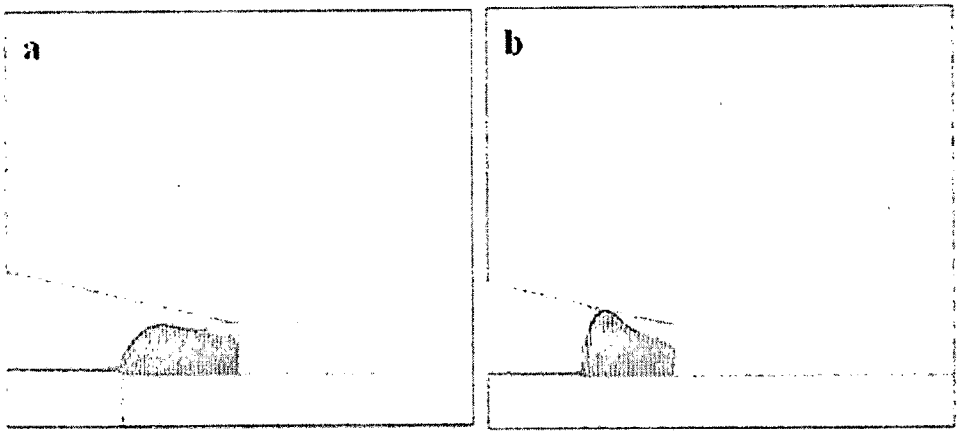
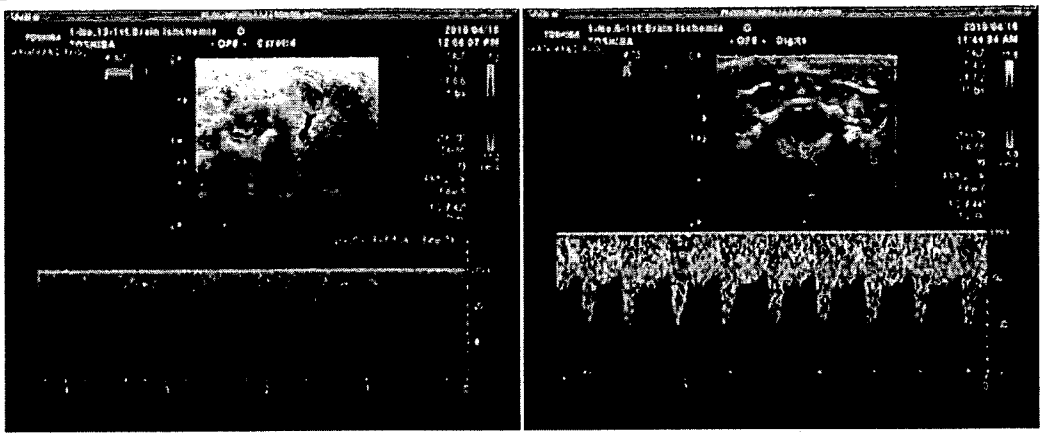


FIG. 3

A B



Nor: BP=120, BBF=11.2; 2W post=121, BBF=12
Crt: BP=154, BBF=15.8; 2W post=148, BBF=14.4
Oegj: BP=137, BBF=26.3; 2W post=128, BBF=21.2

Based on: BP ÷ R = BBF,
For Nor, Rn1=120 ÷ 11.2=10.7; Rn2=121 ÷ 12.0=10.1
For Crt, Rc1=154 ÷ 15.8=9.8; Rc2=148 ÷ 14.4=10.3
For Oegj, Ro1=137 ÷ 26.3=5.2; Ro2=128 ÷ 21.1=6.06

FIG. 4

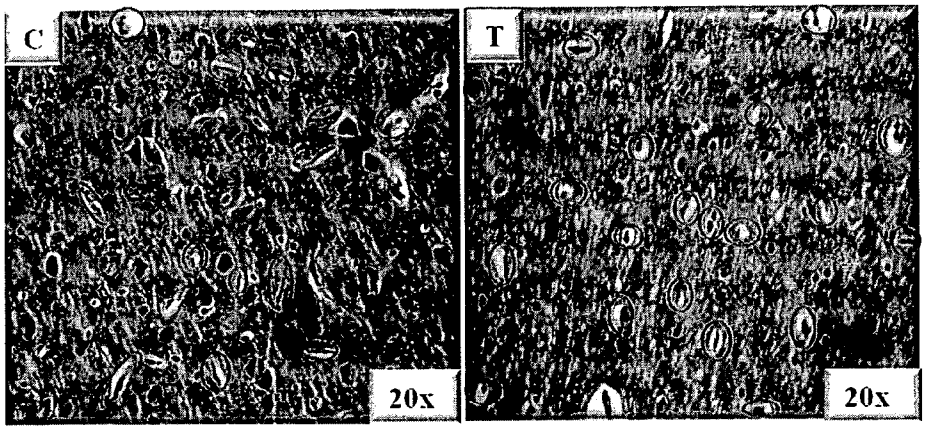

C
T
20x
20x

FIG. 5

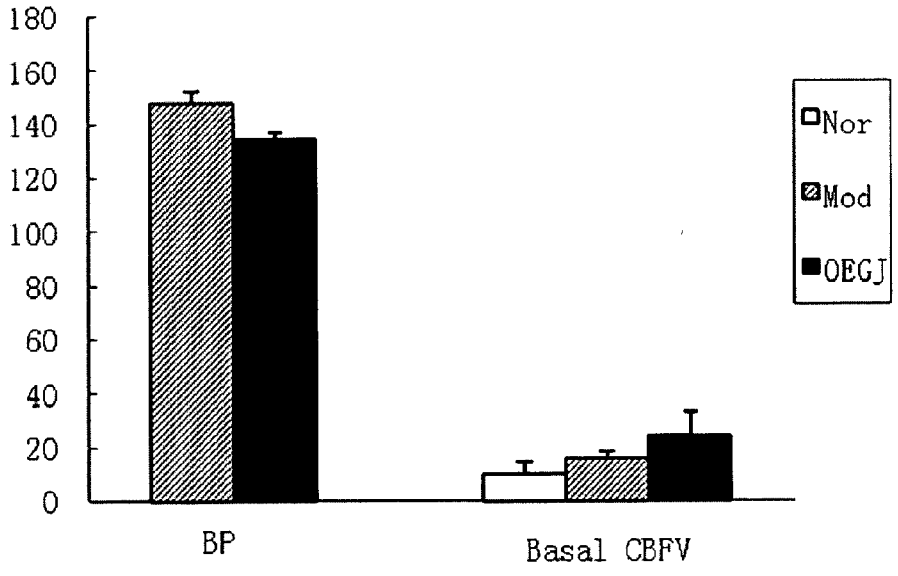

180
160
140
120
100
80
60
40
20
0
BP
Basal CBFV
Nor
Mod
OEGJ

FIG. 6

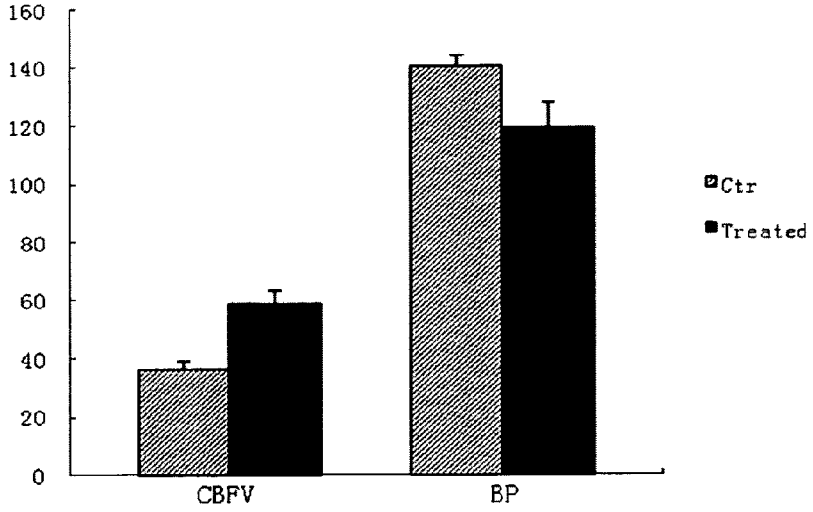
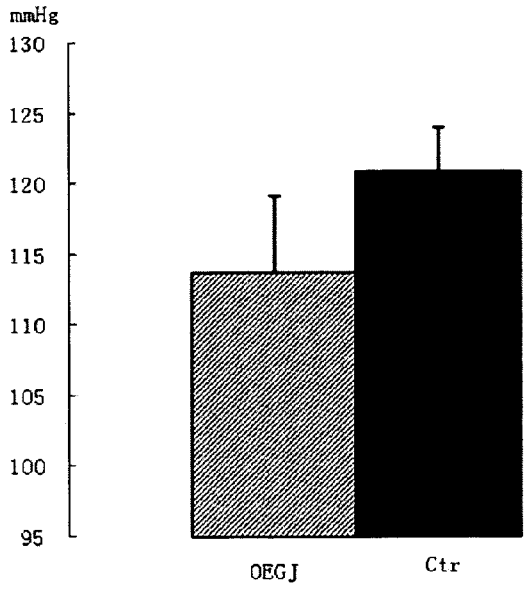

160
140
120
100
80
60
40
20
0
CBFV
BP
Ctr
Treated

FIG. 7


mmHg
130
125
120
115
110
105
100
95
OEGJ
Ctr