Compositions and Methods of Use of Phorbol Esters for the Treatment of Stroke

Related Applications

This application is a United States utility patent application which claims priority benefit of United States Provisional Patent Application Serial No. 61/588,167, filed January 18, 2012, which is incorporataed herein in its entirety by reference.

Additional Disclosure

Additional disclosures relating to the instant application may be found in "Compositions And Methods Of Use Of Phorbol Esters" United States Patent Application Serial No. 12/023,753, filed January 31, 2008, to Richard L. Chang, et al, which claims priority benefit of United States Provisional Patent Application Serial No. 60/898,810, filed January 31, 2007, each of which is incorporated herein by reference in its entirety for all purposes.

Technical Field

The present invention relates generally to the medicinal use of phorbol esters in the treatment and prevention of stroke and the effects of stroke.

Background

Plants have historically served many medicinal purposes. The World Health Organization (WHO) estimates that 4 billion people, 80% of the world's population, presently use herbal medicine for some aspect of primary health care. (WHO Fact sheet Fact sheet N°134, December 2008) However, it can be difficult to isolate the specific compound in a plant that has the desired medicinal effect and to reproduce it on a commercial scale. Additionally, while the active compound may be isolated from a plant, the other parts of a plant such as the minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavanoids, and other substances may also be involved in the functioning of the active compound or the medicinal effect for which the plant is known, making the use, purification and commercialization of plant based pharmaceutical agents a challenge.

Phorbol is a natural, plant-derived organic compound of the tigliane family of diterpenes. It was first isolated in 1934 as a hydrolysis product of croton oil derived from the seeds of *Croton tiglium*, a leafy shrub of the Euphorbiaceae family that is native to Southeastern Asia. Various esters of phorbol have important biological properties including the reported ability to mimic diacylglycerols and activate protein

kinase C (PKC); and to modulate downstream cell signaling pathways including the mitogen-activated protein kinase (MAPK) pathways. Phorbol esters are additionally thought to bind to chimaerins, the Ras activator RasGRP, and the vesicle-priming protein Munc-13 (Brose N, Rosenmund C., JCell Sci;115:4399–411 (2002)). Some phorbol esters also induce nuclear factor-kappa B (NF-κB). The most notable physiological property of phorbol esters is their reported capacity to act as tumor promoters. (Blumberg, 1988; Goel, G *et al.,* Int, Journal of Toxicology 26, 279-288 (2007)).

12-O-tetradecanoylphorbol-13-acetate (TPA), also called phorbol-12-myristate-13-acetate (PMA), is a phorbol ester used in models of carcinogenesis as an inducer for differentiation and/or apoptosis in multiple cell lines and primary cells. TPA has also been reported to cause an increase in circulating white blood cells and neutrophils in patients whose bone marrow function has been depressed by chemotherapy (Han Z. T. *et al.* Proc. Natl. Acad. Sci. 95, 5363-5365 (1998)), and to inhibit an HIV- induced cytopathic effect on MT-4 cells. (Mekkawy S. *et al.*, Phytochemistry 53, 47-464 (2000)). However, due to a variety of factors, including caustic reactions when contacted with the skin and concerns for its potential toxicity, TPA has not been shown to be an effective tool for treating, managing, or preventing disease. Indeed, as phorbol esters play a key role in activation of protein kinase C, which triggers various cellular responses resulting in inflammatory responses and tumor development (Goel *et al.,* Int, Journal of Toxicology 26, 279-288 (2007)), phorbol esters would generally be excluded from possible treatment candidates for conditions that involve inflammatory reactions such as stroke.

Heart disease and stroke kill some 17 million people a year, causing almost one-third of all deaths globally. They are predicted to become the leading cause of both death and disability worldwide, with the number of fatalities projected to increase to over 20 million a year by 2020 and to 24 million a year by 2030. (Atlas of Heart Disease and Stroke, World Health Organization 2004).

While there are over 300 risk factors associated with coronary heart disease and stroke (Atlas of Heart Disease and Stroke, World Health Organization 2004), in developed countries, at least 1/3 of all cardiovascular disease is attributable to tobacco use, alcohol use, high blood pressure, high cholesterol and obesity.

Current treatments for the management and prevention of stroke are generally a combination of medications such as ACE inhibitors, aspirin, beta blockers and lipid lowering medications; devices such as pacemakers, implantable defibrillators, coronary stents, prosthetic valves and artificial hearts; and operations such as coronary artery bypass, balloon angioplasty, valve repair and replacement, heart transplants and artificial heart operations. "Increasingly, high-technology procedures are chosen over less expensive, but nevertheless effective , strategies " (Atlas of Heart Disease and Stroke, World Health Organization 2004) adding to the rising costs of health care and leading to marked disparities in the quality of treatment between different groups of individuals.

However, even where advanced technology and facilities are available, 60% of those who suffer a stroke die or become dependent and each stroke significantly increases the risk of further episodes. "Worldwide, treatment of cardiovascular diseases and their risk factors remains inadequate for most patients." (Atlas of Heart Disease and Stroke, World Health Organization 2004). There is therefore clearly a need for new and more effective measures to treat and prevent strokes and to treat or prevent the long term effects caused by stroke.

Summary

The present invention relates to compositions containing and methods of using phorbol esters. The compositions and methods described herein are effective in preventing and treating stroke and in managing the sequelae of stroke including acute ischemic events.

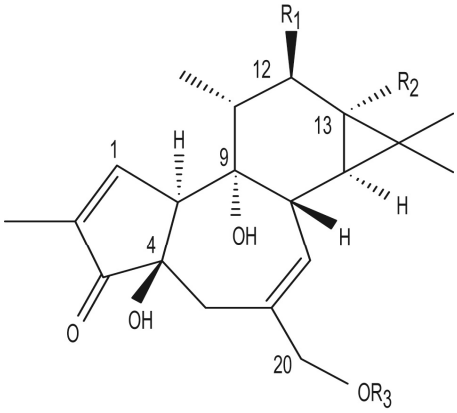
Effects of stroke that may be prevented or treated by using the phorbol esters and derivatives of phorbol esters as described herein include, but are not limited to, paralysis, spatial impairment, impaired judgment, left-sided neglect, memory loss, aphasia, coordination and balance problems, nausea, vomiting, cognitive impairment, perception impairment, orientation impairment, homonymous hemianopsia and impulsivity. In some embodiments, the use of phorbol esters and derivatives of phorbol esters may prevent initial and subsequent strokes from occurring.

Successful treatment will be determined according to conventional methods, such as a reduction in the severity or sequelae of a stroke, a decrease or elimination of

the effects of stroke, a decrease in risk factors that predispose an individual to a stroke, and/or a decrease in the number or severity of strokes including subsequent strokes.

In another embodiment, the phorbol esters and derivatives of phorbol esters as described herein may be used to modulate cell signaling pathways. Such modulation may have a variety of results, for example, in some embodiments, the use of compositions containing phorbol esters and derivatives of phorbol esters may alter the release of Th1 cytokines in mammalian subjects. In a further embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the release of interleukin 2 (IL-2) in mammalian subjects. In an additional embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the release of interferon in mammalian subjects. In yet another embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the rate of ERK phosphorylation.

The invention achieves the foregoing and satisfies additional objects and advantages by providing novel and surprisingly effective methods and compositions for treating and preventing stroke, modulating cell signaling pathways and/or managing, treating and preventing the sequelae of a stroke using compositions containing a phorbol ester of Formula I, below:



FORMULA I

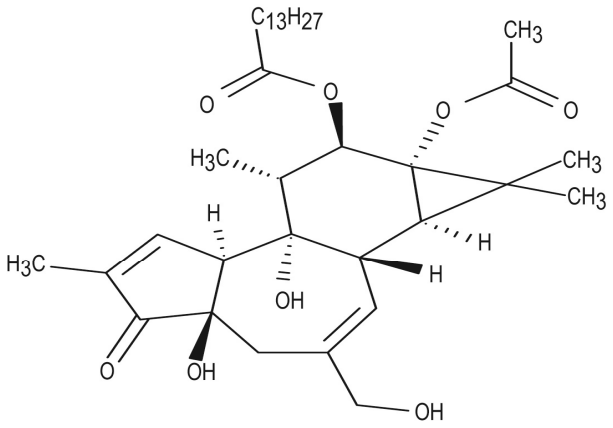
wherein R_1 and R_2 may independently be hydrogen; hydroxyl; $-O-\overset{O}{\overset{\|}{C}}$-alkyl, wherein the alkyl group contains 1 to 15 carbon atoms; $-O-\overset{O}{\overset{\|}{C}}$-lower alkenyl, wherein a lower

alkenyl group contains between 1 to 7 carbon atoms; —O—C(=O)-phenyl; —O—C(=O)-benzyl; and substituted derivatives thereof. R_3 may be independently be hydrogen or —C(=O)-lower alkyl and substituted derivatives thereof. The methods and compositions of the present invention further include any pharmaceutical salts, enantiomers, isomer, polymorphs, prodrugs, hydrates and solvates of the compositions of Formula I.

In some embodiments, at least one of R_1 and R_2 are other than hydrogen and R_3 is hydrogen or —C(=O)-lower alkyl and substituted derivatives thereof. In another embodiment, either R_1 or R_2 is —O—C(=O)—C_1— C_{15} alkyl the remaining R_1 or R_2 is a —O—C(=O)-lower alkyl, wherein a lower alkyl is between 1 and 7 carbons, and R_3 is hydrogen.

The alkyl, alkenyl, phenyl and benzyl groups of the formulas of Formula I herein may be unsubstituted or substituted with halogens, preferably, chlorine, fluorine or bromine; nitro; amino; and/or similar type radicals.

An exemplary phorbol ester composition as used in the methods and compositions as described herein is 12-O-tetradecanoylphorbol-13-acetate (TPA) of Formula II, below:



FORMULA II

Useful phorbol esters of Formula I and related compounds and derivatives within the formulations and methods of the invention include, but are not limited to, other pharmaceutically acceptable active salts of said compounds, as well as active

isomers, enantiomers, polymorphs, glycosylated derivatives, solvates, hydrates, and/or prodrugs of said compounds. Exemplary forms of phorbol esters for use within the compositions and methods of the invention include, but are not limited to, phorbol 13-butyrate; phorbol 12-decanoate; phorbol 13-decanoate; phorbol 12,13-diacetate; phorbol 13,20-diacetate; phorbol 12,13-dibenzoate; phorbol 12,13-dibutyrate; phorbol 12,13-didecanoate; phorbol 12,13-dihexanoate; phorbol 12,13-dipropionate; phorbol 12-myristate; phorbol 13-myristate; phorbol 12-myristate-13-acetate (also known as TPA or PMA); phorbol 12,13,20-triacetate; 12-deoxyphorbol 13-angelate; 12-deoxyphorbol 13-angelate 20-acetate; 12-deoxyphorbol 13-isobutyrate; 12-deoxyphorbol 13-isobutyrate-20-acetate; 12-deoxyphorbol 13-phenylacetate; 12-deoxyphorbol 13-phenylacetate 20-acetate; 12-deoxyphorbol 13-tetradecanoate; phorbol 12-tigliate 13-decanoate; 12-deoxyphorbol 13-acetate; phorbol 12-acetate; and phorbol 13-acetate.

Mammalian subjects amenable to treatment with phorbol esters of Formula I or derivatives of a phorbol ester of the Formula I, particularly TPA, according to the methods of the invention include, but are not limited to, individuals who have suffered or are at risk for a stroke. Subjects amenable to treatment with phorbol esters of Formula I, particularly TPA, additionally include those suffering from the effects of a stroke including, but not limited to, paralysis, spatial impairment, impaired judgment, left-sided neglect, memory loss, aphasia, coordination and balance problems, nausea, vomiting, cognitive impairment, perception impairment, orientation impairment, homonymous hemianopsia and impulsivity.

These and other subjects are effectively treated prophylactically and/or therapeutically, by administering to the subject an effective amount of a phorbol ester of Formula I or derivatives of a phorbol ester of the Formula I sufficient to modulate NF-κB activity, increase Th1 cytokine activity, prevent or treat paralysis, increase spatial awareness, decrease memory loss, decrease aphasia, increase coordination and balance, prevent or decrease the incidence and severity of a stroke, and improve cognition.

Therapeutically useful methods and formulations of the invention will effectively use a phorbol ester of Formula I or derivative of a phorbol ester of the Formula I in a variety of forms, as noted above, including any active, pharmaceutically acceptable salts of said compounds, as well as active isomers, enantiomers, polymorphs,

solvates, hydrates, prodrugs, and/or combinations thereof. TPA of formula II is employed as an illustrative embodiment of the invention within the examples herein below.

Within additional aspects of the invention, combinatorial formulations and methods are provided which employ an effective amount of a phorbol ester of Formula I or derivative of a phorbol ester of Formula I in combination with one or more secondary or adjunctive active agent(s) that is/are combinatorially formulated or coordinately administered with the phorbol ester compound of Formula I to yield an effective response in the subject.

Combinatorial formulations and coordinate treatment methods in the treatment of stroke employ a phorbol ester compound of Formula I or derivative of the phorbol ester of Formula I in combination with one or more additional, stroke preventing, treating or other indicated, secondary or adjunctive therapeutic agents. The secondary or adjunctive therapeutic agents used in combination with a phorbol ester, e.g., TPA, in these embodiments may possess direct or indirect effects on prevention or recovery from stroke, alone or in combination with the phorbol ester, e.g. TPA; may exhibit other useful adjunctive therapeutic activity in combination with, e.g. TPA (such as anti-clotting, anticholesterolemic, vasodilating, antihypertensive, arteriolar resistance lowering, venous capacity increasing, heart oxygen demand reducing, heart rate decreasing, heart rate stabilizing, or neuroprotecting); or may exhibit adjunctive therapeutic activity useful for treating or preventing stroke or associated symptoms alone or in combination with, e.g. TPA. Such secondary or adjunctive therapeutic agents may be administered prior to, simultaneously, or after administration of a phorbol ester of Formula I or derivative of a phorbol ester of Formula I.

Useful adjunctive or secondary therapeutic agents in these combinatorial formulations and coordinate treatment methods for the prevention or treatment of stroke in a mammalian subject include, but are not limited to, tissue plasminogen activators, anticoagulants, statins, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, anti-platelet agents, beta-blockers, aspirin, fibrates, calcium channel blockers, or diuretics. In addition, adjunctive or secondary therapies may be used such as, but not limited to, surgical intervention including carotid endarterectomy, angioplasty, balloon

angioplasty, valve repair and replacement, coronary artery bypass, stent placement, craniotomy, endovascular coil emobilization, or patent foramen ovale closure.

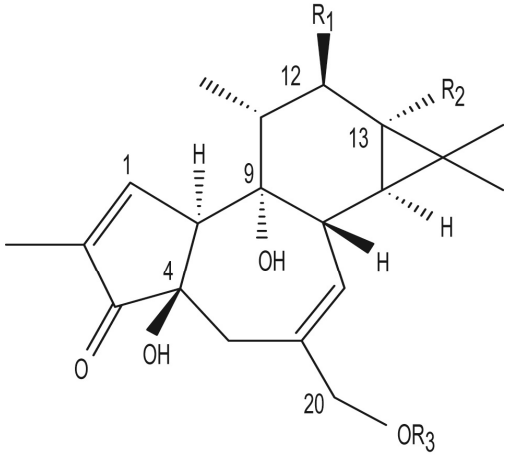
The foregoing and additional objects, features, aspects and advantages of the present invention will become apparent from the following detailed description.

Detailed Description

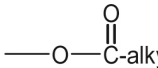
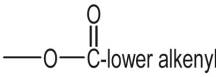
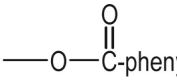
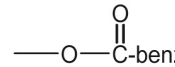
Novel methods and compositions have been identified for use in preventing and/or treating stroke and the sequelae of stroke in mammalian subjects, including humans.

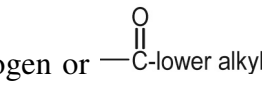
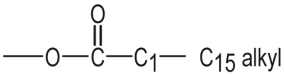
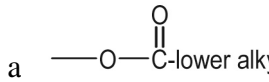
In various embodiments, the composition and methods as described herein may increase the release of Th1 cytokines, increase ERK phosphorylation, modulate NF-κB activity, prevent or treat paralysis, increase spatial awareness, decrease memory loss, decrease aphasia, increase coordination and balance, improve cognition, improve orientation, decrease the prevalence of subsequent strokes, and decrease impulsivity.

Formulations and methods provided herein employ a phorbol ester of Formula I or derivative compound of a phorbol ester of Formula I as more fully described in U.S. Patent Application No. 12/023,753, filed January 31, 2008, which claims priority benefit of United States Provisional patent application Serial No. 60/898,810, filed January 31, 2007 which is incorporated herein in its entirety by reference,



FORMULA I

wherein R_1 and R_2 may independently be hydrogen; hydroxyl; , wherein the alkyl group contains 1 to 15 carbon atoms; , wherein a lower alkenyl group contains between 1 to 7 carbon atoms; ; ; and substituted derivatives thereof. R_3 may be independently be hydrogen or .

In some embodiments, at least one of R_1 and R_2 are other than hydrogen and R_3 is hydrogen or  and substituted derivatives thereof as novel compositions for use in treating chronic or recurring conditions. In another embodiment, either R_1 or R_2 is  the remaining R_1 or R_2 is a , wherein a lower alkyl is between 1 and 7 carbons, and R_3 is hydrogen.

The alkyl, alkenyl, phenyl and benzyl groups of the formulas herein may be unsubstituted or substituted with halogens, preferably, chlorine, fluorine or bromine; nitro; amino; and/or similar type radicals.

Stroke treating formulations and methods provided herein employ a phorbol ester of Formula I or derivative compound of a phorbol ester of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as anti-stroke agents.

Th1 cytokine increasing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as novel Th1 cytokine increasing agents. A broad range of mammalian subjects, including human subjects, are amenable to treatment using the formulations and methods of the invention. These

subjects include, but are not limited to, individuals who have suffered or are at risk for a stroke.

ERK phosphorylation increasing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as novel ERK phosphorylation increasing agents. A broad range of mammalian subjects, including human subjects, are amenable to treatment using the formulations and methods of the invention. These subjects include, but are not limited to, individuals who have suffered or are at risk for a stroke.

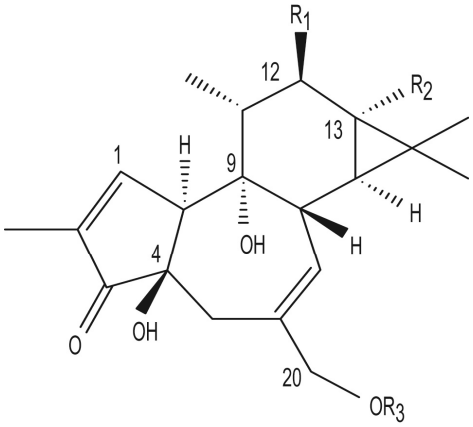
Within the methods and compositions of the invention, one or more phorbol ester compound(s) of Formula I or derivative compounds of a phorbol ester of Formula I as disclosed herein is/are effectively formulated or administered as an agent effective for treating and preventing stroke or the sequelae of stroke. In exemplary embodiments, TPA is demonstrated for illustrative purposes to be an effective agent in pharmaceutical formulations and therapeutic methods, alone or in combination with one or more adjunctive therapeutic agent(s). The present disclosure further provides additional, pharmaceutically acceptable phorbol ester compounds in the form of a native or synthetic compound, including complexes, derivatives, salts, solvates, isomers, enantiomers, polymorphs, and prodrugs of the compounds disclosed herein, and combinations thereof, which are effective as therapeutic agents within the methods and compositions of the invention in the treatment and prevention of stroke and the sequelae of stroke.

Strokes are caused by disruption of the blood supply to the brain. This may result from either blockage (ischaemic stroke) or rupture of a blood vessel (haemorrhagic stroke). The symptoms of a stroke include sudden numbness or weakness, especially on one side of the body; sudden confusion or trouble speaking or understanding speech; sudden trouble seeing in one or both eyes; sudden trouble with walking, dizziness, or loss of balance or coordination; or sudden severe headache with no known cause. Risk factors for stroke include high blood pressure, abnormal blood

lipids, tobacco use, physical inactivity, obesity, stress, diabetes, alcohol use, excess homocystein in the blood, inflammation and abnormal coagulation. There are also non-modifiable risk factors such as age, heredity, gender and ethnicity.

Treatment for stroke has three distinct phases: prevention, therapy immediately after a stroke, and post-stroke rehabilitation. The compositions and methods described herein may be used at any phase of stroke treatment, independently or in conjunction with one or more additional therapies including other pharmaceutical agents, devices or surgical interventions.

Phorbol is a natural, plant-derived polycyclic alcohol of the tigliane family of diterpenes. It was first isolated in 1934 as the hydrolysis product of croton oil derived from the seeds of *Croton tiglium*. It is well soluble in most polar organic solvents and in water. Esters of phorbol have the general structure of Formula I, below:



FORMULA I

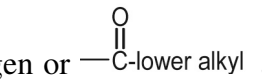
wherein R_1 and R_2 are selected from the group consisting of hydrogen; hydroxyl; —O—C(=O)-alkyl, wherein the alkyl group contains 1 to 15 carbon atoms; —O—C(=O)-lower alkenyl, wherein a lower alkenyl group contains between 1 to 7 carbon atoms; —O—C(=O)-phenyl; —O—C(=O)-benzyl; and substituted derivatives thereof. R_3 may be hydrogen or —C(=O)-lower alkyl and substituted derivatives thereof as well as

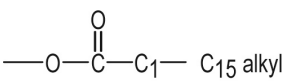
pharmaceutically acceptable salts, enantiomers, polymorphs, prodrugs solvates and hydrates of compounds of Formula I.

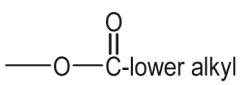
The term "lower alkyl" or "lower alkenyl" as used herein means moieties containing 1 to 7 carbon atoms. In the compounds of the Formula I, the alkyl or alkenyl groups may be straight or branched chain. In some embodiments, either or both R_1 or R_2, are a long chain carbon moiety (i.e., Formula I is decanoate or myristate).

The alkyl, alkenyl, phenyl and benzyl groups of the formulas herein may be unsubstituted or substituted with halogens, preferably, chlorine, fluorine or bromine; nitro; amino and similar type radicals.

Organic and synthetic forms of phorbol esters, including any preparations or extracts from herbal sources such as *croton tiglium*, are contemplated as useful compositions comprising phorbol esters (or phorbol ester analogs, related compounds and/or derivatives) for use within the embodiments herein. Useful phorbol esters and/or related compounds for use within the embodiments herein will typically have a structure as illustrated in Formula I, although functionally equivalent analogs, complexes, conjugates, and derivatives of such compounds will also be appreciated by those skilled in the art as within the scope of the invention.

In more detailed embodiments, illustrative structural modifications according to Formula I above will be selected to provide useful candidate compounds for treating and/or preventing strokes, damage due to stroke and/ or managing the effects or sequelae of strokes in mammalian subjects, including humans, wherein: at least one of R_1 and R_2 are other than hydrogen and R_3 is selected from the group consisting of hydrogen or 

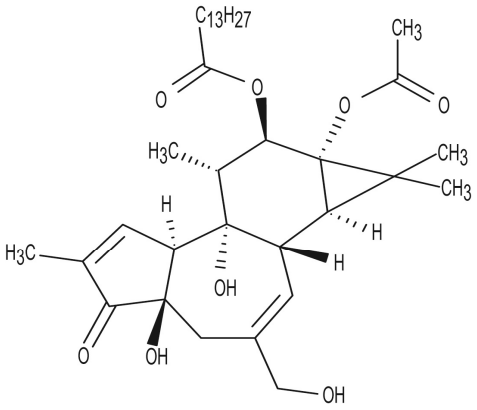
and substituted derivatives thereof. In another embodiment, either R_1 or R_2 is 

the remaining R_1 or R_2 is 

and R_3 is hydrogen.

An exemplary embodiment of a phorbol ester compound of Formula I useful in the treatment of in treating or preventing strokes, damage from strokes, and/or managing the effects or sequelae of strokes in mammalian subjects, including humans,

is found in phorbol 12-myristate-13-acetate (also known as PMA or 12-O-tetradecanoyl-phorbol-13-acetate (TPA)) shown in Formula II, below.

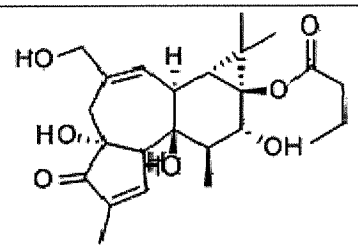
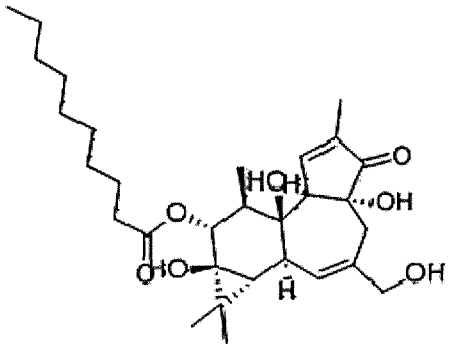
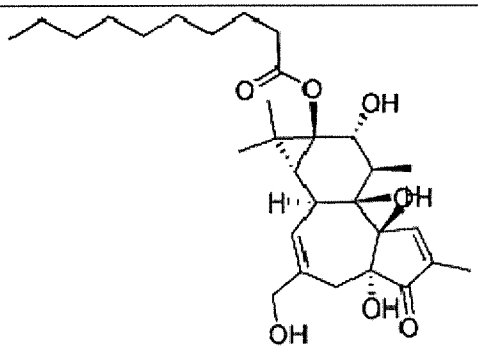
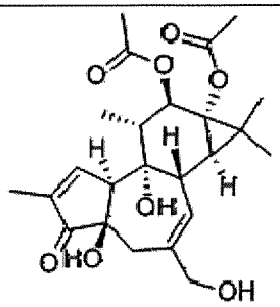
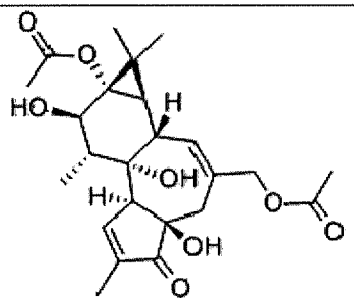


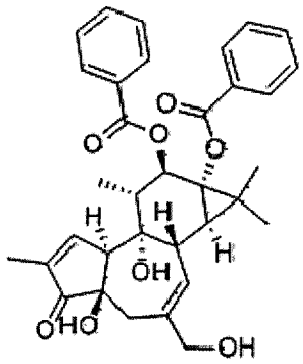
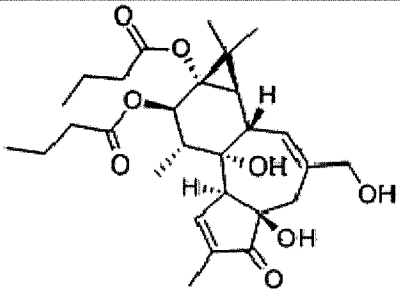
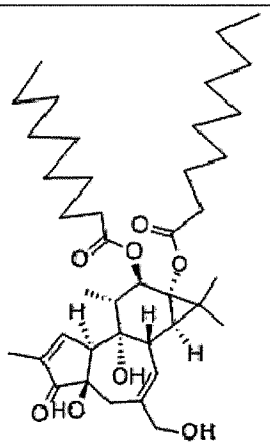
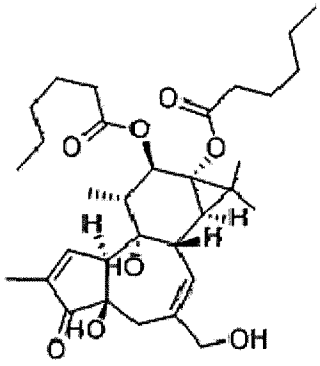
FORMULA II

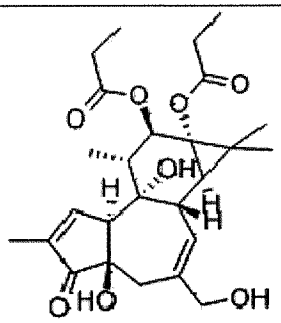
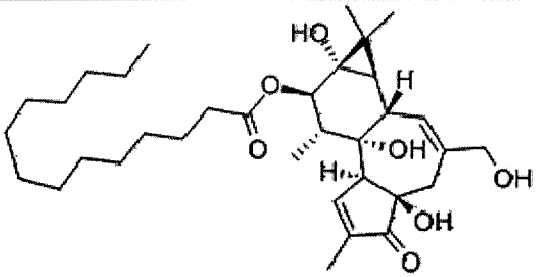
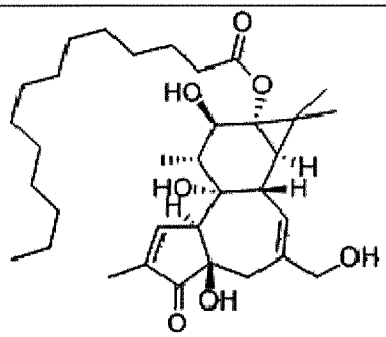
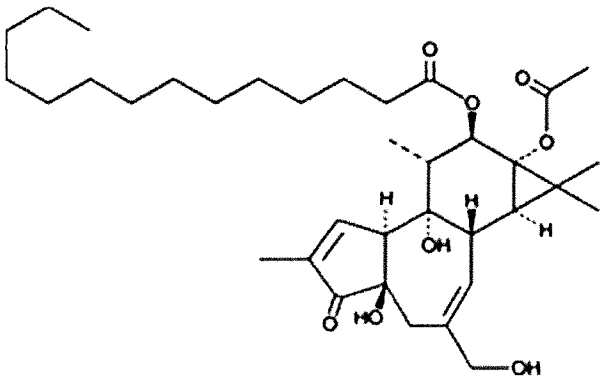
Additional useful phorbol esters and related compounds and derivatives within the formulations and methods of the invention include, but are not limited to, other pharmaceutically acceptable active salts of said compounds, as well as active isomers, enantiomers, polymorphs, glycosylated derivatives, solvates, hydrates, and/or prodrugs of said compounds. Derivatives of phorbol esters of Formula I may or may not be phorbol esters themselves. Further exemplary forms of phorbol esters for use within the compositions and methods of the invention include, but are not limited to, phorbol 13-butyrate; phorbol 12-decanoate; phorbol 13-decanoate; phorbol 12,13-diacetate; phorbol 13,20-diacetate; phorbol 12,13-dibenzoate; phorbol 12,13-dibutyrate; phorbol 12,13-didecanoate; phorbol 12,13-dihexanoate; phorbol 12,13-dipropionate; phorbol 12-myristate; phorbol 13-myristate; phorbol 12,13,20-triacetate; 12-deoxyphorbol 13-angelate; 12-deoxyphorbol 13-angelate 20-acetate; 12-deoxyphorbol 13-isobutyrate; 12-deoxyphorbol 13-isobutyrate-20-acetate; 12-deoxyphorbol 13-phenylacetate; 12-deoxyphorbol 13-phenylacetate 20-acetate; 12-deoxyphorbol 13-tetradecanoate; phorbol 12-tigliate 13-decanoate; 12-deoxyphorbol 13-acetate; phorbol 12-acetate; and phorbol 13-acetate as shown in Table 1.

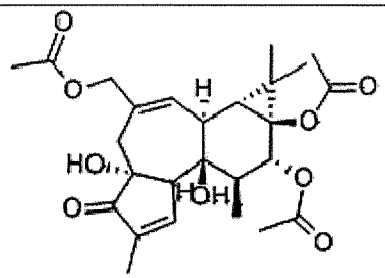
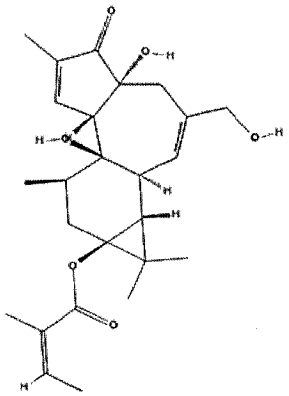
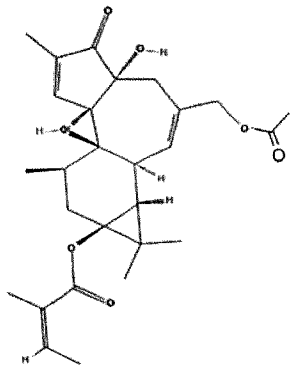
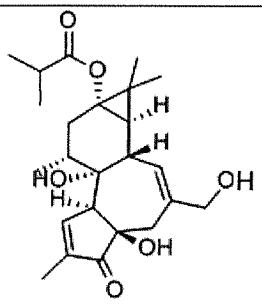
Table 1

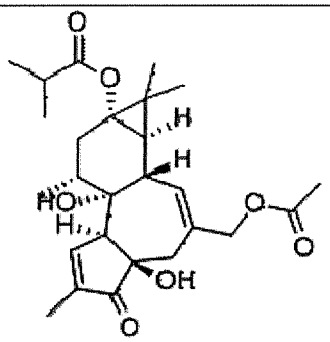
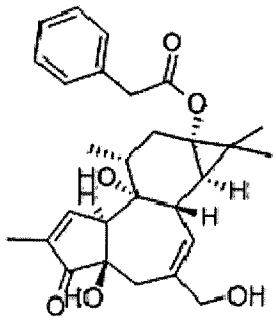
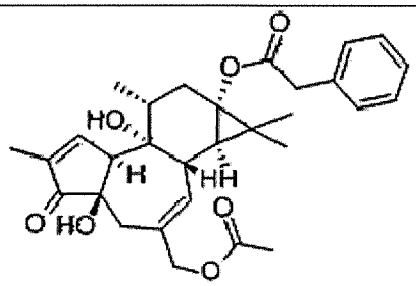
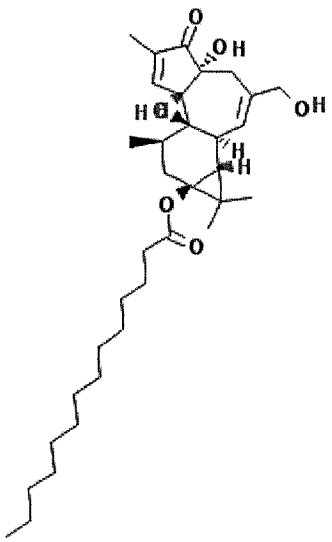
Exemplary Phorbol Esters

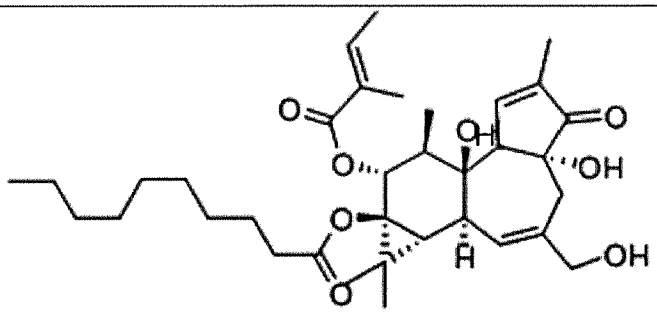
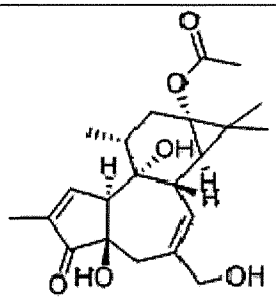
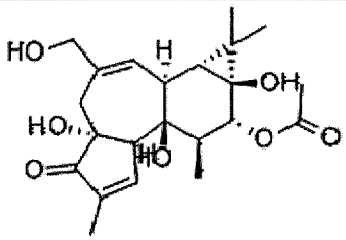
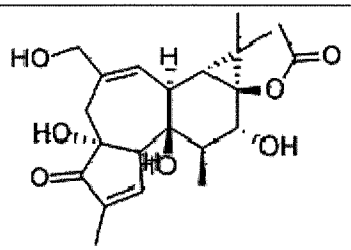
Phorbol 13-Butyrate	
Phorbol 12-Decanoate	
Phorbol 13-Decanoate	
Phorbol 12,13-Diacetate	
Phorbol 13,20-Diacetate	

Phorbol 12,13-Dibenzoate	
Phorbol 12,13-Dibutyrate	
Phorbol 12,13-Didecanoate	
Phorbol 12,13-Dihexanoate	

Phorbol 12,13-Dipropionate	
Phorbol 12-Myristate	
Phorbol 13-Myristate	
Phorbol 12-Myristate-13-Acetate (also known as TPA or PMA)	
Phorbol 12,13,20-Triacetate	

	
12-Deoxyphorbol 13-Angelate	
12-Deoxyphorbol 13-Angelate 20-Acetate	
12-Deoxyphorbol 13-Isobutyrate	

12-Deoxyphorbol 13-Isobutyrate-20-Acetate	
12-Deoxyphorbol 13-Phenylacetate	
12-Deoxyphorbol 13-Phenylacetate 20-Acetate	
12-Deoxyphorbol 13-Tetradecanoate	

Phorbol 12-Tigliate 13-Decanoate	
12-Deoxyphorbol 13-Acetate	
Phorbol 12-Acetate	
Phorbol 13-Acetate	

Compositions as described herein comprise stroke treating compositions comprising a stroke damage alleviating or preventing effective amount of a phorbol ester compound of Formula I or derivative compound of a phorbol esters of Formula I, which is effective for prophylaxis and/or treatment of stroke or stroke related symptoms or sequelae in a mammalian subject. A "stroke treating," "anti-clotting," "anticholesterolemic," "vasodilating," "antihypertensive," "arteriolar resistance lowering," " venous capacity increasing," "heart oxygen demand reducing," "heart rate decreasing," "heart rate stabilizing," or "neuroprotective" effective amount of the active compound is therapeutically effective, in a single or multiple unit dosage form, over a specified period of therapeutic intervention, to measurably alleviate one or more

symptoms or sequelae of stroke in the subject. Within exemplary embodiments, the compositions of the invention are effective in treatment methods to prevent or alleviate symptoms of stroke or sequelae of stroke in human and other mammalian subjects vulnerable to or who have suffered a stroke.

Phorbol ester treating compositions of the invention typically comprise an effective amount or unit dosage of a phorbol ester compound of Formula I or derivative compound of a phorbol ester of Formula I, which may be formulated with one or more pharmaceutically acceptable carriers, excipients, vehicles, emulsifiers, stabilizers, preservatives, buffers, and/or other additives that may enhance stability, delivery, absorption, half-life, efficacy, pharmacokinetics, and/or pharmacodynamics, reduce adverse side effects, or provide other advantages for pharmaceutical use. Effective amounts of a phorbol ester compound or related or derivative compound of Formula I (e.g., a unit dose comprising an effective concentration/amount of TPA, or of a selected pharmaceutically acceptable salt, isomer, enantiomer, solvate, polymorph and/or prodrug of TPA) will be readily determined by those of ordinary skill in the art, depending on

clinical and patient-specific factors. Suitable effective unit dosage amounts of the active compounds for administration to mammalian subjects, including humans, may range from about 10 to about 1500 μg, about 20 to about 1000 μg, about 25 to about 750 μg, about 50 to about 500 μg, about 150 to about 500 μg, about 125 μg to about 500 μg, about 180 to about 500 μg, about 190 to about 500 μg, about 220 to about 500 μg, about 240 to about 500 μg, about 260 to about 500 μg, about 290 to about 500 μg. In certain embodiments, the disease treating effective dosage of a phorbol ester compound or related or derivative compound of Formula I may be selected within narrower ranges of, for example, 10 to 25 μg, 30-50 μg, 75 to 100 μg, 100 to 300 μg, or 150 to 500 μg. These and other effective unit dosage amounts may be administered in a single dose, or in the form of multiple daily, weekly or monthly doses, for example in a dosing regimen comprising from 1 to 5, or 2 to 3, doses administered per day, per week, or per month. In one exemplary embodiment, dosages of 10 to 30 μg, 30 to 50 μg, 50 to 100 μg, 100 to 300 μg, or 300 to 500 μg, are administered one, two, three, four, or five times per day. In more detailed embodiments, dosages of 50-100 μg, 100-300 μg, 300-400 μg, or 400-600 μg are administered once or twice daily. In a further embodiment, dosages of 50-100 μg, 100-300 μg, 300-400 μg, or 400-600 μg are administered every other day. In alternate embodiments, dosages are calculated based on body weight, and may be administered, for example, in amounts from about 0.5μg/m^2 to about 300μg/m^2 per day, about 1 μg/m^2 to about 200 μg/m^2, about 1 μg/m^2 to about 187.5 μg/m^2 per day, about 1 μg/m^2 per day to about 175 μg/m^2 per day, about 1 μg/m^2 per day to about 157 μg/m^2 per day about 1 μg/m^2 to about 125 μg/m^2 per day, about 1 μg/m^2 to about 75 μg/m^2 per day, 1 μg/m^2 to about 50/ μg/m^2 per day, 2 μg/m^2 to about 50 μg/m^2 per day, 2 μg/m^2 to about 30 μg/m^2 per day or 3 μg/m^2 to about 30 μg/m^2 per day.

[047] In other embodiments, dosages may be administered less frequently, for example, 0.5μg/m^2 to about 300μg/m^2 every other day, about 1 μg/m^2 to about 200 μg/m^2, about 1 μg/m^2 to about 187.5 μg/m^2 every other day, about 1 μg/m^2 to about 175 μg/m^2 every other day, about 1 μg/m^2 per day to about 157 μg/m^2 every other day about 1 μg/m^2 to about 125 μg/m^2 every other day, about 1μg/m^2 to about 75 μg/m^2 every other day, 1 μg/m^2 to about 50μg/m^2 every other day, 2 μg/m^2 to about 50 μg/m^2 every other day, 2 μg/m^2 to about 30 μg/m^2 per day or 3 μg/m^2 to about 30 μg/m^2 per day. In additional

embodiments, dosages may be administered 3 times/week, 4 times/week, 5 times/week, only on weekdays, only in concert with other treatment regimens, on consecutive days, or in any appropriate dosage regimen depending on clinical and patient-specific factors.

[048] The amount, timing and mode of delivery of compositions of the invention comprising an (alternatively "stroke treating," "anti-clotting," "anticholesterolemic," "vasodilating," "antihypertensive," "arteriolar resistance lowering," " venous capacity increasing," "heart oxygen demand reducing," "heart rate decreasing," " heart rate stabilizing," "ERK phorsphorylation inducing," "IL-2 modulating" and/or "neuroprotective") effective amount of a phorbol ester compound of Formula I or derivative compound of a phorbol ester of Formula I, will be routinely adjusted on an individual basis, depending on such factors as weight, age, gender, and condition of the individual, the acuteness of the disease and/or related symptoms, whether the administration is prophylactic or therapeutic, and on the basis of other factors known to effect drug delivery, absorption, pharmacokinetics, including half-life, and efficacy.

[049] An effective dose or multi-dose treatment regimen for the instant disease treating (alternatively, "stroke treating," "anti-clotting," "anticholesterolemic," "vasodilating," "antihypertensive," "ERK phorsphorylation inducing," "arteriolar resistance lowering," " venous capacity increasing," "heart oxygen demand reducing," "heart rate decreasing," " heart rate stabilizing," "blood clot decreasing," "neuroprotective," "IL-2 modulating" or "NFκB modulating") formulations of the invention will ordinarily be selected to approximate a minimal dosing regimen that is necessary and sufficient to substantially prevent or alleviate the symptoms of stroke in the subject. A dosage and administration protocol will often include repeated dosing therapy over a course of several days or even one or more weeks or years. An effective treatment regime may also involve prophylactic dosage administered on a day or multi-dose per day basis lasting over the course of days, weeks, months or even years.

[050] Effectiveness of the compositions and methods of the invention in the treatment of stroke may be demonstrated using a variety of model systems including temporary middle cerebral artery occlusion as shown in Example 9, permanent middle cerebral artery occlusion as shown in Example 8, endovascular filament middle cerebral artery occlusion, embolic middle cerebral artery occlusion as shown in Example 7, endothelin-1

-induced constriction of arteries and veins, or cerebrocortical photothrombosis. Use of the phorbol ester compositions of the present invention will decrease the symptoms or long term effects exhibited by the model systems by 0%, 20%, 30%, 50% or more, up to a 75-90%, 96% or greater decrease over control animals.

[051] Effectiveness of the compositions and methods of the invention in the treatment of stroke may further be demonstrated by a decrease in the symptoms exhibited in individuals who have suffered a stroke. Such symptoms include, but are not limited to, paralysis, spatial impairment, impaired judgment, left-sided neglect, memory loss, aphasia, coordination and balance problems, nausea, vomiting, cognitive impairment, perception impairment, orientation impairment, homonymous hemianopsia and impulsivity. Use of the phorbol ester compositions of the present invention will decrease the symptoms exhibited by individuals by 0%, 20%, 30%, 50% or more, up to a 75-90%, 96% or greater decrease over initial states.

[052] Within additional aspects of the invention, combinatorial disease treating ("stroke treating," "anti-clotting," "anticholesterolemic," "vasodilating," "antihypertensive," "ERK phorsphorylation inducing," "arteriolar resistance lowering," " venous capacity increasing," "heart oxygen demand reducing," "heart rate decreasing," " heart rate stabilizing," or "NFκB modulating") formulations and coordinate administration methods are provided which employ an effective amount of a phorbol ester compound of Formula I or a derivative compound of Formula I and one or more secondary or adjunctive agent(s) that is/are combinatorially formulated or coordinately administered with the phorbol ester compound of Formula I to yield a combined, multi-active disease treating composition or coordinate treatment method.

[053] Exemplary combinatorial formulations and coordinate treatment methods in the prevention or treatment of stoke employ the phorbol ester compound of Formula I, or derivative compound of a phorbol ester of Formula I, in combination with one or more additional, neuroprotective or other indicated, secondary or adjunctive therapeutic agents that is/are useful for treatment or prophylaxis of the targeted disease, condition and/or symptom(s). For most combinatorial formulations and coordinate treatment methods of the invention, a phorbol ester compound of Formula I or related or derivative compound is formulated, or coordinately administered, in combination with one or more secondary

or adjunctive therapeutic agent(s), to yield a combined formulation or coordinate treatment method that is combinatorially effective or coordinately useful to prevent or treat stroke, or the effects of stroke. Exemplary combinatorial formulations and coordinate treatment methods in this context employ a phorbol ester compound of Formula I, or derivative compound of a phorbol ester of Formula I, in combination with one or more secondary or adjunctive therapeutic agents selected from tissue plasminogen activator, an anticoagulant, a statin, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitor, anti-platelet agent, fibrate, beta-blocker, calcium channel blocker, or diuretic. Exemplary anticoagulants include, but are not limited to, heparin, warfarin, heparinoids, phenindione, atomentin, acenocoumarol, phenprocoumon, idraparinux, fondaparinux, and thrombin inhibitors. Exemplary statins include, but are not limited to, lovastatin, amlodipine, atorvastatin, rosuvastatin, simvastatin, fluvastatin, pitavastatin, and pravastatin. Exemplary angiotensin II receptor blockers include, but are not limited to, candesartan, eprosartan, irbesartan, losartan, olmesartan, telmisartan, and valsartan. Angiotensin converting enzyme inhibitors include, but are not limited to, enazepril, captopril, enalapril, fosinopril, isinopril, moexipril, perindopril, quinapril, ramipril, and trandolapril. Exemplary beta-blockers include, but are not limited to, alprenolol, bucindolol, carteolol, carvedilol, labetalol, nadolol, oxprenolol, penbutolol, pindolol propranolol, sotalol, timolol, eucommia, acebutolol, atenolol, betaxolol, bisoprolol, celiprolol, esmolol, metoprolol, and nebivolol. Exemplary calcium channel blockers include, but are not limited to, amlodipine, clevidipine, diltiazem, felodipine, isradipine, nifedipine, nicardipine, nimodipine, nisoldipine, and verapamil. Exemplary diuretics include, but are not limited to, chlorothiazide, hydrochlorothiazide, bumetanide, ethacrynic acid, furosemide, amiloride, eplerenone, spironolactone and triamterene. Exemplary fibrates include, but are not limited to, benzafibrate, ciprofibrate, clofibrate, gemfibrozil or fenofibrate. Exemplary anti-platelet agents include, but are not limited to, clopidogrel and ticlopidin.

[054] Coordinate treatment methods may further employ surgical intervention including, but not limited to, the use of pacemakers, implantable defibrillators, coronary stents, prosthetic valves, coronary artery bypass, balloon angioplasty, valve repair and

replacement, carotid endarterectomy, angioplasty, stent placement, craniotomy, endovascular coil emobilization, patent foramen ovale closure and heart transplantation.

[055] In certain embodiments the invention provides combinatorial disease treating ("stroke treating," "anti-clotting," "anticholesterolemic," "vasodilating," "antihypertensive," "ERK phorsphorylation inducing," "arteriolar resistance lowering," " venous capacity increasing," "heart oxygen demand reducing," "heart rate decreasing," " heart rate stabilizing," or "NFκB modulating") formulations comprising a phorbol ester and one or more adjunctive agent(s) having disease treating activity. Within such combinatorial formulations, a phorbol ester of Formula I and the adjunctive agent(s) having disease treating activity will be present in a combined formulation in disease treating ("stroke treating," "anti-clotting," "anticholesterolemic," "vasodilating," "antihypertensive," "ERK phorsphorylation inducing," "arteriolar resistance lowering," " venous capacity increasing," "heart oxygen demand reducing," "heart rate decreasing," " heart rate stabilizing," or "NFκB modulating") effective amounts, alone or in combination. In exemplary embodiments, a phorbol ester compound of Formula I and a non-phorbol ester agent(s) will each be present in a disease treating/preventing amount (i.e., in singular dosage which will alone elicit a detectable alleviation of symptoms in the subject). Alternatively, the combinatorial formulation may comprise one or both the phorbol ester compound of Formula I and the non-phorbol ester agents in sub-therapeutic singular dosage amount(s), wherein the combinatorial formulation comprising both agents features a combined dosage of both agents that is collectively effective in eliciting a disease, condition, or symptom alleviating response. Thus, one or both of the phorbol ester of Formula I, or derivative compound of a phorbol ester of Formula I, and non-phorbol ester agents may be present in the formulation, or administered in a coordinate administration protocol, at a sub-therapeutic dose, but collectively in the formulation or method they elicit a detectable decrease in symptoms of disease, the occurrence or recurrence of stroke, or sequelae from a stroke in the subject. In yet another embodiment, the combinatorial formulation may include one or more neuroprotective agents. In a further embodiment, the combinatorial formulation may include one or more anti-inflammatory agents or other secondary or additional therapeutic agents as described herein.

[056] To practice coordinate administration methods of the invention, a phorbol ester compound of Formula I, or derivative compound of a phorbol ester of Formula I, may be administered, simultaneously or sequentially, in a coordinate treatment protocol with one or more of the secondary or adjunctive therapeutic agents contemplated herein. Thus, in certain embodiments a compound is administered coordinately with a non-phorbol ester agent, or any other secondary or adjunctive therapeutic agent contemplated herein, using separate formulations or a combinatorial formulation as described above (i.e., comprising both a phorbol ester compound of Formula I or related or derivative compound, and a non-phorbol ester therapeutic agent). This coordinate administration may be done simultaneously or sequentially in either order, and there may be a time period while only one or both (or all) active therapeutic agents individually and/or collectively exert their biological activities.

[057] In another embodiment, such coordinate treatment methods may, for example, follow or be derived from various protocols for the treatment of stroke. Coordinate treatment methods may, for example, include a phorbol ester and/or treatments for prevention or treatment of damage caused by a stroke. A distinguishing aspect of all such coordinate treatment methods is that the phorbol ester compound of Formula I or derivative compound of a phorbol ester of Formula I, exerts at least some activity, which yields a favorable clinical response in conjunction with a complementary stroke preventing or treating agent, or distinct, clinical response provided by the secondary or adjunctive therapeutic agent. Often, the coordinate administration of the phorbol ester compound of Formula I, or derivative compound of a phorbol ester of Formula I, with the secondary or adjunctive therapeutic agent will yield improved therapeutic or prophylactic results in the subject beyond a therapeutic effect elicited by the phorbol ester compound of Formula I or derivative compound of a phorbol ester of Formula I, or the secondary or adjunctive therapeutic agent administered alone. This qualification contemplates both direct effects as well as indirect effects.

[058] Within exemplary embodiments, a phorbol ester compound of Formula I, or derivative compound of a phorbol ester of Formula I, will be coordinately administered (simultaneously or sequentially, in combined or separate formulation(s)), with one or more secondary stroke treating compounds or other indicated or adjunctive therapeutic

agents, e.g. tissue plasminogen activator, an anticoagulant, a statin, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitor, fibrate, beta-blocker, calcium channel blocker, lipid-lowering medication, anti-platelet agent or diuretic. In addition, adjunctive or secondary therapies may be used in the treatment of stroke or the effects of stroke such as, but not limited to, pacemakers, implantable defibrillators, coronary stents, prosthetic valves, artificial hearts, coronary artery bypass, balloon angioplasty, valve repair and replacement, heart transplantation, carotid endarterectomy, angioplasty, stent placement, craniotomy, endovascular coil emobilization, or patent foramen ovale closure.

[059] As noted above, in all of the various embodiments of the invention contemplated herein, the disease treating methods and formulations may employ a phorbol ester compound of Formula I in any of a variety of forms, including any one or combination of the subject compound's pharmaceutically acceptable salts, solvates, isomers, enantiomers, polymorphs, solvates, hydrates, and/or prodrugs. In exemplary embodiments of the invention, TPA is employed within the therapeutic formulations and methods for illustrative purposes.

[060] The pharmaceutical compositions of the present invention may be administered by any means that achieve their intended therapeutic or prophylactic purpose. Suitable routes of administration for the compositions of the invention include, but are not limited to, conventional delivery routes, devices and methods including injectable methods such as, but not limited to, intravenous, intramuscular, intraperitoneal, intraspinal, intrathecal, intracerebroventricular, intraarterial, subcutaneous and intranasal routes.

[061] The compositions of the present invention may further include a pharmaceutically acceptable carrier appropriate for the particular mode of administration being employed. Dosage forms of the compositions of the present invention include excipients recognized in the art of pharmaceutical compounding as being suitable for the preparation of dosage units as discussed above. Such excipients include, without intended limitation, binders, fillers, lubricants, emulsifiers, suspending agents, sweeteners, flavorings, preservatives, buffers, wetting agents, disintegrants, effervescent agents and other conventional excipients and additives.

[062] If desired, the compositions of the invention can be administered in a controlled release form by use of a slow release carrier, such as a hydrophilic, slow release polymer.

Exemplary controlled release agents in this context include, but are not limited to, hydroxypropyl methyl cellulose, having a viscosity in the range of about 100 cps to about 100,000 cps or other biocompatible matrices such as cholesterol.

[063] Some phorbol ester compositions of Formula I of the invention are designed for parenteral administration, e.g. to be administered intravenously, intramuscularly, subcutaneously or intraperitoneally, including aqueous and non-aqueous sterile injectable solutions which, like many other contemplated compositions of the invention, may optionally contain anti-oxidants, buffers, bacteriostats and/or solutes which render the formulation isotonic with the blood of the mammalian subject; and aqueous and non-aqueous sterile suspensions which may include suspending agents and/or thickening agents. The formulations may be presented in unit-dose or multi-dose containers. Additional compositions and formulations of the invention may include polymers for extended release following parenteral administration. The parenteral preparations may be solutions, dispersions or emulsions suitable for such administration. The subject agents may also be formulated into polymers for extended release following parenteral administration. Pharmaceutically acceptable formulations and ingredients will typically be sterile or readily sterilizable, biologically inert, and easily administered. Such polymeric materials are well known to those of ordinary skill in the pharmaceutical compounding arts. Parenteral preparations typically contain buffering agents and preservatives, and injectable fluids that are pharmaceutically and physiologically acceptable such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like. Extemporaneous injection solutions, emulsions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described. Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as described herein above, or an appropriate fraction thereof, of the active ingredient(s).

[064] In more detailed embodiments, compositions of the invention may comprise a phorbol ester compound of Formula I or derivative compound of a phorbol ester of Formula I encapsulated for delivery in microcapsules, microparticles, or microspheres, prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly(methylmethacylate)

microcapsules, respectively; in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules); or within macroemulsions.

[065] As noted above, in certain embodiments the methods and compositions of the invention may employ pharmaceutically acceptable salts, e.g., acid addition or base salts of the above-described phorbol ester compounds of Formula I and/or related or derivative compounds. Examples of pharmaceutically acceptable addition salts include inorganic and organic acid addition salts. Suitable acid addition salts are formed from acids which form non-toxic salts, for example, hydrochloride, hydrobromide, hydroiodide, sulphate, hydrogen sulphate, nitrate, phosphate, and hydrogen phosphate salts. Additional pharmaceutically acceptable salts include, but are not limited to, metal salts such as sodium salts, potassium salts, cesium salts and the like; alkaline earth metals such as calcium salts, magnesium salts and the like; organic amine salts such as triethylamine salts, pyridine salts, picoline salts, ethanolamine salts, triethanolamine salts, dicyclohexylamine salts, N,N'-dibenzylethylenediamine salts and the like; organic acid salts such as acetate, citrate, lactate, succinate, tartrate, maleate, fumarate, mandelate, acetate, dichloroacetate, trifluoroacetate, oxalate, and formate salts; sulfonates such as methanesulfonate, benzenesulfonate, and p-toluenesulfonate salts; and amino acid salts such as arginate, asparginate, glutamate, tartrate, and gluconate salts. Suitable base salts are formed from bases that form non-toxic salts, for example aluminum, calcium, lithium, magnesium, potassium, sodium, zinc and diethanolamine salts.

[066] Other detailed embodiments, the methods and compositions of the invention for employ prodrugs of phorbol esters of Formula I. Prodrugs are considered to be any covalently bonded carriers which release the active parent drug *in vivo*. Examples of prodrugs useful within the invention include esters or amides with hydroxyalkyl or aminoalkyl as a substituent, and these may be prepared by reacting such compounds as described above with anhydrides such as succinic anhydride.

[067] The invention disclosed herein will also be understood to encompass methods and compositions comprising phorbol esters of Formula I using *in vivo* metabolic products of the said compounds (either generated *in vivo* after administration of the subject precursor compound, or directly administered in the form of the metabolic product itself). Such

products may result for example from the oxidation, reduction, hydrolysis, amidation, esterification and the like of the administered compound, primarily due to enzymatic processes. Accordingly, the invention includes methods and compositions of the invention employing compounds produced by a process comprising contacting a phorbol ester compound of Formula I with a mammalian subject for a period of time sufficient to yield a metabolic product thereof. Such products typically are identified by preparing a radiolabelled compound of the invention, administering it parenterally in a detectable dose to an animal such as rat, mouse, guinea pig, monkey, or to man, allowing sufficient time for metabolism to occur and isolating its conversion products from the urine, blood or other biological samples.

[068] The invention disclosed herein will also be understood to encompass diagnostic compositions for diagnosing the risk level, presence, severity, or treatment indicia of, or otherwise managing diseases including, but not limited to, stroke, in a mammalian subject, comprising contacting a labeled (e.g., isotopically labeled, fluorescent labeled or otherwise labeled to permit detection of the labeled compound using conventional methods) phorbol ester compound of Formula I to a mammalian subject (e.g., to a cell, tissue, organ, or individual) at risk or presenting with one or more symptom(s) of stroke, and thereafter detecting the presence, location, metabolism, and/or binding state of the labeled compound using any of a broad array of known assays and labeling/detection methods. In exemplary embodiments, a phorbol ester compound of Formula I is isotopically-labeled by having one or more atoms replaced by an atom having a different atomic mass or mass number. Examples of isotopes that can be incorporated into the disclosed compounds include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, and ^{36}Cl, respectively. The isotopically-labeled compound is then administered to an individual or other subject and subsequently detected as described above, yielding useful diagnostic and/or therapeutic management data, according to conventional techniques.

Examples

[069] The experiments described below demonstrate novel and powerful uses for phorbol esters and derivative compounds as stroke treating and preventing agents. These

and additional findings are further expanded and elucidated within the following examples.

Example I

Effect of TPA on the Peripheral White Blood Cells (WBC) And Hemoglobin (Hb) Counts In S180 Cell-Injected Mice:

[070] Sarcoma 180 (S180) cells were injected into Kwen-Ming mice. On the third day, the mice were given TPA interperitoneally (i.p.). at 50, 100 or 200 μg/kg/day for 7 days. On the second day after the treatment was completed, blood samples were taken from the tails of the treated mice for WBC and Hb analyses. The WBC counts for the treated groups (50, 100, or 200 ug/kg/day for 7 days) were 16.1±7.4, 18.7±.3.0 and 20.7±.3.4 $x10^9$ /L, respectively; the WBC count for the control group was 13.6±1.8$x10^9$ /L. The Hb of the treated groups were 136±11, 149±12 and 149±10 g/L, and the Hb of the control group was 134+-15 g/L. The results indicate that i.p. injection of TPA could increase the peripheral WBC counts in mice in a dose-dependent manner, whereas the Hb levels were not greatly affected in TPA treated mice when compared to the control mice.

Example II

Dose Ranging Study.

[071] Due to the strong local irritation caused by TPA application, TPA was given to patients by intravenous (i.v.) infusion. TPA solution in a sterile syringe was injected into 200 ml of sterile saline and mixed well for i.v. infusion.

[072] The Toxicity and Side Effects of Different TPA Doses Administered Clinically:

[073] (1) TPA given at 1 mg/patient/week:

[074] One mg TPA in solution was mixed well with 200 ml of sterile saline for intravenous infusion which was completed in 1 h at the rate of 16 μg/min. One hour after TPA administration, patients started to have chills which lasted for about 30 min, followed by fever, (the patients' temperature reached 37.5-39.5°C. which lasted for 3-5 h, then returned to normal) with light to heavy perspiration. The above symptoms could be alleviated by giving the patients glucocorticoids. TPA at this dose caused a minority of patients to bleed, several patients suffered for a short period of time difficulty in

breathing, and Hb was detected in the urine. However, these side effects were short lived and reversible. The cardiac, hepatic, renal and pulmonary functions were all found to be normal.

[075] (2) TPA given at 0.5 mg/patient x 2/week: (two doses a week)

[076] 0.5 mg of TPA in solution was mixed well with 200 ml of saline for intravenous infusion which was completed in 1 h at the rate of 8 μg/min. The reactions after administration were similar to that of the 1 mg TPA dosage, but to a lesser extent than the 1 mg dose. The patients tolerated the lower dose more easily. Occasionally, Hb was detected in patients' urine. Difficulty in breathing was not observed. The cardiac, hepatic, renal and pulmonary functions were all normal.

[077] (3) TPA given at 0.25 mg/patient x 4/week:

[078] 0.25 mg of TPA in solution was mixed well with 200 ml of saline for intravenous infusion which was completed in 1 h at the rate of 4 μg/min. After administration, symptoms such as chills and fever were also observed, but to a much lesser extent than with the higher dosages. No Hb was detected in the urine, and no patient suffered difficulty in breathing. The cardiac, hepatic, renal and pulmonary functions were all normal.

Example III

Treatment of Relapsed/Refractory Malignancies with TPA

[079] Patients with histologically documented relapsed/refractory hematologic malignancy/bone marrow disorders are treated with a combination of TPA (Xichuan Pharmaceuticals, Nan Yang, Henan, China), dexamethasone and choline magnesium trisalicylate. Comparable methods as set forth below for demonstrating the therapeutic use of TPA in the treatment of Acute Myelogenous Leukemia (AML) will be applied to demonstrate the use of TPA for treating other neoplastic conditions and malignancies. In addition to the specific protocols herein, successful treatment and/or remission will be determined for different targeted neoplastic and malignant conditions using any of a wide variety of well known cancer detection and assessment methods—for example by determining size reduction of solid tumors, histopathological studies to evaluate tumor growth, stage, metastatic potential, presence/expression levels of histological cancer markers, etc.

[080] AML is an aggressive disease that generally warrants urgent and intensive therapy. The average patient age at AML diagnosis is 64-68 years old, and patients over the age of 60 treated with standard chemotherapy are cured of their disease < 20% of the time. Patients who develop AML after an antecedent hematologic disorder or prior leukemogenic chemotherapy/radiation therapy have similarly poor outcomes, as do patients whose disease is associated with specific adverse cytogenetic and clinical features. Hence, most patients diagnosed with AML have patient and/or disease-related features that are associated with a very poor prognosis. For patients with relapsed disease, no standard non-transplant therapy has demonstrated the capacity for cure. For these patients, AML is often a fatal disease. New approaches to the therapy of AML are needed.

[081] Employing the methods and compositions of the instant invention, TPA, is developed as a therapeutic agent for treating patients with AML, based on TPA's novel role in modulating intracellular signaling pathways, it's capacity to induce differentiation and/or apoptosis in cell lines, and clinical data indicating the effectiveness of TPA in treating neoplastic and malignant disorders, including myeloid malignancies.

[082] Thus far clinical evaluation of TPA has demonstrated that TPA exerts direct therapeutic cytotoxic effects in at least a subset of AML cases, as measured by cell viability and apoptosis assays. In all primary cultures analyzed by Western analysis, TPA strongly induced ERK phosphorylation by 1 hour in culture. TPA's cytotoxic effect on primary AML cells is associated with the subsequent loss of the phospho-ERK pro-survival signal after 24 hour *ex vivo* exposure. This observation is in good agreement with other studies that reported decreased primary AML survival after pharmacological interruption of ERK signaling by MEK inhibitors, such as PD98059, U0126 and PD 184352. In our studies, loss of ERK signaling was associated with induction of ERK phosphatases.

[083] In addition to protein kinase C and ERK activation, TPA is a known inducer of NF-κB, a pro-survival transcription factor often constitutively active in AML blasts and leukemic stem cells. Recent work from our laboratory has demonstrated that AML cell NF-κB can be inhibited *in vivo* with 48h of treatment with dexamethasone + choline magnesium trisalicylate (CMT). In addition, we have shown that dexamethasone can

induce MKP-1 ERK phosphatase expression and enhance TPA cytotoxicity on primary AML samples. In this context, we have chosen in exemplary embodiments below to use dexamethasone and CMT as adjunctive medications to be used 24h pre- and 24h post treatment with TPA. These medications are well-tolerated and anticipated to reduce inflammatory adverse effects of treatment and enhance TPA cytotoxicity by increasing ERK phosphatase expression and inhibiting NF-κB. In addition dexamethasone and CMT will be used as adjunctive medications because they are anti-inflammatory, may ameliorate adverse effects, and may enhance anti-leukemic activity by inhibition of the anti-apoptotic effects of constitutive NF-κB expression and induction of phosphatases that decrease signaling pathway activity.

[084] An initial TPA Phase 1 study enrolled 35 patients [23 with relapsed/refractory AML, 2 with other myeloid malignancies (CML-blast crisis, myelodysplasia with excess blasts), 3 with Hodgkin's Disease, 3 with non-Hodgkin's lymphoma and 4 with solid tumors]. The majority of patients had relapsed/refractory AML. Our clinical results include one AML patient with stable disease for > 5 months, who received 8 TPA infusions. In a second AML patient, a pronounced (5-fold) decline in the number of circulating blasts was seen following TPA administration. This decline in leukemic blasts persisted for 4 weeks, and the patient eventually died from a fungal infection. Finally, a patient with relapsed and refractory Hodgkin's disease despite high dose chemotherapy with autologous stem cell rescue had a partial remission of a chest wall mass after TPA administration. TPA dose escalation has been completed, in the last cohort 2 out of 3 patients treated at a dose of 0.188mg/m2 d1-5, 8-12 experienced grade III non-hematologic dose limiting toxicities (DLT), establishing the maximum tolerated TPA dose as a single agent at 0.125mg/m2/d on d1-5 and 8-12.

[085] In the case of AML and other hematologic malignancies, patients are given an initial dose of TPA of 1 mg/week x 3 weeks (days 1, 8, 15) administered with continuous/intermittent pulse oximetry for 6 hours. Twenty four hours prior to initiation of TPA therapy, patients are given 10mg of dexamethasone every six hours and 1500mg of choline magnesium trisalicylate (CMT) every eight hours continuing until 24 hours after administration of TPA. After administration of the initial dose of TPA, patients have a two week rest period after which they may be reevaluated. Those patients that

have a disease response or stabilization from the initial dose of TPA are treated for up to six cycles of twenty-eight days according to the protocol below.

[086] Following the two week rest period, patients are pre-medicated with Tylenol 650 mg and Benadryl 25-50 mg (depending on the patient's size and age) thirty minutes prior to administration of TPA. They are then given an intravenous infusion of TPA through a central venous catheter daily for 5 days a week for two consecutive weeks followed by a 2-week rest period. TPA is administered at a dose of 1 mg in 200 ml of normal saline over 1 hour. Twenty four hours prior to initiation of TPA therapy, patients are given 10 mg of dexamethasone every six hours and 1500 mg of choline magnesium trisalicylate continuing every eight hours until 24 hours after administration of the TPA.

[087] Blood levels of TPA are measured prior to and after infusion using a bioassay that measures organic solvent extractable differentiation activity. 1ml of blood is extracted twice with 5 ml of ethyl acetate, redissolving the extraction residue in 50 μL of ethanol and addition of an aliquot of HL60 cells. After 48 hours, adherent cells are measured.

[088] Tests are also run on blood samples taken prior to and after infusion with TPA to determine levels of white blood cells, platelets, and neutrophils. The samples are additionally analyzed for the presence of myeloblasts and Auer rods. These and continuing experiments will further elucidate the therapeutic cytotoxic and other effects that TPA elicits against neoplastic cells in AML and other neoplastic and malignant conditions.

Example IV

Measurement of the Modulation of ERK Activation

[089] Phospho-ERK levels are measured in circulating malignant cells in patients with leukemia and in peripheral blood mononuclear cells in lymphoma/solid tumor patients. A blood sample is taken from patients treated according to the protocol of Example III both prior to and after administration of TPA.

[090] In leukemia patients with a WBC$\geq$ 1000 per μL, flow cytometry is performed on a blood sample using cell surface antigen-specific and phospho-ERK specific antibodies directly conjugated to flurophores (BD Biosciences, San Jose, CA). Samples are taken pre-administration of TPA and one hour after infusion of TPA on days 1, 2, and 11 in the initial treatment according to the protocol of Example III and days 1 and 11 in subsequent

cycles. In leukemia patients with an absolute leukemic blast number ≥2500 per μL and other non-leukemic patients, peripheral blood samples are taken on days 1, 8 and 15 of the initial cycle according to the protocol of Example III prior to and 1 and 4 hours post infusion. Samples are also analyzed using Western blot analysis for phosphor-ERK, and total ERK1/2 levels to confirm the results obtained from the flow cytometry and correlated to clinical responses.

[091] The foregoing analyses will further elucidate TPA's role in treatment of neoplastic and malignant conditions, including TPA's cytotoxic effect on malignant cells, exemplified by primary AML cells, and the associated reduction by TPA of the phosphor-ERK pro-survival signal.

Example V

Measurement of NF-κB Modulation

[092] In prior studies we have shown that NF-κB activity can be modulated in patients following administration of TPA with dexamethasone. Additionally, dexamethasone has been shown to induce MKP-1 ERK phosphatase expression and enhance TPA cytotoxicity. The following studies are designed to further elucidate how NF-κB activity is therapeutically modulated in patients treated with TPA plus dexamethasone.

[093] NF-κB binding is measured in patient peripheral blood samples at baseline and pre and post infusion from patients treated with TPA according to Example III using ELISA-based assays (BD Bioscience, San Jose, USA). NF-κB levels are quantified using chemiluminescent intensity to detect binging in limiting amounts of cellular extract using a 96-well format. Additionally, electrophoretic mobility shift assays are performed to measure NF- κB binding in peripheral blood samples from leukemia patient with an absolute leukemic blast number ≥2500 per μL and other non-leukemic patients with normal white blood cell counts.

[094] The foregoing studies will further show that TPA is an inducer of NF-κB; however these experiments demonstrate that AML cell NF-κB can be inhibited with treatment with dexamethasone and choline magnesium trisalicylate.

Example VI

Treatment of Individuals Who Have Suffered a Stroke.

[095] Patient N.C., male, 68, suffered a stroke eighteen months prior to treatment with TPA. At the time TPA treatment was initiated, he was unable to walk without a cane, had difficulty with both his left hand and left leg and was tired and weak. He received injections of 1 ampoule containing 0.19mg of TPA (0.125mg/m^2) every other day for four weeks, then 0.24 mg of TPA (1.25 x 0.125mg/m^2) every other day for 2 weeks, and then 0.26 mg of TPA (1.5 x 0.125mg/m^2) every other day for an additional 3 weeks. The patient has recovered fully.

[096] Patient M.C., male, age 65, suffered a stroke seven years prior to beginning treatment with TPA. He received 3-4 injections of 0.19 mg of TPA (0.125mg/m^2) per week for ten weeks for a total of 35 injections. He has regained mobility in his face and had an 80% improvement in the mobility of his right side.

Example VII

Treatment of Embolic Stroke Model with TPA

[097] Male Sprague-Dawley rats (Charles River, Japan) each having a body weight of 280-350 g are used. An embolic stroke is induced following a modification of the method of Kudo, et al. (1982) The rats to be used for the collection of blood are anesthetized with 1.0% halothane (Fluorothane™; Takeda, Osaka, Japan) under spontaneous respiration. A 24-gauge Surflo™ (Terumo Medical Products, Elkton, MD) is secured in the femoral artery and 0.1 mL of arterial blood is taken with a 1-mL syringe for injection (Terumo Medical Products, Elkton, MD). The artery blood in the syringe is incubated at 30°C. for 2 days to form a blood clot. After that, 0.1 mL of physiological saline is added to the syringe for injection and passed through a 26-gauge injection needle (Terumo Medical Products, Elkton, MD) twice so that the blood clot is crushed.

[098] Rats in which a cerebral embolic stroke is induced are anesthetized with 1.0% halothane under spontaneous respiration. The neck of the rats is subjected to a midline incision and external carotid artery, superior thyroid artery, occipital artery and pterygopalatine artery are cauterized with a bipolar coagulator (T-45; Keisei Medical

Industrial Co. Ltd, Tokyo, Japan). Cerebral embolism is induced by injecting 0.1 mL of the crushed blood clot into the internal carotid.

[099] Evaluation of the formation of a cerebral embolism is carried out using a laser Doppler flowmetry (FloC1; Omegawave, Tokyo, Japan). A decrease in cerebral blood flow to a level of 30% or less is taken as a positive evidence of embolism formation. The cerebral blood flow is monitored for 30 minutes after infusion of the blood clot and blood flow is monitored as remaining at 50% or less of the flow prior to the injection of the blood clot. After that, a cannula (PE50) for administration of the medicament is secured in the jugular vein and the animals are woken.

[0100] The rats that have successfully formed a cerebral embolism are divided into four groups. The first group of rats is given a saline injection every other day. Groups 2-4 are given 0.125mg/m^2 injection of TPA every other day for four weeks. Group 2 is then sacrificed. Groups 3-4 are given a further 0.156 mg/m^2 of TPA every other day for two weeks and then Group 3 is sacrificed. Group 4 is given 0.18775 mg/m^2 of TPA every other day for three weeks and then sacrificed.

[0101] The brains are excised after the animals are sacrificed and sliced in ten sections at 1 mm intervals using a McIwain tissue chopper (Mickle Laboratory Engineering, U.K.) and are stained by dipping for 20 minutes in a 2% TTC (2,3,5-triphenyltetrazolium chloride; Tokyo Kasei) at 37°C. Images of the TTC-stained slices are uploaded into a computer using a digital camera (HC-2500; Fuji PhotoFilm) and Phatograb-2500 (Fuji Photo Film) and infarct volume is calculated using Mac Scope (Mitani, Japan). Infarct volume is given by a mean value ± standard error. With regard to the statistical test of the result of the infarct volume, the evaluation is done by carrying out a Dunnett's test for control group and for each of the TPA -administered groups as compared with the control group and then by carrying out the t-test for the TPA-administered group.

[0102] Neurological symptoms are observed daily until sacrifice and the rats are evaluated according to three tests: (1) Rats are held gently by the tail, suspended one meter above the floor, and observed for forelimb flexion; (2) Rats are placed on a large sheet of soft, plastic coated paper that could be gripped firmly by their claws. With the tail held by hand, gentle lateral pressure is applied behind the rat's shoulder until the forelimbs slid several inches; (3) Rats are allowed to move about freely and are observed

for circling behavior. Scoring of the neurological symptoms is carried out according to the scale developed by Bederson et al. (1986) as follows: 0: no observable deficit; 1: forelimb flexion; 2: decreased resistance to lateral push without circling; 3: same behavior as grade 2, with circling.

[0103] Neurological symptoms are evaluated using a Steel's test for the control group and for each of the TPA administered groups as compared with the control group and then by carrying out a Wilcoxon test for the TPA administered group. In any of the tests, the value where $p<0.05$ is defined to be statistically significant.

Example VIII

Effectiveness of TPA in the Treatment of Stroke Using

A Permanent Middle Cerebral Artery Occlusion Model

[0104] Male Wistar rats (250-320 g) are used for this study. Animals are anesthetized with Isoflurane (3% induction, 1-2% maintenance). Anesthesia is monitored by toe pinch. Aseptic technique is used for all procedures during this study. The surgical site is clipped and cleaned with alcohol and surgical scrub. The animal is placed on a warm water heating pad to maintain body temperature. A paramedian incision is made on the neck over the carotid artery. The tissue is bluntly dissected away to reveal the carotid artery and the bifurcation. Sutures are placed around the proximal portion or the common carotid and the external carotid arteries. These sutures are tied off. An incision is made in the common carotid, distal to the ligation. A pre-prepared filament (4-0 monofilament suture or like material) is placed in the carotid and advanced into the internal carotid artery. The filament is advanced about 20 mm past the carotid bifurcation until slight resistance is felt as it wedges in the middle cerebral artery. Care must be taken to not rupture the artery upon insertion of the filament. The filament is tied in place and the skin incision closed. The animal is evaluated when awake for successful occlusion using the Bederson scale. (See Bederson et al., (1986) Stroke, 17:1304-1308.) Body temperature is taken every 15 minutes to maintain normothermia. Animals that have undergone the middle cerebral artery occlusion procedure may have difficulty in thermoregulation for a few hours after surgery Animals are placed in a cooling or heating box as determined by their temperature. Body temperature is maintained at 37.5° C. Animals are

monitored for 6 hours following middle cerebral artery and are then placed in cages overnight.

[0105] The rats are divided into four groups. The first group of rats is given saline injections every other day. Groups 2-4 are given 0.125mg/m^2 injection of TPA every other day for four weeks. Group 2 is then sacrificed. Groups 3-4 are given a further 0.156mg/m^2 of TPA every other day for two weeks and then Group 3 is sacrificed. Group 4 is given 0.18775mg/m^2 of TPA every other day for three weeks and then sacrificed.

[0106] The brains are excised after the animals are sacrificed and sliced in ten sections at 1 mm intervals using a McIwain tissue chopper (Mickle Laboratory Engineering, U.K.) and are stained by dipping for 20 minutes in a 2% TTC (2,3,5-triphenyltetrazolium chloride; Tokyo Kasei) at 37◦C. Images of the TTC-stained slices are uploaded into a computer using a digital camera (HC-2500; Fuji PhotoFilm) and Phatograb-2500 (Fuji Photo Film). Brain slices are photographed and analyzed for infarct size, infarct volume, penumbra, and edema.

[0107] Neurological symptoms are observed daily until sacrifice. Neurological symptoms are observed daily until sacrifice and the rats are evaluated according to three tests. (1) Rats are held gently by the tail, suspended one meter above the floor, and observed for forelimb flexion. (2) Rats are placed on a large sheet of soft, plastic coated paper that could be gripped firmly by their claws. With the tail held by hand, gentle lateral pressure is applied behind the rat's shoulder until the forelimbs slid several inches. (3) Rats are allowed to move about freely and are observed for circling behavior. Scoring of the neurological symptoms is carried out according to the scale developed by Bederson et al. (1986) as follows: 0: no observable deficit; 1: forelimb flexion; 2: decreased resistance to lateral push without circling; 3: same behavior as grade 2, with circling.

[0108] Neurological symptoms are evaluated using a Steel's test for the control group and for each of the TPA administered groups as compared with the control group and then by carrying out a Wilcoxon test for the TPA administered group. In any of the tests, the value where $p<0.05$ is defined to be statistically significant.

Example IX

Effectiveness of TPA in the Treatment of Stroke Using A Temporary Middle Cerebral Artery Occlusion Model

[0109] Male C57B16 mice (25-30 g) are used in this study. Mice are anesthetized with Isoflurane (3% induction, 1-2% maintenance). The surgical site is clipped and cleaned with alcohol and surgical scrub. A midline neck incision is made over the carotid artery and the artery is dissected to its bifurcation. A monofilament suture is introduced into the internal carotid artery and advanced until it lodges in the middle cerebral artery. The suture is tied in placed and the incision is closed. Two hours after occlusion the mice will be re-anesthetized and the suture will be removed from the MCA. Body temperature is maintained by use of a heating pad both during and after surgery. Animals are monitored for 4 hours following middle cerebral artery occlusion.

[0110] The rats are divided into four groups. The first group of rats is given saline injections every other day. Groups 2-4 are given 0.125mg/m^2 injection of TPA every other day for four weeks. Group 2 is then sacrificed. Groups 3-4 are given a further 0.156mg/m^2 of TPA every other day for two weeks and then Group 3 is sacrificed. Group 4 is given 0.18775mg/m^2 of TPA every other day for three weeks and then sacrificed.

[0111] The brains are excised after the animals are sacrificed and sliced in ten sections at 1 mm intervals using a McIwain tissue chopper (Mickle Laboratory Engineering, U.K.) and are stained by dipping for 20 minutes in a 2% TTC (2,3,5-triphenyltetrazolium chloride; Tokyo Kasei) at 37◦C. Images of the TTC-stained slices are uploaded into a computer using a digital camera (HC-2500; Fuji PhotoFilm) and Phatograb-2500 (Fuji Photo Film). Brain slices are photographed and analyzed for infarct size, infarct volume, penumbra, and edema.

[0112] Neurological symptoms are observed daily until sacrifice and the rats are evaluated according to three tests. (1) Rats are held gently by the tail, suspended one meter above the floor, and observed for forelimb flexion. (2) Rats are placed on a large sheet of soft, plastic coated paper that could be gripped firmly by their claws. With the tail held by hand, gentle lateral pressure is applied behind the rat's shoulder until the forelimbs slid several inches. (3) Rats are allowed to move about freely and are observed

for circling behavior. Scoring of the neurological symptoms is carried out according to the scale developed by Bederson et al. (1986) as follows: 0: no observable deficit; 1: forelimb flexion; 2: decreased resistance to lateral push without circling; 3: same behavior as grade 2, with circling.

[0113] Neurological symptoms are evaluated using a Steel's test for the control group and for each of the TPA administered groups as compared with the control group and then by carrying out a Wilcoxon test for the TPA administered group. In any of the tests, the value where $p<0.05$ is defined to be statistically significant.

Example X

Clinical Effectiveness of the Use of TPA to Treat Stroke

[0114] Males and Females between the ages of 30-72 years who suffered a stroke less than one month previously are recruited for participation in a ten week trial of TPA.

[0115] Recruited individuals sign an informed consent form and are evaluated using computed tomography (CT), physical and neurological tests, neurological check, sedation level, National Institute of Health Stroke Survey (NIHSS), 12-lead electrocardiogram, telemetry of electrocardiogram, pulse oxygen measurement, vital sign, body weight, background of the patient, test on pregnancy, measurement of medicament in urine, hematological test, coagulation panel, general clinical test, urine test. Clinical Laboratory Testing includes a Complete Metabolic Panel (Na, K, Cl, CO2, Glu, BUN, Cr, Ca, TP, Alb, TBili, AP, AST, ALT), Hematology CBC (Hgb, Hct, RBC, WBC, Plt, Diff), and Serum hCG for all females.

[0116] Individuals are administered $0.125mg/m^2$ of TPA or placebo every other day for four weeks, then $1.25 \times 0.125mg/m^2$ or placebo every other day for weeks five and six and $1.5 \times 0.125mg/m^2$ or placebo every other day for weeks seven to nine. Individuals are monitored during and for two hours after administration of TPA or placebo.

[0117] At weeks five and week ten, subjects are evaluated using the NIHSS (NIH Stroke Scale), the Barthel ADL index (Granger, 1979), and a modified Rankin scale (Farrell, 1991),

[0118] Efficacy is determined by measuring the change from baseline in the NIHSS in individuals treated with TPA in comparison to placebo. Secondary efficacy variables are

the Barthel ADL index and a modified Rankin scale. Safety measures are collected and evaluated through the trial, specifically measuring changes from baseline visit to week 5. These measures include adverse event reports, physical examinations, vital signs, weight measurements, ECGs, clinical laboratory test results, and vital signs as well as scores for suicidal behaviors and/or ideation. Adverse events are any untoward medical event occurring in a subject administered study drug, irrespective of whether it has a causal relationship to the study drug. An adverse event can therefore be any unfavorable or unintended sign (including abnormal laboratory findings, for example), symptom, or disorder temporarily associated with study drug, whether or not considered related to the study drug.

[0119] Subjects are considered to have completed the study if they complete all of the visits. They may be terminated from the study if they fail to meet inclusion/exclusion criteria; suffer from an adverse event, have an insufficient therapeutic response, withdraw their consent, violate the protocol, stop coming, or die.

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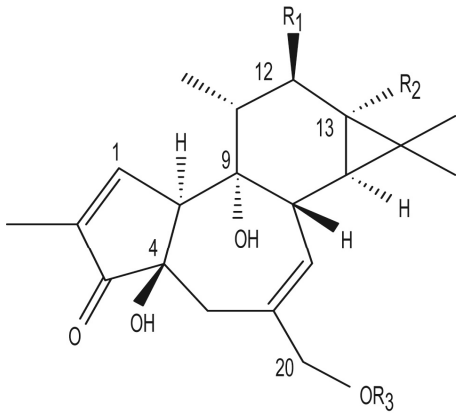
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CLAIMS

1. Use of a phorbol ester of Formula I, or a pharmaceutically-acceptable salt, isomer, or enantiomer, thereof



FORMULA I

wherein R_1 and R_2 are selected from the group consisting of hydrogen, hydroxyl, —O—C(=O)-alkyl, —O—C(=O)-lower alkenyl, —O—C(=O)-phenyl, —O—C(=O)-benzyl, and substituted derivatives thereof; and R_3 is selected from hydrogen, —C(=O)-lower alkyl and substituted derivatives thereof, in the manufacture of a medicament for preventing or treating one or more of the effects or sequelae of stroke in a mammalian subject, wherein said medicament comprises between about 10 µg to about 1500 µg of said phorbol ester of Formula I as maximum daily dose.

2. The use of claim 1, wherein R_1 or R_2 is —O—C(=O)—C_1— C_{15} alkyl the remaining R_1 or R_2 is —O—C(=O)-lower alkyl and R_3 is hydrogen.

3. The use of claim 1, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate,

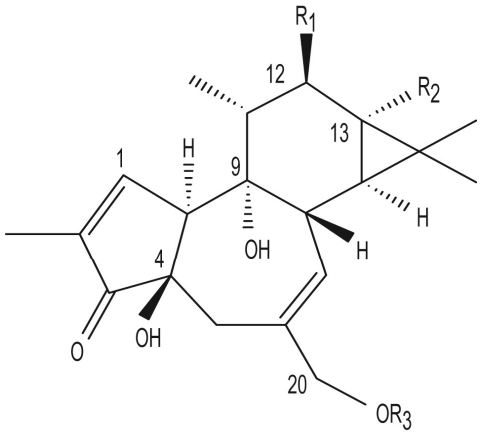
phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

4. The use of claim 1, further comprising use of at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation or coordinate treatment regimen with said phorbol ester of Formula I in the manufacture of the medicament to treat or prevent effects of stroke in said subject.

5. The use of claim 4, wherein the at least one secondary or adjunctive therapeutic agent is tissue plasminogen activator, an anticoagulant, a statin, fibrate, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitor, beta-blocker, anti-platelet agent, calcium channel blocker, or diuretic.

6. The use of claim 1, wherein the one or more effects of stroke are paralysis, spatial impairment, impaired judgment, left-sided neglect, memory loss, aphasia, coordination and balance problems, nausea, vomiting, cognitive impairment, perception impairment, orientation impairment, homonymous hemianopsia, impaired mobility, or impulsivity.

7. Use of a phorbol ester of Formula I, or a pharmaceutically-acceptable salt, isomer, or enantiomer, thereof



FORMULA I

wherein R_1 and R_2 are selected from the group consisting of hydrogen, hydroxyl, —O—C(=O)-alkyl, —O—C(=O)-lower alkenyl, —O—C(=O)-phenyl, —O—C(=O)-benzyl, and substituted derivatives thereof; and R_3 is selected from hydrogen, —C(=O)-lower alkyl and substituted derivatives thereof, in the manufacture of a medicament for preventing a stroke in a mammalian subject at risk for a stroke, wherein said medicament comprises between about 10 µg to about 1500 µg of said phorbol ester of Formula I as maximum daily dose.

8. The use of claim 7, wherein R_1 or R_2 is —O—C(=O)—C_1— C_{15} alkyl the remaining R_1 or R_2 is —O—C(=O)-lower alkyl and R_3 is hydrogen.

9. The use of claim 7, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-

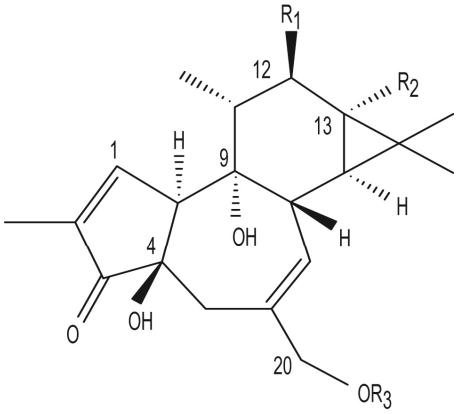
deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

10. The use of claim 7, further comprising use of at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation or coordinate treatment regimen with said phorbol ester of Formula I in the manufacture of the medicament to treat or prevent effects of stroke in said subject.

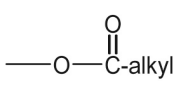
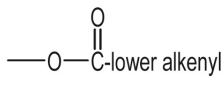
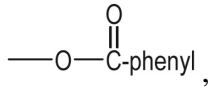
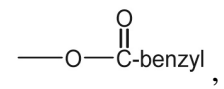
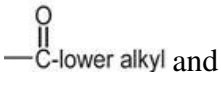
11. The use of claim 10, wherein the at least one secondary or adjunctive therapeutic agent is an anticoagulant, a statin, fibrate, angiotensin II receptor blockers, anti-platelet agent, angiotensin-converting enzyme inhibitor, beta-blocker, calcium channel blocker, or diuretic.

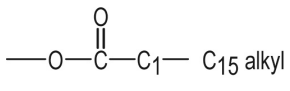
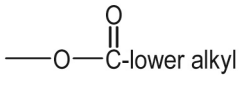
12. The use of Claim 1 or Claim 7, wherein the phorbol ester is 12-O-tetradecanoylphorbol-13-acetate.

13. Use of a phorbol ester of Formula I, or a pharmaceutically-acceptable salt, isomer, or enantiomer, thereof



FORMULA I

wherein R_1 and R_2 are selected from the group consisting of hydrogen, hydroxyl, , , , , and substituted derivatives thereof; and R_3 is selected from hydrogen,  and substituted derivatives thereof, in the manufacture of a medicament for preventing or treating one or more symptoms of stroke in a mammalian subject, wherein said medicament comprises between about 10 µg to about 1500 µg of said phorbol ester of Formula I as maximum daily dose.

14. The use of claim 13, wherein R_1 or R_2 is  the remaining R_1 or R_2 is  and R_3 is hydrogen.

15. The use of claim 13, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

16. The use of Claim 13, wherein the phorbol ester is 12-O-tetradecanoylphorbol-13-acetate.

17. The use of claim 13, further comprising use of at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation or

coordinate treatment regimen with said phorbol ester of Formula I in the manufacture of the medicament to treat or prevent one or more symptoms of stroke in said subject.

18. The use of claim 17, wherein the at least one secondary or adjunctive therapeutic agent is tissue plasminogen activator, an anticoagulant, a statin, fibrate, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitor, beta-blocker, anti-platelet agent, calcium channel blocker, or diuretic.

19. The use of claim 13, wherein the one or more symptoms of stroke are sudden numbness, weakness, sudden confusion, trouble speaking or understanding speech, trouble seeing in one or both eyes, trouble walking, dizziness, loss of balance or coordination, impaired mobility, or sudden headache.

20. The use of claim 13, wherein the medicament is to be administered per day.