PROTECTING NEURONS FROM ISCHEMIA

Background of the Invention

The present invention is directed to the use of EGb 761®, more particularly bilobalide, in protecting neurons from ischemic insults associated with stimulation of mitochondrial gene expression. Ischemia is caused by reduced or severely blocked blood flow, resulting in inadequate supply and loss of function of the affected tissues. The brain is very sensitive to ischemia because it has a high metabolic rate and low oxygen stores and small reserves of high-energy phosphates or carbohydrates. A brief period of global brain ischemia causes cell death in hippocampal CA1 pyramidal neurons days after perfusion (Pulsinelli, W.A., et al., 1982, Ann. Neurol., 11, 491-498 and Schmidt-Kastner, R., et al., 1991, Neuroscience 40, 599-636), whereas, other neurons within the hippocampus, such as the CA3 sector and the dentate gyrus are largely resistant. Recently, factors of neuronal disorganization have been suggested to play an important role in causing selective neuronal death (Rothman, S.M. and Olney, J.W., 1986, Ann. Neurol., 19, 105-111; Widmann, R., et al., 1992, J. Cereb. Blood Flow Metab., 12, 425-433; Rabin, O., et al., 1996, in Advances in Ginkgo biloba Extract Research, Vol. 5, Effect of Ginkgo biloba Extract (EGb 761®) on Neuronal Plasticity. Eds. Y. Christen, M.T. Droy-Lefaix and J. F. Macias-Nunez, Elsevier, Paris, pp. 7-19). These factors include massive accumulation of calcium, release of excitatory amino acids, increased release and incorporation of arachidonic acid and persistent disturbances of protein synthesis.

In a recent study, K. Abe et al., showed that the level of mRNA for cytochrome c oxidase subunit 1 (COX 1), which is encoded by mitochrondrial DNA (mtDNA), progressively decreased in CA1 neurons of gerbils from 1 to 3 h of the reperfusion after 3.5 min transient forebrain ischemia and complete disappeared at 7 days (Abe, K., et al., 1993, Mol. Brain Res., 19, pp. 69-75). The early decrease in COX I mRNA occurs in absence of a decrease in mtDNA. Similar disproportionate decrease in levels in mtDNA-encoded COX subunit mRNA to the levels of mtDNA are also observed in vulnerable brain regions in Alzheimer disease and due to decreased neuronal activity in animal experiments (Chandrasekaran, K., et al., 1994, Mol. Brain Res., 24, 336-340; Hatanpaa, K., et al., 1996, Ann. Neurol., 40, 411-420,

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1996; Wong-Riley, M.T.T., 1989, Trends Neurosci., 12, pp. 94-101). These results suggest that disturbances in mtDNA gene expression occurs early in response to acute changes in neuronal activity and energy demand. Pharmacological agents which stimulate mitochondrial transcription may then help to protect or render neurons less vulnerable to sudden metabolic insult such as ischemia and excitotoxicity.

Recent studies of a leaf extract of *Ginkgo biloba*, termed EGb 761®, have reported medicinal value of the product in the treatment of a variety of clinical disorders including cerebrovascular and peripheral vascular insufficiencies associated with aging and senility. See e.g., Ginkgo biloba Extract (EGb 761®) Pharmacological Activities and Clinical Applications, DeFeudis, F.V., Eds, Elsevier, 1991; and Ullstein Medical 1998, Gingko biloba extract (EGb 761®), Eds. Wiesbaden, DeFeudis, F.V. The extract contains 24% ginkgo-flavone glycosides, 6% terpene lactones (ginkgolides and bilobalide), about 7% proanthocyanidins and several other constituents. See Boralle, N., et al., In: Ginkgolides, Chemistry, Biology, Pharmacology and Clinical perspectives, Ed: Braquet, P., J.R. Prous Science Publishers, 1988. Bilobalide accounts for about 3% of the total extract. See Drieu, K., Presse Med, 1986, 15, 1455-1457. Previously, a protective role by bilobalide on mitochondrial respiration has been shown (Spinnewyn, B., Blavet, N. and Drieu, K. Effects of Ginkgo biloba extract (EGb 761®) on oxygen consumption by isolated cerebral mitochondria, Advances in Ginkgo biloba extract research, Vol. 4, Eds. Y. Christen, Y. Courtois, M-T Droy-Lefaix, pp. 17-22, Elsevier, Paris, 1995). *In vivo* administration of EGb 761® was shown to increase mitochondrial respiration after cerebral ischemia in gerbils. The principal component in Ginkgo biloba extract that increased 'state 3' respiration (maximal rate of respiration achieved in presence of substrate, ADP and Pi) was identified to be bilobalide.

Summary of the Invention

In one aspect, this invention is directed to a method of protecting neurons from ischemic insult in a patient in need thereof, which comprises administering to said patient an effective amount of bilobalide.

In a second aspect, this invention is directed to a pharmaceutical composition useful for protecting neurons from ischemic insult in a patient in need thereof, which

comprises a pharmaceutically acceptable carrier and an effective amount of bilobalide.

In another aspect, this invention is directed to a pharmaceutical composition useful for protecting neurons from ischemic insult in a patient in need thereof, which comprises a gingko biloba extract comprising an effective amount of bilobalide.

In still another aspect, this invention is directed to a method of stimulating mitochondrial gene expression in a patient in need thereof, which comprises administering to said patient an effective amount of bilobalide.

In yet another aspect, this invention is directed to a pharmaceutical composition useful for stimulating mitochondrial gene expression in a patient in need thereof, which comprises a pharmaceutically acceptable carrier and an effective amount of bilobalide.

In yet still another aspect, this invention is directed to a pharmaceutical composition useful for stimulating mitochondrial gene expression in a patient in need thereof, which comprises a gingko biloba extract comprising an effective amount of bilobalide.

In a further aspect, this invention is directed to a method of protecting neurons from ischemic insult in a patient in need thereof, which comprises administering to said patient a gingko biloba extract comprising an effective amount of bilobalide.

In a further still another aspect, this invention is directed to a pharmaceutical composition useful for protecting neurons from ischemic insult in a patient in need thereof, which comprises a pharmaceutically acceptable carrier and an effective amount of bilobalide.

In an even further aspect, this invention is directed to a pharmaceutical composition useful for protecting neurons from ischemic insult in a patient in need thereof, which comprises a gingko biloba extract comprising an effective amount of bilobalide.

In another further aspect, this invention is directed to a method of stimulating mitochondrial gene expression in a patient in need thereof, which comprises administering to said patient a gingko biloba extract comprising an effective amount of bilobalide.

In a furthermore aspect, this invention is directed to a pharmaceutical composition useful for stimulating mitochondrial gene expression in a patient in need

thereof, which comprises a pharmaceutically acceptable carrier and an effective amount of bilobalide.

In another furthermore aspect, this invention is directed to a pharmaceutical composition useful for stimulating mitochondrial gene expression in a patient in need thereof, which comprises a gingko biloba extract comprising an effective amount of bilobalide.

This invention is also directed to a method of treating stroke, head trauma, spinal cord trauma, traumatic brain injury, multiinfarct dementia, Alzheimer's disease, senile dementia of the Alzheimer's type, Huntington's disease, Parkinson's disease, epilepsy, amyotrophic lateral sclerosis, pain, AIDS dementia, drug addictions, or an ischemic event arising from CNS surgery, open heart surgery or any procedure during which the function of the cardiovascular system is compromised, which comprises administering to a patient in need thereof an effective amount of bilobalide.

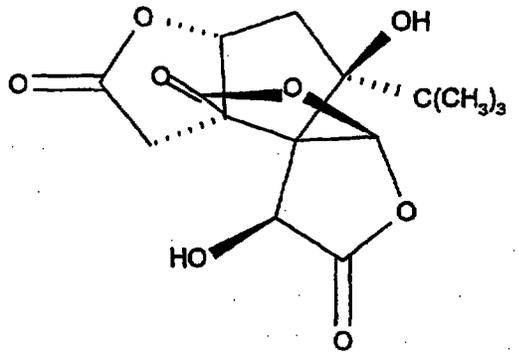
This invention is also further directed to a pharmaceutical composition useful for treating stroke, head trauma, spinal cord trauma, traumatic brain injury, multiinfarct dementia, Alzheimer's disease, senile dementia of the Alzheimer's type, Huntington's disease, Parkinson's disease, epilepsy, amyotrophic lateral sclerosis, pain, AIDS dementia, drug addictions, or an ischemic event arising from CNS surgery, open heart surgery or any procedure during which the function of the cardiovascular system is compromised, which comprises a pharmaceutically acceptable carrier and an effective amount of bilobalide.

This invention is furthermore directed to, a pharmaceutical composition useful for treating stroke, head trauma, spinal cord trauma, traumatic brain injury, multiinfarct dementia, Alzheimer's disease, senile dementia of the Alzheimer's type, Huntington's disease, Parkinson's disease, epilepsy, amyotrophic lateral sclerosis, pain, AIDS dementia, drug addictions, or an ischemic event arising from CNS surgery, open heart surgery or any procedure during which the function of the cardiovascular system is compromised, which comprises a gingko biloba extract comprising an effective amount of bilobalide.

In the foregoing aspects of this invention, it is preferred that the gingko biloba extract comprising bilobalide is EGb 761®.

Detailed Description

The term "ginkgo terpenoid" as used herein includes all of the naturally occurring terpenes which are derived from the gymnosperms tree *Ginkgo biloba* as well as synthetically produced ginkgo terpenoids and pharmaceutically active derivatives and salts thereof and mixtures thereof. Examples of ginkgo terpenoids include ginkgolides and bilobalide. Examples of ginkgo terpenoids are disclosed in Ginkgolides, Chemistry, Biology, Pharmacology, and Clinical Perspectives, J.R. Provs. Science Publishers, Edited by P. Braguet (1988); F.V. DeFeudis, Ginkgo Biloba Extract (EGb 761®); Pharmacological Activities and Clinical Applications, Elsevier, Chapter II (1991). Bilobalide has the following structure:



The term "ginkgolide" and "bilobalide" herein include the various ginkgolides and bilobalide disclosed in the books cited above as well as non-toxic pharmaceutically active derivatives thereof. Examples of ginkgolide and bilobalide derivatives include tetrahydro derivatives, acetyl derivatives, and alkyl esters such as the monoacetate derivatives and triacetate derivatives disclosed in Okabe, et al., J. Chem. Soc. (c), pp. 2201-2206 (1967).

The term "ginkgo biloba extract" as used herein includes a collection of natural molecules, including terpenoids, derived from the ginkgo biloba tree. Preferably, the extract is the ginkgo biloba extract EGb 761®.

Bilobalide can be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous or subcutaneous injection, or implant), nasal, vaginal, rectal, sublingual or topical routes of administration and can be formulated with

pharmaceutically acceptable carriers to provide dosage forms appropriate for each route of administration.

Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, bilobalide is admixed with at least one inert pharmaceutically acceptable carrier such as sucrose, lactose, or starch. Such dosage forms can also comprise, as is normal practice, additional substances other than such inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, the elixirs containing inert diluents commonly used in the art, such as water. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring and perfuming agents.

Preparations according to this invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions. They can also be manufactured in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use.

Compositions for rectal or vaginal administration are preferably suppositories which may contain, in addition to the active substance, excipients such as coca butter or a suppository wax.

Compositions for nasal or sublingual administration are also prepared with standard excipients well known in the art.

The dosage of bilobalide in the compositions of this invention may be varied; however, it is necessary that the amount of the active ingredient be such that a suitable dosage form is obtained. The selected dosage depends upon the desired

therapeutic effect, on the route of administration, and on the duration of the treatment. The dose can be administered as a single dose or divided into multiple doses.

An effective amount of bilobalide depends upon the condition being treated, the route of administration chosen and ultimately will be decided by the attending physician or veterinarian. Bilobalide may be administered in an amount of 0.05 to 2 mg/kg body weight of the patient or, preferably, administered in an amount of 0.1 to 1 mg/kg body weight of the patient.

A pharmaceutical composition of bilobalide includes within its meaning an extract of ginkgo biloba which contains bilobalide, such as EGb 761®. It should be noted that not all extracts of ginkgo biloba contains bilobalide, however, EGb 761® (which is also known as TANAKAN®, R_KAN®, TEBONIN®, TANAKENE®, and TEBOFORTAN®) does.

The use of bilobalide in protecting neurons from ischemic insults associated with stimulation of mitochondrial gene expression is evaluated using a cell culture model system. A morphological variant of rat pheochromocytoma PC12 cells, called PC12S (obtained from Dr. Sayeeda Zain, Dept. of Biochemistry, University of Rochester, School of Medicine and Dentistry, 603 Elm St., Rochester, NY), that retains the ability to grow attached to plastic tissue culture dish, is used. PC12S cells undergo differentiation in presence of nerve growth factor (NGF) and their morphology resemble that of sympathetic neurons. In this cell culture system, the expression of mtDNA-encoded genes can be regulated by changes in intracellular Na^+. Thus, addition of ouabain, an inhibitor of the Na/K-ATPase or sodium pump, or monensin, a sodium ionophore, causes a significant decrease in levels of mtDNA-encoded cytochrome c oxidase subunit III (COX III) gene.

Cell Culture: a morphological variant of rat pheochromocytoma PC12 cells, called PC12S, that retains the ability to grow attached to plastic tissue culture dish, is used. PC12S cells are grown in DMEM medium (Bio Fluids, Rockville, Maryland) containing 2 mM glutamine, 7.5% heat inactivated fetal calf serum, and 7.5% heat inactivated horse serum, with penicillin-streptomycin (Bio Fluids, Rockville, Maryland). Nerve growth factor (NGF); (Life Technologies, MD, USA) was added at a concentration of 50 ng/ml. Two days after addition of NGF, PC12S cells demonstrate prominent neurite outgrowth, and after 5 days, their morphology resembles that of sympathetic

neurons. All experiments are done on cells that are maintained in the presence of NGF for 10 days.

Chemicals: Ouabain and monensin were purchased from Research Biochemicals (Natick, MA, USA). Bilobalide was obtained from Dr. Katy Drieu, (IPSEN, France), it is also available from Sigma (St. Louis, Missouri). Ouabain is dissolved in water, whereas, bilobalide is dissolved in 95% ethanol. When ethanol is used as a solvent, appropriate control experiments are conducted using vehicle alone. Ethanol concentrations are always <0.1%. In studies related to the effect of pretreatment of bilobalide, cells are treated with 10 μg/ml for about 24 hours and then ouabain is added and total RNA is isolated at various time periods.

Experimental Procedure: PC12S cells grown in presence of NGF (on 10-cm dishes) are used. Cells are treated with drugs for various periods of time, 0, 1, 3, 6, 12, 24 and 48 hours after addition of drug. Cells are then washed once with DPBS (Dulbecco's Phosphate Buffered Saline; Bio-Fluids, Rockville, Maryland) without calcium and magnesium and total RNA is isolated using the TRIzol reagent (Life Technologies, MD, USA). One ml of TRIzol reagent is added directly to the dish and placed in a rocker for about 5 min. The suspension is then transferred to a 2 ml eppendorf centrifuge tube containing 0.2 ml of chloroform. The tube is vortexed for about 15 seconds and is centrifuged for about 15 min at about 4 °C. The aqueous phase is removed and total RNA is precipitated with half-volume of isopropanol. The total RNA is pelleted by centrifugation of about 13,000 g for about 15 min at about 4 °C, the pellet is washed once with 70% ethanol, dried and suspended in 100 μl of DEPC (diethyl pyrocarbonate; Sigma, St. Louis, Missouri) treated water.

Northern blot analysis: Ten μg of total RNA is run on a 1.2% formaldehyde agarose gel and transferred onto a GeneScreen membrane as described by the manufacturer (Dupont, New England Nuclear, Massachusetts, USA). Prehybridization is done by about 16 hours at about 42 °C using the hybridizol reagent (Oncor, MD, USA; Hybridizol I and Hybridizol II mixed in the ratio of 4:1). Hybridization is done by about 48 hours at about 42 °C in the same solution with the addition of [^{32}P] labeled cytochrome oxidase subunit III (COX III) probe. Blots are washed in 2 X SSC (1 X SSC = 150 mM sodium chloride, 15 mM sodium citrate), 0.1% sodium dodecyl sulfate (SDS) at room temperature for about 30 min, 2 X SSC, 0.1% SDS at about 42 °C for about 1 hour with one change, and finally at about 65 °C with 0.2 X SSC, 0.1 % SDS for about 30 min. Blots are exposed to X-ray film

(Biomax MS, Kodak, Rochester, NY, USA) with an intensifying screen for about 45 min to about 2 days at about -70 °C. The probe is removed form the blots by placing the blots in boiling DEPC-treated water for about 10 min. The blots are then rehybridized with a [^{32}P] labeled control β actin probe as described above. The level of RNA hybridized is quantified using an image analysis program (NIH image 1.54) from autoradiograms of lower exposure than is used for photography. Ratios of COX III mRNA to β actin mRNA are calculated.

Probe preparation and labeling: Cytochrome oxidase subunit III probe is prepared by polymerase chain reaction (PCR) using rat genomic DNA and primers corresponding to rat mtDNA sequence. The PCR conditions are as follows: 30 cycles for about 1 min at about 94 °C, about 30 sec at about 55 °C, and about 1 min at about 72 °C. The PCR product (565 bp) is purified by agarose gel electrophoresis separation, elution and alcohol precipitation (Quiagen, CA, USA). β actin probe is prepared by isolating the cDNA insert from the plasmid clone (ATCC-# 78554, American Type Culture Collection, MD, USA). The probes are labeled using the random primer method and purified by gel-filtration (Pharmacia, NJ, USA).

Results: Expression of Mitochondrial DNA encoded Cytochrome oxidase subunit III mRNA in differentiated PC12S cells. To examine the effect of chemicals on mtDNA-encoded COX III gene expression, the basal level of COX III mRNA in PC12S cells treated with the vehicle, PC12S cells were differentiated with NGF for 10 days. The cells were treated with the vehicle for various periods of time and total RNA was isolated. The total RNA samples were subjected to northern blot analysis with a probe for the mtDNA-encoded COX III gene and with a probe for β actin gene. Northern blots showed discrete bands for COX III mRNA at 0.8 kb, and for the β actin at 1.9 kb. Expression of nuclear DNA (nDNA)-encoded β actin (1.9 kb) was determined to ensure that equivalent amounts of RNA were loaded and transferred to each lane in Northern Blot analyses. In vehicle treated PC12S cells, the amounts of COX III mRNA and that of the β actin mRNA were found to be roughly equivalent among samples, see Table 1. No significant differences were observed in the ratio of COX III mRNA to β actin mRNA among samples. Thus, in vehicle treated PC12S cells, there was no significant change in the amount of COX III mRNA.

Table 1

Ratio of COX III mRNA to β actin mRNA in vehicle treated cells

Time in hours	Ratio of COX III mRNA to β actin mRNA
0	0.82
1	0.82
3	0.82
6	1.2
12	1.0
24	1.0
48	1.0

Expression of Mitochondrial DNA encoded Cytochrome oxidase subunit III mRNA in differentiated PC12S cells in presence of bilobalide (Lot # BN52023; CP 160.002, IPSEN). In Table 2, the results on COX III mRNA level obtained with the addition of increasing concentration of bilobalide (from 0.2μg/ml to 10μg/ml) are given. PC12S cells were treated for 6 hours with increasing concentrations of bilobalide. Total RNA was then isolated and Northern blot analysis was done for COX III mRNA. Addition of 5 μg/ml and 10 μg/ml bilobalide to differentiated PC12S cells caused a significant increase in the ratio of COX III mRNA to β actin mRNA. The stimulator effect of bilobalide reached two fold increase with these concentrations.

Table 2

Effect of addition of bilobalide on COX III mRNA in PC12S cells.

Concentration of Bilobalide (μg/ml)	Ratio of COX III mRNA to β actin mRNA
0	1.2
0.2	1.3
0.5	1.3
1	1.35
2	1.3
5	2.1
10	2.25

A time course on the expression of Mitochondrial DNA encoded Cytochrome oxidase subunit III mRNA in differentiated PC12S cells in presence of bilobalide (Lot # BN52023; CP 160.002). The time course of the induction of mitochondrial gene expression by bilobalide was examined . Bilobalide was added at a concentration of 10 μg/ml to differentiated PC12S cells and after various periods of treatment, total RNA was isolated. The total RNA samples were subjected to Northern Blot analysis with COX III probe and then with β actin probe. The results are shown in Table 3. Addition of 10 μg/ml bilobalide caused a significant increase in the amount of COX III mRNA during the first 6 hours after addition and remained at a higher level up to 48 hours.

Table 3

Effect of Bilobalide on COX III mRNA levels - time course

Time in hours	Ratio of COX III mRNA to β actin mRNA
0	1.2
3	2.0
6	2.0
12	2.2
24	2.3
48	2.5

Claims



What is claimed is:

1. A method of protecting neurons from ischemic insult in a patient in need thereof, which comprises administering to said patient an effective amount of bilobalide.

2. A pharmaceutical composition useful for protecting neurons from ischemic insult in a patient in need thereof, which comprises a pharmaceutically acceptable carrier and an effective amount of bilobalide.

3. A pharmaceutical composition useful for protecting neurons from ischemic insult in a patient in need thereof, which comprises a gingko biloba extract comprising an effective amount of bilobalide.

4. A method of stimulating mitochondrial gene expression in a patient in need thereof, which comprises administering to said patient an effective amount of bilobalide.

5. A pharmaceutical composition useful for stimulating mitochondrial gene expression in a patient in need thereof, which comprises a pharmaceutically acceptable carrier and an effective amount of bilobalide.

6. A pharmaceutical composition useful for stimulating mitochondrial gene expression in a patient in need thereof, which comprises a gingko biloba extract comprising an effective amount of bilobalide.

7. A method of protecting neurons from ischemic insult in a patient in need thereof, which comprises administering to said patient a gingko biloba extract comprising an effective amount of bilobalide.

8. A method of stimulating mitochondrial gene expression in a patient in need thereof, which comprises administering to said patient a gingko biloba extract comprising an effective amount of bilobalide.

9. A method of treating stroke, head trauma, spinal cord trauma, traumatic brain injury, multiinfarct dementia, Alzheimer's disease, senile dementia of the Alzheimer's type, Huntington's disease, Parkinson's disease, epilepsy, amyotrophic lateral sclerosis, pain, AIDS dementia, drug addictions, or an ischemic event arising from CNS surgery, open heart surgery or any procedure during which the function of the cardiovascular system is compromised, which comprises administering to a patient in need thereof an effective amount of bilobalide.

10. A pharmaceutical composition useful for treating stroke, head trauma, spinal cord trauma, traumatic brain injury, multiinfarct dementia, Alzheimer's disease, senile dementia of the Alzheimer's type, Huntington's disease, Parkinson's disease, epilepsy, amyotrophic lateral sclerosis, pain, AIDS dementia, drug addictions, or an ischemic event arising from CNS surgery, open heart surgery or any procedure during which the function of the cardiovascular system is compromised, which comprises a pharmaceutically acceptable carrier and an effective amount of bilobalide.

11. A pharmaceutical composition useful for treating stroke, head trauma, spinal cord trauma, traumatic brain injury, multiinfarct dementia, Alzheimer's disease, senile dementia of the Alzheimer's type, Huntington's disease, Parkinson's disease, epilepsy, amyotrophic lateral sclerosis, pain, AIDS dementia, drug addictions, or an ischemic event arising from CNS surgery, open heart surgery or any procedure during which the function of the cardiovascular system is compromised, which comprises a gingko biloba extract comprising an effective amount of bilobalide.

12. Use of bilobalide in the manufacture of a medicament for use in the treatment of:

a patient in need of protection of neurons from ischemic insult; and/or

a patient in need of stimulation of mitochondrial gene expresion; and/or

a patient suffering stroke, head trauma, spinal cord trauma, traumatic brain injury, multiinfarct dementia, Alzheimer's disease, senile dementia of the Alzheimer's type, Huntington's disease, Parkinson's disease, epilepsy, amyotrophic lateral sclerosis, pain, AIDS dementia, drug addictions, or an ischemic event arising from CNS surgery, open heart surgery or any procedure during which the function of the cardiovascular system is compromised, which comprises administering to a patient in need thereof an effective amount of bilobalide.

13. Use as claimed in claim 12, wherein said bilobalide comprises ginkgo biloba extract.