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(54) Title: INHIBITION OF REACTIVE OXYGEN SPECIES AND PROTECTION OF MAMMALIAN CELLS

(57) Abstract: Methods and compositions useful for neuronal protection in retinal cells in vitro and the protection of mammalian cells from reactive oxygen species in vivo are provided. UltrafÊne nano-size cerium oxide particles, less than 10 nanometers in diameter, have been provided to decrease reactive oxygen species (ROS) in retina tissue that generates large amounts of ROS. These reactive oxygen species (ROS) are involved in light- induced retina degeneration and age-related macular degeneration (AMD). Cerium oxide nanoparticles have been used to promote the lifespan of retinal neurons and protect the neurons from apoptosis induced by hydrogen peroxide in vitro and in vivo. The neuronal protection in retinal cells is achieved by decreasing generation of intracellular reactive oxygen species (ROS). Thus, cerium oxide particles are used to promote the longevity of retinal neurons in vitro and mammalian cells in vivo.

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INHIBITION OF REACTIVE OXYGEN SPECIES AND PROTECTION OF MAMMALIAN CELLS

This invention claims the benefit of priority from United States Provisional Application Serial No. 60/676,043 filed April 29, 2005 and United States Provisional Application Serial No. 60/716,630 filed September 13, 2005.

FIELD OF THE INVENTION

This invention relates to biological uses of nanoceria particles, and in particular to methods and compositions useful for neuronal protection in retinal cells *in vitro* and the protection of mammalian cells from reactive oxygen species *in vivo* and is supported in part by funding from the National Science Foundation and National Institutes of Health under the Contract numbers: P20 RR17703, FY014427, FY13050, and FY12190.

BACKGROUND AND PRIOR ART

Cerium is a silvery metallic element, belonging to the lanthanide group. Cerium Oxide (CeO_2) is used in precision polishing and lapping applications. Ultra fine nano-size cerium oxide, less than 10 nanometers, is more efficient for coating purposes. Recently, it was reported by B. Rzigalinski et al. that nanoparticles prolong the life of cortical neurons in culture 4 fold over the cells without treatment; decreased the intracellular Ca2+ concentration and prevented UV damage of cortical neurons. See B. Rzigalinski et al., "Cerium Oxide Nanoparticles Extend Cell Longevity and Act as Free Radical Scavengers" at website http://www.med.miami.edu/mnbws/Rzigalinski112.html. Based on its chemical characteristics, this effect is partially due to a decrease of reactive oxygen species (ROS).

Retina tissue generates a large amount of ROS which are involved in light-induced retina degeneration and age-related macular degeneration (AMD). The present invention tests the hypothesis that nanoparticles can promote the lifespan of retinal neurons in culture and protect them from apoptosis induced by hydrogen peroxide (H_2O_2) *in vitro* by decreasing the intracellular concentration of reactive oxygen species.

In 2004, T. H. Margrain et al. discuss the state of research in the treatment of age-related macular degeneration in *Progress in Retinal and Eye Research,* 2004, 23: 523-531, "Do Blue Light Filters Confer Protection Against Age-Related Macular Degeneration?" The problem of apoptosis in the body is discussed in an article by P. Moongkarndi et al. in "Antiproliferation, Antioxidation and Induction of Apoptosis by Garcinia Mangostana (Mangosteen) on SKBR3 Human Breast Cancer Cell Line", *Jl. of Ethnopharmacology*, 2004, 90: 161-166.

Often persons suffering from light-induced retina degeneration and age-related macular degeneration (AMD) are without satisfactory remedies to prevent the eventual outcome of blindness. There are some proteins available for neuronal protection of retinal cells, however, they are big molecules and over time their effect may fade away.

It is desirable to find reliable solutions to prolong the lifespan of retinal neurons so that blindness is avoided for persons with retina degeneration and AMD.

In addition to diseases of the eye, many human diseases are due to the death of cells in specific tissues or organs. The majority of those diseases are due to accumulation of metabolic insults from reactive oxygen species originating within or outside of the cells. These diseases include all forms of blindness whether hereditary, light-induced, or physical damage such as occurs in retinal detachment. In addition, damage due to ageing, stroke, cardiac infarction, burns, etc, which proceed through reactive oxygen species, can be addressed with the nanoceria particles of the present invention.

The present invention promotes a longer lifespan for retinal neurons. The greatest benefit of the nanoceria is its ability to get inside the cells and provide protection from reactive oxygen species (ROS); other body systems and tissues can also be protected from damage due to ROS.

SUMMARY OF THE INVENTION

A primary objective of the present invention is to promote the lifespan of retinal neurons in culture.

A secondary objective of the present invention is to protect retinal neurons from apoptosis induced by hydrogen peroxide (H_2O_2) *in vitro.*

A third objective of the present invention is to protect retinal neurons from apoptosis induced by reactive oxygen species *in vivo.*

A fourth objective of the present invention is to inhibit the rise in the intracellular concentration of reactive oxygen species (ROS).

A fifth objective of the present invention is to provide method for inhibiting apoptosis induced by H_2O_2 of retinal neurons *in vitro* in a dose and time dependent manner.

A sixth objective of the present invention is to provide method for inhibiting apoptosis induced by reactive oxygen species in retinal neurons *in vivo* in a dose and time dependent manner.

A seventh objective of the present invention is to provide a method for preventing an increase in the intracellular reactive oxygen species (ROS) in a dose and time dependent manner.

An eighth objective of the present invention is to manufacture and modify cerium oxide (CeO_2) nanoparticles for effective use in neuronal protection in retinal cells.

A ninth objective of the present invention is to manufacture and modify cerium oxide (CeO_2) nanoparticles for effective use in mammalian cells *in vivo* to inhibit damage caused by reactive oxygen species (ROS).

A preferred composition for promoting longevity of retinal neurons includes at least one of CeO.sub.n1 wherein $0<n1<2$, and $0<n2<3$ in the form of ultra-fine particles. The preferred ultra-fine particles have a diameter in a range between approximately 1 nanometer (nm) and approximately 10 nm and the preferred CeO.sub.n1 is further defined as n1 equals approximately 2.

A preferred composition for inhibiting apoptosis induced by hydrogen peroxide oxidation of retinal neurons includes at least one of CeO.sub.n1 wherein $0<n1<2$, and $0<n2<3$ in the form of ultra-fine particles. The preferred ultra-fine particles have a diameter in a range between approximately 1 nanometer (nm) and approximately 10 nm and the preferred CeO.sub.n1 is further defined as n1 equals approximately 2.

A preferred composition for inhibiting apoptosis of retinal neurons in a dose and time dependent manner includes at least one of CeO.sub.n1 wherein $0<n1<2$, and $0<n2<3$ in the form of ultra-fine particles. The preferred ultra-fine particles have a diameter in a range between approximately 1 nanometer (nm) and approximately 10 nm and the preferred CeO.sub.n1 is further defined as n1 equals approximately 2.

A more preferred composition that decreases the concentration of intracellular reactive oxygen species (ROS) includes at least one of CeO.sub.n1 wherein $0<n1<2$, and $0<n2<3$ in the form of ultra-fine particles. The more preferred composition is used in the treatment of diseases of the retina selected from the group consisting of light-induced retina degeneration and age-related macular degeneration, and is also used *in vivo* for the treatment of diseases in mammalian cells to inhibit damage caused by reactive oxygen species (ROS).

The mammalian cells that can be treated by the composition of the present invention, include, but are not limited to, retinal neurons, brain cells, heart cells, skin cells, liver cells, kidney cells and peripheral nervous system cells.

A preferred method for promoting longevity of retinal neurons includes preparing ultra-fine particles of at least one of CeO.sub.n1 wherein 0<n1<2, and 0<n2<3 in a preselected concentration, and adding the preselected concentration of CeO.sub.n1 wherein 0<n1<2, and 0<n2<3 to primary retinal neurons. The preferred ultra-fine particles have a diameter in a range between approximately 1 nanometer (nm) and approximately 10 nm and the CeO.sub.n1 is further defined as n1 equals approximately 2. The preferred preselected concentrations of CeO_2 are in a range between approximately 3 nanomolar (nM) and approximately fifty nanomolar (nM), more preferably in a range between approximately 3 nanomolar (nM) and approximately twenty nanomolar (nM).

It is also preferred that the preselected concentrations of CeO_2 are added to primary retinal neurons *in vitro* and/or administered to mammalian cells *in vivo* to protect the mammalian body system from damage to any tissue due to reactive oxygen species (ROS).

Further objects and advantages of the present invention will be apparent from the following detailed description of a presently preferred embodiment which is illustrated schematically in the accompanying drawings.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 is a timeline of exposure of a primary retinal neuron culture to treatment with cerium oxide nanoparticles.

Fig. 2 is a graph showing the percentage of apoptotic retinal neurons in culture with and without 5 nanomoles (nM) cerium oxide nanoparticle treatment at different time points.

Fig. 3 shows the initial cerium oxide nanoparticle treatments followed by hydrogen peroxide incubation, and time line of exposure.

Fig. 4A is a flow cytometry plot of the control sample of primary retinal neurons with no cerium oxide nanoparticle treatments and no hydrogen peroxide incubation.

Fig. 4B is a flow cytometry plot of retinal neurons in the presence of 100 micromoles (μM) hydrogen peroxide.

Fig. 4C is a flow cytometry plot of retinal neurons treated with 1nM cerium oxide in the presence of 100 micromoles (μM) hydrogen peroxide.

Fig. 4D is a flow cytometry plot of retinal neurons treated with 3nM cerium oxide in the presence of 100 micromoles (μM) hydrogen peroxide.

Fig. 4E is a flow cytometry plot of retinal neurons treated with 5nM cerium oxide in the presence of 100 micromoles (μM) hydrogen peroxide.

Fig. 4F is a flow cytometry plot of retinal neurons treated with 10nM cerium oxide in the presence of 100 micromoles (μM) hydrogen peroxide.

Fig. 4G is a flow cytometry plot of retinal neurons treated with 20nM cerium oxide in the presence of 100 micromoles (μM) hydrogen peroxide.

Fig. 4H is a flow cytometry plot of retinal neurons treated with 20nM cerium oxide.

Fig. 5 shows relative viable retinal neurons with and without incubation of different concentrations of cerium oxide nanoparticles for time periods between approximately12 hours and approximately 96 hours.

Fig. 6 shows the initial cerium oxide nanoparticle treatments, hydrogen peroxide and DCFH-DA incubation, and time line of exposure.

Fig. 7A is a graph of the intracellular level of reactive oxygen species (ROS) of retinal neurons after 30 minutes incubation with cerium oxide nanoparticles.

Fig. 7B is a graph of the intracellular level of reactive oxygen species (ROS) of retinal neurons after 12 hours incubation with cerium oxide nanoparticles.

Fig. 7C is a graph of the intracellular level of reactive oxygen species (ROS) of retinal neurons after 24 hours incubation with cerium oxide nanoparticles.

Fig. 7D is a graph of the intracellular level of reactive oxygen species (ROS) of retinal neurons after 96 hours incubation with cerium oxide nanoparticles.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Before explaining the disclosed embodiments of the present invention in detail it is to be understood that the invention is not limited in its application to the details of the particular arrangements shown since the invention is capable of other embodiments. Also, the terminology used herein is for the purpose of description and not of limitation.

The present invention has two embodiments describing in detail the *in vitro* and *in vivo* treatment of mammalian cells with ultra fine nano-size cerium oxide particles, less than 10 nanometers in diameter, to protect the "body system" from damage to any tissue due to reactive oxygen species (ROS).

Hydrogen peroxide (H_2O_2) is one of many reactive oxygen species. In the present invention, H_2O_2 is added directly to cultures for the *in vitro* treatments. H_2O_2 is not added to the live tissue samples, since H_2O_2 is one of the ROS products of light damage. The discussion below confirms that nanoceria particles inhibit all forms of reactive oxygen species (ROS).

For example, the nanoceria particles of the present invention can protect the brain against stroke and reperfusion injury, the heart cells from effects of cardiac infarction, the skin from UV rays and burn injuries. Neurodegeneration (e.g., Alzhiemer's, Parkinson's, dementia, amyotrophic lateral sclerosis) and potentially mental retardation (due to loss of brain cells) within the central and peripheral nervous systems can also be inhibited. This protection can extend to diseases which produce chronic problems such as cirrhosis of the liver or kidney or the multi-organ effects of aging itself. The nanoceria can become the universal treatment for all major and minor diseases and events which involve reactive oxygen species.

The nanoceria (CeO_2 nanoparticles) have the ability to destroy toxic products of metabolism known as reactive oxygen species (ROS). It has been shown in one embodiment that the nanoceria particles prevent the ROS induced death of mammalian retinal neurons in culture (*in vitro)* and subsequently prolonged the life of the cells in culture and protected the cells from ROS. In the second embodiment, the ability to provide mammalian cell protection *in vivo* is disclosed.

The first embodiment of the present invention provides a method and composition for promoting the lifespan of retinal neurons and protecting the nerve cells in the eye from apoptosis induced by hydrogen peroxide (H_2O_2) *in vitro* by decreasing generation of intracellular reactive oxygen species. The treatment of the eye with ultra fine nano-size cerium oxide is a significant advance in biological uses of cerium oxide. Persons afflicted with such conditions as, light-induced retina degeneration and age-related macular degeneration (AMD) have hope for brighter, clearer vision.

The examples below provide further detail on the preparation and treatment of retinal nerve cells with CeO_2 nanoparticles.

Example 1

A primary retinal neuron culture is obtained from albino rat pups. Retinae of Sprague-Dawley albino rat pups (0-2-day old) were dissected out and mechanically dissociated in 25ml of DMEM/F12 medium. After being filtered through 230μm and 140μm sieves, the dissociated cells were centrifuged at 1200rpm for 5min. The cell pellets were re-suspended in the medium to 1×10^5 cells/ml. 1ml of the cell suspension was plated in each well pre-treated with 10μg/ml of poly-D-lysine. The cells were maintained in the medium until day 7, when different concentrations of CeO_2 nanoparticles were added to the cultures. The timeline for the addition of CeO_2 nanoparticles is shown in Fig. 1. The treated neuronal cells were harvested on day 14, day 19, day 24 and day 29 after the beginning of treatment on day 7. The percentage of apoptotic retinal neurons in the culture with and without 5nM CeO_2 nanoparticle treatment is shown in Fig. 2 at the 12th day, 14th day, 19th day, 24th day and 29th day. Data are shown in M±S.D. Statistics were collected by Student t-test (n=3, *$p<0.05$, **$p<0.01$). Fig. 2 confirms that at every testing period the control with no CeO_2 nanoparticle treatment had a higher percentage of apoptotic retinal neurons in the culture, in contrast to the decreased percentage of apoptotic retinal neurons in the cells treated with 5nM CeO_2 nanoparticles.

Example 2

The detection of apoptosis by flow cytometry is illustrated in Figures 3, 4A – 4H and 5. After periods of incubation with CeO_2 or H_2O_2, the cells were washed with serum free medium 3 times, followed by treatment of 1ml of 1×trypsin for 2min. After centrifuging, the cell pellet was resuspended in 500μl of 1×PBS containing 5μl of Annexin V-FITC and 25μl of Propidium Iodide (PI). The kit used for the analysis is commercially available from Beckman Coulter and is known as the "ANNEXIN V-FITC Kit." The mixture was incubated on ice for 10 minutes. The fluorescent emissions of FITC and PI were detected by flow cytometry (Beckman Coulter) with the excitation filters of 492 nanometers (nm) and 550nm. The FITC fluorescent emissions signals the presence of cells undergoing apoptosis; whereas, the PI signals with an automatic red color fluorescence the binding to DNA fragments identifying cells in a necrotic stage.

Fig. 3 shows a treatment timeline with CeO_2 incubation after the 7th day of treatment at the time intervals of 12 hours, 24 hours, 72 hours and 96 hours with each treated sample subsequently exposed to 12 hours incubation time with H_2O_2.

Figures 4A – 4H are representative flow cytometry plots of retinal neurons with and without incubation with CeO_2 nanoparticles. Measurements were taken after 96 hours. The cytometry plot shows activity of approximately 10,000 cells in four quadrants, as described below.

C1 represents the percentage of 10,000 cells showing Annexin V positive signals, which are interpreted to indicate the percentage of 10,000 cells undergoing apoptosis.

C2 represents the percentage of 10,000 cells showing both Annexin V and PI positive signals, which is interpreted as the percentage of 10,000 cells which are in late apoptotic or necrotic stage. (AnnexinV signals apoptotic stage; PI signals necrotic stage.)

C3 represents the percentage of 10,000 cells showing *neither* Annexin V nor PI positive signals, which is interpreted as the percentage of 10,000 cells *which are still viable.*

C4 represents the percentage of 10,000 cells showing a PI positive signal, which is interpreted as the percentage of 10,000 cells in a necrotic stage.

Quadrants C2 and C4 show the percentage of 10,000 cells committed to die. In quadrant C1 the percentage of 10,000 cells undergoing apoptosis are shown and some may be salvaged. It is important to observe the percentage of 10,000 cells in quadrant C2 for the efficacy of the cerium oxide treatment of the present invention.

Focusing on the viable cells, the control in Fig. 4A has no CeO_2 nanoparticle treatment and 73.9% of the cell population is viable after 96 hours. Fig. 4B is treated with 100 μM H_2O_2, causing a deadly assault and leaving the lowest percentage (57.7%) of viable cells. The addition of gradually increasing concentrations of cerium oxide with 100 μM H_2O_2 , are shown in Figures 4C – 4G.

Fig. 4C is treated with 1nM CeO_2 nanoparticles and 100 μM H_2O_2 and 60.6% of the cell population remains viable. Fig. 4D is treated with 3nM CeO_2 nanoparticles and 100 μM H_2O_2 and 67.0% of the cell population is viable. Fig. 4E is treated with 5nM CeO_2 nanoparticles and 100 μM H_2O_2, with 68.9% of the cell population remaining viable. Fig. 4F is treated with 10nM CeO_2 nanoparticles and 100 μM H_2O_2 and 72.6% of the cell population is viable. Fig. 4G is treated with 20nM CeO_2 nanoparticles and 100 μM H_2O_2 and 72.3% of the cell population remains viable.

Fig. 4H is treated with 20nM CeO_2 nanoparticles without the addition of H_2O_2 and 73.4% of the cell population remains viable showing that the CeO_2 nanoparticles alone have no negative effect on the cell population.

The data in Figures 4A - 4H can also be summarized from the analysis of cells undergoing apoptosis as shown in quadrant C1. The control, Fig. 4A shows 16.2% of cells undergoing apoptosis under normal conditions, without any treatment, after 96 hours. Fig. 4B shows 26.1% of cells undergoing apoptosis after the 100 μM H_2O_2 challenge. Figures 4C – 4G show there are 28.3%, 21.9%, 22.2%, 18.5% and 15.8% of cells undergoing apoptosis with 1, 3, 5, 10 and 20nM CeO_2 nanoparticle treatment, respectively. Fig. 4H shows 15.7% of cells undergoing apoptosis with 20nM CeO_2 nanoparticle treatment, which is a slight improvement over no treatment at all as shown by the control in Fig. 4A.

Thus, cerium oxide nanoparticles inhibit apoptosis in retinal neurons *in vitro* in a dose and time dependent manner.

Fig. 5 shows relative viable retinal neurons with and without incubation of different concentration of CeO_2 nanoparticles for different time periods. The concentration of CeO_2 nanoparticles were 1nM, 3nM, 5nM, 10nM, 20nM each with 100 μM H_2O_2; one sample was treated with only 100 μM H_2O_2 and another sample was treated with only 20nM CeO_2 nanoparticles. The measurement of relative viable cells for each group was determined after 12 hours, 24 hours, 48 hours, 72 hours and 96 hours. The effects showed dose and time dependency.

5nM cerium oxide nanoparticles started to decrease the apoptosis at 24h of incubation. As incubation time increased, the protective effect from 5nM nanoparticles was more significantly increased. Additionally, 10nM and 20nM nanoparticles began to have effects late with 96h of incubation. (Statistical analysis was done by ANOVA, and Duncan test for post hoc analysis. Data are presented in M±S.D. $n \geq 3$, $*p<0.05$, $**p<0.01$). Fig. 5 shows that among the various treatment doses, 5nM CeO_2

nanoparticle treatment gave the earliest response with moderate protective effect and 20nM CeO_2 nanoparticle treatment gave the highest protective effect with late response.

Example 3

Intracelluar reactive oxygen species (ROS) production was measured in both CeO_2 nanoparticle treated and control cells using 29,79-dichlorofluorescein diacetate (DCFH-DA, Sigma). Briefly, the retinal neurons were exposed to CeO_2 nanoparticles with different concentrations and various incubation times. After incubation, the cells were incubated with 10μM DCFH-DA (dissolved in dimethylsulfoxide (DMSO)) at 37°C for 30 min. The cells then were incubated with 1mM H_2O_2 at 37°C for 30 min after the excess DCFH-DA was washed with PBS. The cells were harvested as described above. The intensity of fluorescence was detected by flow cytometry with the excitation filter of 485nm. The ROS level was calculated as a ratio: ROS = mean intensity of treated cells divided by mean intensity of control cells.

Fig. 6 is an experimental paradigm showing the beginning of the CeO_2 nanoparticle treatment on the 7th day and simultaneous incubation with H_2O_2 and DCFH-DA and a timeline of exposure at 30 minutes, 12 hours, 24 hours, and 96 hours. Figures 7A – 7D show how the CeO_2 nanoparticles decreased the generation of ROS in a dose and time dependent manner. The ROS generated in the groups of 5nM, 10nM, and 20nM nanoparticle incubation were statistically significantly less than the group without treatment at the earliest (12h) of the tested points. 3nM nanoparticle incubation had an effect at 24h. However, 1nM nanopartcles did not show any significant decrease within the tested time point. Statistical analysis was done by ANOVA, and Duncan test for post hoc analysis. Data were shown in M±S.D. n=3, * * $p<0.01$; *$p<0.05$.

The cerium oxide of the present invention includes those cerium compounds that have reacted with atmospheric oxygen to have stable oxide layers identified as CeO.sub.n1, wherein O is less than 1 or equal to 2 $(0<n1<2)$ and $(0<n2<3)$.

The cerium oxide particles of the present invention are characterized as ultra-fine and are preferably in a size range of from approximately 1 nanometer in diameter to approximately 10 nanometers in diameter; more preferably from approximately 1nm to approximately 7 nm. A judicious selection of particle size is required by someone skilled in the art and is not a limitation of the present invention.

The results of testing in the above examples document the ability of cerium oxide nanoparticles to promote the longevity of retinal neurons *in vitro* and inhibit apoptosis induced by hydrogen peroxide on retinal neurons *in vitro* in a dose and time dependent manner. It has also been determined that cerium oxide nanoparticles decrease generation of intracellular reactive oxygen species in a dose and time dependent manner. Thus, the present invention represents a significant advance in the treatment in degenerative diseases of the retina, such as, but not limited to light-induced retina degeneration and age-related macular degeneration (AMD).

In the second embodiment of the present invention a rat "light damage" model for retinal degeneration was used as the test system. The data demonstrate that the nanoceria prevented the death of retinal neurons when given prior to the "light insult". The nanoceria particles protected the cells at the time of exposure as well as prevented the subsequent death seen days later in the untreated animals. The *in vivo* route of administration, including, direct injection into the eye, intravenous, intraperitoneal, intramuscular, oral or topically on the eye or skin may improve the result. Similarly, the time of administration of the nanoparticle, both before or after an insult, is important. Thus, the nanoceria will also prevent the death of retinal cells due to glaucoma, diabetic retinopathy, inherited retinal degeneration (for example, Retinitis Pigmentosa), macular degeneration, retinal detachment or any disease or event which proceeds through the production of ROS. These particles should preserve and prolong vision when administered *in vivo*.

Example 4

Rats were injected intravitreally with 2 microliters (μl) of nanoceria (concentrations from 0.1 to 1.0 micromolar) three days before they were exposed for six hours to a bright light (2700 LUX) in a light box. The animals were returned to normal lighting for 3.5 days, then killed, the eyes enucleated, fixed, processed for paraffin embedding, sectioned, and either stained with H & E or processed for immunocytochemistry.

The number of photoreceptors remaining was determined with the H&E sections by using a microscope connected to a digital camera to record the images and then measuring the thickness of the outer nuclear layer every 240 microns from the optic nerve along both the superior and the inferior retina to the ora seratta. The data was then plotted as retina thickness versus the distance from the optic nerve head. The changes in other layers of retinal cells were recorded with images but not quantified. Cells actively undergoing apoptosis were also visualized using a commercially available "Apoptosis kit" and recording the microscopic images with a fluorescence microscope.

The data demonstrate that the nanoceria at all concentrations tested prevented the immediate death of retinal cells shortly after exposure to light as well as the ongoing death seen days later in the untreated animals. We therefore have demonstrated *in vivo* that these nanoceria particles can protect cells within the rat retina from light-induced cell death.

Prior to the present invention, it was not known that nanoceria particles could be used *in vivo* for preventing blindness or death of retinal cells, or for preventing the death *in vivo* of any other cell type in any disease. The chemical properties of CeO2 nanoparticles enable the destruction of reactive oxygen species (ROS) produced by toxins and/or products of oxygen metabolism within cells.

Thus, nanoceria can protect any cell type from ROS induced damages. Diseases which could possibly be prevented, cured or ameliorated would include hereditary blindness, macular degeneration, glaucoma, diabetic retinopathy, retinal detachment, and other blinding diseases which involve ROS would be potentially solved. Similarly, in other cells within the central nervous system (CNS), neuronal death in strokes, degenerative diseases such as Alzheimer's Disease, Parkinson's, Huntington's Disease, and the death of peripheral nerves are preventable or at least the rate of cell death can be decreased and would result in prolonged function of the cells, tissues, organs, and individual.

While the invention has been described, disclosed, illustrated and shown in various terms of certain embodiments or modifications which it has presumed in practice, the scope of the invention is not intended to be, nor should it be deemed to be, limited thereby and such other modifications or embodiments as may be suggested by the teachings herein are particularly reserved especially as they fall within the breadth and scope of the claims here appended.

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We claim:

1. A composition when used for treating diseases of the retina, said diseases selected from the group consisting of light-induced retina degeneration and age-related macular degeneration, wherein said composition comprises at least one of CeO.sub.n1 wherein $0<n1<2$ or CeO_2 in the form of ultra-fine particles and wherein the ultra-fine particles have a diameter in the range between approximately 1 nanometer (nm) and approximately 10 nm.

2. A method for treating diseases of the retina selected from the group consisting of light-induced retina degeneration and age-related macular degeneration, wherein said method comprises administering a composition comprising at least one of CeO.sub.n1 wherein $0<n1<2$, or CeO_2, in the form of ultra-fine particles, wherein the ultra-fine particles have a diameter in the range between approximately 1 nanometer (nm) and approximately 10 nm, to a subject in need thereof.

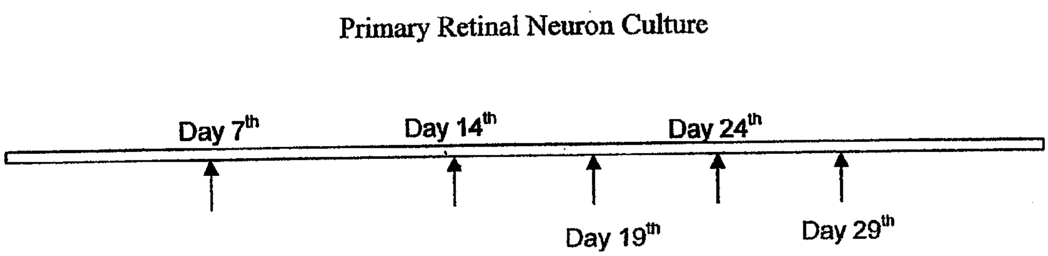
Primary Retinal Neuron Culture
Day 7th
Day 14th
Day 24th
Day 19th
Day 29th

Fig. 1

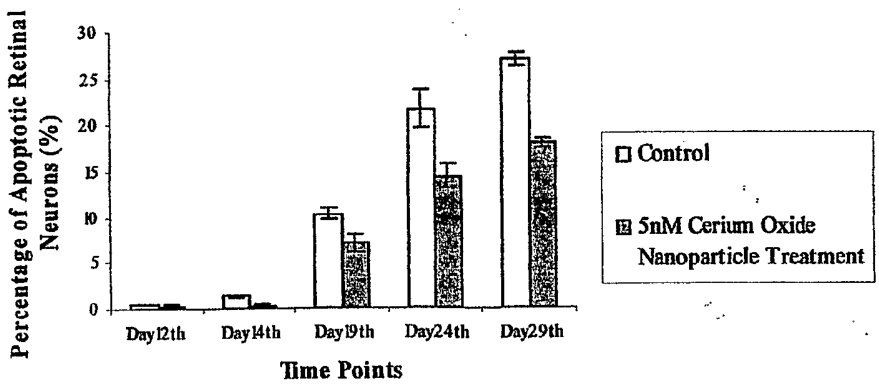
Percentage of Apoptotic Retinal Neurons (%)
30
25
20
15
10
5
0
Day12th
Day14th
Day19th
Day24th
Day29th
Time Points
Control
5nM Cerium Oxide Nanoparticle Treatment

Fig. 2

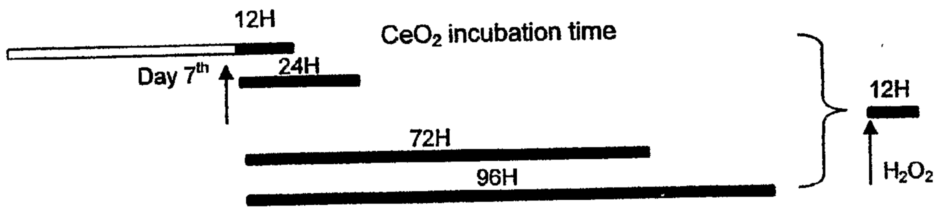

Fig. 3

Control

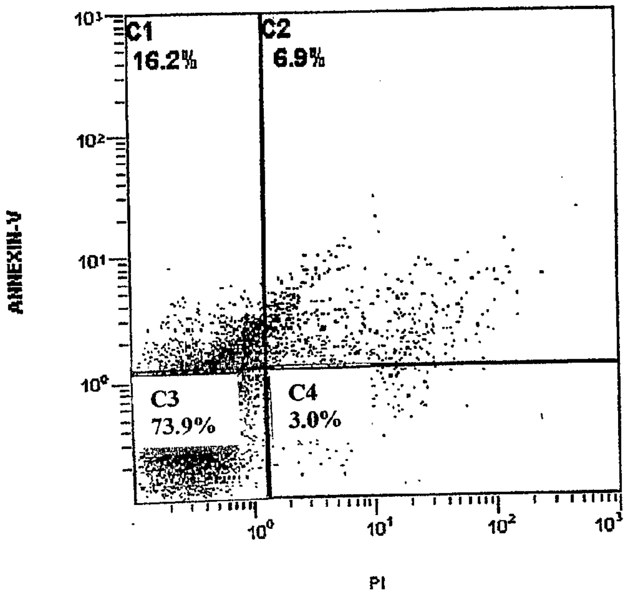

Fig. 4A

100µM H_2O_2

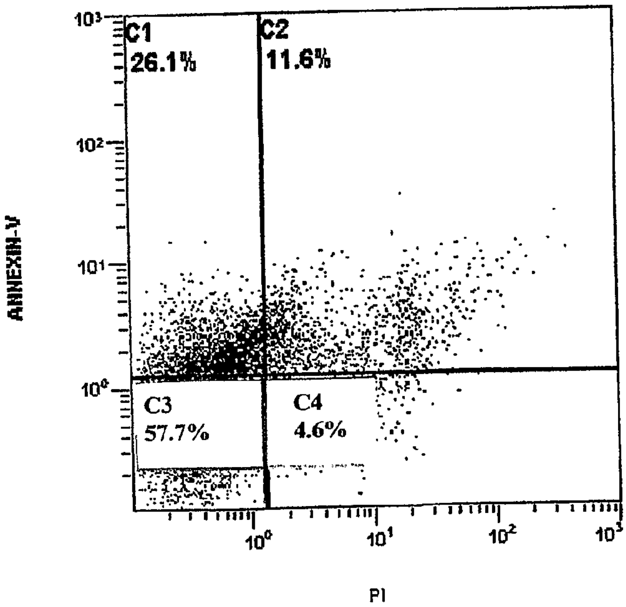

Fig. 4B

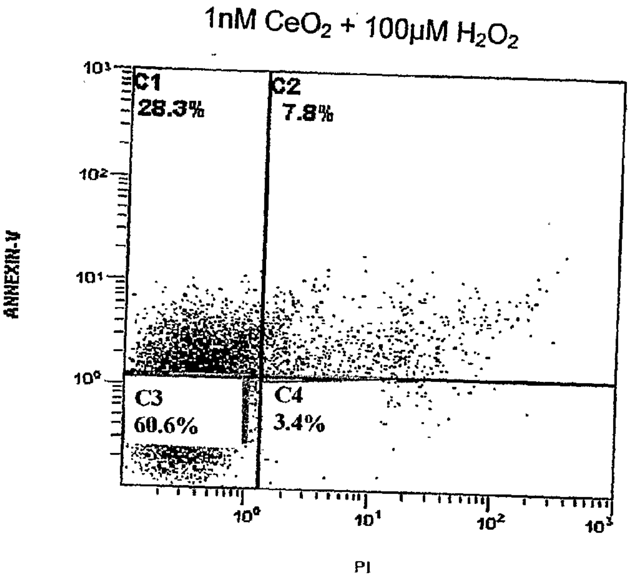
1nM CeO2 + 100µM H2O2
C1
28.3%
C2
7.8%
C3
60.6%
C4
3.4%
ANNEXIN-V
PI
10^0
10^1
10^2
10^3

Fig. 4C

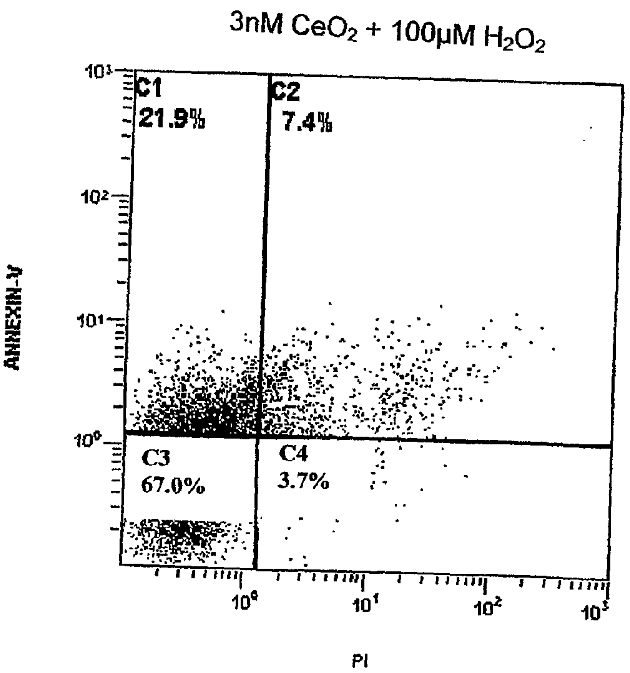
3nM CeO2 + 100µM H2O2
C1
21.9%
C2
7.4%
C3
67.0%
C4
3.7%
ANNEXIN-V
PI
10^0
10^1
10^2
10^3

Fig. 4D

5nM CeO2 + 100µM

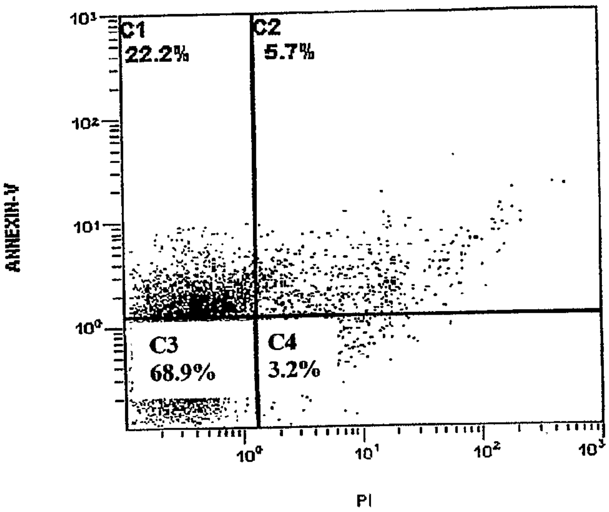

Fig. 4E

10nM CeO_2 + 100µM H_2O_2

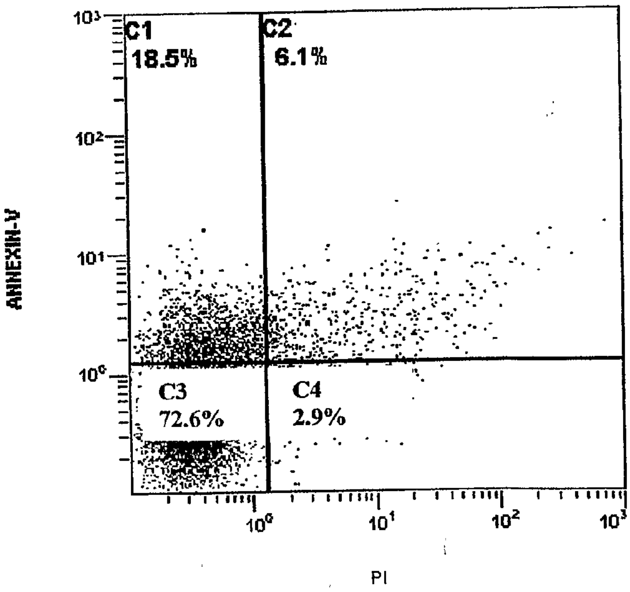

Fig. 4F

20nM CeO_2 + 100μM H_2O_2

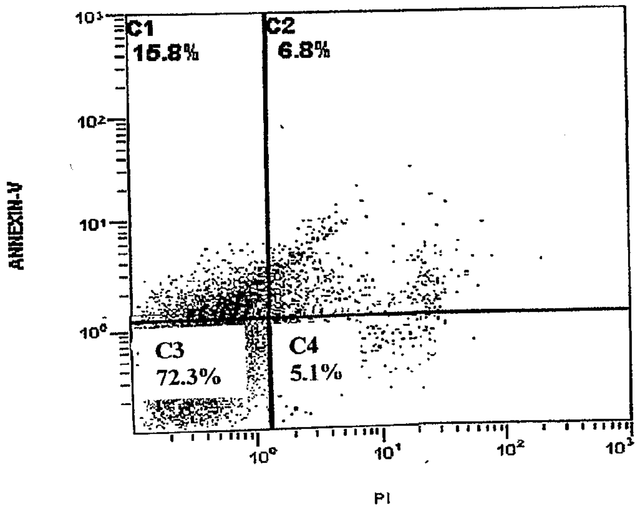

Fig. 4G

20nM CeO_2

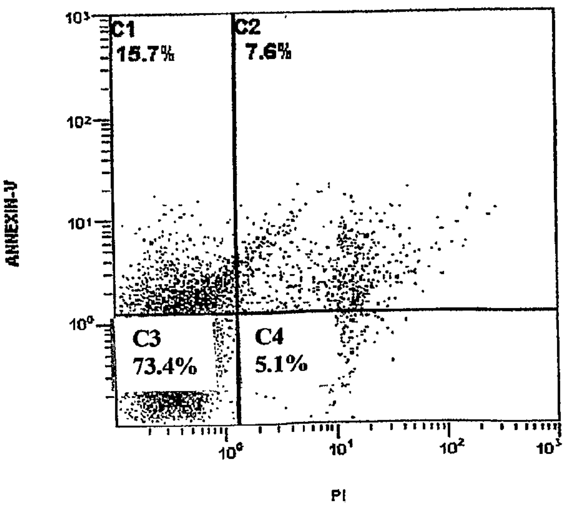

Fig. 4H

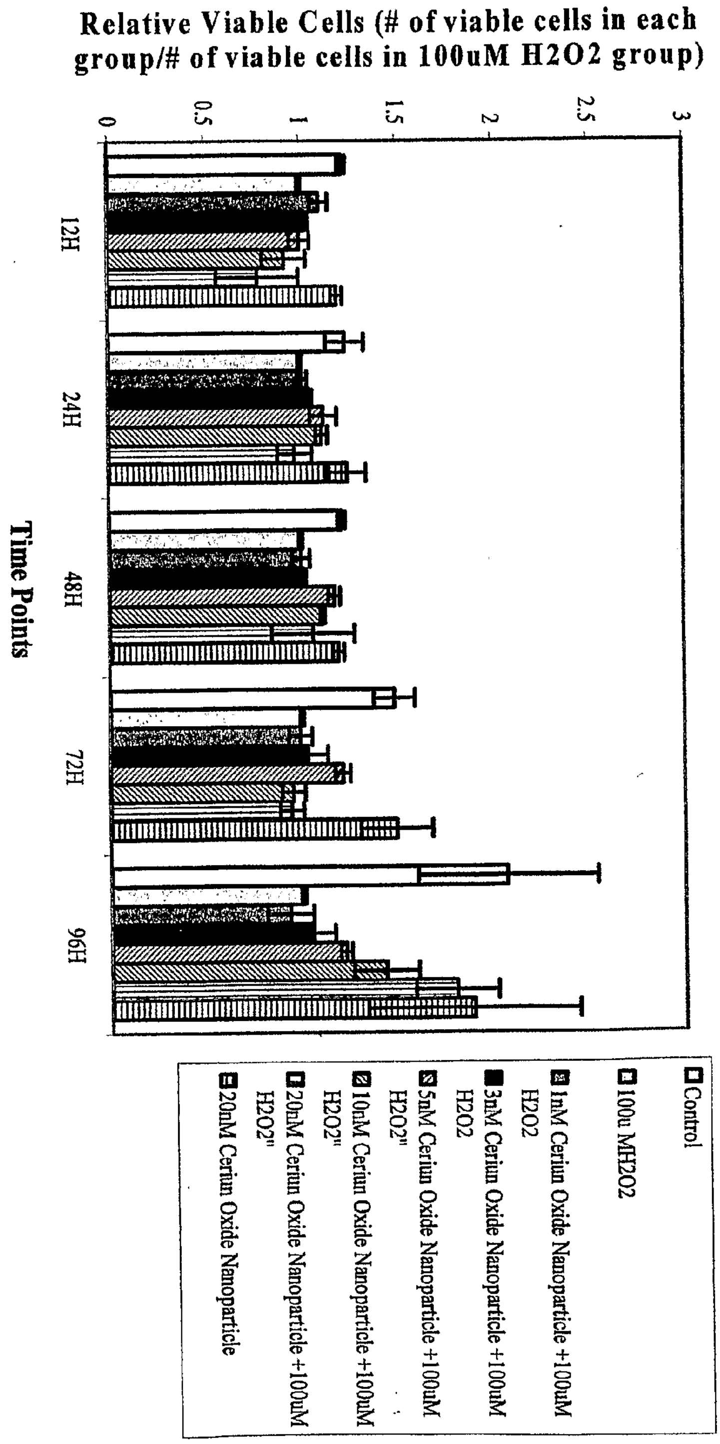
Relative Viable Cells (# of viable cells in each group/# of viable cells in 100uM H2O2 group)
0
0.5
1
1.5
2
2.5
3
12H
24H
48H
72H
96H
Time Points
Control
100u MH2O2
1nM Cerium Oxide Nanoparticle +100uM H2O2
3nM Cerium Oxide Nanoparticle +100uM H2O2
5nM Cerium Oxide Nanoparticle +100uM H2O2"
10nM Cerium Oxide Nanoparticle +100uM H2O2"
20nM Cerium Oxide Nanoparticle +100uM H2O2"
20nM Cerium Oxide Nanoparticle

Fig. 5

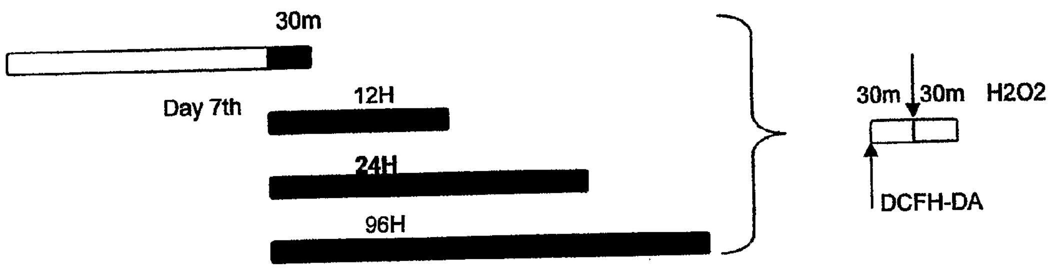

Fig. 6

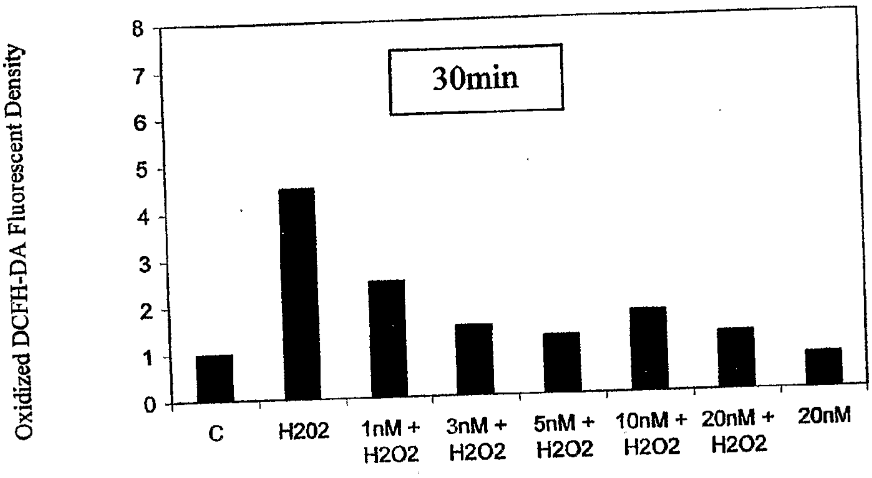
Oxidized DCFH-DA Fluorescent Density
30min
0
1
2
3
4
5
6
7
8
C
H202
1nM + H2O2
3nM + H2O2
5nM + H2O2
10nM + H2O2
20nM + H2O2
20nM

Fig. 7A

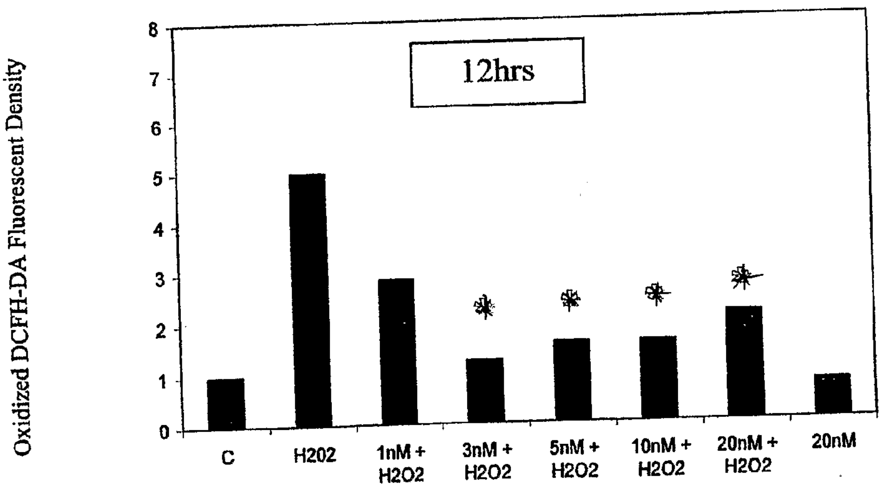
Oxidized DCFH-DA Fluorescent Density
12hrs
0
1
2
3
4
5
6
7
8
C
H202
1nM + H2O2
3nM + H2O2
5nM + H2O2
10nM + H2O2
20nM + H2O2
20nM

Fig. 7B

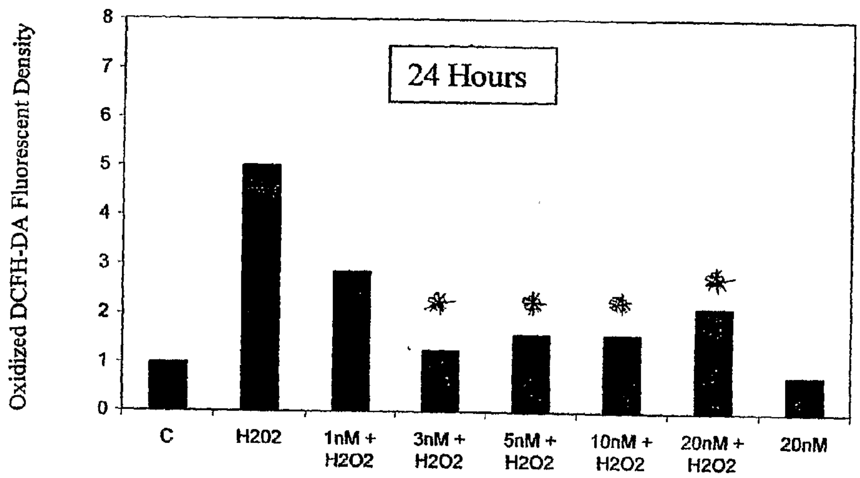
24 Hours
Oxidized DCFH-DA Fluorescent Density
8
7
6
5
4
3
2
1
0
C
H202
1nM + H2O2
3nM + H2O2
5nM + H2O2
10nM + H2O2
20nM + H2O2
20nM

Fig. 7C

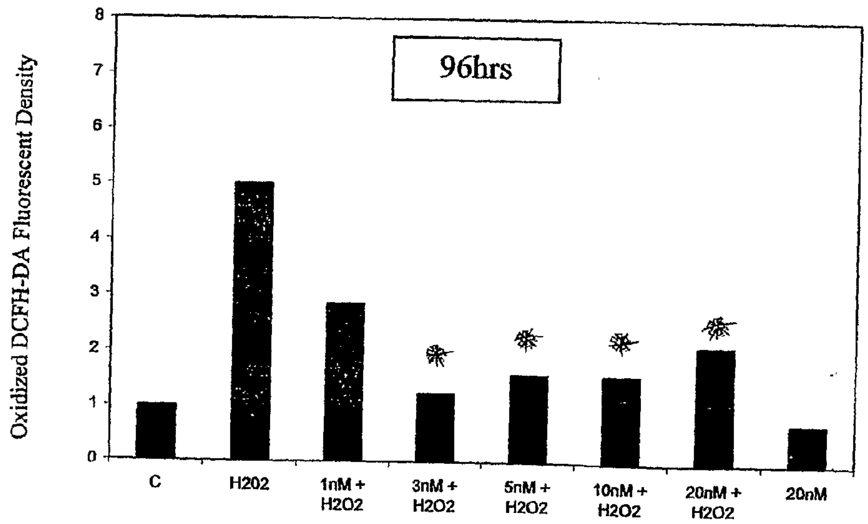
96hrs
Oxidized DCFH-DA Fluorescent Density
8
7
6
5
4
3
2
1
0
C
H202
1nM + H2O2
3nM + H2O2
5nM + H2O2
10nM + H2O2
20nM + H2O2
20nM

Fig. 7D