Cell apoptosis inducer containing chlorine dioxide and application thereof to preparation of cosmetics, or anti-aging or antineoplastic drugs

Patent CN 103720709 A

Abstract
The invention relates to a cell apoptosis inducer containing chlorine dioxide, and further relates to a cell apoptosis inducer kit which comprises two independent components: a first component: chlorine dioxide precursor solid, or a solution containing a chlorine dioxide precursor; a second component: a water solution of an acidity pH adjuster; the two components are stored independently, and can be mixed before use to react with each other on site, so as to prepare the cell apoptosis inducer containing chlorine dioxide; moreover, the amount and concentration of the first component and the second component enable the pH of the mixed solution to be 1.5-6.5. The invention further relates to application of the cell apoptosis inducer containing chlorine dioxide or the cell apoptosis inducer kit containing chlorine dioxide to the preparation of drugs for treating tumors or anti-aging drugs for target tissues of mammals or chemotherapeutic drugs, or being used as cosmetics.

Claims(8) translated from Chinese

A chlorine dioxide comprising apoptosis inducing agent.

Apoptosis inducing agent according to claim 1, which comprises dissolving in water chlorine dioxid e, wherein the chlorine dioxide concentration of 500-2900ppm, based on the quality calculated. Apoptosis inducing agent according to claim 2, further comprising an acidic pH adjusting agent suc h that the pH of apoptosis inducer = 1.5-6.5, the acidic pH adjusting agent selected from at least one of the following set : an organic acid or a salt thereof, which is selected from formic acid, acetic aci d, propionic acid, butyric acid, lactic acid, pyruvic acid, citric acid, malic acid, tartaric acid, gluconi c acid, glycolic acid, fumaric acid, malonic acid, maleic acid, oxalic acid, succinic shoot acid, acryli c acid, crotonic acid, acid, and salts thereof garrison set composed; inorganic acid or a salt thereof s elected from hydrochloric acid, phosphoric acid, boric acid, metaphosphoric acid, pyrophosphoric a cid, an amino acid, phosphoric acid dihydrogenphosphate, hydrogen phosphate composition set. Apoptosis inducing agent according to claim 3, wherein the pH adjusting agent is selected from citri c acid, acetic acid or dihydrogen phosphate.

A chlorine dioxide comprising apoptosis inducing agent kit comprising two separate components: th e first component: chlorine dioxide precursor solid, or a solution containing chlorine dioxide precur sor; first two component: aqueous acidic PH adjusting agent; both stored separately and can be mix ed prior to use to on-site response and prepared contain chlorine dioxide apoptosis-inducing agent; a nd the first component and a second group the amount and concentration points can make the mixed solution PH = L 5-6.5; wherein said chlorine dioxide precursors is selected from sodium chlorite, po tassium chlorite, chlorite, lithium chlorite, calcium chlorite at least one magnesium or barium chlori te; and wherein each of said acidic PH adjusting agent is selected from the set of at least one of the f ollowing: an organic acid or a salt thereof, which is selected from formic acid, acetic acid, propionic acid, butyric acid, lactic acid , pyruvic acid, citric acid, malic acid, tartaric acid, gluconic acid, glyco lic acid, fumaric acid, malonic acid, maleic acid, oxalic acid, succinic shoot acid, acrylic acid, croto nic acid, garrison acid, and their salts inorganic acid or salt thereof, which is selected from hydrochl oric acid, phosphoric acid, boric acid, metaphosphoric acid, pyrophosphoric acid, sulfamic acid, dih

ydrogen phosphate, hydrogen phosphate composition set; set.

Containing chlorine dioxide kit apoptosis inducing agent apoptosis inducing agent 5 or 6 according to any one of claims 1-4 in claim 1 for the manufacture of a medicament inducing apoptosis of. Containing chlorine dioxide apoptosis inducer kit 5 Preparation of a medicament for the treatment o f tumors of use or apoptosis inducers containing chlorine dioxide as claimed in any one of claim 1-4 requirements, or for the preparation of a mammalian target tissue for anti-aging drug use, or as cos metic purposes, or for the preparation of chemotherapeutic agents.

Use of claim 7, wherein the tumor include: brain metastases, meningiomas, tumors of the skull, brai n, pituitary adenomas, acoustic neurinoma, glioma, brain tumor; maxillary sinus carcinoma, larynx cancer, nasopharyngeal cancer, tongue cancer, thyroid cancer, gum cancer, lip cancer; thymoma, lun g cancer, adenocarcinoma, breast sarcoma, lung metastases, breast fibroids, breast cancer; pancreati c cancer, stomach cancer, gallbladder cancer, rectal cancer, pancreatic cancer, esophageal cancer, co lon cancer, liver cancer; renal pelvis cancer, penile cancer, urothelial carcinoma, prostate cancer, uri nary tract cancer, testicular cancer, bladder cancer, Wilms' tumor, kidney cancer; ovarian cancer, fall opian tube cancer, vulvar cancer, vaginal cancer, uterine cancer, cervical cancer, choriocarcinoma, p elvic cancer; skin cancer, liposarcoma, malignant teratoma, fibroma, neurofibroma, melanoma, bile duct cancer, squamous cell carcinoma, basal cell carcinoma; chordoma, bone, cartilage, osteosarco ma, synovial sarcoma, giant cell tumor of bone fibrosarcoma; acute leukemia, malignant lymphoma, chronic leukemia; hepatic hemangioma, islet cell carcinoid, neuroblastoma, myxoma neck metastati c cancer, cardiac; independently, wherein the anti-aging medicine, including prevention memory los s, insomnia, Alzheimer's disease, Parkinson's disease, osteoporosis, diabetes or cardiovascular disea ses: Independent , in which the cosmetic include skin whitening, skin water retention, reduce or eli minate wrinkles, elasticity enhancement, acne, freckle, remove scars or cosmetic skin rejuvenation.

Description translated from Chinese

Containing chlorine dioxide apoptosis-inducing agent and its use in the manufacture of cosmetic or anti-aging or anti-tumor drugs

Technical Field

[0001] The present invention belongs to the field of anti-aging and cancer treatment, particularly to apoptosis by inducing senescence and tumor cells, so old and methods for treating anti-tumor, and c hlorine dioxide for the manufacture of mammalian apoptosis-inducing agent apply cosmetics or dru gs.

Background

[0002] aging body can not be equated to cell aging, but the former is the cumulative result of the latt er, and therefore the aging body can be used to describe the percentage of senescent cells to some ex tent. The total body cells constantly aging and death, and also the proliferation produce new cells to compensate. If the proliferation of new cells produced not only the full complement of dead cells, a nd the ability to replace the aging cell fraction, then the body will show more young. Studies have s hown that: Lifetime remove senescent cells, can delay age-related phenotypes time originally appea red. And, later removal will delay the development has occurred with age-related disorders. This su ggests that senescent cells can indeed cause age-related phenotypes, and their clearance can prevent or delay age-related tissue function loss (Baker DJ, et al.Clearanceof pl6Ink4a-positive senescent ce lls delays ageing-associated disorders.Nature , 2011,479: 232-236). It can be said, clearing senescen t cells, reducing the proportion of senescent cells, the body will be able to show the younger, and ca n prevent or delay age-related tissue function loss. Senescent cells apoptosis is essentially dead. (Wa ng Hui Xiang et al., "Free radicals and apoptosis," "Biochemistry and Biophysics", 1996; 23:12).

[0003] The aging does not mean dying, if the periodic replacement of the culture medium, is still th e long-term survival of senescent cells (Seshadri T, Campisi J.Repression of c-fos transcription and an altered geneticprogram in senescent human fibroblasts.Science, 1990Janl2; 247 (4939): 205-9). However, inhibition of cell proliferation and senescence is irreversible, its growth arrest in the Gl ce ll cycle phase and can no longer enter S phase. This suggests that aging cells may actively proliferat ing young cells than anti-apoptotic, so long-term survival (Huang Ying, Tan Tong Zhangzong Yu Ju n, "people aged fibroblasts apoptosis inducible", "Chinese Journal of Medicine", 2000 In June Vol. 19, No. 3).

[0004] Thus, in theory, to find an anti-aging ideas, that by inducing apoptosis of senescent cells, sen escent cells promote apoptosis, dying, so that it can clear out from the body. Machine experience au tomatically start stem cell regeneration, regeneration of new cells to replace cells are cleared, so that the body younger.

[0005] conscious senescence induced apoptosis, is to remove senescent cells the most appropriate w ay of thinking. Remove excess during embryonic development through apoptosis and cell mission h as been completed, to ensure the normal development of the embryo; in adulthood remove aging an d diseased cells through apoptosis, ensuring the health of the body. For example, in studies in mice we found that when the young Stem cells are placed in a young microenvironment, they can rejuven ate (Rescuing replicationand osteogenesis of aged mesenchymal stem cells by exposure to a younge xtracellular matrix Yun Sun, et al.The FASEB Journal, May 2011, vol.25n0.51474-1485), namely y ounger, said regeneration ability. From another aspect of the understanding, the organization to rem ove senescent cells, due to the presence of specific induction of space, the organization will automat ically regenerate stem cells, tissue regeneration more youthful, so the body's overall performance fo r the young.

[0006] There are many studies indicate delayed apoptosis helps the body associated with aging loss of tissue function. For example, the mark is also characterized by DNA fragmentation Apoptosis, D NA double-strand breaks (DNA double-strand breaks) changes in the relationship between brain acti vity showed that mice: This DNA molecules break even in completely healthy mice neurons also wi ll occur in the process of adaptation to the new environment of mouse brain, DNA breakage rate is t o stay in the old environment that does not move the mice six times (Elsa Suberbielle, et al.pHysiol ogic brain activity causes DNA double-strandbreaks in neurons, with exacerbation by amyloid-β Na ture Neuroscience 16, 613 -621 (2013)). Clearly mice adapt to the new environment more memory and thinking ability, suggesting that the apoptotic process of DNA double-strand breaks can promot e brain younger mice.

[0007] autophagy promotes the ultimate degradation of apoptosis, senescence is the final step in cell clean. Increased autophagy has anti-aging effect (Louis R.Lapierre, et al.The TFEB orthologue HL H-30regulates autopHagy and modulates longevity in Caenorhabditis elegans, NatureCommunicatio ns.4, Article number: 2267). Factors that can cause cellular oxidation can cause apoptosis. Through the study of macrophages and monocytes, Albina also put forward NO apoptosis inducing agent (Al binaJE, Cui S, Mateo RB et al.Nitric oxide-mediated apoptosis in murine peritonealmacropHages.J Immunol, 1993 Jun I; 150 (11): 5080-5) "

[0008] Nitric oxide (NO) for many diseases of aging have a therapeutic effect, for example ,, nitric oxide can prevent Parkinson's disease leads to nerve cell abnormalities role of nitric oxide which pr omotes the decomposition of protein waste, played a role in protecting neurons (Kentaro Ozawa, et al.S-nitrosylation regulates mitochondrialquality control via activation of parkin Scientific Reports3, Articlenumber:. 2202 (July2013) nitric oxide diseases of aging such mitigation mechanism seems t o promote senescence apoptotic cells.

[0009] However, in reality, it did not `how to safely and easily find targeted remove senescent cells, leaving the body rejuvenation technology.

[0010] Chinese Patent Application No. 201110102598.1 of "red sandalwood extract with a combina

tion of nitric oxide in skin care products" mentioned: Nitric oxide itself promote skin regeneration, aging resist a good effect, but also increase the skin of traditional cosmetics absorption of active ing redients.

[0011] Patent Application No. 201210499300.X "a promoter and mammalian stem cells for use in t he manufacture of chlorine dioxide start mammalian stem cell drugs", have the use of chlorine dioxi de used to start stem cells to promote regeneration , but it did not mention how to remove senescent cells make tissue rejuvenation mechanism, nor be used in pharmaceutical or cosmetic anti-aging ap plications. Although the patent mentioned mechanism based on stem cell regeneration to treat some chlorine dioxide senile diseases, but did not give more programs in the prevention of these diseases.

[0012] The root causes of cancer and aging are the same (Carlos Lopez-Otin, et al.The Hallmarks of Aging.Cell, 6June2013). Induce apoptosis of tumor cells have become an important way to treat can cer. If the tumor cells seen in senescent cells, so by inducing apoptosis of tumor cells to treat cancer by inducing apoptosis ideas and senescence to anti-aging idea is the same.

[0013] The tumor cells typically have some mechanism of apoptosis resistance, the use of cell apopt osis mechanism for tumor therapy is the regulation of apoptosis in each level to make pro-apoptotic and anti-apoptotic balance changes, inducing tumor cell wither death.

[0014] Although studies on the mechanism of the classical pathway of apoptosis last ten years has b een great progress, but can exact a detailed explanation of apoptosis, especially the mechanism of a poptosis in mammalian cells is not much, is now generally accepted in There are two: the death rece ptor pathway and mitochondrial pathway.

[0015] For tumor cell specific pH environment, studies have shown that sodium bicarbonate increas es the pH value of the suppression of the spontaneous tumor metastasis month (Ian F.Robey, et al.Bi carbonate Increases Tumor pH and InhibitsSpontaneous Metastases Cancer Res Marchl5, 200 969; 2260), but its effect on tumor cell apoptosis induced by itself is not formed.

[0016] existing tumor cell apoptosis inducing agent has the following disadvantages: the worship of sexual ambiguity, side effects, treatment for complicated and high uncertainty, can cause cancer cell s to resist, only a few types of cancer cells effectively .

[0017] If you induce tumor cell apoptosis is thinking right treatment of cancer, we need to look for a more targeted, less toxic side effects, more universal apoptosis inducing agent. [0018] Chlorine diox ide is internationally recognized as the current generation of high efficiency, broad-spectrum, safe st erilization, preservative, it is the best alternative to chlorine preparations, in developed countries has been widely used. Relevant organizations the United States, Western Europe, Canada, Japan and oth er developed countries such as the US Environmental Protection Agency, the Food and Drug Admin istration, the US Department of Agriculture have approved and recommended chlorine dioxide for d isinfection of food, food processing, pharmaceuticals, hospitals, and other public environment , mil dew and other preservation and food. World Health Organization (WHO) and the World Food Orga nization (FAO) also has chlorine dioxide as a safe and effective disinfectant Al level. To control the water produced in the "three-induced substance" (carcinogenic, teratogenic, mutagenic), the Europe an and American countries have been widely used alternative to chlorine dioxide chlorine to disinfe ct drinking water. But chlorine dioxide as a medicine has not yet been accepted by the market. How ever, there are some patents related to the use of chlorine dioxide for cosmetic or therapeutic use dis orders (e.g., CN102137651A, CN101641104A and CN1199633C, US5750108, CN102441006A), th ese patents have not found chlorine dioxide, or an acidic solution containing chlorine dioxide It has the potential to remove senescent cells.

[0019] Summary of the Invention

[0020] a first aspect, the present invention relates to a chlorine dioxide comprising apoptosis induci ng agent.

[0021] In a preferred embodiment, the apoptosis-inducing agent comprising chlorine dioxide dissol

ved in water, wherein the chlorine dioxide concentration of 500-2900ppm, based on the quality calc ulated.

[0022] In a more preferred embodiment, the apoptosis-inducing agent of the present invention furth er comprises an acidic pH adjusting agent so that the apoptosis inducing agent PH = L 5-6.5, the aci dic pH adjusting agent is selected from Since each of the set of at least one of the following:

[0023] an organic acid or a salt thereof, which is selected from formic acid, acetic acid, propionic ac id, butyric acid, lactic acid, pyruvic acid, citric acid, malic acid, tartaric acid, gluconic acid, glycolic acid, fumaric acid, malonic acid, set composed of maleic acid, oxalic acid, succinic acid, acrylic aci d, crotonic acid, glutaric acid and salts thereof;

[0024] The inorganic acid or salt thereof, selected from hydrochloric acid, the set consisting of phos phoric acid, boric acid, metaphosphoric acid, pyrophosphoric acid, sulfamic acid, dihydrogen phosp hate, hydrogen phosphate.

[0025] Most preferably, wherein the pH adjusting agent is selected from citric acid, acetic acid or di hydrogen phosphate.

[0026] a second aspect, the present invention relates to a chlorine dioxide comprising apoptosis ind ucing agent kit comprising two separate components:

[0027] First component: a solid body before the chlorine dioxide, chlorine dioxide, or former memb er comprising a solution;

[0028] Second Component: an aqueous solution of acidic pH adjusting agent;

[0029] both stored separately, and can be mixed prior to use to on-site response and prepared contai ning chlorine dioxide apoptosis inducing agent; and the amount and concentration of the first comp onent and a second component enables the mixing PH after solution = L 5-6.5;

[0030] wherein before the chlorine dioxide is selected from at least one of sodium chlorite, potassiu m chlorite, chlorite, lithium chlorite, calcium chlorite, magnesium or barium chlorite;

[0031] wherein the acidic pH adjusting agent is selected from the following set of at least one of:

[0032] an organic acid or a salt thereof, which is selected from formic acid, acetic acid, propionic ac id, butyric acid, lactic acid, pyruvic acid, citric acid, malic acid, tartaric acid, gluconic acid, glycolic acid, fumaric acid, malonic acid, set composed of maleic acid, oxalic acid, succinic acid, acrylic aci d, crotonic acid, glutaric acid and salts thereof;

[0033] an inorganic acid or a salt thereof, which is selected from hydrochloric acid, the set consistin g of phosphoric acid, boric acid, metaphosphoric acid, pyrophosphoric acid, sulfamic acid, dihydrog en phosphate, hydrogen phosphate.

[0034] a third aspect, the present invention relates to the use of chlorine dioxide comprising said ap optosis inducing agent comprising chlorine dioxide or said inducer of apoptosis kit for the preparati on of a medicament to induce apoptosis. Preferably, the cell is a mammalian cell.

[0035] The fourth aspect, the present invention relates to the use of chlorine dioxide comprising sai d apoptosis inducing agent comprising chlorine dioxide or said inducer of apoptosis kit for the prep aration of a medicament the treatment of tumors, or for the preparation of lactation animal target tis sue anti-aging drug use, or as cosmetic purposes, or for the preparation of chemotherapeutic agents.

[0036] DETAILED DESCRIPTION

[0037] On the basis of prior art research, an object of the present invention is to provide a chlorine d ioxide used in pharmaceutical or cosmetic preparation for mammalian apoptosis-inducing agent of. According to the application, for anti-aging drugs or cosmetics containing chlorine dioxide have ap optosis-inducing agent, the drug or cosmetic can remove senescent cells in the target tissue, promot e the body's regeneration, regeneration young organization, in order to achieve anti-aging effect. Th

e application has little side effects or no side effects.

[0038] Another object of the present invention is to provide a chlorine dioxide used in preparation o f a medicament for the apoptosis-inducing agent of the mammal. According to the application, for ` promote mammalian tumor cell apoptosis drug-containing chlorine dioxide apoptosis inducing agen t, the drug can be targeted to specific induction of tumor cell apoptosis, to achieve better cancer trea tment and with little side effects or no side effects, less burden on the patient.

[0039] To achieve the above object, the technical scheme of the present invention is used: prepared containing chlorine dioxide apoptosis inducer mammalian target tissue and the step of administering an apoptosis inducing agent, wherein said apoptosis providing an effective amount of chlorine dioxi de when inducing agent is administered to a mammalian target tissue.

[0040] Further, an apoptosis inducing agent containing chlorine dioxide was prepared as follows: T he chlorine dioxide gas is dissolved in pH adjusting agents containing an acidic pH value of the acid ic solution A 1.5 ~ 6.5, a solution of 500 ~ 2900ppm chlorine dioxide solution.

[0041] Further, an apoptosis inducing agent containing chlorine dioxide was prepared as follows: T he chlorine dioxide precursor is dissolved in water to prepare a 1% to 40% aqueous solution, was ad ded to the aqueous solution containing an acidic pH adjusting agent acid solution B, the pH of the m ixed solution adjusted from 1.5 to 6.5.

[0042] Further, an apoptosis inducing agent kit containing chlorine dioxide was prepared as follows: The chlorine dioxide precursor is dissolved in water to a concentration of 1% to 40% aqueous solut ion of C; acidic pH adjusting agent Preparation of the acid solution was dissolved in water D; mixed before use solution C, D, adjusting the pH of a mixed solution of 1.5 to 6.5.

[0043] Still further, the chlorine dioxide precursor is sodium chlorite, potassium chlorite, chlorite, li thium chlorite, calcium chlorite, magnesium chlorite and barium.

[0044] Still further, an acidic pH adjusting agent is citric acid, acetic acid or sodium dihydrogen pho sphate.

[0045] Further, an apoptosis inducing agent through the arteries, intramuscular, subcutaneous, intrac ardiac, intrathecal, intra-articular injection, puncture, rectal, nasal, transdermal administration or inh alation to the drug directly to the organ or tissue damage or give to other target tissues.

[0046] Further, an apoptosis inducing agent may be prepared injections, ointments, inhalants, nasal drops, lotions, suppositories, patches, pastes, tablets, oral liquid, capsules, granules, powder , pills o r syrups.

[0047] The present invention provides a pharmaceutical preparation of chlorine dioxide for chlorine dioxide in the preparation of a mammalian apoptosis-inducing agents, and cosmetic applications, in which the drug or cosmetic for anti-aging mammalian target tissue make it younger.

[0048] Further, this anti-aging including skin rejuvenation namely skin care, body rejuvenation othe r organizations, such as adding memory, improve sleep therapy, prevention of Alzheimer's disease, Parkinson's disease, osteoporosis, diabetes, cardiovascular disease and other age-related diseases, in cluding, but not limited to these.

[0049] Furthermore, the preparation of chlorine dioxide in a mammalian apoptosis-inducing agent f or a pharmaceutical, cosmetic applications, wherein the medicament is for anti-aging body tissues, a nd the prevention of Alzheimer's disease, Parkinson's disease , osteoporosis, diabetes, cardiovascula r disease and other age-related diseases; the cosmetic skin care for skin, remove acne acne or may b e used in oral care mouthwash, including but not limited to these.

[0050] The present invention also provides a pharmaceutical in the manufacture of chlorine dioxide for inducing apoptosis in mammalian cells in the application, wherein the medicament is for the trea tment of cancer, particularly for the induction of apoptosis in mammalian cancer cells.

[0051] Further, by inducing apoptosis, in which the targeted cancer include:

[0052] brain metastases, meningiomas, tumors of the skull, brain, pituitary adenomas, acoustic neuroma, glioma, brain tumor; maxillary sinus cancer, laryngeal cancer, nasopharyngeal cancer, tongue cancer, thyroid cancer , gum cancer, lip cancer; thymoma, lung cancer, adenocarcinoma, breast sarcoma, lung metastases, breast fibroids, breast cancer; pancreatic cancer, stomach cancer, gallbladder cancer, colorectal cancer, pancreatic cancer, esophageal cancer, colon cancer, liver cancer; renal pelvis cancer, penile cancer, urothelial carcinoma, prostate cancer, urinary tract cancer, testicular cancer, bladder cancer, Wilms' tumor, kidney cancer; ovarian cancer, fallopian tube cancer, vulvar cancer, vaginal cancer, uterine cancer, cervical cancer choriocarcinoma, pelvic cancer; skin cancer, liposarcoma, malignant teratoma, fibroma, neurofibroma, melanoma, bile duct cancer, squamous cell carcinoma, basal cell carcinoma; chordoma, bone, cartilage, osteosarcoma , synovial sarcoma, giant cell tumor of bone fibrosarcoma; acute leukemia, malignant lymphoma, chronic leukemia; hepatic hemangioma, islet cell carcinoid, neuroblastoma, myxoma, neck metastasis, cardia. Including but not limited to these cancers.

[0053] Further, the chlorine dioxide in the preparation of the present invention for inducing apoptosis in mammalian cells, the drug application, the application of the drug can be administered at risk of developing cancer was diagnosed with cancer in cancer treatment during or after cancer treatment in individual subjects during recovery, or the drug can be administered as a prophylactic agent of the subject individual to prevent or delay

The development of cancer.

DETAILED DESCRIPTION

[0054] In the following with reference to specific embodiments of the present invention will be further described below.

[0055] The present invention is defined as the apoptotic cell death and degradation cleared without strict distinction belongs to apoptosis (apoptosis), necrosis (necrosis) or from the Liao Jie (autophagy). The present invention induce cell senescence and apoptosis refers to induce senescence in cell death, and tumor cell death and degradation cleared, and the degradation of clear, without careful study of the way and the process of dying. [0056] Before describing particular embodiments of the present invention, the first mechanism of the present invention to provide a method will be described. These are described further in connection with specific examples will help to understand those skilled technical solutions and the protection scope of the invention.

[0057] Studies have shown that: Lifetime remove senescent cells, can delay age-related phenotypes time originally appeared. And, later removal will delay the development has occurred with age-related disorders. This suggests that senescent cells can indeed cause age-related phenotypes, and their clearance can prevent or delay age-related tissue function loss ° (Baker DJ, et al.Clearance of pl6Ink4a-positive senescent cells delaysageing-associated disorders. Nature, 2011, 479: 232-236). It can be said, clearing senescent cells, reducing the proportion of senescent cells, the body will be able to show the younger, and can prevent or delay age-related tissue function loss. Senescent cells apoptosis is essentially dead. (Wang Hui Xiang et al., "Free radicals and apoptosis," "Progress in Biochemistry and Biological Physics", 1996; 23: 12).

[0058] autophagic degradation promote apoptosis, so it can play a clear role in aging cells. The study found that memory loss can be achieved by eating flies polyamine-rich foods reversed. Hey Drosophila eat polyamines can enhance the life of the organism. It seems that autophagy by reversing age-related decline to work. Autophagy means the cells used to clear the debris of the mechanism itself. By gene technology or calorie restriction increase autophagy, it is possible to extend the life of fruit flies. (VarunK Gupta, et al.Restoring polyamines protects from age-1nduced memory impairmentin an autopHagy-dependent manner.Nature Neuroscience, 01 September 2013).

[0059] ROS can induce high levels of apoptosis (Lau AT, Wang Y, Chiu JF.Reactive oxygenspecies:

current knowledge and applications in cancer research and therapeutic.J Cell Biochem.2008Mayl5; 104 (2): 657-67) . Studies have shown that when the production of nitric oxide nematodes feed on b acteria, the life has been significantly increased (Ivan Gusarov, et al.Bacterial Nitric Oxide Extends theLifespan of C.elegans Cell, Volume 152, Issue 4,818-830,14February2013). This shows that the oxide can promote apoptosis.

[0060] Although anti-aging antioxidant effective as the standard has always existed, but in reality ve ry little evidence to support. If you can not promptly remove defective protein, it will lead to the los s of homeostasis within proteins, triggering aging-related diseases. For example, in Alzheimer's dise ase, you can not remove the proteins form plaques, leading to neuronal death. Although the number of free radicals may be harmful, but their presence will trigger a protective response. There is no ge netic evidence shows that enhance the body's antioxidant defense can delay aging. (Carlos Lopez-O tin, et al.The Hallmarks of Aging.Cell, 6June2013).

[0061] In some embodiments, the present invention also proved a strong oxidizing substances (chlor ine dioxide) can promote apoptosis, and helps the body younger.

[0062] On the other hand, in the apoptosis of growth factor-dependent cellular processes within the ubiquitous intracellular acidification, intracellular acidification is an intracellular signal changes dur ing apoptosis, which promote apoptosis has been following facts supported: ① specific Na + / H + e xchange inhibitor by inhibiting Na + / H + exchanger intracellular acidification may induce apoptos is, in addition to Na + / H + exchanger outside, Na + -HCCV collaborative transportation with the C 1_ / HC03_ switch changes also closely related with apoptosis, dose-effect relationship exists cytopl asmic acidification degree and incidence of apoptosis; ② pure alkali treatment by reducing the degr ee of intracellular acidification exhibit against inducers of apoptosis; ③ cut memory cells in the acid ic nucleases, intracellular acidification can activate the enzyme, triggering nucleosome DNA cleava ge enzyme is an important material basis for intracellular acidification mediated apoptosis; ④ many of the enzymes involved in apoptosis and enhanced activity of the protein in an acidic environment (side XIAO Hai et al., "Progress in the intracellular PH value in apoptosis" and "Changzhi Medical College" 2004 4).

[0063] senescence or apoptosis under acidic conditions are more likely to die. Apoptotic cell glycol ysis strengthened acidic metabolites increased, if the external pressure is applied acid (lower PH val ue), apoptotic cells will accelerate to death. For example, cardiac hypertrophy increased myocardial tissue load a defensive reaction to temporarily adapt or change the overall regional myocardial stres s, if desired physiological function of the heart more than the ability to adapt the compensatory myo cardial cells, myocardial cell death occurs suicidal That apoptosis, one of the major mechanisms of failure transformed from cardiac hypertrophy. When hypoxia, cardiac hypertrophy cell glycolysis ra te decreased slightly, but increased rapidly after reoxygenation glycolytic rate, it was the explosive kind of peak then gradually recover to the level before hypoxia. Increased glycolytic rate would ine vitably lead to intracellular lactic acidosis. When normoxia rate of apoptosis in cardiac hypertrophy was significantly higher than the normal cardiomyocytes, both apoptosis in hypoxia were significan tly increased after reoxygenation decreased apoptosis of normal myocardium, and myocardial hyper trophy in reoxygenation Early apoptotic rate continues to rise dramatically, then gradually decrease d. (Feng Bing, Liu Wei, Xu Jing, Zuo-yun, Yang standard, "cardiac hypertrophy apoptosis hypoxia r eoxygenation and energy metabolism pathways relations", ActapHysiologica Sinica, 0ctober25, 200 5, 57 (5): 636- 642)

[0064] Creatine (creatine) in biochemistry, is a naturally occurring vertebrates in a nitrogen-containi ng organic acids, it can assist in providing energy to muscle and nerve cells. The study found that or al creatine can improve G93A transgenic mice (amyotrophic lateral sclerosis) body performance an d extend the life of this kind of action presented dose-effect relationship, but also protects mice mot or neurons and substantia nigra neurons Damage (Klivenyi P, et al.Neuroprotective effects ofcreatin e in a transgenic animal model of amyotropHic lateral sclerosis..NatureMedicine.1999.mar, 5 (3): 3 47 - 350) "The study also found that lipoic acid (lipoic acid) slows the aging process.

[0065] anti-aging effects generally considered L- ascorbic acid is its antioxidant capacity. However, in 2009, an important laboratory experiments by Michael • Risto Jena University in Germany showe d ROS for the maintenance of essential basic functions of the liver. The results show that by increasi ng physical activity from the mitochondria (intracellular production tiny organ can produce the prin ciple part via redox chain) the electron transport chain system within ROS generation, to prevent the occurrence of type II diabetes. However, if at the same time taking antioxidants vitamin C and vita min E daily, exercise, there is no positive effect. February 2009 issue of "Clinical Nutrition" publish ed a review article summarizes the total number of participants up to hundreds of thousands of 22 ra ndomized double-blind study published concluded there was no support, "Antioxidants prevent coro nary artery disease "argument. And a review "Journal of the American Medical Association" publish ed in February 2007 more antioxidants to combat health care products. A total of 230,000 people in volved in the 68 studies summarized analysis of several antioxidants have no effect on mortality. Ex cluding those not high quality studies, only 47 high-quality studies totaling more than 180,000 peop le participate in the analysis, so these types of antioxidants and even a small increase in mortality. T hus, the present inventors believe that the anti-aging effects L- ascorbic acid in its acidic.

`[0066] more acidic substances beauty. For example, 30% higher concentration of salicylic acid che mical peels as an agent, and 70% can achieve the same dilute acid skin pigmentation spots, shrink p ores, remove fine wrinkles and improve the aging caused by the sun and many other effects . Skin c are is to make the skin younger, so generally it can be concluded, the acidic substance can remove s enescent cells, promoting younger skin regeneration. Medium and high doses of citric acid treated mouse testis caused apoptosis (Mediate: Impact of citric acid on apoptosis of mouse testis [D]; Hen an University of Science and Technology: 2012).

[0067] The inventors suspect, L- ascorbic acid, creatine, lipoic acid and other acidic substances can be anti-aging effect, more because of its acidic character can accelerate aging apoptosis, causing the body younger. The inventors have surprisingly found that there is chlorine dioxide solution with a hi gh oxidation potential and can be supplemented by the acidic environment of anti-aging.

[0068] In some embodiments, the present invention is demonstrated by experiment, acidic environm ent of apoptosis contribute to improve the preparation of chlorine dioxide to the cells.

[0069] In the present invention, a plurality of embodiments, acidic chlorine dioxide solution we giv e the target tissue targeting, proven to the target tissue rejuvenation.

[0070] Tumor cells have ten characteristics: self-sufficiency in growth signals (Self-Sufficiency in GrowthSignals); anti-growth signals insensitive (Insensitivity to Anti growth Signals); resistance to cell death (Resisting Cell Death); unlimited potential ability to replicate (Limitless Replicative Pote ntial); sustained angiogenesis (Sustained Angiogenesis); tissue invasion and metastasis (Tissue Inva sion andMetastasis); avoid immune destroy (Avoiding Immune Destruction); promote tumor inflam mation (TumorPromotion Inflammation); Pan quantity abnormal cell compartment (Deregulating C ellular Energetics); genomic instability and mutation (Genome Instability and MutationXHanahan D, Weinberg RA.Hallmarksof cancer: the next generation.Cell.2011Mar4; 144 (5): 646_74). If you s uppress the resistance to cell death, which is the induction of apoptosis, to a large extent suppressed cancer. From Logically, the tumor cells are non-normal cells, the body's own innate should inhibit t hem, that is, should automatically apoptosis in these cells, why not with its own characteristics asso ciated apoptosis. The study found that some cancers may not be entirely attributed to genetic damag e, but tell them to bypass the senescent cells stop growing due to the switching behavior between ag ing and cancerous cells show similarities, if senescent cells managed to escape death They have the potential to become cancer (HazelA.Cruickshanks, et al.Senescent cells harbour features of the canc er epigenome, Nature Cell Biology (2013)). The new view is that the root causes of cancer and agin g are the same (CarlosLopez-Otin, et al.The Hallmarks of Aging.Cell, 6June2013). Tumor cells wer e regarded as the inventor of senescent cells, it was supposed to apoptosis. Therefore, the inventors believe that the tumor cells because of some special ability has allowed not to apoptosis, while long -term retention in the body.

[0071] For the two characteristics of tumor cells may be the best way to eliminate tumors. The two f eatures that resist cell death and abnormal cell energy. Reference to induce apoptosis in senescent ce lls ideas apply equally to tumor cells. Although the tumor cell has its own characteristics, general ca ncer patients is due to the presence of tumor cells in the body's environmental effects are not autom atic to death, but relative to normal cells, tumor cells themselves change, such as more afraid oxide, and more afraid of the acidic environment .

[0072] The oral sildenafil three cases of children suffering from cystic lymphangioma through medi cation, three tumor volume in children are significant narrowing (Glenda L.et al.Swetman, Sildenafi l for Severe LympHaticMalformations N Engl J Med2012; 366: 384_386January26, 2012). Sildenaf il is 5-phosphodiesterase inhibitors, phosphodiesterase NO-cGMP pathway is a negative regulatory factor, sildenafil can thus releasing the bioactive substance by nitric oxide. Accordingly, the present inventors believe that nitric oxide plays a direct role in inhibiting tumor.

[0073] In experiments on breast cancer and melanoma found that nitroglycerin can be enhanced thr ough the production of nitric oxide in general chemotherapy drugs to kill cancer cells (Barsoum IB, et al.Hypoxia induces escape frominnate immunity in cancer cells via increased expression of ADA MlO: role ofnitric oxide.Cancer Res.201 IDec15; 71 (24): 7433-41) "to promote the release of nitric oxide precursor drugs induce death of breast cancer cells, while leaving normal mammary epithelial cells (Vanity McMurtry, et al.JS-K, anitric oxide-releasing pro-drug, induces breast cancer cell deat h while sparingnormal mammary epithelial cells, International of journal of oncology, Publishedonli ne on: Tuesday, January25, 2011, Pages: 963-971) It is generally believed, a high concentration of n itric oxide synthase expression, tumor cells can inhibit the toxic effects of their growth (Xu W, et al. The role of nitricoxide in cancer.Cell Res.2002Dec; 12 (5-6) : 311-20).

[0074] Cell proliferation assays show, 400 μ mol / L, 800 μ mol / L, 1600 μ mol / L Hela cells in vit ro cytotoxic varying degrees, up 89.0 percent. After hydrogen peroxide treatment, the rate of cell se nescence and active oxygen levels with elevated concentrations of hydrogen peroxide increased, sh owing dose-effect relationship. Conclusion: hydrogen peroxide on Hela cells significantly cytotoxic, can induce senescence and influence production levels of reactive oxygen species. [0075] tumor cel ls and senescent cells, more rely on hypoxia glycolysis provide energy. As early as 80 years ago that cancer cells than normal cells glucose metabolism exuberant, and even under aerobic conditions is a lso dependent on glycolysis, the phenomenon known as the "Warburg effect" on their cell membran e mechanism of tumor cells glucose transporter protein (Glut) functionally active and hexokinase ac tivity enhancement. Results "Warburg effect" is a cell to produce a large number of glycolysis produ ct - lactic acid. Off from a large number of H + is not like aerobic oxidation of sugar as through the respiratory chain oxidation of water, a lot of H + accumulation will face the pH (pHi) acidification a nd threatening apoptosis in cells, and tumor cells increased acid secretion make Intracellular PHi m aintained, but the surrounding normal host cells would be adversely affected. Tumor cells "Warburg effect" tumor cells than normal cells to produce more acid, but the tumor cells detected PH value int racellular pHi no significant difference compared with normal cells, and extracellular pH (pHe) and intracellular acidic vesicles pH (ρHν) was significantly lower than in the normal cells, suggesting th at the tumor cells have a strong acid secretion function. (Izumi H, Torigoe T, Ishiguchi H, et al.Cellu larpH regulators: potentially promising molecular targets for cancer chemotherapy.Cancer Treat Rev, 2003,29 (6): 541-549). [0076] In order to avoid toxic acidic microenvironment, tumor cells then ou twardly discharged hydrogen ions, alkaline environment and eventually produce acidic intracellular environment extracellular. Tumor cells through upregulation of plasma membrane H + related trans port proteins, such as sodium hydrogen exchanger (Na + / H +), Na + / K + -ATPase, vesicle-type H + -ATPases, H + / CF symporter and monocarboxylate transporter protein (MCT), etc., to achieve t his mechanism (Harguindey S, et al.The role of pH dynamics andthe Na + / H + antiporter in the eti opathogenesis and treatment of cancer.Two facesof the same coin-one single nature, [J] .Biochim Bi opHys Acta, 2005,1756 (I): 1-24).

[0077] Accordingly, the inventors determine if tumor cells by increasing the pressure so that acid se cretion decreases, it will cause PHi reduce the tumor cells to acidosis, thereby inducing apoptosis of

tumor cells. The increase in pressure, even if the tumor extracellular environment pH (pHe) decreas ed, so that the tumor cells transport H + ions of the proton pump pressure increases, and eventually collapse.

[0078] has more support in the literature, the acidic environment can induce tumor cell apoptosis. F or example, acidic environment, through p53-mediated induction of human gastric adenocarcinoma cell line and apoptosis (AC Williams, et al.An acidic environmentleads to p53dependent induction o f apoptosis in human adenoma and carcinomacell lines: 1mplications for clonal selection during col orectal carcinogenesis, Oncogene.1999May27; 18 (21): 3199-204).

[0079] Experimental results show that: to give a pH of gastric cancer AGS cells cultured 6.0 acidic environment, such acidic environment makes extracellular H + ion concentration, may inhibit tumor cells of NHE-1 Na + -H + exchange feature enables excessive intracellular glycolysis H + ions can not be discharged to the already alkaline intracellular environment significantly acidification, both p layed a changing role of gastric extracellular environment, but also changed the intracellular enviro nment. This acidification of gastric cancer cells to change the pH of the internal and external enviro nment for its growth and proliferation of cells and external environment, so that the majority of gast ric cancer cells stop proliferation (Gao Yan et al., Measurement and acidity in the "pH value of AGS cells to their environment proliferation and apoptosis, "" Progress in Modern Biomedicine "2008 09).

[0080] Rich IN and other proven system and peripheral leukocytes obtained from blood cells in viv o lymphoid leukemia patients with normal hematopoietic cells than universal, high PH value statisti cally significant. It illustrates the existence of a direct relationship between the intracellular pH and normal hematopoietic cells and cell cycle regulation leukemia cells. With this relationship, with thei r Na + / H + exchanger inhibitor 5_ (N, N-hexamethylene) -amiloride (HMA) treated leukemia cells, reducing the intracellular pH value, thereby inducing apoptosis. (Rich IN, Worthington-White D, G arden 0A, MuskP.Apoptosis of leukemic cells accompanies reduction in intracellular pH aftertargete d inhibition of the Na + / H + exchanger.Blood.2000,15,95 (4): 1427 ~ 1434).

[0081] In one embodiment of the present invention, we used to give an acidic chlorine dioxide solut ion targeting tumor cells, tumor cell apoptosis, and with the strengthening of the acidic environment of the tumor cell death increased accordingly.

[0082] skilled in the art are understood strong oxidants and acidic environment damage effects on n ormal cells, that is, an acidic solution of chlorine dioxide to normal cells also accelerate apoptosis. However, the present inventors believe that the damage to normal cells will stimulate the body's reg enerative stem cells, thus completely general injury can be repaired; In addition, aging or cancer cel l is a cell dysfunction, they resist apoptosis induced by oxidant or an acidic environment ability wea ker. So aspect of the present invention is targeted specifically and with fewer side effects of an anti-aging or anti-cancer technology.

[0083] In some embodiments, the present invention demonstrates an acidic chlorine dioxide formul ation of senescent cells or tumor cell apoptosis induced by a stronger, but less damage to normal cel ls, or that this damage can be in make up the extent.

[0084] Chlorine dioxide-containing apoptosis-inducing agent according to the present invention ma y comprise a single unitary dosage form containing chlorine dioxide or a combination of several par ts of chlorine dioxide precursors. Wherein the single dosage form comprises chlorine dioxide alone may be prepared as follows: [0085] Method 1: pH adjusting agent added to the water to prepare an a cidic aqueous solution pH of 1.5 to 6.5. By normative, and the concentration of chlorine dioxide gas produced in the manufacture of more than 99.9% by way of chlorine dioxide gas (chlorite with an a cid preferably). Bubbled and dissolved to prepare a solution of 500 ~ 2900ppm chlorine dioxide by the chlorine dioxide gas to said acidic aqueous solution. The solution used in the present invention p rior to use, it should be sealed in the dark, and has been stored at 4 ° C ~ 15 ° C lower temperature s tate. The method of preparation of the formulation containing chlorine dioxide does not have to be a

solution, it can play only ensure the target tissue when administered to the target tissue for the role o f the main substance chlorine dioxide, and in certain acidic environment.

[0086] Method 2: The chlorine dioxide precursor is dissolved in a 1% to 40% aqueous solution prep ared in water. To this was added an acidic aqueous solution containing a PH adjusting agent (prefera bly from 2% to 50% citric acid solution), adjusting the pH of the mixed solution of 1.5 to 6.5. The s olution used in the present invention prior to use, it should be sealed in the dark, and has been store d at 4 ° C ~ 15 ° C lower temperature state. The method of preparation of the formulation containin g chlorine dioxide does not have to be a solution, it can play only ensure the target tissue when admi nistered to the target tissue for the role of the main substance chlorine dioxide, and in certain acidic environment.

[0087] chlorine dioxide precursors comprising a number of parts of the compositions can be prepare d as follows: The chlorine dioxide precursor is dissolved in a 1% to 40% aqueous solution prepared in water, which is the first solution; pH adjusting agent prepared by acidic solution (preferably from 2% to 50% citric acid solution), which is the second solution. Used in the present invention in use p rior to the above solution mixing site, the final adjustment of the mixed solution of PH value from 1. 5 to 6.5, until the reaction to produce chlorine dioxide, the solution was applied to the target tissue. The method of preparation of the formulation containing chlorine dioxide does not have to be a solu tion, it can play only ensure the target tissue when administered to the target tissue for the role of th e main substance chlorine dioxide, and in certain acidic environment.

[0088] As a precursor of chlorine dioxide can be used in the present invention include, for example alkali metal chlorite, alkali earth metal chlorite. As alkali metal chlorite include, for example sodiu m chlorite, potassium chlorite, lithium chlorite; as chlorite of alkali earth metal, such as chlorite incl ude calcium chlorite, magnesium, Asia barium perchlorate.

[0089] where, not only from the grounds of the ease, and the sustainability of chlorine dioxide from the viewpoint of excellent activity, preferably sodium chlorite, potassium chlorite, more preferably s odium chlorite.

[0090] As the pH adjusting agent can be used in the present invention, as long as the acid has a buff ering property can be very good use.

[0091] As the organic acid or its salt include formic acid, acetic acid, propionic acid, butyric acid, la ctic acid, pyruvic acid, citric acid, malic acid, tartaric acid, gluconic acid, glycolic acid, fumaric aci d, malonic acid, maleic acid, oxalic acid, succinic acid, acrylic acid, crotonic acid, glutaric acid, and their salts.

[0092] Examples of the inorganic acids include hydrochloric acid, phosphoric acid, boric acid, meta phosphoric acid, pyrophosphoric acid, an amino acid. Examples of the inorganic acid salts, for exa mple, dihydrogen phosphate (sodium salt, potassium salt, the same below), a mixture of dihydrogen phosphate and hydrogen phosphate and the like. PH adjusting agents may be used alone I species, a nd may be two or more kinds.

[0093] for use in the human body from safety considerations, pH adjusting agent is preferably citric acid, acetic acid and sodium dihydrogen phosphate, more preferably citric acid.

[0094] In addition, the final chlorine dioxide solution pH of preferably 1.5 to 5.5, more preferably 1. 5 to 3.5.

[0095] The present invention apoptosis-inducing agent is preferably a liquid containing chlorine dio xide.

Dose [0096] The present invention apoptosis-inducing agents due to age, body weight, disease and condition of the patient and the nature of the change. However, in the case of an adult, for example, I mg to 5000 mg per day of chlorine dioxide, preferably I mg per day

To 1000 milligrams of chlorine dioxide.

[0097] The present invention is an apoptosis inducing agent containing chlorine dioxide can be adm inistered in various ways. Containing chlorine dioxide apoptosis-inducing agents may be administer ed systemically, such as by intravenous or intraarterial intraperitoneal administration,; can also be a dministered locally directly to the affected area, the method can be by direct transdermal, puncture methods lesion administration.

[0098] The mode of administration of the present invention, an apoptosis inducing agent containing chlorine dioxide can be produced by any of the routes to reach the expected tissue, for example, ma y be via intravenous drip, intravenous injection, intraarterial injection, intramuscular injection, subc utaneous injection, intradermal injection, intracardiac injection, intraperitoneal injection, intrathecal injection, intra-articular injection, needle injection, rectal administration, sublingual administration, nasal administration, percutaneous administration, inhalation or topical administration to the target organ or tissue, but are not limited to these.

`[0099] When administered by the above manner, due to the size of the target tissue, the scope of th e present invention, an effective amount of the apoptosis-inducing agent is the daily 0.1 ~ 500mg / k g of chlorine dioxide, and sometimes more easily by the area of the target tissue expression, the effe ctive range of the present invention, an apoptosis inducing agent is chlorine dioxide per day 0.1 ~ 5 00mg / IOOcm2, according to the dose administered, effective at least 10 days.

[0100] In any case, containing chlorine dioxide apoptosis-inducing agent of the present invention, t he only guarantee of chlorine dioxide for the target effective dose to the target tissue in an appropria te way into the play apoptosis-inducing action, but not limited to any forms, methods and steps, and help other auxiliary substances.

[0101] "Chinese Pharmacopoeia formulation General" in any of the formulations of the present inve ntion can be used as apoptosis-inducing agent dosage form. The method of the present invention as an apoptosis-inducing agent Examples of pharmaceutical dosage form comprising directly for in viv o injection (including suspensions, emulsions); ointments (including oleaginous ointments, emulsio n ointments (creams), water-soluble ointment etc.), inhalants, liquids formulations (including eye dr ops, nasal drops, etc.), suppositories, patches, pastes, lotions and other topical agents; or tablets (inc luding sugar coating, film, gel coat), liquid formulations , capsules, granules, powders (including fin e grade), pills, syrups, lozenges and the like. These formulations can be prepared according to Chin ese Pharmacopoeia General preparation method.

[0102] In addition, the method of the present invention, the apoptosis-inducing agent is administere d, can have include pharmaceutically acceptable solid or liquid carriers or interventional therapy ma terials. As a pharmaceutically acceptable solid or liquid carriers include solvents, stabilizers, dissolu tion aids, emulsifying agents, suspending agents, buffers, isotonic agents, coloring agents, bases, thi ckeners, excipients, lubricants , binders, disintegrating agents, coating Qi ü, flavoring agents, condit ioners, foaming agents, super absorbent polymers, surface active agents, permeation enhancers and pH adjusting agent, but is not limited to these.

[0103] Specific examples include deionized water, lactose, sucrose, fructose, glucose, mannose, sor bitol and other sugar or sugar alcohol; crystalline cellulose, methyl cellulose, ethyl cellulose, hydrox ypropyl cellulose, Low-substituted hydroxypropylcellulose, hydroxypropylmethylcellulose, hydrox ypropylmethylcellulose phthalate, hydroxypropylmethylcellulose acetate succinate, carboxymethyl cellulose, hydroxymethyl corn starch, wheat starch; cellulose calcium, sodium carboxymethylcellul ose, cross-linked sodium carboxymethylcellulose, hydroxymethyl ethyl cellulose, cellulose acetate phthalate cellulose and its related derivatives , rice starch, potato starch, cyclodextrin, pullulan point effort and its related derivatives; agar, sodium alginate, gum arabic, gelatin, collagen, shellac, yello w potato gum, xanthan gum and other hot high molecule (seaweed, plant mucilage, proteins and the like); polyvinylpyrrolidone, aminoalkyl methacrylate copolymers, methacrylic acid copolymers, hy droxy vinyl copolymer, polyvinyl alcohol, dimethylpolysiloxane and other synthetic polymer; olive oil, cocoa butter, carnauba wax, beef tallow, hardened oil, soybean oil, sesame oil, camellia oil, lins eed oil, paraffin, liquid paraffin, yellow beeswax, white petrolatum, coconut oil, microcrystalline w

ax, etc. fats and oils; stearate, aluminum stearate, calcium stearate, magnesium stearate, triethyl citr ate, triethyl glycerides, medium chain fatty acid triglycerides, stearyl, myristoyl acid isopropyl Qu f atty acid esters and derivatives thereof; ethanol, glycerol, stearyl alcohol, cetyl alcohol, propylene gl ycol, polyethylene glycol, and polyols; zinc oxide, calcium hydrogen phosphate, precipitated calciu m carbonate, synthetic aluminum silicate, silicic anhydride, kaolin, dried aluminum hydroxide gel, s ynthetic hydrotalcite, titanium oxide, talc, bentonite, magnesium aluminum silicate, aluminum potas sium sulfate, sub gallic acid bismuth subsalicylate bismuth, calcium lactate, sodium citrate, sodium chloride , inorganic matter and metal compounds of sodium hydrogencarbonate and the like; sucros e fatty acid ester, polyoxyl stearate, polyoxyethylene hydrogenated castor oil, polyoxyethylene poly oxypropylene glycol, sorbitan monooleate times with , sorbitan ester trioleate, sorbitan monostearat e sorbitan esters, sorbitan esters but palmitate, sorbitan monolaurate ester, polysorbate, glyceryl mo nostearate, sodium lauryl sodium sulfate, lauromacrogol and other surfactants; dimethyl sulfoxide a nd the like, nitrogen-based compounds, pyrrole derivatives, alcohols and fatty acids and other comp ounds permeation enhancer; pigments; perfumes. But it is not limited to these.

[0104] Examples of interventional therapy materials include syringes, stents, artificial blood vessels, syringe needle, catheter, balloon, but are not limited to these.

[0105] The present invention is an apoptosis inducing agent containing chlorine dioxide prior to ad ministration, it can be administered in accordance with the mode of administration anesthetics, such as barbiturates and other injectable anesthetic, nitrous oxide and other inhaled anesthetics , anestheti cs lidocaine and other surfaces, but are not limited to these.

[0106] The present invention in mammalian apoptosis-inducing agent, specific examples of mamma ls, including humans, monkeys, dogs, pigs, cats, rabbits, rats and mice. Among them, those who are preferred. Example

[0107] The following examples are provided to illustrate the present invention and are not in any wa y limit the invention.

[0108] Example 1: chlorine dioxide formulation containing fibroblast apoptosis of hypertrophic scar s skin

[0109] hypertrophic scars affect organ function as a kind, beautiful disease, the cause of a variety, pr ovided that damage involving the deep dermis injury, are likely to have hypertrophic scars. Generall y considered insufficient fibroblast apoptosis is the cause of hypertrophic scars produced (Ogawa, R. The most current algorithms for the treatment and prevention ofhypertropHic scars and keloids [J] . Plast Reconstr Surg, 2010,125: 557-568). Common sense dictates that the same size of the wound, a s opposed to the young, the elderly wound healing is slow and easy to leave scars. Thus, a hypertrop hic scar skin aging can be understood as a phenomenon, by inducing fibroblast apoptosis good way to scar skin may be cured.

[0110] I) apoptosis assay

[0111] Patients with hypertrophic scars 6 patients, 5 males and I female, mean age 42 years. This st udy consisted of four groups: (within a radius of the central 2/3) scar the central portion (1/3 radius of the inner periphery) scar edge portion of the skin surrounding the scar (keloid from the inner edg e of 0.5cm range) of patients in other parts of the scar normal skin. Cultured fibroblasts [0112] to: s pecimens were grouped according to the method described above cut their skin and subcutaneous tis sue under sterile conditions, a small amount of fetal calf serum specimens will cut about Imm3 tissu e blocks, at 37 ° C, under 5% C02 humidified conditioned medium 4-6h, the tissue block adhering t o the sides of the bottle, and then added with 15% fetal bovine serum DMEM (dulbeccc / s modifie deagle medium) medium amount, 3_4 day I was changed times, 2_3 weeks later, primary cells cove red and merged into tablets each day passage 3_5 times I experiment with 6-8 passage cells.

[0113] configured with deionized water to a concentration of a mixed solution of 7.47% sodium chl orite and 1.59% sodium chloride to prepare a first solution; configured with deionized water at a co

ncentration of 16.7% citric acid solution to prepare a first Two solution. Were taken from the same v olume of solution in the container in different parts of the solution, mixed, the mixed solution was st ill waiting for 3-5 minutes, then 0.22 μ m filter membrane bilayer, diluted with deionized water to p repare various concentrations of chlorine dioxide acidic solution.

[0114] Cells were seeded and applying different processing factor induced apoptosis. Take a bottle o f each group were in the logarithmic growth phase cells were seeded in 6-well cell culture plate, eac h of the cells in each plate were inoculated I wells (IX IO5 cells / well) were inoculated six plate 24 hole and continued to train 48_72h at 37 ° C, 5% C02 saturated humidity conditions to a cell covere d the hole bottom, whichever

I plates, medium was aspirated with Hank solution (a balanced salt solution) was washed with DME M medium without FBS was added, the other to take three plates containing chlorine dioxide were a dded to the formulation, the final concentration reached 100ppm, 1000ppm, 2900ppm, take I plates added FasMcAb (Fas monoclonal antibody), to give a final concentration of I μ g / ml, in addition I plates without applying any treatment factors, cultured 24h after detection rate of apoptosis.

[0115] PI staining and flow cytometry to compare the composition of the apoptosis rate of fibroblast s. After digesting the above-treated cells were collected in IOml centrifuge tube, 1000r / min centrif ugal IOmin, post-cold PBS (phosphate buffer solution) to wash times with 70% ethanol fixed overni ght at 4 ° C, and then centrifuged at low speed 10min, PBS After washing the cells were resuspende d by adding RNase A 2μ I (20mg / ml) in the remaining 0.5ml cell suspension, 37 ° C for 30min inc ubation immediately put into an ice bath to stop the role of RNase A, then add 500μ1 PI dye (100 μ g / ml) dark for 30min, to disperse the cells, after 300 mesh filter on DNA analysis by flow cytometr y to detect cell apoptosis rate.

[0116] All the cells in each group by serum-free culture after 24h `its apoptosis rate were increased i n different degrees, but after a growth rate of apoptosis after serum-free culture groups were compar ed and found significantly higher than normal skin keloid edge portion (P <0.01), while the surroun ding skin and keloid keloid central portion between the two, and there was no difference between th e groups was significant (P> 0.05, Table 1). This shows that aging is seen as scar tissue has a signifi cant anti-apoptotic ability, so not only can not be scar tissue automatically cleared, it will hyperplasi a.

[0117] Table 1 serum-free culture after 24h scar and surrounding skin fibroblast apoptosis rate

[01]

取材部位	无血清培养 24h 后	细胞凋亡增长率
瘢痕边缘部	9.44±1.43	84±20
瘢痕中央部	10.52±1.50	109±25
瘢痕周围皮肤	10.90±1.79	106±14
正常皮肤	110.6±1.99	133±28*

[0119] Note: * Compared with the scar edge portion, P <0.01

[0120] Under FasMcAb effect of normal skin fibroblast apoptosis was significantly higher than the scar and surrounding skin fibroblast apoptosis rate (P <0.01, Table 2). This also shows that the scar t issue has anti-apoptotic ability. [0121] Table 2 FasMcAb UNDER scar and surrounding skin fibrobl ast apoptosis rate

[0122]

取材部位	FasMcAb 作用（1μg/ml）
瘢痕边缘部	7.32±2.18
瘢痕中央部	6.52±1.90
瘢痕周围皮肤	13.08±1.97
正常皮肤	63.04±6.59*

[0123] Note: * Compared with the scar edge portion, P <0.01

[0124] Under the effect of chlorine dioxide formulation, four fibroblast apoptosis rate increased wit h the increase of chlorine dioxide concentration gradient gradually increase, the increase was signifi cant (P <0.01, Table 3). This shows that the preparation of chlorine dioxide under acidic environme nt of hypertrophic scar tissue has a significant effect on apoptosis.

[0125] Table 3 under the action of chlorine dioxide scar and surrounding skin fibroblast apoptosis ra te

[0126]

取材部位	二氧化氯作用浓度（ppm）			
	0	*100*	*1000*	*2900*
瘢痕边缘部	*5.10*±0.27	*37.30*±4.18	*91.25*±6.89	*99.12*±1.63
瘢痕中央部	*4.94*±0.42	*36.52*±4.91	*93.24*±7.32	99.65±1.54
瘢痕周围皮肤	*5.28*±0.62	*50.08*±5.68	*90.65*±9.65	*99.42*±1.87
正常皮肤	*4.88*±0.24	*73.21*±7.56	*87.98*±13.60	*99.87*±1.02

[0127] 2) the treatment of scars

[0128] configured with deionized water to a concentration of a mixed solution of 7.47% sodium chl

orite and 1.59% sodium chloride to prepare a first solution; configured with deionized water at a co ncentration of 16.7% citric acid solution to prepare a first Two solution. Were taken from the same v olume of solution in the container in different parts of the solution, mixed, the mixed solution was st ill waiting for 3 to 5 minutes, and then double-membrane filter of 0.22 μ m. Preparation of the chlor ine dioxide formulation.

[0129] The preparation of chlorine dioxide over twice for treatment of hypertrophic scars, over the direct use of the chlorine dioxide preparation, the second time together with the same amount of di methyl sulfoxide. That is twice applied directly scar, an interval of 30min, continuous smear 15 day s.

[0130] immediately after treatment of hypertrophic scars, dark red, gray to yellowish scar surface se e dotted gasification dander evenly distributed, no obvious bleeding, exudate. After the first day of t reatment, the wound dry see the red dot crusting, then to the fifth day after treatment, the wound tre atment was bright red, gradually scab off the wound. And twenty days after the treatment, the woun d is completely healed, crusts all off, while the center of the scar observed papillae height becomes f lat, scar skin was smooth and some pigmentation. 25 days after treatment, with a smooth skin fresh generation, in 40 ± 20 days or so, the original irregularly shaped scar completely flat. [0131] before taking treatment, after lh, 2 days, 7 days, 14 days after treatment, 28 days scar, cut scars full thickne ss, cut tissue in 4% paraformaldehyde after dehydrated, embedded in paraffin, HE staining sections underwent various periods in each group fibroblasts microscopic morphology, density and arrangem ent of collagen fibers, capillary hyperplasia, TUNEL (TerminaldeoxynucIeotidyl transferase dUTP nick end labeling) assay fibroblast apoptosis rate change.

[0132] by TUNEL staining region located in the nucleus. Light microscopy normal fibroblast nuclei were blue, negative reaction, apoptotic cell nuclei were shades of tan, a positive reaction, namely ap optosis. 40 times the light microscope, each slice randomly selected three fibroblast cells most posit ive power fields were counted positive cell response rate (R) = positive cells / total cells, the results were averaged.

[0133] In fibroblasts, keratinocytes, endothelial cells were apoptotic bodies, namely apoptosis. Imm ediately after treatment, that is a significant increase in performance apoptotic fibroblasts after treat ment lh, 2 days, 7 days, 14 days, 28 days treatment fibroblast apoptosis rate gradually increased apo ptosis rate in each period than before treatment , P value <0.01, indicating that the difference was st atistically significant.

[0134] Table 4 scar tissue TUNEL assay to change fibroblast apoptosis rate

治疗前	1h	2天	7天	14天	28天
7.42 ± 2.28	16.54 ± 1.23*	25.13 ± 1.48*	46.65 ± 2.10*	87.32 ± 4.12*	95.87 ± 3.01*

[0136] * before representatives of the treatment compared to P <0.01.

[0137] Therefore, regardless of the in vitro or direct treatment, have shown chlorine dioxide formul ation of the present invention under acidic environment of inadequate tissue can induce apoptosis in which the acceleration of apoptotic cells, making the skin healthy and younger.

2 [0138] Example: cosmetic anti-aging skin test preparation containing chlorine dioxide

Cosmetic preparations [0139] For skin care preparations containing chlorine dioxide: deionized wat er allocation at a concentration of 7.47% sodium chlorite and mixed solution of 1.59% sodium chlor

ide, prepared a first solution; deionized Water configured concentration of 16.7% citric acid solution, to prepare a second solution. Were taken from the same volume of solution in the container in diffe rent parts of the solution, mixed, the mixed solution was still waiting for 3 to 5 minutes, and then do uble-membrane filter of 0.22 μ m. Then add an equal volume of 90% DMSO, then sealed into glass bottles, made against skin aging or rejuvenation cosmetics for skin smear.

[0140] actually applied to the skin by the female observer (100), according to the following evaluati on distinction, evaluate "whether the sticky feeling in use", "astringent sense of the presence or abse nce", "moving on skin Have "and" to wrinkles, sagging (skin stretching feeling) the effect of the pre sence or absence. "

[0141] From the results (Table 5), chlorine dioxide formulation of the present invention as a cosmeti c skin care has a very good role.

[0142] Table 5: Efficacy of skin care use

[0143]

关于黏腻使用感		关于发涩感		对皮肤的密合性		对皱纹、松弛效果	
黏腻非常少	99	无发涩感（光滑感）	98	密合性强	90	有效果	90
黏腻少	1	感到发涩	1	一般	5	稍微有效果	4
略微黏腻	0	发涩	1	弱	5	无效果	6

[0145] The cosmetic preparation of the above example of the present invention efficacy, efficacy tes ting conducted in the crowd observed a user during use of the moisture content, elasticity, fine lines, roughness, improve the situation. Select 30 healthy women and 10 healthy men, daily skin smear fre e choice position, a total of 15 days, prior to use, lh, 7 days (after using Ih), day 14 (after use Ih), the first 28 days of receiving subjects were evaluated skin condition, including clinical judgment and no n-invasive assessment instrument. Clinical evaluation includes the following parameters: skin moist ure content, elasticity, skin gloss, skin roughness / smoothness, color uniformity, improve skin wrin kles. Non-invasive assessment instrument is the use of skin moisture analyzer Corneometer CM820 measuring skin moisture content measured skin elasticity using the skin elasticity tester (Cutometer SEM 575). Experimental data are shown in Table 6.

[0146] Table 6 shows: Use this cosmetics, skin moisture content increased dramatically, indicating t hat the product to improve dry skin effect is very obvious, as time progresses, the first 28 days, the water content of the skin continue to continuous improvement.

[0147] The larger the value, the better skin elasticity, indicating better skin elasticity, can be seen fro m Table 6 for 7 days, elasticity of the skin has been significantly improved, flexible getting better an d there is a significant difference between the baseline and sex.

[0148] Four gloss index value, the less data values, indicating improved better. The results can be se en from the above data, compared with the baseline value, the parameters were improved at the 14th and 28th day, there are between baseline significant difference (reflected in the value of a downwar d trend over time).

[0149] Table 6 contains the skin improvement cosmetic use after chlorine dioxide

[0150]

时间	皮肤含水量（%）	皮肤弹性	光泽度	粗糙度/光滑度	肤色均匀度(美白)	细纹改善
使用前-基础值	30.1±3.5	0.70±0.21	4.55±0.64	3.74±0.47	4.81±0.51	5.16±0.67
1h	48.3±5.5*	0.69±0.19	4.55±0.55	3.72±0.32	4.80±0.43	5.10±0.56
7天	58.6±4.3*	0.74±0.13*	3.68±0.38*	3.19±0.41*	4.35±0.53	4.84±0.53
14天	59.7±3.6*	0.79±0.14*	3.18±0.40*	2.85±0.29*	4.12±0.42	4.56±0.49
28天	47.5±6.3*	0.80±0.23*	3.02±0.29*	2.79±0.31*	4.01±0.39*	4.13±0.56*

[0151] Note: * Compared with baseline, P <0.01. [0152] Accordingly cosmetics containing chlorine dioxide of the present invention includes skin care, water, cream, whitening and wrinkle skin rejuve nation and other cosmetic effect.

Containing chlorine dioxide preparation for the treatment of acne Test Example 3 [0153] Example

[0154] Acne is a skin problem teenagers often recurrent, mainly due to the aging of the stratum corn eum can not be cleared, clogged pores, leading to fat accumulation, the formation of swelling (acne); after the invasion of acne bacteria infection, redness, pustules form. Because chlorine dioxide itself is highly effective fungicide, so only ensure aging skin cells to be cleared, then the chlorine dioxide can treat acne.

Cosmetic preparations [0155] for acne (acne) containing chlorine dioxide preparation: preparation method of Example 2, made acne solution for acne skin smear.

[0156] acne problem to select 30 young people (20 male, 10 female), average age 22 years, every da y above configuration containing chlorine dioxide formulation applied once the acne affected area f or 5 consecutive days. Specific effects in the following table.

[0157] Table 7 Acne Treatment Trial

[0158]

时间	3天	5天	7天	11天
治疗情况	患处变红，肿胀消退	患处有结痂，脱皮现象	患处基本变平。	痤疮消失，皮肤变得平滑

[0159] 30 acne, with 5 consecutive days, and 90% cure acne. Description of chlorine dioxide to trea t acne preparations of the present invention containing non-

Very effective.

[0160] Example 4 containing chlorine dioxide preparation on the life of mice by intravenous injecti on of test

[0161] Production of chlorine dioxide formulation: deionized water allocation at a concentration of 2.49% sodium chlorite and mixed solution of 0.53% sodium chloride, prepared a first solution; deio nized water allocation at a concentration of 5.57% of lemon acid solution, a second solution was pre pared. Were taken from the same volume of solution in the container in different parts of the solutio n, mixed, the mixed solution was still waiting for 5 to 10 minutes, the same amount of 50% DMSO was added, and then double-membrane filter of 0.22 μ m, immediately loaded into the syringe. Prep aration time, once.

[0162] C57BL / 6 male mice were 12 months old, each mouse single cage feed. 100 mice were rand omly divided into two groups: the experimental group (50) and control group (50). Both groups wer e adequate feed and water, since the experimental group after 15 months, once a week, each with 10 ml / kg dose by intravenous formulation of chlorine dioxide prepared by the above method. Each m ouse in each group died of natural causes date detailed records and calculate the average life of each group of mice and maximum service life.

[0163] The results show: the average life expectancy in the control group of mice was 696.5 days, a verage life test group of mice was 778.3 days, weekly injections of chlorine dioxide preparation can extend average lifespan of mice by 11%. Instructions, injection of chlorine dioxide formulation sign ificantly prolong the average life expectancy (see Table 8).

[0164] according to the current method used to calculate the maximum life, that is, the group finally died of natural causes 10% of the average life expectancy for the maximum life span of mice, the re sults showed: control mice maximum lifetime is 932 days, the maximum life test mice It is 987.5 da ys, injectable formulation of chlorine dioxide so that the maximum lifetime prolongation of 5.9%, i ndicating that: the injection of chlorine dioxide formulation has a maximum life span of the mice w as prolonged effect.

[0165] Table 8 Effect of chlorine dioxide preparation on mice injected life

[0166]

组别	N	平均寿命	平均寿命提高	平均最大寿命	平均最大寿命提高
对照组	50	696.5±137.6		932±40.6	
实验组	50	778.3±120.3*	11%	987.5±20.3*	5.9%

[0167] Note: * Compared with the control group, P <0.05

[0168] In the 19-month-old mice a month's time, were randomly selected from the experimental gro up and the control group of 10 mice, Morris water maze test of endurance and balance. Two test inte rvals of 2 weeks.

[0169] water maze test: Before the experiment of continuous training 3 days, 2 times / day. Square water maze device is 50cmX30cmX 15cm black wooden trough, equipped with the starting area, 4 blind-side winding circuit security units. Depth control in 12cm, water temperature (25 ± 2) ° C. Mi ce from the inlet end into the beginning of time, the number of blind-side hit automatically recorded to mouse climbed up security station as the end of time, called the incubation period. Set the maxim um incubation period for the 120s, 120s screening than animals still find the exit.

[0170] balance endurance test: Before the experiment of continuous training 3 days, 2 times / day. B etween two fixing pillars pull a wire having a diameter of 2mm, the distance is 2m, height lm. Lowe r limb tied with a rope, the mice with the upper grip wire, with a stopwatch to calculate the starting clenched fall time. Observation time is set to 30s, the 30s fell screening of animals.

[0171] can be seen from Table 9, all animals on day I was no difference in memory; test on day 3, 6 and 9 days, the test group the number of errors and latency was significantly less than the control gr oup. Visible chlorine dioxide formulation significantly improve the learning ability and memory in mice.

Comparison [0172] Table 9 mice in each group at different times in memory

[0173]

组别	1天	3天	6天	9天
错误次数				
对照组	7.60±2.17	7.80±2.48	7.89±2.56	8.20±2.78
实验组	7.50±2.01	6.80±1.48*	6.30±2.01*	5.30±1.64*
潜伏期				
对照组	50.80±16.89	52.10±24.33	56.40±14.30	60.20±23.96
实验组	50.00±15.69	48.60±24.21*	43.50±17.58*	40.90±25.46*

[0174] Note: * Compared with the control group, P <0.05

[0175] 10 can be seen from the table, the test group mice I days, 3 days, 6 days and 9 days was significantly higher than the balance of stamina. Description of chlorine dioxide formulation significantly improves the body's ability to mice.

Comparison [0176] balance sheet 10 mice in each group at different times of endurance

[0177]

纠别	1天	3天	6天	9天
对照组	88±48.39	93±45.63	102±56.34	112±60.50
实验组	132.90±46.53*	140.30±56.36*	149.35±42.64*	153±60.35*

[0178] Note: * Compared with the control group, P <0.05

[0179] Test data from the above embodiment a plurality, we can conclude that chlorine dioxide formulation acidic environment can significantly improve the animal's life, and with the body's ability to improve memory and its mechanism of chlorine dioxide formulation should be able to thereby induce apoptosis in senescent cells senescent cells to be removed, the body's own regenerative capacity will be restored younger. Thus according to the invention can be made of chlorine dioxide formulation of cosmetics for skin rejuvenation beauty can also be made to make the body younger medicines, thereby preventing the occurrence of age-related diseases.

[0180] Example 5 chlorine dioxide preparation killing effect on human non-small cell lung cancer c ell line A549.

[0181] configured with deionized water to a concentration of a mixed solution of 7.47% sodium chl orite and 1.59% sodium chloride to prepare a first solution; configured with deionized water at a co ncentration of 16.7% citric acid solution to prepare a first Two solution. Were taken from the same v olume of solution in the container in different parts of the solution, mixed, the mixed solution was st ill waiting for 3-5 minutes, then 0.22 μ m filter membrane bilayer, diluted with deionized water to p repare various concentrations of chlorine dioxide solution.

[0182] I) Experimental Method

[0183] PRMI1640 culture medium containing 10% fetal calf serum, and set 37 ° C, 5% C02 incubat or for non-small cell lung cancer A549 cells, digestive cells every three days with 0.25% trypsin liq uid passage , and replace the culture medium. Press

0.7X IO4 / hole concentration were seeded in 96-well plates, set 37 ° C, 5% C02 incubator after 24h, were added to a solution of chlorine dioxide, chlorine dioxide solution each according to a final con centration of 100ppm, 200ppm, 400ppm, 700ppm, 1000ppm, 1500ppm, 2000ppm, 2900ppm added to negative control group cells was added 0.1% (v / v) in DMSO (dimethylsulfoxide), a separate set of zero (i.e., cells in the culture wells without cells, only added to the cells broth). Each group set up four wells, then 96 set 37 ° C, 5% C02 incubator incubated 24h, then press the following method to detect compound killing effect on tumor cells.

[0184] 2) Test Method:

[0185] release by lactate dehydrogenase (LDH) test, using lactate dehydrogenase assay kit tumor ce ll death, specific methods of operation in accordance with the lactate dehydrogenase assay kit instru ctions. With assay buffer (AssayBuffer) resuspended reaction substrate (Substrate Mix). Take a 96- well cell culture plate culture supernatant 3 wells for each group of 50 μ I to a new 96-well plates, e ach group remaining a duplicate wells to a final concentration of 0.9% (V / V) The Triton-xlOO (pr ovided with the kit), and placed in a 37 ° C incubator after 50 minutes to lyse the cells, the supernat ant was added 50 μ I fetch a new 96-well plate, and then each of the new 96- After the hole then add an equal volume of resuspended good reaction substrate solution was incubated for 30 min at room t emperature, each well was added 50 μ I stop solution ie acetic IM's (provided in the kit) to terminat e the reaction, using a microplate reader (490nm wavelength) OD value of each well was measured, cells were lysed by the detected hole OD value as "Cell OD value maximal release", OD value of th e negative control group referred to as "natural release control OD value."

[0186] According 0D490 detected value is calculated according to the following formula cell death, the results mean ± standard deviation to represent, and using SPSS Probit module calculates half kil l the cell concentration, namely IC5Q.

[0187] Tumor cells Mortality (%) = (OD value of experimental group - spontaneous release control OD value) / (maximum release cell OD value - spontaneous release control OD value)

[0188] 3) test results:

[0189] As shown in Table 10, the formulation of chlorine dioxide can effectively kill A549 cells, an d increases the concentration of their killing effect on A549 also dose-dependently increased by SPS S software module calculates Probit, chlorine dioxide A549 cells resulted in the death of half of the formulation of effective dose (IC5tl) is 495ppm.

[0190] Table 10 A549 in vitro cytotoxicity

[0191]

ClO2 浓度（ppm）	0	100	200	400	700	1000	1500	2000	2900
细胞死亡率	0	4.6±1.1	7.0±2.9	23.6±5.9	71.9±4.1	99.0±3.9	99.8±2.4	100±2.1	100±1.4
IC_{50}	495ppm								

Example [0192] This confirms that the acidic chlorine dioxide preparation can significantly A549 kill tumor cells.

[0193] The cytotoxicity of human cervical carcinoma HeLa chlorine dioxide formulation of Example 6

[0194] chlorine dioxide formulation was prepared according to the method of Example 5, according to the cell culture method of Example 5, the human cervical carcinoma HeLa cells were cultured conservation and seeded in 96-well plates. Example 5 The method provided in the experimental group, the control group and zero groups and by lactate dehydrogenase release assay (LDH), the detection of chlorine dioxide solvent caused HeLa cell death, and the calculation results in half of the HeLa cell death dose (IC5 (I).

[0195] Test Results: As shown in Table 11, the formulation of chlorine dioxide can effectively kill HeLa cells, and increases the concentration of the compound, their cytotoxicity of HeLa was dose-dependently increased by SPSS software module calculates Probit , chlorine dioxide preparation HeLa cells leads to the death of half of the effective dose of 405ppm.

[0196] Table 11: preparation of chlorine dioxide in vitro HeLa cell killing

[0197]

二氧化氯浓度（ppm）	0	100	200	400	700	1000	1500	2000	2900
细胞死亡率	0	8.0 ± 1.0	13.1 ± 2.1	43.6 ± 5.9	99.0 ± 4.1	100 ± 3.2	100 ± 1.4	100 ± 0.9	100 ± 0.4
IC_{50}	405ppm								

[0198] Example 7: The LDH release assay to detect chlorine dioxide formulation of a variety of human cancer cells, including breast cancer, ovarian cancer, liver cancer, nasopharyngeal cancer, stomach cancer, laryngeal cancer, pancreatic cancer, melanoma , the killing effect of bladder cancer and leukemia cells.

[0199] Production of chlorine dioxide formulation according to the method of Example 5. Example 5 Method conservation cultured breast cancer cells (MCF-7), ovarian cancer (SK0V3), hepatoma cells (Bel-7402), nasopharyngeal carcinoma cell (HNE), gastric cancer cells (MKN-45), laryngeal cancer cell (! fep-2), pancreatic cancer cells (Pan-I), melanoma cells (A375), bladder cancer (Biu-87), leukemia cells (Jurkat) containing 10% fetal bovine serum using the same PRMI1640, placed in 37 ° C, 5% C02 incubator, according to the conventional way of suspension cell cultures were passaged, namely first centrifuged to remove old at the time of passage of the culture medium, then add a new medium. Then the procedure of Example 5 is provided by the experimental group, the control group and zero groups and release assay of tumor cell death by lactate dehydrogenase (LDH), and the calculation results in tumor cell death half dose (IC5 (I) implemented. [0200] Test Results: As shown in Table 12, the formulation of chlorine dioxide can effectively kill a variety of cancer cells, and with the increasing concentration of chlorine dioxide, which each tumor killing effect of also dose-dependently increases.

[0201] shows that the acidic chlorine dioxide formulations of the present invention provides for a broad spectrum of tumor cells in vitro.

[0202] Table 12 various human tumor cell death at different concentrations of chlorine dioxide (%)

[0203]

二氧化氯浓度（ppm）	100	200	400	700	1000	1500	2000	2900	IC_{50}

[0204]

MCF-7	4.2	30.1	56	84	97	100	100	100	350
SKOV3	5.6	22.3	39	64	87	99	100	100	502
Bel-7402	4.3	23.2	42	84	97	99	100	100	486
HNE	7.2	19.6	32	61	87	98	100	100	550
MKN-45	6.4	26.7	47	61.0	80.0	99	100	100	460
Hep-2	8.9	27.5	46	54.0	76.0	99	100	100	479
Pan-1	9.8	30.9	52	64.3	81.7	100	100	100	387
A375	7.8	29.4	51	63.7	81.3	100	100	100	398
Biu-87	4.5	20.8	37	68.0	84.0	99	100	100	499
Jurkat	11.9	34.5	57	67.3	83.7	100	100	100	360

Example 8 formulation of chlorine dioxide to induce tumor cell apoptosis test [0205] Example

[0206] colon cancer cell line LS174T and breast cancer cell line Cama-1 both death and cell cycle c ell lines were analyzed. Respectively, in the absence or presence of chlorine dioxide IOOppm chlori ne dioxide (prepared by the method of Example 5 embodiment) conditions, cells cultured for 48 hou rs. With I μ g / ml bromodeoxyuridine (BrdU) after pulse 30 minutes, cells were fixed overnight at 4 ° C in 70% ethanol, and then with FITC-conjugated anti-BrdU monoclonal antibody and 3 μ g / ml i odide propidium staining. By flow cytometry (FACS) analysis of cell death and cell cycle. BrdU inc orporation is a proliferation measurement, and propidium iodide staining DNA content can be deter mined, especially near diploid cell populations undergoing apoptosis.

[0207] Data show that the percentage of LS174T cells incorporated BrdU from 27% before treatme nt becomes IOOppm preparation of chlorine dioxide under the presence of 6% after 48 hours. On th e contrary, with near diploid DNA content percentage t dagger LS174T cells, from 4% before the tre atment becomes IOOppm chlorine dioxide formulation under the presence of 23% after 48 hours, in dicating that the cells withered IOOppm chlorine dioxide formulation ability to promote apoptosis.

[0208] The data also show that the percentage of incorporation fcdU of Cama-1 cells, from 15% bef ore the treatment becomes IOOppm presence of 2% chlorine dioxide preparations were incubated fo r 48 hours. In contrast, the percentage of Cama-1 cells have near diploid DNA content from 4% bef ore the treatment becomes IOOppm chlorine dioxide formulation under the presence of 17% after 4 8 hours, indicating IOOppm

Chlorine dioxide preparation trigger apoptosis. [0209] These data suggest that 48 hours of treatment with IOOppm chlorine dioxide formulation, LS174T and Cama-1 cells stop dividing and undergo a poptosis.

Apoptosis chlorine dioxide formulation 9 different pH values for example the case of tumor cells [0 210] Example

[0211] To further investigate the role of chlorine dioxide preparation process of breast tumor cell lin es, in Cama-1 cells by annexin V staining of cell death were analyzed. Cultured in the presence or a bsence of 400ppm chlorine dioxide formulations (prepared by the method of Example 5) cells for 2 4 hours. By Annexin V staining and flow cytometry to detect cell apoptosis. Data show that more th an 70% of Cama-1 cells were annexin V staining, further evidence of 400ppm chlorine dioxide for

mulation induced apoptosis. Other conditions are the same, but the chlorine dioxide preparation 400 ppm by pH adjusting agent, so that the pH value

Increase the value of 3.5 5.5, only more than 50% of the Cama-1 cells were annexin V staining, indicating acidic environment helps improve the rate of cell apoptosis.

[0212] We have also tried to induce apoptosis kinetics of chlorine dioxide measurement. When chlorine dioxide preparation 500ppm (prepared by the method described in Example 5) the presence or absence, pH value 3.5, Cama-1 cells in culture. After 30 hours, the percentage was detected in apoptotic cells (annexin positive cells) in culture. Data show that, after 30 hours, 15% of the untreated cells display spontaneous apoptosis. However, 80% of the formulation with chlorine dioxide treated cells showed cell death. Specifically, Cama-1 cells, nine hours after the preparation of chlorine dioxide was added 500ppm of chlorine dioxide begins induced apoptosis, 30 hours after treatment, 80% of apoptotic cells. Other conditions are the same, but it will be 500ppm chlorine dioxide formulation by a pH adjusting agent to improve the value of PH value from 3.0 6, only more than 45% of the cells showed death, indicating that acidic environment helps improve the rate of cell apoptosis.

[0213] We then tried to chlorine dioxide preparation on human primary breast tumor cells was measured. Newly restored tumor single cell suspension with PBS or chlorine dioxide (IOOppm) preparations were incubated for 48 hours. Apoptosis was measured by PI staining. Expressed as a percentage DN A content ratio of the low content of cells (subGO / Gl cells), that the proportion of apoptotic cells. Data show that 19.5% of the cells treated with PBS DNA having a low content of chlorine dioxide and 38.6% by cells treated with the formulation a low DNA content. Thus, the observed similarity of IOOppm chlorine dioxide preparation for the role of apoptosis in human breast primary tumor cells. Other conditions are the same, but the chlorine dioxide preparation by IOOppm pH adjusting agent to raise the pH value of 4.5 by the 6, only 25% treated with chlorine dioxide formulation DNA having a low content of cells. This shows the acidic environment helps increase the rate of cell apoptosis.

[0214] In vitro anti-tumor combination of the above test, the skilled person can be judged, acidic chlorine dioxide preparation on the tumor cell killing effect has obvious, and this mechanism of action is derived from an acidic chlorine dioxide preparation can significantly induce tumor cell apoptosis.

10 chlorine dioxide directly influence S180 mice tumor effect and immune organ index of cases [0215] Example

[0216] configured with deionized water to a concentration of a mixed solution of 7.47% sodium chlorite and 1.59% sodium chloride to prepare a first solution; configured with deionized water at a concentration of 16.7% citric acid solution to prepare a first Two solution. Were taken from the same volume of solution in the container in different parts of the solution, mixed, the mixed solution was still waiting for 5 to 10 minutes, then add an equal volume of 90% DMSO, and then 0.22 μ m filter membrane bilayer. Quantitative were prepared pH = 5 and pH = 3.5 of lOOppm, 500ppm and 2000ppm of chlorine dioxide formulation.

[0217] Kunming mice were divided into 8 groups of 15, male and female, respectively: control group, cyclophosphamide group, chlorine dioxide, low, medium and high dose groups (pH constant at 5), low doses of chlorine dioxide (pH = 3.5) group, middle dose (pH = 3.5) group and high dose (pH = 3.5) groups. Ip cyclophosphamide dose of 20mg / kg, in the low chlorine dioxide tumor injection, medium and high doses of 100ppm, 500ppm and 2000ppm, the control group received saline. The mice inoculated with S180 solid tumor 24h after dosing volume 0.lml / 10g, l times / d, continuous 14d. The mice were sacrificed on the 5th stopping, stripping tumor masses and spleen and thymus, and weighed to calculate the inhibition rate and the spleen and thymus.

[0218] As can be seen from Table 1, compared with the control group, chlorine dioxide formulation (high pH value) of low, medium and high dose groups, cyclophosphamide chemotherapy group, and chlorine dioxide formulation (low PH value) low, medium and high dose group could significantly inhibit the growth of S180 tumor ($P < 0.05$, $P < 0.01$). Compared with the high pH group, lower pH v

alue the chlorine dioxide preparation group can significantly improve the inhibition of chlorine diox ide preparation on S180 tumor growth (P <0.05).

[0219] Table 13 Inhibitory effect chlorine dioxide preparation on S180 tumor-bearing mice

[0220]

组别	剂量 /(mg/kg)	瘤重 /(g)	抑瘤率 /(%)
空白对照		1.43±0.35	
环磷酰胺	20	0.34±0.14	76.2
二氧化氯（pH=5）	1（100ppm）	0.76±0.21*	46.6
	5（500ppm）	0.44±0.14*	69.4
	20（2000ppm）	0.15±0.06**	86.7
二氧化氯制剂 ((pH=3.5)	1（100ppm）	0.60±0.13*	58.1
	5（500ppm）	0.32±0.11**#	77.6
	20（2000ppm）	0.07±0.05**#	95.1

[0221] Note: * P <0.05, ** P <0.01 compared with the control group; # P <0.05, ## P <0.01 compar ed with a high pH value group

[0222] As can be seen from Table 14, compared with the control group, cyclophosphamide chemoth erapy in mice thymus, spleen index was significantly lower (P <0.01), explain the side effects of che motherapy means a lot. Chlorine dioxide formulation, thymus and spleen index of high dose group were significantly increased (P <0.05). For high and low pH, chlorine dioxide is not significant. Des cription of chlorine dioxide to promote tumor apoptosis in immune organs minimal side effects.

[0223] Table 14 Chlorine Dioxide affect the immune organ index of preparation

[0224]

组别	剂量 /(mg/kg)	脾指数 /(g/kg)	胸腺指数/(g/kg)

[0225]

空白对照		8.77±2.46	2.24±0.39
环磷酰胺	20	5.05±1.81**	0.66±0.12**
二氧化氯（pH=5）	1（100ppm）	8.68±1.09	2.29±0.14
	5（500ppm）	9.73±2.14*	2.39±0.09*
	20（2000ppm）	9.96±1.09**	2.95±0.30**
二氧化氯制剂（（pH=3.5）	1（100ppm）	8.28±1.44	2.23±0.16
	5（500ppm）	9.22±2.02 *	2.70±0.83*
	20（2000ppm）	9.43±1.18 **	2.89±0.54**

[0226] Note: * P <0.05, ** P <0.01 compared with the control group.

[0227] shows that the acidic chlorine dioxide formulations of the present invention provides a significant tumor cells induce apoptosis, and almost no side effects on the body.