Institut National de la Normalisation
et de la Propriété Industrielle

[21] Numéro de dépôt: TN/P/ 2009/493

[22] Date de dépôt: 25/11/2009

[51] CIB: A 61K 31/015, 47/44

[54] Titre:
OILY SOLUTIONS OF C [60] FULLERENE AND THEIR USE FOR PREVENTING DAMAGES CAUSED TO MEATAZOANS BY FREE RADICALS

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[31] [32] [33] Priorités:

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ABREGE

The present invention relates to a stable biocompatible composition comprising: (a) a carrier selected from the group consisting of fats and oils and (b) [60]fullerene, wherein the fullerene is dissolved in said carrier. The above composition may be used to protect metazoans against damages caused by oxidative stress involving free radicals.

TN2009/0493

DESCRIPTION

1. Field of the invention

The present invention concerns stable biocompatible compositions comprising [60]fullerene dissolved in a carrier. The present invention also relates to a method for protecting either metazoans or metazoan cells against oxidative stress involving free radicals, which comprises a step of administering a composition comprising a therapeutically effective amount of [60]fullerene. The present invention further concerns a method for preserving a substance or mixture of substances sensitive to damages caused by free radicals using said composition.

2. Description of related art

The unique properties and potential applications of fullerenes have long been known. Biological applications of fullerene derivatives, in particular as antioxidants, have been reviewed for example in Bioorg. Med. Chem. 1996, 4, 767-779 and Eur. J. Med. Chem. 2003, 38, 913-923. Buckminsterfullerene ([60]fullerene, C60) is a powerful free radical scavenger due to the unique reactivity of its 30 carbon double bonds which characterize it as a radical sponge (Krusic et al., Science 1991, 254, 1183- 1185). Many water-soluble C60 derivatives have been found to retain *in vitro* the free radical scavenger properties of their parent fullerene molecule, allowing these properties to be exploited in biological systems. Many patents already exist for a broad range of biomedical applications and other commercial applications of water-soluble fullerenes, including anticancer and anti-HIV therapies, drugs for neurodegenerative diseases, drug delivery systems, and preparations that retard aging. In particular, a group of hydrophilic C60 derivatives, carboxyfullerenes, were proposed to increase metazoan's lifespan (U.S. Patent Application 2003/0162837) and a stable biocompatible compositions comprising: (a) a carrier selected from the group consisting of fats, oils, waxes and mixtures thereof; and (b) particles of at least one compound selected from the group consisting of water-insoluble fullerenes, wherein said particles are dispersed in said carrier, was proposed for preventing damages caused by free radicals (International application No PCT/EP2005/004963 ; May 4 2005). 2. Until now, availability of biocompatible aqueous or oily pharmaceutical preparations of buckminsterfullerene ([60]fullerene, C60), [70]fullerene (C70) or their derivatives that are insoluble in water have been major obstacles to toxicity and in vivo studies of this new family of compounds. Biological properties of water-insoluble fullerenes are still misunderstood and there are no certified toxicology data about them until now. Most of the fullerenes studied until now are water-soluble derivatives, since study of water-insoluble fullerenes, such as pristine C_{60}, in biological medium proves difficult. It is a common practice to derivatize the fullerene core with substituants such as OH, COOH, NH2 to increase hydrophilicity. Fullerenes are only soluble in a limited number of organic solvents, such as toluene, benzene, chloro-naphtalene and dichlorobenzene. Pristine C_{60} has been shown to be more effective as an antioxidant than certain carboxyfullerenes in Wang, I. et al., J. Med. Chem. 1999, 42, 4614-4620. However, C_{60} has not been employed as an active ingredient to develop an in vivo treating method in this publication. Aqueous suspensions of C_{60} are well known in the art. They are stable for long periods and can be delivered to cells. Moussa et al. described in Fullerene Science & Technology 1996, 4, 21-29 that micronized particles of water-insoluble fullerenes may be administered to mice on the form of a biocompatible aqueous suspension comprising a surfactant (tween 80 or Tween 60) and a suspending agent (carboxymethyl cellulose) which stabilizes the suspension. C_{60} is non toxic, can cross cellular membranes and accumulates in liver. Moussa et al. also disclosed in Fullerene Science & Technology 1995, 3, 333-342 that

partially micronized C60 particles can be incorporated into living human phagocyte cells. C60 was directly suspended in the culture media and did not exhibit acute toxicity. A study of 14C-labeled C-60 reported that it is possible to form suspensions of C60 in water that are stable for long periods and can be delivered to cells (Scrivens, W. A.; Tour, J. M.; Kreek, K. E.; Pirisi, L "Synthesis of C14 labeled C60, its suspension in water, and its uptake by human keratinocytes" J. Am. Chem. Soc. 1994, 116, 4517-4518). However, these suspensions containing very low concentrations of fullerene (typically 0.1 mg per ml) were inadequate to perform in vivo studies, especially toxicity studies and metabolic fate investigations. Other vectorisation methods include the formation of inclusion complexes with cyclodextrins, calixarenes, tween-20, micelles, liposomes, and vesicles; however C60 concentrations reached by such methods are still very low (1 mg/mL at most) and inadequate to perform in vivo toxicity studies. Further, these methods present another drawback because they generally necessitate a preliminary dissolution step of the fullerene in an organic solvent. Another method, disclosed in J. Med. Chem. 2000, 43, 3186-3188 uses polyvinyl-pyrrolidone to solubilize C60; however this vehicle can react with fullerene and the formed complex may cause harmful effects on mice embryos. A group leaded by F Moussa have already used Micronized C60 suspensions as free radical scavenger in vivo (Nano Letters 2005, 5 (12), 2578 - 2585). However, the effective doses were very high (i.e. > 0.5 g/kg of body-weight) and intra peritoneal (i.p.) administration was the only route of administration for such suspensions. Stable biocompatible compositions comprising water insoluble fullerenes dispersed and/or dissolved in an amount ranging from 0.2 to 10 % by weight relative to the total weight of the composition, preferably from 0.1 to 2 % by weight, were already proposed by N Gharbi and F Moussa for preventing damages caused by free radicals (2005/International Application No. PCT/EP2005/004963). However, in such compositions the water-insoluble fullerene is not fully dissolved (i.e. a large part > 90 % remains suspended in the solvent) and their relative bioavailability is very low (i.e. < 1 %). Thus, the in vivo use of water-insoluble fullerenes as free radical scavengers through delivery thanks to a non-aqueous carrier is still not satisfactory. The majority of oils and certain fats used in food, cosmetic and pharmaceutical products is rich in mono-unsaturated or polyunsaturated fatty acids and, because of this, is particularly sensitive to oxidation. Their stability can be improved by the addition of synthetic antioxidants such as, for example, BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene) or TBHQ (te/Y-butyl hydroquinone). Unfortunately, the harmlessness of these compounds is questionable. That is the reason why attempts have been made to replace these synthetic antioxidants with natural antioxidant compounds, either of hydrophobic nature (for example, the tocopherols or ascorbyl palmitate) or of hydrophilic nature (for example, ascorbic acid, vegetable extracts, organic acids or amino acids). However, more efficient radical scavengers are still needed.

SUMMARY OF THE INVENTION

It is in view of the above problems that the present invention was developed. One objective of the invention is to provide stable biocompatible compositions comprising [60]fullerene dissolved in a suitable carrier. A further objective of the invention is to provide a process or method for protecting metazoans against damages caused by oxidative stress involving free radicals, which comprises a step of administering to said metazoans a composition comprising an effective amount of [60]fullerene, which avoids the drawbacks of the prior art processes, and in particular avoids the use of charge transfer complexes. Still, a further objective of the invention is to provide a process or method to preserve a substance or mixture of substances sensitive to damages caused by free radicals, which comprises a step of adding to said substance or mixture of substances a composition comprising an effective amount of [60]fullerene. Now, it has been discovered by the inventors that the compositions comprising [60]fullerene dissolved in a

suitable carrier selected from the group consisting of oils and fats proved suitable to achieve the aforementioned objectives. In particular, not only they allow [60]fullerene to be administered orally or intra peritoneally to protect metazoans against oxidative stress but they are also at least hundred times more active than previous compositions. Besides, the authors discovered that [60]fullerene can cross the brain barrier. Thus, a first embodiment of the instant invention comprises a stable biocompatible composition comprising (a) a carrier selected from the group consisting of fats and oils; and (b) [60]fullerene, wherein [60]fullerene is dissolved in said carrier. The embodiment is further drawn to compositions, in which [60]fullerene is dissolved in the carrier. Another embodiment of the instant invention is a method to protect a metazoan against damages caused by oxidative stress involving free radicals, which comprises a step of administering to said metazoan a stable biocompatible composition comprising an effective amount of [60]fullerene dissolved in a carrier selected from the group consisting of fats and oils. In yet another embodiment, the invention is drawn to a method of preserving a substance or mixture of substances sensitive to damages caused by free radicals, which comprises a step of adding to said substance or mixture of substances a stable composition comprising an effective amount of [60]fullerene dissolved in a carrier selected from the group consisting of fats and oils. In a preferred embodiment, said substance or mixture of substances is food or any nutritional composition. Compositions according to the invention may thus serve as food additives.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, features and advantages of the present invention will become readily apparent to those skilled in the art from a reading of the detailed description hereafter when considered in conjunction with the accompanying drawings wherein: - figure 1 is a representation of the growth rate of rats as a function of time, which were treated or not with a composition according to the present invention. - figure 2 shows whole blood C60 concentrations–time plot (mean ± S.E.M.) following (A) single bolus dose oral administration (4 mg/kg, n = 6) or (B) intra-peritoneal (ip) single bolus injection of the same dose (n = 3) of C60 dissolved in olive oil (0.8 mg/ml). Table 1 summarizes the main pharmacokinetic parameters after oral or i.p. administration of a single bolus dose of C60 dissolved in olive oil (0.8 mg/ml). Figure 3 shows the accumulation of C60 inside the organs after oral and peritoneal administration, figure 4 shows the effects of C60 pre-treatretment (either orally or intra-peritoneally) on CCl4 intoxication in rats showing the hepatoprotective effects of this fullerene against free radicals as reflected in the results of some biochemical tests used as markers of liver injury or oxidative stress, and figure 5 represents the survival percentage of 3 groups of rats (n = 6 per group) treated by gavages (during seven successive days, then every week during one month and then every month until the first control rat died) with C60 dissolved in olive oil (4mg/kg bwt) for group 1 or with water or with olive oil for the control groups (1 and 2, repectively).

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

Free radicals, such as oxygen radicals and other reactive oxygen/nitrogen/chlorine species (hydroxyl, nitric oxide radicals), are constantly formed in vivo. Some of these molecules are physiologically useful, but they can also result in pathological oxidative stress to cells and tissues. Endogenous defences include both antioxidants and repairing systems.

However. excess production of free radicals. their production in inappropriate relative amounts or deficiencies in endogenous defences can have deleterious effects. Free radicals can cause oxidative damage to lipids, DNA. bio molecules. rises in the concentration of intracellular calcium. as well as activation of proteases, nucleases and protein kinases. Considerable evidence supports the view that oxidative damage involving free radicals occurs in most, if not all, human diseases. Oxidative stress is now recognized as an important contributor to the development of many human diseases including liver fibrosis, ischemia-reperfusion, atherosclerosis, neurodegenerative disease and age-related cancer as well as to process of ageing. Thus antioxidants and systems that can protect against oxidative stress are needed to maintain health. This has led to attempts to develop additional antioxidants to supplement the antioxidant defences of cells as potential therapeutic agents. Diet-derived antioxidants and a number of small molecules that can scavenge free radicals as well as super oxide dismutase-mimetics and chelators of transition metal ions were proposed as potential therapeutic agents against oxidative stress. Compositions according to the invention comprising water-insoluble fullerenes have been found to exhibit highly efficient antioxidant properties in vivo. The fullerene cores, i.e. the fullerene skeletons without their lateral substituants, used in the practice of this invention comprise clustered carbon structures generally spherical in shape and having a carbon content generally ranging from about 50 to about 100 carbon atoms, although larger carbon content fullerenes are also known to exist and may be useful in the practice of the invention. Useful fullerene cores are, for example, C60, C70, C74, C76, C78, C84, etc. signifying the number of carbons in the particular fullerene structure. Because the higher carbon number fullerenes are not as easily obtained by present known methods, the preferred fullerene cores in the present invention are C60 and C70, more preferably C60. Pristine C60 is the most interesting compound, since it is much less expensive than chemically functionalized derivatives. Typically, [60]fullerene according to the invention is present in an amount ranging from 0.05 to 0.0.08 % by weight relative to the total weight of the composition, preferably 0.08 % by weight. [60]fullerene is preferably dissolved in the carrier. The stable, biocompatible compositions according to the invention comprise a carrier selected from the group consisting of fats and oils; and [60]fullerene, wherein said fullerene is dissolved in said carrier. The carrier used in the present invention is a pharmaceutically acceptable and biocompatible carrier, selected from the group consisting of fats and oils. The fat or oil may be any natural or synthetic fat or oil suitable for administration to a metazoan. They are not particularly restricted inasmuch as they are components which can be used in pharmaceutical preparations or in foods. Oils and fats can be hydrogenated or partially hydrogenated. They are used at a solid, a semisolid, or a liquid state. Vegetable and animal fats and oils are preferred, vegetable fats and oils are most preferred. Oils and fats include, without limitation fatty acid esters, fatty acids, fatty alcohols and fatty alcohol esters. Synthetic lipids can also be used. Fatty acids, as defined herein, are intended to mean aliphatic monocarboxylic acids having a chain of 4 to 40 carbon atoms, which may be branched or unbranched, saturated or unsaturated, cyclic or acyclic. Fatty acids may be natural or synthetic, polyunsaturated, mono-unsaturated or saturated. Natural fatty acids, which are usually unbranched and C4-C28 even-numbered, are preferred. Examples of fatty acids include, but are not limited to, linoleic acid, arachidonic acid, linolenic acid, gamma-linolenic acid, caprylic acid, stearic acid, myristic acid, a palmitic acid, behenic acid, undecylenic acid, oleic acid, an docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), isostearic acid, 12-hydroxy-stearic acid. Salts thereof [e.g. alkali metal salts (sodium salts, potassium salts, etc.), alkaline earth metal salts (calcium, magnesium salts etc.)] can also be employed. Fatty acid esters are preferably esters of fatty acid as defined hereinabove with C-1-C40 aliphatic or aromatic alcohols, preferably aliphatic, saturated or unsaturated, straight-chain or branched-chain, cyclic or acyclic. Alcohols can be polyols, having preferably up to five

hydroxyl groups. Examples of fatty acid esters include, but are not limited to, triglycerides i.e. tri-esters of glycerol with fatty acids cited above, sterids i.e. esters of sterols with fatty acids cited above, the group consisting of the lower alkyl esters thereof (preferably methyl, propyl, butyl, isopropyl and hexyl), 1 ,2- or 1 ,3-diglycerides, 1- or 2-monoglycerides, polyglycolysed glycerides such as sucrose fatty acid esters, polyglyceryl fatty acid esters, propylene glycol fatty acid esters. Specific examples of fatty acid esters are octyldodecyl behenate; isocetyl behenate; isocetyl lactate; isostearyl lactate; linoleyl lactate; oleyl lactate; isostearyl octanoate; isocetyl octanoate; decyl oleate; isocetyl isotearate; isocetyl laurate; isocetyl stearate; isodecyl octanoate; isodecyl oleate; isononyl isononanoate; isostearyl palmitate; myristyl isostearate; octyl isononanoate; 2-ethylhexyl isononanoate; octyl isostearate; octyldodecyl erucate; isopropyl palmitates, 2-ethylhexyl palmitate, 2- octyldecyl palmitate, branched alkyl myristates such as isopropyl myristate, t-butyl myristate, 2-octyldodecyl myristate, hexyl isostearate, butyl isostearate, isobutyl stearate, hexyl laurate, 2-hexyldecyl laurate, propylene glycol monostearate and distearate. Examples of glycerides (fatty acid esters) include, without limitation, triolein, trilinolein, tripalmitin, tristearin, trimyristin, and triarachidonin. Examples of sterids (fatty acid esters) include, without limitation, cholesteryl oleate, cholesteryl linoleate, cholesteryl myristate, cholesteryl palmitate, cholesteryl arachidate. Examples of fatty alcohols include, without limitation, cetyl alcohol, stearyl alcohol, lauryl alcohol, myristyl alcohol, palmityl alcohol, behenyl alcohol, hexadecyl alcohol, oleic alcohol, isostearyl alcohol, cetostearyl alcohol. They can be used as esters with C4-C40 dicarboxylic, tricarboxylic or tetracarboxylic acids. Oils may be natural oils such as vegetable oils and animal oils (composed predominantly of triglycerides), or mineral oils such as silicon oils, fluorinated oils. Liquid paraffin can also be used. Examples of natural oil include, but are not limited to, oils from plant sources, such as corn oil, wheat germ oil, soybean oil, rice bran oil, rapeseed oil, canola oil, sesame oil, palm (kernel) oil, olive oil, camellia oil, peanut oil, coconut oil, sunflower oil, peanut oil, orange oil, evening primrose oil, borage oil, blackcurrant seed oil, cottonseed oil, beaver oil, pineapple oil, safflower oil, copra oil, oils found in coffee, and animal oils such as turtle oil, fish oil, cod-liver oil. Fats may be mineral fats or natural fats such as vegetable fats and animal fats. Petrolatum, paraffin can also be used. Examples of natural fat include, but are not limited to, butter, cocoa butter, theobroma, peanut butter, lard, beef fat, chicken fat, horse fat, lanolin and lanolin derivatives. Oils and fats can be polyunsaturated such as corn, soybean, safflower oils, or saturated, such as palm, coconut oils and butter, or mono- unsaturated, such as olive oil and canola oil. Other suitable carriers according to the invention are diisopropyl sebacate; diisopropyl adipate; diisostearyl adipate; octyldodecyl stearoyl stearate; pentaerythrityl tetra-isononanoate; pentaerythrityl tetraisostearate; triisopropyl citrate; triisostearyl citrate; and trioctyldodecyl citrate. Preferred carriers according to the invention are butter, cocoa butter, peanut butter, olive oil, soybean oil, cod-liver oil and liquid paraffin. As defined above, carriers may be used each alone or in a combination of two or more species. [60]fullerene is dissolved in the carrier, depending on the nature of the carrier. Some carriers are able to dissolve substantial amounts of water- insoluble fullerenes (more than 1 mg / g of carrier). In one embodiment, at least 0.9 mg of fullerene is dissolved per gram of the carrier (the carrier being a liquid or a solid). As an example, it is possible to dissolve a total weight up to 1 mg of C60 per g of olive or soybean oil in less than one week. The compositions according to the invention may be pharmaceutical compositions comprising the fullerene in a therapeutically effective amount. Preferably, said fullerene can protect against biologically reactive radical species, which means chemicals that are free radicals or contribute to the generation of free radicals. Generally, the biologically reactive radical species are generated from O2 or H2O2. Thus, the invention also concerns a method to protect a metazoan against damages caused by oxidative stress involving free radicals, which comprises a step of administering to said

metazoan a stable biocompatible composition as defined hereinabove. The metazoans preferably are vertebrates, more preferably mammals, and even more preferably humans. The term "metazoan" also encompasses metazoan cells individually, such that the invention also encompasses contacting a metazoan cell with a composition according to the invention to protect it against damages caused by oxidative stress. [60]fullerene, when dissolved in the carriers of the present invention, can be administered to metazoans and this compound is well absorbed by said metazoans. Generally, the at least one water-insoluble fullerene is administered in an amount of at least 0.1 mg/kg of body weight per day. According to the method of the invention, the inventive compositions may be administered orally, intramuscularly, subcutaneously, intra dermally or intra peritoneally, rectally by suppositories, sublingually or by inhalation. For oral ingestion by a metazoan to be treated, the carrier is preferably an edible carrier. In at least one embodiment, said composition is administered in a pure form or as food additive. In another embodiment, it is administered in the form of an emulsion in water. The compositions of the instant invention can be in any liquid or solid conventional pharmaceutical formulation. The carrier enables the fullerene to be formulated as tablets, pills, dragees, capsules, liposome, pomade, ointment, cream, lotion, emulsions, gels, syrups, slurries and the like. The compositions of the present invention are preferably presented for oral administration to metazoans in unit dosage forms, such as tablets, capsules, and oral solutions, containing suitable quantities of [60]fullerene. The compositions may be sterilized and/or may contain some adjuvant such as preservatives, stabilizers, acidity regulators, natural or synthetic flavour, anti-foaming agents, viscosity- control agents, emulsifiers, salts for varying the osmotic pressure and/or other buffers. In addition, compositions may contain other pharmaceutically active agents. The level of free radicals and reactive oxygen species in metazoan cells decreases following treatment as compared to the level of reactive oxygen species in a cell that has not been contacted with a composition according to the invention. Indeed, [60]fullerene according to the invention act as antioxidants and supplement the antioxidant defences of cells. That means they inhibit oxidation or inhibit reactions promoted by reactive oxygen species. Physiologically relevant reactive oxygen species, which contribute to the generation of free radicals, include hydrogen peroxide, super oxide anion, and the like. The protective method of the invention reduces cell damage and death, and thus generally maintains the health of treated metazoans. Further, the inventors discovered that [60]fullerene administered as biocompatible compositions as described herein -1) can be absorbed through the digestive ducts after oral administration with about 25% of relative bioavailability ; -2) they can react inside the liver with vitamin A (retinol) and esters thereof following a Diels-Alder-like reaction without any toxic effect ; -3) they are eliminated through the bile ducts; -4) despite the large amounts administered (weekly oral administration of a composition comprising 0.8 mg of C60 in 1 g of olive oil per 200 g of body weight during several months), no acute, sub-acute or chronic toxicity could be observed in mice and rats. No behaviour or growth disorder could be observed in treated animals either, which can be seen on figure 1. The latter shows growth rate (expressed in % of the initial body weight) of three groups (n = 6) of rats which received weekly per os 1 g of olive oil containing 0.8 mg of C60 or 1 g of olive oil only or 1 ml of water only. Figure 5 shows the % of survival of a group of rats (n= 6) treated by gavages with C60 (0.8 mg) dissolved in olive oil (1 ml) and two control groups (n = 6, per group) treated under the same conditions with water or olive oil according a Kapplan-Meyer plot. The results clearly show that while olive oil increases the survival expectancy, adding a low dose of C60 to olive oil (0.8 mg/ml) further extended considerably lifespan, notably from month 25 after the treatment onset. The in vivo new properties of [60]fullerene are due to fullerenes themselves and/or to the fullerene-retinol and fullerene-retinyl ester adducts formed inside the liver and/or to C60-(O)n resulting from the metabolization of this fullerene by the enzyme CYP-2E1. The invention is

further related to a method of preserving a substance or mixture of substances sensitive to damages caused by free radicals, which comprises a step of adding to said substance or mixture of substances a stable composition as defined hereinabove. Said substance or mixture of substances may be any perishable good, for instance food or a nutritional composition which may contain at least one substance sensitive to oxidation. The preserving method according to the invention is particularly useful for substances rich in compounds derived from unsaturated fatty acids, more particularly polyunsaturated fatty acids. The substance or mixture of substances to be protected against oxidation may be the carrier of the composition itself. Also disclosed herein is a method for preparing a composition according to the present invention, comprising a carrier and particles of of [60]fullerene. Said method comprises the steps of: - (a) Charging a milling vessel with the fullerene, the carrier and balls, said milling vessel and balls being made out of any biocompatible metal or polymer; - (b) Agitating the mixture resulting from step (a) until a homogeneous dissolution is obtained; and - (c) Sterilizing the composition resulting from step (b) by filtration. Direct mechanical milling in the carrier presents the advantages to accelerate the dissolution. Said method comprises the steps of: - (a) Charging a milling vessel with the fullerene, the fat or oil and balls, said milling vessel and balls being made out of any biocompatible metal or polymer; - (b) Agitating the mixture resulting from step (a) until complete homogenization of the solution; - (c) Agitating the composition resulting from step (b) until complete dissolution of the fullerene; and - (d) Sterilizing by filtration the composition resulting from step (c). Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present disclosure. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should be construed in light of the number of significant digits and ordinary rounding approaches. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the disclosure are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements. The invention is further illustrated by the examples described below. These examples are meant to illustrate the invention and are not to be interpreted as limiting the scope of the invention.

EXAMPLES

General considerations

C60 (Purity: 99.98 %) was purchased from Term USA (Fort Bragg, CA, USA). Its purity was tested by HPLC, UV and MS. No impurity could be observed. It was used without further purification as well as after sublimation. All the other reagents were analytical grade and were purchased from Sigma (St Louis, MO). Animals received human care and the study protocols complied with University Paris Sud guidelines for the care and use of laboratory animals. Male Wistar rats (200 ± 10 g, Charles River, France) were housed by groups of 6 in polypropylene cages at constant temperature (22 0C) and humidity (60 %) and with a 12 h light/dark cycle, and fed a standard diet ad libitum. All rats were allowed to acclimate to this facility for at least one week before being used in the experiments. At the end of the experiment, body weights were determined and the animals were sacrificed under the same conditions by bleeding through the thoracic aorta after sodium pentobarbital (1.0 mL/kg of body weight) anaesthesia.

Biochemical tests, C60 determinations and statistics were processed as previously described in N Gharbi, M Pressac, M Hadchouel, H Szwarc, S. R. Wilson and Fathi

Moussa "[60]Fullerene is an in vivo Powerful Antioxidant With no Acute or Sub-acute Toxicity. Nano Letters 2005, 5 (12), 2578 - 2585.

Example 1: Direct dissolution of [60]fullerene in a vegetable oil

In the stainless steel milling vessels of a Pulverisette 7 (Fritsch, Idar- Oberstein, Germany) or a similar device, add 8 mg of [60]fullerene and 10 mL of olive oil or 10 g of butter and 6 stainless steel balls (8 mm of diameter) (the milling vessels and the balls can be made out of any biocompatible metal or polymer such as stainless steel, tempered chrome steel, silicon nitride, corundum, tungsten carbide, agate, oxide of zirconium etc). Agitate the mixture during several hours (at 600 rpm for instance) until complete dissolution. The resulting homogenous solution or paste is then ready for use for oral administration or by any route of administration after appropriate sterilization. Sterilization may be achieved by filtration under vacuum (pore size: 0.2 μm). The sterilized composition is stable for at least 1month in the dark. It is also possible to dissolve water-insoluble fullerenes in natural or mineral oils without stirring however the dissolution may be time consuming (up to several days at room temperature in the dark). Therefore, the former protocol is preferred. The fullerene concentration in compositions according to the invention can be determined by direct UV-Visible spectrophotometry or by HPLC after adequate dilution in mobile phase (toluene/acetonitrile; 50/50, v/v) as described previously [Nano Letters 2005, 5 (12), 2578 – 2585].

Example 2: C60-induced protection of the liver against acute toxicity of carbon tetrachloride (CCI4) in rats and survival percentage in rats

Carbon tetrachloride is a classical hepatotoxicant that causes rapid liver damage progressing from steatosis to centrilobular necrosis. CCI4 intoxication in rodents is an important model for elucidation of the mechanism of action of hepatotoxic effects such as fatty degeneration, fibrosis, hepatocellular death, and carcinogenicity. These effects are consistent with the known induced metabolic activation of CCI4 to reactive intermediates, including CCI3" and CCIO2' free radicals, and mobilization of intracellular calcium. Kupffer cells (liver resident macrophages) participate in the mechanism of toxicity of CCI4 in vivo by release of chemoattractants for neutrophils and a series of chemical mediators (cytokines). Both expression and synthesis of these cytokines are mainly modulated through redox-sensitive reactions. Further, involvement of reactive oxygen species and lipid peroxydation products can be demonstrated in other fundamental events of hepatic fibrogenosis, like activation of hepatic stellate cells (HSC: liver resident nonparenchymal cells also referred to as fat-storing or perisinusoidal cells, lipocytes and lto cells). In a previous work, the effects of C60-pretreatments on acute carbon tetrachloride intoxication in rats, a classical model for studying free-radical-mediated liver injury was reported. The results obtained by the authors leaded by F Moussa (Nano Letters 2005, 5 (12), 2578 – 2585) showed that aqueous C60 suspensions not only have no acute or subacute toxicity in rodents but they also protect their livers in a dose-dependent manner against free-radical damage. The most effective dose of C60 reported in the latter paper was about 2.5 g/kg of body-weight and was administered intra-peritoneally and the better protection was obtained at day 14 after administration. It was now discovered by the inventors that : -1) the relative bio-availability is about 25% (Figure 2), -2) the fullerene can cross the brain barrier (figure 3), -3) the fullerene is about 100 times more active when it is administered in solution than in suspension (Figures 3), and -4) the fullerene prolong the life span in Wistar rats (figure 5).

REVENDICATIONS

1. A stable biocompatible composition comprising: (a) a carrier selected from the group consisting of fats and oils; and (b) one compound selected from the group consisting of water-insoluble fullerenes, wherein said fullerenes are dissolved in said carrier. 2. The stable biocompatible composition of claim 1, wherein the carrier is selected from the group consisting of butter, cocoa butter, peanut butter, olive oil, soybean oil, cod-liver oil, liquid paraffin, and mixtures thereof. 3. The stable biocompatible composition of any one of claims 1 or 2, wherein said water-insoluble fullerenes are present in an amount ranging from 0.01 to 0.3 % by weight relative to the total weight of the composition, preferably from 0.08 to 0.2 % by weight. 4. The stable biocompatible composition of any one of claims 1 or 2, wherein the fullerene core of said water-insoluble fullerenes is C60 or C70. 5. The stable biocompatible composition of any one of claims 1 or 2, wherein said at least one water-insoluble fullerene is comprised in a therapeutically effective amount. 6. The stable biocompatible composition of claim 5, wherein said at least one water-insoluble fullerene can eliminate biologically reactive radical species. 7. The stable biocompatible composition of claim 6, wherein the biologically reactive radical species are generated from O2 or H2O2. 8. A method to protect a metazoan against damages caused by oxidative stress involving free radicals, which comprises a step of administering to said metazoan a stable biocompatible composition according to any one of claims 1 to 7, wherein said composition is administered intravenously, intramuscularly, subcutaneously, intradermally, intrathecally, intraperitoneally, rectally by suppositories, sublingually, by inhalation or orally. 9. The method of claim 8, wherein said composition is administered in a pure form or in the form of an emulsion in water. 10. The method of any one of claims 8 or 9, wherein the at least one water- insoluble fullerene is administered in an amount of at least 0.1 mg/kg of body weight per day. 11. The method of any one of claims 8 or 9, wherein said metazoan is a mammal. 12. The method of claim 11, wherein said metazoan is a human. 13. The method of any one of claims 8 or 11, wherein it maintains the health of said metazoan. 14. A method to preserve a substance or mixture of substances sensitive to damages caused by free radicals, which comprises a step of adding to said substance or mixture of substances a stable composition according to any one of claims 1 to 9. 15. The method of claim 14, wherein said substance or mixture of substances is food or a nutritional composition.

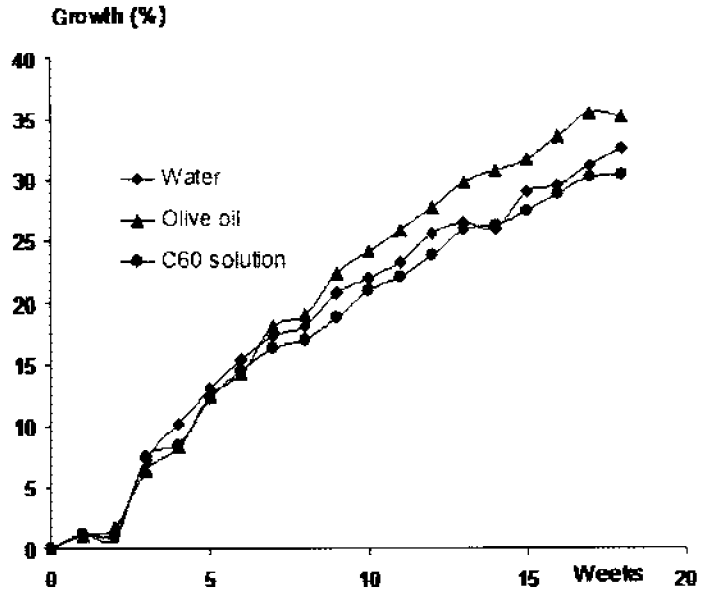

Figure 1. Growth percentage of three groups (n = 6 per group) of rats treated by gavages with C_{60}-olive oil (0.8 (4mg/kg bwt) or water or olive oil (1 ml/kg bwt).

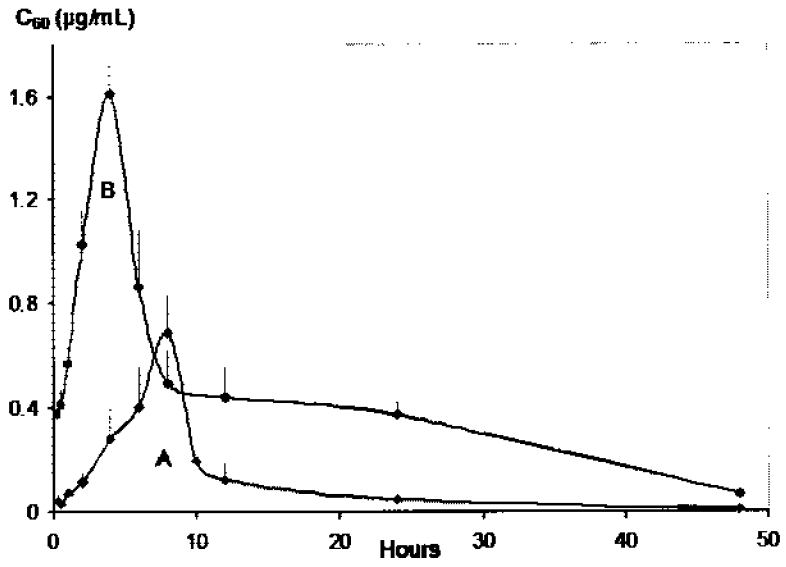

Figure 1. Whole blood C_{60} concentrations–time plot (mean ± S.E.M.) following (A) single dose oral administration (4 mg/kg, n = 6) or (B) intra-peritoneal bolus injection of the same dose (4 mg/kg, n = 3) of C_{60} dissolved in olive oil (0.8 mg/ml).

Table 1. Pharmacokinetic parameters

Parameter	Units	Estimate (Oral admin.)	Estimate (I.p. admin.)
HL_Lambda_z	hr	8.9446	13.0454
Tmax	hr	8.0000	4.0000
Cmax	ug/mL	0.6910	1.6100
AUCINF_obs	hr*µg/mL	5.3384	21.0699
AUC_%Extrap_obs	%	2.1755	6.1634
Vz_F_obs	mL	9669.1002	3572.9843
Cl_F_obs	mL/hr	749.2897	189.8446
AUMCINF_obs	hr*hr*µg/mL	65.4551	369.7917
AUMC_%Extrap_obs	%	10.8065	23.4658
AUMC_%Extrap_pred	%	10.2405	25.3334
MRTINF_obs	hr	12.2612	17.5507

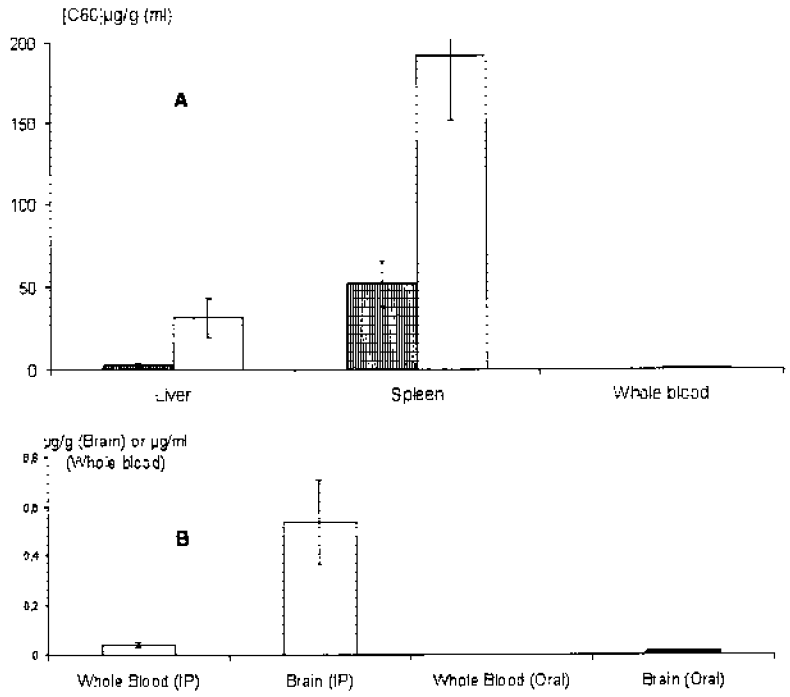

Figure 3. [60]fullerene accumulation in livers, spleens and brains after i.p. (white) or oral (greyish) administration of C_{60}-olive oil (4mg/kg bwt) to rats. (A) 24 hours after administration and (B) 72 hours after administration The insert is a magnification of the graph.

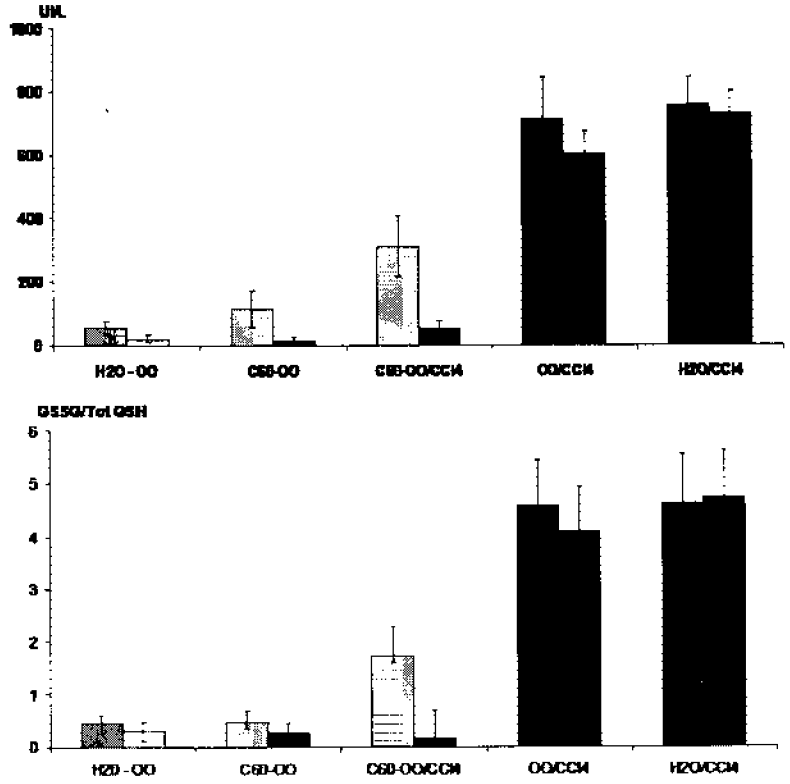

Figure 4. Effects of C_{60} pre treatment (Purple bars = gavages with 4mg/kg bwt during 7 days; and brown bars = intraperitoneal administration of the same dose during the same period) on CCl4 intoxication in rats. The graph in the top represents the serum alanine amino transferase activity used as a biochemical marker of parenchymal cell damage. The graph in the bottom represents the % of oxidized glutathione used as marker of oxidative stress.

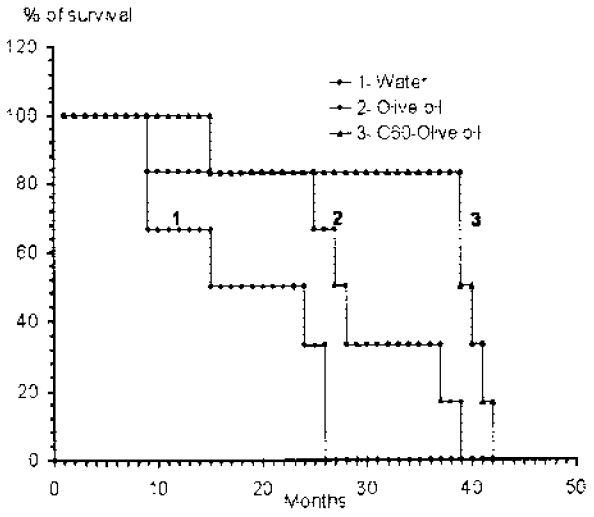

Figure 5. Survival percentage of 3 groups of rats (n = 6) treated by gavages (daily during one week, then weekly during 4 weeks and then monthly during 8 months) with (1) water, (2) olive oil and (3) C_{60} dissolved in olive oil (4 mg/kg of body weight).