A MARINE OIL FORMULATION COMPRISING RESERVATROL OR DERIVATIVES THEREOF FOR USE IN TREATING, DELAYING AND/OR PREVENTING ALZHEIMER'S DISEASE

FISCHÖLFORMULIERUNG MIT RESERVATROL ODER DERIVATEN DAVON ZUR VERWENDUNG BEI DER BEHANDLUNG, VERZÖGERUNG UND/ODER VORBEUGUNG VON MORBUS ALZHEIMER

PRÉPARATION À BASE D'HUILE MARINE COMPRENANT DU RESVÉRATROL OU SES DÉRIVÉS, ET UTILISÉE POUR TRAITER, RETARDER ET/OU PRÉVENIR LA MALADIE D'ALZHEIMER

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).
Remarks:
The file contains technical information submitted after the application was filed and not included in this specification.
The present invention relates to a formulation comprising fresh marine oil in an oil-in-water emulsion and resveratrol or derivatives thereof for use in treating, delaying and/or preventing Alzheimer's Disease. The invention relates further to delaying and/or preventing the onset of symptoms associated with Alzheimer's Disease including Mild Cognitive Impairment (MCI). The present invention also relates to a method for treating, delaying and/or preventing Alzheimer's Disease including MCI by administering said formulation to a human. Such formulation will provide a new drink formula having improved health promoting effects on humans. There is, however, no disclosure of supplements will not induce any health promoting effects in humans. It is known that humans having severe oxidative stress are often deficient in omega-3 fatty acids (DHA and EPA), and possess a low antioxidative status. Oxidative damage and antioxidant deficiency are now regarded as crucial factors to many diseases, and are probably the primary reason for an imperfect replacement of old damaged cells by new cells.

Oxidative stress is a sort of "chemical stress" induced by the presence in our body of abnormal quantities of free radicals. Whatever the cause, oxidative stress is believed to be responsible of early ageing and of a very long series of common diseases - about one hundred - that span from arterial hypertension to atherosclerosis, from infarct toicus, from Parkinson's to Alzheimer's, from colitis to pancreatitis, from obesity to diabetes, from chronic bronchitis to rheumatoid arthritis, from AIDS to several types of cancer. Antioxidants may be vitamins, minerals, and enzymes, either fat soluble or water soluble. In situatons where the body is subjected to enhanced oxidation (a lot of free radicals), the body might not have sufficient antioxidants to neutralize or quench the free radicals. Destructive chain reactions occur, which might cause increased and detrimental oxidative stress. It is also acknowledged that the absorption of antioxidants in the body from antioxidant supplements is a challenge. However, studies have demonstrated that antioxidants in a non-native form or as isolated vitamins are inadequately taken up by the body. Some studies indicate that ingestion of high dosages of isolated vitamins may convert antioxidants to prooxidants, thus leading to elevated oxidation in the body. Studies and literature indicates better absorption and bioavailability of antioxidants naturally present when consumed in foods e.g. as fruits and vegetables.

It is known that humans having severe oxidative stress are often deficient in omega-3 fatty acids (DHA and EPA), and possess a low antioxidative status. Oxidative damage and antioxidant deficiency are now regarded as crucial factors to many diseases, and are probably the primary reason for an imperfect replacement of old damaged cells by new cells. Research work has demonstrated that oxidation products of fatty acids are highly reactive and may affect and interfere with intracellular processes. Many commercially available omega-3 supplements contain fish oil having a significant degree of oxidation, which in turn may induce adverse effects on intracellular processes. Although these dietary supplements often are added antioxidants, this will not reverse the rancidity already present in the dietary supplement. On the other hand, to prevent further oxidation of the unsaturated fish oil, the antioxidants in the supplement will be consumed and finally (after some months) cease. In this case, antioxidants in such commercially available dietary supplements will not induce any health promoting effects in humans. WO2009/120091 provides advantageous compositions combining fresh fish oil and specific antioxidants to provide a new drink formula having improved health promoting effects on humans. There is, however, no disclosure of treating, delaying and/or preventing Alzheimer's Disease including MCI with a composition comprising marine oil and resveratrol. Alzheimer's Disease (AD) is the most prevalent form of dementia. AD impacts millions of people worldwide and at present there is no known cure for the disease. The symptoms of Alzheimer's Disease become progressively more debilitating as the disease advances. Ultimately, Alzheimer's Disease results in the death of the inflicted individual, typically after many years of gradually losing the ability to function in society. Thus, there is a significant need for a treatment to prevent, delay and/or treat Alzheimer's Disease. MCI is regarded as one of the earliest detectable stages of Alzheimer's Disease.
SUMMARY OF THE INVENTION

[0013] The present invention is directed to a formulation for use in treating, delaying and/or preventing Alzheimer’s Disease and preventing and/or delaying the onset of the symptoms of Alzheimer’s Disease including MCI in humans. Said formulation comprises fresh marine oil and the antioxidant resveratrol or derivatives thereof. In a preferred embodiment the formulation is a drink. Preferably the formulation is administered on a daily basis, and most preferably it is a drink formulation administered on a daily basis. As used herein daily basis means administering at least once a day, but also includes multiple administrations in a day, e.g. twice or thrice daily.

[0014] Preferred embodiments of the present invention are set forth in the dependent claims.

DESCRIPTION OF THE FIGURES

[0015] Preferred embodiments of the present invention will now be illustrated in more detail with reference to the accompanying figures.

Figure 1 illustrates the improved phagocytosis of amyloid beta after administration of the drink formulation of Example 1 to AD patients (Group 1) showing down regulation of inflammatory genes.

Figure 2 illustrates the change in baseline expressions of inflammatory genes after administration of the drink formulation of Example 1 to Group 1 AD patients.

Figure 3 illustrates the change in the effect of the exogenous pathogen (sAβ_{1-42}) in Group 1 AD patients after 3 month’s administration of the drink formulation.

Figure 4 illustrates the change in the effect of the exogenous pathogen (sAβ_{1-42}) in Group 1 AD patients after 5 month’s administration of the drink formulation.

Figure 5 illustrates the improved phagocytosis of amyloid beta after administration of the drink formulation of Example 1 to AD patients (Group 2) showing up regulation of inflammatory genes.

Figure 6 illustrates macrophages obtained from Group 1 and Group 2 Patients after administration of the drink formulation.

Figure 7 illustrates the results of patient G from Group 2 after administration of the drink formulation of Example 1.

Figure 8 illustrates the change in baseline expression of inflammatory genes after administration of the drink formulation of Example 1 to Group 2 AD patients.

Figure 9 illustrates a comparison of inflammation in AD patient Group II on a daily regimen of Example 1 drink formulation compared to inflammation in two patients in group 2 who were non-compliant with administration of the drink formulation.

Figure 10 illustrates phagocytosis of FAM-amyloid-beta by fresh monocytes. Note the upper number = mean fluorescence intensity of FAM-Aβ phagocytosis; lower number = % cells positive for Aβ.

A: Cognitively normal subjects or caregivers: RB and DS are caregivers; CE is Parkinson disease patient; DN is diabetic patient with normal cognition; EB is patient with ALS; MF and FD are normal controls.

B: Cognitively-impaired patients with minimental state examination (MMSE) ≥ 19 at baseline receiving supplementation with the drink formulation after the first visit.

C. Patients with Alzheimer Disease (MMSE<19) receiving supplementation with the drink formulation after the first visit.

Fig. 11 illustrates phagocytosis of FAM-Abeta by macrophages of AD patients on supplementation with the drink formulation of the present invention (visit 1 = before intake of the formulation; Visits 2, 3, after intake of the formulation)

Fig. 12 illustrates the in vitro effect of the drink formulation (SMF/Res) on Aβ phagocytosis by macrophages of the
The present invention relates to a formulation for use in treating, delaying and/or preventing Alzheimer’s Disease in humans, said formulation comprises fresh marine oil and the antioxidant resveratrol or derivatives thereof. Said formulation is further used in preventing and/or delaying the onset of the symptoms of Alzheimer’s Disease including MCI. In this context Alzheimer’s Disease and symptoms of Alzheimer’s Disease include the condition Mild Cognitive Impairment (MCI).

The present invention is a formulation of fresh marine oil and resveratrol or derivatives thereof. Preferably the formulation is a drink formulation, although it is also contemplated that the formulation may be presented in other well-known administrations forms, such as a tablet or capsule or gel. For example, a drink formulation may be prepared and then dried, e.g. lyophilized to a powder or granulation, and then presented in a tablet or capsule dosage form. Alternatively, the drink formulation could be concentrated to form a concentrate for use by a patient or gelled using standard gelling techniques to provide an edible gel.

Preferably, the drink formulation may have a base containing natural antioxidants e.g. fruit or vegetable juice, green tea, but any drinkable liquid may be used. Most preferably the base is a fruit juice, such as, for example, those selected from the group consisting of apple concentrate, pear concentrate, pomegranate concentrate, chokeberry concentrate and combinations thereof.

The drink formulation combines a stable omega-3 emulsion known from the prior art, and resveratrol or derivatives thereof. Preparation of the drink formulation used in the present invention is described in US2011/0135745. Such drink formulations show improved delivery, improved uptake and improved effect on oxidative stress.

Both the omega-3 oil and the antioxidants contained in the drink formulation used in the present invention are remarkably stable in the composition, and the progress of rancidity and loss of antioxidant effects are much lower than in known product formulated as separate capsules.

One aspect of the present invention relates to the unexpected and surprising discovery that a drink formulation comprising fresh marine oil in an oil-in-water emulsion wherein the marine oil has a totox value below 15, with a further added at least one antioxidant, of resveratrol (3, 5, 4’-trihydroxy-transstilbene) which is not naturally present in said oil-in-water emulsion, provides a drink formulation that shows promise in the treatment and/or prevention of Alzheimer’s Disease and/or the symptoms associated therewith. As used herein, treatment includes delaying the advance or onset of symptoms of AD, arresting the development of symptoms AD and/or reversing the symptoms associated with AD including MCI. As used herein, preventing means delaying the onset of the symptoms in AD in a person susceptible to AD, e.g., a person at least 55 years old, preferably at 60 years old, more preferably at least 65 years old.

As used herein, derivatives of resveratrol include for example hydrolyzable derivatives such as esters, e.g. C1-6 alkyl esters, of one or more of the hydroxyl groups of resveratrol.

The term fresh marine oil describes an oil prepared from fresh marine species where all process steps are conducted carefully and under strict oxygen control according to functional oil standards in order to prevent oil oxidation. The fresh marine oil will have a low oxidative status, revealing a colourless oil without the characteristic smell or taste of e.g. fish. The level of oxidation given as the totox value (2 times the peroxide value (PV) added with the anisidin value (AV)) should be below 15, preferably below 10, and most preferably below 5. Marine oil present in many food supplements today contains oil with a much higher totox value, typically 20 - 30 or even higher.

The fresh marine oil may be any oil rich in omega-3, e.g. fish oil, seal oil or krill oil. The oil may be mixed with other polyunsaturated oils of vegetable origin such as algae oil and herbal oil such as evening primrose oil and rapeseed oil.

In one preferred embodiment of the present invention the drink formulation is comprised of marine oil, in an amount of about 0.5% to about 10% by weight based on the total weight of the drink formulation, more preferably in the range of, about 0.5% to about 7%, most preferably in the range of about 1.5% to about 4%.

The oil-in-water emulsion is prepared by any conventional method, preferably as described in the applicants own Norwegian applications NO 20044542, 20053136 and 20055620. In said emulsions the antioxidants are present to stabilize the oil during production and storage, not for the purpose of inducing any health promoting effects on humans.

The water phase of the oil-in-water emulsion is preferably a water phase containing natural antioxidants e.g. fruit/vegetable juices, green tea, white tea and herbal tea. The juice may be a fresh pressed juice or juice in the form of juice concentrate or juice puree, or puree diluted to obtain a normal ready-to-use juice. The water phase may also contain proteins such as soy, oat proteins, whey proteins and/or milk proteins. The drink formulation will generally have added water in the amount of about 50 to about 90 weight percent, preferably about 60 to about 80 weight percent. When fruit juice concentrate is added, the drink formulation will contain about 5 to about 30 weight percent fruit juice concentrate,
preferably about 10 to about 20 weight percent fruit juice concentrate.

[0028] It has been surprisingly discovered that a drink formulation containing the fresh marine oil described above in combination with resveratrol or derivatives thereof, may have a beneficial impact on symptoms associated with Alzheimer's Disease or the treatment and/or prevention of Alzheimer's Disease.

[0029] Preferably the resveratrol is present in the drink formulation in an amount of about 0.01 to about 0.5 percent by weight of the formula, more preferably about 0.05 to about 0.25 percent by weight of the formula. In particular preferred embodiments of the invention a one serving drink formulation contains about 70 to 130mg of resveratrol.

[0030] In one embodiment, the formulation may also include one or more vitamins such as, for example, vitamin B, C and/or D and/or one or more minerals such as, for example, selenium, folic acid and/or zinc.

[0031] In one embodiment of the present invention, the formulation may also include prebiotics and/or probiotics.

[0032] In one embodiment of the present invention, formulation may be carbonated.

[0033] The drink formulation may be prepared, for example, by the following steps:

a) resveratrol and flavoring agents, together with emulsifier are added to the oil phase,

b) water soluble additives are added to the water phase,

c) the oil and water phase are mixed to a homogenous emulsion,

d) the emulsion obtained is optionally subjected to pasteurization and/or homogenization processes,

e) the obtained emulsion is cooled down and filled on clean disposable containers;

wherein all steps are performed under strict oxygen control.

[0034] Alternatively, in yet another example, the drink formulation may be prepared according to the following steps:

a) transresveratrol and flavoring agents are added to the oil phase,

b) water soluble additives are added to the water phase,

c) the oil and water phase are mixed and the emulsifier is added, followed by gentle mixing to achieve a homogenous emulsion,

d) the emulsion obtained is optionally subjected to pasteurization and/or homogenization processes,

e) the obtained emulsion is cooled down and filled on clean disposable containers;

wherein all steps are performed under strict oxygen control.

[0035] In the present invention the drink formulation described herein has been found to show properties that suggest it will be useful in the prevention and/or treatment of Alzheimer's Disease. In particular, it has been discovered that individuals drinking the drink formulation described herein on a daily basis showed an improvement in inflammatory gene transcriptions in AD patients. In particular, it was found that the macrophages of the AD patients received the ability to phagocyte amyloid beta. Defective phagocytosis of amyloid beta is a well-known marker of AD.

[0036] A further study measuring the ability of the macrophage to phagocyte amyloid beta and cognitive benefits in individuals with AD showed that the macrophages of the patients as early as 19 days following administration of the drink formulation of the present invention, became functional in uptake of amyloid beta. Also possible cognitive benefits in patients with mild cognitive problems were shown.

[0037] The specific drink formulation used in the present invention is believed to provide significant advantages to humans to which it is administered, including, presenting the essential nutrients and specific health promoting agents (polyunsaturated fatty acids and added antioxidants) to the digestive system and to the cells in a format highly beneficial to the cells and the body.

[0038] While the use of the formulation of the invention is preferably practiced by administration of a drink formulation, it is also contemplated that the drink formulation could be administered concentrated or dried to a granulation or powder. Such a granulation or powder could be formed into a tablet or placed in a capsule using well known procedures. Alternatively the formulation of the invention could take the form of a gel or concentrate that may be prepared from the drink formulation by respectively, employing one or more standard gelling agents or preparing the formulation as a concentrate.

[0039] The drink formulation may be administered on a daily basis in a volume range of 50-300ml, preferably 100ml and more preferably 200ml. The range of each of DHA and EPA in the drink formulation that is administered may be
from about 500mg to about 5000mg per day, preferably about 3000mg per day, more preferably about 2000mg per day and most preferably about 1000mg per day. Generally, the range of resveratrol in the administered formulation may be from about 20 to about 800mg per day, preferably about 50 to about 600mg per day, more preferably about 75 to about 300mg per day and most preferably about 75 to about 130mg and even more preferably about 130mg.

Accordingly a first aspect of the present invention relates to a formulation comprising (i) a fresh marine oil in an oil-in-water emulsion, wherein the marine oil may have a totox value below 15 and (ii) resveratrol or derivatives thereof for use in treatment of Alzheimer’s Disease and/or or delaying the onset of symptoms associated with Alzheimer’s Disease including Mild Cognitive Impairment (MCI).

In a preferred embodiment the formulation the content of the marine oil may be about 0.5% to about 10% by weight of the total weight of the formulation and the content of the resveratrol may be about 0.01% to about 0.5% by weight.

In one embodiment the fruit juice concentrate may be selected from the group consisting of apple juice concentrate, pear juice concentrate and mixtures thereof.

In another embodiment the drink formulation may further comprise pomegranate and/or chookberry.

In a further embodiment the drink formulation may further comprise vitamins selected from the group consisting of Vitamin D, Vitamin C, Vitamin B and mixtures thereof.

In a second aspect of the invention the formulation comprises (i) a fresh marine oil in an oil-in-water emulsion in an amount of about 0.5% to about 10% by weight, wherein the marine oil has a totox value below 15; (ii) resveratrol or derivatives thereof in an amount of about 0.01% to about 0.5% by weight; (iii) fruit juice concentrate; and (iv) one or more vitamins for use in the treatment of Alzheimer’s Disease and/or delaying the onset of symptoms associated with Alzheimer’s Disease including MCI.

In one embodiment the formulation(s) may be a drink formulation.

In a further embodiment the formulation(s) may be used on a daily basis.

In yet a further embodiment the formulation(s) may have a volume in a range of 50 to 300 ml.

In a third aspect the present invention relates to a method of treating a patient with Alzheimer’s Disease comprising administering the formulation as described above to the patient.

In a fourth aspect the present invention relates to a method of delaying the onset of symptoms associated with Alzheimer’s Disease including MCI comprising administering the formulation as described above to the patient.

Having now fully described the present invention in some detail by way of illustration and examples for purpose of clarity of understanding, it will be obvious to one skilled in the art that same can be performed by modifying or changing the invention by a wide and equivalent range of conditions, formulations and other parameters thereof, and that such modifications or changes are intended to be encompassed within the scope of the appended claims.

EXAMPLES

The invention will now be further illustrated with reference to the following non-limiting examples.

Example 1

A 200ml drink formulation providing about 1000mg of each of EPA and DHA per day along with 130mg per day of resveratrol was prepared from a fresh marine oil omega-3 emulsion and resveratrol.

<table>
<thead>
<tr>
<th>wt. %</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, purified</td>
<td>74.3</td>
</tr>
<tr>
<td>Pomegranate</td>
<td>3.5</td>
</tr>
<tr>
<td>Chookberry</td>
<td>1</td>
</tr>
<tr>
<td>Vitamin D₃ (10γg)</td>
<td>-</td>
</tr>
<tr>
<td>Whey Protein Isolat</td>
<td>4.2</td>
</tr>
<tr>
<td>Fiber</td>
<td>0.5</td>
</tr>
<tr>
<td>Apple Juice Conc.</td>
<td>6</td>
</tr>
<tr>
<td>Pear Juice Conc.</td>
<td>5</td>
</tr>
<tr>
<td>Resveratrol (130mg)</td>
<td>0.065</td>
</tr>
<tr>
<td>Marine oil DHA &amp; EPA 1000mg each</td>
<td>4.8</td>
</tr>
</tbody>
</table>
Example 2

Peripheral blood mononuclear cells (PBMCs) of AD patients show either up regulation (Group 2) or down regulation (Group 1) of inflammatory genes in comparison to age-matched controls at baseline, but PBMCs of all AD patients are stimulated by amyloid beta (Abeta) to inflammation. A universal biomarker of AD patients is defective phagocytosis of soluble or fibrillar Abeta by macrophages. In vitro, the lipid modulator from docosahexaenoic acid (DHA) resolvin D1 (RvD1) and the hormonal form of vitamin D3 1,25-dihydroxyvitamin D3 (1,25D3) repair the deregulation of these genes and Abeta phagocytosis (Mizwicki T et al. JAlz Dis 2013). DHA and vitamin D3 are frequently taken as nutritional supplements against dementia, but their biochemical effects in vivo are not well known. Example 1 is a drink formula comprised of marine oil contrary with DHA and eicosapentaenoic acid (EPA) stabilized against oxidation through additive and synergistic effects of components from plants (pomegranate and chokeberry), vitamin D3, transresveratrol, whey protein isolate and fiber.

The inflammatory gene transcription in AD patients who have been consuming this drink daily for over 8 months was tested. In the Group 1 patients the transcription of IL-1 beta increased whereas in the Group 2 IL-1 beta transcription decreased, and the macrophages from both groups recovered the ability to phagocytize Abeta. Minimental state examination (MMSE) scores were stabilized. In conclusion, it was found that nutritional supplementation with a drink of the present invention comprising DHA, EPA, and a resveratrol derivative corrects abnormal transcription and recovers Abeta phagocytosis in AD patients.

Example 3

Methods

Study population: The patients are listed in the order as they enrolled into the study (Table 1). Most subjects were taking approved medication for Alzheimer Disease, such as cholinesterase inhibitor and/or the NMDA inhibitor memantine, and voluntarily the drink formulation of the present invention. Cognitive state was examined by the Minimental State Examination (MMSE).

Formulation of the present invention: The subjects were taking daily 200 ml drink of the invention comprising 1000 mg DHA, 1000 mg EPA, and 75 mg resveratrol.

Lymphocytes isolation and macrophage cultures: Heparinized blood from the AD patients was diluted with PBS (1:1 ratio; vol/vol). Peripheral blood mononuclear (PBMCs) cells were isolated from the diluted blood by Ficoll-hypaque gradient method at 2500 RPM for 20 minutes at room temperature, the mononuclear fraction was collected and washed two times with PBS, and cells were re-suspended with IMDEM medium. Macrophages were differentiated in well chambers from 50,000 mononuclear cells in IMDM medium with 10% autologous serum.

Flow cytometric FAM-Abeta phagocytosis assay: 0.5x10^6 PBMCs were suspended with IMDM medium with 10% autologous serum and were incubated with or without FAM-Abeta (Anaspec, San Jose, CA) overnight at 37° C in 5% CO2 incubator. The cells were then washed two times with FACS buffer and then labeled for 30 minutes at 4° C with anti-CD14 PE. After incubation, cells were washed two times with FACS buffer and fixed with 1% paraformaldehyde. Flow cytometry was performed on FACSCalibur (Becton Dickinson) and data were analyzed using FlowJo software (Ashland, OR) with monocyte gate, based on forward and side scatter.

All patients were evaluated by the flowcytometric test of Aβ phagocytosis, and the Minimental state examination.

Results

Amyloid-P phagocytosis by blood monocytes of AD patients taking the formulation of the present invention

The effects on supplementation of the formulation of the present invention were tested in relation to Aβ phagocytosis and cognition in patients suffering from AD including Mild Cognitive Impairment (Table 2 and Fig. 10). The normal range of the Aβ phagocytosis test in cognitively-normal age-matched subjects was previously established as >450 Mean Fluorescent Intensity (MFI) and in AD patients as ≤450 (Avagyan, Goldenson et al. 2009; J Neuroimmunol 210 (1-2): 67-72).

The results in this study confirmed previous values at baseline. The mean score on the first visit (before
supplementation) was 272 MFI units in the group with advanced dementia (MMSE <19) and in the group with mild dementia or subjective memory complaints (MMSE ≥19) was 280.5 (N.S.). AD patients with advanced dementia taking nutritional supplementation with the formulation of the present invention increased the uptake of amyloid-β on the second visit to 574 MFI units and the patients with mild dementia increased the uptake to 643 MFI units. Cognitively normal subjects and patients with other neurological diseases were also tested (Table 2 and Fig. 10).

**Phagocytosis of amyloid-beta by macrophages of AD patients taking the formulation of the present invention**

[0064] PBMC’s of AD patients were cultured for 10-15 days until macrophages became differentiated from monocytes. The inventors tested these macrophages for amyloid beta phagocytosis by exposing them overnight to FAM-Abeta. In agreement with previous results, the macrophages of AD patients derived from the blood before the first visit were unable to phagocytize Abeta but macrophages isolated from the blood after omega-3 supplementation on subsequent visits improved phagocytosis in most subjects (Fig. 11).

**Minimental state examination (MMSE) scores of AD patients taking the formulation of the present invention**

[0065] In the group with advanced dementia (MMSE <19), the mean MMSE score before supplementation was 9.0 and the score did not significantly change after supplementation (mean score 9.5). In the group with Mild Cognitive Impairment (MMSE ≥19); the MMSE score increased from 23.8 to 27.5 after supplementation (Table 2).

**Increase in amyloid-P phagocytosis by the formulation containing resveratrol**

[0066] Resveratrol was shown to promote proteasome degradation of amyloid-beta in cell lines (Marambaud, Zhao et al. 2005; J Biol Chem 280(45): 37377-37382).

**[0067]** Here we tested in vitro the activity of resveratrol on phagocytosis of amyloid-beta by monocytes. The PBMCs of the AD patient #2, who was supplemented with the formulation of the present invention in vivo, were tested overnight by the amyloid-beta test after in vitro supplementation with:

  a) the formulation of the present invention,

  b) the formulation of the present invention without resveratrol; or

  c) the formulation in b) with added curcumin.

[0068] The phagocytosis was maximally increased with the formulation of the present invention compared to the controls in b) and c) (Fig. 12).

**Table 1. Subjects in the study**

<table>
<thead>
<tr>
<th>A. Cognitively-impaired patient</th>
<th>Age, Sex</th>
<th>Initial MMSE score</th>
<th>Duration of disease before the drink formulation(years)</th>
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<tr>
<td>1</td>
<td>60, M</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>88, F</td>
<td>&lt;5</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>70, M</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
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<td>5</td>
<td>76, F</td>
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<tr>
<td>6</td>
<td>87, M</td>
<td>11</td>
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<td>7</td>
<td>77, M</td>
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<td>8</td>
<td>72, M</td>
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<tr>
<td>10</td>
<td>F</td>
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<tr>
<td>11</td>
<td>78, F</td>
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<td>1</td>
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<tr>
<td>12</td>
<td>8</td>
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<td>5</td>
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<td>MMSE Score at Baseline</td>
<td>Subject #</td>
<td>Duration of AD (yrs)</td>
<td>MMSE</td>
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<tr>
<td>Mean</td>
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</table>

*Patient is complaining of "mental fog"
#Patient is somewhat relieved of "mental fog"
Claims

1. A formulation comprising (i) a marine oil having a totox value below 15 in an oil-in-water emulsion wherein the water phase is fruit or vegetable juice, and (ii) resveratrol or C1-6 alkyl ester derivatives thereof for use in treatment of Alzheimer’s Disease and/or delaying the onset of symptoms associated with Alzheimer’s Disease including Mild Cognitive Impairment (MCI), wherein the content of the marine oil is about 0.5% to about 10% by weight of the total weight of the formulation and the content of the resveratrol is about 0.01% to about 0.5% by weight.

2. The formulation of claim 1, wherein the fruit or vegetable juice are selected from fresh pressed juice or juice concentrate or juice puree.

3. The formulation of claim 2, wherein the fruit juice concentrate is selected from the group consisting of apple juice concentrate, pear juice concentrate and mixtures thereof.

4. The formulation of claim 3, wherein the drink formulation further comprises pomegranate and/or chookberry.

5. The formulation of claim 4, wherein the drink formulation further comprises vitamins selected from the group consisting of Vitamin D, Vitamin C, Vitamin B and mixtures thereof.

6. A formulation comprising (i) a marine oil having a totox value below 15 in an oil-in-water emulsion in an amount of about 0.5% to about 10% by weight wherein the water phase is fruit or vegetable juice; (ii) resveratrol or C1-6 alkyl ester derivatives thereof in an amount of about 0.01% to about 0.5% by weight; (iii) fruit juice concentrate; and (iv) one or more vitamins for use in the treatment of Alzheimer’s Disease and/or delaying the onset of symptoms associated with Alzheimer’s Disease including Mild Cognitive Impairment (MCI).

7. The formulation according to any one of the preceding claims, wherein the formulation is a drink formulation.

8. The formulation according to claim 7, wherein use of the formulation is on a daily basis.

9. The formulation according to claim 8, wherein the drink formulation has a volume in a range of 50 to 300 ml.

Patentansprüche

1. Formulierung umfassend (i) ein Meeresöl mit einem Totoxwert unter 15 in einer Öl-in-Wasser-Emulsion, wobei die Wasserphase Obst- oder Gemüsesaft ist, und (ii) Resveratrol oder C1-6-Alkylesterderivate davon zur Verwendung bei der Behandlung von Alzheimers Krankheit und/oder Verzögerung des Anfangs von Symptomen, die mit Alzheimers Krankheit verbunden sind, einschließlich Milder Kognitiver Beeinträchtigung (MCI), wobei der Gehalt von Meeresöl ca. 0,5 bis ca. 10 Gewichtsprozent des Gesamtgewichts der Formulierung ist, und der Gehalt von Resveratrol ca. 0,01 bis ca. 0,5 Gewichtsprozent ist.

2. Formulierung nach Anspruch 1, wobei der Obst- oder Gemüsesaft aus frisch gepresstem Saft oder Saftkonzentrat oder Saftpüree ausgewählt ist.

3. Formulierung nach Anspruch 2, wobei das Obstsaftkonzentrat aus der Gruppe bestehend aus Apfelsaftkonzentrat, Birnensaftkonzentrat oder Gemischen davon ausgewählt ist.


5. Formulierung nach Anspruch 4, wobei die Getränkeformulierung weiter Folgendes umfasst Vitamine ausgewählt aus der Gruppe bestehend aus Vitamin D, Vitamin C, Vitamin B und Gemischen davon.

6. Formulierung umfassend (i) ein Meeresöl mit einem Totoxwert unter 15 in einer Öl-in-Wasser-Emulsion in einer Menge von ca. 0,5 bis ca. 10 Gewichtsprozent, wobei die Wasserphase Obst- oder Gemüsesaft ist; (ii) Resveratrol oder C1-6-Alkylesterderivate davon in einer Menge von ca. 0,01 bis ca. 0,5 Gewichtsprozent; (iii) Obstsaftkonzentrat; und (iv) ein oder mehrere Vitamine zur Verwendung bei der Behandlung von Alzheimers Krankheit und/oder Verzögerung des Anfangs von Symptomen, die mit Alzheimers Krankheit verbunden sind, einschließlich Milder Kognitiver Beeinträchtigung (MCI).
7. Formulierung nach einem der vorgehenden Ansprüche, wobei die Formulierung eine Getränkeformulierung ist.

8. Formulierung nach Anspruch 7, wobei die Verwendung der Formulierung auf einer täglichen Basis ist.

9. Formulierung nach Anspruch 8, wobei die Getränkeformulierung ein Volumen im Bereich von 50 bis 300 ml aufweist.

Revendications

1. Formulation comprenant (i) une huile marine ayant une valeur de totox inférieure à 15 dans une émulsion huile dans eau dans laquelle la phase aqueuse est un jus de fruit ou un jus végétal, et (ii) le resvératrol ou ses dérivés d’ester d’alkyle en C1-6 à utiliser dans le traitement de la maladie d’Alzheimer et / ou pour retarder l’apparition de symptômes associés à la maladie d’Alzheimer, y compris une déficience cognitive légère (MCI), dans laquelle la teneur en huile marine est d’environ 0,5% à environ 10% en poids du poids total de la formulation et la teneur en resvératrol est d’environ 0,01% à environ 0,5% en poids.

2. Formulation selon la revendication 1, dans laquelle le jus de fruit ou le jus végétal sont choisis parmi le jus fraîchement pressé ou le concentré de jus ou la purée de jus.

3. Formulation selon la revendication 2, dans laquelle le concentré de jus de fruit est choisi dans le groupe constitué du concentré de jus de pomme, du concentré de jus de poire et de leurs mélanges.

4. Formulation selon la revendication 3, dans laquelle la formulation de boisson comprend en outre de la grenade et / ou de la baie d’aronia.

5. Formulation selon la revendication 4, dans laquelle la formulation de boisson comprend en outre des vitamines choisies dans le groupe comprenant la vitamine D, la vitamine C, la vitamine B et leurs mélanges.

6. Formulation comprenant (i) une huile marine ayant une valeur de totox inférieure à 15 dans une émulsion huile dans eau en une quantité d’environ 0,5% à environ 10% en poids, dans laquelle la phase aqueuse est un jus de fruit ou un jus végétal, et (ii) le resvératrol ou ses dérivés d’ester d’alkyle en C1-6 en une quantité d’environ 0,01% à environ 0,5% en poids ; (iii) concentré de jus de fruit ; et une ou plusieurs vitamines à utiliser dans le traitement de la maladie d’Alzheimer et / ou le retardement de l’apparition des symptômes associés à la maladie d’Alzheimer, y compris une déficience cognitive légère (MCI).

7. Formulation selon l’une quelconque des revendications précédentes, dans laquelle la formulation est une formulation pour boisson.

8. Formulation selon la revendication 7, dans laquelle l’utilisation de la formulation est quotidienne.

9. Formulation selon la revendication 8, dans laquelle la formulation de boisson a un volume compris dans l’intervalle de 50 à 300 ml.
Fig. 1

Effect of Example 1 Drink Formulation on Group 1 AD phagocytosis of FAM-Aβ

April 2012  July 2012  September 2012  November 2012

Start
5/8/2012
Fig. 2

Group 1 AD: Change in Baseline Expression of Inflammatory Genes

- An up regulation in the expression of most genes in the Group 1 AD PBMCs has been observed following use of Example 1 Drink Formulation (5/8/12).
Fig. 3

Group 1 AD: Change in the effect of exogenous pathogen (sAβ_{1-42})

Effect of sAβ_{1-42} (4/12)

Effect of sAβ_{1-42} (7/12)
Fig. 4
Group 1 AD: Change in the effect of exogenous pathogen (sAβ\textsubscript{1-42})

Effect of sAβ\textsubscript{1-42} (4/12)

Effect of sAβ\textsubscript{1-42} (9/12)
Fig. 5

Group 2 AD phagocytosis of FAM-Aβ

8/27/2012  10/22/2012  11/21/2012  1/9/2012

Drink Formulation of Example 1 Start
8/28/2012

• (+/-): 2 μg/ml FAM-Aβ_1-42 [Green], marginal increase in Abeta binding following Drink Formulation of Example 1
Fig. 6

In vitro Group 2 AD phagocytosis of FAM-Aβ

- (-/-): At baseline pSTAT3 localized to cytosol
- (+/-): 2 μg/ml FAM-Aβ_{1-42} [Green]
- (+/+): 0.055 mg/ml treatment enhanced binding of FAM-Aβ_{1-42} [Green]
Fig. 7

Effect of Drink Formulation of Example 1 on STAT3 Localization in a Group 2 AD Patient

* (-/-): At baseline pSTAT3 localized to cytosol
* (+/-): 2 μg/ml sAβ_{(1-42)} induces a strong nuclear translocation of pSTAT3
* (+/+): 0.055 mg/ml treatment which attenuates the translocation of pSTAT3 to the nucleus
Fig. 8

Group 2 AD/ Controls: Baseline

- A down regulation in the expression of most genes in the Group 2 AD PBMCs is observed following use of Drink Formulation of Example 1 (start 8/28/12).

- Nearly all of the genes down on 1/9/13 are potently up regulated when the PBMCs are challenged with exogenous sAβ42 (see next slide).
Fig. 9

Group 2 AD: Effect of Drink Formulation of Example 1

- Over the past year we have had at least two follow-ups with three Group 2 or high baseline inflammation AD Patients

- Two of these patients tried the Drink Formulation, but did not continue, the other has been taking the Drink Formulation daily since 8/28/12
Fig. 10 A.

**Phagocytosis of FAM-amyloid-β by freshly-isolated monocytes.**

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Cognitively-impaired patients with MMSE $\geq 19$ at baseline receiving supplementation with the formulation of the present invention drink after the first visit.

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Fig. 10 B
Patients with Alzheimer disease (MMSE<19) receiving supplementation with the formulation of the present invention after the first visit.
Fig.11

Phagocytosis of FAM-Aβ by macrophages of AD patients on supplementation with the formulation of the present invention (visit 1 = before intake of the formulation; Visits 2, 3, after intake of the formulation)
Fig. 12

In vitro effect of curcumin and resveratrol in the drink formulation on Aβ phagocytosis by macrophages of the AD patient #2. Note an increase in mean fluorescent intensity (MFI) (baseline MFI = 563) on addition of the formulation without resveratrol (MFI = 752), said formulation with curcumin (MFI = 660), and the formulation of the present invention (MFI = 890).
REFERENCES CITED IN THE DESCRIPTION

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