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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/24787 A1

(54) Title: USE OF XANTHOPHYLLS, ASTAXANTHIN E.G., FOR TREATMENT OF AUTOIMMUNE DISEASES, CHRONIC VIRAL AND INTRACELLULAR BACTERIAL INFECTIONS

(57) Abstract: The use of at least one type of xanthophylls for the production of a medicament for suppression of excessive Th1 cell mediated immune responses and stimulation of Th2 cell mediated immune responses in a patient during ongoing infection and/or inflammation in said patient is disclosed. Excessive Th1 cell mediated immune responses are caused by such autoimmune diseases and chronic viral and intracellular bacterial infections as Psoriasis vulgaris, Multiple sclerosis (MS), Rheumatoid arthritis, Crohn's disease, Insulin-dependent diabetes mellitus, Tuberculosis (TB), Acute graft-versus-host disease (transplant rejection) and HIV virus infection. The preferred type of xanthophyll is astaxanthin, particularly in a form esterified with fatty acids, obtainable by for example culturing the algae *Haematococcus sp.* Further, a method of suppressing excessive Th1 mediated immune responses and stimulating Th2 cell mediated immune responses in a patient during ongoing infection and/or inflammation in said patient is disclosed.

Use of xanthophylls, astaxanthin e.g., for treatment of autoimmune diseases, chronic viral and intracellular bacterial infections.

The present invention relates to the use and method of treatment concerning utilization of xanthophylls, e.g. astaxanthin, for suppression of excessive Th1 cell mediated immune responses and stimulation of Th2 cell mediated immune responses in a patient during ongoing infection and/or inflammation in said patient.

Background of the invention.

CD4 T lymphocytes can be subdivided into two major subsets - Th1 cells and Th2 cells. These cells release different sets of cytokines that define their distinct actions in immunity. Th1 cells secrete interferon-gamma (IFN-γ) and are mainly involved in activating macrophages and CD8+ cytotoxic T-lymphocytes. Th2 cells secrete the interleukins Il-4, Il-5 and Il-10 and are mainly involved in stimulating B cells to produce antibodies.

There is a balance between the activities of the Th1 and Th2 cells in a normal human body. An excess of Th1 cell activity may be the result of an autoimmune disease, or the result of an ongoing infection. In the normal case, the Th1 cell activity diminishes when the physiological need thereof is reduced. An excess activity is thus seen when the normal reduced level of Th1 cell activity is not achieved as a response to the diminishing presence of the agent that induced the reaction, e.g. the starting point of an autoimmune disease.

Immune modulation aims at altering the balance between different subsets of responding T cells so that damaging responses are suppressed. In many cases autoimmune diseases and intracellular infections are associated with the activation of Th1 cells, which activate macrophages and drive an inflammatory immune response. The drugs currently used to suppress the immune system can be divided into three categories:

1) Powerful anti-inflammatory drugs of the corticosteroid family such as prednisone. Glucocorticoids influence virtually every cellular and humoral mechanism related to inflammation and immune response. However, there are also many adverse effects, including fluid retention, weight gain, diabetes, bone mineral loss and thinning of the skin.

2) Cytotoxic drugs such as azthioprine and cyclophosphamide. Cytotoxic drugs cause immunosuppression by killing dividing cells and they have serious side-effects. The use of these compounds is limited due to a range of toxic effects on tissues that have continuous cell dividing, such as the bone marrow.

3) Cyclosporin A, tacromycin and rapamycin are powerful immunosuppressive agents that interfere with T-cell signaling.

All of these drugs are very broad in their action and inhibit protective functions of the immune system as well as pathological responses that cause tissue injury. Opportunistic infection is therefore a common complication of immune suppressive drugs.

It would be desirable to have an immunosuppressive agent that targets the specific part of the immune response that causes tissue injury. In particular, it would be desirable to obtain a medicament for suppression of harmful, i.e. excessive, Th1 cell mediated immune responses and stimulation of Th2 cell mediated immune responses in a patient during ongoing infection and/or inflammation in said patient.

Description of the invention

The present invention provides a medicament for suppression of excessive Th1 cell mediated immune responses and stimulation of Th2 cell mediated immune responses in a patient during ongoing infection and/or inflammation in said patient.

One aspect of the invention is directed to the use of at least one type of xanthophylls for the production of a medicament for suppression of excessive Th1 cell mediated immune responses and stimulation of Th2 cell mediated immune responses in a patient during ongoing infection and/or inflammation in said patient.

In a preferred embodiment of the invention the excessive Th1 cell mediated immune responses are caused by at least one disease from the group of autoimmune diseases and chronic viral and intracellular bacterial infections.

Examples of diseases that cause excessive Th1 cell mediated immune responses are Psoriasis vulgaris, Multiple sclerosis (MS), Reumatoid arthritis, Crohn's disease, Insulin-dependant diabetes mellitus, Tubercolosis (TB), Acute graft-versus-host disease (transplant rejection) and HIV virus infection.

Xanthophylles, including astaxanthin, is a large group of carotenoids containing oxygen in the molecule in addition to carbon and hydrogen. The carotenoids are produced *de novo* by plants, fungi and some bacteria [Johnson E.A. and Schroeder W.A., 1995, Adv In Biochem Engin. Biotechn. 53: 119-178].

In a preferred embodiment of the invention, the type of xanthophyll is astaxanthin, preferably in a form esterified with fatty acids.

In a particularly preferred embodiment the astaxanthin is derived from a natural source, such as a culture of the algae *Haematococcus sp.*, e.g. *Haemotococcus pluvialis.*

The medicament in the invention is preferably an oral preparation, which optionally comprises an oil of food grade and it is suitably presented in separate unit doses.

The medicament may comprise a mixture of different types of xanthophylls or different forms of the same xanthophyll, such as a mixture of synthetic astaxanthin and naturally produced astaxanthin.

The oral preparation may comprise in addition to the xanthophylls auxiliary ingredients that are pharmacologically acceptable inactive or active ingredients, such as flavoring agents, fillers, emulsifiers, etc.

Examples of separate unit doses are tablets, gelatin capsules and predetermined amounts of solutions, e.g. oil solutions, or emulsions, e.g. water-in- oil or oil-in-water emulsions.

Another aspect of the invention is directed to a method of suppressing excessive Th1 cell mediated immune responses and stimulating Th2 cell mediated immune responses in a patient during ongoing infection and/or inflammation in said patient comprising administration of an Th1 cell response suppressing and Th2 cell response stimulating amount of at least one type of xanthophylls to said patient.

The examples and preferred embodiments described for the use aspect of the invention also apply for this method aspect of the invention.

In particular, excessive Th1 cell mediated immune responses are caused by at least one disease from the group of autoimmune diseases and chronic viral and intracellular bacterial infections, such as Psoriasis vulgaris, Multiple sclerosis (MS), Reumatoid arthritis, Crohn´s disease, Insulin-dependent diabetes mellitus, Tubercolosis (TB), Acute graft-versus-host disease (transplant rejection) and HIV virus infection, and the type of xanthophyll is preferably astaxanthin, particularly in a form esterified with fatty acids, e.g. from a natural source, such as a culture of the algae *Haematococcus sp.*

The daily doses of the active ingredient of the invention will normally be in the range of 0.01 to 10 mg per kg body weight for a human calculated on the amount of astaxanthin, but the actual dose will depend on the immune response of the individual human patient, the reason for suppression of the excessive Th1 cell mediated immune response, such as the type of disease causing the enhanced pathological Th1 cell response, and the recommendations of the manufacturer.

The xanthophyll astaxanthin is commercially produced via culturing of the algae *Haematococcus sp.* by AstaCarotene AB, Gustavsberg, Sweden. It is marketed and sold in Sweden as a dietary supplement

Astaxanthin from other sources, and other xanthophylls as well, are expected to be similarly useful for the purposes of the invention. An advantage of using astaxanthin from

algae is, however, that the astaxanthin exists in a form esterified with fatty acids [Renström B. et al, 1981, Phytochem 20(11) :2561-2564], which esterified astaxanthin thereby is more stable during handling and storage than free astaxanthin.

The naturally produced astaxanthin can be obtained also from fungi and crustaceans, in addition to from algae [Johnson E.A. and Schroeder W.A., ibid].

Case studies

During the last five years reports have been received from patients taking the commercial dietary supplement capsules of the algal meal of *Haematococcus pluvialis,* Astaxin®, containing 4 mg astaxanthin. The daily doses recommended as an antioxidant is one capsule per day. However, 2 – 6 times that dose has been used by some patients without adverse effects. On the contrary, the higher doses have been experienced as beneficial in alleviating symptoms associated with some chronic diseases.

Six patient histories are disclosed more in detail below.

Chron´s disease

Patient 1. Boy, 17 years old, who had suffered from Crohn´s disease for at least four years. He has been treated with anti-inflammatory agents, such as cortisone. He started to take the commercial product Astaxin (two capsules, each containing 4 mg of astaxanthin, per day). In about two months the cortisone treatment was phased out and later on stopped altogether. The patient was asymptomatic for more than a year when he experienced a relapse. He was then received a short-term treatment with cortisone in combination with Astaxin, and the cortisone treatment was again phased out.

Patient 2. Woman, about 50 years of age, who had suffered from Crohn´s disease for a long time. She received treatment with cortisone. Now she has started to take Astaxin in parallel with her steroid medication and she reports that she feels considerably better.

Patient 3. Man, 48 years old, who has suffered from Crohn´s disease for the last 20 years. He has been operated on several times and he has been treated with cortisone. Directly after the last operation he started taking Astaxin (6 capsules per day) and no cortisone. With regard to the circumstances, he has been asymptomatic. He has compared his clinical status after the operation with the status of two other patients who were operated on at the same time and who received conventional treatment with cortisone. In comparison with these two other patients his recovery has been fully equal with theirs, with the positive exception that edema in his colon diminished more quickly than in the two other patients.

Lichen ruber planus.

Patient 4. Woman, more than 70 years of age, who had suffered from the disease for several years. The symptoms of the disease were *inter alia* open wounds which had not healed. She had been treated with anti-inflammatory agents, such as cortisone, for several years, orally and also by injection directly to the local inflammation areas. The treatment has not led to any result. She started to take 4 capsules of Astaxin per day , and after some weeks visible alleviation of the symptoms started to show up. The wounds were healed in slightly more than one month. During this period, the patient herself phased out the cortisone treatment. The dose of Astaxin was lowered to 2 capsules per day when she was asymtomatic. However, the symptoms returned in connection with a common cold. The dose was then increased to 4 capsules per day and the wounds healed again. She says herself that she now feels considerably better.

Psoriasis.

Patient 5. Male, 40 years, who suffers from psoriasis and mainly shows itself in rough skin on the elbows. After treatment with a skin cream enriched with algal meal/astaxanthin (100 mg astaxanthin /kg cream) twice a day for three weeks, the symptoms diminished.

Patient 6. Woman, 45 years old, who suffers from psoriasis and mainly shows itself in rough skin on the elbows. After treatment with a skin cream enriched with algal meal/astaxanthin (100 mg astaxanthin /kg cream) twice a day for three weeks, the symptoms diminished.

Thus, positive reports have been received from several patients suffering from Crohn´s disease, rheumatoid arthritis, psoriasis and lichen planus. All of these diseases are autoimmune diseases which are known to be Th1 cell mediated diseases.

Therefore it is likely that the Th1 mediated response in the patients has been suppressed and that there is a shift of the Th1/Th2 balance of the immune response towards the Th2 response. Further, it is likely that patients suffering from other predominantly Th1 cell mediated diseases would benefit from suppression of excessive Th1 cell responses and stimulation of Th2 cell mediated immune responses during ongoing infection and/or inflammation.

Claims

1. Use of at least one type of xanthophylls for the production of a medicament for suppression of excessive Th1 cell mediated immune responses and stimulation of Th2 cell mediated immune responses in a patient during ongoing infection and/or inflammation in said patient.

2. Use according to claim 1, wherein the excessive Th1 cell mediated immune responses are caused by at least one disease from the group of autoimmune diseases and chronic viral and intracellular bacterial infections.

3. Use according to claim 2, wherein the disease is Psoriasis vulgaris, Multiple sclerosis (MS), Reumatoid arthritis, Crohn's disease, Insulin-dependant diabetes mellitus, Tubercolosis (TB), Acute graft-versus-host disease (transplant rejection), or HIV virus infection.

4. Use according to any one of claims 1 -3, wherein the type of xanthophyll is astaxanthin.

5. Use according to claim 4, wherein the astaxanthin is in a form esterified with fatty acids.

6. Use according to claim 4 or 5, wherein the astaxanthin is derived from a natural source.

7. Use according to claim 6, wherein the natural source is a culture of the algae *Haematococcus sp.*

8. Use according to any one of the claims 1 - 7, wherein the medicament is an oral preparation.

9. A method of suppressing excessive Th1 cell mediated immune responses and stimulating Th2 cell mediated immune responses in a patient during ongoing infection and/or inflammation in said patient comprising administration of an Th1 cell response suppressing and Th2 cell response stimulating amount of at least one type of xanthophylls to said patient.

10. The method according to claim 9, wherein the excessive Th1 cell mediated immune responses are caused by at least one disease from the group of autoimmune diseases and chronic viral and intracellular bacterial infections.

11. The method according to claim 10, wherein the disease is Psoriasis vulgaris, Multiple sclerosis (MS), Reumatoid arthritis, Crohn's disease, Insulin-dependant diabetes mellitus, Tubercolosis (TB), Acute graft-versus-host disease (transplant rejection), or HIV virus infection.

12. The method according to claim 9, wherein the type of xanthophyll is astaxanthin.

13. The method according to claim 12, wherein the astaxanthin is in a form esterified with fatty acids.

14. The method according to claim 12 or 13, wherein the astaxanthin is derived from a natural source.

15. The method according to claim 14, wherein the natural source is a culture of the algae *Haematococcus sp.*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01923

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 31/122, A61P 37/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STN International, File CA, Chemical abstracts, volume 119, no. 11, 13 September 1993, (Columbus, Ohio, US), Date, Yukio et al: "Lipid peroxide-lowering compositions"; & JP,A2,05124958, 19930521, Heisei ---	1-4
X	Nutr Cancer, Volume 26, 1996, Harumi Jyonouchi et al, "Effects of Various Carotenoids on Cloned, Effector-Stage T-Helper Cell Activity" page 313 - page 324	1
Y	---	2-15

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 February 2001	14 -02- 2001
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Anna Sjölund/EÖ Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01923

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Clinical and Diagnostic Laboratory Immunology, Volume 6, No 3, May 1999, Sami T. Azar et al, "Type I (Insulin-Dependent) Diabetes Is a Th1 - and Th2-Mediated Autoimmune Disease" page 306 - page 310 -- --------	2-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE00/01923

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 9-15
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE00/01923

Claims 9-15 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.