"A pharmaceutical composition for the treatment of autoimmune diseases".

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The present invention relates to a pharmaceutical composition comprising an anti-inflammatory drug capable of suppressing the production of cytokines (CSAID), an immunosuppressant and a pharmaceutically acceptable excipient.

It is known that the autoimmune diseases form a wide group of pathologies characterized by inflammatory phenomena and destruction of tissues caused by the production, by the immune system, of body's own antibodies. Examples of diseases considered to be autoimmune in nature are: rheumatoid arthritis, glomerulonephritis, Hashimoto's thyroiditis, systemic lupus erythematosus, myasthenia gravis, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, autoimmune disorders and type 1 diabetes.

Presently, in the autoimmune diseases therapy there are used steroidal and non-steroidal anti-inflammatory drugs, gold compounds, penicillins and immunosuppressants.

Non-steroidal anti-inflammatory drugs (NSAID) show, together with the anti-inflammatory activity, also an antipyretic and a non-narcotic analgesic effect. They are widly used both in the acute inflammatory therapy and in chronic inflammatory treatment. For this reason they are currently used in the treatment of autoimmune pathologies, wherein the inflammatory process is often very important. Even if other mechanisms of action may not be excluded, their activity is mainly due to the capability of inhibiting the enzymes responsible for prostaglandins (PG) and leucotriens synthesis, in particular cyclooxygenases and lipooxygenases. This

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mechanism of action is mostly responsible also for side effects on different organs (mainly gastrointestinal and renal) that occur as a consequence of the prolonged use of said drugs.

Further, to the mainly symptomatic effects that are obtained by the anti-inflammatory drugs in the acute phase of autoimmune pathologies, the most important therapeutical effects are obtained by non-steroidal drugs such as, for example, cyclophosphamide.

However, this kind of drug can not be administered for prolonged periods of time because of the onset of different type of side effects having considerable side effects.

In fact, that treatment must be stopped because of the onset of toxicity on organs or of systemic nature in more than 20% of patients within 12 months. The remission phases last for a very variable period of time (from 1 to 18 months on average) and, although a second and often a third therapy cycle may give positive results, more than 50% of patients that initially responded to the therapy must stop it after 3-6 years because of relapses and/or because of the late toxicity. The organs that are most frequently involved are kidneys, liver, blood and reproductive apparatus.

Therefore, toxic effects of therapies employed in autoimmune phatologies are a serious obstacle to their use and thus there is a serious need of a product capable of reducing the undesired side effects of drugs employed in said therapies, thus reducing the doses or the number of administrations.

Recently, there have been identified compounds capable of affecting the immune system activity via regulation, at transcriptional or translational level by a variety of mechanisms of action, the capability of immunoresponsible cells

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to produce cytokines. Further, it is known that cytokines, particulary the so called proinflammatory cytokines (Tumor Necrosis Factor, interleukine-1 and -6) are involved in maintaining and sometimes inducing inflammatory phenomena. The above mentioned compounds, designated as CSAID (anti-inflammatory suppressive cytokine drugs) are different from conventional anti-inflammatory drugs because of lack of activity on those enzymes that regular the arachidonic acid methabolism and because they do not inhibit the proliferation of immunoresponsible cells and the secretion of immunoglobulins from lymphocytes B (1,2). Further, they are not very active on the inflammation acute phase and are active mostly in the second phase of the inflammatory processes.

Typical examples of CSAIDS are SK and F 86002 (2,3), tenidap (4), BB 2284 (5), GI 129471 (6), pentoxyfylline (7), vivigrol (8), suramin (9), thalidomide (10), romazarit (11) and bindarit known also as AF 2838 or 2-((1-benzyl-indazol-3-yl)--methoxy)-2-methyl propyonic acid (12).

Now, it has been unexpectatebly found that CSAIDs allow reducing the immunosuppressants dose in the prolonged treatment of autoimmune diseases, without reducing the therapeutical efficacy thus improving the tolerability.

Therefore, it is a first object of the present invention to provide a pharmaceutical composition comprising an anti-inflammatory drug capable of suppressing the cytokines (CSAID) production, an immunosuppressant and a pharmaceutically acceptable excipient.

Further, it is a second object of the present invention to provide a method of treating autoimmune diseases characterized by the concurrent administration of an anti-inflammatory drug

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capable of suppressing the production of cytokines (CSAID) and an immunosuppressant.

Typical examples of immunosuppressants according to the present invention are: cyclosporin, azatioprin, methotrexate, cyclophsphamide, FK 506, cortisol, betametasone, cortisone, desametasone, flunisolide, prednisolone, methylprednisolone, prednisone, triamcinolone, alclometasone, amcinonide desonide and desoxymetasone.

Typical examples of diseases that can benefit from the concurrent treatment with a CSAID and an immunosuppressant are: rehumatoid arthritis, glomerulonephritis, Hashimoto's thyroiditis, systemic lupus erythematosus, myasthenia gravis, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, autoimmune hepatitis, type 1 diabetes and similar.

The amount of CSAID will range depending on factors which are well known to the person skilled in the art such as, for example, the type of autoimmune disease, the severity of said disease, the body weight of the patient, the pharmaceutical dosage form, the route of administration, the number of dosage forms administred daily and the efficacy of the used compounds. However, the optimum amount may be easly determined by routine procedures of "dose-finding".

Generally, the amount of the CSAID compound will be such that it insures an administration level of from 0.01 to 100 mg/Kg/day.

For example, in the particular case of Bindarit it will be of from 1 to 50 mg/Kg/day. More preferably, it will be of from 4 to 35 mg/Kg/day.

Also the amount of the immunosuppressant drug will range depending on factors well known to the person skilled in the

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art such as, for example, the type of autoimmune disease, the severity of said disease, the body weight of the patient, the pharmaceutical dosage form, the route of administration, the number of dosage forms administred daily and the efficacy of the used compounds. However, the optimum amount may be easly determined by routine procedures particularly considering that the usual dosages of immunosuppressants are known in the literature (Goodman and Gilman 8th ed.).

Generally, also the amount of the immunosuppressant drug will be such that it insures an administration level of from 0.01 to 100 mg/Kg/day.

For example, in the particular case of cyclophosphamide it will be about 30-40% lower compared to the usual one. Therefore, it will prefearably be of from 0.01 to 10 mg/Kg/day. More preferably, it will be of from 0.05 to 5 mg/Kg/day.

In its turn, in the case of prednisone, said amount will be also 30-40% lower compared to the usual one. Therefore, it will preferably be of from 0.01 to 1 mg/Kg/day. More preferably, it will be of from 0.05 to 0.5 mg/Kg/day.

Example of suitable dosage forms are tablets, capsules, coated tablets, granules, solutions and syrups for oral administration, gels, ointments, creams and medicated patches for topic administration, suppositories for rectal administration and sterile solutions for injectable, aerosolic and ophthalmic administration.

In addition to usual excipients, the compositions may comprise suitable additives for pharmaceutical use such as preservatives, stabilizers, surface active agents, emulsifiers, salts for the regulation of the osmotic pressure, buffers, flavouring agents and colouring agents.

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They may also comprise liposomes, vesicles and other forms useful to obtain a controlled release of pharmacologically active compounds. Further, they can be formulated in form of stratified tablets containing layers which have a different speed of disintegration.

If particular treatments require it, the compositions of the present invention may comprise other compatible active ingredients whose concurrent administration is therapeutically useful.

The pharmaceutical compositions can be producted according to conventional techniques of the pharmaceutical chemist comprising mixing, granulating and compressing, when needed, or various mixings and dissolutions of the ingredients, depending on what is appropriate for obtaining the desired compound.

EXPERIMENTS

1. Bindarit effects as a CSAID.

Bindarit effect on the production of inflammatory cytokines has been evaluated in a mouse model wherein, after administration of concanavaline A (0.3 microg/mouse i.v.), there have been measured serum levels of interleukine-6 (IL-6) both in control animals and in animals treated with 200 mg/Kg of bindarit orally. The experiment results (Table 1) show that in the animals receiving bindarit, the IL-6 serum levels are about 40% lower compared to the untreated controls (10.2 $\pm$ 2.1 vs. 16.5 $\pm$ 6.4 nanogram/ml; 6 animals in each group).

Bindarit capability to reduce the inflammatory cytokines production allows to count this product among CSAID drugs, being already known that bindarit is devoid immunosuppressive activity and that it is not active on cyclooxygenase and lipooxygenase (13).

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animals treated with bola of cyclophosphamide at high doses (2x90 mg/Kg ip).

These results prove that bindarit reduces the amount of immunosuppressors to be administered with consequent reduction of those toxic phenomena that often limit the use thereof in this class of pathologies.

3. Bindarit and corticosteroides in man.

10 patients (8 male and 2 female of from 17 to 60 years old) suffering from lupus nephritis of III and IV class (according to WHO classification) have been treated with bindarit (600 mg) twice a day and with prednisone (5 mg twice or trice a day) for 8 weeks.

Before beginning treatment and at the end thereof UAE (Urinary Albumin Excretion) and interleukine-6 (IL-6) in urine have been measured.

At the end of the study; UAE levels were about 60% of the starting ones. In their turn, the urinary levels of IL-6 were about 10 picograms/ml also in those patients whose starting levels were 200 and 500 picograms/ml.

These results show that bindarit significantly reduces the severity of nephritis complications occuring in patients suffering from systemic lupus erythematosus treated with corticosteroids.

TABLE 1

Bindarit effect on the production of IL-6 induced by concanavaline A

treatment	ng/ml	reduction (%)
concanavaline A	16.5 ± 6.4	
bindarit	10.2 ± 2.1	38.2

TABLE 2

Effects on the median of survival

treatment	average (days)	increase median (days)
control	254	
cyclophosphamide (22.5)	259	4
cyclophosphamide (45)	294	40
cyclophosphamide (90)	330	76*
bindarit	387	133*
bindarit + cyclophosphamide (22.5)	408	154*
bindarit + cyclophosphamide (45)	403	149*

* p less than 0.001, Kaplan-Meier followed by LogRank test

TABLE 3

Effects on Tmax of proteinuria

treatment	average (months)	p-value vs. controls*
control	7	
cyclophosphamide (22.5)	8	n.s.
cyclophosphamide (45)	9	0.0018
cyclophosphamide (90)	10	0.00006
bindarit	9	0.0078
bindarit + cyclophosphamide (22.5)	9	0.0168
bindarit + cyclophosphamide (45)	10	0.00006

* Mann-Whitney test with corrections for multiple comparisons

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CLAIMS

1. A pharmaceutical composition comprising an anti-inflammatory drug capable of suppressing the cytokines (CSAID) production, an immunosuppressant and a pharmaceutically acceptable excipient.
2. A pharmaceutical composition according to claim 1, characterized in that the CSAID compound is selected from the group comprising SK&F 86002, tenidap, BB 2284, GI 129471, pentoxyfylline, vinigrol, suramin, talidomide, romazarit and bindarit.
3. A pharmaceutical composition according to any of claims 1 and 2, characterized in that the immunosuppressant is selected from the group comprising: cyclosporine, azatioprine, methotrexate, cyclophosphamide, FK 506, cortisolo, betametasone, cortisone, desametasone, flunisolide, prednisolone, methylprednisolone, prednisone, triamcinolone, alclometasone, amcinomide desonide and desoxymetasone.
4. A pharmaceutical composition according to any of claims from 1 to 3, characterized in that said composition contains such an amount of CSAID compound to insure an administration level of from 0.01 to 100 mg/Kg/day.
5. A pharmaceutical composition according to any one of claims from 1 to 4, characterized in that said composition contains such an amount of immunosuppressant to insure an administration level of from 0.01 to 100 mg/Kg/day.
6. A pharmaceutical composition according to claim 2, characterized in that said composition contains such an amount of bindarit to insure an administration level of from 1 to 50 mg/Kg/day.

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