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(54) Titre : COMPOSITION POUR LE TRAITEMENT DE MALADIES INFLAMMATOIRES  
 (54) Title: LIPOSOMAL COMPOSITION COMPRISING WATER-SOLUBLE CORTICOSTEROIDS FOR THE  
 TREATMENT OF INFLAMMATORY DISORDERS

(57) **Abrégé/Abstract:**

A pharmaceutical composition for parenteral administration, comprising liposomes composed of non-charged vesicle-forming lipids, including up to 20 mole percent of an amphipathic vesicle-forming lipid derivatised with polyethyleneglycol, and optionally including not more than 10 mole percent of negatively charged vesicle-forming lipids, the liposomes having a selected mean particle diameter in the size range between about 40-200 nm and containing a water soluble corticosteroid for the site-specific treatment of inflammatory disorders, is provided.

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(54) Title: COMPOSITION FOR TREATMENT OF INFLAMMATORY DISORDERS

(57) Abstract: A pharmaceutical composition for parenteral administration, comprising liposomes composed of non-charged vesicle-forming lipids, including up to 20 mole percent of an amphipathic vesicle-forming lipid derivatised with polyethyleneglycol, and optionally including not more than 10 mole percent of negatively charged vesicle-forming lipids, the liposomes having a selected mean particle diameter in the size range between about 40-200 nm and containing a water soluble corticosteroid for the site-specific treatment of inflammatory disorders, is provided.



**WO 02/045688 A3**

## Liposomal Composition Comprising Water-Soluble Corticosteroids For The Treatment Of Inflammatory Disorders

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The present invention relates to a pharmaceutical composition for parenteral and in particular intravenous administration, comprising liposomes composed of non-charged vesicle-forming lipids, including up to 20 mole percent of an amphipathic vesicle-forming lipid derivatised with polyethyleneglycol (PEG) and optionally including not more than 10 mole percent of negatively charged vesicle-forming lipids, the liposomes having a selected mean particle diameter in the size range between about 40 – 200 nm and containing a corticosteroid for the site-specific treatment of inflammatory disorders.

### BACKGROUND OF THE INVENTION

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Intravenous administration of compositions based on PEG-containing liposomes for the site-specific treatment of inflamed tissues and regions is already disclosed in EP-0662820. It is well-known that long-circulating small-sized liposomes, which contain non-charged or slightly negatively charged vesicle-forming lipids, such as PEG-liposomes, after intravenous administration can circulate for many hours in a volume not larger than the general circulation and therefore, in theory, are able to deliver relatively high portions of anti-inflammatory agents via extravasation at sites of enhanced vascular permeability common to inflamed regions. Such liposomes are of particular interest in the treatment of inflammatory diseases, e.g. rheumatoid arthritis, which is a chronic autoimmune disorder, causing joint inflammation and progressive cartilage destruction. Although several types of antirheumatic drugs are available for use, the treatment of severe, persistent synovitis and acute exacerbations may require the use of several intravenous injections containing high doses of glucocorticoids. Although systemic corticosteroids can suppress the symptoms of the disease, adverse effects limit their use. In addition to this, glucocorticoids suffer from unfavourable pharmacokinetic behaviour: short plasma half-life values and a large distribution volume require high and repeated administration in order to reach a therapeutically effective concentration of the drug at the desired site of action. Intra-articular injection of steroids into the affected joints is often used to increase the (local) efficacy of the glucocorticoids and diminish the systemic adverse effects, but this way of administration is less comfortable for the patients and not feasible when multiple small joints are affected.

Also, a significant incidence of painless destruction of the joint may be associated with  
5 repeated intra-articular injections of glucocorticoids. According to EP-0662820-B preferred  
compounds for entrapment in PEG-containing liposomes are the steroidal anti-inflammatory  
compounds, such as prednisone, methylprednisolone, paramethazone, 11-fludrocortisol,  
triamcinolone, betamethasone and dexamethasone. The steroids listed belong to the group of  
10 glucocorticoid-containing PEG-liposome in this patent and relates to the preparation of  
beclomethasone dipropionate-containing PEG-liposomes. However, no in vivo data were  
provided. On preparing dexamethasone-containing PEG-liposomes according to the  
disclosure in EP-0662820 and on intravenous administration of the same in an in vivo  
15 experimental arthritis model, the present inventors noted that the beneficial effects, as taught  
in EP-0662820, could not be observed at all. There was no difference in pharmacokinetic  
profile between a suspension containing the glucocorticoid and the PEG-liposomes in which  
the same glucocorticoid had been encapsulated.

Since glucocorticoids often are the most effective drugs in the treatment of  
inflammatory disorders, there is a need to provide liposomal compositions which after  
20 parenteral administration can more efficiently deliver effective amounts of glucocorticoid at  
the inflamed region or tissue for enhanced and prolonged local activity.

#### SUMMARY OF THE INVENTION

25 It is an object of the present invention to provide a pharmaceutical composition for  
parenteral administration, comprising liposomes composed of non-charged vesicle-forming  
lipids, including up to 20 mole percent of an amphipathic vesicle-forming lipid derivatised  
with polyethyleneglycol and optionally including not more than 10 mole percent of  
negatively charged vesicle-forming lipids, the liposomes having a selected mean particle  
30 diameter in the size range between about 40 – 200 nm and containing a water soluble  
corticosteroid for the site-specific treatment of inflammatory disorders.

A further object of the present invention is to provide a use of a pharmaceutical  
composition, comprising liposomes composed of non-charged vesicle-forming lipids,  
including up to 20 mole percent of an amphipathic vesicle-forming lipid derivatised with  
35 polyethyleneglycol and optionally including not more than 10 mole percent of negatively

charged vesicle-forming lipids, the liposomes having a selected mean particle diameter in  
5 the size range between about 40 – 200 nm and containing a corticosteroid, for the  
preparation of a medicament effective in the site-specific treatment of inflamed tissues or  
regions after parenteral administration, characterised in that the corticosteroid is used in the  
medicament in a water soluble form.

## 10 DETAILED DESCRIPTION OF THE INVENTION

It has now been found that by incorporating a water soluble form of a corticosteroid in  
long-circulating liposomes, composed of non-charged vesicle-forming lipids, including up to  
20 mole percent of amphipathic vesicle-forming lipids derivatised with polyethylene glycol  
15 (PEG) and optionally including not more than 10 mole percent of negatively charged  
vesicle-forming lipids, the liposomes having a selected mean particle diameter in the size  
range between about 40 – 200 nm, an increased localisation and improved retention of the  
corticosteroid at inflamed tissue after one single intravenous injection of a pharmaceutical  
composition, comprising the said liposomes, can be reached as compared with one single  
20 intravenous injection of an aqueous solution containing the same corticosteroid compound.

The long-circulation liposomes according to the present invention have a circulation  
half life of at least 6 hours, the circulation half life being defined as the time at which the  
second linear phase of the logarithmic liposomal clearance profile reaches 50% of its initial  
concentration, which is the extrapolated plasma concentration at  $t=0$ .

25 The particle size of the liposomes is an important feature, which was demonstrated by  
the fact that administration of a water-soluble corticosteroid, such as prednisolone disodium  
phosphate, in PEG-liposomes, having a mean particle diameter  $>500$  nm, did not result in a  
significant decrease of paw inflammation in the rat adjuvant arthritis model.

A water soluble corticosteroid in accordance with the present invention is a compound  
30 which is soluble 1 in  $\leq 10$  (w/v), as assessed in water or water buffered at physiologic values,  
e.g. at  $\text{pH} > 6.0$ , at a temperature between 15 and 25°C.

Water soluble corticosteroids which can be advantageously used in accordance with  
the present invention are alkali metal and ammonium salts prepared from corticosteroids,  
having a free hydroxyl group, and organic acids, such as ( $\text{C}_2 - \text{C}_{12}$ ) aliphatic saturated and  
35 unsaturated dicarbonic acids, and inorganic acids, such as phosphoric acid and sulphuric

acid. Also acid addition salts of corticosteroids can advantageously be encapsulated in the  
5 long-circulating PEG-liposomes. If more than one group in the corticosteroid molecule is  
available for salt formation, mono- as well as di-salts may be useful. As alkaline metal salts  
the potassium and sodium salts are preferred. Also other, positively or negatively charged,  
derivatives of corticosteroids can be used. Specific examples of water soluble corticosteroids  
are betamethasone sodium phosphate, desonide sodium phosphate, dexamethasone sodium  
10 phosphate, hydrocortisone sodium phosphate, hydrocortisone sodium succinate,  
methylprednisolone disodium phosphate, methylprednisolone sodium succinate,  
prednisolone sodium phosphate, prednisolone sodium succinate, prednisolamate  
hydrochloride, prednisone phosphate, triamcinolone acetonide disodium phosphate and  
triamcinolone acetonide dipotassium phosphate.

15 The above-mentioned corticosteroids normally are used in systemic treatment of anti-  
inflammatory diseases and disorders. Since it has been proven by the present inventors that  
by using a water-soluble form of a corticosteroid in PEG liposomes, having a specified small  
mean particle diameter, effective targeting of the drug to arthritic sites - by systemic  
administration - occurs, the present invention can advantageously be applied to  
20 corticosteroids, which - for a variety of reasons - normally are used for topical use. Such  
corticosteroids include for example alclomethasone dipropionate, amcinonide,  
beclomethasone monopropionate, betamethasone 17-valerate, ciclomethasone, clobetasol  
propionate, clobetasone butyrate, deprodone propionate, desonide, desoxymethasone,  
dexamethasone acetate, diflucortolone valerate, diflurasone diacetate, diflucortolone,  
25 difluprednate, flumetasone pivalate, flunisolide, fluocinolone acetonide acetate,  
fluocinonide, fluocortolone pivalate, fluormetholone acetate, fluprednidene acetate,  
halcinonide, halometasone, hydrocortisone acetate, medrysone, methylprednisolone acetate,  
mometasone furoate, parametasone acetate, prednicarbate, prednisolone acetate,  
prednylidene, rimexolone, tixocortol pivalate and triamcinolone hexacetonide. Topical  
30 corticosteroids, which undergo fast, efficient clearance as soon as these drugs become  
available in the general circulation, are of special interest. Examples thereof are budesonide,  
flunisolide and fluticasone propionate. By preparing a water soluble form of the above-  
mentioned topical steroids and encapsulating this into PEG liposomes in accordance with  
the present invention it is now possible to systemically administer such corticosteroids in  
35 order to reach site-specific drug delivery, thereby avoiding adverse effects associated with

systemic treatment and overcoming problems, which are inherent to the corticosteroid, such  
5 as a fast clearance. In this respect budesonide phosphate has appeared to be a salt of great  
interest.

The liposomes in accordance with the present invention may be prepared according to  
methods used in the preparation of conventional liposomes and the PEG-liposomes, as  
disclosed in e.g. EP-0662820. Passive loading of the active ingredients into the liposomes by  
10 dissolving the corticosteroid in the aqueous phase is sufficient in order to reach an  
encapsulation as high as possible, but other methods can also be used. The lipid components  
used in forming the liposomes may be selected from a variety of vesicle-forming lipids, such  
as phospholipids, sphingolipids and sterols. Substitution (complete or partial) of these basic  
components by e.g. sphingomyelins and ergosterol appeared to be possible. For effective  
15 encapsulation of the water-soluble corticosteroids in the liposomes, thereby avoiding leakage  
of the drug from the liposomes, especially phospholipid components having saturated,  
rigidifying acyl chains have appeared to be useful.

The beneficial effects observed after one single injection of the water soluble  
corticosteroid containing PEG liposomes according to the invention are very favourable  
20 when compared with the results obtained after single, but also repeated injections of the non-  
encapsulated water soluble corticosteroid in different concentrations. Administration of the  
non-encapsulated water soluble corticosteroid was much less effective than the encapsulated  
corticosteroid, which can be easily understood if the total amount of free versus  
encapsulated corticosteroid is considered: the free corticosteroid was always by a factor of at  
25 least 5-10 less effective, even when injected on 7 consecutive days. These effects have been  
observed in two different animal models, viz. a rat adjuvant arthritis model and a mouse  
model of collagen induced arthritis. The favourable effects may be a complete and long-  
lasting remission of all arthritis-associated symptoms, dependent of the dose. In addition  
thereto, in the mouse model a reduced cartilage erosion was observed one week after  
30 treatment at the time the inflammation had returned again.

Since in Experimental Autoimmune Encephalomyelitis ultra high doses of  
methylprednisolone have been shown to be more efficient in induction of T cell apoptosis  
than the "standard" dose of 10 mg/kg used in multiple sclerosis therapy, it has been  
investigated whether a long-circulating liposome formulation containing prednisolone is

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superior to methylprednisolone pulse therapy in induction of T cell apoptosis *in situ*.  
5 It has been observed that liposomal prednisolone phosphate given at 10 mg/kg augments T  
cell apoptosis *in situ* rapidly and the reduced infiltration of T cells and macrophages leads to  
an ameliorated disease activity of Adoptive Transfer Experimental Autoimmune  
Encephalomyelitis (AT-EAE). As the liposomes can extravasate and accumulate in inflamed  
10 tissue with a disrupted blood-brain-barrier, liposomal prednisolone phosphate could be a  
therapeutical alternative to methylprednisolone, which needs a higher dosage and would  
therefore cause more systemic side-effects. These findings may have implications for the  
treatment of inflammatory autoimmune diseases of the Central Nervous System (CNS) such  
as multiple sclerosis.

Surprisingly, although small, rigid PEG-containing liposomes are known to hardly  
15 release their contents, the PEG-liposomes according to the present invention were able to  
effectively deliver the water soluble corticosteroid at the desired site of action resulting in a  
complete remission of the inflammation.

The following examples further illustrate the invention.

## 7

## EXAMPLES

5

Example 1**Preparation of prednisolone phosphate-containing PEG-liposomes**

750 mg of dipalmitoyl phosphatidylcholine (DPPC) (Lipoid Ludwigshafen), 250.8 mg of cholesterol (Sigma Aldrich) and 267.6 mg of PEG-distearoylphosphatidylethanol-  
10 amine (PEG-DSPE) (Avanti Polar Lipids) were weighed and mixed in a 100 ml round-bottom flask. The lipids were dissolved in about 30 ml of ethanol. Thereafter evaporating to dryness in a Rotavapor during 1 hour under vacuum at 40°C, followed by flushing with nitrogen gas during 1 hour took place.

1200 mg of prednisolon disodium phosphate (OPG Nieuwegein) were weighed and  
15 dissolved in 12 ml of sterilised PBS. The solution was added to the dry lipid film and shaken during one hour in the presence of glass beads in order to enable complete hydration of the lipid film.

The liposomal suspension was transferred to an extruder (Avestin, maximum volume 15 ml) and extruded under pressure, using nitrogen gas, 6 times through 2 pore  
20 filters one placed on top of the other, having a pore size of 200 and 100 nm respectively, 100 and 50 nm respectively and 50 and 50 nm respectively. Subsequently the liposomal suspension was dialysed in a dialysing compartment (Slide-A-Lyzer, 10.000 MWCO) 2 times during 24 hours against 1 liter of sterilised PBS.

The mean particle size of the liposomes was determined by means of light scattering  
25 (Malvern Zeta-sizer) and was found to be  $93.1 \pm 1.2$  nm, the polydispersity index being  $0.095 \pm 0.024$ . The encapsulation efficiency of the prednisolone phosphate was determined by means of a HPLC method and was found to be between 3 and 4%. The suspension of liposomes was stored in a nitrogen atmosphere at 4°C and found to be stable for about 2 months.

30

Example 2**Preparation of other water soluble corticosteroid containing PEG-liposomes**

Example 1 was repeated but instead of prednisolone disodium phosphate dexamethasone  
35 disodium phosphate (OPG Nieuwegein), betamethasone disodium phosphate (Sigma-

Aldrich) or budesonide 21-phosphate (prepared by Syncom, Groningen, the Netherlands) respectively was used.

### Example 3

#### **Assessment of therapeutic efficacy in rat adjuvant arthritis model**

Lewis rats were immunized subcutaneously at the tail base with heat-inactivated *Mycobacterium tuberculosis* in incomplete Freund's adjuvant. Paw inflammation started between 9 and 12 days after immunization, reached maximum severity approximately after 20 days, and then gradually resolved.

Assessment of the disease was performed by visually scoring paw inflammation severity, maximum score 4 per paw, and measuring disease-induced body weight loss. The therapeutic efficacy of liposomal prednisolone phosphate, prepared according to example 1, on these variables was compared with equal doses unencapsulated drug. Rats were treated when the average score > 6 (at day 14 or 15 after disease induction).

A complete remission of the inflammation process in 4 out of 5 rats was observed within 3 days after treatment with a single dose 10 mg/kg liposomal prednisolone phosphate (average score  $0.1 \pm 0.1$ , compared to  $11.8 \pm 1.9$  of the PBS-treated rats). Unencapsulated prednisolone phosphate did not significantly alter the course of the disease ( $p > 0.05$ , Kruskal-Wallis test, non-parametric). In contrast, also 1 mg/kg liposomal prednisolone phosphate was effective ( $p < 0.05$ , Kruskal-Wallis, non-parametric). With respect to weight loss, only 10 mg/kg liposomal prednisolone phosphate had a significant effect ( $p < 0.05$ , one-way ANOVA). Within 8 days these rats regained initial weight. Other treatment groups continued suffering from progressive weight loss.

No effect of a single injection unencapsulated prednisolone phosphate was observed. Therefore it was decided to inject 10 mg/kg and 20 mg/kg daily for 7 days. Both treatment regimens reduced inflammation scores from an average of  $6.5 \pm 0.56$  (day 14) to average values around 5.0 from day 15 until day 21 (control treatment with daily saline reached a maximum of  $10.6 \pm 1.3$  on day 20). However, single injections of 10 mg/kg and 20 mg/kg liposomal prednisolone phosphate at day 14 resulted in disappearance of adjuvant arthritis (AA) symptoms until day 20. Control treatment with empty liposomes did not result in an

altered progression of the disease ( $p > 0.05$ ).

5 The liposomal compositions of example 2 were also tested in the adjuvant arthritis model. Liposomal dexamethasone phosphate proved to be more effective than either liposomal prednisolone phosphate or liposomal betamethasone phosphate. A dose of 2 mg/kg was equally effective as 10 mg/kg liposomal prednisolone phosphate. 2 mg/kg liposomal betamethasone phosphate proved to be more effective than 2 mg/kg liposomal  
10 prednisolone phosphate, however, in contrast to 2 mg/kg liposomal dexamethasone phosphate, no complete reversal was obtained. Liposomal budesonide 21-phosphate (1 mg/kg) produced at least equally effective disease suppression as compared to liposomal dexamethasone phosphate.

15

#### Example 4

#### **Assessment of therapeutic efficacy in AT-EAE**

Adoptive Transfer Experimental Autoimmune Encephalomyelitis (AT-EAE) was induced in female Lewis rats by intravenous injection of  $10^7$  MBP-specific T cells.

20 10 mg/kg prednisolone phosphate containing liposomes, as prepared according to example 1, were applied intravenously at 42 hours and 18 hours prior to sacrifice. Another group received 50 mg/kg methylprednisolone intravenously at 18 hours and 6 hours before perfusion. Control rats received empty liposomes and/or saline at equivalent time points.

T cells or macrophages in spinal cord were detected immunohistochemically in paraffin  
25 embedded tissue and apoptosis was assessed by the TUNEL assay and by morphological criteria. Student t test for grouped data was used for statistical analysis. The rate of T cell apoptosis in spinal cord tissue was significantly augmented by liposomal prednisolone phosphate ( $39.4 \pm 6.8\%$ ,  $p < 0.0001$  vs.  $16.1 \pm 4.3\%$  in the control group, all data given as mean  $\pm$ SD). Methylprednisolone as an internal control lead to a rate of  $30.8 \pm 8.0\%$   
30 T cell apoptosis ( $p < 0.01$  vs. Controls). As a result of the increase in apoptosis T cell infiltration was clearly reduced by liposomal prednisolone phosphate ( $45 \pm 12$  T cells/ $\text{mm}^2$ ), which was statistically significant compared to controls ( $115 \pm 51$  T cells/ $\text{mm}^2$ ,  $p < 0.05$ ) as well as compared to methylprednisolone ( $96 \pm 19$  T cells/ $\text{mm}^2$ ,  $p < 0.05$ ). As another aspect of inflammation the macrophage infiltration was significantly reduced by liposomal  
35 prednisolone phosphate ( $31 \pm 13$  macrophages/ $\text{mm}^2$ ) compared to controls ( $78 \pm 37$

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macrophages/mm<sup>2</sup>,  $p < 0.05$ ) and compared to methylprednisolone ( $66 \pm 25$   
5 macrophages/mm<sup>2</sup>,  $p < 0.05$ ). Even though the Adoptive Transfer model was chosen to  
investigate rapid mechanisms, a therapeutical benefit from liposomal prednisolone  
phosphate could be observed within 42 hours, achieving a clinical score of  $2.8 \pm 0.2$   
compared to controls ( $3.2 \pm 0.3$ ,  $p < 0.01$ ), which was superior to methylprednisolone  
( $3.2 \pm 0.3$ ,  $p < 0.05$  vs. liposomal prednisolone phosphate).

10

**CLAIMS**

1. A pharmaceutical composition for parenteral administration, the composition comprising liposomes composed of non-charged vesicle-forming lipids, including up to 20 mole percent of an amphipathic vesicle-forming lipid derivatised with polyethyleneglycol and optionally including not more than 10 mol% of negatively charged vesicle-forming lipids in the liposomes, which liposomes have a selected mean particle diameter in the size range between about 40 - 200 nm and contain a corticosteroid for site-specific treatment of inflammatory disorders, characterised in that the corticosteroid is present in a water soluble form which is soluble 1 in  $\leq 10$  (w/v) as assessed in water or water buffered at physiologic values.
2. Composition according to claim 1, characterised in that the corticosteroid is a corticosteroid normally used in systemic treatment of anti-inflammatory disorders.
3. Composition according to claim 2, characterised in that the corticosteroid is prednisolone, dexamethasone or methylprednisolone.
4. Composition according to claim 1, characterised in that the corticosteroid is a corticosteroid normally used for topical use.
5. Composition according to claim 4, characterised in that the corticosteroid is budesonide, flunisolide or fluticasone proprionate.
6. Use of a pharmaceutical composition, comprising liposomes composed of non-charged vesicle-forming lipids, including up to 20 mole percent of an amphipathic vesicle-forming lipid derivatised with polyethyleneglycol and optionally including not more than 10 mole percent of negatively charged vesicle-forming lipids in the liposomes, the liposomes having a selected mean particle diameter in the size range between about 40 - 200 nm and containing a corticosteroid, for the preparation of a medicament effective in site-specific treatment of inflamed tissues or regions

after parenteral administration, characterised in that the corticosteroid is used in the medicament in a water soluble form which is soluble 1 in  $\leq 10$  (w/v) as assessed in water or water buffered at physiologic values.

7. Use according to claim 6, characterised in that as the corticosteroid, a corticosteroid is used which is normally used in systemic treatment of anti-inflammatory disorders.

8. Use according to claim 6, characterised in that the corticosteroid which is normally used for topical use is used in the medicament.

9. Use of a composition according to any one of claims 1 to 5 in site-specific treatment of rheumatoid arthritis.

10. Use of a composition according to any one of claims 1 to 5 in site-specific treatment of multiple sclerosis.