Title: CORTICOSTEROIDS ADDUCTS WITH NATURAL POLYSACCHARIDE POLYMERS

Abstract: Adducts of natural polysaccharides with corticosteroid drugs in the form of aqueous solutions or solid state in powder form, possess an identical therapeutic efficacy to that of the active ingredient alone, used in the same amounts. Hence these show lower toxicity with equal anti-inflammatory activity. These new adducts are obtained through a process of simple realization, therefore also their cost is lower.
CORTICOSTEROIDS ADDUCTS WITH NATURAL POLYSACCHARIDE POLYMERS

FIELD OF THE INVENTION

The present invention concerns adducts of natural polysaccharide polymers with active ingredient belonging to the class of corticosteroids having improved therapeutic efficacy.

STATE OF THE ART

Natural corticosteroids are steroidal molecules produced and released from the adrenal cortex, some of which possess a hormonal activity. Among these the glucocorticoids act on the metabolism of the carbohydrates, lipids and proteins. Approximately 95% of the circulating hormone is bound to a globulin synthesized in the liver, while the remainder is available to realize its effects on target cells in which it binds to intracellular receptors. The macromolecular complex thus formed is then transported into the nucleus and interacts with the chromosomal constituents.

The number of natural and synthesized glucocorticoids, and esters thereof is constantly increasing. The properties for which they find greatest application are their anti-inflammatory and immunosuppressor effect. The former is due to the increase in neutrophils and the decrease in lymphocytes, monocytes, eosinophils and basophils, but it is also due to the lower release of histamine from the basophils, and to the reduction of bacterial toxins and quinines involving a decrease in the capillary permeability.

The control of rejection is caused by the decreased release of antigen from the implanted tissue and by the delayed vascularization. For this purpose they are frequently used in the case of anaphylactic reactions, in asthma, in tissue and ligament inflammations, in rejections and in dermatological disorders.

Such properties have stimulated the research and development of synthesized steroids of different strength and action duration depending on the therapy to be performed.

There are considerable undesired effects and entail, for example, rounding of the face (moon-face), weight increase, the appearance of acne and down. At high posologies they can reduce the production of antibodies and considerably
increase the production of acid and pepsin in the stomach, causing peptic ulcers.
The glucocorticoids used as anti-inflammatories are not, in any case, able to cure
the pathological process, therefore its administration should be reduced to a
minimum because of the widespread toxic effects that are dose-dependent.

SUMMARY
The Applicant has now surprisingly found some adducts of corticosteroids with
polysaccharide polymers, characterized in that the percentage of said
corticosteroids in the adduct is between 40 and 80% in weight as regards the total
weight of the adduct.
The adducts according to the present invention are in powder form or in aqueous
solution that preferably contains them in a concentration of between 0.2 and 20%
by weight calculated on the total weight of the aqueous solution.
The present invention further relates to the process for preparing the aforesaid
adducts which, in particular, comprises the following steps:
a) preparing the solution of the polysaccharide polymer in water in a percentage of
between 20 and 60% by weight on the total weight of the adduct and adding
afterwards the corticosteroid in salified form, so that the adduct concentration in
the final aqueous solution is preferably comprised between the aforesaid intervals;
b) filtering the aqueous solution thus obtained, thereby achieving the adduct in
aqueous solution form;
c) removing water thereby obtaining the adduct in powder form according to the
present invention.
An additional object of the present invention are pharmaceutical compositions
containing the adducts object of the present invention in association conjunction
with suitable excipients and/or diluents.

DETAILED DESCRIPTION OF THE INVENTION
In the present invention adducts are described formed by of a cortisonic active
ingredient bound to a natural and biocompatible polysaccharide, these substance
being able to interact without forming covalent and ionic bonds.
The selected drugs are the water soluble esters of hydrocortisone,
methylprednisolone, betamethasone and of dexamethasone, preferably used are
hydrocortisone 21-emissuccinate, methylprednisolone 21-emissuccinate and
methylprednisolone 21-phosphate, betamethasone 21-phosphate and the dexamethasone 21-phosphate.

Hydrocortisone 21-emisuccinate can be administered intramuscularly and intravenously at doses of 100-500 mg/die. The acetate or butyrate type esters are only limited to topical use since they are insoluble.

Methylprednisolone as such is orally administered, in the form of 21-emisuccinate, parenterally with attack doses of 20-40 mg/die, whereas as an acetate ester it is used for slow release injectable forms and for cutaneous applications. Betamethasone 21-phosphate is used parenterally at doses of 1.5-4 mg.

As dipropionate, valerate, valeracetate and benzoate it is insoluble and therefore cannot be used for injectable purposes.

Dexamethasone is a powerful analogue of cortisol and depending on the ester used it is available for systemic or topical use. For instance, dexamethasone 21-phosphate is administered by intramuscular or intravenous parenteral route in doses of 4-8 mg/die or for cutaneous, ophthalmological and otorhinolaryngological use in percentages of 0.1-0.2%.

In the present invention the percentage of corticosteroids and in particular glucocorticoids is preferably 60% calculated on the total adduct weight.

Among the natural polysaccharide polymers dextrans, inulin and, for oral preparations, maltodextrins of pharmaceutical grade are preferred.

Dextrans are polymers formed by linear chains of α-D-glucose molecules and present very different molecular weights, ranging from 1000 Dalton (dextran 1) to 110000 Dalton (dextran 110) and do not undergo enzymatic degradation. Hydrophilicity and therefore the solubility in water decreases by increasing the molecular weight. Dextrans with molecular weights lower than 4000 are completely excreted in the urine within 48 hours, while those having higher molecular weights remain in circulation for longer periods of time. The dextrans of from 4 to 70 are preferred in the present invention.

Maltodextrins are formed by a branched polymer of maltose and dextrans in which the D-glucose units are mainly bound by α bond (1-4), but also α (1-6) in the branched sections. They have molecular weights varying between 900 and 9000 Dalton.
Also inulin is a natural linear structure polysaccharide consisting of fructose molecules with a molecular weight of 5000, it does not undergo enzymatic degradation and is excreted in the urine, so that it is used in the medical field as a diagnostic means of renal functionality.

The selected natural polysaccharides are therefore biocompatible and inert and therefore their adducts with glucocorticoids. In particular, dextran 5 and inulin were used in the present invention.

The preparation of the adducts envisages the solution of the polymer in water, in a percentage of between 20 to 60% as regards the total of the adduct, and the subsequent addition of the corticosteroid in saltified form, allowing the hydrophilic interaction of hydroxyl groups of the active principles with those of the numerous polymer. Thus hydrogen type bonds are formed weaker than covalent or ionic type bonds (Remington's Pharmaceutical Science 18th ed. p. 186).

The polymer and the drug are dissolved in distilled water in the presence of buffers and preservatives if necessary, filtering is performed to obtain a clear solution. Conversely for obtaining the adduct in powder form, the solvent is removed from the adduct through a process of freeze-drying or nebulization (spray drier) obtaining the adduct in solid form. For the preparation of sterile forms (injectable or ophthalmic) depyrogenated and w.f.p. sterile distilled water is used, and the solution is filtered, with filters of 0.1 to 0.2 μm porosity, then placed in depyrogenated and sterilized phialoids in a sterile environment, and preferably freeze-dried. The solution is thus able to be quickly reconstituted with the addition of an aqueous solvent such as w.f.p. water or physiological solution.

Distilled water is used for the preparation of oral forms and the solution is filtered on filters of 0.45 μm porosity. The solvent is removed preferably through nebulization, as a solid and a porous adduct is obtained, suitable for the preparation of oral use pharmaceutical forms, such as tablets, capsules and granules.

The adducts in the form of aqueous solutions according to the present invention can be prepared with a process that in particular comprises the following steps:

a') dissolving the active ingredient in distilled water,
b') filtering the aqueous solution coming from the previous step,
c') removing water, preferably by freeze-drying, from the product obtained in the previous step,
d') reconstituting the aqueous solution by the addition of water or other w.f.p. solvent., in which the polysaccharide polymer is dissolved, so that the concentration of the adduct in said aqueous solution is preferably between 0.2 and 20% in weight.

These adducts favourably alter the pharmacokinetic and pharmacological characteristics of the cortisonic drug. In fact, an adduct with a low molecular weight polysaccharide can increase the solubility of a poorly soluble corticosteroid and accordingly, if orally administered its bioavailability. In the event of parenteral administration an adduct with a high molecular weight polysaccharide can prolong the time the drug remains in circulation and therefore its effect. Moreover, the presence of reduced doses of active ingredient reduces the toxicity with equal anti-inflammatory activity.

The adducts of the present invention are obtained with simple, quick and economic processes if compared to the traditional synthetic approaches, which envisage the formation of a covalent bond between the corticosteroid and the polymer, and therefore they also result less expensive.

Some examples of the preparation of the adducts according to the present invention are reported hereinbelow for illustrative but not limitative purposes.

EXAMPLE 1 - Preparation of solution of the adduct: 60% hydrocortisone 21-emisuccinate - 40% dextran

4 g of dextran 5 are dissolved under strong shirring in 40 ml of w.f.p. water and maintained at 55°C, by means of a thermostat. 7.7 g of hydrocortisone emisuccinate acid are added salifying with 1.4 g of sodium bicarbonate and buffering to pH 7.35 with bibasic sodium phosphate. Then the final volume is adjusted to 60 ml with w.f.p. water and the solution, brought to room temperature of 20-25°C, is filtered with a 0.2 µm porosity sterilizing filter and placed in depyrogenated and sterilized vials, in a ratio of 3 ml/vial. The hydrocortisone content in the adduct is 59.6%, and is determined by HPLC, using a HypersylC8 mobile phase column formed by KH₂PO₄ 0.067M, pH 2.6-2.8, 50% methanol at a flow rate of 1 ml/min. and with detection at λ = 254nm.
EXAMPLE 2 - Preparation of the solid adduct: 60% hydrocortisone 2-emisuccinate 40% - dextran

The vials containing the adduct solution of the previous example are finally placed in a freeze-dryer programmed to perform a freeze-drying cycle at the following temperatures: -33°C for 6 hours, -10°C for 5 hours, +25°C for 7 hours and +42°C for 10 hours; the vacuum is kept at 4.4·10^-2 mbar.

The lyophilized adduct is compact, white in colour, and is readily soluble with the addition of 3 ml of w.f.p. water. The hydrocortisone content by weight in the adduct, determined with the HPLC method described in example 1, is 59.6%

EXAMPLE 3 - Preparation of the solution of the adduct: 50% methylprednisolone 21-emisuccinate - 50% dextran

800 mg of dextran 5 are dissolved in approx. 30 ml of w.f.p. distilled water and added with 1 g of buffered methylprednisolone sodium succinate, corresponding to 800 mg of methylprednisolone. The solution is then brought to 40 ml, filtered with a 0.2 μm sterilizing filter, placed in vials a maintaining a filling of 1 ml for each vial.

Methylprednisolone titre is 50.2% and was detected by HPLC on a HypersilC8 mobile phase column formed by 70% KH₂PO₄ 0.067M, pH 2.6-2.8 and of 30% CH₃CN at a flow rate of 1.5 ml/min and with the detection at λ= 240 nm.

EXAMPLE 4 - Preparation of the solid adduct: 50% methylprednisolone 21-emisuccinate - 50% dextran

The vials of the adduct prepared as described in example 3 are set in a freeze-dryer programmed to perform the freeze-drying cycle already described in example 2. The lyophilized dried substance is compact, titrated with the HPLC method described in example 3 and has a methylprednisolone content of 50.2%, and with the addition of 1 ml of w.f.p. water readily reconstitutes an injectable solution containing 20 mg of active ingredient.

EXAMPLE 5: Preparation of the solution of the adduct: 50% methylprednisolone 21-emisuccinate - 50% inulin

1 g of buffered methylprednisolone sodium succinate, equal to 800 mg of methylprednisolone is dissolved in approx 50 ml of distilled w.f.p. water. The solution is then brought to 80 ml and filtered with a 0.2μm sterilizing filter, placed in sterile and depyrogenated vials, maintaining a filling of 2 ml for each vial and
freeze-dried as in example 2.
The freeze-dried adduct, titrated with the HPLC method described in example 3, has a content in methylprednisolone of 50%. With the addition of 2 ml of solvent containing 20 mg of inulin in distilled w.f.p. water, 40 mg of adduct are constituted by 50% of active ingredient.

EXAMPLE 6: Preparation of the solution of the adduct: 60% dexamethasone 21-phosphate - 40% dextran.
50 mg of sodium metabisulphite, 50 mg of phenol and 32 mg of dextran 5 are solubilized in a volume of approx. 10 ml water. Under stirring 58.2 mg of disodic dexamethasone 21 phosphate (48 mg dexamethasone 21 phosphate) are added while buffering with 0.1 N NaOH until complete solubilization of the active ingredient. The solution, brought to a final volume of 20 ml with w.f.p. water (pH = 7.5), is filtered with a sterilizing filter of 0.2 μm porosity and placed in depyrogenated and sterilized vials, in a ratio of 1ml/vial. The dexamethasone 21-phosphate titre is 60.12% by weight in the adduct solution.

The HPLC analysis for determining the titre is carried out using a MicroBondapakC18 mobile phase column formed by 55% of KH₂PO₄ 0.01M and by 45% of CH₃OH, at a flow rate of 1.5 ml/min, with the detection at λ = 236 nm.

EXAMPLE 7- Preparation of the solid adduct: 60% dexamethasone 21-phosphate - 40% dextran.
50 mg of sodium metabisulphite, 50 mg of phenol and 32 mg of dextran 5 are dissolved in a volume of approx 10 ml of w.f.p. water. Under stirring 58.2 mg of sodium dexamethasone 21 phosphate (48 mg dexamethasone 21 phosphate) are added, while buffering with 0.1N NaOH until complete solubilization of the active ingredient. The solution, brought to a final volume of 20 ml with w.f.p. water (pH = 7.5), is filtered with a sterilizing filter of 0.2μm of porosity and freeze-dried in bulk with the cycle described in example 2. 184 mg of compact freeze-dried substance are obtained, containing 80 mg of adduct with a dexamethasone 21-phosphate titre of 59.9% by weight determined with the HPLC method reported in example 6, hence equal to 48 mg.
The bulk freeze-dried substance is then placed in containers under sterile conditions under hood with a laminar flow rate.
EXAMPLE 8 - In vivo activity of the adduct with 60% hydrocortisone 21-emisuccinate

Hydrocortisone 21-emisuccinate and the corresponding adduct of example 1 were administered to cats with an acute attack of bronchial asthma, following the protocol proposed by the veterinary therapeutic manual SCIVAC (SCIVAC Editions 1995, pp. 273-74).

7 common European cats, of male gender and of varying ages between 5 and 7 years were treated. More precisely, 3 cats were intravenously injected with 2 mg/Kg of hydrocortisone 21-emisuccinate, and 4 cats with 2 mg/Kg of adduct. In both groups, the attack dose decreased to 1 mg/Kg in the two day maintenance period. Remission of the clinical symptomatology of the cough was observed in all subjects.

EXAMPLE 9 - In vivo activity of the adduct with 50% methylprednisolone 21-emisuccinate

Methylprednisolone 21-emisuccinate and the corresponding 50% active ingredient adduct of example 3 were administered to dogs with an acute attack of bronchial asthma, following the protocol proposed by M. Gogny and O. Souille in "The cough and its treatment in carnivores," Summa, 8, 1994, pp. 15-18.

Approx 4 years old hybrid dogs of male gender were treated. More precisely 4 dogs were intravenously injected with 3 mg/Kg of methylprednisolone suspension, and 4 dogs with 3 mg/Kg of adduct. In both groups the attack dose decreased to 1.5 mg/Kg in the two day maintenance period.

Clinical recovery was observed in all subjects.

EXAMPLE 10 - In vivo activity of the adduct with 60% dexamethasone 21-phosphate

The dexamethasone 21-phosphate and the corresponding adduct containing 60% of active ingredient of example 6 were administered to cats with an acute attack of bronchial asthma, following the protocol proposed by M. Gogny and O. Souille in the "The cough and its treatment in carnivores", Summa, 8, 1994, pp. 15-18.

9 common European cats of male gender, and of varying ages between 2 and 5 years were treated. More precisely, 4 cats were intravenously injected with 1 mg/Kg of dexamethasone 21-phosphate, and 5 cats with 1 mg/Kg of adduct. In
both groups, the attack dose decreased to 0.5 mg/Kg in the two day maintenance period. Remission of the clinical symptomatology of the cough was observed in all subjects.

The adducts of the present invention between corticosteroids and natural polysaccharide polymers have shown, in the in vivo activity tests in cats and dogs, a clinical efficacy comparable to that of the active ingredient alone, although they contain it in lower amounts, thus reducing the cost of the therapy. This translates into a reduction of the adduct toxicity, with equal efficacy.

In addition, this adduct is obtained with a quick economic process and which is suitable for the subsequent preparation of parenteral forms and, with suitable excipients also of oral forms for the treatment of various inflammatory states and of anaphylactic reactions in animals and humans.
CLAIMS

1. A corticosteroid adduct with natural type polysaccharide polymers, wherein the percentage of said corticosteroid in the adduct is comprised between 40 and 80% by weight based on the total weight of the adduct.

2. Adduct according to claim 1 in the form on an aqueous solution.

3. Adduct according to claim 2 wherein it is contained in said aqueous solution in concentrations of between 0.2 and 20% by weight based on the total weight of said aqueous solution.

4. Adduct according to claim 1, in powder form.

5. Adduct according to claim 4, wherein it is obtained by spraydrying or freeze-drying of the aqueous solution according to claims 2 and 3.

6. Adduct according to any one of the claims 1-5 wherein the glucocorticoid content is equal to 60% by weight based on the total weight of said adduct.

7. Adduct according to any one of claims 1-5, wherein the glucocorticoid is selected from the group consisting of water soluble esters of: hydrocortisone, methylprednisolone, betamethasone and dexamethasone.

8. Adduct according to claim 7, wherein said soluble esters are selected from the group consisting of hydrocortisone 21-emisuccinate, methylprednisolone 21-emisuccinate and methylprednisolone 21-phosphate, betamethasone 21-phosphate and dexamethasone 21-phosphate.

9. Adduct according to any one of the claims 1-8, wherein said natural type polysaccharide polymers are selected from the group consisting of dextrans, inulin, maltodextrins of pharmaceutical grade.

10. Adduct according to claim 9, wherein dextrans from 4 to 70 are used.

11. Adduct according to claims 9 and 10 wherein the natural type polysaccharide polymer is selected from the group consisting of dextran 5 and inulin.

12. A process for preparing the adduct according to any one of the claims 1-11, comprising the following steps:
   a) preparing the solution of the polysaccharide polymer in water in a percentage of between 20 and 60% by weight based on the total weight of the adduct and adding the corticosteroid in salified form;
   b) filtering the aqueous solution thus obtained, thereby achieving adduct in
aqueous solution form according to claim 2 or 3;
c) optionally removing water, by freeze-drying or spraydrying for obtaining the
adduct in powder form according to claim 4.
13. The process according to claim 12 for preparing the adduct in the form of an
aqueous solution as in claim 3, characterized in that in step (a) the concentration
of the adduct in said aqueous solution is between 0.2 and 20% in weight.
14. The process according to claim 12, wherein for the preparation of the adduct
in the form of sterile aqueous solution for ophthalmic or injectable use in step (a)
depyrogenated and w.f.p. sterile distilled water is used, in step (b) the solution is
filtered with a filter of porosity of from 0.1 to 0.2 μm and step (c) is performed by
freeze-drying.
15. The process according to claim 12, wherein for the preparation of the adduct to
be used orally in step (a) distilled water is used, in step (b) the solution is filtered
on filters of 0.45 μm porosity and step (c) is performed by spraydrying.
16. A process for preparing the adduct in aqueous solution form according to
claims 2 or 3, comprising the following steps:
a') dissolving the active ingredient in distilled water,
b') filtering the aqueous solution obtained in the previous step,
c') removing water preferably by freeze-drying, from the product obtained in the
previous step,
d') reconstituting the aqueous solution by the addition of water or other w.f.p.
solvent, in which the polysaccharide polymer is dissolved.
17. The process according to claim 16, wherein the concentration of the adduct in
the aqueous solution obtained in step (d') is comprised between 0.2 and 20% in
weight.
18. A pharmaceutical composition containing, as the active ingredient at least one
adduct according to any one of the claims 1-11 in association with suitable
excipients and/or diluents.
19. The pharmaceutical composition according to claim 18, for oral use in the form
of tablets, capsules and granules.
20. The pharmaceutical composition according to claim 19, wherein the adduct
contains pharmaceutical grade maltodextrin as the polysaccharide polymer.
21. The pharmaceutical compositions according to claim 18 for ophthalmic and injectable use in the form of aqueous solutions that can be prepared at the moment of use by the addition of w.f.p. water or physiological solution, to the adducts prepared with the process according to claim 13.