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(12) (19) (CA) **Demande-Application**

(21) (A1) **2,302,886**
(22) 2000/03/29
(43) 2000/09/30

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(51) Int.Cl.[7] A61K 31/454, A61K 9/08
(30) 1999/03/31 (199 14 621.7) DE

(54) **SOLUTION AQUEUSE STABLE D'EM 12**
(54) **A STABLE AQUEOUS SOLUTION OF EM 12**

(57) An aqueous solution of EM 12 is described which is suitable as a parenteral form of application, particularly as an intravenous form of application of EM 12 for the therapy of immunological and haematological-ontological diseases, and a method of producing the corresponding solution of EM 12 is also described.

Abstract

An aqueous solution of EM 12 is described which is suitable as a parenteral form of application, particularly as an intravenous form of application of EM 12 for the therapy of immunological and haematological-oncological diseases, and a method of producing the corresponding solution of EM 12 is also described.

Supplementary Patent Application, of Grünenthal GmbH,
D-52078 Aachen, to Main Patent Application DE 197 43 968.3
(internal reference G 2905)

A stable aqueous solution of EM 12

This invention relates to a parenteral form of application of the thalidomide derivative EM 12 and to a method of producing the latter. This form of application can be used for the therapy of inflammatory and haematological-oncological diseases.

The excess formation of the proinflammatory cytokine TNF-α (tumour necrosis factor) and of interleukin (IL)-12 by phagocyte cells (e.g. monocytes) plays a central

part in the pathogenesis of various inflammatory diseases (Trinchieri 1995, Ann. Rev. Immunol. 13: 251).

One approach to the therapy of these diseases consists of deliberately suppressing the formation of these proinflammatory cytokines by the administration of immunomodulating active ingredients, such as dexamethasone or thalidomide, or the thalidomide derivative EM 12, for example. Whereas corticoids such as dexamethasone exist in injectable forms, this has not hitherto been the case for the derivative EM 12, which has an immunomodulatory effect. A parenteral form of application of thalidomide has been proposed in DE 197 43 968.3.

Thalidomide has proved to be superior to classical immunosuppressants for the treatment of severe aphthous stomatitis. Other examples of diseases in which thalidomide has been shown to exhibit good efficacy without resulting in general immunosuppression include cutaneous lupus erythematodes, pyoderma gangrenosum and urogenital ulcers in Bechet's syndrome, as well as ulcerations, in those infected with HIV, which do not differ histologically from aphthous ulcers and in which - as distinct from most HIV-associated mucocutaneous lesions - no microbial causative agents can be detected. As distinct from aphthous stomatitis, these lesions, which can also sometimes assume the size of major aphthae, can occur in the entire digestive tract, and when they are located in the pharyngeal cavity or the gullet they can make the ingestion of food difficult and can also make the ingestion of oral medication difficult due to the pain which they cause.

In severe cases of pharyngeal or oesophageal ulcers in which oral ingestion is made difficult or may even be impossible, and in cases of HIV-associated pathology in which severe symptoms of diaorrhea make oral ingestion unpredictable, parenteral administration provides a solution. However, the low solubility in water of thalidomide (Arch. Pharm. 321, 371 (1988)) constitutes an obstacle to the parenteral administration of this active ingredient. There has therefore been no lack of attempts aimed at the development of water-soluble forms of application.

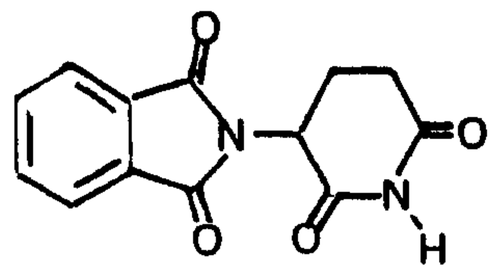
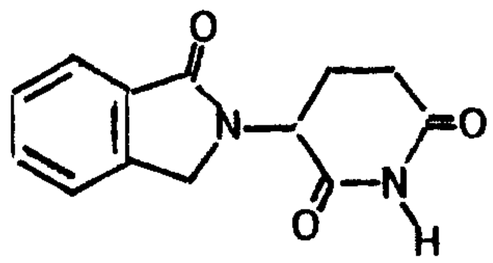
Water-soluble thalidomide derivatives, which exhibit a considerably higher solubility in water than that of thalidomide and which are suitable for parenteral application, are known from DE 42 11 812.

Furthermore, thalidomide prodrugs have been proposed for parenteral application which can be administered in water-soluble form within the physiological pH range and are toxicologically harmless (DE 196 13 976). It is disadvantageous that the production of both types of compounds mentioned above is more costly than the production of thalidomide.

Since the thalidomide derivative EM 12 exhibits similar disadvantageous properties to thalidomide as regards its solubility in an aqueous medium and its tendency to undergo spontaneous hydrolysis, the underlying object of the present invention was to develop a water-soluble form of application of this thalidomide derivative, which has an immunomodulatory effect. The object was further that the form of application to be developed should be stable in its aqueous dissolved form, and that it should have no physiologically incompatible physicochemical properties which would result in toxicological effects.

It has been found that the production of aqueous solutions is not practicable, due to the tendency of EM 12 to undergo spontaneous hydrolysis. However, if the pH of aqueous solutions falls within a range of pH which is less than or equal to 5.5, hydrolysis does not occur.

Table 1. Structure of the substances thalidomide and EM 12

Substance	Structure	Name
Thalidomide		2-(2,6- dioxo-piperidin-3-yl)-isoindole-1,3-dione
EM 12		3-(1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione

Accordingly, the present invention relates to a solution of EM 12 which is suitable for parenteral application, wherein said solution is an aqueous solution with a pH less than or equal to 5.5 and contains glucose as a constituent. According to the invention, EM 12 is dissolved, either as a racemate or in the form of one of its enantiomers, in an isotonic glucose solution. Solutions of this type can be used for parenteral application, particularly for intravenous application.

Forms of application of EM 12 which have an active ingredient content of at least 0.2 mg/ml are suitable as injectable forms of application.

The present invention further relates to a method of producing said aqueous solution. According to the invention, EM 12 is added to an isotonic glucose solution with a pH of 4 to 5 and this mixture is shaken until dissolution of the EM 12 is complete and/or is subsequently treated with ultrasound in order to shorten the time of production, and is filtered under aseptic conditions.

The form of application according to the invention is toxicologically harmless, both when administered as a rapid infusion and when administered as a slow infusion (10 ml/min).

Apart from EM 12, drugs according to the invention also contain glucose. Other adjuvant substances can also optionally be added to the EM 12 solution. The choice of said further adjuvant substances and the amounts thereof which are used depend on exactly how the drug is to be administered.

The amount of active ingredient to be administered to the patient, which depends on the weight of the patient, on the indication and on the degree of severity of the disease, usually ranges between 0.1 and 1 mg/kg.

Parenteral EM 12 solutions can be used, as can thalidomide solutions also, for the therapy of diseases in which an excess production of TNF-α and JL-12 is responsible for pathogenesis (including, amongst others, diseases of the bowels, of the skin, of the mucous membranes and of the vessels, as well as autoimmune diseases). Furthermore, due to their anti-angiogenetic effect, they can also be used for the therapy of haematological diseases and other oncological diseases.

The diseases of the aforementioned groups include, amongst others, inflammations of the skin (e.g. atopical dermatitis, psoriasis, eczema), inflammations of the respiratory tracts (e.g. bronchitis, pneumonia, bronchial asthma, ARDS (adult respiratory distress syndrome), sarcoidosis, silicosis/fibrosis), inflammations of the gastrointestinal tract (e.g. gastroduodenal ulcers, Crohn's disease, ulcerative colitis), and also include diseases such as hepatitis, pancreatitis, appendicitis, peritonitis, nephritis, aphthosis, conjunctivitis, keratitis, uveitis, rhinitis.

Autoimmune diseases comprise, for example, diseases of the arthritic type (e.g. rheumatoid arthritis, diseases associated with HLA-B27), and also include multiple sclerosis, juvenile diabetes and lupus erythematosus.

Further indications include sepsis, bacterial meningitis, cachexia, transplant rejection reactions and graft-versus-host reactions, as well as reperfusion syndrome and atherosclerosis.

Other diseases which can be treated include haematological diseases such as multiple myeloma and leukaemia, as well as other oncological diseases such as glioblastoma, cancer of the prostate and beast cancer.

Example

In order to produce an infusion solution in a concentration of 200 µg/ml, 70 mg of racemic EM 12 in 350 ml of a 3 % glucose solution for infusions (pH 4 to 5) were introduced into a glass infusion bottle.. The mixture was thoroughly shaken and was treated with ultrasound for 15 minutes. Since the concentration of dissolved EM 12 depended upon the intensity of shaking and on the ultrasonic treatment, both steps were repeated until complete dissolution was achieved. The water temperature in the ultrasonic bath reached a maximum of 33°C. The solution was filtered under aseptic conditions through a Millex GS sterile filter with a pore size of 0.22 µm (Millipore S.A., Molsheim, France) into a sterile glass infusion bottle. The solution was stored at room temperature.

The pH of the final solution was 5.5.

Testing of stability

Portions of the solutions were repeatedly taken as samples for analysis over a period of 2 weeks.

After 2 weeks, the EM 12 was still present and retained its full biological efficacy.

Testing of immunomodulatory efficacy

In order to investigate the immunomodulatory efficacy of the solutions produced, humane monocytes were isolated from peripheral blood mononuclear cells (PBMCs) and were activated with bacterial LPS (lipopolysaccharide). The concentration of TNF-α and IL-12 in the supernatant liquors from the cell cultures was determined by means of sandwich ELISAs (Biosource Europe, Fleurus, Belgium).

Table 2. The effect of an EM 12 solution produced according to the above Example on the TNF-α and IL12 production of LPS-activated monocytes.

	Ø		EM 12 10 µg/ml		EM 12 2 µg/ml	
	pg/ml	% inh*	pg/ml	% inh*	pg/ml	% inh*
TNF-α	23678	0	9548	61	9915	60
IL-12	2534	0	464	79	528	74

* % inh. = % inhibition of TNF-α or IL-12 production in the control without inhibitors

Claims

1) A stable aqueous solution according to Patent Application DE 197 43 968.3, containing EM 12 for parenteral administration, wherein the EM 12 is dissolved in an isotonic glucose solution and the pH of the solution is less than or equal to 5.5.

2) A stable aqueous solution according to claim 1, characterised in that it has an active ingredient content of at least 0.2 mg/ml.

3) A method of producing the stable aqueous solution according to claim 1, characterised in that EM 12 is added to an isotonic glucose solution with a pH of 4 to 5 and this mixture is shaken until dissolution is complete and/or is subsequently treated with ultrasound in order to shorten the time of production, and is filtered under aseptic conditions.