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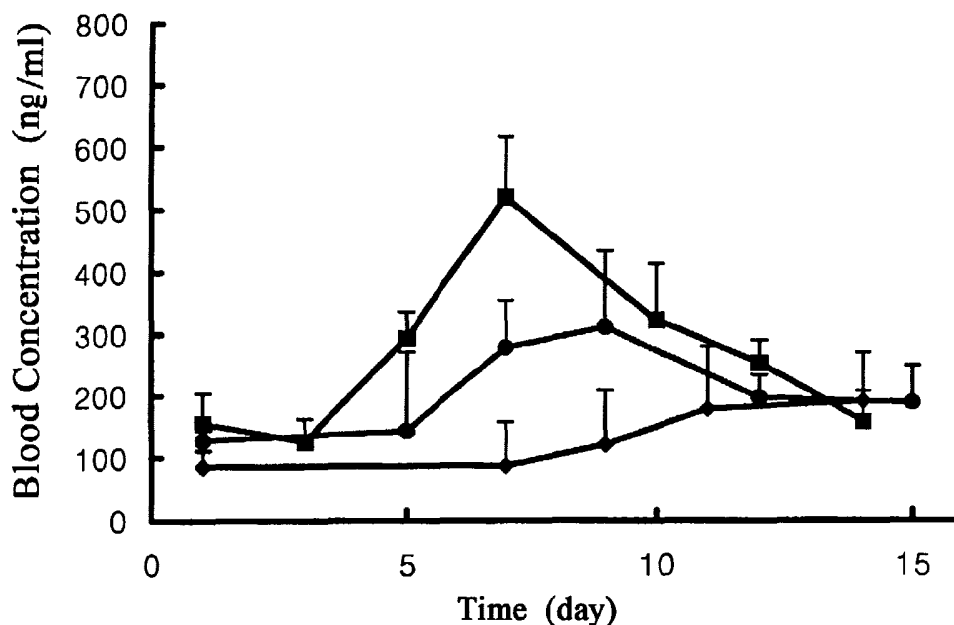
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(54) Title: CYCLOSPORIN-CONTAINING SUSTAINED RELEASE PHARMACEUTICAL COMPOSITION



(57) Abstract: The present invention relates to a cyclosporin-containing sustained release pharmaceutical composition. More particularly, the present invention is directed to a cyclosporin-containing sustained release pharmaceutical composition essentially comprising a biodegradable polymer, cyclosporin and a release modifier encapsulated therein, in which cyclosporin and the release modifier are encapsulated in the biodegradable polymer and the release modifier is at least one member selected from the group consisting of hydrophilic release modifiers and lipophilic release modifiers.

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CYCLOSPORIN-CONTAINING SUSTAINED RELEASE PHARMACEUTICAL COMPOSITION

Technical Field

5 The present invention relates to cyclosporin-containing sustained release pharmaceutical compositions.

Background Art

10 Until now, a main area of clinical research on cyclosporin has been regarded with its use as an immunosuppressive agent, particularly its application to recipients of organ transplants such as heart, lung, combined heart-lung, liver, kidney, pancreas, bone marrow, skin and corneal transplants and specifically allogeneic organ transplants. In this field, the application of cyclosporin has achieved remarkable success.

15 At the same time, applicability of cyclosporin to various autoimmune diseases and inflammatory conditions, particularly, induced by an etiologic factors including an autoimmune component in arthritis and rheumatic diseases, has been emphasized. Many reports and results in vitro, in animal models and in clinical trials are widely disclosed in the literature. Specific auto-immune diseases for which cyclosporin therapy has been proposed or applied, include autoimmune hemolytic diseases
20 (including, for example, hemolytic anemia, aplastic anemia, normocytic anemia and idiopathic thrombocytopenia), systemic lupus erythematosus, polychondritis, scleroderma, Wegener's granulomatosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, psoriasis, Stevens-Johnson syndrome, idiopathic sprue, autoimmune inflammatory bowel diseases (including, for example, ulcerative colitis and Crohn's
25 disease), endocrine opthalmopathy, Graves' disease, sarcoidosis, multiple sclerosis,

primary biliary cirrhosis, juvenile diabetes mellitus (genuine diabetes type I), uveitis (anterior and posterior), keratoconjunctivitis sicca, vernal keratoconjunctivitis, interstitial pulmonary fibrosis, psoriatic arthritis and glomerulonephritis (with or without nephrotic syndrome, e.g. including idiopathic nephrotic syndrome or minimal lesion nephritic syndrome). Further areas of research on cyclosporin has introduced its potential applicability as an antiparasitic, particularly anti-protozoal agent, and suggested use for treatment of malaria, coccidiomycosis and schistosomiasis. More recently, cyclosporin is used as an agent for reversing or eliminating antineoplastics-resistance of tumors and the like.

Cyclosporin is the most widely used one of various immunosuppressive agents until now, however, it has a serious defect of low bioavailability. When cyclosporin is administered, 10 to 27% of the total absorbed amount is subjected to the first pass effect in liver. The distribution half-life is 0.7 to 1.7 hours and the elimination half-life is 6.2 to 23.9 hours. Such pharmacokinetic parameters of cyclosporin show large individual difference, depending on the secretion level of bile acid, conditions of patients and the types of transplanted organs. Also, cyclosporin shows renal adverse effects such as reduction of glomerular filtration rate, increase of proximal renal tubular reabsorption, and the like. It has been reported that about 30% of patients taking cyclosporin-containing formulations show the adverse effects of nephrotoxicity due to the high level of cyclosporin in blood. Therefore, cyclosporin is classified as one of the drugs that should be subjected to a periodic therapeutic drug monitoring for blood level of patient.

Since cyclosporin has such specific properties, that is, very low solubility, low bioavailability and a great variation in absorption level among individuals, high dosage unit and narrow therapeutic index, and the fact that the conditions of the patients treated with cyclosporin may be unstable, it is very difficult to establish optimum drug dosage

regimen to ensure survival of transplanted patients by maintaining constantly efficient blood concentration at the level that can avoid adverse effects and rejections. Due to the poor and variable bioavailability of cyclosporin, it is necessary to adjust the daily dose of cyclosporin for achieving desired blood concentration according to the dosage form and indispensable to monitor the blood concentration simultaneously. Currently, a dose of cyclosporin is determined on the basis of the data obtained from analysis of blood concentration pattern for each patient after administering the drug prior to a transplantation operation. In the future, getting in to step with the development of the medical technology and the advancement of learning, organ transplantation will steadily increase and hence, use of immunosuppressive agents such as cyclosporin will also increase. Then, medical expenses for analysis of blood concentration pattern of cyclosporin, which is required to determine a daily dose of each individual, and for the therapeutic drug monitoring after surgical operations will increase. Moreover, as the number of patients increases, the quality of medical care may be deteriorated.

Therefore, there is ultimate need for a novel formulation that has high bioavailability and can maintain a constant blood concentration without individual difference.

Actually, there have been attempts to improve the bioavailability of cyclosporin, resulting in improvement of formulations of cyclosporin. Such attempts were mainly focused on means to solubilize cyclosporin. Typical examples of such means include the use of liposomes, microspheres, and mixed solvent systems consisting of general vegetable oils and surfactants, formation of powdery compositions using adsorption complexes, inclusion complexes, solid dispersions, etc., and other various formulations. They are mainly formulations for oral administration.

One of the most important attempts to improve the bioavailability of

cyclosporin by the change of formulation is US PAT. No. 5,342,625. This technology discloses a microemulsion pre-concentrate comprising a three-phase system, i.e. (1) a hydrophilic phase component, (2) a lipophilic phase component, and (3) a surfactant. The composition includes alcohol as an essential component and provides an oil-in-water microemulsion having an average particle size of less than about 100 nm upon dilution with water. Such an increased surface area leads to improved bioavailability of cyclosporin compared to conventional dosage form. Comparison of the microemulsion formulation, which is available in vivo (Composition I from US PAT. No. 5,342,625) with conventional formulations based on ethanol and oil, which has been previously reported in US PAT. No. 4,388,307 (Composition X), was conducted on healthy volunteers and described in US PAT. No. 5,342,625. Composition I records bioavailability level of 149.0% ($\pm$ 48) compared with Composition X (for which bioavailability achieved is set as 100%). Although the average AUC value of Composition I is 40% higher than that of Composition X, its deviation is too large of 20% to use practically for medicinal preparation.

US PAT No. 5,641,745 discloses microspheres which comprise cyclosporin entrapped in a biodegradable polymer, and are capable of releasing more than 80% of the entrapped cyclosporin within an 8 hours, thereby maximizing absorption of cyclosporin in the small intestine. This technology presents preparations with improved bioavailability by maximizing the release of cyclosporin entrapped in poly(lactide) in the upper small intestine, where cyclosporin is predominantly absorbed. For this preparation, however, the phenomenon that more than 80% of the drug is released within 8 hours is considered to be due to the initial burst of drug, which is typical for microsphere-type preparations, rather than release regulation by a biodegradable polymer. Also, it is suggested that the release amount varies according

to the poly(lactide) content in the polymer. However, it is considered to be because the solubility of cyclosporin depends on its form, that is, amorphous and crystalline, which is variable according to the types of polymer, not to be because of the controlled release of cyclosporin by the biodegradable polymer. In practice, an additional drug release
5 was not observed during the remaining test period after the initial release in 8 hours.

Therefore, the said formulation type is not suitable for controlled release preparations that should release continuously a drug for a long period of time, although it is suitable for oral preparations which should complete release in a targeted organ (upper small intestine). Moreover, it is hard to expect long-term drug delivery by oral
10 administration. Low and non-uniform absorption level of cyclosporin is due to the individual difference upon oral administration, so it is expected that administration of cyclosporin through other routes than oral administration may overcome the problems.

At present, cyclosporin injection preparations are commercially available. However, since they include as a solubilizers polyoxyethylated castor oil derivatives,
15 which may show a risk of inducing hypersensitive reactions, their applications are limited to patients who cannot be orally administered. In order to combat this problem, US PAT No. 5,527,537 discloses a pharmaceutical composition containing cyclosporin for intravenous administration, which does not contain polyoxyethylated castor oil derivatives. However, considering that cyclosporin should be administered for a long
20 period of time, the preparation for intravenous administration, which should be administered every day, is not considered to be a good substitute for oral preparations.

Recently, several researchers have studied a biodegradable microsphere preparation that can continuously release cyclosporin for a long period of time using poly(lactide) or poly(lactide-co-glycolide), and reported their results. It was reported
25 that microspheres containing cyclosporin showed rapid release of drug in vitro at the

early stage, followed by sustained-release with the maximum of 50% for 4 weeks (Int. J. Pharmaceut. 99 (1993) 263-273). Even in case of regulation of the particle size, which is one of the general methods for regulation of drug release pattern, only initial burst was increased, failing to lead to increase of releasing rate. It is believed to be due to the fact that release is restricted by the interaction between cyclosporin and poly(lactide-co-glycolide) at the later release stages. The phenomenon that in-vitro release of drug almost never occurs at the later release stages is often observed in not only hydrophobic drugs but also hydrophilic protein drugs. Considering biodegradable characteristics of polymers, it is difficult to reproduce the in-vivo release pattern into the in-vitro release test perfectly. In any case, the release rate of less than 50% for 4 weeks suggests that there is a need for the promotion of the additional release.

T. Urata et al. conducted a research to improve release of cyclosporin in vitro by adding various fatty acid esters and demonstrated the possibility of increasing the release in vitro using the materials (*J. Controlled Release* 58 (1999) 133-141). They described that lipophilic cyclosporin was considered to be mainly solubilized in the fatty acid ester and the fatty acid ester was dispersed in poly(lactide), and the solubilized drug was released through water channels formed by the fatty acid ester. All of the fatty acid esters employed in the study are liquid at room temperature, except for ethyl stearate having a carbon number of 18. However, as ethyl stearate has melting point of 33 to 35 °C, it also becomes liquid at 37 °C, which is the temperature of human body as well as of in-vitro release test temperature. That is, since only cyclosporin dissolved in liquid phase can be released over time, a desired increase of releasing rate can be attained when the content of the fatty acid ester based on the total weight of preparation is 30% or more, in which cyclosporin is sufficiently dissolved. They have prepared the microspheres using poly(lactide) or polylactide co-glycolide by

the solvent evaporation method, and it has a problem that, when the liquid phase is contained at a high concentration of 30% or more, the liquid phase is liable to volatilize during the preparation process, leading to difficulty in encapsulating the fatty acid ester of desired amount in the microspheres reproducibly. This means that the encapsulation efficiency of cyclosporin, which is dissolved in the fatty acid ester, may be affected and there may be difficulty in obtaining microspheres of a uniform composition. Also, a relatively large amount of fatty acid esters are needed in terms of the mechanism for achieving increase of release, which consequently acts as a limiting factor in encapsulating cyclosporin in biodegradable polymer microspheres. According to the result of the study, the amount of cyclosporin which can be encapsulated in practice is less than 20%. Considering that the dose of cyclosporin is relatively large, the fact that the amount of encapsulated drug is small suggests that there will be difficulty in utilization as a sustained release preparation. The required daily dose of cyclosporin in human beings is 60 mg/60 kg to 120 mg/60 kg. Supposing that the drug content is 20%, it means that the converted amount on the basis of cyclosporin-containing microspheres lasting only for one week, 2.1 g to 4.2 g of microspheres should be administered, resulting in problems in application to human bodies. In case of injection administration, a convenient administration route, the dose amount of the said preparation is too large to be utilized for application as an injection preparation. Moreover, since fatty acid esters, of which pharmaceutical acceptability has not yet been established, should be contained in a large amount, the possibility of inducing adverse effects such as topical irritation and necrosis therefrom cannot be completely excluded.

Therefore, the present inventors intended to develop a cyclosporin preparation

based on new concept, which minimizes adverse effects that may occur due to non-uniform bioavailability and individual difference in case of the oral administration, accomplishes reduction of medical expenses incurred for a preliminary monitoring, improves patient compliance, and establishes a reliable drug administration regimen.

That is, the present invention has object to provide an injectable cyclosporin preparation, particularly a cyclosporin-containing sustained-release pharmaceutical composition that is capable of regulating and maintaining the blood concentration of the drug in the effective range for several days to several weeks by continuously releasing the drug for several days to several weeks.

Brief Description of Drawings

The above objects, and other features and advantages of the present invention will become more apparent after a reading of the following detailed description when taken in conjunction with the drawings, in which:

Fig. 1 is the scanning electron micrograph of microspheres prepared from Example 5;

Fig. 2 show the results of the in-vitro release test of cyclosporin from microspheres of Comparative Example 1 (◆) and Examples 1 (△), 2 (▲), 3 (●), 4 (□) and 5 (■), in which Tween 80 was added to the release medium (at a concentration of 0.025% in Fig. 2a and 0.05% in Fig. 2b) and the test tube was positioned perpendicular to a vibrating direction; and

Fig. 3 is the blood concentration-time profiles of cyclosporin following the subcutaneous injections of microspheres of Comparative Example 1 (◆) and Examples 3 (●) and 5 (■) to SD rat.

Disclosure of the Invention

As used herein, the term "cyclosporin" refers to cyclosporin A and analogues of Cyclosporin A having similar physical properties.

The present invention relates to a cyclosporin-containing sustained release pharmaceutical composition. More particularly, the present invention is directed to a cyclosporin-containing sustained release pharmaceutical composition essentially comprising a biodegradable polymer, cyclosporin and a release modifier encapsulated therein, in which cyclosporin and the release modifier are encapsulated in the biodegradable polymer and the release modifier is at least one member selected from the group consisting of hydrophilic release modifiers and lipophilic release modifiers.

Said biodegradable polymer, said cyclosporin and said release modifier may form microspheres or nanospheres.

In the pharmaceutical composition of the present invention, the amounts of cyclosporin, the biodegradable polymer and the release modifier are preferably 15 to 70%, 25 to 80% and 0.01 to 20%, more preferably 25 to 60%, 35 to 70% and 0.1 to 10%, respectively.

The biodegradable polymer used in the composition of the present invention may be any injectable or implantable biodegradable polymer, preferably being selected from the group consisting of hydroxy acids such as polylactide (PLA) and polyglycolide (PGA); poly(lactide-co-glycolide) (PLGA), poly β -hydroxy butyric acid (PHB), polycaprolactone, polyanhydride, polyorthoester, polyurethane, poly(butyric acid), poly(valeric acid) and poly(lactide-co-caprolactone); and derivatives, copolymers and mixtures thereof.

The present inventors have discovered that the rate of drug release in vivo upon injection could be regulated by using the release modifier that can prevent the

interaction between cyclosporin and a biodegradable polymer and can promote the drug release from the biodegradable polymer, thereby have completed the present invention.

The release modifier used in the composition of the present invention may be at least one member selected from the group consisting of hydrophilic release modifiers and lipophilic release modifiers. Preferably, the hydrophilic release modifier and lipophilic release modifier may be properly combined with each other to ensure that the drug can be continuously released at a constant rate in vivo.

The hydrophilic release modifier that can be used in the present invention includes, for example, polyoxyethylene sorbitan fatty acid esters, glyceryl monooleate, sorbitan fatty acid esters, poly(vinyl alcohol), poloxamers, poly(ethylene glycol), glyceryl palmitostearate, benzyl benzoate, ethyl oleate, α -cyclodextrin, β -cyclodextrin, γ -cyclodextrin, hydroxypropyl β -cyclodextrin and the like.

The hydrophilic release modifier contains hydrophilic groups such as hydroxy, ester, ethylene oxide, propylene oxide and the like and are pharmaceutically acceptable while not carrying an electric charge. They induce an initial drug release by producing proper small pores inside of the microsphere at the early stage of drug release. That is, they do not affect the solubility of cyclosporin but do form appropriate small pores in the structure of the microspheres, whereby they do not induce an excessive initial drug release. The type and amount of the hydrophilic release modifier used to induce the initial release can vary depending on the kinds of the biodegradable polymer and the lipophilic release modifier used.

The lipophilic release modifier that can be used in the present invention include for example, pharmaceutically acceptable natural oils such as soybean oil, cotton seed oil, sesame oil, peanut oil, canola oil, corn oil, coconut oil, rapeseed oil, theobroma oil and the like. They can continuously induce the drug release at the later stages by

reducing the hydrophobic interaction between cyclosporin and biodegradable polymers, which is believed as a main cause of obstruction of the release at the later stages. The natural oil can function as a kind of buffer between cyclosporin and hydrophobic biodegradable polymers, thereby inhibiting the obstruction of the drug release due to the hydrophobic interaction. Also, they are harmless to the human body and are widely used for injections now. The type and amount of the lipophilic release modifier can vary depending on the kinds of the biodegradable polymer and the hydrophilic release modifier used.

The hydrophilic and the lipophilic release modifiers can be used alone or in combination of at least two thereof to effectively regulate the release of cyclosporin encapsulated in a biodegradable polymer.

The compositions according to the present invention can be administered by an injection or an implantation method. More specifically, the injection method includes subcutaneous injection, intramuscular injection and the like. Also, examples of the applicable formulations thereof include formulations for injection such as injection solutions, powders for reconstitution into injection solution just before injection and the like, and implant.

Therefore, the compositions according to the present invention may further comprise excipients, stabilizers, pH modifiers, isotonic agents and the like, according to the requirement in preparing the foregoing formulations for practical application.

The compositions according to the present invention may be prepared by methods such as freeze-drying, evaporation drying, spray drying, vacuum drying and the like. The production of the microspheres containing cyclosporin according to the present invention can be performed by the method such as W/O single emulsion solvent evaporation and solvent extraction using an appropriate mixer commonly used, or by

spray drying. In order to prepare the composition of the present invention having a desired release-controlling effect, it is important to produce microspheres in a short time under relatively mild conditions.

The compositions according to the present invention may maintain cyclosporin blood concentration of 100 to 500 ng/ml in vivo for 7 to 28 days through the sustained release of cyclosporin.

The compositions according to the present invention do not show a temporary increase in the blood concentration of cyclosporin, while uniformly maintaining a pharmaceutically effective concentration, thereby resulting in the reduction of drug toxicity. The temporary increase is generally observed immediately after oral administration of other preparations. Because the composition of the present invention, also, does not show individual difference in absorption ratio, it is possible to predict the blood concentration. As a result, it is possible to omit procedures for unnecessary drug administration to determine the dose of cyclosporin preparations and blood concentration assay for the therapeutic drug monitoring (TDM). In addition, as the compositions may release the drug at a constant concentration for several days to several weeks, it is expected that inconvenience of having to take a medicine every day can be eliminated, thereby improving patient compliance for medication.

[Release test of cyclosporin]

The present inventors have confirmed that, in the in-vitro release test for cyclosporin-containing microsphere preparation, when the composition of the release medium was changed, the in-vitro release pattern was also altered. With this result, considering that the target formulation of the present invention was not intended for oral administration (but for injection or implant), we have come to expect that the in-vitro

release patterns obtained by the conventional method might not reflect the in vivo release patterns of the formulations of the present invention. Therefore, we have established an in vitro release test method suitable for the compositions of the present invention, taken a screening of the candidate compositions by analyzing the in vitro release patterns of cyclosporin and administered them to rats. So, we completed the present invention on the basis of the results of a blood concentration assay.

From the experiments with various release media to establish optimal releasing conditions of microspheres in vitro, the present inventors have found that a release medium with polysorbate 80, i. e. Tween 80, was the most effective. According to the recent report of AAPS PharmSciTech 2001:2(1) article 2, as the concentration of Tween 80 was increased 20 times, cyclosporin solubility was increased 60 to 160 times through micellization by Tween 80. Therefore, the release pattern can be modulated through the control of a solubilization of cyclosporin encapsulated in microspheres, by adjusting the concentration of Tween 80 to the range of 0.025 to 0.1%, in the release medium of sodium phosphate buffered saline of pH 7.5 containing 0.01% sodium azide. 10 mg of freeze-dried microspheres with encapsulated cyclosporin were dispersed in sodium phosphate buffered saline of pH 7.5 containing 0.025 to 0.1% (W/V) Tween 80 and 0.01% sodium azide, followed by being subjected to the release test in vitro. A test tube for measurement of the released amount was placed in a water bath vibrating in a fixed direction at 37°C and, such that the test tube was positioned perpendicular or horizontal to the vibrating direction. In the apparatus for the release test, it was observed that placement of the test tube in a perpendicular or horizontal direction to the vibrating direction in the water bath resulted in different cyclosporin release profiles. Particularly, when the test tube was placed in a horizontal direction to the vibrating direction in the water bath, the microspheres in the tube did not settle down due to the

rapid movement of medium, but remained in the form of separate particles. As a result, water channels can be formed relatively readily and cyclosporin encapsulated in the microspheres can be dissolved out rapidly through the water channels of the hydrophobic microspheres. On the other hand, when the test tube was placed in a perpendicular direction to the vibrating direction in the water bath, the microspheres settled down and agglomerated with each other by gravity, due to the weight of the microspheres. The cyclosporin was found to be released slowly. This is believed to be the results from the fact that the agglomerated microspheres lying in the bottom of the test tube had difficulty in forming water channels inside of the microspheres. Moreover, it is also believed to be the results from the fact that cyclosporin should be released from such conglomerates.

In the present invention, in order to predict the in-vivo release pattern of cyclosporin, a system simulating circumstances in vivo upon administration of the microspheres was established by varying the concentration of Tween 80 in in-vitro release medium between 0.025 and 0.1% while placing the test tube in a perpendicular direction to a vibrating direction in the water bath, and used for this study.

Best Mode for Carrying Out the Invention

Now, the present invention will be described in detail based on the following examples, but it should be understood that the present invention is not limited thereto in any way.

Examples 1-5 and Comparative Example 1

Preparation of microsphere using PLGA 5015 as a biodegradable polymer - solvent evaporation method

Microspheres were prepared by solvent evaporation method using W/O single emulsion, according to the formulations given in Table 1 below.

Table 1. Formulations of microspheres using PLGA 5015 as a biodegradable polymer

	Comp. Exam. 1 (CyA- PLGA)	Exam. 1 (RP5)	Exam. 2 (RP10)	Exam. 3 (RP2S2)	Exam. 4 (RP5S5)	Exam. 5 (RP10s10)
Cyclosporin	160 mg	160 mg	160 mg	160 mg	160 mg	160 mg
Poly(lactide-co-glycolide) (PLGA5015)	240 mg	220 mg	200 mg	224 mg	200 mg	160 mg
Poloxamer 188	-	20 mg	40 mg	8 mg	20 mg	40 mg
Sesame oil	-	-	-	8 mg	20 mg	40 mg

In Comparative Example 1 and Examples 1 to 5, poly(lactide-co-glycolide) (PLGA) [PLGA5015, Wako Pure Chemical Industry, Japan] having a molecular weight of 15000 (lactic acid:glycolic acid = 50:50) was used.

A stirring apparatus was designed by fixing a blade with a diameter of 45 mm at a height of 30 mm from the bottom in a cylindrical container with a diameter of 70mm and a height of 105 mm, which had 3 partitions with a thickness of 10 mm mounted on the surface of the cylindrical wall at 120 degree intervals, and used for preparation of microspheres.

Cyclosporin, poly(lactide-co-glycolide), Poloxamer 188 and sesame oil were weighed, separately, in the amounts shown in Table 1, and added to a lidded container of appropriate dimensions. 4 ml of dichloromethane was added to the container and the container was sealed tightly, followed by stirring to completely dissolve the contents

to obtain an oily solution (Solution 1). 150 ml of aqueous solution (Solution 2) containing 0.3% polyvinyl alcohol and 0.3% Tween 80 was added to the container for preparation of microspheres and then Solution 1 was added to the Solution 2 while being stirred at 1000 rpm, followed by stirring at 1000 rpm for 30 minutes to form an O/W emulsion. The resulting emulsion was stirred for one more hour at 300 rpm to solidify microspheres. The solidified microspheres were separated by filtering through a cellulose acetate membrane of 0.22 μm , washed three times with distilled water, and freeze-dried for 24 hours. Thus, the preparations of the microspheres of Comparative Example 1 and Examples 1 to 5 was completed. All the processes described above were performed on a clean bench, and the level of aseptic conditions was maintained as high as possible.

Examples 6-10 and Comparative Example 2

Preparation of microspheres using PLGA 5015 as a biodegradable polymer - sonication method

These examples were performed using the same Solutions 1 and 2 as in Examples 1 to 5. Solution 1 was added to Solution 2. The resulting suspension was promptly dispersed by sonication at 70 mW for 3 minutes and stirred at 700 rpm for 2 hours by a magnetic stirrer to solidify microspheres. The solidified microspheres were separated by filtering through a cellulose acetate membrane of 0.22 μm , washed three times with distilled water, and freeze-dried for 24 hours. All the processes described above were performed on a clean bench and aseptic conditions were maintained as much as possible.

Experimental Example 1. Scanning Electron Microscopy of microspheres

Fig. 1 shows the result of the scanning electron microscopy of microspheres prepared from Example 5. It was confirmed that uniform microspheres having particle size of less than 30 μm could be conveniently prepared by the method according to the present invention, even when 20% of a release modifier was added.

5

Experimental Example 2. Encapsulation efficiency of cyclosporin in microspheres

In this example, the inventors used the physicochemical properties of methanol, that is, it can dissolve cyclosporin well while can not dissolve the biodegradable polymeric carriers for cyclosporin such as poly(lactide-co-glycolide), poly(lactide), and the like. It is an efficient method in that it can conveniently and precisely measure an encapsulated amount of cyclosporin in microspheres with high encapsulation amount of cyclosporin.

10

10 mg of microspheres containing cyclosporin in a large proportion (30 to 60%) were dispersed in 50 ml of methanol. The dispersion was subjected to sonication for 1 hour so that encapsulated cyclosporin was fully and rapidly extracted. The extracted cyclosporin in methanol was measured by reverse-phase high pressure liquid chromatography at a detection wavelength of 215 nm. Also, in order to confirm that cyclosporin contained in the microspheres had been completely extracted, the biodegradable polymers transformed into gel were measured using nuclear magnetic resonance spectroscopy.

15

20

The encapsulation efficiencies of cyclosporin in the microspheres prepared in Comparative Example 1 and Examples 1 to 5 are shown in Table 2. It was found that at least 95% of cyclosporin was completely encapsulated into the microspheres prepared in Comparative Example 1 and Examples 1 to 5. The encapsulation efficiency was calculated by the following equation ($n=3$).

25

Encapsulation Efficiency (%) = (amount of cyclosporin in 10 mg microspheres/4 mg) X 100

(4 mg: Theoretical loading amount of cyclosporin)

Table 2. Encapsulation efficiency of microspheres

	Comp. Exam. 1	Exam. 1 (RP5)	Exam. 2 (RP10)	Exam. 3 (RP2S2)	Exam. 4 (RP5S5)	Exam. 5 (RP10S10)
Encapsulation efficiency	99% (± 2)	105% (± 3)	103% (± 2)	95% (± 4)	98% (± 5)	102% (± 3)

5

Experimental Example 3. In-vitro release test of drug from microspheres containing cyclosporin

10 mg of freeze-dried cyclosporin-containing microspheres were dispersed in sodium phosphate buffer of pH 7.5 containing 0.025 to 0.1% (W/V) Tween 80 and 0.01% sodium azide, followed by subjection to a release test in vitro. A test tube for measurement of the released amount was placed in a water bath vibrating in a fixed direction at 37°C and, at right angles to the vibrating direction.

In order to measure the released amount of cyclosporin, the test tube was centrifuged at a speed of 3000 rpm for 15 minutes at fixed time intervals, 50 ml of supernatant was obtained and then fresh medium of an equal volume was added promptly to the test tube. Using the release medium obtained from the supernatant, the released amount and the stability of cyclosporin was measured by reverse-phase high pressure liquid chromatography with UV detector at a wavelength of 215 nm. The reverse-phase high pressure liquid chromatography system is described as follows:

Waters 510 HPLC pump system was connected to Waters 484 UV detector, the temperature of the column was kept at 70°C and the mobile phase was a mixed solution of acetonitrile and water (80:20). As a column, a Phenomenex Column-Luna, RP-18

(4.6 X 250 mm, particle size 5 μ m, USA) was used.

Upon examining the drug release patterns in vitro shown in Fig. 2a and Fig. 2b, when the concentration of Tween 80 was 0.025%, the compositions of Examples 1 to 5, which contain the release modifier, differed by about 15% in the amount of released cyclosporin from the composition of Comparative Example 1, which did not contain a release modifier, at the third day of test. However, it fails to show clearly the difference of release patterns depending on the content of the release modifier. Furthermore, it was not observed any increase of release amount of cyclosporin after the third day. On the other hand, when the concentration of Tween 80 was increased to 0.05%, the difference of the drug release patterns depending on the content of the release modifier was shown to reach a maximum of 40% at the third day. In the present invention, the medium containing 0.05% Tween 80 was selected as an in-vitro release medium for the use in the formulation screening test.

Experimental Example 4. In-vivo release test of drug from microspheres containing cyclosporin

For in-vivo drug release test, 200 g male Sprague-Dawley rats was subcutaneously injected with cyclosporin-containing microspheres suspended in a solvent for injection with amount of 37.5 mg/kg. The solvent for injection was 1.5% sodium carboxymethylcellulose solution in distilled water for injection containing 0.9% sodium chloride and 0.1% Tween 20. Sodium chloride was used to make the injection solution isotonic for the alleviation of pain around the injection site. Sodium carboxymethylcellulose was used as a thickener to maintain the viscosity of the injection solution at 200 to 400 cps in order that microspheres can be effectively suspended in the solvent for injection, the injection solution can be maintained in the

form of a homogeneous suspension during injection and the microspheres can be remained around the injection site after injection. Any thickener that is injectable and nontoxic can be employed, but the obtained injection solution is required to maintain the foregoing range of the viscosity. The solvent for injection was sterilized before use.

5 Cyclosporin-containing microspheres were suspended at a concentration of 50 mg/ml just before use and then injected to SD rat in a converted amount on the basis of the weight of the rat. Here, a 22-gauge needle was used. The blood concentration of cyclosporin in the white mouse was determined by the cyclosporin monoclonal whole blood assay (TDx system, Abbott Lab., USA) with a fluorescence polarization
10 immunoassay (FPIA) using whole blood.

As a consequence of the administration of cyclosporin-containing microspheres, it was shown that the blood concentration of cyclosporin varied considerably according to the content of the release modifier (Fig. 3). The group that did not contain a release modifier maintained a blood concentration of about 100 ng/ml, falling short of the
15 effective blood concentration (Comparative Example 1: ◆). On the other hand, Examples 3 (●) and 5 (■) that contained the release modifier according to the present invention appeared to maintain much higher blood concentration on the whole.

In addition, it was observed that Example 5, which contained Poloxamer 188 and sesame oil as a release modifier in an amount of 10% separately, showed a
20 maximum blood concentration of 500 ng/ml or higher, whereas Example 3 (RP2S2), in which the content of the release modifier was regulated to 2%, showed effective and constant blood concentration between 150 ng/ml to 350 ng/ml. These results indicate that the blood concentration can be controlled by adjusting the content of the release modifier. The type and amount of a release modifier can vary according to the type of
25 a used biodegradable polymer and the cyclosporin content.

Industrial Applicability

The sustained-release microspheres containing high concentration of cyclosporin, prepared according to the present invention, can release the whole quantity of cyclosporin encapsulated in microsphere at a constant rate while uniformly maintaining the therapeutically effective concentration of cyclosporin for several days to several weeks, which is required in cyclosporin preparations, and it is possible to minimize adverse effects that may occur due to non-uniform bioavailability caused by the oral administration, thereby accomplishing reduction of medical expenses incurred for a preliminary monitoring and improving patient compliance for medication.

CLAIMS

1. A cyclosporin-containing sustained release pharmaceutical composition comprising a biodegradable polymer, cyclosporin and a release modifier encapsulated therein, in which said cyclosporin and said release modifier are encapsulated in said biodegradable polymer and said release modifier is at least one member selected from the group consisting of hydrophilic release modifiers and lipophilic release modifiers.
2. The pharmaceutical composition of claim 1, wherein said biodegradable polymer, said cyclosporin and said release modifier form microspheres or nanospheres.
3. The pharmaceutical composition of claim 1, wherein contents of cyclosporin, the biodegradable polymer and the release modifier are 15 to 70%, 25 to 80% and 0.01 to 20%, respectively.
4. The pharmaceutical composition of claim 3, wherein contents of cyclosporin, the biodegradable polymer and the release modifier are 25 to 60%, 35 to 70% and 0.1 to 10%, respectively.
5. The pharmaceutical composition of claim 1, wherein the biodegradable polymer is selected from the group consisting of polylactide and polyglycolide; poly(lactide-co-glycolide), poly β -hydroxy butyric acid, polycaprolactone, polyanhydride, polyorthoester, polyurethane, poly(butyric acid), poly(valeric acid) and poly(lactide-co-caprolactone); and derivatives, copolymers and mixtures thereof.
6. The pharmaceutical composition of claim 1, wherein the hydrophilic release modifier

is at least one member selected from the group consisting of polyoxyethylene sorbitan fatty acid esters, glyceryl monooleate, sorbitan fatty acid esters, poly(vinyl alcohol), poloxamers, poly(ethylene glycol), glyceryl palmitostearate, benzyl benzoate, ethyl oleate, α -cyclodextrin, β -cyclodextrin, γ -cyclodextrin and hydroxypropyl β -cyclodextrin.

7. The pharmaceutical composition of claim 1, wherein the lipophilic release modifier is at least one member selected from the group consisting of soybean oil, cottonseed oil, sesame oil, peanut oil, canola oil, corn oil, coconut oil, rapeseed oil and theobroma oil.

8. The pharmaceutical composition of claim 1, wherein the composition is an injection preparation.

9. The pharmaceutical composition of claim 1, wherein the composition is an implant preparation.

10. The pharmaceutical composition of claim 1, wherein the composition performs sustained release so as to maintain a blood cyclosporin concentration of 100 to 500 ng/ml in vivo for 7 to 28 days.

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Fig.1

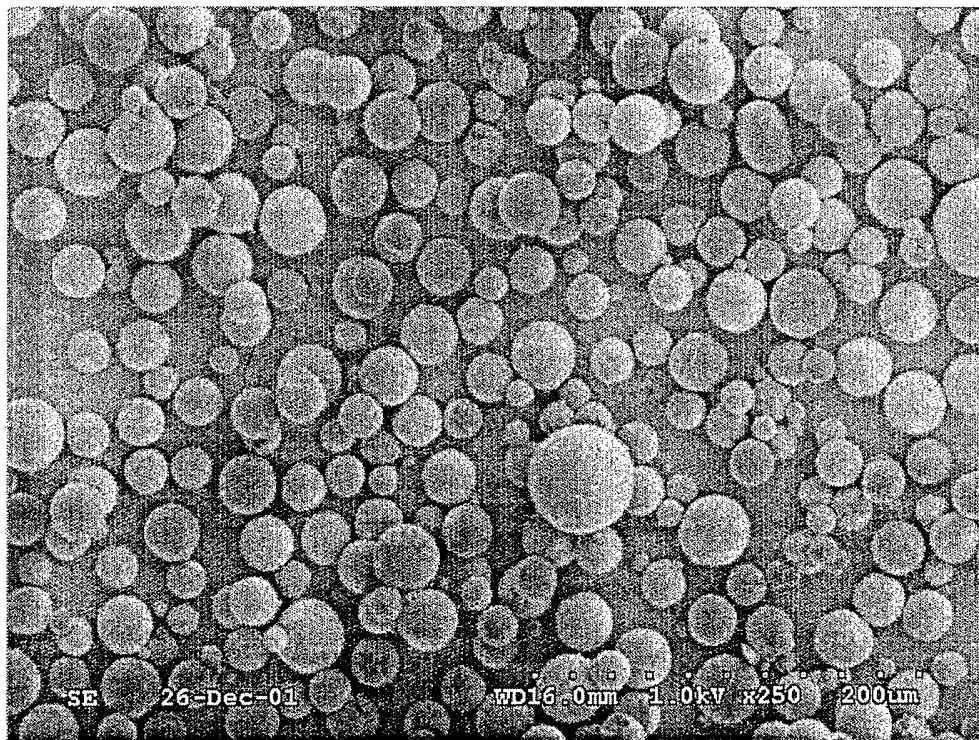
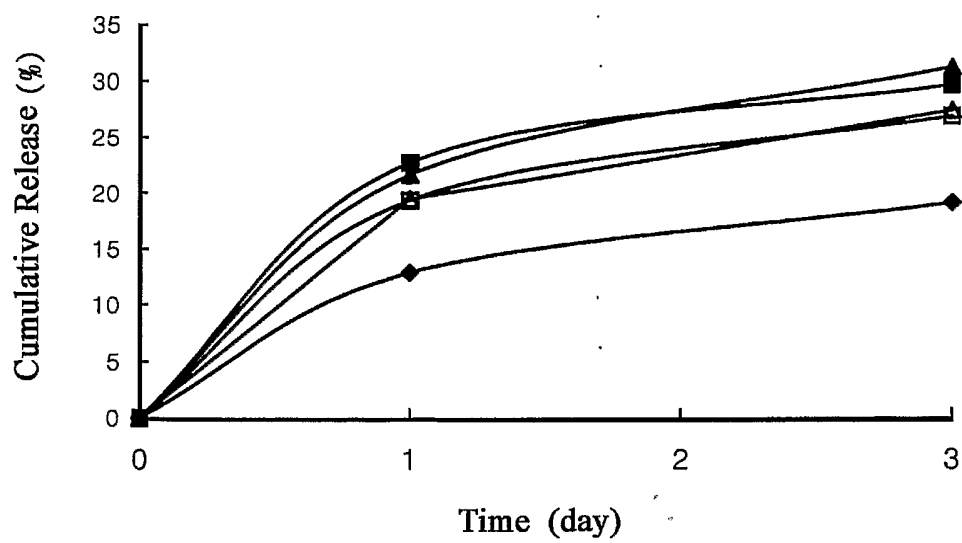


Fig. 2a



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Fig. 2b

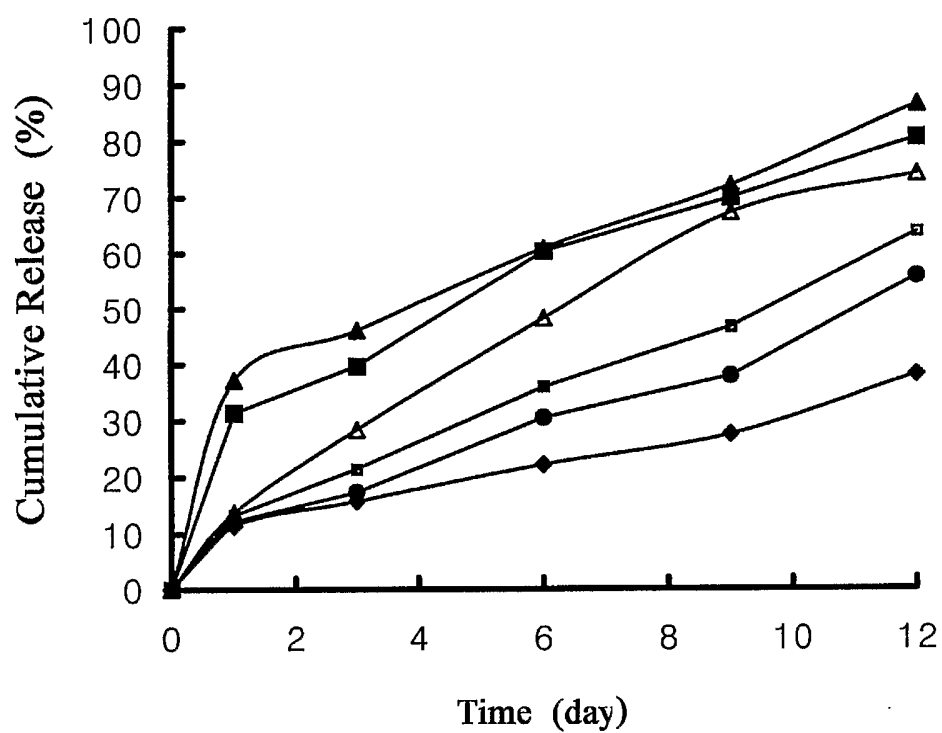
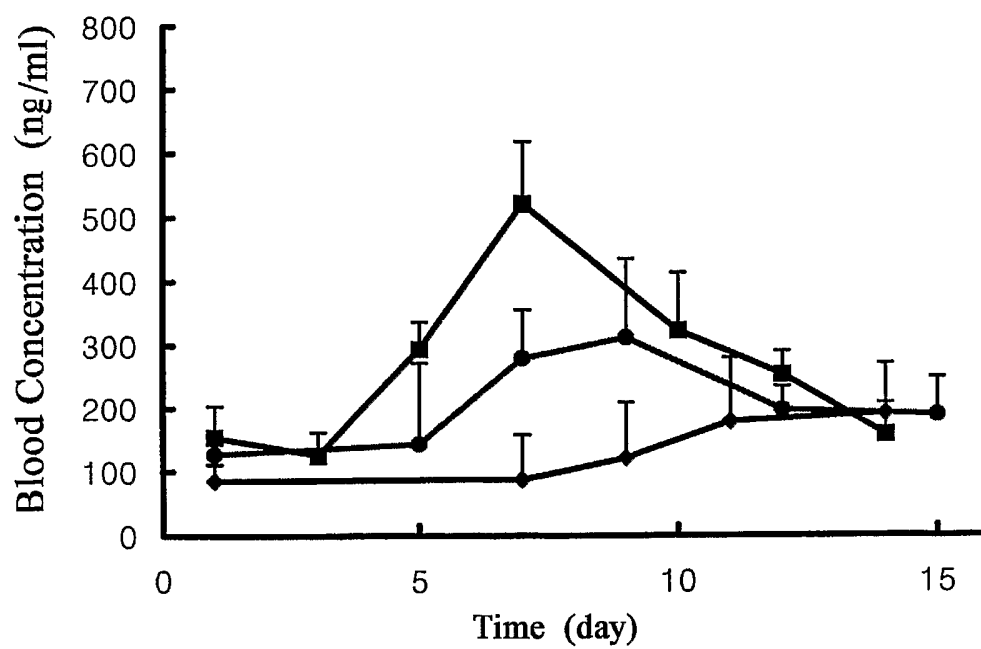


Fig. 3



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR03/00138

A. CLASSIFICATION OF SUBJECT MATTER**IPC7 A61K 9/52**

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

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

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

CA-Online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96-31202 A1 (ELAN CORP PLC) 10 October 1996 (10. 10. 1996) see examples 1-3 and claims 1-10.	1-10
Y	US 6322805 B1 (SAMYANG CO) 27. November 2001 (27. 11. 2001) see column 14 example 2 and claims 1-47.	1-10
Y	Molpeceres et al. "Stability of cyclosporin-loaded polycaprolactone nanoparticles" In Journal of Microencapsulation (1997), 14(6), pages 777-787.	1-10
Y	Urata et al. "Modification of release rates of cyclosporin A from poly(L-lactic acid) microspheres by fatty acid esters and in-vivo evaluation of the microspheres" In Journal of Controlled Release (1999), 58(2), pages 133-141.	1-10

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

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"&" document member of the same patent family

Date of the actual completion of the international search

26 FEBRUARY 2003 (26.02.2003)

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR03/00138

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 96-31202 A1	10. 10. 1996	US 5641745 A CA 2217462 AA AU 9652866 A1	24. 06. 1997 10. 10. 1996 21. 01. 1998
US 6322805 B1	27. 11. 2001	WO 9710849 A1	27. 03. 1997