5α-ANDROSTANE-3β,5,6β-TRIOL INJECTION AND PREPARATION METHOD THEREFOR

FIELD OF THE INVENTION

The present invention is in the field of pharmaceutics and relates to 5α-androstane -3β,5,6β-triol injection and its preparation method.

BACKGROUND OF THE INVENTION

5α-androstane-3β,5,6β-triol (hereinafter YC-6) is a newly-found neuronprotective compound. Currently, Acute Ischemic Stroke (AIS) is treated mainly by thrombolytic or neuronprotective therapy. Neuronprotective agents can reduce cerebral infarction area and avoid hemorrhage complication that may occur during thrombolytic or anticoagulant therapy. Moreover, it can be used even without any aetiological diagnosis and make early treatment possible. Neuronprotective agents have therefore attracted increasing attention in AIS research.

However, no neuronprotective agent has been proven to be safe and effective so far. A lot of compounds with potential value for clinical application are under clinical trials, including calcium channel blockers (CCB), calcium channel modulators, glutamate release inhibitors, γ-aminobutyric acid (GABA) receptor agonists, free radical scavengers, anti-intercellular adhesion molecule antibodies, and so on.

Among a large number of compounds, neuroactive steroids are increasingly attractive due to their extensive effect of neuron protection. In particular, the effect of compound YC-6, as a newly-found neuroprotective chemical entity, is not limited to neuron protection. It is effective against not only cerebral ischemia but also spinal cord ischemia at daily dose of 50-100 mg.

YC-6 is insoluble in water. Although its solubility is increased in conventional non-aqueous solvents or mixtures thereof, these solvents cause irritation, and the precipitation of YC-6 might occur when diluted by water. The efficacy and safety of YC-6 injection are therefore adversely affected and its use thereof is limited.

SUMMARY OF THE INVENTION

To overcome the deficiencies set forth above, YC-6 injections and their preparation methods are provided by the present invention. The present invention uses hydroxypropyl-β-cyclodextrin as a solubilizing agent to prepare YC-6 injections. The irritation caused by non-aqueous solvents is successfully cut down while the solubility of YC-6 is increased.

To achieve this, YC-6 injections are provided in liquid or solid form. The injections

have at least one soluble excipient including hydroxypropyl-β-cyclodextrin. The at least one soluble excipient can also comprise an isotonic adjusting agent or a freeze drying filler.

Preferably, YC-6 is present at a part by weight ratio of 1~20:40~500 to hydroxypropyl-β-cyclodextrin.

The injections can also be prepared by the following components (by weight): 1~20 parts of YC-6, 40~500 parts of hydroxypropyl-β-cyclodextrin, 1~100 parts of an isotonic adjusting agent, 0~200 parts of a freeze drying filler, and 0~2000 parts of a solvent.

The isotonic adjusting agent is selected from the group consisting of sodium chloride, glucose, mannitol, lactose, xylitol, sorbitol, maltitol and mixtures thereof.

The freeze-drying filler is selected from the group consisting of sodium chloride, glucose, mannitol, lactose, xylitol, sorbitol, maltitol and mixtures thereof.

If a liquid injection is to be prepared, the solvent is selected from the group consisting of propanediol, ethanol, polyethylene glycol 400, polyethylene glycol 200, glycerol, water and mixtures thereof.

The injection of the present invention can be prepared by a method comprising steps of dissolving hydroxypropyl-β-cyclodextrin, YC-6 and at least one soluble excipient in water for injection in sequence to obtain a raw solution; and subjecting the raw solution to decolorization, filtration and sterilization in sequence to obtain the injection of the present invention.

Freeze-drying powder is prepared by filling a filtrate produced by the filtration step in the above into an ampoule and undergoing freeze drying.

Sterile powder is prepared by spray drying a filtrate produced by the filtration step in the above followed by packaging.

The decolorization can be performed by using 0.1~ 0.3% activated carbon and the sterilization can be performed at 115°C for 30 min or at 121°C for 15 min.

It will be appreciated that the YC-6 can also be formulated into YC-6-loaded infusions, through mixing YC-6 injection with conventional drug-free infusions such as glucose infusion, sodium chloride infusion or glucose and sodium chloride infusion.

The present invention is advantageous over conventional techniques. Uses of hydroxypropyl-β-cyclodextrin or non-aqueous solvents/mixed solvents increase the

solubility of YC-6, so that YC-6 can be prepared into injection solution, sterile powder, freeze drying powder, YC-6-loaded glucose infusion, sodium chloride infusion, or glucose and sodium chloride infusion, which makes possible for YC-6 to be administrated by intravenous injection in case of urgency. In addition, YC-6 has sufficient solubility and efficacy without any irritation when formulated into the present injection. The preparation process is also simple and widely available.

DETAILED DESCRIPTION OF THE INVENTION

Example 1 Preparation of 200 ampoules of YC-6 injection (specification 5ml: 50mg)

Formula:

YC-6	10 g
2-hydroxypropyl-β-cyclodextrin	200 g
Sodium chloride	1.25 g
Water for injection, added to	1000 ml

Preparation: Dissolve 2-hydroxypropyl-β-cyclodextrin in 80% of the fresh water for injection and add YC-6, followed by stirring at room temperature for 10~20 min to make YC-6 completely dissolved. Add sodium chloride and make it dissolved by stirring, then add water for injection to 1000 ml. Add 0.1% activated charcoal into the above solution, stir for 15 min at 60℃ and then cool naturally the solution to room temperature. A 0.22μm microporous membrane filter is used to filtrate. The filtrate is collected and filled to prepare 5 ml injection, and subject to sterilization at 121°C for 15 min.

Example 2 Preparation of 200 ampoules of YC-6 injection (specification 10ml: 80mg)

Formula:

YC-6	16 g
3-hydroxypropyl-β-cyclodextrin	400 g
Glucose	13.9 g
Water for injection, added to	2000 ml

Preparation: Dissolve 3-hydroxypropyl-β-cyclodextrin in 80% of the fresh water for injection and add YC-6, followed by stirring at room temperature for 10~20 min to make YC-6 completely dissolved. Add glucose and make it dissolved by stirring, then add water for injection to 2000 ml. Add 0.1% activated charcoal into the above solution, and stir for 15 min at 60℃ and then cool naturally the solution to room temperature. A 0.22μm microporous membrane filter is used to filtrate. The filtrate is collected and filled to prepare 10 ml injection, and subject to sterilization at 121°C for

15 min.

Example 3 Preparation of 200 ampoules of YC-6 injection (specification 5 ml: 100 mg)

Formula:

YC-6	20 g
2-hydroxypropyl-β-cyclodextrin	400 g
Water for injection, added to	1000 ml

Preparation: Dissolve 2-hydroxypropyl-β-cyclodextrin in 80% of the fresh water for injection and add YC-6, followed by stirring at room temperature for 10~20 min to make YC-6 completely dissolved. Add water for injection to 1000 ml. Add 0.1% activated charcoal into the above solution, and stir for 15 min at 60℃ and then cool naturally the solution to room temperature. A 0.22μm microporous membrane filter is used to filtrate. The filtrate is collected and filled to prepare 5 ml injection, and subject to sterilization at 115°C for 30 min.

Example 4 Preparation of 200 ampoules of YC-6 sterile powder (specification 80 mg/bottle)

Formula:

YC-6	16 g
2-hydroxypropyl-β-cyclodextrin	400 g
Sodium chloride	2.5 g
Packaged into	200 ampoules

Preparation: Dissolve 2-hydroxypropyl-β-cyclodextrin in 80% of the fresh water for injection and add YC-6, followed by stirring at room temperature for 10~20 min to make YC-6 completely dissolved. Add sodium chloride and make it dissolved by stirring, and add water for injection to 2000 ml. Add 0.1% activated charcoal into the above solution, and stir for 15 min at 60℃ and then cool naturally the solution to room temperature. A 0.22μm microporous membrane filter is used to filtrate. The filtrate is subject to spray drying and then packaged into 200 ampoules.

Example 5 Preparation of 200 ampoules of YC-6 freeze drying powder (specification 5 ml: 60 mg)

Formula:

YC-6	12 g
3-hydroxypropyl-β-cyclodextrin	200 g
Glucose	7 g
Water for injection, added to	1000 ml

Preparation: Dissolve 3-hydroxypropyl-β-cyclodextrin in 80% of the fresh water for injection and add YC-6, followed by stirring at room temperature for 10~20 min to make YC-6 completely dissolved. Add glucose and make it dissolved by stirring, and add water for injection to 1000 ml. Add 0.1% activated charcoal into the above solution, and stir for 15 min at 60℃ and then cool naturally the solution to room temperature. A 0.22μm microporous membrane filter is used to filtrate. The filtrate is packaged into 5 ml ampoules and then subject to freeze drying.

Example 6 Compatible Stability of YC-6 injection and conventional infusions

Two ampoules of YC-6 injection (5 ml X 2) of Example 1 are added to conventional infusions to evaluate the compatible stability of YC-6 therein in 8 hours. Evaluating items of compatible stability include color, clarity, pH and YC-6 content. Results are shown in the following tables.

Table 1. Compatibility tests of YC-6 injection and conventional infusions

ID	Compatibility tests （25-30°C）
A	YC-6 injection 5ml X 2+5% glucose injection 250 ml
B	YC-6 injection 5ml X 2+0.9% sodium chloride injection 250 ml
C	YC-6 injection 5ml X 2+glucose and NaCl injection 250 ml
D	YC-6 injection 5ml X 2+compound NaCl injection 500 ml
E	YC-6 injection 5ml X 2+5% sodium bicarbonate injection 250 ml

Table 2. Changes in color and clarity of conventional infusions

		After adding (h)			
ID	Prior to adding	0	2	4	8
A	Colorless, clear	Colorless, clear	Colorless, clear	Colorless, clear	Colorless, clear
B	Colorless, clear	Colorless, clear	Colorless, clear	Colorless, clear	Colorless, clear
C	Colorless, clear	Colorless, clear	Colorless, clear	Colorless, clear	Colorless, clear
D	Colorless, clear	Colorless, clear	Colorless, clear	Colorless, clear	Colorless, clear
E	Colorless, clear	Colorless, clear	Colorless, clear	Colorless, clear	Colorless, clear

Table 3. pH changes of conventional infusions

ID	Prior to adding	After adding (h)

		0	2	4	8
A	4.05	4.06	4.05	4.02	4.08
B	5.60	5.62	5.58	5.46	5.58
C	4.02	4.04	4.03	4.00	4.04
D	5.64	5.62	5.60	5.59	5.59
E	7.99	7.94	7.90	8.04	8.00

Table 4. Changes of YC-6 concentration in conventional infusions

ID	0h	2h	8h	24h
A	375.4	364.7	367.7	363.4
B	379.2	373.5	380.4	386.1
C	385.6	387.6	383.4	384.5
D	382.0	383.7	387.2	380.8
E	386.7	375.1	381.3	376.5

Example 7 Preliminary Evaluation on the Security of YC-6

Kunming mice are caged by weight and randomly divided into 5 groups. Each group has 10 mice, with half males and half females. The YC-6 injection prepared in Example 3 (20 mg/ml) is *i.v.* administrated via tail vein at different doses. All mice are sacrificed after one-week observation. The toxic reaction and number of death of the animals are recorded every day. LD_{50} and 95% confidence are calculated. LD_{50} of YC-6 is more than 400 ± 121 mg/kg.

Blood cells are prepared according to conventional methods with fresh blood obtained from New Zealand rabbits. The blood cells are diluted with saline to a 2% suspension. The YC-6 injection prepared in Example 1 is then added to the 2% suspension and incubated at 37°C for 3 hours. Hemolysis rate is determined by using colorimetric method. Hemolysis rate of YC-6 injection is less than 1%.

Albino guinea pigs are subject to anaphylactic test according to conventional procedures. No anaphylactic reaction is observed after intravenous administration of the YC-6 injection prepared in Example 1.

New Zealand rabbits are used to perform vascular stimulation test of intravenous administration of the YC-6 injection prepared in Example 1. The results show that tissue changes of ear edge vein are similar between treatment group and control group. Each rabbit has integral vascular wall of ear edge vein and normal vein structure. No

pathological change such as endothelial cells damage or surrounding tissue edema is observed.

CLAIMS

1. An injectable composition in the form of a liquid having a solvent or in the form of a solid, the injectable composition comprising: 5α-androstane-3β,5,6β-triol and at least one soluble excipient, wherein the at least one soluble excipient includes hydroxypropyl-β-cyclodextrin.

2. The injectable composition of claim 1, wherein the weight ratio of 5α-androstane-3β,5,6β-triol to hydroxypropyl-β-cyclodextrin is 1-20: 40-500.

3. The injectable composition of claim 1 or claim 2, wherein the soluble excipient further comprises an isotonic adjusting agent and/or a freeze drying filler.

4. The injectable composition of claim 3, wherein the isotonic adjusting agent is selected from the group consisting of: sodium chloride, glucose, mannitol, lactose, xylitol, sorbitol, maltitol and mixtures thereof.

5. The injectable composition of claim 3, wherein the freeze-drying filler is selected from the group consisting of: sodium chloride, glucose, mannitol, lactose, xylitol, sorbitol, maltitol and mixtures thereof.

6. The injectable composition according to any one of claims 1 to 5, wherein the solvent is selected from the group consisting of: propanediol, ethanol, polyethylene glycol 400, polyethylene glycol 200, glycerol, water and mixtures thereof.

7. The injectable composition according to any one of claims 3 to 5, wherein the injectable composition consists of (by weight): 1-20 parts of the 5α-androstane-3β,5,6β-triol, 40-500 parts of the hydroxypropyl-β-cyclodextrin, 1-100 parts of the isotonic adjusting agent, 0-200 parts of the freeze drying filler, and 0-2000 parts of the solvent.

8. A method for preparing the injectable composition of claim 1, comprising steps of:

(a) dissolving hydroxypropyl-β-cyclodextrin, 5α-androstane-3β, 5,6β-triol and additional soluble excipients in water for injection in sequence to obtain a raw injectable solution; and

(b1) subjecting the raw injectable solution to decolorization, depyrogenation, filtration and sterilization to obtain the injectable composition; or

(b2) subjecting the raw injectable solution to decolorization, depyrogenation, filtration and filling a filtrate produced by the filtration into an ampoule, followed by freeze drying to obtain a freeze dried powder; or

(b3) subjecting the raw injectable solution to decolorization, depyrogenation, filtration and spray drying a filtrate produced by the filtration to produce a powder,

and packaging the powder.

9. The method of claim 8, wherein the decolorization is achieved by using activated charcoal at an amount of 0.05-0.3 wt% of the injectable composition.

10. The method of claim 8 or claim 9, wherein the sterilization is performed at 115 °C for 30 min.

11. The method of claim 8 or claim 9, wherein the sterilization is performed at 121 °C for 15 min.