

A pharmaceutical composition for regulating the potassium channel in cardiac muscle cell and its preparation method and its uses

Technical Field

[1] The present invention relates to a pharmaceutical composition for regulating the potassium channel in cardiac muscle cell and its preparation method and its uses.

Background Art

[2] Cardiovascular diseases, such as hypertension, coronary disease, arrhythmia, have become major threat to human health, the morbidity and mortality of which are the highest among all the diseases worldwide. Severe arrhythmia, such as fast ventricular tachycardia (VT), ventricular fibrillation (VF) and sudden cardiac death (SCD), is the major cause of cardiovascular disease-associated death.

[3] The normal blood pumping function of heart relies on the rhythmic movement, which is alternately contract and relax, of cardiac muscle. The formation and conduction of excitability on cardiac muscle cell membrane are the initial causes for the rhythmic contract and relax movement of the heart. The formation and conduction of excitability on cardiac muscle cell membrane are based on the change of electrical potential on cardiac muscle cell membrane. The abnormal electrical potential change of membrane leads to the change of rhythm of the heart, i.e., arrhythmia. Arrhythmia severely affects the blood pumping function of the heart, which needs to be treated as early as possible.

[4] Based on the diseased site, the arrhythmia can be classified into

sinus arrhythmia, atrial arrhythmia, atrioventricular junctional arrhythmia, ventricular arrhythmia and heart blocks. Atrial arrhythmia includes premature atrial contractions, atrial tachycardia, atrial flutter, atrial fibrillation. Ventricular arrhythmia includes premature ventricular contractions, ventricular tachycardia, ventricular flutter, ventricular fibrillation (Ren-gao Ye, *Internal medicine*, People's medical publishing house, Oct.1984, 2nd edition, p.172-212).

[5] Currently, based on the effect on cardiac muscle electrophysiology and the mechanism, the anti-arrhythmia agents for clinical use and in development can be classified into 4 groups: I - sodium channel blockers, II - β receptor blockers, III - the agent selectively prolonging action potential duration (APD) and effective refractory period (ERP) in cardiac muscle cells, and the agent selectively blocking potassium channel in cardiac muscle, IV - calcium channel blockers. Agents from groups I, II and IV can reduce conduction velocity and even lead to conduction block, which can increase the possibility of reentry activation and may thereby lead to arrhythmia. Agents from group III have no substantial effect on automaticity (Rui Li, *Pharmacology* (4th edition), People's medical publishing house, Nov. 1999, 14th print, p159-171).

[6] A pharmaceutical composition and its preparation method have been disclosed in Chinese patent ZL02146572.X, which is just directed to the research of premature ventricular beat of coronary heart.

Summary

[7] Surprisingly, the applicant found a pharmaceutical composition

for regulating the potassium channel in cardiac muscle cell, which can inhibit the potassium channel, inhibit inward rectifying potassium current and transient outward potassium current in a voltage-dependent manner, and can delay the rectifying potassium current. The anti-arrhythmia effect is achieved by decreasing the excitability of cardiac muscle cell, inhibiting the repolarization of action potential (AP) in cardiac muscle cell, and prolonging action potential duration (APD). The pharmaceutical composition of the present invention is useful for various atrial, ventricular and supraventricular arrhythmia.

[8] Another object of the present invention is to provide a preparation method of the pharmaceutical composition.

[9] Another object of the present invention is to provide the uses of the pharmaceutical composition.

[10] The present invention provides a pharmaceutical composition for regulating the potassium channel in cardiac muscle cell comprising the following components by weight:

[11] ginseng 45-180 parts, ophiopogonis 50-200 parts, corni 125-450 parts, miltiorrhizae 125-450 parts, fried semen ziziphi spinosae 95-400 parts, taxilli 95-400 parts, rubra 45-200 parts, steleophaga 35-150 parts.

[12] The pharmaceutical composition also comprises the following components by weight: Rhizoma Nardostachyos 45-200 parts, Rhizoma Coptidis 25-90 parts, Fructus Schisandrae Sphenanthenae 35-150 parts, Os draconis 75-300 parts.

[13] The pharmaceutical composition preferably comprises the following components by weight:

[14] ginseng 45-90 parts, ophiopogonis 112-135 parts, corni 224-270 parts, miltiorrhizae 200-225 parts, fried semen ziziphi spinosae

150-186 parts, taxilli 150-186 parts, rubra 89-100 parts, steleophaga 35-100 parts, Rhizoma Nardostachyos 45-95 parts, Rhizoma Coptidis 45-60 parts, Fructus Schisandrae Sphenanthenae 67-75 parts, Os draconis 145-150 parts.

[15] The pharmaceutical composition preferably comprises the following components by weight: ginseng 89 parts, ophiopogonis 112 parts, corni 224 parts, miltiorrhizae 224 parts, fried semen ziziphi spinosae 186 parts, taxilli 186 parts, rubra 89 parts, steleophaga 75 parts, Rhizoma Nardostachyos 89 parts, Rhizoma Coptidis 45 parts, Fructus Schisandrae Sphenanthenae 67 parts, Os draconis 149 parts.

[16] The pharmaceutical composition preferably comprises the following components by weight:

[17] ginseng 45 parts, ophiopogonis 112 parts, corni 224 parts, miltiorrhizae 225 parts, fried semen ziziphi spinosae 186 parts, taxilli 186 parts, rubra 89 parts, Rhizoma Nardostachyos 45 parts, steleophaga 35 parts, Rhizoma Coptidis 45 parts, Fructus Schisandrae Sphenanthenae 67 parts, Os draconis 149 parts.

[18] The dosage form of the pharmaceutical composition is capsule, tablet, medicinal granules, powder or oral solution formulation.

[19] The present invention also provides the use of the pharmaceutical composition in the preparation of medicament for treating supraventricular arrhythmia, ventricular tachycardia, ventricular flutter, ventricular fibrillation, premature atrial contractions, atrial tachycardia, atrial flutter or atrial fibrillation.

[20] The present invention also provides the method for preparing the pharmaceutical composition, comprising the following steps:

a) adding suitable amount of 70% ethanol to 45-180 parts (by weight) of ginseng, performing reflux extraction 3 times, pooling the

extract solution, filtering, concentrating, drying, and grounding it into fine powder;

b) adding suitable amount of 70% ethanol to 125-450 parts (by weight) of corni and 125-450 parts (by weight) of miltiorrhizae, performing reflux extraction 3 times, pooling the extract solution, filtering, concentrating it into concrete;

c) grounding 35-150 parts (by weight) of steleophaga into fine powder, drying it for further use;

d) adding suitable amount of water to 50-200 parts (by weight) of ophiopogonis, 95-400 parts (by weight) of fried semen ziziphi spinosae, 95-400 parts (by weight) of taxilli, 45-200 parts (by weight) of rubra, boiling twice, pooling the extract solution, concentrating it into concrete;

e) pooling the concrete from step b) and d), adding the pharmaceutical fine power from step c) and the pharmaceutical power from step a) thereinto in turn, mixing the powder and subjecting the homogenized powder to preparation process to obtain the pharmaceutical composition.

[21] The raw material in step b) further comprises 45-200 parts (by weight) of Rhizoma Nardostachyos, 35-150 parts (by weight) of Fructus Schisandrae Sphenanthenae, 25-90 parts (by weight) of Rhizoma Coptidis. The raw material in step d) further comprises 75-300 parts (by weight) of Os draconis.

[22] The preparation method of the pharmaceutical composition preferably involves the following components by weight: ginseng 89 parts, ophiopogonis 112 parts, corni 224 parts, miltiorrhizae 224 parts, fried semen ziziphi spinosae 186 parts, taxilli 186 parts, rubra 89 parts, steleophaga 75 parts, Rhizoma Nardostachyos 89 parts, Rhizoma Coptidis 45 parts, Fructus Schisandrae Sphenanthenae 67

parts, *Os draconis* 149 parts.

[23] In the application of the present invention, the dosage form of the pharmaceutical composition selected from: capsule, tablet, medicinal granules, powder or oral solution formulation. In order to obtain the above dosage form, a pharmaceutically acceptable adjuvant can be supplemented in the preparation of such dosage form, such as bulking agents, disintegrants, lubricants, suspending agents, binding agents, sweeteners, flavoring agents, preservatives and the like. Bulking agents include starch, pregelatinized starch, lactose, mannitol, chitin, microcrystalline cellulose, sucrose etc. Disintegrants include starch, pregelatinized starch, microcrystalline cellulose, sodium carboxymethyl starch, cross-linked polyvinylpyrrolidone, low-substituted hydroxypropyl cellulose, cross-linked sodium carboxymethyl cellulose and the like. Lubricants include magnesium stearate, sodium dodecyl sulfate, talc, silica and the like. Suspending agents include polyvinylpyrrolidone, microcrystalline cellulose, sucrose, agar, hydroxypropyl methylcellulose etc. Binding agents include starch pulp, polyvinylpyrrolidone, hydroxypropyl methyl cellulose etc. Sweeteners include sodium saccharin, aspartame, sucrose, sodium cyclamate, glycyrrhetic acid etc. Flavoring agents include sweetener and various kinds of fragrances. Preservatives include nipagins, benzoic acid, sodium benzoate, sorbic acid and the salt thereof, benzalkonium bromide, chlorhexidine acetate, eucalyptus oil and the like.

[24] The pharmaceutical composition according to the present invention can inhibit inward rectifying potassium current and transient outward potassium current in cardiac muscle cell in a voltage-dependent manner, and can delay the rectifying potassium

current. Said pharmaceutical composition decreases the excitability of cardiac muscle cell and inhibits the repolarization of action potential (AP) in cardiac muscle cell by regulating the potassium channel of the cardiac muscle cell membrane. It prolongs action potential duration of cardiac muscle cell by regulating the potassium channel in cardiac muscle cell membrane.

[25] The present invention also provides the use of the pharmaceutical composition in the preparation of the anti-arrhythmia drugs.

[26] The arrhythmia includes supraventricular arrhythmia, ventricular tachycardia, ventricular flutter, ventricular fibrillation, premature atrial contractions, atrial tachycardia, atrial flutter or atrial fibrillation.

[27] The pharmaceutical composition according to the present invention can inhibit the I_{K1} of cardiac muscle cell membrane, and can reduce the current density of I_{K1} .

[28] The pharmaceutical composition according to the present invention can inhibit the transient outward potassium current (I_{to}) of cardiac muscle cell membrane, which can accelerate the inactivation of the current.

[29] The pharmaceutical composition according to the present invention can inhibit the inward part of the inward rectifying potassium current I_{K1} (I_{K1} current), without affecting the reversal potential and rectifying property of the channel, and slightly increase the outward part of the current, which is useful for the stabilization of cell membrane and the elimination of early afterdepolarization.

[30] The amount of the pharmaceutical composition according to the present invention (by the total weight of active components in the raw material) is 3-28 g/day. It can be administrated once a day,

preferably in 2-4 divided dosage.

Description of the drawings

[31] Fig.1 shows the *ex vivo* heart perfusion system (Langendorff perfusion system).

[32] Fig.2 shows the effect of the pharmaceutical composition according to the present invention on I_{K1} current. A. control. B. The drug can inhibit I_{K1} with the concentration of 0.5%. C. Effect of the pharmaceutical composition according to the present invention on current density-voltage relationship.

[33] Fig .3 shows the features of the current I_{to} and the effect of the pharmaceutical composition according to the present invention on current. A. The outward current recorded on a single rat ventricular muscle cell, $V_h = -90\text{mV}$, in which a pre-stimulus (-40mV 20ms) is firstly given and then a depolarization to $+60\text{mV}$ is carried out with a step of 10mV , and the clamp time is 400ms; the stimulated outward current comprises two parts: transient current and holding current. B. The drug can inhibit I_{to} when the concentration is 0.5%. C. The curve of I_{to} current-voltage relationship. ●: total peak current, ▲: holding current, ■: transient current (■=●-▲). D. Current density-voltage relationship plot of the peak of transient current. ●: before drug treatment, ▲: After drug treatment; the drug inhibites I_{to} , which is manifested by the decrease of current density.

[34] Fig.4 shows the effect of the pharmaceutical composition according to the present invention on the time dependent-inactivation of I_{to} . A. before drug treatment; B. Drug concentration is 0.5%; and the inactive part of I_{to} current is fitted using single exponential equation.

[35] Fig.5 shows the effect of the pharmaceutical composition

according to the present invention on the I_{to} steady-state inactivation. A. before drug treatment. B. Drug concentration is 0.5%. C. Voltage dependency of different parts of outward current inactivation. ●: peak current, ▲: holding current, ■: transient current (■=●-▲). D. the curve of steady-state inactivation of the transient outward current I_{to} before and after drug treatment, ●: before drug treatment, ▲: after drug treatment, the solid lines in the plot indicate the fitting using Boltzmann equation.

[36] Fig. 6 shows the effect of the pharmaceutical composition according to the present invention on the recover of I_{to} current after inactivation. A. Control. B. Drug concentration is 0.5%. C. Plot of time-dependent recovery after I_{to} inactivation. ●: before drug treatment, ▲: After drug treatment, the solid lines in the plot indicate the fitting using single exponential equation.

[37] Fig.7 shows the voltage-dependent inhibition of the pharmaceutical composition according to the present invention on delayed rectifying potassium current (I_K). A, $V_H = -40\text{mV}$, stepwise depolarizing to 60mV , with 5s duration, and the voltage-dependent and time-dependent activation of the outward current I_K is obtained in a single guinea pig ventricular muscle cell. B. Drug concentration is 0.5%, the current changes for a 5s duration at $V_T = 50\text{mV}$ before and after drug treatment. C. I-V relationship plot (current density of peak tail current (under various test voltage) vs. voltage) before and after drug treatment.

[38] Fig.8 shows the inhibition of the pharmaceutical composition according to the present invention on I_K current which is activated in a time-dependent manner. A. $V_H = -40\text{mV}$, $V_T = 50\text{mV}$, for a 500ms~5s duration, increased with 500ms intervals. B. I_K current and tail current under the same test voltage and time after drug

treatment (drug concentration: 0.5%). C. Time-dependent activation plot (peak tail current vs. time of activation under various time of activation) before and after drug treatment.

Detailed description of the preferred embodiments

[39] The following examples illustrates the preparation of the pharmaceutical composition according to the present invention. These examples do not limit the scope of the present invention in any way.

Example 1

[40] The pharmaceutical composition is prepared as a capsule to facilitate the application of such pharmaceutical composition as a potassium channel regulator, comprising:

ginseng 89g, ophiopogonis 112g, corni 224g, miltiorrhizae 224g, fried semen ziziphi spinosae 186g, taxilli 186g, rubra 89g, steleophaga 75g.

[41] Preparation method:

a) reflux extracting from ginseng 3 times using 70% ethanol, 3h for the first extraction, 2h for the second and the third extraction, pooling the extract solution, filtering, recovering ethanol, concentrating, drying, and grounding it into fine powder, for further use;

b) reflux extracting from corni and miltiorrhizae 3 times using 70% ethanol, pooling the extract solution, filtering, recovering ethanol, concentrating it into concrete, for further use;

c) grounding steleophaga into fine powder, drying for further use.

d) adding water to ophiopogonis, fried semen ziziphi spinosae, taxilli and rubra, and boiling twice, pooling the extract solution, filtering, concentrating it into concrete, for further use;

e) pooling the concrete from step b) and d), adding the pharmaceutical fine power from step c) thereinto, drying, grounding it into fine powder, and then adding the pharmaceutical fine power from step a), mixing and adding adjuvant for the preparation of 1000 capsules.

[42] Indications: supraventricular arrhythmia, ventricular tachycardia, atrial flutter.

[43] Dosage and amount: oral, 2~4 capsules per time, 3 times a day.

Example 2

[44] ginseng 45g, ophiopogonis 50g, corni 125g, miltiorrhizae 125g, fried semen ziziphi spinosae 95g, taxilli 95g, rubra 89g, steleophaga 35g, Rhizoma Nardostachyos 45g, Rhizoma Coptidis 25g, Fructus Schisandrae Sphenanthenae 35g, Os draconis 75g.

[45] Preparation method:

a) reflux extracting from ginseng 3 times using suitable amount of 70% ethanol, 3h for the first extraction, 2h for the second and the third extraction, pooling the extract solution, filtering, recovering the ethanol, concentrating, drying, and grounding it into fine powder, for further use;

b) reflux extracting from corni, miltiorrhizae, Fructus Schisandrae Sphenanthenae, Rhizoma Nardostachyos and Rhizoma Coptidis 3 times using suitable amount of 70% ethanol, pooling the extract solution, filtering, recovering the ethanol, concentrating it into concrete, for further use;

c) grounding steleophaga into fine powder, drying the powder for further use;

d) adding proper amount of water to ophiopogonis, fried semen ziziphi spinosae, taxilli, rubra, and boiling twice, pooling the extract

solution, concentrating it into concrete, for further use;

e) pooling the concrete from step b) and d), in which the pharmaceutical fine power from step c) is added, drying and grounding it into fine powder, then adding the pharmaceutical fine power from step a) thereinto, mixing and formulating it into medicinal granules by conventional method.

Example 3

[46] ginseng 180g, ophiopogonis 200g, corni 450g, miltiorrhizae 450g, fried semen ziziphi spinosae 400g, taxilli 400g, rubra 200g, steleophaga 150g, Rhizoma Nardostachyos 200g, Rhizoma Coptidis 90g, Fructus Schisandrae Sphenanthenae 150g, Os draconis 300g.

[47] Preparation method:

a) reflux extracting from ginseng 3 times using proper amount of 70% ethanol, 3h for the first extraction, 2h for the second and the third extraction, pooling the extract solution, filtering, recovering the ethanol, concentrating, drying, and grounding it into fine powder, for further use;

b) reflux extracting from corni, miltiorrhizae, Fructus Schisandrae Sphenanthenae, Rhizoma Nardostachyos and Rhizoma Coptidis 3 times using proper amount of 70% ethanol, pooling the extract solution, filtering, recovering the ethanol, concentrating it into concrete, for further use;

c) grounding steleophaga into fine powder, drying the powder for further usage.

d) adding proper amount of water to ophiopogonis, fried semen ziziphi spinosae, taxilli, rubra, and boiling twice, pooling the extract solution, concentrating it into concrete, for further use;

e) pooling the concrete from step b) and d), adding the

pharmaceutical fine power from step c) thereinto, drying and grounding it into fine powder, then adding the pharmaceutical fine power from step a) thereinto, mixing and formulating it into tablet by conventional method.

Example 4

[48] ginseng 60g, ophiopogonis 135g, corni 270g, miltiorrhizae 200g, fried semen ziziphi spinosae 150g, taxilli 150g, rubra 100g, steleophaga 100g, Rhizoma Nardostachyos 95g, Rhizoma Coptidis 55g, Fructus Schisandrae Sphenanthenae 75g, Os draconis 145g.

[49] Preparation method:

a) reflux extracting from ginseng 3 times using proper amount of 70% ethanol, 3h for the first extraction, 2h for the second and the third extraction, pooling the extract solution, filtering, recovering the ethanol, concentrating, drying, and grounding it into fine powder, for further use;

b) reflux extracting from corni, miltiorrhizae, Fructus Schisandrae Sphenanthenae, Rhizoma Nardostachyos and Rhizoma Coptidis 3 times using proper amount of 70% ethanol, pooling the extract solution, filtering, recovering the ethanol, concentrating it into concrete, for further use;

c) grounding steleophaga into fine powder, drying the powder for further use.

d) adding proper amount of water to ophiopogonis, fried semen ziziphi spinosae, taxilli, rubra, and boiling twice, pooling the extract solution, concentrating it into concrete, for further use;

e) pooling the concrete from step b) and d), adding the pharmaceutical fine power from step c) and step a), mixing and formulating it into oral solution by conventional method.

Example 5

[50] Prescription:

ginseng 89g, ophiopogonis 112g, corni 224g, miltiorrhizae 224g, fried semen ziziphi spinosae 186g, taxilli 186g, rubra 89g, steleophaga 75g, Rhizoma Nardostachyos 89g, Rhizoma Coptidis 45g, Fructus Schisandrae Sphenanthenae 67g, Os draconis 149g.

[51] Preparation method:

a) by the above prescription, reflux extracting from ginseng 3 times using 70% ethanol, 3h for the first extraction, 2h for the second and the third extraction, pooling the extract solution, filtering, recovering the ethanol, concentrating, drying, and grounding it into fine powder, for further use;

b) by the above prescription, reflux extracting from Fructus Schisandrae Sphenanthenae, corni, miltiorrhizae, Rhizoma Coptidis and Rhizoma Nardostachyos 3 times using 70% ethanol, pooling the extract solution, filtering, recovering the ethanol, concentrating it into concrete, for further use;

c) by the above prescription, grounding steleophaga into fine powder, drying the powder for further use.

d) by the above prescription, adding water to ophiopogonis, fried semen ziziphi spinosae, taxilli and rubra, boiling twice, pooling the extract solution, filtering, pooling this filtrate with the above extract solution involving Fructus Schisandrae Sphenanthenae etc., concentrating it into concrete, for further use;

e) pooling the concrete from step b) and d), adding the pharmaceutical fine power from step c) thereinto, grounding it into fine powder, then adding the pharmaceutical fine power from step a), mixing and filling 1000 capsules therewith.

Experiments

[52] The following is the pharmacological activity experiment of the pharmaceutical composition according to the present invention.

[53] In order to illustrate the sodium potassium-channel-regulating-activity of the pharmaceutical composition according to the present invention, the following animal experiments was performed using the powder inside the capsules from Example 2 (referring to as “capsule power according to the present invention” hereafter) .

1. Material and Methods

1.1 Material

[54] 1) Reagents: The capsule power according to the present invention is provided by SHIJIAZHANG YILING PHARMACEUTICAL CO. LTD. Collagenase II is from Gibico. Pronase E is from Merck. N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), ethylene glycol bis-tetraacetic acid (EGTA), L-glutamic acid, taurine, aspartate, disodium creatine phosphate, choline-Cl, CaCl_2 , K_2ATP are from Sigma. 4-aminopyridine (4-AP) is from Fluka. The other agents are of analytical grade and provided by Beijing chemical reagent company.

[55] 2) Solutions

① Calcium-free Krebs solution (mmol/L): NaCl 136, KCl 5.4, MgCl_2 1.0, NaH_2PO_4 0.33, HEPES 10, Glucose 10, adjusted to pH 7.4 with NaOH .

② KB solution (mmol/L): KCl 40, KH_2PO_4 20, MgSO_4 3.0, KOH 80, L-glutamic acid 50, taurine 20, HEPES 10, Glucose 10, EGTA 0.5, adjusted to pH 7.4 with KOH .

③The perfusate for detecting potassium current in the whole cell (mmol/L): Choline-Cl 136, MgCl_2 1.2, KCl 5.4, NaH_2PO_4 0.33, CaCl_2 1.8, CdCl_2 0.15, HEPES 10, Glucose 10, adjusted to pH 7.4 with LiOH. Add 1mmol/L BaCl_2 to block I_{K1} current to detect I_K .

④ Electrode fill solution for detecting potassium current in the whole cell (mmol/L): potassium aspartate 120, KCl 20, HEPES 5, MgCl_2 1.0, $\text{K}_2\text{-ATP}$ 4, EGTA 10, disodium creatine phosphate 2, adjusted to pH 7.3 with KOH. The electrode fill solution used is filtered by micro filter membrane with a 0.22 μm diameter.

⑤ The preparation of capsule power solution according to the present invention: making the capsule power according to the present invention into a 5% solution (w/w) with KCl free extracellular perfusate, then adjusting the concentration of K^+ to 5.4mmol/L, subjecting the solution to centrifugation and obtaining the supernatant for further use.

[56] 3) Animals: male SD rat, weighing 250-300g, provided by Beijing WEI TONG LI HUA experimental animal technology Co. LTD.

1.2 Methods

[57] 1.2.1 Isolation of single cardiac muscle cell: The single ventricular muscle cell is prepared by acute enzymatic method. A rat was anesthetized with 50mg/kg mebumal sodium via intraperitoneal injection, and the heart was removed by thoracotomy. Then, the fat and pericardium were discarded in calcium-free Tyrode solution at 4°C, and aorta was isolated and cannulated to perform Langendorff heart perfusion (Fig.1) with the speed of 6~8ml/min. Perfusion was carried out for 3 min using calcium-free Krebs solution (the perfusion pressure was 20 cmH₂O) to clear out the blood in the coronary, then low-calcium enzymatic solution (low-calcium Krebs

solution with CaCl_2 0.05mmol/L, containing 0.4mg/ml collagenase II and 0.04mg/ml protease E) was used to perform recirculating perfusion. Around 20min later, the heart was taken from Langendorff system, placed into RT (room temperature) KB solution, the atrial and cardiac base tissue was cut out, the ventricular tissue was gently torn with ophthalmic tweezers and slowly pipetted with wide-tip pipette to detach cardiac muscle cell from cardiac muscle tissue to obtain single ventricle muscle cell. The supernatant is filtered by nylon filter (120 meshes/inch), and the cardiac muscle cell is kept in a tube filled with KB solution. The temperature of the whole perfusion system is kept at 37°C, and all perfusate and KB solution are saturated by 100% O_2 for more than 30 min. The cells were left at room temperature for about half an hour to recalcify to 0.25mmol/L, and then kept them on room temperature for future use. The isolation method of guinea pig cardiac muscle cell is substantially the same.

[58] 1.2.2 Experiment of whole cell patch clamp

Before the experiment, small amount of KB suspension with abundant cells was pipetted into 0.5ml perfusion chamber and left still for 5~10min. Once the cells precipitate and are attached to the chamber, surface infusion (with the rate of 2ml/min) was performed for 5~10min with extracellular fluid saturated by 100% O_2 . The uncontracted cells with clear border, smooth surface and clear stripe were selected under inverted microscope (OLYMPUS IX70). The experiment was performed at room temperature (22~24°C). The channel current was recorded under voltage clamping by standard whole cell patch clamp. Patch clamp amplifier (Axopatch 700B, Axon Co. U.S.) was associated with the computer through A/D and D/A data converter (Digidata 1322, Axon Co. U.S.). The software

(pClampex 10.0, Axon Co. U.S.) controlled the capture of stimulus signal and voltage, current input signal. The chunk glass tube (provided by Institute of Physics, Chinese academy of sciences) was made by microelectrode puller (p-97, Sutter Co. U.S.) with 4 steps, and polished with microforge system (MF-830, Narishige Co. Japan) leading to the diameter of the tip 2~3 μ m. The resistance in electrode fill solution was 1.5~3M Ω . After the compensation liquid was charged with potential, the electrode tip was moved to cell surface via the three-dimensional manipulator, forming giga seal, with the seal resistance above 1G Ω . The fast capacity was compensated and cell membrane was broken to generate the whole cell recording mode. A 10mV step stimulation was provided when determining the capacity, and the capacity of the membrane is calculated by $C_m = \tau \cdot I_{ss} / \Delta V$). The slow capacity compensation and tandem resistance compensation was adjusted 80%~90% to reduce the current and clamp error due to transient charge and discharge. Signals pass through the fourth-order low pass Bessel filter with the cutoff frequency of 1kHz, and the sampling rate was 10kHz.

[59] While recording, ATP was added to the electrode fill solution to avoid the influence of current decay, and to finish the experiment within 25 min after the cell membrane broken. Based on the protocol, the prepared drug solution was perfused to cell perfusion chamber by a peristaltic pump with a constant speed of 2ml/min. And then the waste solution was sucked out by a negative pressure suction device. 5min after the membrane break, the current before drug treatment is recorded, and 5min after drug treatment, the current after the drug treatment is recorded. The obtained data was stored on the hard disc of a computer for further measurement and analysis. In order to eliminate the statistical error between cells, the value of the current

was presented as current density (pA/pF).

[60] 1.2.3 The design of current stimulation schemes and the observation index: 1) the stimulation scheme for I_{K1} recording: holding potential (V_h)-40mV, command potential -120mV~0mV, step 10mV, clamping duration 400ms, stimulation frequency 0.2Hz; the observation index is the change of constant current density of I_{K1} (under the test voltage -100mV) before and after drug treatment; 2) the stimulation scheme for recording I_{to} current-voltage relationship: holding potential (V_h)-90mV, a pre-stimulus (-40mV 20ms) and then a depolarization stimulus of -40mV~+60mV is carried out, step 10mV, clamping duration 400ms, stimulation frequency 0.2Hz; the observation index is the change of peak current density of I_{to} (under the test voltage 60mV) before and after drug treatment; 3) the stimulation scheme for recording I_{to} steady-state inactivation: holding potential -90mV, a pre-stimulus (400ms) was given from -120mV to 60mV with 10mV step, then clamp at 60mV for 300ms to record I_{to} current, and compare the current before and after drug treatment; 4) the stimulation scheme for recording the time-dependent recovery after I_{to} inactivation: a double-pulse stimulus was given, the holding potential was -90mV, then two depolarization stimulus (to 60mV, duration 200ms) P1 and P2 were given in turn at following increasing intervals (Δt): 1, 3, 5, 7, 10, 15, 20, 30, 40, 60, 90, 120, 150, 180, 210, 250, 300 (ms). The peak current amplitude I corresponding to P2 was recorded. The plot of the recovery after steady-state inactivation was made as I/I_{max} vs Δt . 5) The voltage-dependent activation of I_k is determined: V_H =-40mV, the depolarization was carried out from -40mV gradually to +60mV with the step of 10mV and the stimulus wave width of 5s, when back to V_H , hold 2.5s to record tail current. 6) The time-dependent

activation of I_K tail current (envelop of tails test): $V_H = -40\text{mV}$, the depolarization was carried out to $+50\text{mV}$ then back to V_H , 10 continuous pulses were given with the wave width of $500\sim 5000\text{ms}$, increasing 500ms each time.

1.3 Data analysis and statistics

[61] The raw current data was detected and collected by Clampfit 10.0 (Axon Co. U.S.), and the collected data was analyzed, fitted and plotted using Origin 6.0 data process software (Microcal Software Co. U.S.). The data from the experiment was presented as $\text{mean} \pm \text{standard deviation}$ ($\bar{x} \pm s$), paired t-test was performed for the before drug treatment and after drug treatment group. The difference is significant if $P < 0.05$.

2. Results

2.1 The effect of the pharmaceutical composition according to the present invention on the I_{K1} of rat cardiac muscle cell membrane

[62] A rapidly activated inward current was recorded on rat single ventricular muscle cell, without obvious inactivation within 400ms (holding potential (V_h)- 40mV , command potential $-120\text{mV} \sim 0\text{mV}$, step 10mV , clamping duration 400ms , stimulation frequency 0.2Hz) (see Fig.2A). Such a current can be completely blocked by 1mmol/L BaCl_2 , indicating that the current was I_{K1} . The inward part of the I_{K1} current decreased as the depolarization occurs, the reversal potential was about -40mV (with the properties of inward rectifier). The capsule power according to the present invention was resolved by potassium extracellular liquid into a 0.5% solution which can inhibit I_{K1} (see Fig. 2B). Under -100mV , the average peak current density was -10.78 ± 1.80 (pA/pF) before drug treatment, the average inhibition rate was $33.1 \pm 16.85\%$ ($n=11$, $P < 0.05$). The current density under various pulses was plotted as the function of corresponding

membrane potential, generating an I-V plot. Under every command current, the drug can decrease the current density of I_{K1} , while the reversal potential and the shape of the curve remained substantially unchanged (see Fig. 2C). After drug treatment, I_{K1} current was inhibited under every clamping voltage, manifested by the moving-up of the current density-voltage curve, without significantly affecting the reversal potential and rectification.

2.2 The effect of the pharmaceutical composition according to the present invention on the I_{to} of rat cardiac muscle cell membrane

2.2.1 The effect of the pharmaceutical composition according to the present invention on the I_{to} current-voltage (I-V) curve

[63] The potassium channel extracellular and endocellular recording solution was used, the holding potential (V_h) was -90mV, first a pre-stimulus (-40mV 20ms) was given, then a depolarization stimulus of -40mV to +60mV (with a step of 10mV), clamping duration was 400ms, stimulation frequency was 0.2Hz. The outward potassium current recorded in rat ventricular muscle cell comprises two parts. One part is transient current (I_{to}) that is rapidly activated and inactivated, with a sharp "A" shape, which becomes active at 0mV and reaches the peak at about 10ms, and then becomes inactive rapidly to a steady-state within 200ms. The activation and inactivation process are voltage- and time-dependent. The current can be completely blocked by 2mM 4-AP, indicating that the current is I_{to} . The other part is maintained current ($I_{maintained}$), which is activated at -10mV and increases as the voltage increases within -10~60mV (see Fig. 3A, C). The current density under various pulses was plotted as the function of corresponding membrane potential, generating an I-V plot. Transient current, i.e. I_{to} , is obtained by subtracting maintained current from peak current. The

0.5% capsule power solution according to the present invention can inhibit I_{to} current (see Fig. 3B), the peak current decreases when the test voltage is 0~60mV, and the I-V curve is moving down. The average current density at 60mV decreased from -19.82 ± 7.10 (pA/pF) to -10.02 ± 3.93 (pA/pF), with the average inhibition rate of $50.60 \pm 10.77\%$ ($n=6$, $p<0.05$) (see Fig.3D). The inactive part of I_{to} is fitted using single exponential equation to obtain the time constant of time-dependent inactivation (τ). The pharmaceutical composition according to the present invention can accelerate the inactivation of the current (see Fig.4), the τ value before and after drug treatment are 29.06 ± 4.66 and 21.44 ± 3.12 , respectively ($n=6$, $P<0.05$).

2.2.2 The effect of the pharmaceutical composition according to the present invention on the I_{to} steady-state inactivation curve

[64] The stimulation scheme for recording I_{to} steady-state inactivation are: holding potential -90mV, a pre-stimulus (400ms) was given from -120mV to 60mV with a step of 10mV, then clamp at 60mV for 300ms to record I_{to} current, and compare the current before and after drug treatment (Fig. 5A, B). It can be observed from obtained current plot that the inactivation of outward current can be divided into two parts, the inactivation of maintained current with low inactivation voltage; then the inactivation of the peak current with inactivation voltage higher than the former one. The amplitude of I_{to} depends on the difference between the peak current and the maintained current measured at the end of test pulse (Fig. 5C). The amplitudes of each current were standardized, and each membrane potential is plotted as a function of corresponding current to obtain the steady-state inactivation curve. Half-inactivation voltage ($V_{1/2}$) and slope factor of the inactivation curve (k) is obtained by fitting with Boltzmann equation ($I/I_{max}=1/\{1+\exp[(V_m-V_{1/2})/k]\}$) (Fig.

5D). The 0.5% capsule power solution according to the present invention results in the left movement of I_{to} steady-state inactivation curve, with an increased slope. The $V_{1/2}$ before and after drug treatment were $-15.67 \pm 2.52 \text{ mV}$ and $-26.45 \pm 3.88 \text{ mV}$, respectively, k value changed from 3.41 ± 0.67 to 6.38 ± 2.02 ($n=7$, $P<0.05$) (see Fig. 5).

2.2.3 The effect of the pharmaceutical composition according to the present invention on the recovery curve after I_{to} inactivation

[65] A double-pulse stimulus was given, the holding potential was -90 mV , then two depolarization stimulus (to 60 mV , duration 200 ms) P1 and P2 were given in turn at following increasing intervals (Δt): 1, 3, 5, 7, 10, 15, 20, 30, 40, 60, 90, 120, 150, 180, 210, 250, 300 (ms). The peak current amplitude I corresponding to P2 was recorded. The plot of the recovery after steady-state inactivation was made as the I/I_{max} vs Δt . The curve is fitted by using single exponential equation to obtain the time constant of the recovery curve (τ). When the concentration of the drug is 0.5%, the recovery time can be prolonged from 12.86 ± 0.31 to 18.52 ± 3.76 ($n=5$, $P<0.05$) (see Fig. 6).

2.2 The effect of the pharmaceutical composition according to the present invention on the I_K of guinea pig cardiac muscle cell

[66] Fig.7 shows the recorded outward current I_K , which is activated in a voltage- and time-dependent manner, in guinea pig single ventricular muscle cell ($V_H = -40 \text{ mV}$, depolarization to 60 mV with 10 mV step, for 5 s). The pharmaceutical composition according to the present invention inhibits I_K current in a voltage-dependent manner (Fig. 7C). The observation index is the recorded I_K when $V_T = 50 \text{ mV}$, when the drug concentration is 0.5%, the current density of peak tail current decreased to $30.77 \pm 1.11\%$ ($n=5$, $p<0.05$). Fig 8A,

B shows the I_K tail current activated along with time by the envelope stimulus scheme, as well as the inhibition of the drug on time-dependently activated I_K .

[67] Now, the I_{to} is found to include two types, I_{to1} (which is sensitive to 4-aminopyridine and insensitive to the intracellular calcium) and I_{to2} (which is calcium-dependent and can be blocked by caffeine and Co^{2+}). In the present study, there are high concentration of calcium complexing agent EGTA (10mmol/L) in pipette solution and the perfusate contains 0.15 mmol/L calcium blocker $CdCl_2$. Thus, the transient outward potassium current recorded in this study does not contain the I_{to2} part. We have observed in present study that the pharmaceutical composition according to the present invention can significantly inhibit I_{to} , the reduction of I_{to} may arise from the influence of phase 1 repolarization of action potential, particularly in species with higher I_{to} (such as dogs, rats and human etc.), to prolong APD. In addition, I_{to} is the major current responsible for the uneven trans-myocardial wall repolarization, the inhibition of which will reduce the uneven trans-myocardial wall repolarization, and limit the formation of trans-wall reentry micro-circle, to avoid arrhythmia such as torsades de pointes ventricular tachycardia etc. Currently, there has been no I_{to1} channel-targeted drug among the anti-arrhythmia drugs for clinical use.

[68] In the present study, we observed the effect of the drug on I_K current with guinea pig single cardiac muscle cell. The I_K current in guinea pig can be divided into 2 types: the rapidly-activated and slowly-activated one, we use the tail current recorded 5s after test pulse as a observation index, which corresponds to the slowly-activated part of I_K (I_{KS}). Actually, when heart rate increases, I_{KS} plays a major role in repolarization. We observed in the study

that when the drug can inhibit I_K with a concentration of 0.5%, leading to the prolonging of APD and thereby exerting the anti-arrhythmia effect during tachycardia.

[69] The inhibition of the drug on the inward part of I_{K1} current is also observed in present study, without affecting the reversal potential and rectifying property of the channel, but slightly increasing the outward part of the current. Although there is no significant difference between such increase before and after drug treatment, the increase of the outward part of I_{K1} can promote the repolarization of phase 3 action potential of cell, and it facilitates the cell membrane stabilization, eliminates the early afterdepolarization and inhibits the arrhythmia caused by trigger mechanism.

[70] Taken together, the 5% solution prepared by the capsule powder according to the present invention can inhibit inward rectifying potassium current and transient outward potassium current in a voltage-dependent manner, and delay the rectifying potassium current. Therefore, it can exert the anti-arrhythmia effects by decreasing the excitability of cardiac muscle cell, inhibiting the repolarization process of AP in cardiac muscle cell, and prolonging APD.

Claims

1. A pharmaceutical composition for regulating the potassium channel in cardiac muscle cell, comprising the following components by weight:

ginseng 45-180 parts, ophiopogonis 50-200 parts, corni 125-450 parts, miltiorrhizae 125-450 parts, fried semen ziziphi spinosae 95-400 parts, taxilli 95-400 parts, rubra 45-200 parts, steleophaga 35-150 parts.

2. The pharmaceutical composition for regulating the potassium channel in cardiac muscle cell according to claim 1, further comprising the following components by weight:

Rhizoma Nardostachyos 45-200 parts, Rhizoma Coptidis 25-90 parts, Fructus Schisandrae Sphenanthenae 35-150 parts, Os draconis 75-300 parts.

3. The pharmaceutical composition for regulating the potassium channel in cardiac muscle cell according to claim 2, comprising the following components by weight:

ginseng 45-90 parts, ophiopogonis 112-135 parts, corni 224-270 parts, miltiorrhizae 200-225 parts, fried semen ziziphi spinosae 150-186 parts, taxilli 150-186 parts, rubra 89-100 parts, steleophaga 35-100 parts, Rhizoma Nardostachyos 45-95 parts, Rhizoma Coptidis 45-60 parts, Fructus Schisandrae Sphenanthenae 67-75 parts, Os draconis 145-150 parts.

4. The pharmaceutical composition for regulating the potassium channel in cardiac muscle cell according to claim 2, comprising the following components by weight: ginseng 89 parts, ophiopogonis 112 parts, corni 224 parts, miltiorrhizae 224 parts, fried semen ziziphi spinosae 186 parts, taxilli 186 parts, rubra 89 parts, steleophaga 75 parts, Rhizoma Nardostachyos 89 parts, Rhizoma

Coptidis 45 parts, Fructus Schisandrae Sphenanthenae 67 parts, Os draconis 149 parts.

5. The pharmaceutical composition for regulating the potassium channel in cardiac muscle cell according to claim 2, comprising the following components by weight:

ginseng 45 parts, ophiopogonis 112 parts, corni 224 parts, miltiorrhizae 225 parts, fried semen ziziphi spinosae 186 parts, taxilli 186 parts, rubra 89 parts, Rhizoma Nardostachyos 45 parts, steleophaga 35 parts, Rhizoma Coptidis 45 parts, Fructus Schisandrae Sphenanthenae 67 parts, Os draconis 149 parts.

6. The pharmaceutical composition according to any of claims 1-5, wherein the dosage form of the pharmaceutical composition is capsule, tablet, medicinal granules, powder or oral solution formulation.

7. Use of the pharmaceutical composition according to claim 6 in the preparation of medicament for treating supraventricular arrhythmia, ventricular tachycardia, ventricular flutter, ventricular fibrillation, premature atrial contractions, atrial tachycardia, atrial flutter or atrial fibrillation.

8. A method for preparing the pharmaceutical composition according to claim 1, comprising the following steps:

a) adding proper amount of 70% ethanol to 45-180 parts (by weight) of ginseng, reflux extracting 3 times, pooling the extract solution, filtering, concentrating, drying, and grounding it into fine powder;

b) adding proper amount of 70% ethanol to 125-450 parts (by weight) of corni and 125-450 parts (by weight) of miltiorrhizae, reflux extracting 3 times, pooling the extract solution, filtering, concentrating it into concrete;

c) grounding 35-150 parts (by weight) of *steleophaga* into fine powder, drying the powder for further use.

d) adding proper amount of water to 50-200 parts (by weight) of *ophiopogonis*, 95-400 parts (by weight) of fried *semen ziziphi spinosae*, 95-400 parts (by weight) of *taxilli*, 45-200 parts (by weight) of *rubra*, boiling twice, pooling the extract solution, concentrating it into concrete;

e) pooling the concrete from step b) and d), adding the pharmaceutical fine power from step c) and the pharmaceutical fine power from step a) thereinto in turn, mixing and formulating it into the pharmaceutical composition.

9. The method for preparing the pharmaceutical composition according to claim 8, wherein the raw material of step b) further comprises 45-200 parts (by weight) of *Rhizoma Nardostachyos*, 35-150 parts (by weight) of *Fructus Schisandrae Sphenanthenae*, 25-90 parts (by weight) of *Rhizoma Coptidis*, and wherein the raw material of step d) further comprises 75-300 parts (by weight) of *Os draconis*.

10. The method for preparing the pharmaceutical composition according to claim 9, involving the following components by weight: ginseng 89 parts, *ophiopogonis* 112 parts, *corni* 224 parts, *miltiorrhizae* 224 parts, fried *semen ziziphi spinosae* 186 parts, *taxilli* 186 parts, *rubra* 89 parts, *steleophaga* 75 parts, *Rhizoma Nardostachyos* 89 parts, *Rhizoma Coptidis* 45 parts, *Fructus Schisandrae Sphenanthenae* 67 parts, *Os draconis* 149 parts.