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Laboratory Models for Cardiotonic Drugs Screening

A. Sai Datri*, A. Lakshmana Rao

Department of Pharmaceutical Analysis, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, AP, India

***Corresponding author:** A. Sai Datri
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Abstract | **Review Article**

The human heart is an organ that pumps blood throughout the body via the circulatory system, supplying oxygen and nutrients to the tissues and removing carbon dioxide and other wastes. Thus, to maintain a healthy heart is a crucial factor for overall health and well-being. But because of today's food habits and stress conditions can eventually lead to various heart ailments. These conditions can be cured with cardiotonic agents. Before introducing drugs into market, that drug has to check for its safety and efficacy. For studying the drug activity, both in vitro and in vivo screening models have been developed in the past years. These Systems measures the ability of the test drugs to prevent or cure heart problems in laboratory conditions and on experimental animals. This review reveals some of such animal model to check the activity of cardiotonic drugs.
Keywords: Heart, circulatory, ailments, cardiotonic agents.

INTRODUCTION

The heart (Fig. 1) is a muscular organ in humans, which pumps blood through the blood vessels of the circulatory system [1]. Blood provides the body with oxygen and nutrients, as well as assists in the removal of metabolic wastes [2]. In humans, the heart is located between the lungs, in the middle compartment of the chest [2]. The heart pumps blood with a rhythm determined by a group of pacemaking cells in the sinoatrial node. These generate a current that causes contraction of the heart, traveling through the atrioventricular node and along the conduction system of the heart. If any malfunction of this conducting system causes heart diseases.

Heart diseases [4-6] can be primarily grouped into three major disorders: cardiac failure, ischemia and cardiac arrhythmia. Cardiac failure can be described as the inability of the heart to pump blood effectively at a rate that meets the needs of the metabolizing tissues. This occurs when the muscles that perform contraction and force the blood out of heart are performing weakly. Thus cardiac failures primarily arise from the reduced contractility of heart muscles, especially the ventricles. Reduced contraction of heart leads to reduced heart output but new blood keeps coming in resulting in the increase in heart blood volume. The heart feels congested. Hence the term congestive heart failure. Congested heart leads to lowered blood pressure and poor renal blood flow. This results in the development of edema in the lower extremities and the lung (pulmonary edema) as well as renal failure.

For the treatment of these heart problems, cardiotonic drugs[7] are used. They can treat the heart problems by increase the strength of the muscle contractions, which facilitates the pumping of more blood from the heart.

Cardiac action potential – the electrophysiology of heart [2-9]

The cardiac action potential is a brief change in voltage (membrane potential) across the cell membrane of heart cells [1]. This is caused by the movement of charged atoms (called ions) between the inside and outside of the cell, through proteins called ion channels. The cardiac action potential differs from action potentials found in other types of electrically excitable cells, such as nerves. Action potentials also vary within the heart; this is due to the presence of different ion channels in different cells. The action potential (Fig. 2) in typical cardiomyocytes is composed of 5 phases (0-4), beginning and ending with phase 4.

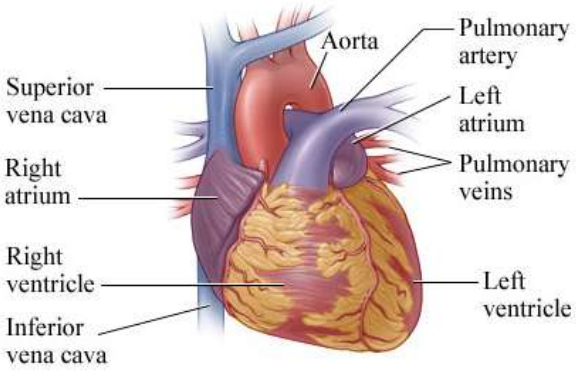

Fig-1: Human Heart

Phase 4: The resting phase

- The resting potential in a cardiomyocyte is −90 mV due to a constant outward leak of K^+ through *inward rectifier channels.*
- Na^+ and Ca^{2+} channels are closed at resting TMP.

Phase 0: Depolarization

- An action potential triggered in a neighbouring cardiomyocyte or pacemaker cell causes the TMP to rise above −90 mV.
- Fast Na^+ channels start to open one by one and Na^+ leaks into the cell, further raising the TMP.
- TMP approaches −70mV, the threshold potential in cardiomyocytes, i.e. the point at which enough fast Na^+ channels have opened to generate a self-sustaining inward Na^+ current.
- The large Na^+ current rapidly depolarizes the TMP to 0 mV and slightly *above* 0 mV for a transient period of time called the overshoot; fast Na^+ channels close (recall that fast Na^+ channels are *time-dependent*).
- L-type ("long-opening") Ca^{2+} channels open when the TMP is *greater than* −40 mV and cause a small but steady influx of Ca^{2+} down its concentration gradient.

Phase 1: Early repolarization

- TMP is now slightly positive.
- Some K^+ channels open briefly and an outward flow of K^+ returns the TMP to approximately 0 mV.

Phase 2: The plateau phase

- L-type Ca^{2+} channels are still open and there is a small, constant inward current of Ca^{2+}. This becomes significant in the *excitation-contraction coupling* process described below.
- K^+ leaks out down its concentration gradient through *delayed rectifier* K^+ channels.
- These two countercurrents are electrically balanced, and the TMP is maintained at a *plateau* just below 0 mV throughout phase 2.

Phase 3: Repolarization

- Ca^{2+} channels are gradually inactivated.
- Persistent outflow of K^+, now exceeding Ca^{2+} inflow, brings TMP back towards resting potential of −90 mV to prepare the cell for a new cycle of depolarization.
- Normal transmembrane ionic concentration gradients are restored by returning Na^+ and Ca^{2+} ions to the extracellular environment, and K^+ ions to the cell interior. The pumps involved include the sarcolemmal *Na^+-Ca^{2+} exchanger*, *Ca^{2+}-ATPase* and *Na^+-K^+-ATPase*.

Cardio-tonic herbs [10-13]

Cardio tonic herbs are used to support cardiac function. They have observable beneficial actions on the heart but do not contain cardiac glycosides found in our more dramatic acting plants. Although generally safe they can interact with some pharmaceutical drugs.

- Hawthorne (Craetagus spp.)
- Linden (Tilia spp.) - Buy Linden at Mountain Rose Herbs
- Arjuna (Terminalia arjuna)
- Motherwort (Leonurus cardiaca)

Phyto-products used to treat the congestive heart failure [14-16]

- *Digitalis lanata* (Digoxin)
- *Digitalis purpura* (Digitoxin)
- *Stropanthus gratus* (Stropanthin)
- *Stropanthus kombe* (Ouabian)

Methods for screening [17-33]

- Frog method
- Pigeon method
- Hatcher's cardio toxicity in cats
- Cardiac insufficiency induced in guinea pigs
- Cat papillary muscle method
- Loss of K^+ ion from isolated Guinea pig heart

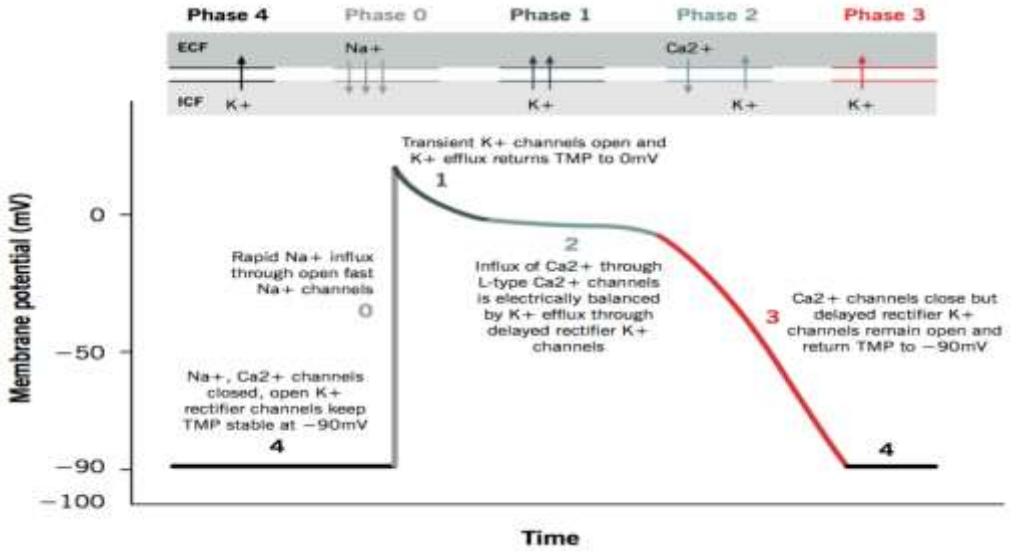

Fig-2: Action potential in cardiac muscle

Frog method

This test is a quantal assay (all or none). In this test, the test animals are divided into two groups, which are of similar weight (20-30g) and sex and used for standard and test preparations to minimize biological variation. Individually place the frogs into the wire cage of depth of 1cm. Digitalis preparation (standard) is injected into the dorsal ventricle lymph sac of the frogs at a dose of 1mg/kg through i.v. route for one group of animals and sample is injected for other group. A positive test is indicated if the frog dies within 1 hour in the old USP and within 3-12 hours in the old BP. The number of dead frogs is counted after opening the chest to confirm the heart arrest in systole (dilated hearts are not counted). The potencies of the test and standard preparations are compared.

Evaluation criteria

Systolic ventricle arrest and Wide dilated atrium

Pigeon method

It is an indirect method in which the effect of digitalis like compounds is estimated by its effect on vomiting center of pigeon. In this test, Pigeons of similar sex and weight (300-400g) are used. Inject the digitalis solution (standard) into the alar vein (wing vein at axillaries side), at a rate of 1 ml/kg at 5 min intervals for one group of animals and sample is injected for other group. After the injection, observe the pigeon for emesis. Compare the standard and test results.

Evaluation criteria: Emesis within 15min

Hatcher's cardio toxicity in cats

This test is a graded assay as it determines the volume causing death. In this test, Cats are anesthetized with ether and continue the anesthesia with chlorolone 70mg/kg. Lie down the cat on thermostatically controlled table and the limbs of cat is tied to corners of table. Tracheotomy is performed, for respiration artificial aeration is allowed by cannulation of trachea. The digitalis (standard) preparation is infused into the femoral vein at a rate of 1ml/min until the cat dies for one group of animals and sample is infused for other group. The progress of digitalis and sample effects are monitored, using a stethoscope, in the form of extrasystoles, increased heart rate, cardiac arrhythmia, and ventricular fibrillation until no palpations are heard. A positive test is indicated when the cat dies with heart arrest in systole within 30-55 min of the infusion. Measure the volume of the digitalis preparation injected. Compare the test and standard preparations.

Evaluation criteria: Ventricular fibrillation

Cardiac insufficiency induced in guinea pigs

Take male guinea pigs of weight 300 – 400g for this test. Shave the far at ventral thorax region. Disinfected the animal and then anaesthetize it. Open the thorax at 4^{th} rib of the intercostals muscle. Remove the pericardium carefully then extrude the heart from thorax with pressure. Apply the round clamp around the heart without blocking the circulation. Thread which is soaked in the disinfectant is allowed to make as loop and then that loop is tied to cover the $1/3^{rd}$ of ventricle. The knout is such that it is not too tight to block the circulation at the same time it is not too loose to slip from the ventricle. After the procedure remove the clamp and again the place the heart into the thoracic region and disinfect it and close all the external openings with the sutures. Animals shows the symptoms of cardiac arrest along with death occur in 80% within 14 days. Animals treated with cardiac glycosides in a period of 2 weeks shows less symptoms or dimensions symptoms of cardiac insufficiency.

Evaluation criteria

Edema of body parts, Increase in thoracic fluid and Hematological and histological studies are done.

Cat papillary muscle method

Take either sex of cat of weight 2 – 3 kgs for this test. Anaesthetize the cat with pentobarbitone (50mg/kg). Carefully open the thorax region of cat and isolate the strip of papillary muscle (Fig. 3). Mounted the strip to organ bath containing ringer solution and temperature is adjusted to 35 – 37^0C using the thermostat. One end of muscle is tied to electrical guage and other to tissue holder. Muscle is electrically stimulated to 4 – 6v per 2ms. Contractions are recorded after 1hr the contraction of the muscle is diminishes. At this stage, add cardiac glycosides. Record the contortions and calculate the increase in the contractions over the previous dose. Repeat the procedure with the test. Statistical compare the results of the test and standard.

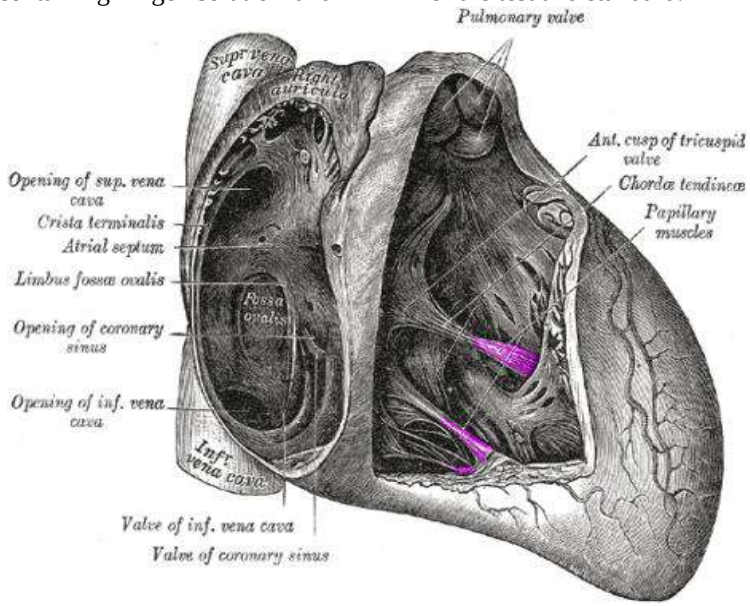

Fig-3: Inner view of cat heart

Loss of k^+ ion from isolated guinea pig heart [34-39]

In this test, Seven (7) guinea pigs of either sex, weighing between 600–800gm were injected with 1000 units of heparin in the ear vein to avoid irreparable damage by clots forming inside the heart before giving a sharp to the head. The throat was cut, the chest was opened and the heart was carefully removed. It was placed as quickly as possible in a dish containing Tyrode solution at room temperature. The preparation was gently squeezed several times in order to remove as much blood as possible. The aorta was located and dissected free and all other fascia tissue connected to the heart was trimmed away. To screen for the cardiotonic effect of *sample* Langendorff preparation was used. The aorta was cut just below the point where it divides and the heart was transferred to the perfusion apparatus containing tyrode solution, constantly oxygenated and maintained at 37°C, where the aorta was tied onto the glass cannular. Care was taken to ensure that air bubbles did not enter the aorta, and any bubbles that did were immediately removed. A funnel was placed beneath the suspended heart in order to allow for the collection of fluid flow from the heart to determine the flow rate with a graduate measuring cylinder and stopwatch. A fine nylon thread was attached to the ventricle by a hook and to the auricle by a small spring clip. The thread was connected to spring levers to record the heart contractions. The heart was allowed to stabilize for a period of about 20 minutes. Readings of the rate of beating and of the coronary flow were most conveniently taken over a period of 30 seconds. Drugs: digoxin Acetylcholine, Adrenaline and the extract were added to the preparation by injection through the rubber tubing into the perfusion fluid. Any noted heart block was reversed by the administration of 0.1µg atropine.

Evolution parameters

Cardiac muscle contraction, Coronary output, K^+ ion loss and Tone of cardiac muscle

CONCLUSION

Despite tremendous advances in modern medicine, Cardiotonic drug remains a worldwide health problem; thus the search for new medicines is still ongoing. Numerous formulations of medicinal plants are used to treat heart disorders in traditional medicine. The cardiotonic activity of the plants majorly due to the presence of alkaloids and glycosides. Active extracts, fractions or mixture of fractions/extracts of plants may prove very effective drugs. Plant drugs (combinations or individual drug) for cardiac diseases should possess sufficient efficacy to cure severe cardaic diseases caused by toxic chemicals and today's life style. Effective formulations have to be developed using indigenous medicinal plants, with proper pharmacological experiments and clinical trials.

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