國家科學委員會專題研究計劃報告

整合型總計劃名稱：心臟衰竭之研究與藥物探索群體計劃

本計劃名稱：

Carvedilol **與數種植物粹取成份對雜種狗慢性心臟衰竭之治療功效：頻脈性心臟衰竭模式之研究(第三年)**

Global Cardiac Therapeutic Effect of Carvedilol and Several Plant-Principle Pharmaceutics on Chronic Canine Congestive Heart Failure Induced by Rapid Ventricular Pacing (The Third Year)

NSC 89-2320-B-002-008-M48

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Introduction

Congestive heart failure represents the end stage of global cardiac performance and the collapse of all compensatory mechanisms, including rennin-angiotensin-aldosterone and sympathetic nervous activations (1, 2). The worsening of the cardiac failure is aggravated not only by the loss of functioning myocardium but also by the direct toxic adverse effects of catecholamines and angiotensin released from supposedly supporting compensatory mechanisms (3-4). Basing on the aforementioned concept, contemporary large-scale clinical trials have been conducted and confirmed long-term benefits of angiotensin-converting enzyme inhibitor and beta-blocker in the management of mild, moderate and even advanced congestive heart failure in humans (5-8). On the contrary, drugs simply augmenting the cardiac contractile performance, by catecholamine-like or inhibition of phosphodiesterase inhibitor (i.e. to increase intracellular C-AMP) or the release of calcium store etc, have unanimously proved to be harmful to the already compromised cardiac function and ironically deteriorated patients, survival (9, 10). Among the causes of death, the majority has been sudden arrhythmic events, particularly ventricular tachyarrhythmias (4). Therefore, the search of effective therapeutic agents for the treatment of congestive heart failure remains a task of high difficulty, needing a lot more efforts in multiple research fields.

Recently, several plant principles of aporphine alkaloid group have been isolated and proved to be effective in suppressing cardiac arrhythmias, as well as preserving or augmenting cardiac contractile performance. Two examples are thaliporphine (11) and liriodenine (12, 13). Thaliporphine is a phenolic aporphine alkaloid isolated from plants of several families such as Lauraceae. Thaliporphine can inhibit markedly the action potential upstroke and prolonging the action potential duration by blocking the I_{to}, I_K and

I_{Na}. Meanwhile, thaliporphine is a weak Ca^{++} channel agonist and may augment ventricular contractile performance. As to liriodenine, it's an aporphine derivative isolated from the plant *Fissistigma glaucescens*. Liriodenine is a known M3 muscarinic receptor acting mainly on smooth muscle cell, e.g. trachea. In addition, liriodenine can inhibit Na^{+} and I_{to} channels on cardiac myocytes, which result in antiarrhythmic effect and mild positive inotropism by prolongation of action potential duration. In the third year of the project, we tested the feasibility of thaliporphine and liriodenine in the control of chronic congestive heart failure induced by rapid ventricular pacing in dogs. The hypothesis is that the dual effects of thaliporphine and liriodenine, i.e. antiarrhythmic and positively inotropic, be helpful to the improvement of survival of canine congestive heart failure.

Materials and Methods

Canine model of congestive heart failure

Eighteen mongrel dogs of either sex were included in the study. The mean body weight was 12±1 kg (8 to 15 kg). All the dogs were pre-conditioned to exclude incidental infection of cardiac filarisis.

On the first day, all the dogs received transthoracic echocardiography to evaluate baseline cardiac chamber size, left ventricle contractile performance and to detect associated cardiac anomaly (if any). Then, under pentobarbital anesthesia, artificial pacemaker (Medtronic, VVI) was implanted and the generator located at the back of the neck. The pacing threshold was tested and pacing amplitude established at 5 times of diastolic threshold. The right ventricular overdrive pacing was constantly set at 240 bpm (as previously reported). The dogs were returned to the dormitory after the recovery from general anesthesia.

Serial echocardiography and functional status monitoring during thaliporphine treatment

All the dogs successfully paced by right ventricle pacemaker were fed with capsule-filled thaliporphine (5 mg), produced by the department of Pharmacy. The intake of the capsule was facilitated by mixing with dog food and checked by vetenarians. Every day, the intake, output and body weight of the dogs were recorded by vetenarians.

Serial ECG and echocardiographic examinations were performed in the animal dormitory-neighbored preparation room. The sizes of all 4 cardiac chambers, left ventricle contractile performance, pericardial effusion (if any) and the basic sinus rhythm ECG were recorded.

Final study of cardiac hemodynamics and tissue sampling

After pacing for a total of 4 weeks or the reach of marked left ventricular dysfunction, i.e. LVEF < 30%, the animals were brought to the animal laboratory. Under general anesthesia and endotracheal intubation for ventilation support, a Millar catheter with micromanometer tip was introduced into the left ventricle via retro-aortic approach to record the left ventricle systolic and end-diastolic pressure, and the derived dp/dt value in basic sinus rhythm. After the recording of cardiac hemodynamics, atrial extrastimuli and atrial overdrive pacing were conducted to provoke atrial arrhythmias (if any). At the end of electrophysiologic study, the animals were euthanized by high-dose barbiturate and the hearts were removed and sliced, before superfusing for in vitro studies of ion channels, contractility and molecular derangement.

Results

Survival of chronic heart failure dogs treated with thaliporphine

Out of the 18 dogs, 5 died suddenly within 1 week in the dormitory and 9 died of

rapidly progressive heart failure within 2-4 weeks. Only 4 dogs survived up to 4 weeks to receive final evaluation after 2 follow-up echocardiographic studies, which revealed steady increase of cardiac chamber dimensions and decrease of LV contractile performance. The poor survival rate was unexpectedly low (4/18, 22.2%) in the thaliporphine-treated dogs, much less than that in carvedilol-treated dogs in our previous study (66.7% of survival). Apparently, the addition of Na^+ channel and I_{to} blocking effects as well as mild inotropic effect by I_{Ca} enhancement in the pharmacological functions of thaliporphine didn't improve the survival of pacing-induced congestive heart failure. Instead, the antiadrenergic function including â- and á-blocking effects seemed more beneficial in reversing the cardiac deterioration.

Serial echocardiographic studies

For the 4 dogs surviving for a total of 4 weeks, serial echocardiographic studies showed the progressive increase of cardiac chamber size and deterioration of cardiac contractile performance: LV end-diastolic dimension increased from 39±7 mm to 49±5 mm ($p < 0.05$); LV end-systolic dimension increased from 24±5 mm to 41±4 mm ($p < 0.05$); and LV fractional shortening decreased from 38±5% to 16±5%, LV ejection fraction decreased from 62±2% to 26±2% ($p < 0.05$). All 4 hearts had moderate (3 dogs) to marked-grade (1 dog) pericardial effusion accumulation.

Cardiac hemodynamic study after 4 weeks of rapid pacing

During anesthesia and intubation, one dog couldn't even tolerate the procedure and succumbed to ventricular fibrillation, which's failed to be converted. The LV end-diastolic pressures for the remained 3 dogs were 20, 26, 28 mmHg, with LV systolic pressures of 100, 104, 104 mmHg respectively.

Atrial programmed stimulation by single to 3 extrastimuli coupling to atrial drive-CL

400 msec induced nonsustained atrial fibrillation in 2 dogs, and none in 1 dog.

Discussion

The third year of the project failed to demonstrate a beneficial effect of aporphine alkaloid, e.g. thaliporphine, in the improvement of cardiac contractile performance or the reduction of sudden death in the canine model of pacing-induced congestive heart failure. The hypothesis of combination of antiarrhythmic plus positive inotropic effects is rejected.

Ideal medicine for congestive heart failure

The failure of thaliporphine in the reversal of congestive heart failure deterioration is not totally a surprise. The combination of Na^+, I_{to}-blocking plus Ca^{++} channel agonist effects of thaliporphine may still not be enough to manipulate the complicated myocardial and/or electrophysiological milieu in heart failure. The Na^+ channel suppression could deteriorate the cardiac functional downhill more than the upheaving effect of weak Ca++-channel agonist. The prolongation of action potential duration by the blocking of I_{to} may likewise be overwhelmed by the combined contribution of pacing-induced myocyte dysfunction and Na^+-channel blocking effect of thaliporphine, not to mention the uneven distribution of I_{to} in the heart and subsequently the inhomogeneous repolarization after thaliporphine. The marked surge of sudden death in the dogs, early after rapid pacing, might well indicate the unexpected derangement of electrophysiologic environment, in addition to the superimposed myocardial suppression.

The apparent benefit of carvedilol over thaliporphine in regards of the evolution of congestive heart failure highlighted several pivotal mechanisms in the design of best anti-heart failure medicine(s). First, the negative influence of sympathetic nervous system, probably also the rennin-angiotensin-aldosterone, has to be removed or modulated (3, 4, 14); Second, the better antiarrhythmic approach should be the homogeneous prolongation

of action potential duration, without the sacrifice of Na^{+}-channel function; Third, whether or not the facilitation of Ca^{++} ion entry helps the failing myocardium remains obscure, and has to be proved by chronic animal models in the future.

Conclusion. Our pioneer study of applying aporphine alkaloid derivative, thaliporphine, in the management of chronic congestive heart failure in dog model has failed to demonstrate an acceptable result. The augmentation of cardiac inotropic function plus Na^{+}-channel-blocking or I_{to}-blocking effects (thought to be antiarrhythmic) is harmful to a failing heart. However, whether thaliporphine will be suitable for the control of atrial or ventricular tachyarrhythmias in structurally normal hearts is unknown.

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