行政院國家科學委員會專題研究計畫成果報告
Preparation of NSC Project Reports

† 八十六年度及以前的一般國科會專題計畫(不含產學合作研究計畫)亦可選擇適用，惟較特殊的計畫如國科會規劃案等，請先洽得國科會各學術處同意。

心電生 理特性、抗心律不整效力及不整脈誘發性之測定，包括carvedilol及thalidoparhine與liriodenine等新衍發物

Third year: Electrophysiological modulation by liriodenine

計畫編號：NSC 89-2314-B-002-253-M48
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一、中文摘要

1. 本實驗室工作群長期致力於由天然植物萃取有效之化合物。經評估後，目前有兩大類化合物，一為thalidiporphine，一為liriodenine，有收縮促進效果及抗心律不整效力。
2. 因此本群體計劃之目的在(1)將此兩大類化合物予以改變部份結構，使具有部份α與β拮抗作用(2)且能維持充份之心臟收縮促進效果及抗心律不整效力。
3. 本子計劃之主要工作在執行(1)第一年：先界定carvedilol是否有直接抗心律不整效力，或對心臟電生理特性有直接影響，若有，其機轉為何?(2)其次，第二年：將予界定thalidoporplinie各類衍生物之抗心律不整之效力及對心臟傳導系統之影響(3)第三年：將予界定liriodenine各類衍生物之抗心律不整之效力及對心臟傳導系統之影響。
4. 方法：(1)心臟電生理特性之評估：以Langendorff灌流之離體心臟作評估，以自製特別之電極導線放入心房、心室及希氏束處，記錄並刺激，以得到各項組織之電生理特性資料(2)抗心律不整之效力檢定：以Langendorff定壓灌流之模式，經綁住再放鬆左冠狀動脈支以誘發ischemia-reperfusion不整脈，比較liriodenine treatment與control之差異。
5. 本年度計劃結果顯示：較低濃度(0.3與1μM)之liriodenine對於心臟傳導系統並無顯著之影響，然而較高濃度之liriodenine則可延緩心臟之傳導，包括經過心房、心室結與希氏束之傳導。此外，心臟組織包括房室結、希氏束及心室組織的refractory period都會延長，這些變化在心室組織最為明顯。然而在10μM liriodenine的實驗有10次可誘發心室頻脈。

二、ABSTRACT

Background. We have identified the compound, liriodenine, to possess an antiarrhythmia potential and a positive inotropic effect.
Methods. The direct modification by various interventions on cardiac conduction system was performed by intracardiac recording and stimulation in isolated, Langendorff perfused hearts. The changes were measured in the hearts isolated from rats and rabbits.
Results. The changes of the electrophysiological parameters after liriodenine were determined in 10 rats. At low concentrations, 0.3 to 1 μM, the cardiac conduction and the refractoriness of the cardiac conduction system were not significantly altered by liriodenine. At higher concentrations, 3 and 10 μM, liriodenine could significantly prolong the conduction over the heart, including the intratrial conduction (SA interval), atrioventricular conduction (AH interval), conduction over the His-Purkinje system (HV interval). The refractory period of the atrioventricular nodal, His-Purkinje system and ventricular tissue were also significantly prolonged. But, the sinus cycle length and the atrial refractory period were not changed. However, ventricular tachyarrhythmias could be induced by ventricular extrastimulation at 3 μM (one rat) and 10 μM (4 rats) liriodenine infusion. In the ischemia-reperfusion model, liriodenine could convert the reperfusion ventrciualr arrhythmia with an EC_{50} of 0.3 μM.
Conclusions. After the delineation of the inhibitory effects of liriodenine on the ionic currents ($I_{Na,}$ $I_{Ca,}$ $I_{to,}$ and I_{K1}) in isolated rat ventricular myocytes, the electrophysiological study in Langendorff-perfused isolated rat hearts defined its electrophysiological modulation by drugs. Liriodenine at low concentrations could effectively convert the reperfuion arrhythmias, but at high concentration that 30 folds higher than the EC_{50} for conversion of the reperfusion arrhythmias might induce ventricular arrhythmia via extremely prolonged centrciualr refractoriness and QT intervals.

三、緣由與目的

Medical treatment for the heart failure usually include: digoxin, diuretics, angiotensin converting enzyme inhibitor and phosphodiesterase. In recent years, research has been directed toward the understanding of the pathological mechanisms involved in the progression of congestive heart failure. Much of this research has focused on the various neuroendocrine systems that are activated in this syndrome and the roles they play in the progression of congestive heart failure. The activation of neurohumoral pathways which lead to peripheral vasoconstriction and then enhances the afterload. Furthermore, the down-regulation of ß-receptor in the presence of chronic heart failure and the possible "catecholamine cardiotoxicity" prompted the rational of ß-blocker for chronic heart failure. Several recent multicenter double blind, placebo-controlled clinical trials have proved the clinical benefits of carvedilol, a "third generation" ß-blocking agent. Carvedilol, at therapeutic doses, blocks all three adrenergic receptors, with a rank order of potency of $\beta_1 > \alpha_1 > \beta_2$. Because of its α_1 blocking effect, carvedilol is a moderate vasodilator on acute administration and therefore has a good initial tolerability in patients with heart failure. Besides, carvedilol has been shown as a strong antioxidant. Clinical benefits have been well documented in patients with chronic heart failure from several multicenter clinical trials and hence carvedilol had been approved for the treatment of heart failure.

In our team, we have identified several novel compounds derived from natural alkaloids which possess adequate positive inotropic effects and antiarrhythmia efficacy. The positive inotropic effect is independent of adrenergic stimulation and is attributed to the relative degree inhibition of I_{to} and I_{Ca}. The

ionic current inhibition include mainly the I_{to}, I_{Na} and partly I_{Ca}. Via the inhibition, the electrophysiological properties of the cardiac conduction system are modified and consequently may terminate the arrhythmias. Therefore, such compound will be potential drugs for the treatment of arrhythmia and heart failure. However, based on the clinical experiences that the differential blocking effects on various sympathetic receptors, such as carvedilol, provide chronic benefits for heart failure. Our further aim for such drug development will be the modification of these compounds to contain the structure bone with various α and ß-receptor blockade and optimally antioxidation effects. Then, we may have the chance to obtain the ideal novel anticongestive drug; acutely and chronically positive inotropic and antiarrhythmic.
After the initial tests for the positive inotropic effects by muscle-strip experiment and neurotransmitters blocking assessment, the index compound, liriodenine, had been identified.

We have identified the compound, ***liriodenine***, to possess an antiarrhythmia potential and a positive inotropy. This third-year project sought to determine the direct electrophysiological modulation in isolated Langendorff perfused hearts.

四、方法與結果

Methods. The direct modification by various interventions on cardiac conduction system was performed by intracardiac recording and stimulation in isolated, Langendorff perfused hearts.

Results. The changes of the electrophysiological parameters after liriodenine were determined in 10 rats. At low concentrations, 0.3 to 1 μM, the cardiac conduction and the refractoriness of the cardiac conduction system were not significantly altered by liriodenine. At higher concentrations, 3 and 10 μM, liriodenine could significantly prolong the conduction over the heart, including the intratrial conduction (SA interval), atrioventricular conduction (AH interval), conduction over the His-Purkinje system (HV interval). The refractory period of the atrioventricular nodal, His-Purkinje system and ventricular tissue were also significantly prolonged. But, the sinus cycle length and the atrial refractory period were not changed. However, ventricular tachyarrhythmias could be induced by ventricular extrastimulation at 3 μM (one rat) and 10 μM (4 rats) liriodenine infusion. In the ischemia-reperfusion model, liriodenine could convert the reperfusion ventrciualr arrhythmia with an EC_{50} of 0.3 μM.

五. 討論

Liriodenine, an aporphine drivative isolated from the plant *Fissistigma glaucescens*, possessed selective M3 muscarinic receptor antagonistic activity in guinea-pigs and in canine tracheal smooth muscle cells. In isolated rat ventricular myocytes, liriodenine could inhibit the I_{Na} (IC_{50}= 0.7 μM), I_{Ca} (IC_{50}= 2.5 μM) and I_{to} (IC_{50}= 2.8 μM) and I_{K1} . As compared to quinidine, the degree of inhibition on I_{Na} by liriodenine was higher. In Langendorff perfused hearts, liriodenine can slowed down the cardiac conduction through the whole cardiac tissues. The refractoriness of cardiac tissue was increased and the changes were greatest were found in the ventricular tissue. Ventricular tachyarrhythmias may be induced at highest test concentration. The safety range needs to be defined in further studies.

REFERENCES

1. Cohn JN: Overview of pathophysiology of clinical heart failure. In: Congestive heart failure. Hosenpund JD, Greenberg BA (Eds). Springer-Verlag: Berlin 1994, 11-6.
2. Cohn JN, Rector JS: Prognosis of congestive heart failure and predictors of mortality . Am J Cardiol 1988;62:25A-30A.
3. Waggstein F, Caidahi K, Wallentin I, Bergh CH, Hjalmarson A: Long-term ß-blockade in dilated cardiomyopathy. Circulation 1989;80:551-563.
4. Rosenbaum JS, Ginsburg R, Billingham ME, Hoffman BB: Effects of adrenergic receptor antagonists on cardiac morphological and functional alterations in rats harboring pheochromocytoma. J Pharmacol Exp Ther 1987;241:354-360.
5. Ruffulo RR, Gelai M, Heible JP, Willette RN, Nichols AJ. The pharmacology of carvedilol. Eur J Clin Pharmacol 1990;38:S82-S88.
6. Yue TL, Cheng HY, Lysko PG, McKenna PJ, Feuerstein R, Gu JL, Lysko KA, Davis LL, Feuerstein G. Carvedilol, a new vasodilator and beta-adrenoceptor antagonist, is and antioxidant and free radical scavenger. J Pharmacol Exp Ther 1992;263:92-98.
7. Colucci WS, Packer M, Bristow MR, Gilbert EM, Cohn JN, Gowler MB, Krueger SK, Hershberger R, Uretsky BF, Bowers JA, Sackner- Bernstein JD, Young ST, Holcslaw TL, Lukas MA: Carvedilol ingibits clinical progression in patients with mild symptoms of heart failure. Circulation 1996;94:2800-2806.
8. Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilbert EM, Shusterman NH: The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. N Engl J Med 1996;334:1349-1355.
9. Bristow MR, Gilbert EM, Abraham WT, Adams KF, Fowler MB, Hershberger RE, Kubo SH, Narahara KA, Ingersoll H, Krueger S, Young S, Shusterman N: Carvedilol produces dose-related improvements in left ventricular function and survival in subjects with chronic heart failure. Circulation 1996;94:2807-2816.
10. Australia/New Zealand heart failure research collaborative group. Randomised, placebo-controlled trial of carvedilol in patients with congestive heart failure due to ischaemic heart disease. Lancet 1997;349:375-380.
11. Wu MH, Su MJ, Lee SS, Lin LT, Young LT: Electrophysiogical basis for antiarrhythmia efficacy, positive inotropy and low proarrhythmic potential of (-)-caryachine. Br J Pharmacol 1995;3211-3218.
12. Chang GJ, Wu MH, Wu YC, Su MJ: Electrophysiological mechanisms for antiarrhythmic efficacy and positive inotropy of liriodenine, a natural aporphine alkaloid from Fissistigma glauscens. Br J Pharmacol 1996;118:1571-1583
13. Wu MH, Su MJ, Lee SS, Young LT: The electrophysiogical effects and antiarrhythmic potential of a secoaporphine-N-allylsecoboldine. Br J Pharmacol 1994;113:221-227.
14. Wu MH, Su MJ, Lue HC: Age-related quinidine effects on ionic currents of rabbit cardiac myocytes. J Mol Cell Cardiol 1994;26:1167-1177.