

## Frequency dependent prolongation of effective refractory period by a complex class III antiarrhythmic agent CPU-86017<sup>1</sup>

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**KEY WORDS** CPU-86017; myocardium; lorcaïnide; dofetilide; verapamil; amiodarone; antiarrhythmic agents; papillary muscles

### ABSTRACT

**AIM:** To compare the prolongation of the effective refractory period (ERP) of a novel complex Class III agent CPU-86017 with compounds blocking  $I_{Na}$  (lorcaïnide, Lor),  $I_K$  (dofetilide, Dof),  $I_{Ca}$  (verapamil, Ver) and a complex Class III multiple channel blocking agent (amiodarone, Ami) at different frequency levels. **METHODS:** The ERP of the guinea pig right papillary muscle and the anoxic left atrial muscle treated with high  $K^+$  at pH 6.6-6.8, in concentrations ranging 0.03-30  $\mu\text{mol/L}$  was compared at frequency levels ranging from 0.5-4.0 Hz. **RESULTS:** CPU-86017 showed a positive frequency dependence (PFD) with respect to ERP in the papillary muscles and a mild reverse frequency dependence (RFD) in the diseased atrium. The potency of ERP prolonging effect of various agents at 4.0 Hz was Dof > CPU-86017 > Ver > Ami > Lor, and Dof > CPU-86017 > Ami > Lor > Ver in the normal papillary and diseased atrial muscle, respectively. **CONCLUSION:** The profile and potency of prolonging the ERP by CPU 86017 is similar to Dof which blocks  $I_{Kr}$  in the diseased atrium, and to Ver which blocks  $I_{Ca}$  in guinea pig ventricle.

### INTRODUCTION

The development of novel antiarrhythmic agents for treating ventricular dysarrhythmia of diseased heart has not been successful as is evident by the CAST (Cardiac Arrhythmias Suppressing Trial) and SWORD (Survival

With Oral *d*-Sotalol Trial)<sup>(1-3)</sup>. Recent awareness about involvement of multiple channel disorders in diseased heart may lead to the development of novel antiarrhythmic agent based on these blocking action on one or more ion channels<sup>(5,6)</sup>. CPU 86017, a novel class III agent developed by China Pharmaceutical University, has been found to effectively suppress various arrhythmias in animal model<sup>(7)</sup> by exerting a blocking action on multiple ion channels<sup>(8)</sup>. The compound has been classified as a complex class III agent as its blocking effect is not limited only to  $I_{Kr}$  but also extends to  $I_{Ks}$ ,  $I_{Na}$ , and  $I_{Ca}$ . Effectiveness of an antiarrhythmic action can be corrected with a prolongation in the effective/functional refractory period (ERP/FRP), which can be further classified into responses at the high frequency or low frequency. Dofetilide, a novel pure Class III,  $I_{Kr}$  blocking agent, shows a reduced effect on the ERP at a high frequency dependence which is known as a reverse frequency dependence (RFD)<sup>(9)</sup>. It was, therefore, interesting to investigate the profile of prolongation of ERP of CPU-86017 and to see whether it is related to the blockage of either of  $I_{Na}$ ,  $I_{Kr}$ ,  $I_{Ca}$ , or to a complex blockade of all three. In the present study, we explored the ERP prolonging effect of CPU-86017 determined both at the low and high frequency with lorcaïnide (Class I<sub>c</sub> agent,  $I_{Na}$  blocker), dofetilide (pure Class III,  $I_{Kr}$  blocker), verapamil (Class IV agent,  $I_{Ca}$  blocker), and amiodarone (complex Class III agent,  $I_K$ ,  $I_{Na}$ , and  $I_{Ca}$  blocker).

### MATERIALS AND METHODS

**Animals** Guinea pigs (368 ± 36) g, Grade II, either sex were obtained from the Animal House of the University (Certificate No 98004).

**Drugs** CPU-86017 and dofetilide (Dof, UK 68798) synthesized and obtained from the Center of Research and Development of New Drugs, China Pharmaceutical University, amiodarone (Ami) from Shanghai Hongqi Pharmaceutical Factory, and verapamil (Ver)

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from Guangdong Liming Pharmaceutical Factory, and lorcaïnide (Lor) from Department of Pharmaceutical Chemistry in our University were used. Other reagents were of AR grade.

**ERP assay** Guinea pigs were stunned. The left atrial and right papillary muscles were separated and equilibrated for 45 min in oxygenated Ringer-Locke's solution (RLS) in mmol/L: NaCl 154, KCl 5.6, NaHCO<sub>3</sub> 6.5, CaCl<sub>2</sub> 1.6, glucose 2.3, pH 7.4 at (33.0 ± 0.5) °C and thereafter the RLS of the left atrial muscle was replaced by anoxic, high K<sup>+</sup> and acidic Ringer's solution in mmol/L: NaCl 153, KCl 8.45, CaCl<sub>2</sub> 3.06, NaHCO<sub>3</sub> 1.19, glucose 0.3, pH 6.6–6.8, aerated with pure nitrogen. Contractile responses were recorded for a series of 8 stimuli at frequencies of 0.5, 1.0, 2.0, and 4.0 Hz at 150 % threshold voltage and a stabilizing time of 30 min was given between each stimulus. The interval of the eighth stimulus was lessened at 10 ms interval until loss of response was observed. Concentrations were used as: 0.3–30 μmol/L for CPU-86017, Lor, Ver, and Ami, and 0.03–3 μmol/L for Dof.

**Positive frequency dependence (PFD) and reverse frequency dependence (RFD)** The ERP determined at the lowest frequency (ERP<sub>L</sub>) and the highest frequency (ERP<sub>H</sub>) were compared. The PFD was defined as the FRP<sub>H</sub> > FRP<sub>L</sub>, and the RFD was defined as ERP<sub>L</sub> > ERP<sub>H</sub>. The PFD and RFD were assessed in both the normoxic and anoxic condition at different concentrations. No response to high frequency at higher concentrations due to the negative inotropism caused by I<sub>Na</sub> (lorcaïnide) or I<sub>Ca</sub> (verapamil) blockade was recognized as the RFD.

**Statistical analysis** The results were expressed as  $x \pm s$  and the *t* test was applied.

## RESULTS

**Effect of CPU-86017 on ERP in normal guinea pig ventricular muscles** The ERP of the guinea pig right papillary muscles responded at frequencies at 0.5, 1.0, 2.0, and 4 Hz before medication were (213 ± 24) ms, (203 ± 13) ms, (175 ± 15) ms, and (148 ± 16) ms, respectively. Prolongation of ERP after treated with the five compounds was presented as % of the control in Fig 1A. CPU-86017 showed a PFD at stimulating frequencies of 0.5–4.0 Hz as a prolongation in ERP was observed in right papillary muscles. The increment in ERP at 30 μmol/L was 18.8 % ± 0.9 % and

30 % ± 5 % (*P* < 0.01) at 0.5 Hz and 4.0 Hz, respectively. A loss of response to 2.0 Hz and 4.0 Hz with Lor 30 μmol/L was caused by a potent negative inotropic effect due to its marked I<sub>Na</sub> blocking action. The maximal prolongation of ERP by Lor (I<sub>Na</sub> blocker), Ami at 30 μmol/L (complex blocker on I<sub>Na</sub>, I<sub>Kr</sub>, and I<sub>Ca</sub>), Dof at 3 μmol/L (I<sub>Kr</sub> blocker), and Ver at 30 μmol/L (I<sub>Ca</sub> blocker) at 0.5 and 4.0 Hz was 22 % ± 2 % (30 μmol/L) and 35 % ± 3 % (10 μmol/L), 10.4 % ± 0.9 % and 9.0 % ± 0.9 %, 54 % ± 9 % and 34 % ± 5 %, and 16.5 % ± 1.4 % and 28 % ± 4 %, respectively (Fig 1A). RFD induced by Dof was noticed as compared to the other four compounds. The ability of Ami to prolong the ERP was mild. The order of the ERP prolongation in ventricular muscles (at 30 μmol/L, except Dof 3 μmol/L) at low frequency (0.5 Hz) as Dof > Lor > CPU-86017 > Ver > Ami, and changed at high frequency (4.0 Hz) as: Dof > CPU-86017 > Ver > Ami > Lor (Fig 1A).

**Effect of CPU-86017 on ERP in diseased atrium** The control ERP of normal guinea pig left atrial muscles in response to frequencies at 0.5, 1.0, 2.0, and 4.0 Hz were (95 ± 14) ms, (90 ± 22) ms, (82 ± 26) ms, and (80 ± 20) ms, respectively. After being treated at anoxic, high K<sup>+</sup>, and acidic medium the ERP of the diseased atrium were prolonged at frequencies as the follows: (111 ± 20) ms, (103 ± 20) ms, (101 ± 20) ms, and (100 ± 22) ms, respectively. The relative values (%) of ERP prolonging effect by the five compounds were compared with the diseased one, and plotted in Fig 1B. There was a RFD of prolongation of ERP in guinea pig left atrial muscle in response to the five compounds in the anoxic, acidic, and high K<sup>+</sup> condition. Ver 30 μmol/L caused a loss of contraction due to its negative inotropic effect. The maximal effect of CPU-86017 on ERP at 0.5 Hz and 4.0 Hz was 55 % ± 3 % to 42 % ± 6 %. The maximal prolongation of ERP at 0.5 Hz and 4.0 Hz by I<sub>Na</sub> blocker (Lor) 10 μmol/L, I<sub>Kr</sub> blocker (Dof) 3 μmol/L, complex blocker (Ami) 30 μmol/L, and I<sub>Ca</sub> blocker (Ver) were 30.0 % ± 2.8 % and 11.30 % ± 0.27 %, 58 % ± 10 % and 44 % ± 7 %, 39 % ± 6 % and 26.1 % ± 1.7 %, and 50 % ± 7 % (30 μmol/L) and 42 % ± 4 % (10 μmol/L), respectively. The order of the ERP prolongation (at 30 μmol/L, except Dof at 3 μmol/L) in the diseased atrium at 0.5 Hz was Dof > CPU-86017 > Ver > Ami > Lor, and at 4.0 Hz: Dof > CPU-86017 > Ami > Lor > Ver (Fig 1B).

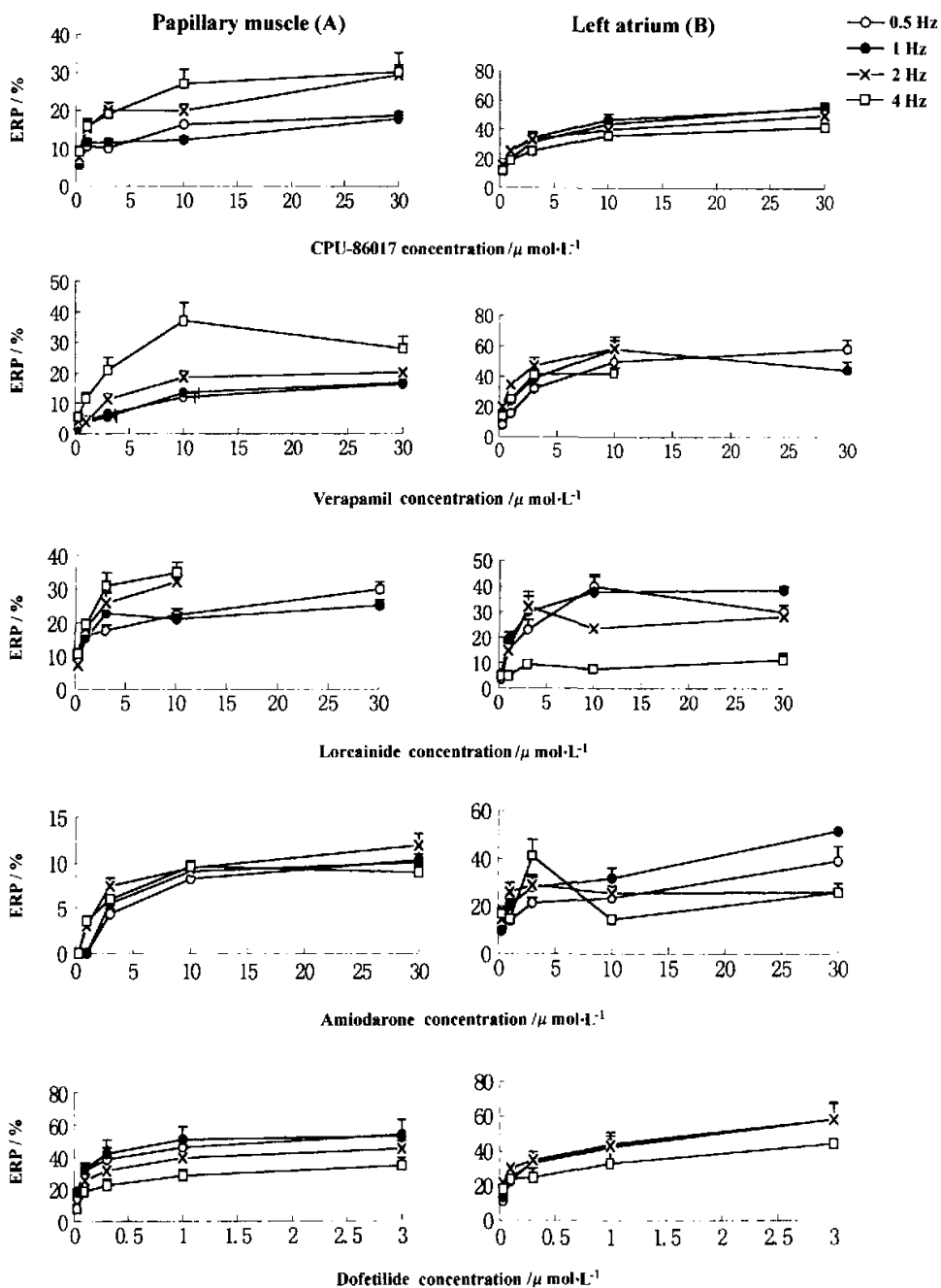


Fig 1. Effective refractory period (ERP) of isolated guinea pig right papillary muscle and diseased atrium stimulated at 0.5, 1.0, 2.0, and 4.0 Hz in the presence of CPU-86017, lorcaïnide, dofetilide, verapamil, and amiodarone, respectively.  $n=6-8$ .  $\bar{x} \pm s$ .

## DISCUSSION

The measurement of ERP by recording of the contractions is dependent on not only the action potential duration (APD), but also contractility. So it is more relevant regarding clinical evaluation of antiarrhythmic agents. The ERP, like APD, is also affected by multiple ion currents involved in the repolarization process<sup>[10]</sup>. Dof suppressing the  $I_{Kr}$  causes a prolongation of ERP, but shows characteristic RFD feature<sup>[9,11]</sup>. The effect by CPU-86017 on ERP is possibly induced by either a balanced effect on the three ion channels, or mainly due to a single channel in the ventricle or atrium. In ventricular muscle the pattern of ERP prolongation by CPU 86107 was similar to that of Ver. In the diseased atrial muscle, however, the profile of prolongation of ERP by CPU-86017 was more prominent and similar to Dof which is reported as the most effective agent to treat atrial fibrillation/flutter (AF/AFI)<sup>[12]</sup>. Therefore, CPU-86017 seems to be a promising drug for suppressing AF/AFI in clinical settings. ERP prolonging effect of Ami was found to be mild in the present study, and this is in agreement with a report by Gill, *et al*<sup>[13]</sup>.

A negative inotropic effect was observed with Lor and Ver resulting in a loss of contractile response in the ventricular papillary or atrial muscles at 4.0 Hz, respectively. This negative inotropic property of an  $I_{Ca}$  agent exerts a toxic effect on the compromised cardiac function and thus is not suitable for treating ventricular tachycardia<sup>[3,12]</sup>. CPU-86017, blocker of  $I_{Na}$ ,  $I_{Kr}$ , and  $I_{Ca}$ , resulted in a much less negative inotropic effect, and favorably reduced *torsades de pointes*<sup>[14]</sup>, and resembled azimilide<sup>[15]</sup> in its control of tachyarrhythmia.

In conclusion, the ERP of guinea pig papillary muscles was prolonged by CPU 86017 in a manner similar to Ver, however, in the atrial muscles it resembled Dof, thus implying that blocking of  $I_{Ca}$  and  $I_{Kr}$  played a relatively important role in the ventricle and atrium, respectively.

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## 复合型Ⅲ类抗心律失常药 CPU-86017 延长有效不应期的频率依赖性<sup>1</sup>

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**关键词** CPU-86017; 心肌; 劳卡尼; 多非利特; 维拉帕米; 胺碘酮; 抗心律失常药; 乳头状肌

**目的:** 比较复合型Ⅲ类抗心律失常药 CPU-86017 与劳卡尼(Lor)、多非利特(Dof)、维拉帕米(Ver)及复合型Ⅲ类抗心律失常药胺碘酮(Ami)延长心肌有效

不应期(ERP)的频率依赖性。方法: 梯度浓度(0.03-30  $\mu\text{mol/L}$ )对豚鼠右乳头状肌和在缺氧、高钾、酸中毒状态下左房肌在 0.5-4.0 Hz 时延长 ERP 的比较。结果: CPU-86017 延长右乳头状肌 ERP 呈正性频率依赖性, 对病变左房肌有弱负性频率依赖性。高频(4.0 Hz)时对正常右乳头状肌及病变左房肌延长 ERP 的强度: 分别为 Dof > CPU-86017 > Ver > Ami > Lor, 及 Dof > CPU-86017 > Ami > Lor > Ver。结论: CPU-86017 延长病变左房肌 ERP 的强度及类型与 Dof 相似, 但对心室肌的作用与 Ver 相似。

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