Plasma CPU-86017 concentrations regarding suppression of ouabain-induced cardiac arrhythmias and decrease of heart rate in guinea pigs

Khan Hussien Hamed, Dai De-Zai, Xiao Da-Wei, Lin Sheng, Wang Zi-Zheng, Lou Sheng, Qing Qing

(Research Division of Pharmacology, China Pharmaceutical University, Nanjing 210009, China; The First Municipal Hospital of Nanjing, Nanjing 210006, China)

KEY WORDS CPU-86017; high pressure liquid chromatography; ouabain; amiodarone; thromboxane $A_2$; prostaglandins F; arrhythmia; heart rate

ABSTRACT

AIM: To determine the effective plasma levels of CPU-86017 which could suppress the cardiac arrhythmias induced by iv ouabain in guinea pigs. METHODS: The cardiac arrhythmias and the heart rate were monitored by ECG traces. Blood samples were collected to determine plasma levels using HPLC assay. TXB$_2$ and 6-keto-PGF$_{10}$ were measured in plasma. RESULTS: The plasma concentrations of CPU-86017 which were effective to suppress ventricular fibrillation (VF) and heart rate were 0.13 - 0.23 mg/L and 0.13 - 0.31 mg/L, respectively. A reduction of TXB$_2$ levels and an elevation of 6-keto-PGF$_{10}$ levels were observed after CPU-86017 iv administration. CONCLUSION: The arrhythmia-suppressing and heart rate-slowing effect of CPU-86017 followed a linear relationship with its concentrations in plasma.

INTRODUCTION

Malignant arrhythmias contribute predominantly to the occurrence of sudden cardiac death. The development of new antiarrhythmic agents has not yet been successful$^{[1,2]}$, with the failure of the class III drugs in CAST (Cardiac Arrhythmias Suppressing Trial)$^{[3]}$ (1989)

and the pure class III drugs in SWORD (Survival With Oral d-Sotalol) (1994)$^{[4,5]}$. Following lead compound from a medicinal plant origin, a novel compound, CPU 86017 was developed, which possessed marked antiarrhythmic effects in various animal models$^{(6)}$ and exerted a suppressive effect on multiple ion channels$^{(7)}$. Prior attempts have been made to determine the effective plasma levels of this compound in animals$^{(8)}$. In this paper we intended to measure the plasma levels of the compound in relation to its suppression of cardiac arrhythmias and heart rate in guinea pigs.

[Chemical structure diagrams are shown here.]

MATERIALS AND METHODS

Animals Guinea pigs, 40 either sex, (weighing 250 - 300 g, Grade I), were obtained from the Animal Experimental Center of the University (Certificate No 97004).

Chemicals CPU-86017, p-chloro-benzyl-tetrahydro-berberine chloride, white crystal powder, purity > 99 % and mp 206 - 209 °C, was kindly offered by the Center of New Drug Research and Development of China Pharmaceutical University. Urethane was obtained from...
RESULTS

HPLC assay of CPU-86017  The resolution time of CPU-86017 and internal standard (THB) was 10.3 and 13.1 min, respectively. The precision of intra- and inter-day assay with 4 doses of CPU-86017 was 2.0 % - 10.3 % and 1.6 % - 10.7 %, respectively (Tab. 1). The recovery rate of the 4 doses ranged from 74.5 % - 95.9 % (Tab 2). The calibration curve of CPU-86017 was set up ranging from 0.05 - 1.0 mg/L. (Fig 1).

CPU-86017 plasma levels and arrhythmia
The time of occurrence of PB, VT, and VF was recorded during the ouabain infusion in anesthetized guinea pigs (Tab 3), with amiodarone as the positive reference drug. The arrhythmic action of CPU-86017 was evidenced by a significant prolongation in the time of ouabain-induced PB, VT, and VF. CPU-86017 delayed the time of occurrence of ouabain-induced VF by 20 %, 140 %, and 130 %, at the doses of 0.5, 1.0, and 2.0 mg/kg. The blood samples were drawn at the appearance of the VF.

<table>
<thead>
<tr>
<th>Dose /mg·L⁻¹</th>
<th>Intra-day /mg·L⁻¹</th>
<th>CV/%</th>
<th>Inter-day /mg·L⁻¹</th>
<th>CV/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.098 ± 0.004</td>
<td>3.6</td>
<td>0.004 ± 0.009</td>
<td>10.7</td>
</tr>
<tr>
<td>0.4</td>
<td>0.36 ± 0.04</td>
<td>10.3</td>
<td>0.41 ± 0.04</td>
<td>9.8</td>
</tr>
<tr>
<td>1.0</td>
<td>0.86 ± 0.09</td>
<td>10.0</td>
<td>0.91 ± 0.09</td>
<td>10.2</td>
</tr>
<tr>
<td>2.0</td>
<td>1.91 ± 0.04</td>
<td>2.0</td>
<td>1.91 ± 0.03</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Tab. 2. Recovery of CPU-86017 from plasma of guinea pig.  n = 5.  x ± s.

<table>
<thead>
<tr>
<th>Dose/mg·L⁻¹</th>
<th>Recovered/mg·L⁻¹</th>
<th>Average/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.067 ± 0.009</td>
<td>86.8</td>
</tr>
<tr>
<td>0.2</td>
<td>0.15 ± 0.03</td>
<td>74.5</td>
</tr>
<tr>
<td>1.0</td>
<td>0.87 ± 0.06</td>
<td>86.8</td>
</tr>
<tr>
<td>2.0</td>
<td>1.92 ± 0.03</td>
<td>95.9</td>
</tr>
</tbody>
</table>

CPU-86017 plasma levels and the heart rate
The R-R interval measured was plotted against the plasma concentrations of CPU-86017 (Fig 2). A marked reduction in heart rate was observed after the iv administration of CPU-86017 in a concentration-dependent manner. The HR-slowing effect was caused by plasma levels of...
Fig 1. Calibration curve of CPU-86017, ranging from 0.05 - 1.0 mg/L. A₁ = Area of sample CPU-86017, A₂ = Area of tetra-hydro-berberine (THB), an internal standard.

Tab 3. Effect of CPU-86017 on ventricular arrhythmias induced by ouabain and plasma concentrations were determined on the occurrence of VF in guinea pigs. n = 8 guinea pigs. x ± s. *P > 0.05, **P < 0.05, ***P < 0.01 vs control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses /mg·kg⁻¹</th>
<th>TXB₂ /pg·kg⁻¹</th>
<th>6-Keto-PGF₁₀ /pg·kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12 ± 3</td>
<td>1.13 ± 0.05</td>
<td>1.20 ± 0.03</td>
</tr>
<tr>
<td>CPU-86017</td>
<td>0.5</td>
<td>1.58 ± 0.40</td>
<td>0.52 ± 0.22</td>
</tr>
<tr>
<td>1</td>
<td>1.36 ± 0.04</td>
<td>1.86 ± 0.35</td>
<td>3.34 ± 0.32</td>
</tr>
<tr>
<td>2</td>
<td>1.10 ± 0.17</td>
<td>1.27 ± 0.37</td>
<td>3.14 ± 0.32</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>11 ± 3</td>
<td>1.15 ± 0.06</td>
<td>1.27 ± 0.37</td>
</tr>
</tbody>
</table>

CPU-86017 within the 0.13 - 0.31 mg/L range and a linear relationship existed between the plasma levels and slowing effect of HR.

Fig 2. Negative linear relationship of heart rate with plasma levels of CPU-86017 ranging from 0.13 - 0.31 mg/L in guinea pigs.

Tab 4. Influence of CPU-86017 on TXB₂ and 6-keto-PGF₁₀ in guinea pig plasma. n = 6 guinea pigs. x ± s. *P > 0.05, **P < 0.01 vs control.

DISCUSSION

The precision of HPLC assay of CPU-86017 has been found to be accurate enough to measure its plasma levels. Earlier in our laboratory the assay for berberine was established⁹-¹⁰ and its plasma levels have been assayed by HPLC for many years. The new antiarrhythmic drugs require an accurate measurement of effective plasma levels and this study is the first to measure effective plasma concentrations of CPU-86017 with regards to its ability to suppress arrhythmias and slow down the heart rate¹¹-¹². A high concentration of CPU-86017 caused a prolongation in the time taken by the arrhythmias to develop after an iv infusion of ouabain. The effects of CPU-86017 were observed to be concentration-dependent. The suppression of the ouabain-induced VF correlated well to the plasma CPU-86017 concentrations. The ouabain-mediated arrhythmias is due to its toxic effect on...
the myocardium leading to an insult of cardiac myocytes as evidenced by increased TXβ levels and decreased 6-keto-PGF₁α levels. The antiarrhythmic action of CPU-86017 is accompanied with its protective effect on myocardium, a phenomenon observed similarly with amiodarone. This cardiac protective effect has been attributed to its (whose) multiple ion channel blocking action, particularly to its calcium channel blocking effect[13]. The slowing down of the heart rate after iv administration of CPU-86017 also correlates with its I_ca blocking action. The above effect was concentration-dependent within the range of 0.13 – 0.31 mg/L. Considering that the plasma levels of most antiarrhythmic agents lie within a range of 1 – 5 mg/L[14], CPU-86017 with its effective plasma levels well below 1 mg/L can be considered as a potent antiarrhythmic agent.

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REFERENCES
2. Dai DZ. The type and characteristics of ion channel disorder inducing cardiac arrhythmia and development of the therapeutic agents. Adv Pharm Sci 1998; 14; 181 – 4.

CPU-86017 血浆浓度与抑制哇巴因诱发豚鼠
心律失常和心率减慢的关系

关键词 CPU-86017；高血压、色胞体；哇巴因；胺碘酮；血栓素 A2；前列腺素 F 类；心律失常；心率

目的：测定 CPU-86017 抑制哇巴因诱发的豚鼠心律失常和心率减慢的有效血浆浓度。方法：CPU-86017 体外抑制哇巴因诱发心律失常，心率由 ECG 监护。用 HPLC 法测 CPU-86017 有效血浆浓度，用宏免法测血浆中 TXB2 及 6-酮-PGF1α。结果：CPU-86017 抑制哇巴因诱发的心律失常及心率减慢的血浆药浓度分别为 0.13 — 0.23 mg/L 及 0.13 — 0.31 mg/L。CPU-86017 体外抑制心律失常及降低心率作用与血浆浓度呈相关性。

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