EFFECTS OF AMILORIDE ON OUABAIN INDUCED ARRHYTHMIAS IN VIVO IN GUINEA PIGS

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SUMMARY

Objectives: To examine the effects of amiloride on ouabain induced arrhythmias in anaesthetised adult, healthy, guinea-pigs.

Methods: In acute studies, amiloride (10 mg/kg) was given intravenously before the onset of constant ouabain (8 µg/100 µl/min) infusion, to induce ventricular premature beats (VPB), ventricular tachycardia and/or fibrillation with sudden fall in blood pressure (VT/F) and cardiac arrest (CA). Propranolol (2mg/kg, i.v.) was also studied against ouabain induced arrhythmia as a positive control. Changes in blood pressure (BP) and heart rate (HR) were monitored simultaneously in anaesthetised animals. In chronic studies, amiloride (10 mg/kg) was given intraperitoneally for three days followed by intravenously on fourth day, before ouabain infusion, as described above.

Results: In acute as well as in chronic studies, no changes were observed in the quantity of ouabain required to produce arrhythmias after amiloride pretreatment. On the contrary, propranolol increased ouabain requirement very significantly. There were significant changes in BP and HR after amiloride administration in acute studies. In chronic studies also, similar changes were observed in BP and HR after intravenous administration of amiloride.

Conclusion: Amiloride in the dosage studied in vivo did not protect the animal from ouabain induced arrhythmias.

KEY WORDS
Amiloride ouabain propranolol ventricular arrhythmia

INTRODUCTION

Cardiac glycosides are the most frequently used class of drugs in the treatment of cardiac failure and certain arrhythmias. They have regained their status in congestive cardiac failure after controversies surrounding their role for sometime in the past and this reappraisal has brought back the component of their cardiac toxicity in the clinical forefront once again. Digitalis intoxication is thought to be the most prevalent adverse effect encountered in clinical practice that often presents with cardiac arrhythmias as the most serious and potentially fatal complication.

Cardiac glycosides produce their therapeutic and toxic cardiac effects by their direct as well as indirect effects. The pharmacological profile of direct toxicity is well substantiated whereas indirect toxicity involves participation of autonomic nervous system. The clinical use of beta-adrenoceptor blockers is well established in digitalis induced tachyarrhythmias and is very well supported by animal studies also.

The cellular mechanisms for direct effects of cardiac glycosides are not yet fully resolved. Inhibition of sarcolemmal Na+, K+- ATPase (sodium pump) is involved in toxicity and probably to a large extent in therapeutic efficacy. Sodium pump inhibition by cardiac glycosides leads ultimately to increased intracellular Ca²⁺ concentration through Na⁺/Ca²⁺ exchange and associated increase in slow inward Ca²⁺ current as well as transient Ca²⁺ current. Increased activity of Na⁺/H⁺ exchange is implicated in amplifying the intracellular Ca²⁺ concentration as a result of digitalis induced increased inotropy with consequent fall in intracellular pH. Na⁺/H⁺ exchange also works as a major pathway of Na⁺ entry and subsequent intracellular Ca²⁺ overload in myocardial tissue, especially in ischaemia/reperfusion dysfunction.

Amiloride, a pyrazine antikaliuretic agent, is commonly used as part of digitalis-diuretic therapy of cardiac failure with possible clinical interactions between them. Conflicting information is available on the effects of amiloride and its derivatives on cardiac
performance as such. Multiple transmembrane blocking actions, with different \textit{in vitro} dose requirements, have been described for amiloride. As a corollary, amiloride can inhibit cardiac cell membrane ionic processes, viz, Na$^+/H^+$ exchange, Na$^+/Ca^{2+}$ exchange, L-type Ca$^{2+}$ channels, Na$^+$ channels and K$^+$ channels. The above diverse actions of amiloride can modify the cardiac glycoside, ouabain-induced arrhythmias, as circumstantial evidence does support the theory that amiloride counteracts the di-goxin effects.

As such few \textit{in vivo} and \textit{in vitro} studies in the past were reported on amiloride-digitalis interactions with controversial results. The \textit{in vivo} studies addressed directly to the role of amiloride in digitalis toxicity are scant in literature. However, amiloride and its analogs were reported to produce consistent protection in ischaemia/reperfusion dysfunction and arrhythmias, the most striking being intracellular Ca$^{2+}$ overload and increased Na$^+/H^+$ exchange activity.

It is thus envisaged that amiloride may protect the heart against ouabain induced arrhythmias \textit{in vivo} studies also. The present study was undertaken to investigate the effects of amiloride on ouabain induced arrhythmias in guinea pigs.

\section*{MATERIALS AND METHODS}

\textbf{Animals:} Guinea-pigs of either sex (350 to 500 gms body weight) were used in all experiments. The method as described by Thomas and Tripathi was employed with some modifications. In brief, fasting animals were anaesthetised with urethane (1.5 gm/kg, i.p.). The trachea and jugular vein were cannulated to allow artificial respiration and drug administration respectively. Positive pressure ventilation with room air was provided by a rodent respiratory pump (INCO), at a rate of 45 strokes/minute and a stroke volume of 1.0 ml/100 gm of body weight. Right common carotid artery was catheterized and connected to physiological pressure transducer with a polythene cannula to record systemic arterial blood pressure which was monitored continuously with standard limb lead II ECG on a polyrite (INCO) recorder, run at a speed of 25 mm/sec. Rectal temperature was maintained approximately at 37$^\circ$C. Twenty minutes stabilization time period after surgery was given before the start of experimental protocol. Heart rate was calculated from the average of RR intervals, determined for the three successive beats on ECG signals for corresponding BP recordings at any given point in all experiments. For each experiment, amiloride was dissolved in the vehicle, dimethyl sulfoxide (DMSO), 99.5% in purity, and was further diluted in 0.9% saline so that the required dose of amiloride for each experiment could finally be administered in 1 ml of 5% DMSO-saline solution either i.v. or i.p. as required in each experiment.

\textbf{Acute studies:} Amiloride (1, 10 and 100 mg/kg), or vehicle (5% DMSO) as control and propranolol (2 mg/kg) were given i.v. 30 min before constant infusion of ouabain. Ouabain (80 µg/ml) solution was continuously infused at a rate of 100 µg/min with a continuous slow infusion pump to induce ventricular premature beats (VPB), ventricular tachycardia and/or fibrillation with sudden fall in blood pressure (VT/F) and cardiac arrest (CA) as end points successively. Amount of ouabain in µg/kg of body weight, required to produce above end points were determined and compared with the control (DMSO, 5%) group. To rule out the \textit{per se} effect of DMSO (5%), both 0.9% saline and DMSO (5%) pretreatment were also studied.

\textbf{Chronic studies:} Animals were treated either with amiloride (10 mg/kg, i.p.) or by vehicle (DMSO 5%, i.p.) in controls, for three consecutive days as per the method of Dafnis \textit{et al} in which significant diuretic induced increase in serum potassium levels were observed in rats. On 4th day, amiloride (10 mg/kg, i.v.) or vehicle (DMSO 5% i.v.) in controls were administered 30 min before ouabain infusion as described for acute studies. Animal care and feeding were identical in both the groups.

\textbf{Drugs:} Amiloride was obtained as a free gift from Hoechst India Ltd, Bombay. Ouabain octahydrate, DMSO, and propranolol Hcl were obtained from Sigma chemical Co. ST. Louis, MI, U.S.A. DMSO was used as vehicle for amiloride, as it is sparingly soluble in water particularly towards higher concentrations. Propranolol and ouabain were dissolved in distilled water and diluted with normal saline. All solutions of drugs were prepared freshly, just before injection. Total fluid volume administered prior to ouabain infusion, was kept constant for all experiments.
Table 1. Effect of drug pre-treatment on the doses (mean ± SEM) of ouabain required to produce ventricular arrhythmias and cardiac arrest.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>n</th>
<th>ouabain (µg/kg) required to induce</th>
<th>Difference in reading (µg/kg of ouabain)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>VPB</td>
<td>VT/F</td>
</tr>
<tr>
<td>Acute studies:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (DMSO, 5%)</td>
<td>7</td>
<td>150.58</td>
<td>169.22</td>
</tr>
<tr>
<td>±7.34</td>
<td></td>
<td>±5.18</td>
<td>±4.76</td>
</tr>
<tr>
<td>Amiloride (10 mg/kg)</td>
<td>7</td>
<td>148.85</td>
<td>156.02</td>
</tr>
<tr>
<td>±9.29</td>
<td></td>
<td>±8.21</td>
<td>±9.91</td>
</tr>
<tr>
<td>Propranolol (2 mg/kg)</td>
<td>6</td>
<td>223.95**</td>
<td>273.17**</td>
</tr>
<tr>
<td>±12.8</td>
<td></td>
<td>±15.03</td>
<td>±11.73</td>
</tr>
<tr>
<td>Chronic studies:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (DMSO, 5%)</td>
<td>5</td>
<td>170.91</td>
<td>186.2</td>
</tr>
<tr>
<td>±12.61</td>
<td></td>
<td>±7.12</td>
<td>±12.28</td>
</tr>
<tr>
<td>Amiloride (10 mg/kg)</td>
<td>6</td>
<td>171.79</td>
<td>192.75</td>
</tr>
<tr>
<td>±10.49</td>
<td></td>
<td>±10.88</td>
<td>±19.46</td>
</tr>
</tbody>
</table>

VPB: Ventricular premature beats; VT/F: Ventricular tachycardia and/or fibrillation with sudden fall in blood pressure; CA: Cardiac arrest. *P <0.05; **P <0.01, versus control and amiloride groups.

All drugs except ouabain were given as bolus injections.

Statistical analysis: All data were expressed as means ± SEM. The results were analysed by Student’s ‘t’ test (two tailed) for between group comparisons and paired ‘t’ test for within group comparison. P<0.05 was considered to be statistically significant.

RESULTS

Acute studies

Dose of ouabain required to produce VPB, VT/F and CA as end points: No changes were observed with amiloride pretreatment on the amount of ouabain required to produce any of the above end points, while propranolol pretreatment produced very significant increase in the ouabain requirement in all above end points, as compared to control values. It was also observed that propranolol significantly increased the dose of ouabain required to convert VPB to VT/F but was not able to increase the dose required to convert VT/F to CA, which were comparable in all the three groups (Table 1). Effect of saline or DMSO, pretreatment on ouabain arrhythmias were comparable as observed in separate experiments (Data not shown).

BP and HR change: In all groups of animals, baseline BP and HR were comparable. Amiloride significantly decreased the BP and HR after 30 minutes of its administration, a change that was not observed in vehicle treated control animals. Propranolol decreased HR very significantly. Group comparisons in pre-ouabain BP were comparable while pre-ouabain HR was significantly less in amiloride versus control group. Pre-ouabain HR decreased very significantly in propranolol group in comparison to control or amiloride groups (Table 2). In separate experiments, no difference was observed in BP and HR either with saline or DMSO, after 30 min of their administration (Data not shown).

In general, initial dose dependent increase in BP was observed after ouabain infusion in all the groups. This trend was more marked in propranolol pretreated group, which became significant in comparison to amiloride group just before the appearance of VPB. A dose dependent decrease in HR was observed only with control group after ouabain infusion, that reached to a significant level just before the appearance of VPB (Table 2).

Acute pilot study done on three animals with amiloride (100 mg/kg, i.v.) also showed no changes in ouabain induced arrhythmias (mean values of 151.61; 204.18;250.29 µg/kg of ouabain for three experiments versus 177.33; 193.31; 261.77 µg/kg of ouabain in two control experiments for VPB, VT/F and CA
Table 2. Effect of drugs on blood pressure (BP) and heart rate (HR) before (Pre-ouabain) and after ouabain (Post-ouabain) infusion in guinea-pigs.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>n</th>
<th>Pre-drug/baseline</th>
<th>Post-drug/pre-ouabain</th>
<th>Post-ouabain readings at just before ventricular premature beats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BP (mmHg)</td>
<td>HR (Beats/min)</td>
<td>BP (mmHg)</td>
</tr>
<tr>
<td>Acute studies:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (DMSO, 5%)</td>
<td>7</td>
<td>47.5 ± 3.0</td>
<td>368.6 ± 4.3</td>
<td>45.4 ± 2.1</td>
</tr>
<tr>
<td>Amiloride (10 mg/kg)</td>
<td>7</td>
<td>50.4 ± 3.6</td>
<td>367.0 ± 7.2</td>
<td>42.9* ± 2.6</td>
</tr>
<tr>
<td>Propranolol (2 mg/kg)</td>
<td>6</td>
<td>46.0 ± 2.5</td>
<td>354.2 ± 10.3</td>
<td>49.6 ± 3.1</td>
</tr>
<tr>
<td>Chronic studies:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (DMSO, 5%)</td>
<td>5</td>
<td>42.5 ± 1.6</td>
<td>330.2 ± 12.6</td>
<td>40.0 ± 1.4</td>
</tr>
<tr>
<td>Amiloride (10 mg/kg)</td>
<td>6</td>
<td>48.3 ± 2.8</td>
<td>349.5 ± 9.9</td>
<td>41.7 ± 2.1</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. *P <0.05, versus baseline values; **P <0.001, versus baseline values and corresponding control and amiloride group values; ***P <0.01, versus corresponding amiloride group values. ††P <0.05, versus control group values; †††P <0.01, versus pre-ouabain values; ††††P <0.05, versus pre-ouabain values.

respectively). However, amiloride (100 mg/kg, i.v.) did produce a sharp decrease in BP and HR (49 to 35 mm Hg and 351 to 218/min respectively) after 30 min of its administration (mean data of three values). Preliminary studies of amiloride (1 mg/kg, i.v.) also did not produce any significant change in ouabain arrhythmias (Data not shown).

**Chronic studies**

**Dose of ouabain required to produce VPB, VT/F and CA, as end points:** No changes were observed in the above end points after ouabain infusion in chronically treated animals with amiloride in comparison to control group (Table 1).

**BP and HR changes:** BP tend to decrease after amiloride (10 mg/kg, i.v.) administration but results were not statistically significant, while BP remained unchanged in control group after 30 min of DMSO (5%) administration. Pre-ouabain between group comparisons in BP were insignificant. Significant decrease in HR was observed with amiloride after 30 min of i.v. administration while no such changes in HR were observed in controls. After infusion of ouabain, comparable dose dependent initial rise in BP was observed in both the groups, even just before the appearance of VPB. HR changes after ouabain infusion showed significant decrease in amiloride group, while it tended to decrease in control group, just before the appearance of VPB (Table 2).

**DISCUSSION**

In the present study, amiloride failed to modify cardiac toxicity of ouabain in guinea pigs. Amiloride had been shown to influence digitalis induced cardiac toxicity by its direct actions, indirect actions, and probably by the complex kinetic interactions, when used together chronically. Possible kinetic interference is ruled out in the present study as ouabain was not used chronically along with amiloride.

Acute studies done in vivo does exclude the slow and weak indirect antikaleuretic effects of amiloride. Amiloride had been shown to produce antiarrhythmic effects against arrhythmias induced by acetylstropanthidin in pentobarbitone anaesthetised dogs, where each dog was used its own control, while in a separate study reported from the same laboratory, amiloride failed to protect against acetylstropanthidin induced arrhythmias in the same animals. Inconsistent in vitro antiarrhythmic and proarrhythmic
actions based on electrophysiological studies\textsuperscript{19} have also been documented for amiloride. However limitations imposed due to a difference in the cardiac glycoside, method of its administration, species variation and anaesthetic used, can not be excluded for the failure herein the present study.

Similar results were obtained in the chronic studies, which were planned to include the possible indirect effects of amiloride on ouabain arrhythmias. Although K\textsuperscript+ levels were not monitored in this study, the results indicate that any possible change in K\textsuperscript+ levels, as documented in some past studies in human volunteers\textsuperscript{16} and in animals\textsuperscript{17,22} was unable to influence the toxicity of ouabain. Surprisingly, one study in the past observed no change in K\textsuperscript+ levels with chronic oral amiloride administration\textsuperscript{24} and in other study, K\textsuperscript+ levels were significantly raised in patients with digitalis toxicity in comparison to non-toxic patients taking digitalis therapy\textsuperscript{2}. Moreover it is well known that amiloride does not increase K\textsuperscript+ levels much in normal healthy individuals except in hypokalemic situations\textsuperscript{23}, which may equally be true for the present study done on normal healthy adult animals.

A significant decrease in B.P. and H.R. with amiloride (10 mg/kg and 100 mg/kg) in acute studies was observed. This confirms the various earlier in vitro and in vivo observations on electrophysiologic\textsuperscript{19,23} and pharmacological\textsuperscript{12,18,25} effects of amiloride. Similar observations were repeated for H.R. changes with intravenous amiloride in chronic studies, although B.P. changes were insignificantly decreased. These changes with amiloride seems to be short-lasting as baseline values in chronic control and amiloride treated animals were comparable. Such short-lasting decrease in B.P. with i.v. amiloride was also observed in dogs\textsuperscript{18} and is further strengthened by the earlier observations showing no change in B.P. after chronic oral administration of amiloride in healthy human subjects\textsuperscript{16}.

Observed decrease in H.R. and insignificant increase in B.P. with propranolol in chronic studies are in confirmatory with the known effects of propranolol when given intravenously.

Changes in B.P. and H.R. just before the onset of VPBs after ouabain infusion in controlled acute experiments reflects reported known direct and indirect effects of cardiac glycosides\textsuperscript{1,18}. Changes in H.R. with ouabain infusion were blunted by pretreatment with both amiloride (10 mg/kg, i.v.) and propranolol, while B.P. after ouabain infusion was insignificantly blunted by pretreatment with amiloride in contrast to expected and observed further increase in B.P. after propranolol pretreatment. Such changes in B.P. and H.R. after ouabain infusion by pretreatment of amiloride were not seen in chronic studies, the reasons for which are not clear.

It is thus concluded that although amiloride has multiple diverse cellular actions, it has no protective role in ouabain induced arrhythmias and toxicity in the dosage range studied in vivo in healthy adult guinea pigs.

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