Angiotensin II Type 1 Receptor Blockade Prevents Alcoholic Cardiomyopathy

Che-Ping Cheng, MD, PhD; Heng-Jie Cheng, MD, PhD; Carol Cunningham, PhD; Zakariya K. Shihabi, PhD; David C. Sane, MD; Thomas Wannenburg, MD; William C. Little, MD

Background—Activation of the renin-angiotensin system (RAS) may contribute to the development of alcoholic cardiomyopathy. We evaluated the effect of angiotensin II (Ang II) type 1 receptor (AT₁) blockade on the development of alcoholic cardiomyopathy.

Methods and Results—We serially evaluated left ventricular (LV) and cardiomyocyte function and the RAS over 6 months in 3 groups of instrumented dogs. Eight animals received alcohol (once per day orally, providing 33% of total daily caloric intake); 6 received alcohol and irbesartan (5 mg · kg⁻¹ · d⁻¹ PO); and 8 were controls. Compared with controls, alcohol ingestion caused sustained RAS activation with progressive increases in plasma levels of Ang II, renin activity, LV angiotensin-converting enzyme activity, and LV myocyte Ang II AT₁ receptor expression. The RAS activation was followed by a progressive fall in LV contractility (Ees, alcohol-fed dogs 3.9±0.8 versus control dogs 8.1±1.0 mm Hg/mL); reductions in the peak velocity of myocyte shortening (78.9±5.1 versus 153.9±6.2 μm/s) and relengthening; and decreased peak systolic Ca²⁺ transient ([Ca²⁺]ᵢT) and L-type Ca²⁺ current (IᵥCa,L; P<0.05). Irbesartan prevented the alcohol-induced decreases in LV and myocyte contraction, relaxation, peak [Ca²⁺]ᵢT, and IᵥCa,L. With alcohol plus irbesartan, plasma Ang II, cardiac angiotensin-converting enzyme activity, and AT₁ remained close to control values.

Conclusions—Chronic alcohol consumption produces RAS activation followed by progressive cardiac dysfunction. The cardiac dysfunction is prevented by AT₁ receptor blockade. (Circulation. 2006;114:226-236.)

Key Words angiotensin • alcohol • hemodynamics • myocytes • contractility

C hronic alcohol consumption is a common cause of dilated cardiomyopathy that produces congestive heart failure. The underlying mechanisms through which alcohol produces this condition remain poorly understood.¹² Although the central role of the renin-angiotensin system (RAS) in the pathophysiology of heart failure and the importance of interrupting this pathway have been well established in both clinical and experimental studies,³⁴ the role of RAS in the development of alcoholic cardiomyopathy (ACM) has not been defined.

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Because alcohol consumption is a potent activator of RAS,¹⁶⁻⁹ we hypothesized that angiotensin II (Ang II), acting through Ang II type 1 receptors (AT₁), plays an important role in the development of ACM. To help evaluate this hypothesis, we examined the effect of chronic alcohol consumption on the RAS, myocyte AT₁ expression, and left ventricular (LV) and cardiomyocyte functional performance in chronically instrumented dogs. We also assessed the effect of chronic AT₁ blockade on the development of ACM.

Methods

Experimental Model

Instrumentation

Twenty-two healthy, adult, heartworm-negative mongrel dogs (age 2 to 3 years; weight 28 to 30 kg) were instrumented to measure 3 LV internal dimensions and LV and left atrial (LA) pressure. Hydraulic occluders were placed around the venae cavae by a technique that was described previously.⁴¹⁰

Induction and Verification of Experimental Alcoholism

After full recovery from instrumentation, dogs were randomly assigned to 3 groups: (1) alcohol-fed (n=8; alcohol administered as 22% alcohol, once per day orally, providing 33% of total daily caloric intake); (2) alcohol/angiotensin receptor blocker irbesartan (ARB) (n=6; animals received alcohol plus irbesartan, 5 mg · kg⁻¹ · d⁻¹ PO); and (3) control (n=8; animals received no alcohol). Each dog also served as its own control. The data collected in the dog before alcohol was administered were compared with those collected in the same dogs after prolonged alcohol intake. The same procedure was followed for the animals in alcohol/ARB and control groups. Ang II animals were maintained for 6 months under identical conditions. The dose of irbesartan used abolished Ang II–induced pressor responses but had minimal effect on resting baseline blood pressure.
pressure and hemodynamics in our initial dose-selection studies and was consistent with previous studies.11

**Diet**

Each dog was well nourished and consumed an ~2000-calorie balanced diet along with a vitamin supplement, Palavite (Virbac Laboratory, Division of Vizlac, Inc, Fort Worth, Tex). The fat (12%) and protein (30%) content was kept constant in all groups. In the control group, the carbohydrates were 58%, which included an isocaloric amount (33%) of glucose. In the alcohol-fed dogs, carbohydrates were reduced to 25%, and alcohol was added, providing 33% of the total daily caloric intake.

**Alcohol Administration**

Each day in the early morning at 8 AM, alcohol (400 mL of a 22% solution) was added to the food and drinking water, and the dogs consumed this amount within 1 hour. To ensure similarities of physical activity and nutritional intake between the alcohol-treated control animals, each animal was walked for 20 minutes in the afternoon. All animals were weighed twice weekly. Venous blood samples were collected at the onset of the study and at intervals of 1 month thereafter for nutritional status assessment. Random blood samples were obtained from the experimental animals at various times of the day to estimate the average level of alcohol in the blood.

**Experimental Protocol**

**Determination of LV Functional and RAS Responses**

**Measurement of LV Functional Performance**

**Studies Before Alcohol.** Before initiation of alcohol in all animals, baseline steady state and caval occlusion data were acquired, and blood was collected for biochemical assay from the LA catheter at rest.4,10

**Studies After Alcohol.** The protocol as outlined above was repeated early morning the next day, 1 to 2 hours before alcohol intake, twice per week after alcohol initiation, and throughout the development of alcoholism over 6 months. Blood plasma levels of plasma renin activity (PRA) and Ang II were measured 2 weeks apart at the onset of the study and at intervals of 1 month thereafter. Angiotensin-converting enzyme (ACE) activity was measured at the beginning and end (6 months) of the treatment period. To minimize the acute influence of the last administered dose, all measurements were taken after alcohol had been temporarily withheld for 26 to 28 hours. LV volume, rate of LV relaxation (β), LV end-systolic pressure (PES)– end-systolic volume (Ves) relations and their slope (Ees), and stroke work (SW)–end-diastolic volume (Ved) relations and their slope (Emv) were analyzed.4 LV arterial coupling was quantified as the ratio of Ees to arterial elastance (Ea) determined as Pes/stroke volume.10

**Measurement of the Components of RAS**

As previously described,6 blood samples collected during the interventions were immediately placed in iced tubes containing the appropriate inhibitors and centrifuged at 4°C. The levels of PRA, Ang II, and myocardial ACE activity were measured by the Wake Forest University Health Sciences Hypertension Core Laboratory and by the Inter Science Institute (Ingleside, Calif). As previously described, myocardial ACE activity was determined with the synthetic substrate Hip-His-Leu by a modified procedure.12 All assays were performed in duplicate.

**Myocyte Isolation**

Using well-established techniques in our laboratory,4,10 myocardial specimens were collected at the time of surgery and again before the animals were euthanized. To further examine whether the altered myocyte functional response to Ang II occurs in the early phase of the development of alcoholism, in the subgroups of 5 control and 5 alcohol-fed animals, myocardial specimens were also collected at 2 months after the beginning of the study by endomyocardial biopsy with the use of 50-cm Cordis BioPal 7 biopsy forceps (502-402B, Cordis, Miami, Fla) through the left carotid artery guided by fluoroscopy to the LV.13 Reproducible, high-yield (>85%), calcium-tolerant myocytes were isolated from these myocardial specimens. At room temperature (22°C), the isolated myocytes maintained rod-shaped morphology for more than 18 to 24 hours.

**Myocyte Functional Performance**

**Measurement of Contractile Performance**

Response to Ang II and Isoproterenol. After 2 hours of stabilization, isolated myocytes were placed in superfused culture dishes. The myocyte being studied was displayed on the computer monitor with an IonOptix MyoCam camera (IonOptix, Milton, Mass), which rapidly scans the image area every 8.3 ms such that the amplitude and velocity of shortening/relengthening are recorded with good fidelity. The edge-detection software (IonOptix) was used to measure cell length during shortening and relengthening. Briefly, myocyte contractions were elicited by field stimulation at a frequency of 1.0 Hz. After stabilization, steady state data were recorded. Myocytes were randomly exposed to Ang II (10^{-8} mol/L) or isoproterenol (ISO, 10^{-8} mol/L), and data were acquired after 3 to 5 minutes of drug exposure and 5 to 10 minutes after drug washout. In a subset of myocytes obtained from the animals after chronic alcohol intake of 2 months, the Ang II study was repeated in the presence of losartan (10^{-8} mol/L) in the superfusion or preincubated with pertussis toxin (2 μg/mL, 36°C, 5 hours).4,14 As described previously,4,10 measurements of myocyte dimensions and functional responses were made in 60 to 80 randomly selected, rod-shaped cells from each experiment. Systolic amplitude or percentage shortening (SA) was determined as the percentage difference between the maximum and minimum cell length of each contraction. The peak velocity of shortening (dL/dtmax) and the peak velocity of relengthening (dR/dtmin) were obtained by differentiating the digitized contractile profiles.4,10,14

**Simultaneous Measurement of Calcium Transient and Contractile Response**

In the second series of experiments, peak systolic Ca^{2+} transient ([Ca^{2+}]_i) and contraction responses in a single myocyte were measured simultaneously with a dual-excitation fluorescence photomultiplier system (IonOptix). When myocytes were loaded with indo-1-AM, compartmentalization of the indicator may have occurred in the mitochondria, and thus, the absolute value of [Ca^{2+}]_i was not used. The ratio of the emitted fluorescence (410/490) was used to represent the relative changes in peak intracellular calcium ([Ca^{2+}]_i) before and after interventions.10,14

**Myocyte Calcium Current Response**

To determine whether the alcohol-induced alterations in [Ca^{2+}]_i might be secondary to alteration in the inward calcium current, in the third series of experiments, the I-type calcium current (ICa,L) response was measured by whole-cell patch-clamp technique.13 After baseline ICa,L was determined, data were obtained during the 0 to 10 minutes of Ang II (10^{-6} mol/L) superfusion.

**Myocyte Ang II mRNA and Protein Levels**

AT1 and AT2 Receptor mRNA. LV myocyte total RNA was extracted by RNAsolue phenol-free total RNA isolation kit (Ambion, Austin, Tex) and treated with RNase-free DNase I (GIBCO BRL, Carlsbad, Calif). The reverse-transcription reaction was performed with RETROscript first-strand synthesis kit (Ambion) using the antisense primer. The cDNA produced was amplified by polymerase chain reaction with the published primers for canine AT1 (210 bp) and AT2 (180 bp) receptor DNA fragments.16 GAPDH was coamplified as an internal control.14,16

**Western Blot.** LV myocytes were briefly washed with prechilled PBS before the addition of a membrane protein-extraction reagent (Pierce, Rockford, Ill) with a protease inhibitor cocktail. Cell membrane lysate (50 μg) was blotted to a PVDF membrane and then incubated with polyclonal IgG to AT1 and AT2 (1:1500 dilutions, Santa Cruz Biotechnology Inc, Santa Cruz, Calif) at 4°C overnight.
ANOVA for repeated measures. If the ANOVA revealed significance, pairwise tests of individual group means were compared by use of Bonferroni procedure. Treatment effects were determined by ANCOVA on the outcome measures adjusted for baseline values. Repeated-measures ANOVA was used for the time effect with separate paired t tests in the presence of a significant interaction. In each animal, measurements of myocyte contractility, \([\text{Ca}^{2+}]_{\text{cyt}}\), and \(I_{\text{Ca,L}}\) were averaged and treated as a single data point. Then, the mean differences in cell dimensions, cell dynamics, \([\text{Ca}^{2+}]_{\text{cyt}}\), and \(I_{\text{Ca,L}}\) obtained from the animals before and after a 2- or 6-month study period were obtained. Significance was established as \(P<0.05\).

**Statistical Analysis**

Data were summarized as mean±SD. Indices of LV function, systemic hemodynamics, neurohormonal profiles, and isolated myocyte function were compared among the treatment groups by ANOVA for repeated measures. If the ANOVA revealed significant differences, pairwise tests of individual group means were compared by use of Bonferroni procedure. Treatment effects were determined by ANCOVA on the outcome measures adjusted for baseline values. Repeated-measures ANOVA was used for the time effect with separate paired t tests in the presence of a significant interaction. In each animal, measurements of myocyte contractility, \([\text{Ca}^{2+}]_{\text{cyt}}\), and \(I_{\text{Ca,L}}\) were averaged and treated as a single data point. Then, the mean differences in cell dimensions, cell dynamics, \([\text{Ca}^{2+}]_{\text{cyt}}\), and \(I_{\text{Ca,L}}\) obtained from the animals before and after a 2- or 6-month study period were obtained. Significance was established as \(P<0.05\).

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**TABLE 1. LV Function and General Hemodynamic Variables With Chronic Alcohol Consumption: Effects of AT1 Blockade**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=8)</th>
<th>Alcohol (n=8)</th>
<th>Alcohol/ARB (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (C)</td>
<td>After 6 Months (C)</td>
<td>Baseline (A)</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>116±4</td>
<td>109±7</td>
<td>106±6</td>
</tr>
<tr>
<td>Maximum dP/dt, mm Hg/s</td>
<td>2837±149</td>
<td>2946±168</td>
<td>2995±181</td>
</tr>
<tr>
<td>Minimum dP/dt, mm Hg/s</td>
<td>-2099±168</td>
<td>-2189±188</td>
<td>-2327±146</td>
</tr>
<tr>
<td>Stroke volume, mL</td>
<td>15.6±2.4</td>
<td>16.0±2.2</td>
<td>15.6±2.9</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
<td>8.2±1.8</td>
<td>8.5±1.9</td>
<td>8.0±2.1</td>
</tr>
<tr>
<td>LV end-systolic pressure, mm Hg</td>
<td>106±6</td>
<td>109±5</td>
<td>110±12</td>
</tr>
<tr>
<td>Minimum LV pressure, mm Hg</td>
<td>1.2±1.7</td>
<td>1.3±1.9</td>
<td>1.9±1.4</td>
</tr>
<tr>
<td>Mean LA pressure, mm Hg</td>
<td>6.2±1.9</td>
<td>6.9±2.0</td>
<td>6.7±1.6</td>
</tr>
<tr>
<td>LV end-diastolic volume, mL</td>
<td>43.8±6.3</td>
<td>44.2±7.2</td>
<td>45.1±7.4</td>
</tr>
<tr>
<td>LV end-systolic volume, mL</td>
<td>28.2±6.1</td>
<td>28.2±6.3</td>
<td>29.5±8.8</td>
</tr>
<tr>
<td>Maximum dv/dt, mL/s</td>
<td>193±58</td>
<td>195±43</td>
<td>187±52</td>
</tr>
<tr>
<td>Cardiac output, mL/min</td>
<td>1809±184</td>
<td>1728±290</td>
<td>1654±269</td>
</tr>
<tr>
<td>Emax, mm Hg/mL</td>
<td>6.7±1.4</td>
<td>6.8±1.8</td>
<td>7.0±1.5</td>
</tr>
<tr>
<td>Time constant of relaxation, ms</td>
<td>27.5±1.2</td>
<td>27.2±2.0</td>
<td>27.8±1.8</td>
</tr>
<tr>
<td>LV chamber stiffness, mm Hg/mL</td>
<td>1.55±0.27</td>
<td>1.59±0.34</td>
<td>1.60±0.38</td>
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<tr>
<td>WSS, g/cm²</td>
<td>62.1±15.0</td>
<td>61.2±15.1</td>
<td>60.4±10.9</td>
</tr>
<tr>
<td>Emax, mm Hg/mL</td>
<td>8.0±1.1</td>
<td>8.1±1.0</td>
<td>8.4±1.1</td>
</tr>
<tr>
<td>Mmax, mm Hg</td>
<td>86.7±7.8</td>
<td>88.6±8.0</td>
<td>85.6±6.2</td>
</tr>
</tbody>
</table>

n indicates number of dogs; WSS, mean end-systolic circumference stress of LV. Values are mean±SD.

\*P<0.05, C vs A and C vs T;
†P<0.05, A vs C and A vs T;
‡P<0.05, A vs T.

After washes, the membrane was incubated with horseradish peroxidase–conjugated anti-rabbit IgG (1:3000 dilutions, Sigma, St. Louis, Mo). For normalization, the same blot was stripped and reprobed with polyclonal IgG to actin at 1:2500 dilutions (Santa Cruz Biotechnology Inc).14,16

Figure 1. Examples of PES-VES relationships obtained from 1 alcoholic dog after the 6-month protocol. During the progression of alcoholism, there were serially progressive rightward shifts of the PES-VES relations with decreased slopes, which indicates progressively depressed LV contractility.
The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

**Results**

A small increase in body weight occurred at the conclusion of the 6-month observation period that was similar in all 3 groups of animals. Hematocrit, serum albumin, and fasting blood glucose, as well as minerals (eg, iron and zinc) and vitamins (eg, vitamin A and thiamin), were also within normal limits in all of the groups. The average blood alcohol concentration in the alcohol-fed groups exhibited a peak value of 149 mg/100 mL 2 hours after alcohol intake with no significant difference between alcohol and alcohol/ARB groups. Before the daily alcohol dose, on the next morning, the blood alcohol concentration dropped to 18 mg/100 mL.

Compared with animals in both control and alcohol/ARB groups, only the animals in the alcohol group demonstrated clinical symptoms of heart failure, including anorexia, edema, and pulmonary congestion. Five of these animals also had ascites. Chest radiograph showed a markedly increased ratio of the heart to thoracic diameter (alcohol-fed 0.74±0.04 versus control 0.49±0.06%), and echocardiographic examination showed significantly increased LV systolic and diastolic dimensions with a concomitant reduction in fractional shortening (24.6±1.9% versus 41.4±1.8%). In alcoholic dogs, the LV weight increased by 39% (185.8±3.1 versus 133.6±2.2 g), with a 35% increase in the calculated ratio of LV to body weight (9.76±0.07 versus 7.24±0.09 g/kg). The calculated ratios of wet lung to body weight were all significantly increased.

**LV Function With Chronic Alcohol Consumption: Effects of AT1 Blockade**

LV function and hemodynamic alterations measured for the 3 groups of animals are summarized in Table 1 and displayed in Figures 1 and 2. Compared with controls, 6 months after alcohol intake, LV end-diastolic pressure (PED), mean LA pressure, LV end-systolic and diastolic volume, and the time constant of LV relaxation were all significantly increased, accompanied by reduced LV peak filling rate and stroke volume. The slopes of LV pressure-volume relations, load-insensitive measures of LV contractile performance, were decreased by 50% (Ees 3.9±0.8 versus 8.1±0.8 mm Hg/mL; Msw 43.9±4.1 versus 88.6±8.0 mm Hg). The relations shifted to
the right. As clearly illustrated in Figures 1 and 2A, in the alcohol group, there were progressive decreases in LV contractility with chronic alcohol ingestion. Compared with the control group, the depression of LV contractility was apparent 4–6 months after initiation of alcohol ingestion, with 30% decreases in EES and a further decrease of 51% at 6 months, with clinical signs of heart failure. The degree of these depressed pressure-volume relations was similar to the changes we observed in the dogs after 4 weeks of rapid LV pacing.4,10 As shown in Figure 2B, alcohol intake for 6 months also caused clearly upward and rightward shifts of the early diastolic portion of LV pressure-volume loops. In contrast, LV function and general hemodynamics were similar between the control and alcohol/ARB groups. Concomitant ARB with alcohol prevented alcohol-induced decreased LV contractility and the abnormal upward and rightward shifts of LV pressure-volume loops. Mean LA pressure, τ, and stroke volume were all close to control values (Table 1; Figure 2).

RAS Activation With Chronic Alcohol Consumption: Effects of AT1 Blockade

As shown in Figure 4, compared with normal control myocytes and alcohol/ARB myocytes, only alcoholic myocytes had a significantly higher signal ratio of Ang II AT1 mRNA to GAPDH (alcoholic myocytes 0.69±0.08; control 0.33±0.04; alcohol/ARB myocytes 0.39±0.06). By using Western blot analysis, a single band of Ang II AT1 protein (≈40 kDa) was detected. Only in alcoholic myocytes, the signal ratio of Ang II AT1 protein to actin was increased 2-fold (alcoholic myocytes 0.79±0.05; control 0.37±0.04; alcohol/ARB myocytes 0.40±0.08; \( P < 0.05 \)), which indicates that chronic alcohol intake increased AT1 protein levels. Conversely, the signal ratios of AT2 mRNA to GAPDH (alcoholic myocytes 0.18±0.01; control 0.16±0.01; alcohol/ARB myocytes 0.17±0.01) and AT2 protein to actin (alcoholic myocytes 0.16±0.02; control 0.14±0.06; alcohol/ARB myocytes 0.14±0.03) were similar in myocytes obtained from the 3 groups of animals, which indicates that cardiac AT2 receptor expression was unaffected by chronic alcohol ingestion.

Myocyte Function With Chronic Alcohol Consumption: Effects of AT1 Blockade

As shown in Figure 3, compared with normal control myocytes, both PRA and Ang II continued to increase and reached 6 months, with 30% decreases in EES and a further decrease of 51% at =6 months, with clinical signs of heart failure. The degree of these depressed pressure-volume relations was similar to the changes we observed in the dogs after 4 weeks of rapid LV pacing.4,10 As shown in Figure 2B, alcohol intake for 6 months also caused clearly upward and rightward shifts of the early diastolic portion of LV pressure-volume loops. In contrast, LV function and general hemodynamics were similar between the control and alcohol/ARB groups. Concomitant ARB with alcohol prevented alcohol-induced decreased LV contractility and the abnormal upward and rightward shifts of LV pressure-volume loops. Mean LA pressure, τ, and stroke volume were all close to control values (Table 1; Figure 2).

Figure 3. Group mean of RAS response with and without chronic irbesartan treatment before and after chronic alcohol consumption. Serial measures of plasma levels of Ang II and PRA were obtained in each group during the 6-month study period. All measurements were taken after alcohol had been temporarily withheld for 26 to 28 hours.
Importantly, chronic alcohol intake also altered the myocyte response to an inotropic stimulus. As displayed in Figures 5A and 6, in control myocytes, Ang II (10⁻⁶ mol/L) increased SA (24%), dL/dt max (35%), [Ca²⁺] iT (23%), and I c,-L (24%). In contrast, in myocytes collected from the alcoholic group, Ang II stimulation produced direct inhibition of cell contraction with significantly reduced SA (42%), dL/dt max (36%), and dR/dt max (37%) and decreased [Ca²⁺] iT (23%) and I c,-L (28%). These effects were completely reversed after washout of the Ang II (data not shown). As shown in Figure 5B, in both control myocytes and alcohol/ARB myocytes, isoproterenol (10⁻⁴ mol/L) resulted in similar increases in SA (control 59% versus alcohol/ARB myocytes 61%), dL/dt max (48% versus 46%), dR/dt max, and [Ca²⁺] iT (29%). In contrast, in alcoholic myocytes, the isoproterenol-induced increases in SA (24%), dL/dt max (26%), dR/dt max, and [Ca²⁺] iT (13%) were reduced significantly.

We found that the altered myocyte functional response to Ang II occurred in the early phase of the development of alcoholism. As shown in Figure 7, after 2 months of alcohol intake (at a time when clinical heart failure was absent), isoproterenol resulted in similar increases in cell contractile force.

### TABLE 2: Myocyte Contraction and [Ca²⁺] iT Transient With Chronic Alcohol Consumption: Effects of AT1 Blockade

<table>
<thead>
<tr>
<th></th>
<th>Control (n=8)</th>
<th>Alcohol (n=8)</th>
<th>Alcohol/ARB (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (C₀)</td>
<td>After 6 Months (C₁)</td>
<td>Initial Control (A₁)</td>
</tr>
<tr>
<td>Resting length, µm</td>
<td>120.4±4.9</td>
<td>122.1±4.8</td>
<td>121.8±5.4</td>
</tr>
<tr>
<td>Percent shortening (SA), %</td>
<td>12.2±0.7</td>
<td>12.4±0.9</td>
<td>12.6±0.9</td>
</tr>
<tr>
<td>Velocity of shortening (dL/dt max), µm/s</td>
<td>150.3±6.2</td>
<td>153.9±6.2</td>
<td>149.2±7.1</td>
</tr>
<tr>
<td>Velocity of re-lengthening (dR/dt max), µm/s</td>
<td>114.2±3.8</td>
<td>116.6±3.4</td>
<td>122.0±5.8</td>
</tr>
<tr>
<td>Peak systolic [Ca²⁺] transient</td>
<td>1.20±0.02</td>
<td>1.21±0.03</td>
<td>1.20±0.01</td>
</tr>
<tr>
<td>Diastolic [Ca²⁺]</td>
<td>0.82±0.03</td>
<td>0.79±0.04</td>
<td>0.79±0.02</td>
</tr>
<tr>
<td>I c,-L, PA/PF</td>
<td>3.2±0.3</td>
<td>3.3±0.2</td>
<td>3.4±0.3</td>
</tr>
</tbody>
</table>

*P<0.05, T₁ vs C₀ and T₂ vs C₀. †P<0.05, A₁ vs C₀ and A₂ vs T₁. ††P<0.05, A₂ vs A₁.

n indicates number of dogs. Values are mean±SD.

0.79±0.04) was markedly higher than in normal control myocytes. The peak systolic [Ca²⁺] iT (1.12±0.01 versus 1.21±0.03) was markedly reduced, and the decline of [Ca²⁺] was also slower in the alcoholic myocytes. In alcoholic myocytes, membrane capacitance was significantly increased, whereas the current density was significantly lower compared with control values. The resting [Ca²⁺], systolic peak [Ca²⁺] iT levels, and I c,-L were also similar to those of the control group (Table 2; Figures 5 and 6).

In chronic alcohol consumption, Ang II (10⁻⁶ mol/L) increased SA (24%), dL/dt max (35%), [Ca²⁺] iT (23%), and I c,-L (24%). In contrast, in myocytes collected from the alcohol group, Ang II stimulation produced direct inhibition of cell contraction with significantly reduced SA (42%), dL/dt max (36%), and dR/dt max (37%) and decreased [Ca²⁺] iT (23%) and I c,-L (28%). These effects were completely reversed after washout of the Ang II (data not shown). As shown in Figure 5B, in both control myocytes and alcohol/ARB myocytes, isoproterenol (10⁻⁴ mol/L) resulted in similar increases in SA (control 59% versus alcohol/ARB myocytes 61%), dL/dt max (48% versus 46%), dR/dt max, and [Ca²⁺] iT (29%). In contrast, in alcoholic myocytes, the isoproterenol-induced increases in SA (24%), dL/dt max (26%), dR/dt max, and [Ca²⁺] iT (13%) were reduced significantly.

We found that the altered myocyte functional response to Ang II occurred in the early phase of the development of alcoholism. As shown in Figure 7, after 2 months of alcohol intake (at a time when clinical heart failure was absent), isoproterenol resulted in similar increases in cell contractile force.
performance with marked increases in SA (alcoholic myocytes 60% versus control 59%) and dL/dtmax (62% versus 60%) in both normal myocytes and myocytes obtained from animals after alcohol intake for 2 months. However, the nature of myocyte functional response to Ang II was altered. Before alcohol intake, Ang II (10^{-6} mol/L) caused a positive inotropic action, with increased SA (26%) and dL/dtmax (32%) and relatively unchanged dR/dtmax. After 2 months of alcohol administration, this positive response was reversed, and the abrupt exposure to Ang II caused a rapid decrease in cell contraction, with markedly reduced SA, dL/dtmax, dR/dtmax, and [Ca^{2+}]_i. In the alcoholic myocytes, the ISO-induced positive action was reduced.

Figure 5. Examples of myocyte contractile function and [Ca^{2+}]_i responses to Ang II and isoproterenol (ISO). Myocytes isolated from heart tissue of LV obtained by biopsy from 1 control dog, 1 alcoholic dog, and 1 alcohol/ARB-treated dog after 6-month study period. A, Analog recordings in electrically stimulated myocytes before (Baseline) and after (Ang II) superfusion of Ang II (10^{-6} mol/L). Myocyte baseline contraction and relaxation (measured as dL/dtmax and dR/dtmax) and peak systolic [Ca^{2+}]_i were similar in both control and alcohol/ARB-treated myocytes. Superfusion of Ang II (10^{-6} mol/L) had a similar positive inotropic effect, with increased dL/dtmax and [Ca^{2+}]_i. In contrast, compared with controls, after 6 months of alcohol intake, SA, dL/dtmax, dR/dtmax, and [Ca^{2+}]_i were markedly reduced. Moreover, Ang II caused a negative inotropic effect, with markedly decreased dL/dtmax, dR/dtmax, and [Ca^{2+}]_i, which indicates myocyte contractile dysfunction and [Ca^{2+}]_i homeostasis impairment as presented in ACM exacerbated by Ang II. B, In the control or alcohol/ARB-treated myocytes, superfusion of ISO (10^{-6} mol/L) caused positive inotropic responses with markedly increased SA, dL/dtmax, dR/dtmax, and [Ca^{2+}]_i. In the alcoholic myocytes, the ISO-induced positive action was reduced.
Several clinical studies indicate that the main effect of alcohol ingestion is loss of cardiac contractility, an effect that progressively induces the development of a dilated cardiomyopathy that leads to heart failure. However, the experimental evaluation of cardiac contractility performance as alcoholism progresses has been difficult in the past due to the lack of animal models that more closely resemble or mimic the human condition in terms of the amount and pattern of alcohol consumption. Although acute alcohol consumption has been demonstrated to have a direct cardiodepressant effect, chronic alcohol intake has been variably reported to have no direct effect or to impair LV performance. Previous investigations with dogs administered alcohol for 6 months or longer demonstrated a clear impairment of LV relaxation and diastolic filling and an abnormal response to angiotensin infusion, which is consistent with our findings. However, these earlier investigations failed to show chronic alcohol-induced depression in LV contractility, LV dilatation, or clinical congestive heart failure. In the present study, the animals consumed a large amount of 22% alcohol (400 mL) in a short time period (< 1 hour). Previous studies have used a lower amount of alcohol administered in the drinking water over a longer time period, and the actual intake per animal was estimated only at biweekly intervals. There was uncertainty as to the quantity of alcohol intake per day and per animal. Additionally, in the previous investigations, LV contractility assessment was performed under anesthesia and with load-sensitive measures of LV contractility. In contrast, we serially evaluated LV contractile performance in conscious animals with pressure-volume analysis. We found that chronic alcohol ingestion produces direct inhibition of LV contractility and causes progressive LV systolic and diastolic functional impairment and LV structural remodeling. These findings are similar to the clinical syndrome of ACM.

Importantly, we found that chronic alcohol-induced progressive LV dysfunction and remodeling are associated with parallel depression in cardiomyocyte function and increased resting myocyte length. By using freshly isolated single myocytes from the LV of chronic alcoholic dogs, we observed progressively depressed peak systolic [Ca\(^{2+}\)]\(_{cyt}\), elevated diastolic [Ca\(^{2+}\)]\(_{cyt}\), and decreased I\(_{Ca,L}\) (Table 2; Figures 5 and 6). This suggests that a defect in cardiomyocyte contractile performance and [Ca\(^{2+}\)]\(_{cyt}\) regulation may be the primary cause of ACM. Past studies in dogs fed 25% alcohol for 6 months and turkeys fed 5% alcohol (25% of calories consumed) for 58 days provided evidence that chronic alcohol consumption–induced alterations in [Ca\(^{2+}\)]\(_{cyt}\) may result from diminished Ca\(^{2+}\) uptake and Ca\(^{2+}\) binding by the sarcoplasmic reticulum and reduced Na\(^+\), K\(^+\)-stimulated AT-Pase. The present study indicates chronic alcohol intake results in significantly reduced inward calcium current, which also contributes to the abnormal [Ca\(^{2+}\)]\(_{cyt}\) in ACM. More information is needed on the changes of intracellular Ca\(^{2+}\) handling properties, such as measures of protein levels of sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\)-ATPase, phospholamban, and Na/Ca exchanger during alcohol ingestion.

The chronic alcohol-induced LV dysfunction and cardiac failure were prevented by administration of an ARB during ingestion of alcohol. In these alcohol/ARB-treated animals, LV function, resting myocyte lengths, myocyte systolic [Ca\(^{2+}\)]\(_{cyt}\), diastolic [Ca\(^{2+}\)]\(_{cyt}\), and I\(_{Ca,L}\) remained close to control

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**Discussion**

We found the daily consumption of a large amount of alcohol (providing 33% of total daily caloric intake) produced sustained activation of the RAS and cardiac AT\(_1\) upregulation followed by LV dilation and dysfunction. This was associated with parallel changes in myocyte structure and function and altered responses to Ang II and adrenergic stimulation. These effects of alcohol were prevented by the administration of an ARB. Thus, the present study demonstrates the key role of Ang II acting through AT\(_1\) receptors in the development of LV dilation and dysfunction after the chronic consumption of large amounts of alcohol.

**LV and Isolated Myocyte Function and Chronic AT\(_1\) Receptor Blockade**

Several clinical studies indicate that the main effect of alcohol ingestion is loss of cardiac contractility, an effect that...
levels. These findings indicate that chronic administration of a specific AT↓ receptor antagonist provided a protective effect on LV and myocyte contractile performance and [Ca$^{2+}$]$_i$ homeostasis in these alcoholic animals.

**RAS Activation and Chronic AT↓ Receptor Blockade**

Increased circulating levels of Ang II and elevated PRA commonly occur with the development of severe heart failure in patients. In the present study and consistent with past reports, chronic alcohol ingestion was accompanied by a similar profile of RAS activation. However, contrary to heart failure due to other causes, changes in RAS occurred early in the course of chronic alcohol consumption. Before clinical signs of heart failure appeared, both circulating and cardiac Ang II increased to very high levels, and the LV and myocyte responses to exogenous and endogenous elevations in circulating Ang II were altered. These findings suggest that AT↓ receptor activation may be an initial event in the development of ACM.

Previous studies demonstrated that acute ingestion of alcohol produces transient RAS activation and that alcoholics have an enhanced RAS activation after acute ethanol ingestion. Alcoholic also have enhanced serum ACE activity. The early RAS activation in the present study may have been caused by a direct effect of alcohol on the RAS or through an alcohol-induced reduction of plasma volume, a reduction in blood pressure, an alteration in sodium balance or sympathetic activation, and/or by production of cardiac depression and cardiac hemodynamic overload (including increased LV wall stress). It is evident that myocardial stretch and/or the increase in shortening load on the myocardium turns on the genes of the RAS. However, our present observation is inconsistent with the effects of alcohol on myocardial ACE activity in rats. In that rat study, 12 months but not 8 months of alcohol consumption was associated with increased LV ACE activity, which suggested that activation of the tissue RAS does not seem to be involved in the initiation of ACM. This discrepancy may result from species differences, because the angiotensin-forming mechanism, Ang II receptor
distribution, and Ang II receptor–mediated cardiac effects are different in the rat than in humans and dogs.\textsuperscript{23,25,26} For example, heart chymase, an important intracardiac Ang II–forming mechanism in human and canine hearts, is not present in the rat heart.\textsuperscript{23} In contrast with human, dog, and other mammals, there is an absence of a cardiac contractile response to Ang II in the hearts of adult rats.\textsuperscript{27} The absence of significant cardiac dysfunction and structural changes in a rat model fed alcohol may also contribute to this discrepancy. By contrast, in the present chronically instrumented dog model during 6 months of alcohol consumption, we observed that both circulating levels of PRA and Ang II and intracardiac ACE activity and AT\textsubscript{1} receptor expression were all increased with increasing diastolic wall stress and were negatively correlated with the LV functional state.

We found that in normal cardiomyocytes, Ang II causes a positive modulation of cardiac contraction and [Ca\textsuperscript{2+}], regulation, but in cells from alcohol-fed dogs, Ang II stimulation produced depression of cardiac contraction and [Ca\textsuperscript{2+}], regulation, which is paralleled by an increase in cardiac AT\textsubscript{1} expression. Changes in myocyte contractile response to Ang II occurred early in the course of chronic alcohol consumption, at a time when clinical heart failure was absent, and both LV and myocyte responses to β-adrenergic receptor stimulation were preserved. This abnormal myocyte response to Ang II was reversed by AT\textsubscript{1} receptor blockade. It is likely that this normalization may be attributable to the prevention of chronic alcohol-induced upregulation of cardiac AT\textsubscript{1} receptor and early changes in the cardiac RAS signal transduction system in chronic alcohol intake. It was demonstrated previously that chronic alcohol ingestion upregulates G\textsubscript{i} protein, increases protein kinase C activity, and alters the protein kinase C isoforms.\textsuperscript{1,28} However, chronic alcohol-induced Ang II–mediated intracellular signal transduction pathways with and without ARB treatment remain to be determined.

It should be recognized that a potent antioxidant action of irbesartan may also account for its protective effect in the development of ACM. Oxidative stress plays an integral role in myocardial dysfunction in ACM and could be mediated through AT\textsubscript{1}.\textsuperscript{2,29} Past reports have shown that alcohol-induced increases in reactive oxygen species impair myocyte calcium handling and mechanical function,\textsuperscript{17} reactive oxygen species decrease expression and activity of sarcoplasmic reticulum Ca\textsuperscript{2+} ATPase-2,\textsuperscript{30} and Ang II generates reactive oxygen species.\textsuperscript{29} Thus, AT\textsubscript{1} receptor blockade may have limited or prevented the chronic alcohol ingestion–caused increased oxidative stress, thereby preserving normal myocyte [Ca\textsuperscript{2+}], regulation and myocyte functional performance.

Study Limitations

Several methodological issues should be considered in interpreting the present data. We used chronically instrumented animals. Instrumentation may produce some LV damage, although the instrumented animals demonstrated well-preserved cardiovascular function and good exercise tolerance.\textsuperscript{4,5} This canine model reproduces many of the functional, structural, and neurohormonal features of clinical ACM; however, it must be recognized that any animal model will not fully represent the complex clinical spectrum of human ACM.

Conclusions

The conscious instrumented dog model represents a clinically relevant, “long-term binge” model for an ACM study. By using this model, the present study demonstrates that chronic alcohol ingestion produces progressive LV and myocyte functional impairment, structural remodeling, and sustained RAS activation accompanied by Ang II stimulation–induced negative inotropic response with an exacerbated negative modulation on [Ca\textsuperscript{2+}], regulation, which is paralleled by increased cardiac AT\textsubscript{1} expression. All of these adverse effects were prevented by AT\textsubscript{1} blockade. This indicates a key role for Ang II acting through AT\textsubscript{1} receptors in the development of ACM (LV and myocyte dilation and dysfunction) after the chronic consumption of a large amount of alcohol. It suggests that ARBs may be useful in preventing the development and progression of ACM.

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Disclosures

Dr Little has received research support for other studies from Merck Pharmaceuticals and serves as an end point committee member for Bristol-Myers Squibb. The remaining authors report no conflicts.

References


**CLINICAL PERSPECTIVE**

Chronic alcohol abuse can result in heart failure due to a dilated cardiomyopathy. The mechanism through which alcohol produces this condition is poorly understood. Because the acute ingestion of large amounts of alcohol is a potent stimulus for the renin angiotensin system (RAS), and the RAS plays an important role in the progression of other forms of heart failure, we investigated the role of the RAS in the production of alcoholic cardiomyopathy in a canine model. We found that the daily ingestion of a large amount of alcohol results in sustained RAS activation in these animals, followed by progressive left ventricular and myocyte functional impairment, structural remodeling, altered myocyte calcium regulation, and an increase in cardiac angiotensin II type I receptor (AT1) expression. All of these adverse effects were prevented by AT1 blockade. These findings demonstrate the importance of the RAS in the pathogenesis of alcoholic cardiomyopathy and suggest that interrupting the RAS may have clinical benefit in patients with alcoholic cardiomyopathy.
Angiotensin II Type 1 Receptor Blockade Prevents Alcoholic Cardiomyopathy
Che-Ping Cheng, Heng-Jie Cheng, Carol Cunningham, Zakariya K. Shihabi, David C. Sane, Thomas Wannenburg and William C. Little

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