Verapamil Prevents the Development of Alcoholic Dysfunction in Hamster Myocardium

JEFFREY S. GARRETT, MD, JOAN WIKMAN-COFFELT, PhD, RICHARD SIEVERS, BS, WALTER E. FINKBEINER, MD, WILLIAM W. PARMLEY, MD, FACC
San Francisco, California

Ethanol causes depression of cardiac function. A new model in hamsters was developed for studying ethanol-induced myocardial dysfunction and the effects of verapamil in preventing the functional and metabolic derangements caused by ethanol ingestion were evaluated. Ethanol was added to the drinking water of hamsters in increasing amounts, reaching 50% from 5 weeks on. A control group received plain water only. A third group had verapamil (1.75 mg/cc) added to the ethanol-water mixture to evaluate its potential protective effect. After 5, 7 and 12 weeks, the animals were killed and the hearts perfused using a Langendorff heart preparation. Pressures were recorded and metabolic analysis was performed by the freeze-clamp technique.

From the Department of Medicine, Cardiovascular Research Institute, University of California, San Francisco, California 94143. Dr. Garrett is a recipient of Individual Research Fellowship HL-06944 from the National Institutes of Health, Bethesda, Maryland. This work was supported in part by the Susan and Don Schleicher Fund and the George D. Smith Fund, San Francisco.

Manuscript received June 16, 1986; revised manuscript received September 29, 1986, accepted October 22, 1986.

Address for reprints: William W. Parmley, MD, University of California, San Francisco, Division of Cardiology, Moffitt Hospital, San Francisco, California 94143.

©1987 by the American College of Cardiology

Compared with control hearts, the hearts from hamsters ingesting ethanol showed significant depression of developed pressure and maximal rate of rise in pressure. There was also significant depression of high energy phosphates and adenosine. The animals drinking the ethanol-verapamil mixture had preservation of left ventricular performance and high energy phosphates, with measurements indistinguishable from those of the control group. In summary, verapamil prevented the development of myocardial depression and preserved normal energy metabolism in hearts of hamsters drinking 50% ethanol.

(J Am Coll Cardiol 1987;9:1326-31)

Ethanol causes both acute and chronic depression of cardiac function (1-5). The reasons for this: loss of contractility are not clear, although impaired handling of calcium by the myocardial cells is thought to play a central role (6). These defects may occur at the cell membrane, sarcoplasmic reticulum, mitochondria or at the level of the contractile apparatus (7,8). Verapamil, a calcium entry blocker, has demonstrated salutary effects on contractility while preserving the nucleotide pool in the inherited cardiomyopathy of the Syrian hamster (9,10). It is unclear whether this verapamil effect is specific to the hereditary cardiomyopathic model or could be demonstrated in a cardiomyopathy of another origin. Accordingly, we developed and characterized a new model of cardiac depression caused by ethanol ingestion in the hamster. The purpose of this study was to assess the functional and metabolic effects of verapamil on this new model.

Specifically, the goals of this study were to develop a new model of ethanolic cardiac dysfunction in the Golden hamster; evaluate the mechanical, biochemical and histologic correlates of this model over time; and assess the effects of verapamil on preventing the functional and metabolic derangements caused by ethanol ingestion.

Methods

Study groups. Forty-five 2 month old 100 g Golden hamsters were entered into the study and were separated into nine groups of five hamsters each. There were three control groups, three groups receiving ethanol and three receiving ethanol mixed with verapamil. The ethanol was delivered in drinking water according to the following protocol: week 1, 10% ethanol; week 2, 20% ethanol; week 3, 30% ethanol; week 4, 40% ethanol; week 5 to termination of study, 50% ethanol. Ethanol plus verapamil (1.75 mg/cc) was administered to three groups in honey-flavored drinking water to counter the bitter taste of verapamil, using an identical ethanol regimen as in the ethanol groups. Honey was not added to the drinking water of the control or ethanol...
groups. The average fluid intake per animal was 13 cc/day in the control groups and 8 cc/day in the other groups. All animals were fed standard chow. Three groups were sacrificed and studied at 5 weeks and three parallel groups were studied at 7 and 12 weeks, respectively.

**Isolated perfused heart studies.** The hamsters were anesthetized with ether and the heart was removed. The isolated heart preparation was perfused by the Langendorff method. The heart was perfused in a modified Krebs-Henseleit solution containing 117 mM sodium chloride, 4.3 mM potassium chloride, 3.5 mM calcium chloride, 0.1 mM potassium dihydrogen phosphate (KH₂PO₄), 25 mM sodium bicarbonate, 0.6 mM sodium ethylenediaminetetraacetic acid, and 15 mM glucose. The perfusion pressure was 100 mm Hg. Pacing wires leading to a Grass stimulator were inserted into the base of the right ventricle and the heart was paced at 250 beats/min. A cannula exiting from the apex of the left ventricle was connected to a pressure transducer for left ventricular pressure measurements. The heart was perfused for 20 minutes before freeze-clamping, during which time physiologic measurements were made. In earlier studies, it was shown that the heart reached a stable energy level with this perfusion time (11).

**Termination of metabolic processes at a predetermined phase of the cardiac cycle** was accomplished using the stimulator-triggered freeze-clamp technique. Anvils, cooled by liquid nitrogen, were driven closed at 80 psi allowing the temperature in the center of the heart to drop to −80°C in 5 ms (12), instantaneously terminating metabolic processes. All processes described in this study were terminated during diastole. When the anvils were released, the frozen flakes of heart tissue dropped into liquid nitrogen. These studies were performed in accordance with the animal welfare regulations at this institution and with the guiding principles of the American Physiological Society.

**Biochemical analysis.** Extracts of heart tissue were prepared according to previously described methods (13–16). The frozen tissue was pulverized under liquid nitrogen; a 100 mg sample was removed, weighed, dried (110°C) and weighed again to assess wet to dry weight. Acid (−200°C, 3 ml 10% perchloric acid) was pulverized separately and added to the pulverized tissue. Pulverization under liquid nitrogen continued. The frozen mixture was transferred to a Sorvall tube and weighed and then transferred to a mortar. The weight of the cold tube and acid was used as a factor in calculating the final tissue weight (wet). The frozen mixture was brought to 0°C while pulverizing. After centrifugation of the mixture (20,000 g for 5 minutes), the extract was weighed, neutralized with 5 N potassium hydroxide and weighed again for determining volume. The mixture was centrifuged to remove potassium chloride, and the supernatant was stored at −80°C.

Analysis of nucleotide has been described in detail earlier (11). For separation of adenosine triphosphate (ATP) and adenosine diphosphate (ADP), a Beckman high performance liquid chromatograph with a C-18 reverse phase column was used. For the mobile phase 19% acetonitrile in 0.03 M KH₃PO₄ with 0.01 M tetrabutyammonium (TBA) phosphate (pH 2.65) was used. Elution was 1 ml/min and detection by a Beckman ultraviolet spectrophotometer at 254 nm. Adenosine monophosphate (AMP) was analyzed on the same column using the same conditions except elution was with 5% acetonitrile in 0.02 M TBA and 0.02 M KH₃PO₄. Adenosine was analyzed by injecting 10 µl of extract onto the same column using a 10 minute gradient of 0 to 20% methanol in water, followed by a 10 minute elution time at 20% methanol. Detection and volume elution were the same. Phosphocreatine and creatine were analyzed on the same column except elution was 1.5 ml/min with 0.2% KH₃PO₄ and 0.1% TBA, pH 2.65. Detection was at 210 nm. Cyclic adenosine monophosphate was determined by radioimmunoassay.

**Histologic analysis.** Histologic studies were performed on three randomly selected hearts from each group at 7 and 12 weeks to ascertain whether any structural changes occurred in the hamster myocardium that could be correlated with the associated mechanical and biochemical characteristics. The heart was cut into 2 mm slices and fixed at 4°C in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Tissue processing and plastic embedding were performed according to the method of Beckstead (17). Sections (2 to 3 µm) were stained with a modified Maximow’s stain (hematoxylin-eosin-azure) before examination with a light microscope. For electron microscopy, tissue was fixed overnight in a solution of 2.5% glutaraldehyde, 0.08 sodium cacodylate, 5 mM calcium chloride and 1% sucrose (pH 7.4) and postfixed for 2 hours in 1.5% osmium tetroxide in 30 mM barbital acetate buffer (pH 7.4). After dehydration in ethanol and embedding in Epon 812, semithin sections were cut and stained with toluidine blue for light microscopy. Representative areas from these sections (areas that appeared to have all of the normal elements of myocardium) were chosen for thin sectioning and were stained with uranyl acetate and lead citrate and examined in a JEOL 100S electron microscope.

**Statistical analysis.** Mechanical and biochemical characteristics were evaluated by analysis of variance using a linear regression model to test for the independent effects of time and assigned treatment and to assess the possibility

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Control</th>
<th>Ethanol</th>
<th>Ethanol Plus Verapamil</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>190 ± 15</td>
<td>205 ± 20</td>
<td>190 ± 24</td>
</tr>
<tr>
<td>7</td>
<td>215 ± 7</td>
<td>194 ± 24</td>
<td>190 ± 24</td>
</tr>
<tr>
<td>12</td>
<td>185 ± 7</td>
<td>195 ± 25</td>
<td>172 ± 30</td>
</tr>
</tbody>
</table>
of interactions between assigned treatment and time (for example, was there a change in response to the ethanol regimen over time in the hamsters that received ethanol alone). Differences were considered significant at probability ($p < 0.05$).

**Results**

There was no significant difference in body weight among the control, ethanol plus verapamil and ethanol groups at any time period (Table 1). Hamsters that were either in the ethanol or the ethanol plus verapamil group were subjectively different from the control animals; they appeared unkempt and had an unsteady gait.

**Hemodynamic measurements (Fig. 1).** Assessment of performance characteristics of the hamsters in the ethanol group at 7 and 12 weeks revealed a significant decrease in peak left ventricular pressure, maximal rate of rise in pressure and developed pressure when compared with control hamsters. There was no significant difference between ethanol and control groups in time to peak pressure, end-diastolic pressure or half-time to relaxation ($RT_{1/2}$). A comparison of the performance characteristics between 7 and 12 week hamsters in the ethanol group versus the verapamil plus ethanol group demonstrated preserved function in the verapamil-treated hamsters. Furthermore, verapamil-treated animals had higher peak pressures, rate of rise in pressure and developed pressures as compared with the ethanol group. No statistical differences were demonstrated in these variables between the verapamil plus ethanol and control groups at 7 and 12 weeks.

**Biochemical characteristics (Fig. 2).** There were significant decreases in phosphocreatine/creatine ratio, absolute adenosine triphosphate (ATP), ATP/adenosine diphosphate (ADP) ratio and adenosine for ethanol hamsters at 7 and 12 weeks compared with control values. Cyclic AMP in the ethanol group was 50% over the control value although this was not significant at the $p = 0.05$ level.

Biochemical characteristics of the verapamil plus ethanol group were significantly better than those of the ethanol group at 7 and 12 weeks for ATP/ADP, adenosine and phosphocreatine/creatine ratio (7 weeks only). Hamsters in the verapamil plus ethanol group at 7 and 12 weeks had biochemical characteristics that were not significantly different from control values. The data suggest that the effects of ethanol and the protective effects of verapamil did not become apparent until 7 weeks and remained stable at 12 weeks.

**Histology.** There were no histologic or ultrastructural abnormalities noted on either light or electron microscopy in the hearts from either the ethanol or the verapamil plus ethanol groups.

**Discussion**

The present study demonstrates depression of ventricular function in hearts removed from hamsters drinking ethanol for 7 to 12 weeks. The 7 week hamster group demonstrated

![Figure 1. Performance characteristics are shown for the three groups of hamsters (control, ethanol and verapamil plus ethanol) at 5, 7 and 12 weeks. Dev. = developed; dP/dt = maximal rate of rise in pressure; EDP = end-diastolic pressure; ETOH = ethyl alcohol; Peak Pressure = peak left ventricular pressure; $RT_{1/2}$ = half-time to relaxation; Time to Peak = time to peak pressure.](image-url)
the greatest depression in cardiac function and high energy phosphates. However, even hamsters in the 5 week group which was almost up to 50% ethanol ingestion, showed some evidence of cardiac dysfunction and a decrease in high energy phosphates. There was a moderate improvement in cardiac function and high energy phosphates by 12 weeks which may have been due to an adaptive increase in ethanol metabolism. Myocardial adaptation may have been due to induction of new isoenzymes or a shift in lipid synthesis, a compensatory mechanism allowing the animals to better tolerate the large doses of ethanol (18). Administration of verapamil concurrently with ethanol resulted in improved physiologic and biochemical characteristics at all stages. Pilot studies with verapamil in normal animals have suggested no effect of verapamil on function or metabolism. Thus, no verapamil control group was studied.

Effects on cardiac performance. Chronic ethanol ingestion may lead to decreased cardiac performance. This effect can occur independent of other nutritional factors and has been observed in vivo and in isolated muscle preparations (19–21). Alcohol is not metabolized by myocytes and the circulating concentration of acetaldehyde, the primary metabolite of ethanol, is negligible. It is unknown (18,22) whether ethanol ingestion damages the heart through direct metabolic interactions. The deleterious effects of ethanol on myocardial performance may be explained on the basis of a physical interaction of ethanol with cell membranes and proteins.

Effects on myocardial metabolism. Ethanol affects myocardial cells at a variety of sites. It is known that ethanol fluidizes biologic membranes and alters organelles (23). The fluidized membranes may affect the function of ionophores, preventing a normal ion flux (24). It has been demonstrated (7,25,26) that the presence of ethanol interferes with calcium transport by the sarcoplasmic reticulum (27). This causes increased hydrolysis of ATP, and wasting of cellular energy reserves. In the presence of ethanol, the sodium-potassium activated ATPase of the cardiac plasma membrane is inhibited (28–31).

By disturbing the cells’ normal homeostatic controls for calcium, energy production and contraction may both be directly interfered with by ethanol. Ethanol may therefore lead to a state of intracellular calcium overload due to leaky membranes or relative calcium deficiency in organelles such as the sarcoplasmic reticulum which require ATP to maintain normal calcium stores.

Depression of cardiac function by ethanol is not restricted to membrane effects. It has been shown that the association of the contractile proteins actin and myosin is inhibited in vitro in the presence of ethanol (32). Ethanol also blocks ATP production by directly inhibiting oxidative phosphorylation (33,34). Thus, ethanol seems to affect membrane-bound proteins, calcium transport proteins, mitochondrial respiration and contractile proteins (35–38).

Mechanisms of verapamil effects. Lindenmayer et al. (39) demonstrated that in the hereditary cardiomyopathy of the Syrian hamster, electron transport and calcium uptake were inhibited. The magnitude of these changes appeared to be related to the severity of the cardiomyopathy. Jasmin and Proschek (40) demonstrated that in this model verapamil was effective at maintaining mitochondrial function and protecting myocardial cells from necrosis.
We have previously demonstrated (9,10) that in the myopathic Syrian hamster ATP and cyclicAMP levels were depressed and these changes were associated with a 50% reduction in cardiac performance. Verapamil was found to preserve myocardial contractility (41,42) as well as the overall nucleotide pool (43) in the hereditary cardiomyopathy of the Syrian hamster.

Verapamil has been shown to have the following mechanisms of action: 1) Verapamil directly blocks the slow calcium channels (44). 2) Verapamil directly protects membranes and indirectly activates membrane-selective proteins such as sodium/potassium adenosine triphosphatase (AT-Pase), calcium ATPase and sodium-calcium exchanges (45).

Thus, the various mechanisms of action reported for verapamil correlate with the characteristics adversely influenced by ethanol ingestion. An ethanol-induced membrane disturbance can influence levels of high energy phosphates and cardiac function. Future studies with other calcium entry blockers may provide mechanistic clues. For example, in the inherited cardiomyopathy of the Syrian hamster, verapamil but not diltiazem or nifedipine preserved myocardium (46). This suggests that the beneficial effect of verapamil was not due to calcium entry blockade alone, but also to one of the mechanisms discussed previously.

Conclusion. We have demonstrated that oral ingestion of ethanol in hamsters produces depression of cardiac performance at 7 and 12 weeks. Biochemical correlates of this effect support the hypothesis that normal myocardial handling of high energy compounds is altered by ethanol administration; that ethanol-induced myocardial depression is likely to be associated with disorders in calcium transport by the myocardium; and that verapamil prevents depression of mechanical function by ethanol and preserves myocardial energetics.

Verapamil was kindly supplied by Knoll Pharmaceuticals.

References
30. Williams JW, Tada M, Katz AM, Rubin E. Effects of ethanol and...


