Original article

IMPACTS OF ETHANOL ON THE DEVELOPMENT OF VENTRICULAR FIBRILLATION IN HYPOTHERMIA

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Abstract  This study aimed to explore the acute effects of ethanol on the development of ventricular fibrillation induced in hypothermic condition and to determine how this was related to its concentration. Groups of Sprague-Dawley rats were given an intravenous bolus of either saline or ethanol (0.33, 0.66, 1 g/kg. body wt) following hypothermia induction, and a ventricular fibrillation threshold (VFT) was evaluated by means of an electrical-induced VF. The VFT was significantly decreased ($p<0.001$) in response to a high ethanol dose, whereas, it remained unaltered with low and moderate doses when compared to untreated hypothermic controls. There was a tendency for the decrease in serum $K^+$ and $Ca^{2+}$, which was caused by hypothermia, to return toward baseline following low and moderate ethanol infusion, but the statistical significance ($p<0.01$) was reached only for serum $Ca^{2+}$ in the moderate ethanol-treated group. Compared with the untreated hypothermic rats, high doses of ethanol increased serum $Mg^{2+}$, whilst low and moderate doses were ineffective. The results suggest that the impacts of ethanol on ventricular fibrillation induced in a hypothermic condition are dose-related. Low to moderate ethanol concentrations might have a beneficial role in increasing VFT by the restoration of serum $K^+$ and $Ca^{2+}$ from hypothermic consequences, whereas, a high ethanol concentration promotes the development of ventricular fibrillation, which appears not to be mediated by a deficit in serum $Mg^{2+}$ level. Chiang Mai Med Bull 2001;40:(3):119-126.

Hypothermia has been defined as a condition in which the core temperature is less than 35 °C.(1) In most instances, it can be developed following prolonged accidental exposure to extremely low environmental temperature. However, it may occasionally be intentionally induced as adjunctive therapy during some surgical procedures, such as neurosurgery and cardiovascular surgery, to protect the tissues from ischaemia caused by decreased tissue perfusion.(2-4) This advantageous effect of hypothermia is based on the findings that tissue oxygen requirement is concomitantly reduced with a decreasing body temperature.²³ Despite the beneficial outcome of hypothermia induction, ventricular fibrillation (VF) emerges as its detrimental effect, which proves to be a fatal consequence of this manipulation.(1,4)

Several mechanisms have been put forward to account for this serious complication. Decreased myocardial resting membrane potential, increased action potential duration, decreased myocardial ATP production and excess myocardial catecholamines have all been reported to be involved in the pathogenesis of hypothermia-induced ventricular fibrillation.(5) Furthermore, differentiation in a relative refractory period of various parts of the ventricles, caused by a temperature...
gradient, add another piece to the rational development of ventricular fibrillation in the hypothermic heart.\(^6\) Nevertheless, the underlying mechanisms responsible for ventricular fibrillation-associated hypothermia are thought to be multifactorial and, up until now, remain a matter of debate.

The contribution of ethanol to ventricular fibrillation has been suggested in both human and animal studies. Numerous epidemiological data have revealed that ingestion of low concentrations of ethanol might have cardioprotective properties, whereas, high doses increase risks for ischaemic heart disease and sudden cardiac death.\(^7\)\(^-\)\(^9\) Over the past several years, animal experiments have provided proof of this hypothesis. Evidence has accumulated to indicate that ethanol can produce differential haemodynamic and metabolic effects on a normothermic heart as well as differential mechanical and divalent cation effects on diverse mammalian coronary vessels, depending upon the concentration used.\(^5\)\(^,\)\(^8\)\(^,\)\(^9\)\(^,\)\(^10\)\(^7\) At a low concentration, ethanol clearly displays its beneficial effect through an increase in cardiac performance, coronary blood flow, and magnesium content together with a decrease in exchangeable and membrane-bound the calcium in the coronary vascular muscle. A low dose of ethanol has also been shown to reduce the contraction of the coronary vascular muscle, which is induced by vasoactive substances. In parallel, ethanol at a high concentration possesses a detrimental effect. It decreases coronary blood flow that leads to a loss of cellular magnesium, hypoxia, metabolic acidosis of the myocardium, cell membrane damage, and calcium overload, which could result in ventricular fibrillation and, hence, cardiac failure.

Since ethanol is shown to play a significant role in the normothermic heart and coronary vascular muscle, depending upon the concentration that consequently affects the ventricular fibrillation threshold (VFT), the question has arisen as to whether it could possibly do so in a hypothermic condition. From this view point, we assume that if the impacts of ethanol in normothermic and hypothermic conditions were comparable, the incidence of ventricular fibrillation during hypothermia might have been diminished by its low concentration, while the occurrence of ventricular fibrillation could possibly be potentiated with an increasing concentration. To test this hypothesis, three different concentrations of ethanol were applied and the VFT was evaluated.

**Materials And Methods**

**Animal preparation**

Male Sprague-Dawley rats weighing 350–370 g were purchased from the National Animal Center, Salaya Campus, Mahidol University. They were housed for a week before initiation of the experiments under controlled conditions at a temperature of 24–25 °C and light of 12 h light: 12 h darkness with free access to pelleted food and tap water.

**Surgical procedures**

Under sodium pentobarbital anaesthesia (45 mg/kg. body wt, IP), a tracheostomy was performed on each rat and the animal was allowed to breathe spontaneously. Both femoral arteries were cannulated. The left arterial cannula was connected to a Statham P23db pressure transducer for continuously monitoring the systemic arterial blood pressure on a Grass polygraph (model 7D), and the right cannula was used for arterial blood sampling. Another cannula was introduced into the left femoral vein for ethanol administration and/or to supplement the additional pentobarbital, as necessary. Lead II electrocardiographic electrodes were placed and an electrocardiogram (EKG) was continuously recorded. A rectal temperature probe was inserted and the core temperature was thus monitored via a Yellow Springs Telethermometer. A 30-min stabilisation period was allowed before the experiments began.
Experimental designs

Series 1: Effects of ethanol on VFT

To determine the potential impacts of ethanol on the development of ventricular fibrillation induced in a hypothermic condition, four different conditions were studied. These consisted of an intravenous bolus of either saline (n=12) or ethanol at a dose of 0.33 (n=11), 0.66 (n=9), or 1 g/kg body wt (n=7) to the hypothermic animals.

The surface cooling technique was used to lower the body temperature and hypothermia was considered complete when a core temperature reached 25 °C over a 30-min period. Cooling, however, was continued to maintain the core temperature at 25±0.5 °C for the duration of the experiment. At this time, the animals were artificially ventilated with room air by using a rodent respirator with a tidal volume set at approximately 10 mL kg body wt⁻¹ and 38 strokes min⁻¹. A left lateral thoracotomy was performed, the heart was exposed and a 1 mL of saline or ethanol was intravenously administered. Five minutes after the completion of the ethanol or saline injection, an arterial blood sample (0.5 mL) was collected for ethanol determination, and ventricular fibrillation was induced by a modified method of Debressine.(11) Briefly, bipolar electrodes, with were connected to an electrical stimulator (Model A385, World Precision Instrument, U.S.A), were positioned on the left ventricular anterior wall so as the anode was approximately 3 mm below the atrioventricular ring and the cathode on or near the apex. Square wave pulses (100-msec train of 0.8-msec pulses at 50 Hz) were passed through a constant current stimulus isolator unit. The initial current intensity was 0.1 mA and then increase at steps of 0.1 mA until ventricular fibrillation developed. The ventricular fibrillation threshold was defined as at least five irregular positive and negative deflections on the EKG, accompanied by zero arterial blood pressure following electrical stimulation.(12) The minimum current at which fibrillation occurred was considered to be the ventricular fibrillation threshold.

Series 2: Effects of hypothermia and ethanol on acid-base status and electrolytes

To investigate the influences of ethanol and hypothermia further on acid-base and electrolyte homeostasis, the experiments in Series 1 were repeated, but, in another set of rats. The experiments in Series 2 had to be performed to avoid the confounding effects of the haemodynamic disturbance following a large amount of blood sample withdrawal that could possibly affect the study outcome. Unlike Series 1, an additional group of normothermia was included, while the induction of ventricular fibrillation was excluded. Five minutes after the completion of the saline or ethanol injection, an arterial blood sample (2.5 mL) was collected for the determination of arterial blood pH, ethanol, Na⁺, K⁺, Ca²⁺ and Mg²⁺ concentrations. The serum sample was taken and stored at –20 °C until the day of assay.

Analytical methods

The blood ethanol concentration was determined using the gas chromatography head-space technique. Arterial blood pH was measured using a Radiometer Acid-Base Laboratory (Radiometer ABL3). Serum concentrations of Na⁺ and K⁺ were analysed by an Na-K electrode analyser (Model Nucleus, NOVA), and Mg²⁺ as well as Ca²⁺ by an Automated chemistry analyser (Model MEGA, Merck).

Statistical analysis

Data were expressed as mean ± SE. A one-way analysis of variance followed by a Scheffe’s post-hoc test was used to compare the mean values between the groups, and significance was taken at p<0.05.
Results
Surface cooling caused the body temperature to decrease, and the mean core temperature of all hypothermic groups was found to be in the range of 24.97–25.10 °C throughout the experimental period. Ethanol administration produced a dose-dependent increase in blood ethanol concentration, which was very similar between the two series of experimental studies. In the first series, mean blood ethanol concentrations were 59.82±10.87, 141.11±6.74 and 208.57±10.00 mg% for low, moderate and high ethanol-treated hypothermic animals, respectively, whereas, they were 63.00±2.48, 132.63±4.91 and 187.6±5.48 mg%, respectively, in the second series. It should be noted that the values of blood ethanol concentration in the groups of rats in the first series were not significantly different from those of the corresponding groups in the second series.

The effects of hypothermia and three distinct ethanol concentrations on heart rate and mean arterial blood pressure are demonstrated in Table 1. The initial values of these cardiovascular parameters between the four groups were comparable. Induction of hypothermia resulted in a significant decrease in heart rate of 35–39% \((p<0.001)\) and mean arterial blood pressure of 10–16% \((p<0.05)\) in all groups used in this study. In addition, heart rate and mean arterial blood pressure did not significantly alter following ethanol administration. This observation was evident in all ethanol-treated hypothermic groups. These values of cardiovascular variables in each ethanol-treated hypothermic group were found at the same extent as those seen in the untreated hypothermic group.

Figure 1 depicts the comparisons of VFTs obtained in all the experimental groups examined in this study. A high dose of ethanol given to hypothermic rats markedly and significantly decreased the VFT by some 57% \((p<0.001)\), in comparison with the VFT seen in the untreated hypothermic rats \((1.10±0.26 \text{ mA})\). In contrast to the high dose, administration of low or moderate doses of ethanol seemed to exert no influence on the VFT. It was noticeable that the VFT recorded in the low \((0.95±0.34 \text{ mA})\) and moderate \((0.98±0.21 \text{ mA})\) ethanol-treated hypothermic rats was comparable to that of the untreated hypothermic controls.

The arterial blood pH was slightly, but significantly, decreased \((p<0.05)\) after hypothermia induction and remained relatively constant at this level following three ethanol infusion doses (Table 2). Comparison between the ethanol-treated groups revealed that the value of arterial blood pH achieved from each individual group was very similar to each other.

The effects of hypothermia and ethanol on serum electrolytes were summarised in Table 2. It was apparent that neither hypothermia nor ethanol influenced the

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Heart rate (beat/min)</th>
<th>Mean arterial blood pressure (mmHg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Before hypothermia</td>
<td>After hypothermia</td>
</tr>
<tr>
<td>H (n=12)</td>
<td>348.67±15.54</td>
<td>215.00±0.29</td>
</tr>
<tr>
<td>HL (n=11)</td>
<td>365.45±0.42</td>
<td>232.55±5.92</td>
</tr>
<tr>
<td>HM (n=9)</td>
<td>348.00±13.34</td>
<td>225.11±3.83</td>
</tr>
<tr>
<td>HH (n=7)</td>
<td>377.14±10.18</td>
<td>228.00±4.14</td>
</tr>
</tbody>
</table>

Values are means ± SE. H, untreated-hypothermic group; HL, low dose ethanol treated-hypothermic group; HM, moderate dose ethanol treated-hypothermic group; HH, high dose ethanol treated-hypothermic group; \(^a\), \(p<0.05\); \(^b\), \(p<0.001\) vs. corresponding before hypothermic values.
Fig. 1. Effects of hypothermia and acute ethanol administration on the ventricular fibrillation threshold (VFT). H, untreated-hypothermic group; HL, low dose ethanol treated-hypothermic group; HM, moderate dose ethanol treated-hypothermic group; HH, high dose ethanol treated-hypothermic group. All values are reported as means± SE.

Table 2. Effects of hypothermia and acute ethanol administration on arterial blood pH and serum electrolyte concentrations.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Arterial blood pH</th>
<th>Serum electrolyte concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Na⁺ (mmol/L)</td>
</tr>
<tr>
<td>N (n=8)</td>
<td>7.41</td>
<td>141.88±0.85</td>
</tr>
<tr>
<td>H (n=10)</td>
<td>7.36*</td>
<td>143.50±0.67</td>
</tr>
<tr>
<td>HL (n=7)</td>
<td>7.33*</td>
<td>143.14±0.85</td>
</tr>
<tr>
<td>HM (n=8)</td>
<td>7.33</td>
<td>141.75±0.56</td>
</tr>
<tr>
<td>HH (n=5)</td>
<td>7.32</td>
<td>141.40±1.08</td>
</tr>
</tbody>
</table>

Values are means ± SE. N, normothermic group; H, untreated-hypothermic group; HL, low dose ethanol treated-hypothermic group; HM, moderate dose ethanol treated-hypothermic group; HH, high dose ethanol treated-hypothermic group; *p<0.05; †p<0.01 vs. corresponding normothermic group; ‡p<0.01, untreated vs. ethanol treated-hypothermic groups.

Serum Na⁺ concentration in any of the experimental groups. On the contrary, hypothermia produced a decrease in serum K⁺ by some 10%, albeit not significant, from that of the normothermia. The response of serum K⁺ to the administration of three ethanol doses showed a tendency to increase and return toward the normothermic value. Although serum K⁺ concentrations were numerically higher in all ethanol-treated
hypothermic rats than those of the untreated hypothermic rats, the differences did not reach a statistically significant level. The value of serum $K^+$ was also not significantly different among the three ethanol-treated groups. Similar findings were observed in serum $Ca^{2+}$ concentrations. It appeared that hypothermia reduced serum $Ca^{2+}$ from normothermia by 9%. The already low value of serum $Ca^{2+}$ tended to increase with low and moderate ethanol infusions, but the increment proved to be significant only with the moderate dose, being increased by 39% from the untreated hypothermic group ($p < 0.01$). The marked increment in serum $Ca^{2+}$ of the moderate ethanol dose was obviously higher than that of the low and high ethanol doses ($p < 0.01$). With respect to serum $Mg^{2+}$, the lower serum $Mg^{2+}$ from the normothermic value was noticed after the induction of hypothermia. Serum $Mg^{2+}$ did not decrease any further with the low and moderate ethanol doses, but it increased by 15%, albeit not significantly, with the high ethanol dose.

**Discussion**

The present study provides evidence to suggest that ethanol can produce differential effects on the incidence of ventricular fibrillation in hypothermic condition depending upon the concentration used. A high concentration of ethanol clearly reduces the VFT and promotes ventricular fibrillation, whereas, low to moderate concentrations seem to play no prominent role on the development of ventricular fibrillation in hypothermia.

The surface cooling technique was utilised to produce hypothermia in the current investigation and all animals enrolled had their core temperature maintained at about 25 °C over the course of the experiment. This core temperature was shown to be the temperature level in which ventricular fibrillation spontaneously occurred at a high incidence.\(^{(13)}\) Nevertheless, the approach of electrical-induced ventricular fibrillation was employed to assess the potential impact of ethanol in the present study. This design was selected in order to avoid the inconsistency of spontaneous ventricular fibrillation induced by hypothermia, which may affect the outcome of the experiment. The influence of ethanol on the development of ventricular fibrillation during hypothermia was evaluated on the basis that if ethanol has a protective effect against ventricular fibrillation, it should cause the VFT to increase during hypothermia. If not, the VFT should be unaltered and, if ethanol facilitates the occurrence of ventricular fibrillation, the decreased VFT should be noticed.

The results obtained, herein, demonstrated that there were no significant changes in the VFT following ethanol infusion, in either low or moderate concentrations, compared to the VFT recorded in hypothermia alone, whereas ethanol at a high concentration significantly decreased the VFT. Since ethanol has been shown to alter cardiovascular dynamics depending upon the concentration used,\(^{(5,10)}\) haemodynamic changes were the primary mechanism that could possibly account for these observations. **In vitro** studies using isolated heart and coronary vessels have suggested that low concentrations of ethanol increased stroke volume, cardiac output and reduced vascular contractions induced by vasoactive substances, while high concentrations produced the opposite effects.\(^{(5)}\) **In vivo** studies have also demonstrated that ethanol evoked alterations in heart rate, stroke volume, cardiac output, systemic vascular resistance and systemic blood pressure that are dose-related.\(^{(10)}\) In the current study, despite hypothermia causing a significant decrease in heart rate and mean arterial blood pressure, the parameters did not change further following ethanol administration regardless of the concentrations used. The findings implied that any changes in the VFT caused by ethanol in this study were
not mediated by alterations in these cardiovascular variables.

Disorder of the acid-base status is another point of consideration. Previous publications have shown that the VFT was decreased by acute metabolic acidosis and increased by metabolic alkalosis.\(^{(14-15)}\) Increased excitability of the myocardium has been proposed as the mechanism by which acidosis favoured ventricular fibrillation.\(^{(14-15)}\) Our data provided additional supporting evidence of a contribution of acidosis in mediating ventricular fibrillation during hypothermia through the observation that arterial blood pH was significantly decreased after hypothermia induction. However, the current finding did not support a role for ethanol-induced acidosis in modulating the VFT during hypothermia, since arterial blood pH following ethanol administration remained relatively constant at the same level as hypothermia alone.

Substantial evidence exists of the involvement of electrolyte abnormalities in the etiology of ventricular fibrillation. Decreased serum K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) have all been reported to associate with an increased incidence of ventricular fibrillation.\(^{15,16,17}\) A decreased serum K\(^+\) was noticed in the present study once hypothermia was induced, but there was a tendency of serum K\(^+\) to return toward baseline level after ethanol infusion. These K\(^+\) alterations were observed in all ethanol-treated groups. The changes in serum Ca\(^{2+}\) were found to follow the same pattern as K\(^+\), with the exception of the effect of ethanol to raise serum Ca\(^{2+}\) from hypothermic consequences, which was not apparent in the high dose ethanol-treated group. These pieces of evidence called attention to the possibility that ethanol, at low to moderate concentrations, might have the beneficial effect of dampening the incidence of ventricular fibrillation down by blunting the decrease in K\(^+\) and, in particular, Ca\(^{2+}\) that is caused by hypothermia. Consequently, the counterbalance between decreasing the VFT by hypothermia and increasing it by ethanol could result in an unaltered VFT, as observed in the present study.

The available data from the current investigation provided no clear consensus regarding the decreased VFT obtained in high ethanol concentration-treated hypothermic animals. However, it is unlikely that a deficit in serum Mg\(^{2+}\) is involved, since serum Mg\(^{2+}\) was shown to be slightly, albeit not significantly, increased following ethanol administration.

The outcomes of this study not only extended our knowledge on the mechanisms of ventricular fibrillation-associated hypothermia, but also gave rise to several interesting points. Firstly, it supported the view that low ethanol concentrations possessed cardioprotective properties, whereas, high concentrations increased risks of the development of VF. Secondly, though further studies are needed, it opened up the likelihood that ethanol may be another candidate that is valuable in the prevention of hypothermia-induced ventricular fibrillation. Ethanol could possibly be applied to clinical use where hypothermia had to be manipulated and ventricular fibrillation was the undesirable fatal consequence. Finally, methanol toxicity patients should be aware of accidental ventricular fibrillation, where intravenous ethanol infusion was used as a therapeutic strategy at the same hight dose concentration in this study. In view of this, careful monitoring of the core body temperature may be helpful to lessen the potential effect of hypothermia on ethanol-induced ventricular fibrillation.

**Conclusion**

The results obtained, herein, demonstrate that the impacts of acute ethanol administration on ventricular fibrillation induced in a hypothermic condition are dose-related. At a high concentration, ethanol facilitates the development of ventricular fibrillation, as
exemplified by a decrease in the VFT. Although the mechanism whereby ethanol exerts this detrimental effect remains uncertain, it appears not to be mediated by a deficit in serum Mg$^{2+}$ level. The effects of low to moderate concentrations of ethanol on the VFT in the hypothermic heart are not obvious, but they might have the beneficial role of increasing the VFT by restoring serum K$^+$ and Ca$^{2+}$ from hypothermic consequences. This possibility merits further investigation.

Acknowledgement
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References