Effects of Propofol on Ischaemia-Induced Ventricular Arrhythmias in Rats

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Abstract

The effects of anesthetics on ischemia-induced ventricular arrhythmias are still poorly studied.

The Aim of the Study: Is to investigate the effects of propofol on ischaemia-induced ventricular arrhythmias, and defined a possible mechanism for the arrhythmogenic effect of propofol during acute myocardial ischemia.

Under anesthesia with intraperitoneal sodium pentobarbital male albino rats were divided into 4 groups (each 10 rats); The first group was the control group, second group was sham operated group, the third group was propofol-treated group (given propofol 39mg/Kg/hr), and the fourth group was propofol (39mg/Kg/hr), and atropine (1mg/kg) treated group.

Results: In propofol-treated group marked reduction of ventricular arrhythmia was observed; in propofol atropine-treated group moderate reduction of ventricular arrhythmia was observed.

Conclusion: Propofol has beneficial effects on ischemia-induced arrhythmias via modulation of the autonomic nervous system and/or a principal gap-junction protein, connexin 43 (Cx43).

Key Words: Coronary artery ligation – Propofol – Connexin43.

Introduction

PROPOFOL is widely used during anesthesia in patients with ischemic heart disease. These patients are at high risk for perioperative myocardial ischemia (MI), which is a major factor leading to lethal ventricular arrhythmias. Halothane, enflurane, and isoflurane are known to facilitate reentrant excitation in subjects with MI [1]. However, the effects of propofol on ventricular arrhythmias related to MI remain unknown, even though many studies have suggested that propofol has cardioprotective effects on functional, metabolic, and histologic changes caused by ischemia or reperfusion injury [2].

In the ischemic myocardium there is a reduction of tissue pH, an increase in interstitial potassium levels and intracellular calcium concentration, and neurohumoral changes that all contribute to the development of electrical instability and lethal cardiac arrhythmias [3]. In particular, cell-to-cell electrical uncoupling of ventricular myocytes is important in arrhythmogenesis during acute MI [4,5].

Although the ischemia-induced arrhythmias is the result of a various interacting factors, sympathetic overactivity is important in the generation of acute [6] and chronic [7] ischemia-induced arrhythmias.

MI causes increase in sympathetic nerve activity in the heart, resulting in regional variation in the release and, consequently, variations in tissue levels of sympathetic neurotransmitters (epinephrine and norepinephrine) [3].

Connexin 43 (Cx43) is remarkably reduced in ischemia and heart failure [8,9].

The aim of study is to investigate the effects of propofol on the survival rate and morbidity as a result of ventricular arrhythmias, and defined a possible mechanism for the arrhythmogenic properties of anesthetics during acute myocardial ischemia.

Material and Methods

A- Drugs & reagents:
1- Propofol: Propofol sandoz is a milky, white liquid in a clear glass vial with an aluminum and violet plastic cap. It is available as a sterile emulsion.
2- **Atropine sulfate**: Sigma chemical co. USA, powder dissolved in distilled water.

B- **Animals**:
- Male albino rats weighing 250-300g are used for the experiment.

**Animal grouping and design of the work**:
I- Normal control rat (10 rats).
II- Sham operated group: Ligated non-treated group (10 rats).
III- Propofol treated group: 39mg/kg/hr (10 Rats).
IV- Propofol and Atropine treated group: Atropine 1mg/kg was given just before propofol which was continuously infused IV at a dose 39 mg/kg/hr (10 Rats).

This study was done in Pharmacology Department, Faculty of Medicine, Cairo University in period 2011 – 2013.

**Surgical preparation and coronary artery ligation**:
After induction of anesthesia with 50mg/kg intraperitoneal sodium pentobarbital, the rats were intubated and ventilated artificially with a volume-controlled rodent respirator at 65-80 strokes/min.

After induction of anesthesia, the femoral vein was cannulated for drug administration.

A thoracotomy in the fourth intercostal space, and a suture will loosely tied around the left anterior descending (LAD) coronary artery. Electrodes placed to allow the measurement of a Lead II electrocardiogram. Mean blood pressure (MBP) and heart rate (HR) recorded. Body temperature monitored with a rectal thermometer and maintained at 37°C using a heating pad and overhead lamp. After preparation for LAD ligation, drugs administered according to the group assignment. The rats allowed to stabilize for 15min after administration of all drugs, and then MI induced for 30min by LAD ligation. MI in the groups with LAD ligation confirmed visually by the appearance of regional cyanosis and by ST segment changes in the electrocardiogram.

1- **ECG (power lab, ad instrument)** arrhythmia study:**

**Arrhythmia study**:
- Each arrhythmia was defined according to the guidelines of the Lambeth Convention [10].
- Each arrhythmia was defined according to the method described by Leenen et al., [11].
- 0 for normal sinus rhythm, 1 for premature ventricular contractions, 2 for nonsustained ventricular tachycardia (VT) within 10 beats, 3 for spontaneously reversible VT over 10 beats or reversible VF within 10s, 4 for sustained VT or reversible VF with precordial taps, and 5 for irreversible VF causing death. The highest arrhythmia score was recorded for each 5-min period during 30min of LAD ligation (Walker et al., 1988).

2- **Arterial blood pressure (Ugo Basile, Italy)**:
- The hemodynamic parameters recorded before ligation, 15 minutes after drug/anesthetic administration and 30min after LAD ligation.

3- **Determination of ischemic areas**:
- The ischemic area was determined by negative staining with Evans blue.
- The heart was removed and perfused retrograde-ly with 10ml of 0.9% saline to wash out blood from the coronary circulation. Then, 2ml of 2% Evans blue was injected to confirm the lack of perfusion of the ischemic area. The ischemic area was measured.

4- **Biochemical parameters**:
- Cardiac enzymes (Troponin, Ck-MB, LDH) were measured 2 hours after coronary artery ligation.

**Statistical analysis**:
- All collected questionnaires were revised for completeness and consistency. Pre-coded data was entered on the computer using “Microsoft Office Excel Software” program (2010) for windows. Data was then transferred to the Statistical Package of Social Science Software program, version 21 (SPSS) to be statistically analyzed.
- Data was summarized using mean, and standard deviation for quantitative variables and frequency and percentage for qualitative ones.
- Comparison between groups was performed using one way ANOVA with Tukey’s post hoc test for quantitative variables and Chi square or Fissure exact test for qualitative ones.
- P-values less than 0.05 were considered statistically significant, and less than 0.01 were considered highly significant.
Results

Group I: Normal control rat:
Serum troponin, CK-MB and LDH level (Mean±SD) after 2 HOURS was NEGATIVE, 3.8±2ng/ml, 175±67.7 IU/L (Fig. 3, Table 3).

Heart rate was 391±9.9 and mean blood pressure was 120±11.5 (Figs. 1, 2, Tables 1, 2).

Histopathologically the heart showed no difference after 2 hours & Revealed Score 0 arrhythmia (Fig. 4).

Group II: Sham operated (ligated non treated) control group:
Serum troponin, CK-MB and LDH level (Mean±SD) after 2 HOURS positive, 82.5±14.2 ng/ml, 475±151.4IU/L with highly significant difference in comparison to the normal control group $p<0.001$ (Fig. 3, Table 3).

Heart rate was 401±14.7 insignificant $p$-value 0.1 and mean blood pressure was 121±11.5 with no significant difference in comparison to the normal control $p$-value 1. (Figs. 1, 2, Tables 1, 2).

Histopathologically the heart showed infarcted area 55.7±6.2% LV & Revealed Score I, II, III, IV 40% of cases arrhythmia 60% no arrhythmia (Fig. 5).

Group III: Propofol treated group:
Propofol was given at sedative dose 39mg/kg hr$^{-1}$ (confirmed by the tail-clamp technique).

Serum troponin, CK-MB and LDH level (Mean±SD) after 2 HOURS positive, 73.0±11.6 ng/ml, 340.0±73.8IU/L with highly significant difference in comparison to the normal control ($p<0.001$) ($p$-value <0.001) (Fig. 3, Table 3).

Heart rate was 343.0±8.2 and mean blood pressure was 98.5±8.2 with highly significant difference in comparison to the normal control ($p<0.001$). (Figs. 1, 2, Tables 1, 2).

Histopathologically, showed infarcted area 51.0±5.9% LV after 2 hours & Revealed Score 0 arrhythmia.

Group IV: Propofol atropine group:
Atropine was given as 1mg/kg bolus followed by a continuous infusion of propofol 39mg/kg/hr.

Serum troponin, CK-MB and LDH level (Mean±SD) after 2 HOURS was positive, 74.5±13.8ng/ml, 320.0±42.2 IU/L with highly significant difference in comparison to the normal control ($p<0.001$ (Fig. 3, Table 3).

Heart rate was 389±22, and mean blood pressure was 103±10 with no significant difference in comparison to the normal control ($p$-value 0.9, 0.6). (Figs. 1, 2, Tables 1, 2).

Histopathologically the heart showed infarcted area 51.3±4.4% LV after 2 hours & Revealed Score 0 arrhythmia in 90% of cases and score I in 10% of cases.

Table (1): Comparison between the 4 groups as regards mean blood pressure mmhg after 30 minutes of coronary artery ligation.

<table>
<thead>
<tr>
<th></th>
<th>G I</th>
<th>G II</th>
<th>G III</th>
<th>G IV</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Bl. Pre.</td>
<td>121.0±11.5</td>
<td>121.0±11.5</td>
<td>98.5±8.2</td>
<td>103.0±10</td>
<td>&lt;0.001</td>
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</tbody>
</table>

Table (2): Comparison between the 4 groups as regards heart rate/min.

<table>
<thead>
<tr>
<th></th>
<th>G I</th>
<th>G II</th>
<th>G IIIb</th>
<th>G IVb</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td>391.0±9.9</td>
<td>401.0±13.7</td>
<td>343.0±8.2</td>
<td>389±22</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Histopathologically, showed infarcted area 51.0±5.9% LV after 2 hours & Revealed Score 0 arrhythmia.
Table (3): Comparison between the 4 groups as regards CK-MBng/ml.

<table>
<thead>
<tr>
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<th>G I</th>
<th>G II</th>
<th>G III</th>
<th>G IV</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB</td>
<td>3.8±2.0</td>
<td>82.5±14.2</td>
<td>73.0±11.6</td>
<td>74.5±13.8</td>
<td>&lt;0.001</td>
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Discussion

Antiarrhythmic effect of propofol including the action on Cx43, a reduction in sympathetic tone leading to a dominance of parasympathetic tone, to inhibit action potential duration (APD) [12] sarcolemmal L-type Ca\(^{2+}\) channels, K\(^{+}\) channels [18] and the Ca\(^{2+}\) uptake capacity of the sarcoplasmic reticulum [14]; so, propofol has multiple sites of action in the cardiac cells that could contribute to antiarrhythmic effects in our study.

Intralipid, as a solvent for propofol, has been reported to have no effect on ischemia-reperfusion injury [15,16]. Intralipid itself did not affect hemodynamics and ischemia-induced arrhythmias.

Anesthetics and connexin 43:

Many studies have demonstrated that Cx43 is remarkably reduced in ischemia and heart failure.

Gene targeting studies show that reduced expression of Cx43 increases the incidence of ventricular tachyarrhythmias [17] and causes a significant reduction in conduction velocity in mice during acute MI [18]. Beardslee et al., reported that reversible Cx43 dephosphorylation could also contribute to myocardial cellular uncoupling, and thus play a role in arrhythmogenesis during acute ischemia. One potential mechanism of ischemia-induced accumulation of dephosphorylated Cx43 is decreased intracellular ATP concentration and decreased thermodynamic driving force for phosphorylation (the free energy change of ATP hydrolysis). The decrease in the free-energy change of ATP hydrolysis during ischemia is biphasic, with a moderate immediate decrease and a marked secondary decrease that coincides with cell-to-cell uncoupling [19].

Propofol prevented the loss of P-Cx43 and the increased expression of NP-Cx43, regardless of the dose, during 30min of LAD ligation.

Many studies have demonstrated that vagal nerve stimulation prevented ventricular fibrillation after myocardial infarction in rats [20] and dogs [21]. Ando et al., [20]. Reported that vagal nerve stimulation exerted an antiarrhythmic effect during acute MI by preserving phosphorylated Cx43. Several mechanisms might be involved in the linkage between vagal nerve stimulation and the preservation of protein levels of phosphorylated Cx43 during MI. Vagal nerve stimulation may activate several protein kinases and induce phosphorylation of Cx43 through muscarinic receptors.
Conclusion:

The present study stated that Propofol has differential effects on ischemia-induced arrhythmias via modulation of the autonomic nervous system and/or a principal gap junction protein, Cx43. One of the mechanisms of propofol’s antiarrhythmic effect during myocardial ischemia might be preservation of phosphorylated-Cx43 protein during myocardial ischemia.

Results of the present study are in agreement with Hirata et al., 2009 which stated Differential Effects of Propofol and Sevoflurane on Ischemia-induced Ventricular Arrhythmias and Phosphorylated Connexin 43 Protein in Rats [23].

Recommendation:

The results of the present study recommend to use propofol in patient with IHD in the induction and maintenance of general anesthesia, sedation for mechanically ventilated adults, and procedural sedation and for inducing sedation and amnesia before medical procedures.

References