# Experimental Membrane-Destructive Model of Cardiac Arrhythmia

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We propose a method for experimental modeling of cardiac arrhythmias. The method consists in intravenous injection of LPO inductors: 5% ascorbic acid (50 mg/kg), 1 min later 1% iron sulfate (10 mg/kg), and after the appearance of giant *T* waves on ECG infusion of 10% calcium chloride in a nonarrhythmogenic dose 100 mg/kg. Cardiac arrhythmias were induced in 100% animals. A significant relationship between increased permeability of erythrocyte membranes and development of fatal cardiac arrhythmias was detected. We assumed that this methodologically simple membrane-destructive model of cardiac arrhythmia is pathogenetically close to arrhythmogenesis in patients with coronary heart disease.

**Key Words:** *cardiac arrhythmia; experimental simulation of arrhythmia; membrane-destructive cardiac arrhythmia*

Experimental cardiac arrhythmia (CA) induced by cardiotoxins (aconitine, veratrine, barium chloride, *etc.*) is widely used in preclinical studies of the efficiency and safety of antiarrhythmic agents. However, disturbances in electrophysiological processes and appearance of CA as a result of cardiotoxin effects on the myocardium can hardly be considered as simulation of the natural pathological processes. A more adequate experimental model of CA is reperfusion CA pathogenetically associated with activation of LPO and free-radical damage to cardiomyocyte sarcolemma [6]. This model is associated with impairment of the barrier function of cell membranes, $Ca^{2+}$ and $Na^{+}$ entry into myocardial cells, and $K^{+}$ and $Mg^{2+}$ leakage along the electrochemical gradient of these ions. Intracellular ionic imbalance is responsible for the impairment of electrophysiological properties of myocytes and electrical instability of the myocardium [1,4,6]. Reproduction of reperfusion CA in animals requires occlusion of the coronary artery with subsequent restoration of the blood flow, which involves inevitable thoracotomy and artificial ventilation. Severe mechanical injuries of the thorax and artificial ventilation, in turn, modulate the biochemical processes, *e.g.* LPO. Moreover, the creation of a model of reperfusion CA is technologically difficult and time-consuming.

The aim of this study was to create an experimental model of CA maximally close to natural conditions of CA development in coronary patients, when destruction of the sarcolemma by LPO products plays the key role in the formation of foci of ectopic arrhythmogenesis.

## MATERIALS AND METHODS

Experiments were carried out on 30 random-bred albino rats of both sexes (120-180 g). The animals were divided into control ($n$=10) and experimental ($n$=20) groups. ECG in lead II was recorded under Nembutal narcosis (40 mg/kg intraperitoneally); after that experimental animals were injected (into the femoral vein) with LPO inductors: 5% ascorbic acid in a dose of 50 mg/kg, and after 1 min freshly prepared 1% iron sulfate (10 mg/kg) was slowly infused through the same needle. Giant *T* waves were recorded on ECG as soon as 1.5-2.0 min after the end of infusion in all rats. These shifts reflected impaired ventricular repolarization due to LPO-induced damage to the sarcolem-

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ma. At this moment 10% calcium chloride (100 mg/kg) was intravenously infused (over 4-5 sec) to all rats and ECG was recorded continuously until animal death. Calcium chloride dose used in the experiment did not induce CA in intact animals.

Serum MDA was measured routinely (photometry) and the results were expressed in optical density units. For evaluation of LPO-induced changes in plasma membrane permeability and investigation of the pathogenetic relationship between membrane destruction and CA development, the blood was collected during ventricular fibrillation. Osmotic resistance of erythrocytes (ORE) was evaluated by Libik and Rabir method, erythrocyte membrane permeability (EMP) was measured using working solutions of urea and sodium chloride [3]. Hemolysis was evaluated in percent of optical density of the reference sample with 100% hemolysis. Optical density of hemolyzed blood was evaluated by photometry using green filter against distilled water at $\lambda$=540 nm in 2-ml cuvettes. The data were statistically processed using routine methods.

## RESULTS

No appreciable changes in ECG were recorded after intravenous infusion of ascorbic acid. In 8 rats a short-term (2-3 sec) increase in *R* wave amplitude by 2-3 mm was observed. Subsequent infusion of iron sulfate led to progressive changes in the shape and amplitude of ventricular complex in all animals: *R* wave amplitude increased, *QRS* complexes became wider because of decelerated intraventricular conduction, the amplitude of *T* waves progressively increased, approaching a height of *R* waves in 12 rats. However after 1.0-1.5 min the amplitude of *T* waves suddenly decreased, which indicated impaired cation-transporting function of cell membranes. After a slow (over 3-4 sec) intravenous infusion of CaCl solution second- and third-degree AV block was recorded in 14 rats; signs of decelerated intraventricular conduction reappeared in all animals. The conduction gradually recovered over subsequent 20-25 sec, regular sinus rhythm was restored, the amplitude and shape of ventricular complexes approached those before injection of LPO inductors. It seems that sarcolemma was destroyed during this period, because 2-3 min later the animals developed polytopic extrasystolic arrhythmia, *"R* to *T"* extrasystoles (in 50% animals), which soon transformed into pirouette tachicardia, ventricular palpitation and fibrillation in all rats. In 40% animals ventricular fibrillation developed suddenly without previous cardiac arrhythmia. All animals died because of fatal heart rhythm disorders.

LPO activation and lipid oxidation products had a pronounced effect on erythrocyte membrane permeability in samples with initially low urea concentration, and their significance decreased with increasing urea concentration. Therefore we took into consideration the values in samples with 1:3 volume ratio of the urea to 0.9% NaCl solution.

Ascorbic acid and iron sulfate 3.3-fold increased the content of MDA, which confirms sharpe activation of LPO *in vivo* (Table 1). Evaluation of EMP in the presence of these concentrations of MDA indicate its damaging effect on cell membranes, which is confirmed by a pronounced trend to a decrease in erythrocyte osmotic resistance under the effect of LPO activators.

The pathogenetic significance of LPO activation and its effects on impairment of the barrier function of cell membranes in the development of CA are confirmed by numerous experimental and clinical studies [1,4,7,8]. In addition, LPO products modulate the sensitivity of cardiomyocyte receptor system, which is also significant for excitability and potentiates ectopic automatism [4,5]. We hypothesized that CA in experimental animals were not caused by ascorbate-dependent enzyme reaction of superoxide radical generation. This radical is little active towards the molecular components of myocardial cells. At the same time, initiation of the Haber—Weis reaction with the formation of hydroxyl radicals is possible in the presence of iron ions [1,4]. The mechanism of damage caused by hydroxyl radicals includes modification of protein structures, polysaccharide and lipid components of cell membranes leading to fragmentation and destruction of the sarcolemma with the formation of hydroxyl clusters enabling the entry of $Ca^{2+}$ excess into cardiomyocyte sarcoplasma by the common diffusion mechanism along with the electrochemical gradient of these ions [1]. These channels can spontaneously increase in size, and ion current through the membrane

**TABLE 1.** MDA Levels, Osmotic Resistance of Erythrocytes, and Erythrocyte Membrane Permeability (*M*±*m*)

| Group | MDA | ORE | EMP, opt. density units | EMP, % |
|---|---|---|---|---|
| Control | 0.063±0.010 | 0.45-0.65 | 0.041±0.030 | 12.75±7.10 |
| Experiment | 0.216±0.020* | 0.5-0.7 | 0.136±0.030* | 25.2±5.1* |

**Note.** *$p$<0.05 compared to the control.

increases until its mechanical destruction and complete loss of its barrier function. This leads to electrical auto-breakdown of the membrane, impairment of the electrical activity, and cell death. Additional intravenous infusion of calcium chloride augments intracellular ionic imbalance and accelerates the development and progress of CA.

Hence, intravenous infusion of $Fe^{2+}$-ascorbate-dependent LPO inductors to experimental animals impairs electrical activity of cardiac myocytes, which manifests in the development of polytopic extrasystole and fatal arrhythmias. We detected a relationship between accumulation of MDA, increase in erythrocyte membrane permeability after LPO induction, and development of cardiac arrhythmia.

The experimental model of membrane-destructive cardiac arrhythmias is reproducible in 100% animals and can be used for evaluation of the efficiency of antioxidants and membrane-protective antiarrhythmic drugs. The proposed CA model is easy and can be used under laboratory conditions for screening studies of antiarrhythmic effects of membrane protective drugs and antioxidants.

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