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Pharmaco-Electrophysiology of Isolated Perfused Rat Heart Assessed with Flexible Microelectrode Arrays

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Abstract

Background: Heart slices and enzymatically dissociated cardiomyocytes are used in cardiac safety pharmacology for extracellular recording using microelectrode array (MEA). The aim of this study was to set up and validate a vitro cardiac surface mapping system for studies pharmaco-electrophysiology effects in Langendorff perfused rat hearts by flexible MEA.

Materials and Methods: Hearts isolated from Sprague-Dawley (SD) male rat of either sex weighing 200-250 g were perfused by the Langendorff method with Tyrode's solution. A cardiac surface mapping system suitable for recordings from Langendorff-perfused rat hearts using the Class III antiarrhythmic agents has been developed. In 48 Langendorff-perfused rat hearts, after obtaining baseline data, ibutilide, amiodarone or dofetilide were infused. The field potentials (FP) and heart rhythm by Multi-channel flexible MEA were monitored throughout the experiments.

Results: Langendorff perfusion enabled the autorhythmicity of the rats' heart last about 240 min. Simultaneous 64 channels FP graphs could be recorded stably. FP duration revealed significant, dose-dependent prolongation more than 2 fold upon administration of three drugs, but present a different proarrhythmic properties (dofetilide> ibutilide>amiodarone). Dofetilide and ibutilide lead to early afterdepolarizations (EAD) and ventricular tachycardia (VT) but not amiodarone. Amiodarone led to atrial ventricular block, atrial flutter and junctional escape rhythm, in the presence of ibutilide or dofetilide, neither EAD nor VT occur.

Conclusion: Our model with isolated rat heart and flexible MEA represents a novel and reliable tool for application in cardiac safety pharmacology and preclinical studies of electrophysiological effects of various pathophysiological concepts.

Keywords: Flexible microelectrode array; Cardiac safety pharmacology; Langendorff perfused rat heart

Introduction

It is well known that drug-induced QT interval prolongation predisposes patients to develop potential life-threatening torsades de pointes (TdP). To date, the QT intervals still are the most accepted surrogates by the authorities [1,2]. Safety pharmacology and the development of novel antiarrhythmic drugs (AAD) needs new systems for the detection of toxic side effects on the heart in very early stages of preclinical development [3-5]. These model systems rang from cellular to organic level [6-8].

To date, there is not system available which can show the complex electrophysiology of the heart. So there exists the great need for suitable and improved model systems.

MEA enable non-invasive long-term extracellular recordings of action potentials from multiple channel [9]. MEA system consist of multiple electrode, thus heart slices and enzymatically dissociated cardiomyocytes have been used in cardiac safety pharmacology for extracellular recording using MEA [10-12], such as extracellular action potential signal shapes, cardiac rhythmicity and conductivity [9,13,14]. The correlation of field data with MEA and monoaction potential as well as QT has been shown by previous studies [15,16]. In contrast to conventional *in vitro* electrophysiological technologies, a sophisticated and labor-intensive nature, invasive characteristics and absence of cell-cell-interactions were not needed. The isolated Langendorff perfused rat heart has been used to test the cardiac physiological and pharmacological parameter in numerous experiments [17-19]. But until now, it still lack of the technique to use Langendorff perfused heart to assess the cardiotoxicity on flexible MEA. Another advantage of the flexible MEA system is it can detect cardiac conductibility and arrhythmia *in vitro* and *in vivo*.

Recently, we have reported MEA was used for detecting electrical-conduction function following myocardial infarction in rats [20]. It is well known that III class AAD are known to have proarrhythmic potential, QT prolongation are a surrogate marker for cardiotoxicity.

Therefore, in the present study, we aimed to illustrate the effect of three known class III AAD on FP parameters and their proarrhythmic potential in Langendorff perfused rat heart, so as to characterize suitability of this model in pharmaco-electrophysiology effects by using cardiac surface mapping using flexible MEA system.

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Materials and Methods

This investigation protocol was reviewed and approved by the institutional Animal Care and Use Committee of the First Affiliated Hospital of Xinjiang Medical University. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Animals

Forty eight adult male Sprague-Dawley (SD) rats weighing 200-250 g were used for the experiment. Rats were acclimatized for at least 7 days before any experiments started and were housed at approximately 25°C in 12-h light/dark cycles.

Langendorff perfusion

Isolated rat hearts were essentially prepared as described previously [6,21,22]. Briefly, all the animals were anticoagulated with heparin (1000 Units/kg) by marginal ear vein, and sacrifice by cervical dislocation. After a midsternal incision, the pericardium was opened, the hearts were removed rapidly and put into ice-cold Tyrode's solution. Langendorff retrograde perfusion was started after removing the heart from thorax. A metal cannula was inserted into the ascending aorta to start coronary perfusion in situ. The aorta was rapidly cannulated and connected to a modified Langendorff system. The hearts were perfused retrogradely with pre-warmed (36.8-37.2°C) Tyrode's solution saturated with 95% O_2 and 5% CO_2 at a constant flow rate of 30 ml/h without recirculation. A coronary perfusion pressure of 70 cm H_2O was achieved at the beginning of each experiment [23].

Solutions and drugs

The Tyrode's solution contained the following composition, (mM) NaCl (140), $CaCl_2$ (3), $MgCl_2$ (1), KCl (4), HEPES (14), and glucose (12) at a pH of 7.36 (adjusted with NaOH).

In this study, Amiodarone (sanofi Aventis, 0A067, China), Dofetilide (DZX, Sigma, MO, USA), and ibutilide (Fengyuan, 100204-1, China) were used to the heart tissues.

The working concentrations of all drugs were made using Tyrode's solution. pH was adjusted to 7.36 with NaOH solution.

Electrophysiological study MEA recordings

Flexible MEA measurements and record: Hearts were electrophysiologically assessed using two 36-electrode arrays flexible MEA (EcoFlexMEA 36, System and equipment obtained from Multi Channel Systems, Reutlingen, Germany (Figure 1A)) system consisting of the Flexible MEA chip, preamplifiers, filter amplifier, data acquisition board and software, which offers non-invasive synchronous 64-channel recording of extracellular FP and stored in a binary file for off-line processing.

Data were sampled at 10 kHz per channel with simultaneous data acquisition. The flexible MEA chip consists of 36 gold Polyimide 2611 foil microelectrodes, with a 30 μm electrode diameter and an 300 μm inter-electrode distance, with 2 internal reference electrodes and 2 ground electrodes (Figure 1A).

Langendorff-perfused rat heart were placed on the MEA chip while recording the field potentials (Figure 1A), the hearts were perfused and continued beat through the whole experiment. We can analyze the data offline, every field potential stand for one heart beat, so we can calculate the heart rate (Figure 1B).

For quantitative analysis, field potential duration (FPd), was defined as the duration from the depolarization peak (FP_{min}) to the repolarization peak (FP_{max}) (Figure 1B), indicate monoaction potential duration at 90% of repolarization [12]. All FPd data were determined off-line by hand. In some channels, field potentials is hard to determine because of the low amplitude, we generally choose the channel with highest amplitude and capture FP properties. Myocardial electrical activation mapping was determined by epicardial activation mapping with two 32-electrode arrays.

Experimental protocol

The rats were randomly assigned to four groups, Group 1: Tyrode's solution; Group 2: amiodaronen; Group 3: dofetilide; Group 4: ibutilide.

After 5 min of stabilization in continuously oxygenated Tyrode' solution at 37°C, a baseline recording was obtained for 5 minutes. Then the preparations were exposed to ascending concentrations (5 minutes recording at each concentration) of amiodarone, ibutilide or dofetilide. The 10-minute interval was sufficient for the wash-in of the drug.

Data analysis

Statistical analysis was performed using SPSS 17.0 (SPSS Inc, Chicago, IL). Data are given as mean ± S.E.M. Statistical comparisons between groups were obtained by ANOVA. Repeated-measures ANOVA with a post-hoc Bonferroni test were used to evaluate drug effects in different time. The level of significance was $P<0.05$.

Results

The FP with cardiac surface mapping by flexible MEA

In line with previous reports using human ES cells and native embryonic cardiomyocytes [1,13,14], embryonic stem cells [16] and engineered heart tissue [15], Figure 2A demonstrates a typical cardiac FP morphologies paralleled by spontaneous contractions performed in a Langendorff perfused heart. The recordings of isolated heart on flexible MEA chip revealed distinct FP morphology with comparable signal noise ratio which are comparable to the P wave, QRS complex and T wave of ECG. In fact, a small A wave (Figure 2B), representing the atrial depolarization, followed a V complex wave (Figure 2B), representing the ventricular depolarization. Langendorff perfusion enabled the autorhythmicity of the rats' heart last about 240 min. Flexible MEA system is a kind of cardiac surface mapping therefore enables non-invasive and continuous investigations. Simultaneous 64 polar electrographic recordings were acquired, an accurate measurement of frequency, amplitude and FPd was determined using Cardio2D+. The FPd represented the ventricular depolarization and repolarization. The average FPd was 124 ± 25 ms. Amplitude in recorded FPs ranged from about 1.5 to 4 mV.

FPd prolongation is induced by amiodarone, ibutilide or dofetilide

The Tyrode's solution served as negative control. Concentrations were chosen according to published in isolated, perfused rabbit hearts [23]. As shown in Figure 3, no differences in FPd were seen between the groups in baseline.

In line with clinical observations, in fact, using isolated heart models, we demonstrate here that amiodarone, ibutilide or dofetilide, well known for their potential QT-prolonging effects [24-27], produced significant, dose-dependent increase in the cardiac repolarization phase FPd (Figure 3D). Epicardial activation propagation velocity (APV) was

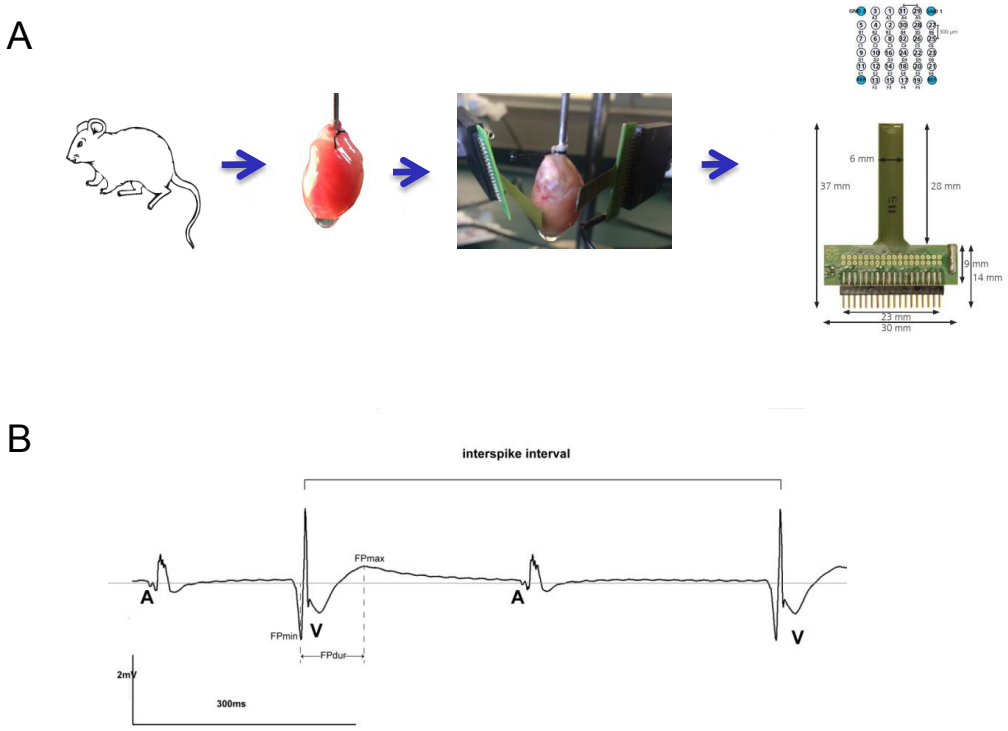

Figure 1: A: Experimental setup and data acquisition. The heart is removed and cannulated via the aorta and retrogradely perfused, the Flexible MEA chip was positioned at both ventricles during the whole experiment, the FP signal can be recorded simultaneously. **B:** Flexible Microelectrode Array with 36 electrodes (interelectrode distance: 300 µm, electrode diameter: 30 µm) arranged in an 6 × 6 Matrix Without Corner Electrodes.

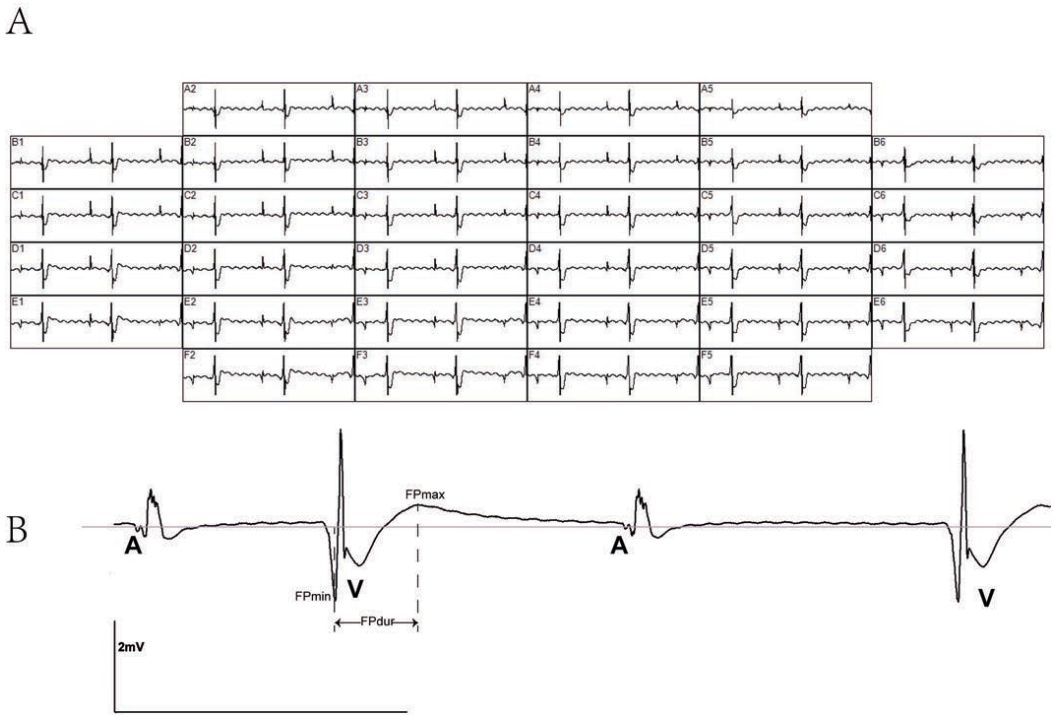

Figure 2: A: Overview of field potentials (FPs) raw data recorded simultaneously on Langendorff perfused rat heart by the software MC-Rack. **B:** Representative FP waveform. A wave stand for atrial signal, V wave stand for ventricular wave. FPMin, FPMax and FPdur indicate characteristic components that can be distinguished in the FP, FPMin, largest negative peak; FPMax, last positive peak in a cycle; FPdur, duration of the FP; FPdur characterizes the repolarization phase. Abbreviations: A, atrial signal; V, ventricular signal.

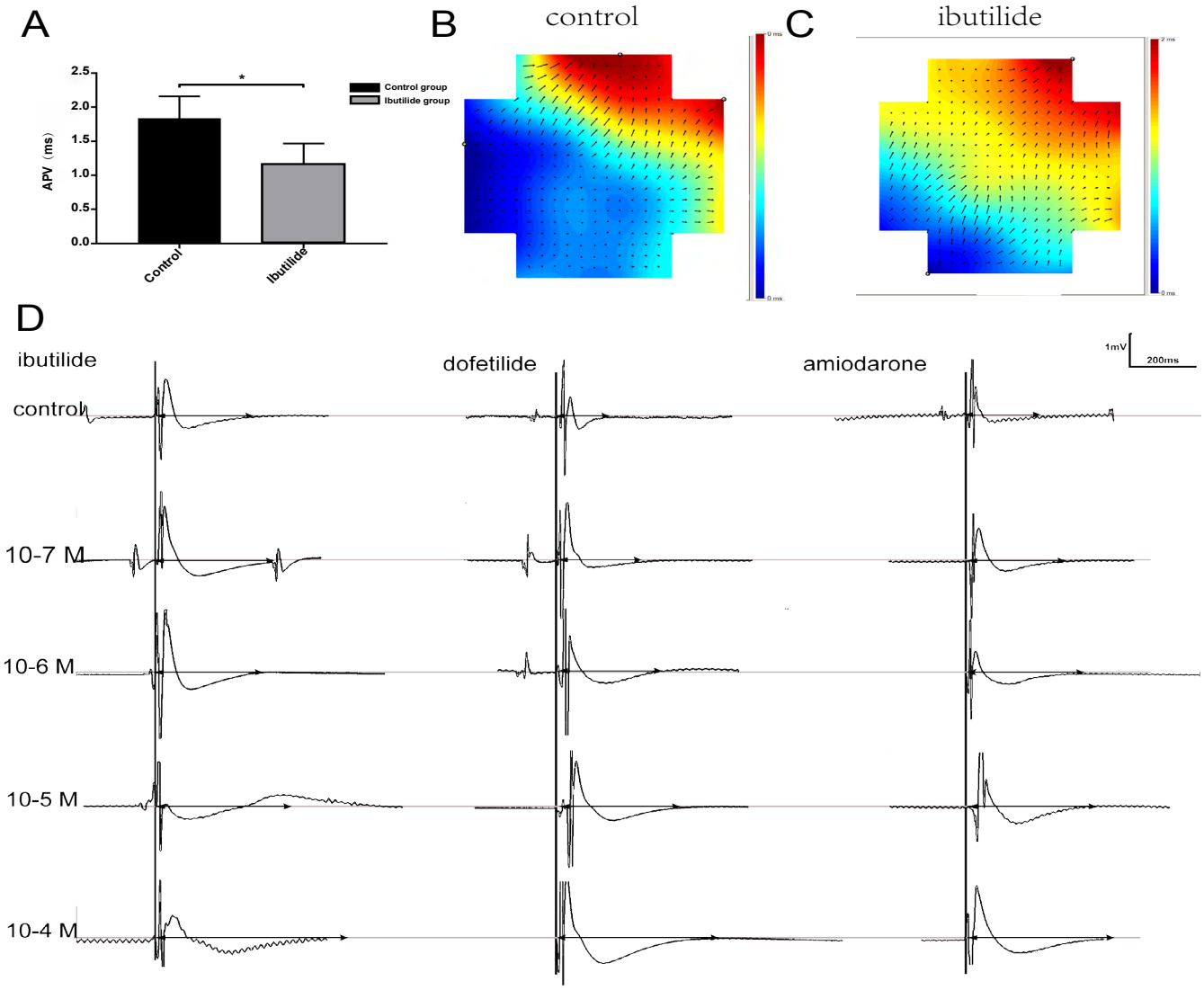

Figure 3: A: Epicardial activation propagation velocity (APV) was decreased by III class anti-arrhythmia drugs as depicted in the colour-map of epicardial multi-electrode activation. **B, C:** Control group and ibutilide (10-4) solution, Blue displays areas of earliest activation, red of latest. **D:** Prolongation of FPd in perfused heart using amiodarone, ibutilide or dofetilide. Note the prolongation of the FPd with dose response increase of the three drugs in cumulative manner from low to high concentrations.

decreased by III class anti-arrhythmia drugs as depicted in the colour-map of epicardial multi-electrode activation (Figures 3A-3C).

Baseline FPd values ranged from 94 to 132 ms and did not differ significantly between groups (Figure 4A). The administration of amiodarone, ibutilide or dofetilide, three well-known III class anti-arrhythmia drugs, led to a concentration dependent exclusive increase of FPd to 429 ± 48, 517 ± 29 and 566 ± 20 ms for concentrations of 10^{-4} M, respectively (mean values for n=12 experiments). For concentrations of 10^{-7} M and above, these alterations were significant (Figure 4B; Table 1). Amiodarone, ibutilide and dofetilide, three well-known blocker of rapidly activating delayed rectifier K+ current [28-30], increased the FPd in a dose-dependent manner. This result demonstrated that FPd can be analyzed using this system.

Arrhythmia

A rhythmic activity of the heart is observed under control group (Figure 5), none of the rats showed arrhythmias in baseline. On the MEA isolated heart is challenged by the drug.

Second degree AV block and junctional premature beat bigeminy, atrial flutter and junctional escape rhythm have been shown on the experiments using amiodarone (Figure 5). However, using ibutilide or dofetilide did not present this effect. Figure 6 shows atrial arrhythmia caused by 10^{-6} M dofetilide. A typical example of VT, induced by ibutilide is shown in Figure 6.

Discussion

Major findings

In the present study, we demonstrated a preparation real-time recordings of electrical signals with Langendorff-perfused contracting heart on flexible MEA in order to evaluate it as a potential tool for non-invasive electrophysiological parameter of interest in cardiac safety

	BS	10^{-7} M	P	10^{-6} M	P	10^{-5} M	P	10^{-4} M	*P*
Saline	124.7 ± 25.0	125.6 ± 23.7	P>0.05	121.3 ± 18.0	P>0.05	132.8 ± 25.1	P>0.05	119.9 ± 15.4	P>0.05
Amiodarone	113 ± 10.5	286.9 ± 21.5	P<0.001	337.5 ± 34.5	P<0.001	406.3 ± 16.9	P<0.001	429.6 ± 48.1	P<0.001
Ibutilide	136.5 ± 20.7	327.5 ± 75.4	P<0.001	451.9 ± 34.2	P<0.001	492.5 ± 49.8	P<0.001	517.5 ± 29.2	P<0.001
Dofitilide	126.3 ± 19.3	316.3 ± 56.3	P<0.001	446.3 ± 67.6	P<0.001	491.3 ± 49.8	P<0.001	566.3 ± 20.7	P<0.001

Data are mean ± S.E.M. BS=baseline, P value compared with BS

Table 1: Field potential duration (FPd) during different concentration of amiodarone, ibutilide and dofitilide.

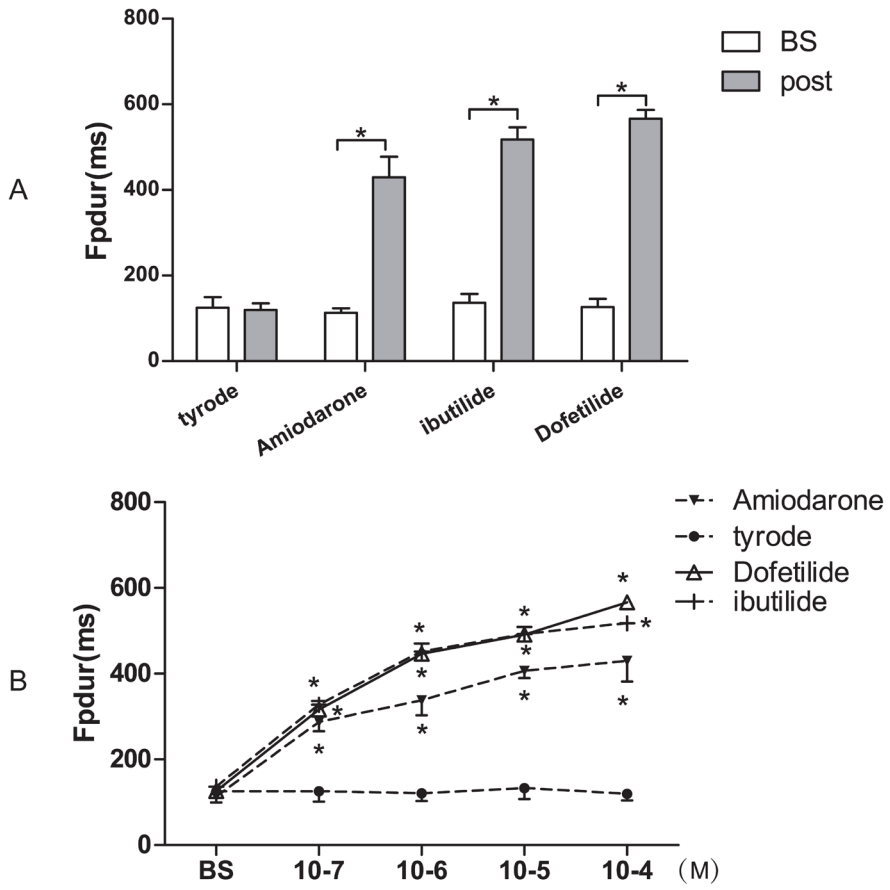

Figure 4: A: Effects of three III class AAD on FPd in comparison with untreated control. **B:** Dose–response curves showing the influences of saline, amiodarone, dofetilide and ibutilide on the field potential of perfused heart recorded with the MEA. Administration of amiodarone, dofetilide and ibutilide increased the FP_{dur} in a dose-dependent manner. The III class anti-arrhythmia drug was applied in concentrations ranging from 10^{-7} M to 10^{-4} M, which resulted in a dose-dependent increase in FP_{dur}. *: p<0.05.

pharmacology, while maintaining the advantages of perfused heart.

Cardiac pharmacological safety research needs reliable data about the toxic and adverse side effects in a model system [31]. Cardiomyocytes [32] and heart slices [12] have been used in cardiac safety pharmacology for extracellular recording using MEA. In comparison to heart slices or cardiomyocytes, the Langendorff perfused heart model, with wide species applicability (mouse, rat, rabbit), has proven invaluable in elucidating the mechanisms of contractile and electrical / conduction properties in physiological, pathological or pharmacological investigations [33]. In a study of isolated perfused hearts from cardiac myocardial infarction model rat it was shown that the conduction velocity and anisotropy of conduction can be determined in Langendorff perfused heart [15]. Our previous studies showed that FP can be recorded directly in different myocardial segments (right, anterior, lateral, posterior) [20]. The advantages of this set-up are the method's longevity, reproducibility and the ability to study the conduction velocity and anisotropy of conduction.

Previously studies has demonstrated that the significant QT corresponding to FPd, MEA have been used in assessing potential side effects of cardioactive drugs such as QT prolongation or proarrhythmic risk [34], which can provide reproducible and accurate data. These microelectrodes offer a real-time and noninvasive tool for monitoring electrical activity of the heart with a high level of temporal and spatial resolution (10~20 μm) in long-period. Furthermore, the flexible MEA technique allows the evaluation of arrhythmia and signal propagation.

Recently, we have reported MEA was used for detecting electrical-conduction function following myocardial infarction in rats [19]. It

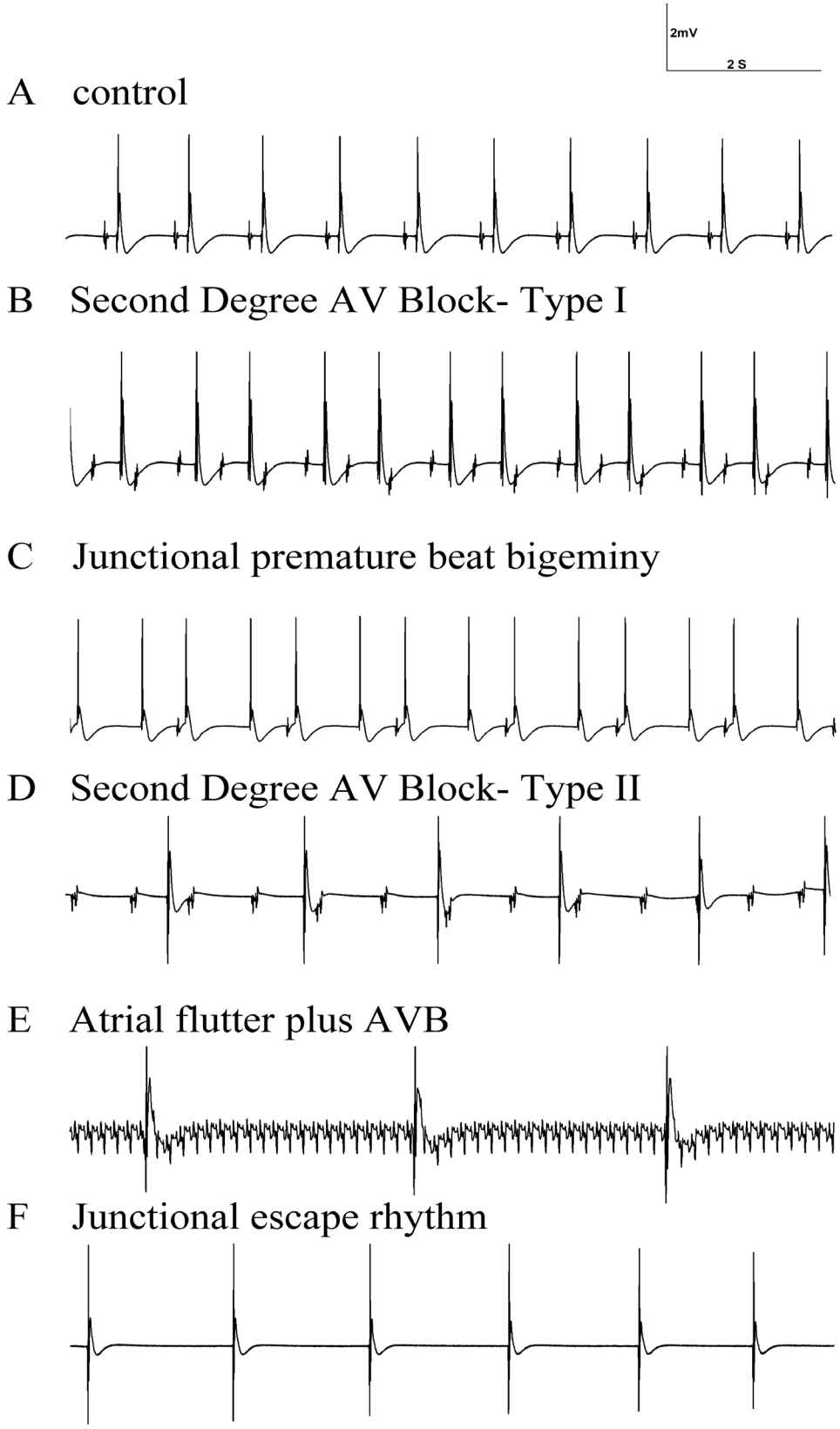

Figure 5: During the 'pretreatment' period a rhythmic activity of the heart is observed (A), amiodarone evoked arrhythmias -- second degree AV block (B, D), junctional premature beat bigeminy (C), atrial flutter (E) and junctional escape rhythm (F).

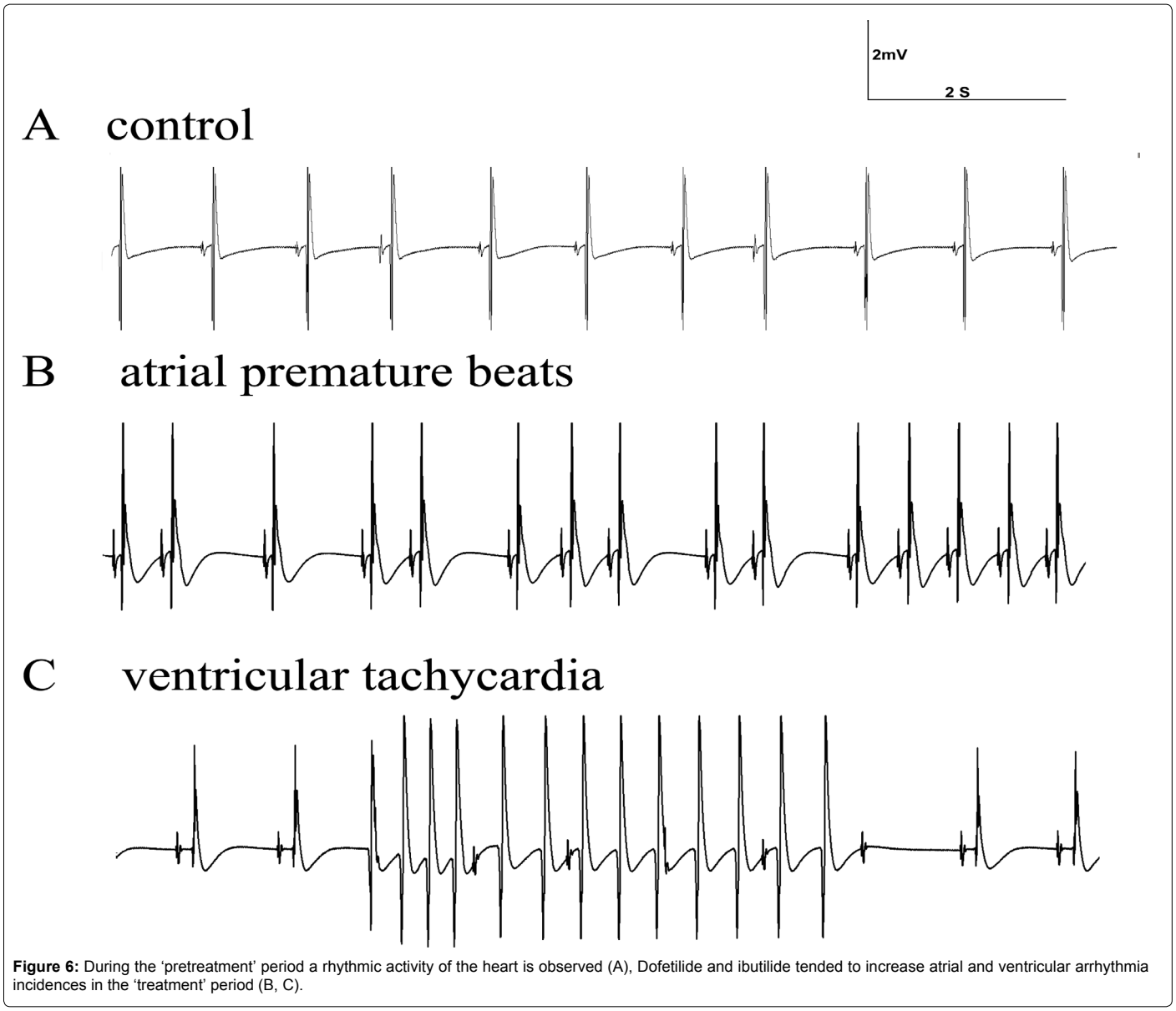

Figure 6: During the 'pretreatment' period a rhythmic activity of the heart is observed (A), Dofetilide and ibutilide tended to increase atrial and ventricular arrhythmia incidences in the 'treatment' period (B, C).

is well known that III class AAD are known to have proarrhythmic potential, QT prolongation are a surrogate marker for cardiotoxicity. Therefore, in the present study, we aimed to illustrate the effect of three known class III AAD on FP parameters and their proarrhythmic potential in Langendorff perfused rat heart, so as to characterize reproducibility and suitability of this model in drug-induced cardiotoxicity by using flexible MEA.

In our study isolated heart were plated onto micro-electrode arrays (MEAs) to record the extracellular FP as well as effects of several antiarrhythmic drugs. Three class III pharmacological agents, amiodarone, ibutilide and dofetilide, were used to demonstrate the effectiveness of this model for drug screening. All of these compounds are known to have cardiac side effects (ie. TdP). Electrophysiological studies of the drug showed that ibutilide prolongs repolarization are mediated via the rapidly activating delayed rectifier potassium currents (IKr) [35], Lee [36] proposed that Na^+ current through the L-type Ca^{++} channel mediates ibutilide's potent clinical class III antiarrhythmic action. Reiffel also demonstrated that ibutilide have the effect on the slow sodium channel [37].

In our study, we demonstrate that amiodarone, ibutilide and dofetilide induced dramatic dose-dependent FPd prolongations (Figure 3B) in the isolated, perfused rat heart. Furthermore, the present study shows that both ibutilide and dofetilide have significantly increased ventricular tachycardia. These results indicated that prolongation of ventricular repolarization and arrhythmias induced by class III antiarrhythmic, can be evaluated using this system. This property of QT-prolonging induced by amiodarone, ibutilide or dofetilide may facilitate early depolarizing postpotentials and increase the risk for ventricular tachycardia.

Limitations

This model is *in vitro*, in fact, measurement *in vivo* should be better by placing the flexible MEA on the surface of the hearts [38,39].

Perspectives

Perfused isolated heart on flexible MEA is a promising model for several hours electrophysiological monitoring in safety pharmacological studies, which is minimally invasive for the surface ECG acquisition. Alternatively, measurements *in vivo* by placing the flexible MEA on the surface of the hearts has been applied in zebrafish hearts [37]. With the development of the MEA equipment, this system could provide important pharmaco-electrophysiology mechanistic insights into how fatal arrhythmias occur for future investigations.

Conclusion

We conclude that isolated perfused rat heart on a MEA chip can provide a novel preparation suitable for preclinical studies of drug screening and electrophysiological effects of various pathophysiological concepts.

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Conflict of Interest

None.

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