# Guinea Pig ECG Changes under the Effect of New Drug Candidate TP28b

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## Abstract

*Anesthetized guinea pig represents a valuable model for evaluation of arrhythmogenic potential of various drugs. Aim of this study was to evaluate arrhythmogenic potential of the TP28b, a new drug candidate with possible beta-adrenergic action. Guinea pigs were anesthetized by isoflurane and jugular vein was cannulated. ECG was recorded by needle electrodes fixed subcutaneously on the chest. Rectal probe was used for continual monitoring of body temperature. Animals were divided into positive control, negative control and test group. The experiment consisted of stabilization, 3 phases of tested drug or vehiculum administration by continual i.v. infusion and recovery. The ECG was recorded and analysed using LabChart 8 Pro software. The heart rate, PR intervals, QRS duration, and QT intervals were measured. QT was corrected according to Framingham study. Our experimental model proved to be stable during the whole experiment since the HR, PR interval, QRS interval, and QTc do not change in negative control in all phases. Esmolol administration led to HR decrease, PQ interval and QRS duration prolongation, whereas only small variations of QTc were detected. Our model proved to be valuable and suitable for testing of newly synthetized drugs with cardiovascular effects.*

## 1. Introduction

Anesthetised guinea pig represents a valuable model for evaluation of potential arrhythmogenic effects of various drugs, since it allows to evaluate complex reactions of cardiovascular system [1]. Next to this fact, action potential of guinea pig cardiomyocyte is well comparable to that of human and the results are therefore well translatable to clinical medicine [2].

Aim of this study was to evaluate arrhythmogenic potential of the TP28b, a new drug candidate with possible beta-adrenergic action, in the model of anesthetised guinea pig.

## 2. Methods

### 2.1. Animal model

All animal experiments were carried out according to the recommendations of the European Community and according to experimental protocol approved by the Committee for Ensuring the Welfare of Laboratory Animals at Masaryk University.

Guinea pigs were purchased from certified provider (Velaz, Czech Republic). They were housed in the Animal Breeding and Experimental Facility, Masaryk University, Faculty of Medicine, in the environment with controlled atmospheric pressure, humidity, temperature, and light cycle 12/12 (12 hours light, 12 hours dark). They were fed with standard diet; water was accessible *ad libitum*.

Ten guinea pigs were anesthetised using isoflurane (3% induction, 2% maintaining) and fixed to the heated pad. Neck and chest of the animal was shaved. Jugular vein was cannulated to ensure *i.v.* access for dosing of tested substances. For recording of ECG, needle electrodes were fixed subcutaneously on the chest. For continual monitoring of body temperature, rectal probe for small animals was used. Animals were allowed to stabilize for approx. 25 minutes.

### 2.2. Experimental groups, phases of the experiment

Animals were divided into three experimental groups: positive control, negative control and test group. As positive control, esmolol, cardioselective beta-1 receptor blocker, was used. As negative control, vehiculum was used. Animals in test group were exposed to newly synthetized substance TP28b (Figure 1), a potential beta receptor blocker. The substance was synthetized at the Department of Chemical Drugs, Faculty of Pharmacy, Masaryk University [3].

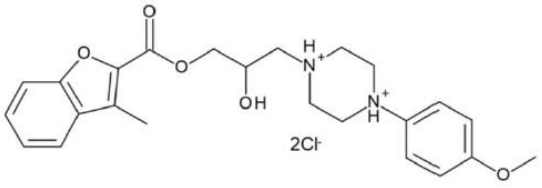


Figure 1.
Chemical structure of TP28b, according to IUPAC: 1-[2-hydroxy-3-(3-methylbenzofuran-2-karbonyloxy)propyl]-4-(4-methoxyfenyl)piperazin-1,4-diium dichlorid.

The stabilisation period (phase 0) was followed by 3 phases (phase 1 – 3) of drug or vehiculum administration.

Both drugs and vehiculum were administered by continual *i.v.* infusion using infusion pump (B. Braun, Germany). Drugs were administered in doses of 1, 5 and 10 mg/kg/min (phase 1, 2 and 3, respectively). The vehiculum was administered at equivalent rate (1, 5 and 10 ml/hour; phase 1, 2 and 3, respectively). Each of the experimental phases 1 – 3 took 5 minutes. After the end of phase 3, the ECG recording continued (phase 4) in order to detect restoration of the heart rate (HR). Maximal duration of phase 4 was 20 minutes.

## 2.3. Data acquisition and analyses

ECG and body temperature were continually recorded during the whole experiment using PowerLab 16/35. One lead ECG was recorded using needle electrodes and Bio Amp amplifier with sampling rate of 1 kHz and range of 10 mV (AD Instruments Ltd., CO, USA). The ECG was recorded and analysed using LabChart 8 Pro software (AD Instruments Ltd., CO, USA). Heart rate, PR intervals, QRS duration, and QT intervals were measured. QT was corrected according to Framingham study [4].

Statistical analyses of obtained data were provided in GraphPad Prism 5 (GraphPad Software, CA, USA). The normality and homoscedasticity of the parameters were checked with Shapiro-Wilk test and Levene's test, respectively. It was confirmed that both assumptions are not precisely hold. Non-parametric statistical test was therefore performed. Unpaired Mann-Whitney U-test was performed to compare measured parameters between the groups as well as particular phases within each group. P-value below 0.05 was considered as significant. Results are expressed as mean ± SD.

## 3. Results

Our experimental model proved to be stable during the whole experiment since the HR, PR interval, QRS interval, and QTc do not change in negative control in all phases (Figures 2 – 5, Table 1).

On the contrary, esmolol (as positive control) decreased HR, as expected (Figure 2). HR at the end of stabilization was 262.16±22.64 bpm, which is physiological frequency of guinea pig heart. During esmolol administration, HR significantly decreased in a dose-dependent manner (210.84±16.97, 183.60±10.45 and 166.53±28.71, for phase 1, 2 and 3, respectively; $p<0.001$ for all phases as compared to phase 0). As expected, the effect of esmolol was partially washed out in phase 4: the HR increased to 192.13±11.81 bpm.

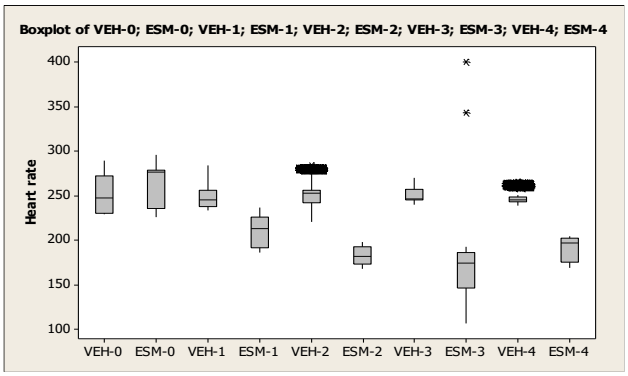


Figure 2. Boxplots of HR median for negative control (VEH) and positive control (ESM) in stabilization (0), three doses of either vehiculum or drug (1-3) and washout (4) phases.

Esmolol administration also significantly increased PR interval in a dose-dependent manner from 56.099±5.221 ms at the end of stabilization to 63.933±7.903 ms, 69.606±10.795 ms and 69.358±8.541 ms at the end of phase 3, respectively ($p<0.001$). These values indicate atrio-ventricular conduction time prolongation. The effect of esmolol was partially washed out in phase 4 (Figure 3).

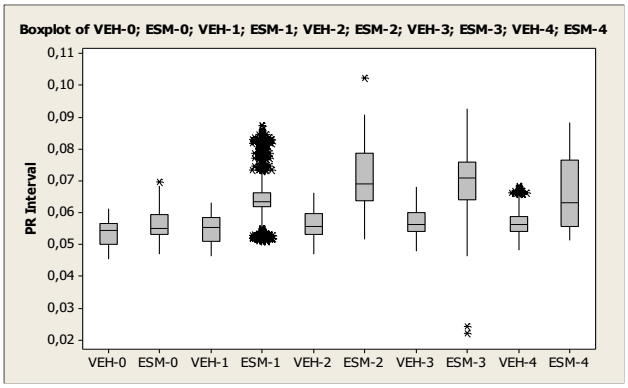


Figure 3. Boxplots of PR interval median for negative control (VEH) and positive control (ESM) in stabilization (0), three doses of either vehiculum or drug (1-3) and washout (4) phases.

Esmolol administration led to QRS prolongation in phase 3 (18.891±2.874 ms at the end of phase 0 *vs.* 29.231±10.370 ms at the end of phase 3). The effect of esmolol administration was covered by high dispersity of data during phases 1 and 2 (19.953±3.402 ms and 22.037±4.103 ms). The effect was again partially washed out (Figure 4).

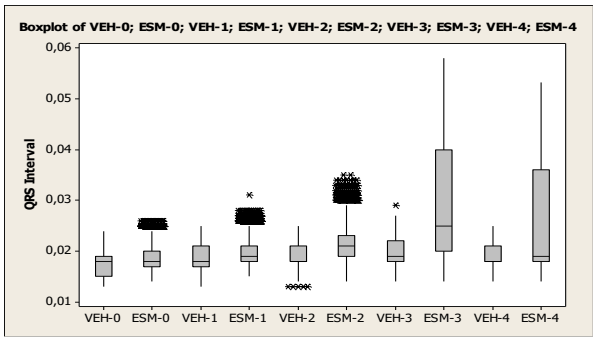


Figure 4. Boxplots of QRS interval median for negative control (VEH) and positive control (ESM) in stabilization (0), three doses of either vehiculum or drug (1-3) and washout (4) phases.

Corrected QT interval (QTc) under the effect of esmolol kept stable during the whole experiment (Figure 5), only small variations were detected (phase 0: 276.17±16.45 ms, phase 1: 280.86±0.73.6 ms, phase 2: 277.90±10.78 ms, phase 3: 269.23±6.66 ms, phase 4: 280.12±9.61 ms).

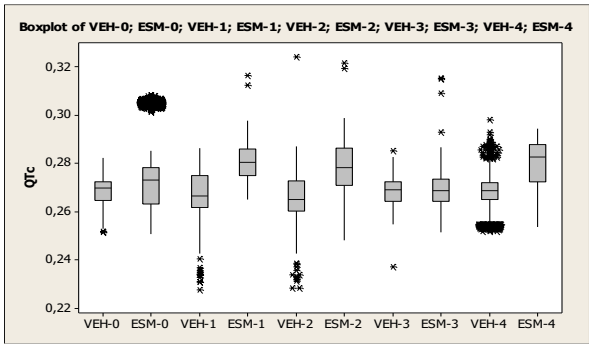


Figure 5. Boxplots of QTc median for negative control (VEH) and positive control (ESM) in stabilization (0), three doses of either vehiculum or drug (1-3) and washout (4) phases.

## 4. Discussion

Anaesthetized guinea pig represents golden standard in preclinical phase of drug testing. Especially, it is valuable biomodel for evaluation of cardiovascular effects of tested drugs. It is used not only for testing of drugs with expected cardiovascular effects, but also for testing of cardiovascular side effects of drugs which primarily target another organ or system. Frequently it is used for evaluation of arrhythmogenic potential of newly synthetized substances [5].

The data from this biomodel can be well transferred to clinical sphere, since guinea pig cardiac cells are quite close to human ones. It concerns mainly the electrical properties of cardiac muscle, the ionic channels and currents of which are similar to those expressed and recorded in human cardiomyocyte [2].

This work was focused on effects of beta-adrenergic receptor blockers. These effects include bradycardia, slowing of atrioventricular conduction and slowing of intraventricular depolarization. In our experimental setup, HR decreased, PQ interval and QRS duration were prolonged. These effects were observed after administration of positive control, esmolol. Also, QT interval was evaluated and corrected.

| | HR | PR | QRS | QTc |
|---|---|---|---|---|
| VEH-0 - VEH-1 | 1 | -2 | -6 | 0 |
| VEH-0 - VEH-2 | 0 | -4 | -9 | 1 |
| VEH-0 - VEH-3 | 1 | -6 | -9 | 0 |
| VEH-0 - VEH-4 | 3 | -7 | -8 | 0 |
| VEH-1 - VEH-2 | -1 | -2 | -2 | 0 |
| VEH-1 - VEH-3 | 0 | -4 | -3 | 0 |
| VEH-2 - VEH-3 | 1 | -2 | -1 | -1 |
| ESM-0 - ESM-1 | 20 | -14 | -6 | -2 |
| ESM-0 - ESM-2 | 30 | -24 | -17 | -1 |
| ESM-0 - ESM-3 | 36 | -24 | -55 | 3 |
| ESM-0 - ESM-4 | 27 | -17 | -32 | -1 |
| ESM-1 - ESM-2 | 13 | -9 | -10 | 1 |
| ESM-2 - ESM-3 | 9 | 0 | -33 | 3 |
| ESM-1 - ESM-3 | 21 | -8 | -46 | 4 |
| VEH-0 - ESM-0 | -3 | -5 | -7 | -3 |
| VEH-1 - ESM-1 | 16 | -17 | -7 | -5 |
| VEH-2 - ESM-2 | 28 | -25 | -15 | -4 |
| VEH-3 - ESM-3 | 34 | -22 | -51 | 0 |
| VEH-4 - ESM-4 | 23 | -15 | -31 | -5 |

Table 1. Relative changes (normalized to previous phases or to negative control) of the median of the measured parameters between particular phases within each group and between the groups.

QT interval evaluation is routinely used as preliminary screening of arrhythmogenicity of drugs and drug candidates [5]. Its prolongation is considered as independent risk factor for ventricular arrhythmias development. Since the QT interval duration depends on the HR (RR interval), it must be corrected. In this work, it is corrected according to clinically accepted method. Small variations of QTc were detected in our data. Therefore, we can conclude that neither vehiculum nor esmolol represent potential arrhythmogenic substance.

It is necessary to emphasize that correction validated for human QT interval may bring certain inaccuracy when used in guinea pig ECG. There are approaches enabling subject-specific QT interval correction [6, 7]. Such approach may uncover subtle changes of RR and QT interval relationship changes.

The preclinical testing of drug includes *in silico* testing, which is then followed by *in vitro* testing on cell lines,

tissue cultures and isolated cells and/or organs. Next step represents *in vivo* testing on animal models. Originally promising substance may be proven ineffective, harmful or even toxic at any step of testing.

Such fate will most probably meet also TP28b, newly synthetized substance which had been proven to block cardiac beta-adrenergic receptors and which was tested in this work. Although this drug looked quite promising at *in silico* and *in vitro* steps, its application to whole-body model led to serious side effects. It seems that solubility of TP28b represents a big challenge which has to be solved before the testing will continue.

It is necessary to design other experiments and to use other approaches to decide definitely whether this promising substance might proceed to clinical phase of testing.

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