Anesthetized Guinea Pig as a Model for Drug Testing

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Summary

Based on the World Health Organization statistics, cardiovascular diseases represent the major cause of death worldwide. Although a wide range of treatment approaches and pharmaceuticals is available, the therapy is often not effective enough and therefore health risks for the patient persist. Thus, it is still essential to test new drug candidates for the treatment of various pathophysiological conditions related to cardiovascular system. *In vivo* models represent indispensable part of preclinical testing of such substances. Anesthetized guinea pig as a whole-body model allows to evaluate complex reactions of cardiovascular system to tested substance. Moreover, action potential of guinea pig cardiomyocyte is quite comparable to that of human. Hence, the results from this model are then quite well translatable to clinical medicine. Aim of this paper was to summarize the methodology of this model, including its advantages and/or limitations and risks, based on the effects of two substances with adrenergic activity on the ECG parameters. The model of anesthetized guinea pig proved to be valuable and suitable for testing of drugs with cardiovascular effects.

Key words

Adrenergic agents • Animal model • Cardiovascular system • Drug testing • Guinea pig

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Introduction

Although medical science has made a great progress recently, therapy often remains behind and thus certain health risks for patients persist. Namely, treatment of cardiovascular diseases, which are the main cause of death worldwide, represents a great challenge [1]. Although a wide range of drugs with the effect(s) on cardiovascular system is available, there are still many cases where a need of targeted therapy is obvious. Therefore, testing of new drug candidates for treatment of various cardiovascular disorders remains in the center of interest in physiological and pharmacological laboratories.

Preclinical phase of drug candidate testing consists of three parts: initial *in silico* tests, followed by *in vitro* testing on isolated cells, tissues and organs, and *in vivo* testing, using whole-body animal models. Testing on animals brings valuable data about the complex reactions, e.g. of cardiovascular system, to tested substance [2,3].

As the heart is concerned, both *in vitro* and *in vivo* tests enable the researcher study two manifestations of its function - electrophysiological and mechanical processes. For evaluation of cardiac electrophysiological changes, either recording of action potential or electrocardiogram (ECG) are mostly employed. ECG recording and evaluation is crucial step in testing of a new drug candidate with potential cardiovascular effect(s).

When using animal models in drug testing, interspecies differences must be considered. Most frequently used species in drug testing are rat, guinea pig and rabbit. Although rat is the most used model in such type of experiment, there is a fact which cannot be omitted: rat ECG curve differs from that of human quite markedly. Although rabbit heart shows electro-physiological properties quite resembling those of human heart, experiments are limited by greater demands on breeding as compared to rat. On the other hand, guinea pig represents good compromise: its breeding requirements are lower on one side and on the other side

electrophysiology of its heart is well comparable to that of human [4,5].

Based on abovementioned, anesthetized guinea pig constitutes a valuable model for evaluation of cardiac electrophysiology changes and the results are consequently well translatable to clinical medicine. Therefore, aim of this study was to evaluate/summarize the methodology of the anesthetized whole-body guinea pig model with its advantages and limitations/risks comprehensively, clearly, and truthfully.

Methods

Animal model and experimental groups

All animal experiments were performed in agreement with the recommendations of the European Community and based on experimental protocol approved by the Committee for Ensuring the Welfare of Laboratory Animals at Masaryk University, Brno, Czech Republic.

Animals were purchased from certified provider (Velaz, Czech Republic) and housed in the Animal Breeding and Experimental Facility, Masaryk University, Faculty of Medicine, Brno. They were kept in the environment with controlled atmospheric pressure, humidity, temperature, and light cycle 12/12 (12 h light, 12 h dark). Standard diet and water were accessible *ad libitum*. At the day of experiment, animals´ body mass varied around 300 g.

Twenty-two guinea pigs were divided into three groups. Vehiculum group (VEH, 5 % glucose solution, n=7) served as a negative control. Two groups for drug testing were esmolol group (ESM, n=7) and dobutamine group (DOB, n=8).

Substances

Isofluranum (Aerrane, Baxter SA), heparinum natricum (Heparin Léčiva, Zentiva), Glucosum 5 % (Glucose 5B. Braun Intravenous Solution for infusion), esmolol (Esmocard Lyo, HIKMA Italia S.P.A.), and dobutaminum (Dobutamin ADMEDA, Admeda Arzneimittel GmbH).

Experimental procedure

Animal was anesthetized by inhalation anesthesia with isoflurane (3 % for induction, then 2 % for maintaining). It was fixed to the pad, which was heated to prevent drop in body temperature during the experiment. Rectal probe for small animals (AD Instruments Ltd., CO, USA) was used for continual monitoring of body temperature. Next, the neck and the chest were shaved, and jugular vein was exposed by blunt preparation. It was cannulated using Vasofix cannula (B. Braun) to ensure *i.v.* access for administration of solutions. Cannula was pre-treated with heparin to prevent blood clotting.

ECG needle electrodes (AD Instruments Ltd., CO, USA) were fixed subcutaneously on the guinea pig chest (Fig. 1). Before the experiment, each animal was allowed to stabilize for approximately 20 min.

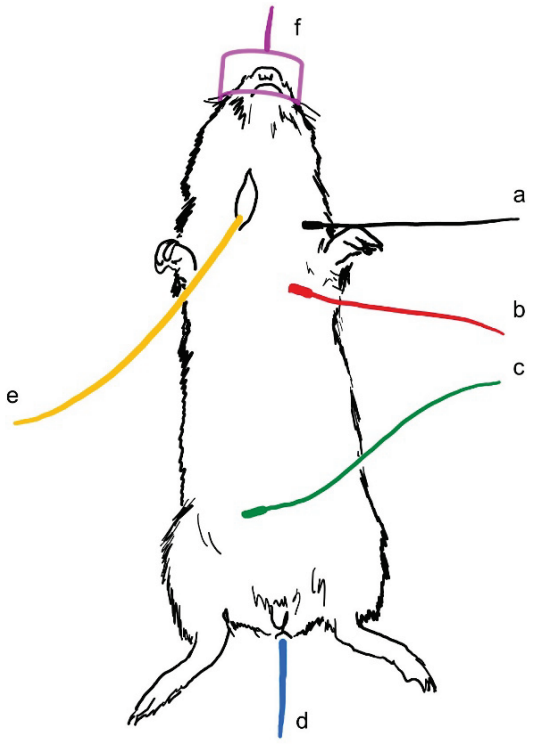

Fig. 1. Schematic picture of anesthetized guinea pig model. (**a-c**) ECG electrodes (a – negative electrode, b – positive electrode, c – ground); (**d**) rectal probe for body temperature monitoring; (**e**) intravenous cannula in the right jugular vein; (**f**) face mask for isoflurane inhalation anesthesia.

The experiment consisted of 5 phases (Fig. 2). The stabilization period (phase 0), 3 phases (phase 1-3) of vehiculum or drug administration, followed by recovery period (phase 4). Infusion pump (B. Braun, Germany) was employed for solutions administration by continual *i.v.* infusion *via* cannulated jugular vein. Based on previous published literature, doses of esmolol and dobutaminum were determined. Esmolol was administered in doses of 1, 5 and 10 mg/kg/min (phase 1, 2 and 3, respectively) [6]. Dobutamine was used in doses of 0.01, 0.05 and 0.1 mg/kg/min (phase 1, 2 and 3,

respectively) [7]. The vehiculum (5 % glucose solution) was administered at equivalent rate (1, 5 and 10 ml/h; phase 1, 2 and 3, respectively). No solutions were administered in phase 0 and in phase 4. Each experimental phase (1-3) lasted 5 min. ECG recording was performed during the whole experiment, e.g., from phase 0 to phase 4. During the phase 4, heart rate (HR) recovery to resting values was followed. Duration of phase 4 never exceeded 20 min.

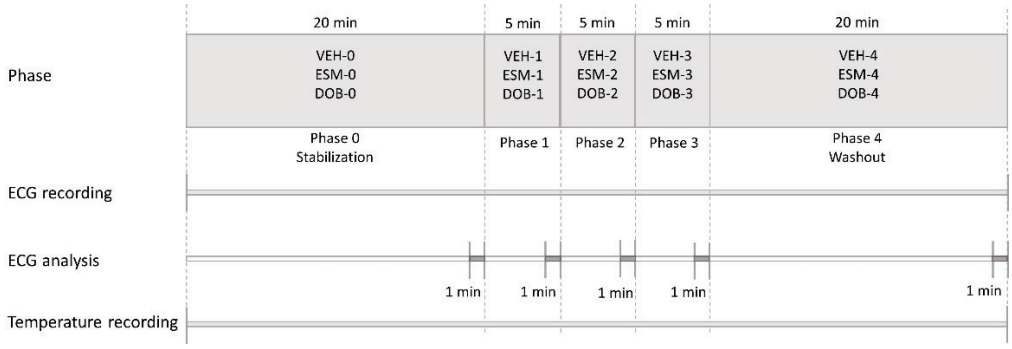

Fig. 2. Experimental protocol. Description in the text.

Data acquisition and analyses

Continual recording of rectal temperature and ECG curve was performed throughout the whole experiment using PowerLab 16/35 (AD Instruments Ltd., CO, USA). One-lead ECG was recorded by needle electrodes and Bio Amp amplifier with sampling rate of 1 kHz and range of 10 mV (AD Instruments Ltd., CO, USA). LabChart 8 Pro software (AD Instruments Ltd., CO, USA) was used both for recording and subsequent analyzing of ECG curve (Fig. 3). RR intervals, PR intervals, QRS duration, and QT intervals were measured during the last minute of each consecutive phase of the experimental protocol (Fig. 2). The RR intervals were used for heart rate (HR) calculation. The QT intervals were corrected according to Framingham study [8].

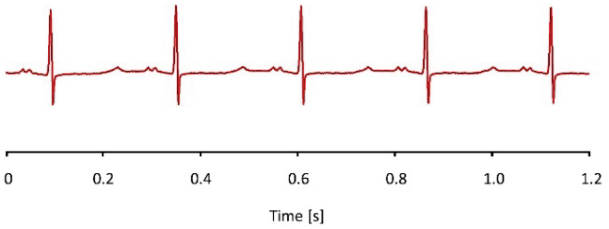

Fig. 3. Representative ECG curve recorded during the last minute of stabilization phase in anesthetized guinea pig model.

Statistical evaluation

Statistical analyses of recorded data were performed 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. Since it was confirmed that both assumptions are not precisely hold, non-parametric statistical test was used for further evaluation. Unpaired Mann-Whitney U-test was employed for comparison of measured parameters between the groups as well as between the particular phases within each group. P-value below 0.05 was considered as significant. Results are expressed as mean ± SD.

Results

Stability and reliability of this model was tested by evaluation of measured parameters in the group VEH. All followed parameters proved to be stable during the whole experiment. Moreover, QTc interval did not change in any of the phases ($p<0.05$ for all phases as compared to phase 0, Figs 4-7).

HR changes

Average HR at the end of stabilization period was 261.89±21.50 bpm, which corresponds to physiological values of HR in guinea pig. After administration of esmolol, HR significantly decreased (Fig. 4) in a dose-dependent manner (phase 1: 219.50±24.88 bpm, $p=0.0152$; phase 2: 187.31±12.41 bpm, $p=0.0022$; phase 3: 165.2±28.2 bpm, $p=0.0022$; always compared to phase 0). This effect was partially washed out in phase 4, where HR increased up to 194.06±15.71 bpm. This HR restoration, although partial, is significant as compared to phase 0.

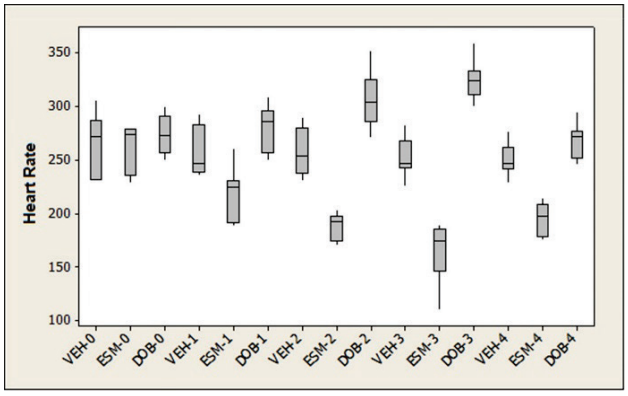

Fig. 4. Boxplots of HR median [bpm] for esmolol group (ESM), dobutamine group (DOB) and vehiculum group (VEH) in stabilization (0), three doses of either vehiculum or drug (1-3) and washout (4) phases. The edges of the box indicate 25th-75th percentiles.

Dobutamine administration led to gradual increase in HR during all experimental phases (Fig. 4). This effect was statistically significant in all phases except the phase 1 (the lowest dose), nevertheless the trend of HR increase is undeniable (phase 1: 279.86±21.06 bpm, p=0.6365; phase 2: 307.28±25.83 bpm, p=0.0136; phase 3: 324.85±18.05 bpm, p=0.0014; always compared to phase 0). This effect was fully cancelled in phase 4, where HR decreased to 268.44±16.11 bpm (NS as compared to phase 0).

PR interval changes

The effect of esmolol on PR interval was mostly insignificant. Nevertheless, dose-dependent PR interval increase was observed from 55.58±4.92 ms at the end of phase 0 to 61.90±8.29 ms, 67.27±10.63 ms and 64.50±10.02 ms at the end of phase 1, 2 and 3, respectively. This effect was partially washed out in phase 4 (Fig. 5).

The effect of dobutamine was significant only in phase 2 and 3. Its administration led to PR interval shortening in a dose-dependent manner, from 51.94±7.70 ms at the end of phase 0 to 50.73±8.02 ms, 48.99±7.59 ms and 47.70±7.08 ms at the end of phase 1, 2 and 3, respectively (Fig. 5). The effect of dobutamine was washed out in phase 4, where PR interval prolonged to 52.91±8.86 ms (NS).

QRS interval changes

Esmolol administration led to a gradual prolongation of QRS interval in a dose-dependent manner (Fig. 6). QRS was 18.69±2.83 ms at the end of phase 0. Statistically significant effect was observed in phase 3 (27.91±10.03 ms, p=0.0106 as compared to phase 0), nevertheless the trend of QRS prolongation is indisputable in all phases (19.49±3.33 ms, 21.38±3.91 ms and 27.91±10.03 ms at the end of phase 1, 2 and 3, respectively; NS). The effect of esmolol was partially washed out in phase 4, where the QRS interval shortening to 22.98±4.39 ms was observed.

Dobutamine administration led to insignificant dose-dependent QRS interval shortening from 19.74±2.94 ms at the end of phase 0 to 18.81±3.16 ms, 18.35±3.06 ms and 17.79±3.46 ms at the end of phase 1, 2 and 3, respectively. The effect of dobutamine was washed out in phase 4 (18.62±3.32 ms).

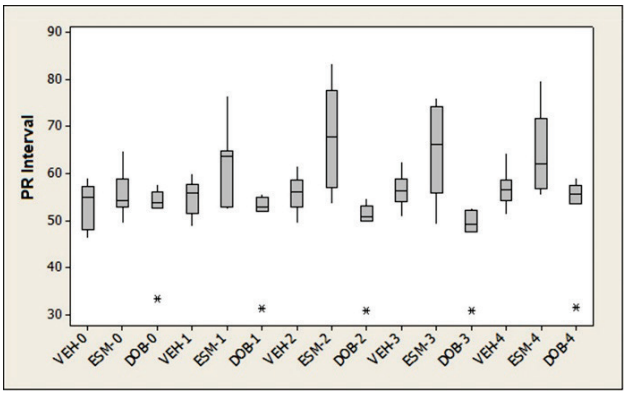

Fig. 5. Boxplots of PR interval median [ms] for esmolol group (ESM), dobutamine group (DOB) and vehiculum group (VEH) in stabilization (0), three doses of either vehiculum or drug (1-3) and washout (4) phases. The edges of the box indicate 25th-75th percentiles. The stars indicate the outliers.

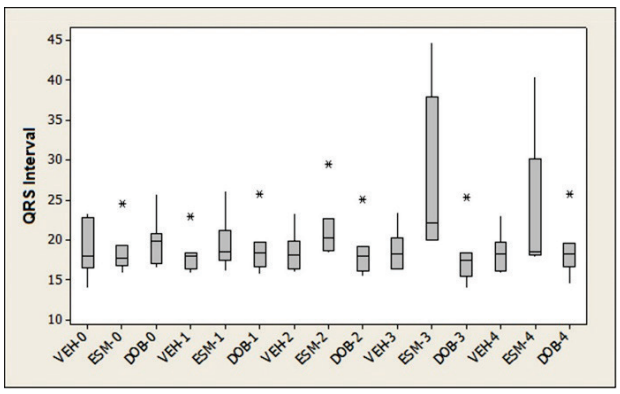

Fig. 6. Boxplots of QRS interval median [ms] for esmolol group (ESM), dobutamine group (DOB) and vehiculum group (VEH) in stabilization (0), three doses of either vehiculum or drug (1-3) and washout (4) phases. The edges of the box indicate 25th-75th percentiles. The stars indicate the outliers.

QT_c interval changes

QT intervals were first corrected according to Framingham study according to the following formula [9,10]:

$$QT_{cFra} = QT + 0.154 \times (1 - RR) \text{ [ms]}$$

Corrected QT intervals (QT_c) under the effect of esmolol kept stable during the whole experiment (Fig. 7), only small insignificant variations were observed (271.97±15.97 ms, 277.40±8.90 ms, 274.70±10.70 ms, 266.23±6.23 ms, and 277.82±8.66 ms at the end of phase 0, 1, 2, 3, and 4, respectively).

Dobutamine affected QT_c interval in insignificant manner (255.32±2.43 ms, 254.06±3.55 ms, 247.52±8.71 ms, 240.00±5.82 ms, and 257.36±8.11 ms, at the end of phase 0, 1, 2, 3, and 4, respectively).

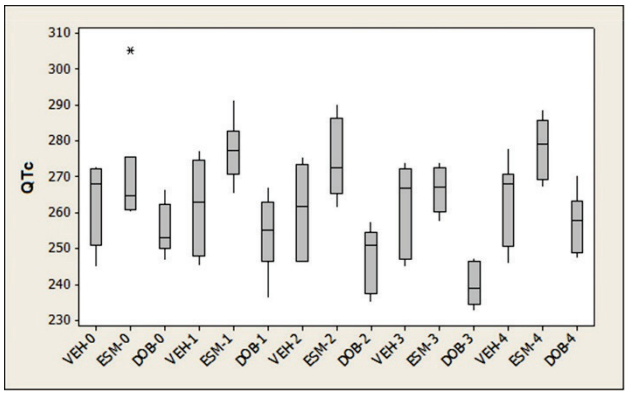

Fig. 7. Boxplots of QTc median [ms] for esmolol group (ESM), dobutamine group (DOB) and vehiculum group (VEH) in stabilization (0), three doses of either vehiculum or drug (1-3) and washout (4) phases. The edges of the box indicate 25th-75th percentiles. The star indicates the outliers.

Discussion

Anesthetized guinea pig represents golden standard in preclinical phase of new candidate drug testing. It is valuable biomodel not only for evaluation drugs with expected cardiovascular effects, but it can be employed also in testing of cardiovascular side effects of drugs which are primarily heading for another organ or system. Also, it is frequently used for evaluation of arrhythmogenic potential of newly synthetized substances [11]. The main advantage is that the data obtained in this model can be well transferred to clinic. It is since guinea pig cardiomyocytes are quite like those in humans, especially as the electrical properties are concerned. The ionic channels and currents of guinea pig cardiac cell are quite similar to those expressed in and recorded from human cardiomyocyte [4]. Certain differences are well described by both *in vivo* studies [12,13,14,15] and mathematic models [16].

However, as any other model, limitations and risks can be identified here, and it is necessary to take them in consideration when planning experiment on this biomodel. Main drawbacks are the effect of anesthesia, including changes of body temperature and consequent effects on electrophysiological parameters of the heart. Next, the administration of anesthesia and tested substance(s) and eventually also their volume must be considered.

Thermal stability

Guinea pig is prone to decrease in body temperature during anesthesia, which may lead to change(s) of ECG parameters (namely the heart rate). Due to this fact, it is necessary to monitor the animal's temperature continuously and, if necessary, heat it up. Rectal probe for continual temperature monitoring, heating pad and blankets are essential for this experimental set-up. It is recommended to properly fix the rectal probe. Otherwise, it may change its position in the rectum during the experiment and the recorded values might not be reliable.

Anesthesia

Recording of ECG in whole-body model requires general anesthesia of the experimental animal. Numerous substances used for general anesthesia exhibit all sorts of cardiovascular effects, from cellular to organ level. Therefore, their use in experiments focused on cardiac electrophysiology is limited or even improper. Effects not only on the heart, but also on the vessels and cardiovascular regulations have been reported [17,18,19,20].

Probably most frequently used type of anesthesia is injection anesthesia – either intramuscular or intraperitoneal. There is no need of special device, it is reasonably cheap, and its onset is rapid. On the other side, there are certain disadvantages – bad control of anesthesia depth as well as its duration [20].

In small rodents, intraperitoneal administration of ketamine and xylazine combination is quite common. However, both drugs are known to affect cardiovascular system. Bradycardia was repeatedly reported in rodents under ketamine/xylazine anesthesia. The direct cardiodepressive effect of ketamine was described in guinea pig isolated heart [21]. However, the relative bradycardia in a whole-body animal model could be also attributed to xylazine's direct effects on the central nervous system, as reported by Redfors *et al.* [22]. Nevertheless,

the cardiovascular effect of the ketamine/xylazine anesthesia did not greatly differ from that of isoflurane [22,23].

Due to the duration of the experiment and need of better control of the anesthesia depth, in our experimental set-up isoflurane inhalation anesthesia was used [24,25]. The use of isoflurane is advantageous since the cardiodepressive effect is dose dependent [22]. Therefore, proper control of isoflurane dose is required during the experiment. As the inhalation anesthesia is concerned, there is a need to administer the substance in such a way that it will ensure well-functioning, deep anesthesia. Basically, there are two possibilities: application *via* orotracheal tube or using of face mask.

A lot of practice is needed for safe orotracheal intubation in guinea pig. Even in case the orotracheal intubation seems to be performed properly, there is still certain risk of failure and consequent hypoxia of the experimental animal. Therefore, in the present study the custom-made face mask covering both animal´s nose and mouth was used (Fig. 1).

Intravenous pump

There are variety of intravenous pumps available on the market. When selecting a pump, it is crucial to pay attention to the range of flow rate which can be adjusted. Too large pump, which must be set to the minimal flow values, represents a risk. In such setting, it may happen that the solution will not flow smoothly when even a small blood clot appears.

Volume of liquid administered intravenously

The guinea pig can withstand only a relatively small volume of administered solution per time – up to 5 ml/kg administered as a short-term infusion (bolus) [26]. Higher volume may increase the intravenous compartment and thus change some of the monitored parameters.

Substances

In this study, two beta-adrenergic receptor ligands were used. Esmolol is clinically used as an ultra-short cardioselective β_1 receptor antagonist. By blocking the adrenergic activity, it decreases heart rate, conduction time and contractility. Intravenous administration is essential because of its extremely short elimination half-life [27,28]. Dobutamine is clinically used in the short-term treatment as a racemic mixture of two stereoisomers with dose-dependent positive chronotropic and inotropic effects. Stereoisomers differ in their effect on the heart. (-)-dobutamine is a powerful adrenergic α_1 agonist, (+)-dobutamine predominantly stimulates β_1 and β_2 adrenoreceptors and exhibits α_1 antagonist activity. Due to this fact, (±)-dobutamine mainly exerts inotropic effect [29,30,31]. In the present study, only two doses of both substances were applied. In case the dose-dependent effects of a tested substance are studied, number of doses must be increased. However, it was not the case of the present study.

Statistical analysis

The main challenges in statistical evaluation of measured ECG parameters are high variability of the data and high number of similar values. Therefore, it is necessary to choose appropriate statistical approach. Moreover, the values of parameters in this study are small numbers, thus expression in milliseconds is necessary. In Figures 5-7, there are numerous outlying values.

The above stated drawbacks of anesthetized guinea pig do not at all misclassify it as a suitable model for testing of newly synthesized substances with putative cardiovascular effect. It has been proven in our experiment, evaluating the impact of two drugs with known adrenergic activity on several parameters obtained from ECG curves. HR changed in an expected way – it was slowed down significantly by esmolol and increased by dobutamine. The effect of dobutamine proved to be significant later (from the phase 2), which reflects its dose dependent behavior. Both drugs caused mostly insignificant changes of PR interval. Since no significant prolongation of atrioventricular conduction time can be expected in these doses, this is also non-surprising result. Mostly insignificant changes of QRS interval – prolongation by esmolol and shortening by dobutamine – were observed. This again reflects dose dependent effect of these adrenergic ligands. Last but not least, QT intervals were measured and corrected.

Evaluation of QT interval is an important step in preliminary screening of arrhythmogenicity of drugs and drug candidates [11]. QT interval lengthening is known as an independent risk factor for ventricular arrhythmias formation. Heart rate dependence of QT interval duration leads to the necessity of its correction. In this study, it was corrected according to clinically accepted method. Only small variations of QTc were detected in the data. In such situation, it can be concluded that the tested substance does not possess arrhythmogenic potential. However, it must be emphasized that correction validated for human QT interval may cause certain inaccuracy when used in

guinea pig ECG. Some approaches enable subject-specific QT interval correction [32,33], which may eventually reveal subtle changes of RR and QT interval relationship.

In conclusion, ECG recording in anesthetized guinea pig brings valuable data for *in vivo* phase of preclinical testing, regardless its limitations. However, it must be always taken in consideration that originally promising tested substance may be proven ineffective, harmful or toxic even at this step.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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References

1. Bartáková A, Nováková M. Secondary metabolites of plants as modulators of endothelium functions. Int J Mol Sci 2021;22:2533. https://doi.org/10.3390/ijms22052533
2. De Clerck F, Van de Water A, D'Aubioul J, Lu HR, van Rossem K, Hermans A, Van Ammel K. In vivo measurement of QT prolongation, dispersion and arrhythmogenesis: application to the preclinical cardiovascular safety pharmacology of a new chemical entity. Fundam Clin Pharmacol 2002;16:125-140. https://doi.org/10.1046/j.1472-8206.2002.00081.x
3. Umscheid CA, Margolis DJ, Grossman CE. Key concepts of clinical trials: a narrative review. Postgrad Med 2011;123:194-204. https://doi.org/10.3810/pgm.2011.09.2475
4. Varró A, Lathrop DA, Hester SB, Nánási PP, Papp JG. Ionic currents and action potentials in rabbit, rat, and guinea pig ventricular myocytes. Basic Res Cardiol 1993;88:93-102. https://doi.org/10.1007/BF00798257
5. Joukar S. A comparative review on heart ion channels, action potentials and electrocardiogram in rodents and human: extrapolation of experimental insights to clinic. Lab Anim Res 2021;37:25. https://doi.org/10.1186/s42826-021-00102-3
6. Shibata S, Okamoto Y, Endo S, Ono K. Direct effects of esmolol and landiolol on cardiac function, coronary vasoactivity, and ventricular electrophysiology in guinea-pig hearts. J Pharmacol Sci 2012;118:255-265. https://doi.org/10.1254/jphs.11202FP
7. Marks L, Borland S, Philp K, Ewart L, Lainée P, Skinner M, Kirk S, Valentin JP. The role of the anaesthetised guinea-pig in the preclinical cardiac safety evaluation of drug candidate compounds. Toxicol Appl Pharmacol 2012;263:171-183. https://doi.org/10.1016/j.taap.2012.06.007
8. Dawber TR, Meadors GF, Moore FE. Epidemiological approaches to heart disease: the Framingham Study. Am J Public Health Nations Health 1951;41:279-281. https://doi.org/10.2105/AJPH.41.3.279
9. Sagie A, Larson MG, Goldberg RJ, Bengtson JR, Levy D. An improved method for adjusting the QT interval for heart rate (the Framingham Heart Study). Am J Cardiol 1992;70:797-801. https://doi.org/10.1016/0002-9149(92)90562-D
10. Vandenberk B, Vandael E, Robyns T, Vandenberghe J, Garweg C, Foulon V, Ector J, Willems R. Which QT Correction Formulae to Use for QT Monitoring? J Am Heart Assoc 2016;5:e003264. https://doi.org/10.1161/JAHA.116.003264
11. Testai L, Calderone V, Salvadori A, Breschi MC, Nieri P, Martinotti E. QT prolongation in anaesthetized guinea-pigs: an experimental approach for preliminary screening of torsadogenicity of drugs and drug candidates. J Appl Toxicol 2004;24:217-222. https://doi.org/10.1002/jat.975
12. Busch AE, Malloy K, Groh WJ, Varnum MD, Adelman JP, Maylie J. The novel class III antiarrhythmics NE-10064 and NE-10133 inhibit IsK channels expressed in Xenopus oocytes and IKs in guinea pig cardiac myocytes. Biochem Biophys Res Commun 1994;202:265-270. https://doi.org/10.1006/bbrc.1994.1922
13. Testai L, Calderone V, Salvadori A, Breschi MC, Nieri P, Martinotti E. QT prolongation in anaesthetized guinea-pigs: an experimental approach for preliminary screening of torsadogenicity of drugs and drug candidates. J Appl Toxicol 2004;24:217-222. https://doi.org/10.1002/jat.975

14. Testai L, Breschi MC, Martinotti E, Calderone V. QT prolongation in guinea pigs for preliminary screening of torsadogenicity of drugs and drug-candidates. II. J Appl Toxicol 2007;27:270-275. https://doi.org/10.1002/jat.1208
15. Brouillette J, Lupien MA, St-Michel C, Fiset C. Characterization of ventricular repolarization in male and female guinea pigs. J Mol Cell Cardiol 2007;42:357-366. https://doi.org/10.1016/j.yjmcc.2006.11.004
16. O'Hara T, Rudy Y. Quantitative comparison of cardiac ventricular myocyte electrophysiology and response to drugs in human and nonhuman species. Am J Physiol Heart Circ Physiol 2012;302:H1023-H1030. https://doi.org/10.1152/ajpheart.00785.2011
17. Grund F, Tjomsland O, Sjaastad I, Ilebekk A, Kirkebøen KA. Pentobarbital versus medetomidine-ketamine-fentanyl anaesthesia: effects on haemodynamics and the incidence of ischaemia-induced ventricular fibrillation in swine. Lab Anim 2004;38:70-78. https://doi.org/10.1258/00236770460734425
18. Williams WR, Johnston MS, Higgins S, Izzo AA, Kendall LV. Blood profiles in unanesthetized and anesthetized guinea pigs (Cavia porcellus). Lab Anim (NY) 2016;45:35-41. https://doi.org/10.1038/laban.911
19. Akata T. General anesthetics and vascular smooth muscle: direct actions of general anesthetics on cellular mechanisms regulating vascular tone. Anesthesiology 2007;106:365-391. https://doi.org/10.1097/00000542-200702000-00026
20. Schmitz S, Tacke S, Guth B, Henke J. Repeated anaesthesia with isoflurane and medetomidine-midazolam-fentanyl in guinea pigs and its influence on physiological parameters. PLoS One 2017;12:e0174423. https://doi.org/10.1371/journal.pone.0174423
21. Kim SJ, Kang HS, Lee MY, Lee SJ, Seol JW, Park SY, Kim IS, ET AL. Ketamine-induced cardiac depression is associated with increase in [Mg2+]i and activation of p38 MAP kinase and ERK 1/2 in guinea pig. Biochem Biophys Res Commun 2006;349:716-722. https://doi.org/10.1016/j.bbrc.2006.08.082
22. Redfors B, Shao Y, Omerovic E. Influence of anesthetic agent, depth of anesthesia and body temperature on cardiovascular functional parameters in the rat. Lab Anim 2014;48:6-14. https://doi.org/10.1177/0023677213502015
23. Plante E, Lachance D, Roussel E, Drolet MC, Arsenault M, Couet J. Impact of anesthesia on echocardiographic evaluation of systolic and diastolic function in rats. J Am Soc Echocardiogr 2006;19:1520-1525. https://doi.org/10.1016/j.echo.2006.06.011
24. Prys-Roberts C. Isoflurane. Br J Anaesth 1981;53:1243-1245. https://doi.org/10.1093/bja/53.12.1243
25. Eger EI. Isoflurane: a review. Anesthesiology 1981;55:559-576. https://doi.org/10.1097/00000542-198111000-00014
26. Turner PV, Brabb T, Pekow C, Vasbinder MA. Administration of substances to laboratory animals: routes of administration and factors to consider. J Am Assoc Lab Anim Sci 2011;50:600-613.
27. Wiest D. Esmolol. A review of its therapeutic efficacy and pharmacokinetic characteristics. Clin Pharmacokinet 1995;28:190-202. https://doi.org/10.2165/00003088-199528030-00002
28. Pevtsov A, Kerndt CC, Ahmed I, Fredlund KL. Esmolol; StatPearls Publishing, 2022.
29. Dubin A, Lattanzio B, Gatti L. The spectrum of cardiovascular effects of dobutamine - from healthy subjects to septic shock patients. Rev Bras Ter Intensiva 2017;29:490-498. https://doi.org/10.5935/0103-507X.20170068
30. Tuttle RR, Mills J. Dobutamine: development of a new catecholamine to selectively increase cardiac contractility. Circ Res 1975;36:185-196. https://doi.org/10.1161/01.RES.36.1.185
31. Ashkar H, Adnan G, Makaryus AN. Dobutamine; StatPearls Publishing, 2022.
32. Vesely P, Halamek J, Stracina T, Krejcirova L, Novakova M. QT Interval analysis in electrograms of isolated guinea pig hearts treated with haloperidol. *Computing in Cardiology Conference.* Zaragoza, Spain, 2013;40:69-72.
33. Vesely P, Stracina T, Hlavacova M, Halamek J, Kolarova J, Olejnickova V, Mrkvicova V, Paulova H, Novakova M. Haloperidol affects coupling between QT and RR intervals in guinea pig isolated heart. J Pharmacol Sci 2019;139:23-28. https://doi.org/10.1016/j.jphs.2018.11.004