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Principal Investigator: Dr. István Baczkó
Department of Pharmacology and Pharmacotherapy
University of Szeged
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Double transgenic LQT2-5 rabbits – understanding reduced repolarization reserve for more reliable testing of pro-arrhythmic side effects

Background for the studies

Sudden cardiac death (SCD) is among the leading causes of death in developed countries and occurs mostly in patients with underlying acute or chronic ischemic heart disease, however, a significant number of SCD cases have no apparent structural heart disease in the background and can also occur in seemingly healthy individuals [1-3]. An important part of SCD in young patients is caused by inherited arrhythmogenic diseases, including congenital long QT syndromes (LQTS) [4]. Most of these patients have a markedly prolonged QT interval and may develop Torsades des Pointes (TdP) polymorphic ventricular tachycardia that can degenerate into ventricular fibrillation and lead to SCD. So far, mutations in 13 different genes that encode for cardiac ion channels and channel modifying proteins have been described underlying congenital LQT. Phenotypic penetrance is highly variable in patients with inherited LQT syndromes, and the individual arrhythmogenic risk varies greatly between different LQTS patients, even in those carrying the same mutation [5]. Individuals who have normal QT intervals at baseline but carry clinically silent LQT mutations can develop TdP with subsequent SCD following the administration of drugs that prolong repolarization [6]. The assessment of an individual LQTS patient's arrhythmogenic risk therefore remains a difficult task in daily clinical practice [7]. In this regard, the concept of repolarization reserve has emerged [8-9] and has been demonstrated experimentally [10-11]. According to this concept, the loss or impairment (congenital or acquired) of the function of only one ventricular repolarizing ionic current does not always lead to clinically manifest repolarization prolongation and arrhythmia, since other repolarizing currents can compensate [9]. However, when additional, even mild hits on cardiac repolarization occur (e.g. non-cardiovascular drugs, certain dietary constituents with potassium channel inhibitory effects), marked QT prolongation and serious ventricular arrhythmia development can follow.

Importantly, acquired drug-induced LQTS, mechanistically closely related to congenital LQTS, is a more frequently occurring LQTS variant that is mostly caused by compounds blocking cardiac repolarizing currents, including I_{Kr} and I_{Ks}. Certain antiarrhythmic drugs have been known for a long time to induce life-threatening TdP arrhythmia in 3–5% of patients. These drugs are expected to modulate cardiac transmembrane ion channels and

electrophysiological properties of the heart as part of their therapeutic effects. However, there has been increasing concern about TdP arrhythmia and SCD caused by non-cardiovascular drugs, and several of them including astemizole, cisapride, grepafloxacin, terfenadine, terolidine have been withdrawn from the market due to their torsadogenic properties thus preventing their further therapeutic application in patients not susceptible to TdP and leading to the loss of several billion dollars in drug development costs. Therefore, the need for minimizing the pro-arrhythmic risk of novel developmental drugs is urgent and represents a significant challenge [12-13].

The reliable evaluation of pro-arrhythmic risk associated with novel pharmaceutical compounds under development is essential, however, it remains unsatisfactory. Current international guidelines recommend that candidate compounds have to be subjected to cardiovascular safety testing. However, these tests, including studies on isolated cardiomyocytes, Langendorff-perfused rabbit hearts, and *in vivo* experiments in anesthetized dogs, concentrate mainly on detecting I_{Kr}-blocking effects [14]. Unfortunately, none of these currently available experimental methods are sufficiently sensitive and selective to reliably assess the risk for drug-induced TdP and/or SCD development *in vivo*. Therefore, novel models with better predictive value for the identification of pro-arrhythmic risk, paying special attention to pathologies and factors leading to impaired repolarization reserve, are needed.

In this regard, models with impaired repolarization reserve where the increased temporal short-term beat-to-beat variability of the QT interval (STV_{QT}) has been able to more reliably predict subsequent serious cardiac arrhythmia development, have been published by others and by our own group [15-16]. An important aspect of these models is the down-regulation or pharmacological block of I_{Ks} [16-17]. Changes in STV_{QT} correlate better with later ventricular arrhythmia development compared to more conventional ECG parameters in the clinical setting [18-20].

Another approach to impair repolarization reserve in animal pro-arrhythmia models is the modification of genes (mimicking known mutations in humans) encoding for proteins that build up ion channels responsible for cardiac repolarization. So far, in species with cardiac repolarization that is relevant for human cardiac electrophysiology, only two models exist, and both were created by our cooperative partner [21].

The present international co-operative project aimed at the generation, in vivo and in vitro characterization of LQT5 and double transgenic LQT2-5 rabbit models in order to increase our understanding of cardiac ventricular repolarization reserve and to develop models that can provide data for (i) more reliable testing of the pro-arrhythmic adverse effects of candidate compounds in development; (ii) identification of electrophysiological biomarkers for future use in screening patients with latent congenital LQT syndromes and (iii) assessment of risk for serious ventricular arrhythmia development in patients suffering from diseases that affect cardiac electrophysiology.

Brief description of the international co-operative nature of the project

The project was exclusively based on and would not have been viable without co-operation: the constructs and proven methodology for the breeding of LQT5 transgenic rabbits was available at the Hungarian partner's site and for LQT2 rabbits at the German partner's site for the subsequent crossbreeding. The activities and experiments took place at both locations. The two project leaders, Dr. Baczkó and Dr. Odening supervised experiments at their respective sites and held weekly discussions on the advancement of the project. They made personal visits to each other's laboratories. Tibor Hornyik, the PhD student of the PI travelled to the other partner's laboratory to master experimental techniques and to perform experiments. The funding for this co-operation was provided by this present NKFIH NN project and by another project sponsored by the German Foundation of Heart Research (Deutsche Stiftung für Herzforschung, DSHF F/02/14) by Dr. Odening, entitled „Double transgenic LQT2-5 rabbits – A novel *in vivo* model of reduced repolarization reserve for more reliable testing of pro-arrhythmic side effects", having started in January, 2014.

Results

Studies on differences in repolarization reserve and arrhythmia susceptibility in two commonly used species for arrhythmia research: dogs and rabbits

For the evaluation the effects of ipaired repolarization reserve and the combined modification of different potassium currents on arrhythmias *in vivo* in rabbits and dogs, the two most commonly used larger animal species used for arrhythmia research, differences in their arrhythmia susceptibility and repolarization characteristics must be investigated. A large number of animal experimental and clinical studies suggest that the degree of repolarization prolongation does not show a close correlation with subsequent ventricular arrhythmia development. In these cases, without marked prolongation of the QT interval, repolarization reserve may be reduced with a consequent increase in arrhythmia susceptibility. According to the concept of repolarization reserve, normal cardiac repolarization is controlled by different potassium currents in a redundant way, and congenital or acquired (e.g. mild potassium current inhibition by a non-cardiovascular drug) decrease in the function of a single repolarizing current does not always lead to marked repolarization prolongation, since other currents can compensate for the lost function. In the case of reduced repolarization reserve, additional inhibition of another repolarizing current can result in excessive prolongation of repolarization and can provoke serious ventricular arrhythmias. Evidence points to a critically important role for the slow component of the delayed rectifier potassium current (I_{Ks}) in ventricular repolarization reserve, however, other potassium currents may also significantly contribute to repolarization reserve. There is considerable variation in the expression of key repolarizing potassium channels in different mammalian species, including dog and rabbit that are frequently used species in pro-arrhythmia models. Therefore, it is reasonable to assume that species specific ion channel expression profiles may result in species dependent alterations in responses to potassium channel blockers. Such differences may significantly

influence the value of data obtained in these models for human extrapolation, however, it is unclear how species specific potassium channel expressions translate into differences in arrhythmia development in dogs and rabbits. A possibly important role for I_{K1} has been suggested in repolarization reserve. We studied the effects of combined pharmacological inhibition of I_{K1} and I_{Ks}, as well as I_{K1} and I_{Kr} on ECG parameters and the incidence of TdP in conscious dogs and anesthetized rabbits. We also investigated whether TdP development was paralleled by increased short-term variability of the QT interval, a novel ECG parameter suggested for more reliable prediction of drug-induced ventricular arrhythmias. We investigated the effects of repolarization reserve impairment by pharmacological block of I_{K1} in combination with I_{Ks} and I_{Kr} on the incidence of the typical drug-induced arrhythmia, TdP, and different ECG parameters. Heart rates were significantly decreased by combined I_{K1}+I_{Kr} block in both species, while I_{K1}+I_{Ks} inhibition reduced heart rate only in rabbits. Inhibition of I_{Ks} alone as well as I_{K1} alone significantly prolonged the QTc interval in dogs but did not do so in rabbits. Increased QTc intervals by combined potassium channel inhibitions did not appear to be informative on subsequent TdP development in either species. We found that combined pharmacological inhibition of I_{K1}+I_{Kr} and I_{K1}+I_{Ks} led to repolarization reserve impairment and high incidence of TdP in conscious dogs and anesthetized rabbits. However, dogs and rabbits exhibited markedly different patterns of TdP suggesting that at least some of these currents may play different relative roles in repolarization reserve in the two species. In contrast, our laboratory showed in previously published experiments that both species responded with a high incidence of TdP paralleled by significant increases of short-term variability of the QT interval (STV_{QT}) following I_{Ks}+I_{Kr} inhibitor administration. In this study, a high TdP incidence was observed following inhibition of I_{K1}+I_{Ks} in dogs (67% vs 14% in rabbits). Rabbits exhibited higher TdP incidence after I_{K1}+I_{Kr} block (72% vs 14% in dogs). Increased TdP incidence was associated with significantly larger STV_{QT} in both models.

We concluded that rabbit pro-arrhythmia models based on pharmacologically impaired repolarization reserve may present greater arrhythmia susceptibility and may be more useful than canine models in predicting human electrophysiological responses to drugs affecting cardiac ventricular repolarization. These results also warrant cautious evaluation of the potential pro-arrhythmic adverse effects and cardiovascular safety of candidate compounds in rabbit and dog models.

A novel transgenic rabbit model with reduced repolarization reserve: long QT syndrome caused by a dominant-negative mutation of KCNE1 gene

We created a rabbit model for long-QT syndrome type-5 (LQT5) with cardiac-specific overexpression of a mutant (G52R) KCNE1 β-subunit of the slow delayed-rectifier K^+-channel (I_{Ks}). ECG parameters, including short-term variability of the QT interval (STV_{QT}), a biomarker for proarrhythmic risk, and arrhythmia development were recorded. In vivo arrhythmia-susceptibility was evaluated by i.v. administration of the I_{Kr} blocker dofetilide. K+ currents were measured with the patch-clamp technique. Patch-clamp studies in isolated ventricular myocytes revealed accelerated I_{Ks} and I_{Kr} deactivation-kinetics in LQT5 rabbits. At baseline, LQT5 animals exhibited slightly but significantly prolonged heart-rate corrected

QT index (QTi) and increased STV_{QT}. Dofetilide provoked Torsade-de-Pointes arrhythmia in a greater proportion of LQT5 rabbits, paralleled by a further increase in STV_{QT}. Conventional ECG parameters characterizing repolarization duration, the QT and frequency corrected QT intervals (QTc), were not different in the two groups at baseline (QT: 146.9 ± 3.18 in WT vs. 145.7 ± 2.87 ms in TG; QTc: 156.8 ± 2.63 in WT vs. 155.6 ± 2.12 ms in TG, all $p>0.05$). Following dofetilide administration, QT and QTc intervals were significantly prolonged in both groups to a similar extent (QTc: 169.4 ± 3.37 in WT and 167.8 ± 3.17 ms in TG). However, the short-term variability of the QT interval, a novel ECG parameter suggested for estimation of repolarization temporal instability and proarrhythmic risk, was higher in KCNE1 TG rabbits compared to WT at baseline (4.8 ± 0.26 in TG vs. 2.8 ± 0.15 ms in WT, $p<0.05$). Following the administration of dofetilide, the incidence of the typically drug induced Torsades des Pointes arrhythmia was significantly higher in TG rabbits compared to WT (77% in TG vs. 51.8% in WT, $p<0.05$). We have created a novel transgenic LQT5 rabbit model with increased susceptibility to drug-induced arrhythmias that may represent a useful model for testing proarrhythmic potential and for investigations of mechanisms underlying arrhythmias and sudden cardiac death due to repolarization disturbances.

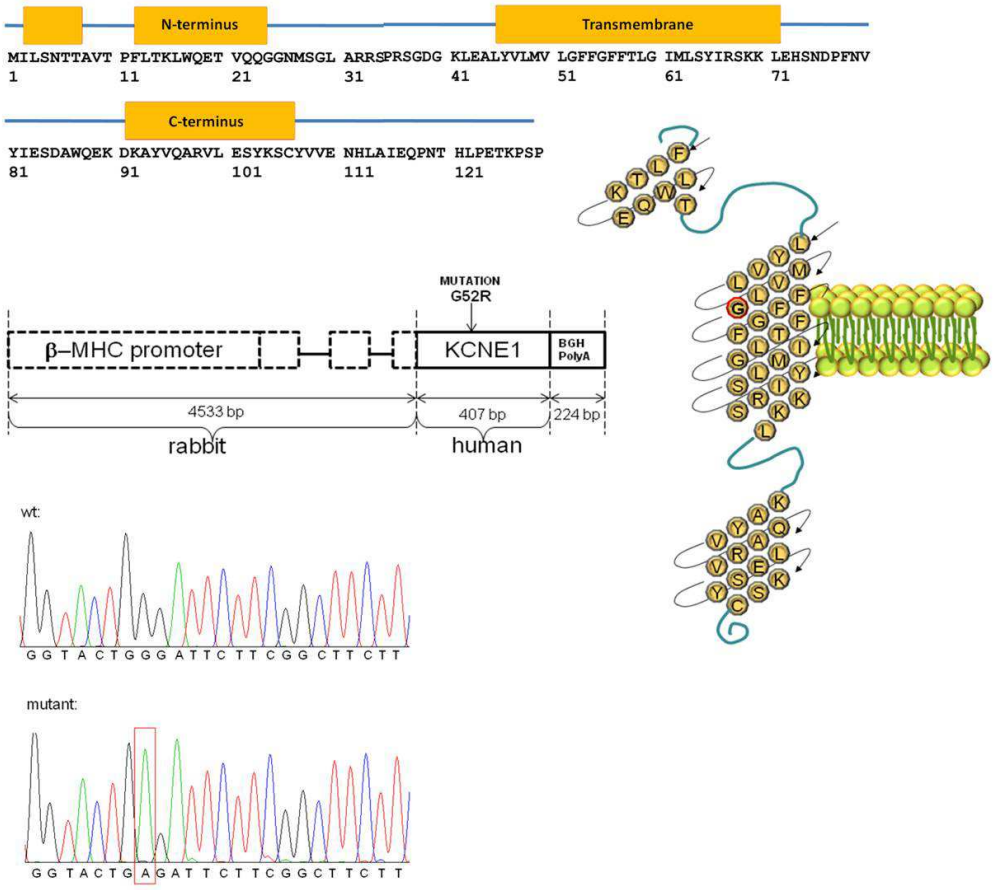

Fig. 1. The G52R mutation and the transgene construct. Schematic drawing of the mutation in KCNE1 polypeptide (top panel) and the transgene construct (middle panel). Mutation G to A at position 154 of human KCNE1 cDNA and wild-type sequence. Sense-strand sequences are shown (bottom panel).

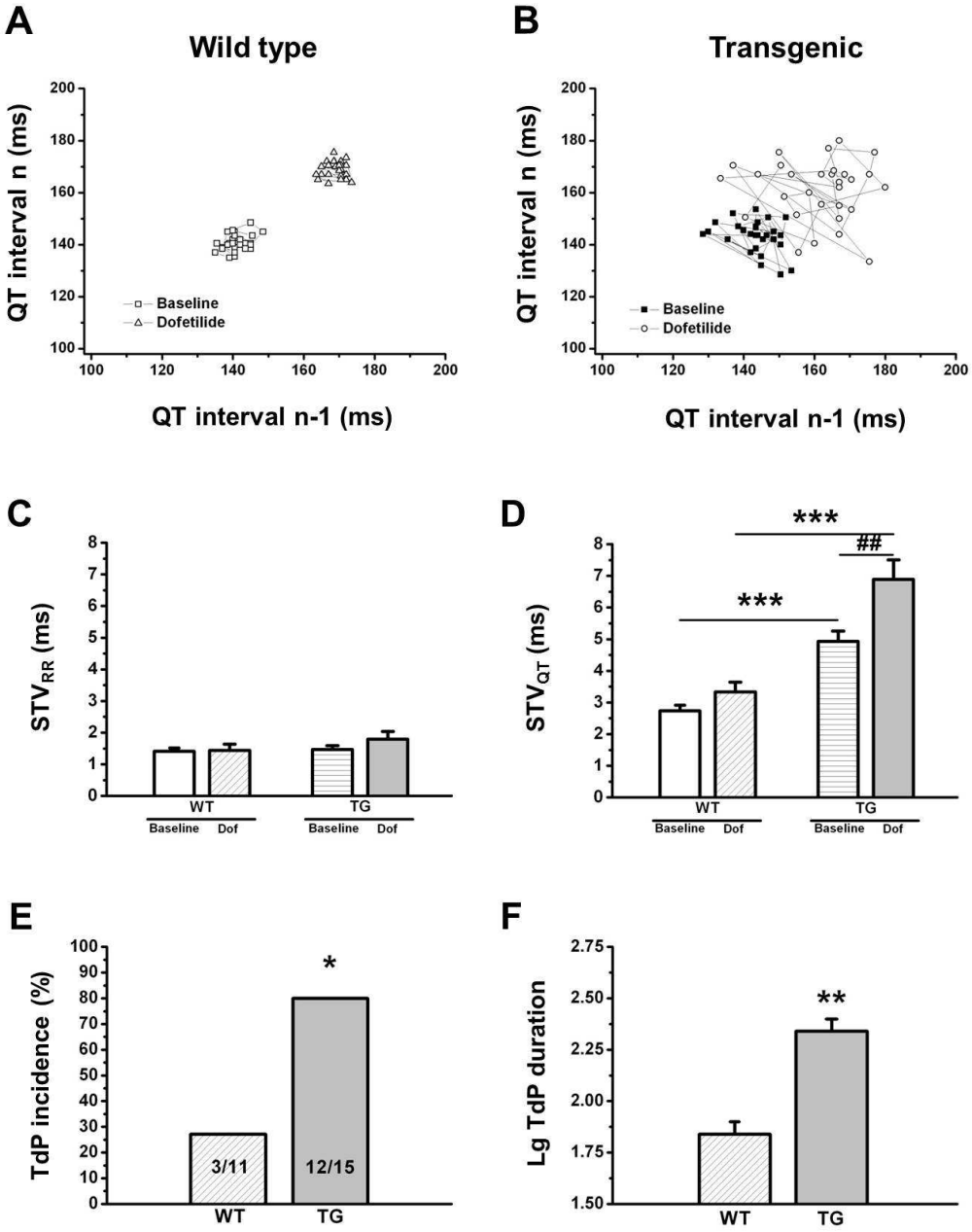

Fig. 2. Short-term variability of the RR and QT intervals and Torsade-de-Pointes in thiopental anesthetized rabbits. (A-B) Representative Poincaré plots demonstrate higher short term beat-to-beat variability of the QT interval (STV_{QT}) in anesthetized transgenic rabbits at baseline conditions compared to wild-type animals. Following the administration of the I_{Kr} blocker dofetilide, STV_{QT} further and markedly increased only in transgenic animals. (C) There were no differences in short-term variability of the RR interval (STV_{RR}) between wild-type (WT) and transgenic (TG) rabbits either at baseline conditions or following the administration of the I_{Kr} blocker dofetilide. (D) Short-term variability of the QT interval (STV_{QT}) was higher in TG animals at baseline conditions and following dofetilide infusion, indicating increased temporal instability of repolarization in transgenic rabbits. (E) Accordingly, transgenic animals exhibited Torsade-de-Pointes (TdP) with significantly higher incidence. (F) The duration of TdP episodes was significantly longer in TG rabbits, expressed as the log10 of duration in seconds (to allow statistical comparison of data with normal distribution). Dof: dofetilide (20 μg/kg, i.v.); n = 11 and 15 animals in WT and TG groups, respectively on panels (C) to (E); n = 3 and 12 animals on panel (F); *p<0.05 vs. wild-type; #p<0.05 vs. baseline in the same group.

Breeding of LQT5, LQT2 and 2-5 transgenic rabbits

In cooperation with Dr. Katja Odening in Freiburg, successful breeding of transgenic LQT2, LQT5 and LQT2-5 rabbits according to the research plan was carried out and was continuously performed during the project – and it is still going on to continue the co-operation. Most of the inseminations were successful and resulted in the birth of a number of offsprings.

Combined use of transgenic LQT2, LQT5 and LQT2-5 rabbit models with decreased repolarization reserve as a novel tool for pro-arrhythmia research

Different transgenic LQTS rabbit models with impaired repolarization reserve were generated by overexpressing the loss-of-function mutants of human HERG (HERG-G628S, loss of I_{Kr}; LQT2) or KCNE (KCNE1-G52R, decreased I_{Ks}; LQT5) or both transgenes (LQT2-5) in the heart. *In vivo* telemetric ECG (QTc, QTi (QT observed/QT expected)) and ex vivo monophasic action potential (MAP) measurements in Langendorff-perfused hearts (action potential duration (APD_{75}), triangulation (APD_{90}-APD_{30}) and reverse use-dependence (APD_{75} at 2Hz-4Hz) were performed to assess the effects of several K^+ channel blockers on cardiac repolarization in wild type (WT), transgenic LQT2, LQT5, and LQT2-5 rabbits. At baseline, QTc (ms) was similar in LQT5 (132.1±6.5) as in WT (135.7±4.8) but was significantly prolonged in LQT2 and LQT2-5 rabbit models (163.9±9.2 and 165.4±12.9; $p<0.05$ vs. WT). Slight I_{Kr}-blockade by low-dose dofetilide (0.02 µg/kg, i.m.) prolonged QT *in vivo* only in LQT5 (QTi (%), 104.5±3.5, $p<0.05$ vs. baseline) but not in WT, nor in LQT2 and LQT2-5 rabbits that lack I_{Kr}. The I_{K1}-blocker $BaCl_2$ (0.3mg/kg, i.m.) prolonged QT in all groups but this effect was particularly pronounced in LQT2 (QTi (%), 110.8±4.8; $p<0.05$ vs. WT, LQT5 and LQT2-5). I_{Ks}-block alone (0.1 mg/kg HMR-1556, i.m.) did not show any significant effect on QT duration in any genotype but combined block of I_{K1} and I_{Ks} resulted in increased QT in all groups. *Ex vivo* at baseline, LQT2 and LQT2-5 shown significantly prolonged APD_{75} compared to WT or LQT5 and significant apico-basal APD heterogeneities were measured in all transgenic animals but not in WT. APD_{75} prolongation induced by I_{Ks}-blocker HMR-1556 (100nM) was more pronounced in LQT2 and LQT2-5 as in WT or LQT5 (changes (ms±SEM), LQT2:+14.7±2.3, LQT2-5:+12.8±2.9 vs. WT:+6.9±1.2 or LQT5:+6.3±1.2). I_{K1}-blocker $BaCl_2$ (10µM) or combined I_{K1}/I_{Ks}-blockade by $BaCl_2$+HMR prolonged APD_{75} significantly more in LQT2 and LQT2-5 than in WT (changes (ms±SEM), $BaCl_2$: LQT2:+29.4±2.8, LQT2-5:+31.7±4.7 vs. WT:+17.7±2.5; $BaCl_2$+HMR: LQT2:+36.8±4.9, LQT2-5:+32.2±3.5 vs. WT:+15.7±2.4; all $p<0.05$). Importantly, triangulation of APD and reverse use-dependence were also more pronounced upon I_{K1}-blockade or combined I_{K1}/I_{Ks}-blockade in LQT2 and LQT2-5 than in WT. In conclusion, LQT2 and LQT2-5 rabbit models with pronounced reduction of repolarization reserve are very sensitive to K^+ channel blockers demonstrating not only QT prolongation but also AP triangulation and reverse use-dependence. The combined use of different transgenic LQTS rabbit models may provide further insights into pro-arrhythmic mechanisms of K^+ channel blocking drugs.

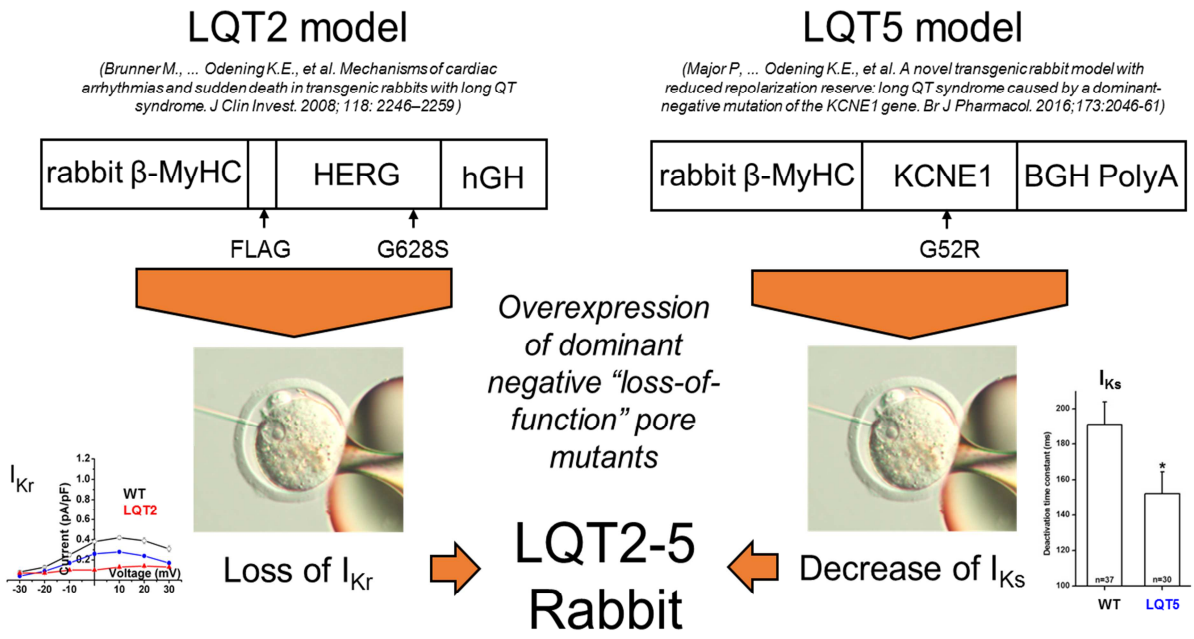

Fig. 3. Schematic illustration of the generation of LQT5 and LQT2 rabbits by pronuclear injection of DNA constructs containing the loss-of-function mutant channel under the control of rabbit β-MyHC promoter (see above) into rabbit embryos. LQT2-5 animals were generated by crossbreeding of LQT5 and LQT2.

Genotype-specific beneficial QT-shortening effects of docosahexaenoic acid (DHA) in transgenic LQT2, LQT5 and LQT2-5 rabbit models

Current treatment strategies in long QT syndrome (LQTS) are symptom-directed, aimed at reducing pro-arrhythmic triggers and arrhythmias (beta blockade and implantation of ICD). These therapies, however, fail to prevent arrhythmia events in up to 40% of affected individuals. Thus, there remains an unmet need for novel, genotype/mutation-specific and mechanism-directed therapies in LQTS. Polyunsaturated fatty acid such as docosahexaenoic acid (DHA) have been demonstrated to increase the repolarizing I_{Ks} current. DHA's I_{Ks} activating and repolarization shortening effect depends on the functionality of the channel alpha-subunit KvLQT1 and beta-subunit KCNE1. We have generated different transgenic LQTS rabbit models (LQT2, HERG-G628S, loss of I_{Kr}; LQT5, KCNE1-G52R, decreased I_{Ks}; double-transgenic LQT2-5, loss of I_{Kr}/decreased I_{Ks}) to investigate genotype-specific benefitial effects of DHA in various subtypes of LQTS. In wild type (WT), transgenic LQT2, LQT5, and LQT2-5 rabbits *in vivo* telemetric ECG analyses were performed at baseline and after 70µM DHA i.m. to assess changes in heart rate corrected QT (QTc, QTi (QT observed / QT expected)). *Ex vivo* monophasic action potential measurements in Langendorff-perfused hearts were performed to investigate DHA-induced changes (20µM) in action potential duration (APD_{75}). At baseline, QTc (ms) was similar in LQT5 (132±6.5, n=10) as in WT (136±4.8, n=7) but was significantly prolonged in LQT2 and LQT2-5 rabbit models (164±9.2, n=8 and 163±13.5, n=8; $p<0.05$ vs. WT). The I_{Ks}-activator DHA significantly shortened QTc (ms) *in vivo* only in rabbits with functional alpha- and beta-subunits of I_{Ks} channels, i.e., WT (-10.46±3.2, n=4, $p<0.01$) and more pronouncedly in LQT2 rabbits (-22.18±5.2, n=3, $p<0.01$).

In contrast, DHA had no effect on QTc in LQT5 (-5.89±4.5, n=5) and LQT2-5 rabbits (+0.34±5.4, n=3) that are harbouring loss-of-function mutations in the beta-subunit KCNE1. Similarly, *ex vivo*, DHA significantly shortened APD_{75} (ms) in WT (-16±3.7, n=4, $p<0.05$) and more pronouncedly in LQT2 rabbits (-22.7±2.3, n=3, $p<0.01$), but had no effect on QTc in LQT5 (-2.2±7.2, n=6) and LQT2-5 rabbits (-4.2±3.4, n=5). The I_{Ks}-activator docosahexaenoic acid exerts genotype-specific beneficial QT/APD shortening effects in LQT subtypes with intact alpha- and beta-subunits of I_{Ks} channels. In types of LQTS with loss-of-function mutations in KCNE1 β-subunits, however, no beneficial effects can be observed. Future studies investigating potential anti-arrhythmic effects of this genotype-specific acceleration of repolarization are warranted.

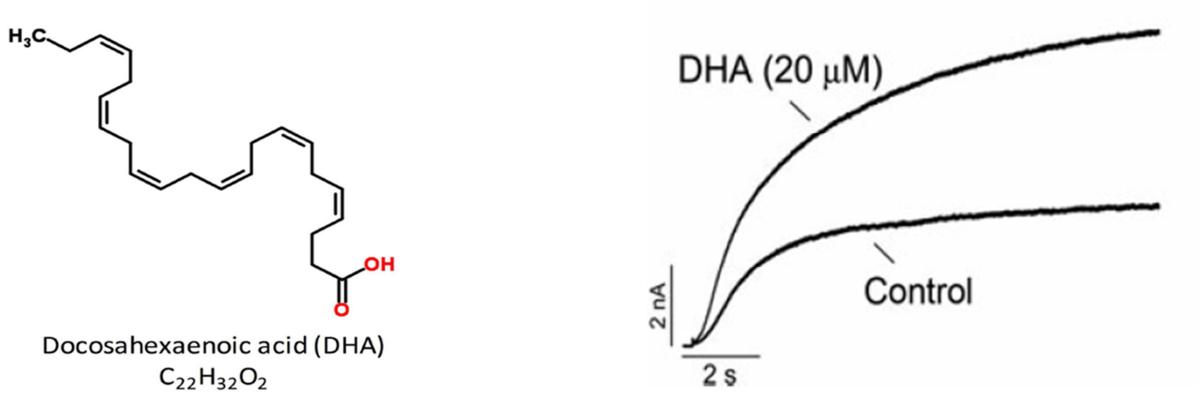

Fig. 4. Structural formula of docosahexaenoic acid (DHA), and DHA effects on I_{Ks} current: patch-clamp original traces of I_{Ks} current obtained in the absence and in the presence of DHA.

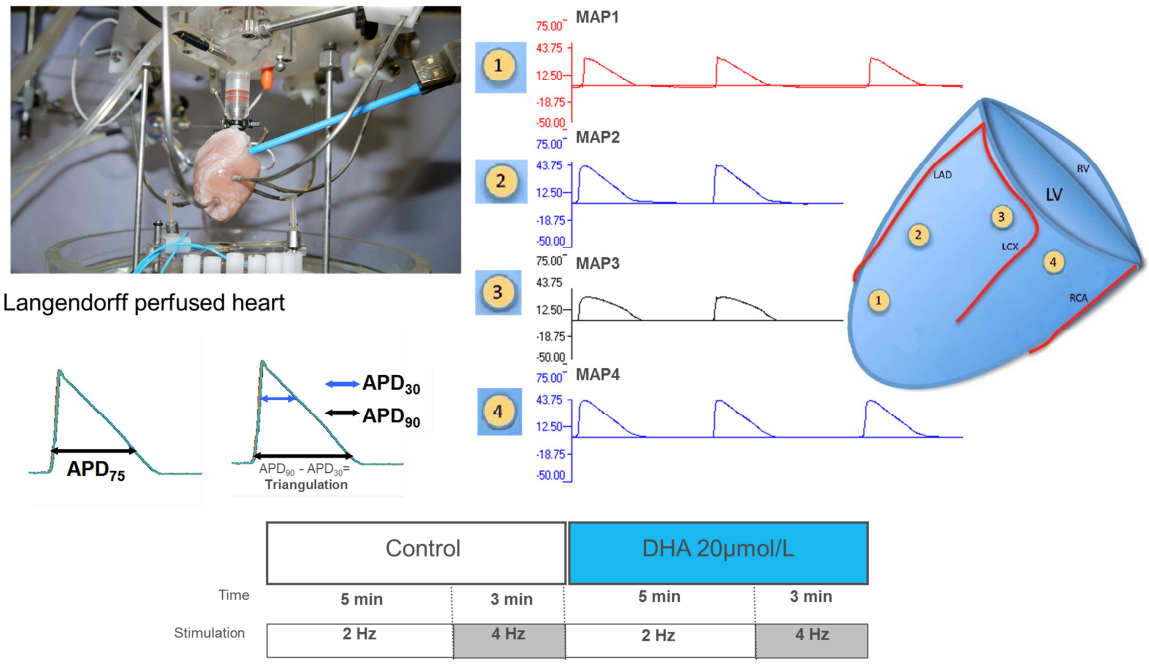

Fig. 5. Illustration of the epicardial mapping during Langendorff perfusion. Four electrodes measured the epicardial action potentials in pre-established regions of the left ventricle (upper panel). Protocol of *ex vivo* part of the study with the applied concentration of DHA (lower panel).

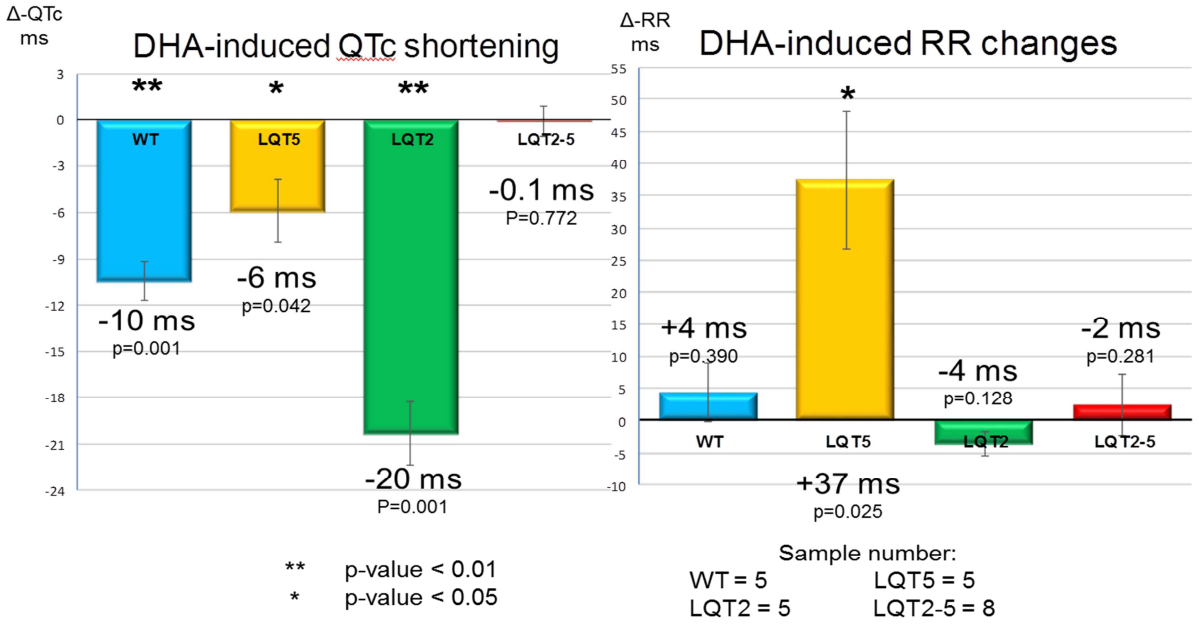

Fig. 6. QTc and RR interval variation observed *in vivo* in the 4 different genotypes after intramucular administration of DHA.

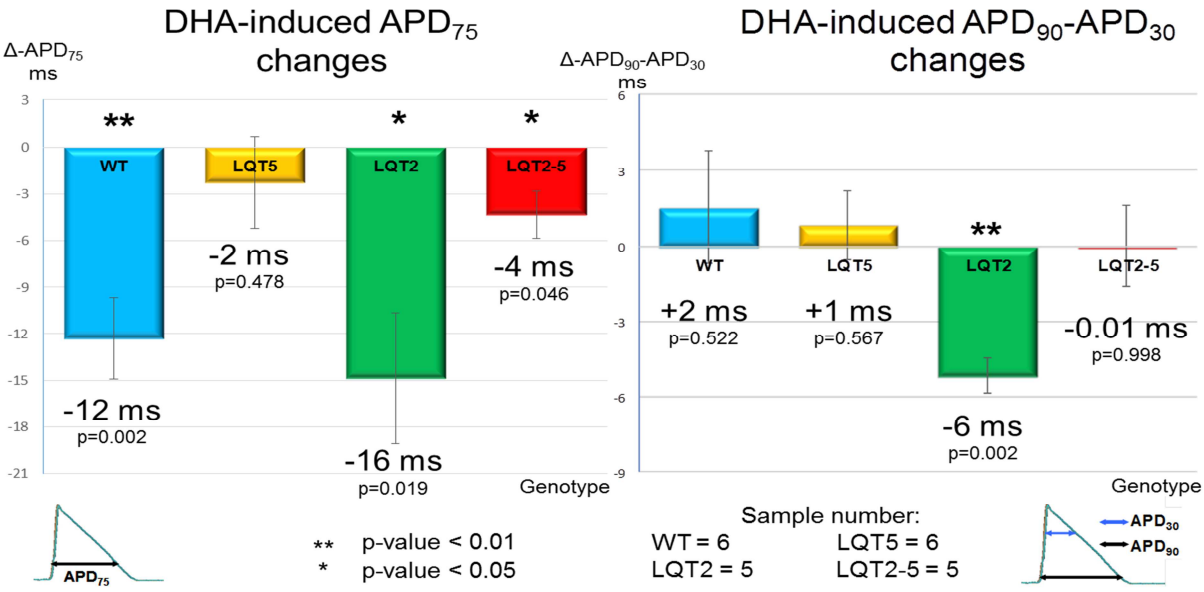

Fig. 7. APD_{75} variation observed in the 4 different genotypes after DHA administration in Langendorff-perfused hearts *ex vivo* and AP triangulation variation observed in the 4 different genotypes after DHA administration in Langendorff-perfused hearts *ex vivo*.

Dissemination of results

The results of the studies were presented at national and international scientific meetings (Hungarian Society of Cardiology, Hungarian Physiological Society, European Working Group for Cellular Cardiac Electrophysiology – European Society of Cardiology, Gordon

Research Conferences – Cardiac Arrhythmia Mechanisms Meetings, Frontiers in Cardiovascular Biology, International Academy for Cardiovascular Sciences: European Section and American Section meetings, 17th World Congress of Basic and Clinical Pharmacology). The concepts and results were, and will be published in high impact international scientific journals (2 more papers are in the pipeline):

I. Baczkó I, Leprán I, Kiss L, Muntean DM, Light PE. Future perspectives in the pharmacological treatment of atrial fibrillation and ventricular arrhythmias in heart failure. Curr Pharm Des, 2015, 21(8): 1011-1029. *IF (2015) = 3.052*

II. Husti Z, Tábori K, Juhász V, Hornyik T, Varró A, Baczkó I. Combined inhibition of key potassium currents differently affects cardiac repolarization reserve and arrhythmia susceptibility in dogs and rabbits. Can J Physiol Pharmacol, 2015, 93(7): 535-544. *IF (2015) = 1.704*

III. Bősze Z, Major P, Baczkó I, Odening KE, Bodrogi L, Hiripi L, Varró A. The potential impact of new generation transgenic methods on creating rabbit models of cardiac diseases. Progress in Biophysics and Molecular Biology, 2016, 121: 123-130. *IF (2016) = 3.227*

IV. Baczkó I, Jost N, Virág L, Bősze Zs, Varró A. Rabbit models as tools for preclinical cardiac electrophysiological safety testing: importance of repolarization reserve. Progress in Biophysics and Molecular Biology, 2016, 121: 157-168. *IF (2016) = 3.227*

V. Major P*, Baczkó I*, Hiripi L, Odening KE, Juhász V, Kohajda Zs, Horváth A, Prorok J, Seprényi Gy, Kovács M, Ördög B, Doleschall Z, Nattel S, Varró A, Bősze Zs. A novel transgenic rabbit model with reduced repolarization reserve: long QT syndrome caused by a dominant-negative mutation of KCNE1 gene. British Journal of Pharmacology, 2016, 173(12): 2046-2061. *shared first authorship. *IF (2016) = 5.491*

The NKFI grant ID was acknowledged in all publications. There are two additional manuscripts in preparation on the results obtained from the double transgenic LQT2-5 model (based partly on results presented in the last 3 abstracts in the publication list), entitled „Double transgenic LQT2-5 rabbits with decreased repolarization reserve as novel tools for more reliable identification of pro-arrhythmic biomarkers" and „Genotype-specific beneficial QT-shortening effects of docosahexaenoic acid in transgenic LQT2, LQT5 and LQT2-5 rabbit models". Dr. Tibor Hornyik, the PhD student of the PI received the Margaret Moffat Award for the best poster in biomedical sciences at the 5th North American Section IACS Meeting in Orlando, Florida, in 2017, for his presentation, entitled „Transgenic LQT2, LQT5 and LQT2-5 rabbit models with decreased repolarization reserve as novel tools for more reliable identification of pro-arrhythmic biomarkers".

Possible exploitation of the results

Our results can provide invaluable data for the better understanding of cardiac ventricular repolarization and repolarization reserve, and of the physiological and pathophysiological roles of different cardiac potassium channels. The results of these studies have significant future clinical translational implications as follows:

1. The transgenic rabbit models can possibly be used for more reliable testing of pro-arrhythmic side effects of candidate compounds in drug development.

2. The electrophysiological results can facilitate the development of novel strategies for screening congenital LQT patients with latent LQT.

3. Based on the electrophysiological results (both spatial dispersion, and temporal variability) the results may help to identify and characterize putative „biomarkers" for arrhythmia risk assessment and arrhythmia prediction in patients with reduced repolarization reserve.

Also, we aim at future economic exploitation of our results: following further research and development, characterization and validation of these transgenic rabbit models, they may represent improved models for testing the proarrhythmic adverse effects of new drugs under development for pharmaceutical companies.

Alterations in the budget

The foreign conference travel and accomodation costs were not exactly as originally planned, due to unforseen changes in the number and increasing costs of conference participations. Importantly, the overall budget spending did not exceed the planned amount. These alterations did not affect the completion of the studies.

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