Research report

Most sudden cardiac death events in athletes are associated with cardiac muscle structural disorders. However, the underlying cause remains unclear in 3-6% of such death events. Apart from the structural disorders, functional remodeling (e.g. reduced repolarization reserve) might also lead to life-threatening ventricular tachyarrhythmias. Our first aim was to establish an exercise-induced hypertrophy animal model. Rodents repolarize with a different set of ion channels causing much shorter action potential durations compared to humans. For this reason, we planned to use a rabbit model. There was no evidence in the literature of an exercise-induced rabbit model to mimic human athlete's heart. Therefore, a new treadmill system had to be developed. After a long planning period, we could achieve to develop a special treadmill system dedicated especially for rabbit training. After a long test phase, a series of experiments was performed with 10 rabbits which lasted 16 weeks. The applied training protocol started with an increasing intensity running and continued with a regular steady-state daily training at 2.5 km/h for 20 minutes. 'Trained' group had running sessions 5 days a week, while 'Sedentary' group did not participate in the training. We succeeded in doing echocardiography measurements under anaesthasia in every 4 weeks. Since fibrosis in the ventricles renders them susceptible to arrhythmias, providing substrate of polymorphic ventricular tachycardia, e.g.Torsades de Pointes, at the end of the experiment the hearts were excised and a histological investigation was done in order to quantify the grade of fibrosis. Echocardiography did not show significant difference between the groups before the training started. After 8 weeks of training, the diastolic internal diameter of the left ventricle (LVIDd) was significantly greater in the 'Trained' group than in the 'Sedentary' group (17.1±0.5 mm vs. 14.8±0.8 mm, $p<0.05$). The diastolic interventricular septal thickness significantly increased in the 'Trained' group as compared with the baseline values. Ejection fraction did not change considerably, however, resting heart rate was lower in the 'Trained' group. Lower heart rate and increased LVIDd indicate a morphological and functional remodeling in the trained rabbit heart. Semi-quantitative histological examination showed higher grade of fibrosis in the 'Trained' group vs. 'Sedentary' group, although the difference was not significant.

To continue our investigation about the athlete's heart a second series of study was performed investigating the effect of the long-term exercise training was tested on the electrical activity of the myocardium in our new rabbit athlete's heart model. New-Zealand white rabbits were randomized into a 'Sedentary' and to an 'Exercised' group again. Both groups contained 7 rabbits. Animals of the 'Exercised' group were trained during a 12-week long treadmill-running protocol. Echocardiography and resting ECG recording were performed under ketamine anaesthesia. At the end of training protocol, proarrhythmic sensitivity were tested with dofetilide (50 nM) in Langendorff-perfused rabbit hearts. ECG repolarization parameters and sinus variability of ECG parameters were evaluated. Tissue samples were taken from the left ventricle and messenger RNA (mRNA) expression level of TGF-ß, fibronectin-1, collagen-I,-III, MMP-2 and TIMP-1 were quantified with RT-qPCR to determine the collagen metabolism. Echocardiography on the 12th week showed significant increase in the internal end-diastolic diameter of the left ventricle (LVIDd) in the 'Exercised' group (17.4±0.3 vs. 14.7±0.8 mm, $p<0.05$) compared to the 'Sedentary' group. Resting heart rate was significantly lower (198±4 vs. 253±8, $p<0.05$), PQ, QT, RR, Tpeak-Tend intervals and variability parameters of the RR and Tpeak-Tend intervals *in vivo* were significantly greater in the 'Exercised' group.

Dofetilide tended to increase the QTc interval in the 'Exercised' group *in vitro*, however, there was no difference in the incidence of proarrhythmia between the two groups. RT-qPCR showed significantly greater mRNA expression of TIMP-1 in the 'Exercised' group. We concluded that the increased LVIDd and the decreased heart rate are characteristics of the exercise-induced athlete's heart. Increased parasympathetic tone of the autonomic nervous system was manifested by the extended PQ and RR intervals and their variability parameters. Greater variability and repolarization parameters may indicate the sensibility of the athlete's heart to arrhythmia. Increased TIMP-1 indicated structural remodelling in our model. We did not find a significant increase in proarrhythmia incidence, thus further investigations are warranted. Our results have already been submitted in a form of a scientific article which under consideration for publication.

Currently we have many experimental and clinical guidelines in arrhythmia research, but these are not unified, and that is a notable problem when we try to objectively evaluate our knowledge. As our research group has been doing proarrhythmia experiments evaluating arrhythmias under experimental conditions, it is really important to follow arrhythmia guidelines. The aim of one this study was to examine whether the systems of arrhythmia definitions of the most frequently used arrhythmia diagnosis guideline, Lambeth Convention I. (published in 1988) and the updated Lambeth Convention II (published in 2013) are compatible with each other, and yield the same qualitative arrhythmia results by applying the two systems of definitions. Male rat hearts were perfused on a Langendorff perfusion system by Class I antiarrhythmic drugs (quinidine, lidocaine or flecainide). One lower and one higher concentration of quinidine (0.79 and 7.90 M), lidocaine (3.88 and 12.93 *M*), and flecainide (0.74 and 1.48 *M*), representing the peak unbound plasma and total blood concentrations, respectively, at "therapeutic" dosage were applied. The present investigation showed that the antifibrillatory effect of flecainide seen with the VF definition of LC I was masked completely, when the new VF definition of LC II was applied. Also it was shown that application of the VF definition of LC II increased VF detection not only in the flecainide-treated group, but even in the quinidine- and lidocaine-treated groups, and also in the control group. Furthermore, it was demonstrated that application of VF definition of LC II substantially changed the conclusion about the physiological and pathophysiological effects of K^+ concentration on VF development. These are the first clear cut evidences that by changing the guidelines and the definition of VF the analysis of the same ECG recordings will yield completely different conclusion. Thus, it can be deducted that the change in VF definition will retrospectively influence the results of many pharmacological, physiological and pathophysiological studies previously made. The manuscript summarizing our results is under construction.

As we are really interested in the proarrhythmia detection, a whole in vitro study was performed with different repolarisation prolonging drugs in order to set-up a new proarrhythmia screening assay. Preclinical *in vivo* QT measurement as a proarrhythmia essay is expensive and not reliable enough. The aim of that study was to develop a sensitive, cost-effective, Langendorff perfused guinea pig heart model for proarrhythmia safety screening. Low concentrations of dofetilide and cisapride (inhibitors of the rapid delayed rectifier potassium current, I_{Kr}) were tested alone and co-perfused with HMR-1556 (inhibitor of the slow delayed rectifier potassium current, I_{Ks}) in Langendorff perfused guinea pig hearts. The electrocardiographic rate corrected QT (QTc) interval, the T_{peak}-T_{end} interval and the beat-to-beat variability and instability (BVI) of the QT interval were determined in sinus rhythm. Dofetilide and HMR-1556 alone or co-perfused, significantly prolonged the QTc interval by

20%, 10% and 55%, respectively. Similarly, cisapride and HMR-1556 alone or co-perfused, significantly prolonged the QTc interval by 11%, 11% and 38%, respectively. Catecholamines increased heart rate and abolished the QTc prolonging effects of the I_{Kr} inhibitors. I_{Ks} inhibition antagonized the QTc shortening effects of cathecolamines. None of the drug perfusions increased significantly the T_{peak}-T_{end} interval and the sinus BVI of the QT interval. I_{Ks} inhibition increased the QTc prolonging effect of I_{Kr} inhibitors in a super-additive manner, and QTc was superior to other proarrhythmia biomarkers measured in sinus rhythm in isolated guinea pig hearts. The effect of catecholamines on the QTc facilitated differentiation between I_{Kr} and I_{Ks} inhibitors. Thus, QTc measurement in Langendorff perfused guinea pig hearts with pharmacologically attenuated repolarization reserve and periodic catecholamine perfusion seems to be suitable for proarrhythmia screening. We published this study in the Journal of Cardiovasculat Pharmacology.

Predicting lethal arrhythmia liability from beat-to-beat variability and instability (BVI) of the ECG intervals is a useful technique in drug assessment. Most investigators use only arrhythmia-free ECGs for this reason. Recently, it was shown that drug-induced torsades de pointes (TdP) liability can be predicted more accurately from BVI measured irrespective of rhythm, even during arrhythmias (absolute BVI). The present study tested the broader applicability of this assessment by examining whether absolute BVI parameters predict another potential lethal arrhythmia, ischaemia-induced ventricular fibrillation (VF). Langendorff-perfused rat hearts were subjected to regional ischaemia for 15 min. Absolute BVI parameters were derived from ECG intervals measured in 40 consecutive ventricular complexes (irrespective of rhythm) immediately preceding VF onset and compared with time-matched values in hearts not expressing VF. Increased frequency of non-sinus beats and 'R on T' arrhythmic beats, shortened mean RR and electrical diastolic intervals, and increased BVI of cycle length and repolarization predicted VF occurrence. Absolute BVI parameters that quantify variability of repolarization (e.g. 'short-term variability' of QT interval) had the best predictive power with high sensitivity and specificity. In contrast, VF was not predicted by any BVI parameter derived from the last arrhythmia-free interlude before VF. The novel absolute BVI parameters that predicted TdP in rabbit also predict ischaemia-induced VF in rat, indicating a diagnostic and mechanistic congruence. Repolarization inhomogeneity represents a pivotal biomarker of ischaemia-induced VF. The newly validated biomarkers could serve as surrogates for VF in pre-clinical drug investigations. These important results were published in the British Journal of Pharmacology.

In another interesting study we investigated the relationship between heart rate and coronary flow in isolated guinea pig hearts, and to examine whether the relationship is affected by variability of heart rate. We recorded the ECG and coronary flow during control perfusion in isolated, Langendorff perfused guinea pig hearts. The average heart rate was calculated and the beat-to-beat variability of heart rate was quantified. Hearts were retrospectively allocated to a “Low” or a “High” heart rate variability group. We found a linear correlation between the average heart rate and the coronary flow in the “Low” as well as the “High” heart rate variability group. The slope of the regression line was significantly greater in the “High” than in the “Low” heart rate variability group; this resulted in higher coronary flow values in the “High” heart rate variability group in the physiological heart rate range. There is a linear correlation between the average heart rate and the coronary flow, but the variability of heart rate influences the correlation. In the physiological heart rate range, an autonomic-independent coronary flow autoregulation occurs in the *ex vivo* - denervated guinea pig heart, mediated by increases in

heart rate and by heart rate irregularity. We have just published our results in the Journal of Pharmacological and Toxicological Methods.

The OTKA support was really important for us to achieve the above mentioned results. Financial support allowed us to develop a special treadmill system, to use infusion pumps for the experiments and to acquire and evaluate the really important ECG, left ventricular pressure, flow and temperature data throughout our various studies. This support contributed to the preparation of a PhD thesis under my supervision. We could achieve our goals, however, a significant amount of work requires to definitely prove our concept about sudden cardiac death in athletes. Other research studies were done during that period allowed the investigation of proarrhythmias in other aspects with newer biomarkers. Three papers are under preparation which will be submitted soon to international scientific journals.