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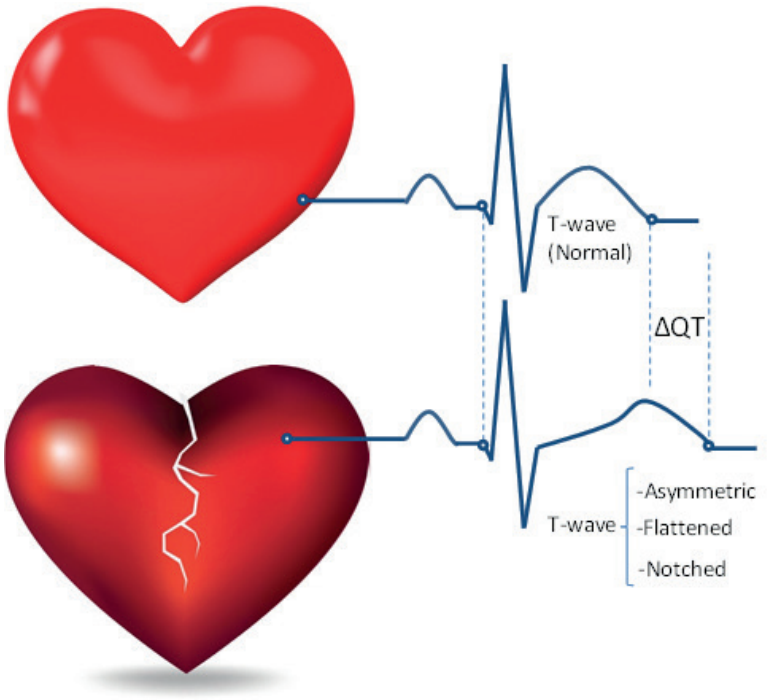

ECG-BASED MEASUREMENTS OF DRUG-INDUCED REPOLARIZATION CHANGES

BY
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ECG-Based Measurements of Drug-induced Repolarization Changes

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Ph.D. Dissertation

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Abstract

Drug induced abnormality in cardiac repolarization poses a major threat to the vulnerable patients with the risk of triggering the Torsades de Pointes arrhythmia- which is potentially lethal and can be accounted for sudden cardiac death. Repolarization abnormality at the cardiac myocyte is reflected on the surface ECG as a prolonged QT interval, altered morphology of the T-wave. The underlying cellular mechanism for prolonged repolarization duration is the inhibition of the rapid component of delayed rectifier potassium current (I_{Kr}) which is encoded by KCNH2 (hERG) gene.

Although the proarrhythmic liability of drug is quantified by their degree of QT prolonging potential, QT is a mediocre marker of repolarization and a poor predictor of drug induced arrhythmia. Therefore other repolarization marker such as the T-wave contains useful information of repolarization abnormality which might aid the quantification of cardiotoxicity of drugs. The LQTS2 type T-wave morphology descriptor has opened a new insight in analysing the cardio-toxic potential of drug.

The purpose of this thesis is to investigate the abnormal repolarization both in cellular and surface ECG and their relationship. It has been identified that the certain morphological changes of the monophasic action potential are predictor of TdP arrhythmia. Therefore the proportional changes of the surface ECG which corresponds to the arrhythmia-triggering MAP morphology is warranted to increase the confidence of determining cardiotoxicity of drugs.

In this thesis, simultaneous recording of MAP and surface ECG has been analysed to quantify and develop ECG parameters which are proportional to the MAP morphology. Furthermore the T-wave morphology has been shown to be more efficient over the QT interval in addressing the repolarization reserve of the vulnerable patients and repolarization measurement of the patients with Atrial Fibrillation (AF). Finally, Other T-wave based marker (e.g. TpTe interval) has been investigated.

Collectively, this work offers new insights into the understanding of the T-wave morphology as a superior predictor of abnormal repolarization than the QT interval and will improve the characterization of the proarrhythmic potential of drugs.

Resumé

Medicin-induceret abnormitet i hjertets repolarisering udgør en stor trussel mod sårbare patienter med risiko for at udløse torsades de pointes arytmi, som kan medføre døden, og kan forklare pludselig hjertedød. Abnorm repolarisering af hjertets muskelceller kan ses på overflade-EKGet som et forlænget QT-interval og en ændret morfologi af T-bølgen. Den underliggende cellulære mekanisme for en forlænget repolariserings varighed er inhibering af den såkaldte "rapidly activating delayed rectifier potassium current (IKr)", en repolariserende kaliumstrøm, som kodes af KCNH2 (hERG) genet.

Selv om proarytmiske lægemidler kvantificeres ved deres grad af QT-forlængende potentiale, så er QT-intervallet en middelmådig markør for repolarisering og en dårlig indikator for medicin-induceret arytmi. T-bølgens form derimod, indeholder nyttige oplysninger om repolariseringsprocessen og -abnormitet, som kan hjælpe med kvantificering af kardiotoksicitet af medicin. Den tidligere udviklede LQTS-2 type T-bølge morfologi deskriptor har åbnet nye muligheder for analyse af potentialt kardiotoksiske lægemidler.

Formålet med denne afhandling er, at undersøge den unormale repolarisering både cellulært og på overflade EKGet og at undersøge forholdet mellem disse metoder. Det er blevet konstateret, at bestemte morfologiske ændringer af monofasiske aktionspotentiale (MAP) er prædikative for TdP arytmi. Hvis tilsvarende ændringer nu også kan vises på overflade-EKGet, så kan EKGet også bruges til bestemmelse af kardiotoksicitet af medicin, forhåbentligt med en bedre præcision, end når kun QT-intervallet bruges som markør.

I denne afhandling beskrives derfor analyser af synkrone optagelse af MAP og overflade-EKG, for at kvantificere og udvikle EKG parametre, der er associeret med MAP morfologi. Desuden viser det sig, at T-bølge morfologi er mere effektiv end QT-intervallet i evalueringen af repolariserings reserven af sårbare patienter og i repolariserings målinger af patienter med atrieflimmer (AF). Derudover er andre T-bølge baserede markører (f.eks. TpTe intervallet) undersøgt.

Dette arbejde giver nye indsigter i, og forståelse af T-bølgen's morfologi som en bedre markør for abnorm repolarisering end QT-intervallet, og kan forbedre karakteriseringen af den proarytmiske risiko af lægemidler.

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Contents

Introduction

Background

This thesis investigates the electrocardiographic markers of drug induced abnormal repolarization both in monophasic action potential (MAP) and surface electrocardiogram (ECG). Drug induced cardio-toxicity poses a major threat to the patients by its propensity to trigger fatal arrhythmia and sometimes sudden cardiac death (SCD). Therefore newly developed drugs (whether cardiac or non-cardiac) need to go through cardiac safety screening to identify any potential adverse impact on cardio physiology. Therefore robustness in developing and identifying ECG parameters to enhance the efficiency of the screening is warranted.

The Clinical Problem

Markers of Abnormal Repolarization: QT interval

The most available and conventional way of detecting abnormality in the cardiac activity is the surface Electrocardiogram (ECG). Since 1903, when Einthoven first recorded the first ECG of heart, it has become the most efficient and non-invasive way of detecting cardiac abnormality which eventually extended its purpose towards the drug safety studies. However, it is still ambiguous how the repolarization process inside the heart manifests on the surface ECG. Dealing with the repolarization abnormality, the mostly used parameter from the surface ECG is the heart rate corrected QT interval (QTc). QTc interval has received a significant attention for its apparent association with triggering of the life threatening Torsades de Pointes (TdP) arrhythmia (TdP) (1). TdP is a polymorphic ventricular tachycardia first described by Dessertene (2). It resembles on the ECG as the twisting of QRS complex around the isoelectric baseline. Although the TdP is a non-sustained arrhythmia, later it may degenerate into ventricular fibrillation and sudden cardiac death. Heterogeneity in ventricular repolarization causes the triggering episode of TdP (3, 4). Studies show that the prolongation of QT interval has been found to be correlated with the risk of TdP (1, 5, 6). Therefore prolongation of QT interval and concomitant TdP risk is a factor to be considered while manufacturing new drugs which have the propensity of QT prolongation, although aimed at non-cardiac medication. Therefore newly manufactured drugs having the QT prolonging potential are often withdrawn from the market. There are several ‘safety boundaries’ of QT interval in the literature considering the onset of TdP. A drug-induced QTc prolongation (QT corrected for

changes in heart rate) of less than 5 ms is unlikely to induce TdP whereas prolongations greater than 20 ms are associated with substantially higher risk (7) . It was seen in a cohort of patients that for every 10 ms increase in QTc beyond 440 ms, there was a 5% exponential increase in risk of TdP (8). The Gender specific threshold of normal QTc interval beyond which there is a risk of Long QT syndrome (LQTS) is set to 470ms for men and 480 ms for women. Despite these limitations, a QTc interval of more than 500 ms for an individual patient or an increase of more than 60 ms from baseline are commonly regarded as thresholds for increased risk of TdP (9).

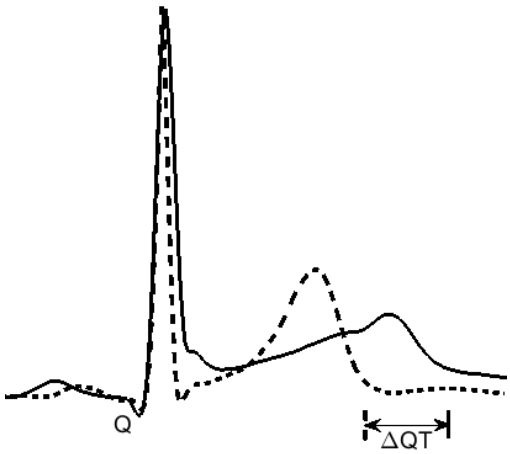

Fig. 1 Drug induced QT prolongation

Drugs which exert their QT prolonging effect do so by blocking the rapid component of the delayed rectifier potassium channel (I_{Kr}) (10) which is encoded by the KCNH2 (the human ether-á-go-go-related gene)(11). The I_{Kr} is primarily responsible for the repolarization or cardiac myocytes. Inhibition of I_{Kr} slows down phase-3 which results in a prolonged MAP duration thereby reflecting on the surface ECG as prolonged QT interval.

In the cardiac myocytes, drugs having the propensity of triggering TdP alter the morphology of Cardiac MAP which might or might not associate the prolongation of APD. It has been shown that the torsadogenic potential of drugs is related to the triangulation of the MAP, not just the increase of APD/QT interval (12).

Markers of Abnormal Repolarization: MAP

The monophasic action potential (MAP) has been used as a significant biomarker to study cardiac repolarization. The MAP is a representation of the transmembrane potential which is recorded from the exterior of the cell. The MAP is recorded by either unipolar or bipolar configuration on which one of the electrodes is at intimate contact with 'injured myocardium'. Therefore the MAP is the representation of the change in voltage of the neighbouring tissues

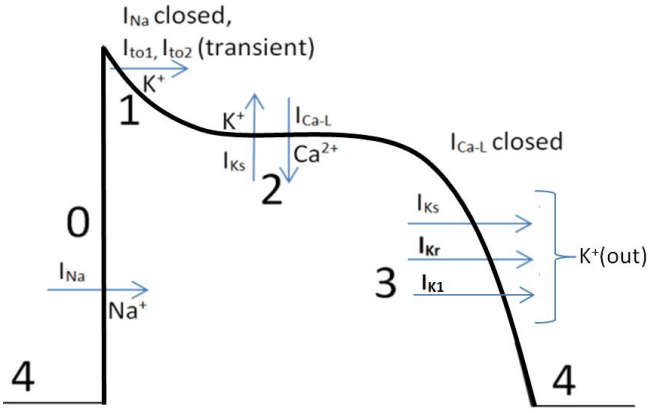

Fig. 2 Illustration of Ion channels associated with AP

rather than the actual measurement of the transmembrane potential (13). Generally it is of smaller amplitude than the transmembrane action potential (10-40 mV), but it accurately reflects the onset of depolarization and of the entire repolarization phase of the transmembrane potential.

Drugs which exert their QT prolonging effect most often do so by blocking the rapid component of the delayed rectifier potassium channel (I_{Kr}) (10). The I_{Kr} is encoded by the KCNH2 (the human ether-á-go-go-related gene)(11). The I_{Kr} is primarily responsible for the repolarization or cardiac myocytes. Inhibition of I_{Kr} slows down phase-3 which results in a prolonged MAP duration thereby reflecting on the surface ECG as prolonged QT interval. Drug induced repolarization changes which might trigger the TdP arrhythmia is more apparent on the cardiac MAP. Altered repolarization can be seen on the MAP, not only as prolonged APD, but also by its change in morphology. The morphological changes of the T-wave has been quantified by Hondeghem et al. (12, 14) by the degree of Triangulation, Reverse use of dependence, Instability and Dispersion which is defined as TRIaD. It has been reported that prolongation of APD is itself anti-arrhythmic provided it is not contaminated by the Triangulation (12). Fig.2 shows a MAP from a pre-clinical study with dogs where the MAP, just before TdP onset, exhibits a slowing down of phase 3 that manifests as triangulation.

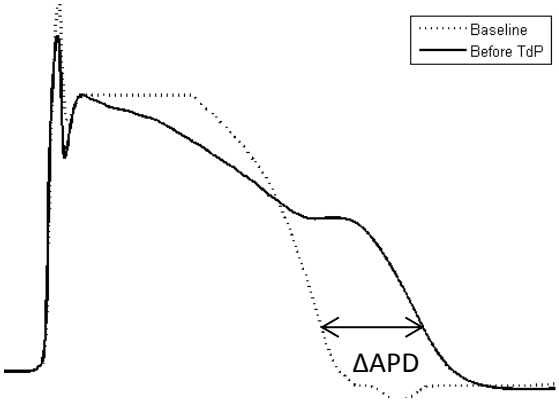

Fig. 3 Changes in MAP morphology (Triangulation) with prolongation.

Markers of Abnormal Repolarization: T-wave

Addressing the repolarization abnormality, the most interesting segment of ECG that reflects the cardiac repolarization is T-wave. T-wave has been a major qualitative source of information regarding abnormal repolarization which was primarily seen at the patients with Long QT Syndrome (LQTS). The Congenital LQTS is a disease which is caused by the mutation of certain genes that affect the repolarizing current. The most prevalent LQTS account for the mutation of these two genes- KvLQT1 (affecting the slowly activating potassium rectifier current I_{Ks}) and hERG (affecting the rapid component of delayed rectifier potassium current). The hERG (the human ether-á-go-go-related gene) is the primary responsible gene for encoding the I_{Kr} current and type 2 LQTS is primarily caused by the loss-of-function mutation of hERG (15). Therefore its

mutation puts the vulnerable patients at the risk of TdP (16). The patients with LQTS have a distinctive T-wave morphology. It was seen that KvLQT1 (accounts for LQTS type-1) patients show a T-wave with relatively high amplitude without a distinct T-wave onset and for hERG patients (accounts for LQTS type-2), T-wave is asymmetric, flattened and has distinctive presence of notch (17). The T-wave morphology featuring such genetic abnormalities shows its necessity to be a surrogate marker for drug induced T-wave changes. Therefore drug inhibiting the I_{Kr} channel is expected to manifest on the T-wave. Hence, it is imperative to investigate the proarrhythmic risk of drugs which exert the similar I_{Kr} inhibition as LQTS by quantifying their effect on T-wave morphology.

Quantification of the T-wave can be categorized under three groups- Mathematical modelling, waveshape models based on physiological process, and parameters describing T-wave shapes. Kanters *et. al* quantified T-wave by the mathematical model using Hills transformation by which there was a perfect discrimination between KvLQT1 and hERG patients was possible, although overlap existed between KvLQT1 and Normals as well as hERG and Normals (18) . In a computer model, the shape of the T-wave also has been linked to the dispersion of repolarization of and repolarization duration AP (19). A number of T-wave shape describing parameters has been established which are mostly used in pattern classification. The normalized loop area of the T-wave (calculated from the area of the vector loop of the T-wave) and T-wave loop dispersion (average angle between all vectors of different ECG leads) have been used for risk stratification of post myocardial infarction (20). A slightly different form of measurement of T-wave known as early repolarization duration (ERD) and the late repolarization duration (LRD) which is done by measuring the ascending and descending part of the first eigen value of the T-wave, reported to be associated with the risk of TdP (21). T_{peak}-T_{end} interval (TpTe) is another T-wave based biomarker that has also drawn the potential interest at risk stratification. TpTe is considered as the index of transmural dispersion of repolarization (22) as explained from the wedge model. TpTe is prolonged in congenital LQTS (23) and predicts TdP in acquired LQTS (24). Prolonged TpTe is correlated with the sudden cardiac death (25)-as confirmed by the studies with a population for US. However, there are several conflicting results which make TpTe a dubious marker of cardiac risk. It has been reported that the TpTe failed to distinguish asymptomatic and symptomatic patients in Congenital Long QT Syndrome (26). Drug induced transmural heterogeneity quantified by TpTe shows conflicting result between wedge model and clinical trial with Amiodarone (26). In intact canine heart, TpTe was observed

without any significant transmural dispersion of repolarization (27). Therefore, T-wave descriptors in several specific points of views have been developed to be surrogate markers yet with shortcomings.

A more systematic and robust T-wave morphology was developed to identify the difference between healthy subjects and LQT2 patients by quantifying certain apparent T-wave morphological features-asymmetry, flatness and notch. The linear relationship of these parameters is termed as Morphology Combination Score (MCS) (28, 29). The drug induced repolarization abnormalities can be quantified by analysing T-wave morphology in terms of MCS. The components of MCS are as follows:

Asymmetry

The average of the square of the difference between the slopes of the ascending and descending part of the T-wave is defined as asymmetry (29-31). The difference was calculated by mirroring the slope of the descending part of the T-wave to the slope of the ascending part to compare the corresponding points. The average squared difference (d) between the slope segments was defined as asymmetry.

$$\text{Asymmetry} \approx \frac{\sum_{i=1}^{N} d(i)^2}{N}$$

Flatness

A modified version of the standard Kurtosis measure, commonly used in statistics to describe the peakedness of the probability distribution function, has been used to calculate the flatness of the T-wave. In Brief, Fourth central moment (M_4) was normalized with the squared second moment (M_2) and subtracted from 1 to let increasing values to be proportionate with the degree of flatness (29-31).

$$M_k = \left[\sum_{i=1}^{N} (i - M_1) ECG(i) \right]^{\frac{1}{k}}, M_1 = \sum_{i=1}^{N} i(ECG(i))$$

Notch

The notch categorically estimated by calculating the curvature signal (29-31)-

$$Curvature = -\frac{\frac{d^2y}{dx^2}}{\left[1 + \left(\frac{dy}{dx}\right)^2\right]^{\frac{3}{2}}}$$

The deflection of the curvature signal was used to quantify notches within three categories as suggested previously (32): no notch=0, moderate notch (having perceptible bulge) = 0.5 and pronounced notch 1.0 (distinct protuberance on apex).

Morphology Combination Score (MCS)

All three morphology parameters were standardized by their relative variances to give equal importance each of those and a linear combination was formed by their weighted sum to yield the overall description of T-wave morphology, termed as Morphology Combination Score (MCS) (29-31):

$$MCS = Asymmetry + Notch + 1.6 \times Flatness$$

It has been found that newly development parameter provides higher confidence in detecting drug induced abnormal repolarization and better and provides higher confidence in determining drug induced cardiac safety profile of drugs (28, 29).

Several drug studies with MCS have proven more reliability over QTc interval. MCS showed significantly higher effect size over QTc to show drug effect (29, 30). Moxifloxacin, which has a cardiac safety profile despite having QT prolonging propensity and handful number of proarrhythmic history, has been validated with MCS (28). It was seen that covariate analysis of MCS and QTcF can reveal complex effect on cardiac repolarization which are not described by QT interval (31) .

Linkage Between Cellular MAP to Surface ECG: Beyond QT Prolongation

As we know the MAP duration of the cardiac myocytes reflects on the ECG as the QT interval. Although QT interval has been used as a surrogate marker of TdP risk, its relation with the etiology of TdP is still unclear (33, 34). The reason is partly because QT interval is subjected to measurement error (35) and drug induced changes of T-wave might complicate its measurement. It is thus ambiguous if mere prolongation can be generalized to indicate the proarrhythmic risk of drugs at the similar manner. It can be concluded from the extrapolation that drugs prolonging the QTc interval at the same level sometimes results in different triggering incidence of TdP (e.g. sotalol versus dofetilide or amiodarone versus almokalant). Moreover, a similar incidence of TdP episode associated with certain drugs does not necessarily prolong QT interval at the similar degree (36, 37). In some studies, smaller QT prolongation has been associated with the drug induced TdP (38,

39). In some cases, no correlation between QT prolongation and torsadogenic potential has been reported as well (40).

Since, QT interval is not a robust surrogate for stratification of TdP risk. Therefore it would aid the drug safety screening if a relationship between the known MAP morphologies (those account for the triggering of TdP) with the surface ECG can be developed as human MAP data is generally not accessible in non-invasive way. Antzelevitch et al. showed the relation between transmembrane APs from epicardial, endocardial, and M-cell regions with the T-wave (22). However the specific changes of morphology characteristics of MAP were not quantified which can show the proportional change/effect on the T-wave. Since, the phase-3 duration of MAP

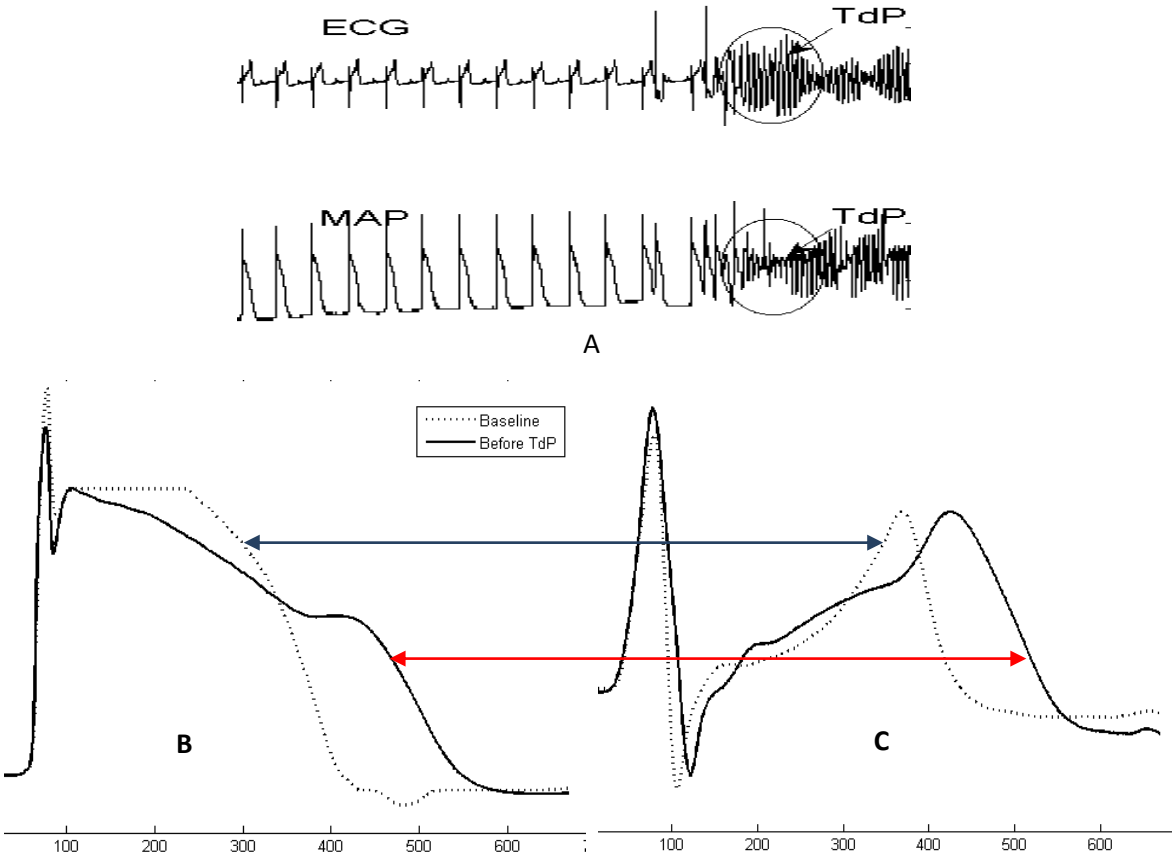

Fig. 4 Simultaneous recording of MAP and ECG baseline and just before TdP onset. Top panel shows the longitudinal data (A) and bottom panel shows the median beats of MAP (B) and ECG (C) at baseline and before TdP onset. Triangualtion of MAP which is a marker of TdP onset reflects on surface ECG as flattened T-wave.

accounts for the cardiac repolarization, an association between MAP morphology and the surface representation of cardiac repolarization (T-wave) is expected.

Not only is it important to quantify the repolarization changes in ECG, rather it is imperative to identify those ECG characteristics that trigger the TdP arrhythmia. In that attempt, both the ECG and MAP data are to be accessible simultaneously. Fig. 4 shows the simultaneous recording of the MAP and ECG from dog which was treated with supratherapeutic dose of sertindole just before the TdP onset. The most prominent morphological change MAP other than the prolonged APD is the triangulation which manifests as the flattening of the T-wave of corresponding ECG. Interestingly, Triangulation may be accompanied by either shortening or lengthening of the cardiac action potential duration which indicates that drug-induced T-wave morphology changes may be independent of QT interval measurements**.**

Therefore it is quite clear that, T-wave contains the information of TdP risk which is not available directly from the QT prolongation.

Perspective

Assurance of a safe cardiac profile is the primary concern during drug development. The pharmaceutical companies often discontinue the development of drugs that prolong the QT interval in pre-clinical studies although those drugs might be the breakthrough medication for their respective purpose (41). There are drugs which have the QT prolonging effect without history of inducing the TdP arrhythmia. Therefore it is clear that the drug safety estimation by only from the result of Thorough QT study might put a grey region of stratifying true torsadogenic risk. The safety limit of QT set by the thorough QT study is primarily the regulators' previous experience with approving QT prolonging drugs which might or might not reflect the drug's actual safety profile. On the other hand, several drugs in animal model that prolong QTc to an equal extent have been associated with markedly different torsadogenic potential although, clinically, much smaller QTc prolongations than expected have been reported in patients with drug-induced TdP; Whereas the onset of TdP was frequently preceded by marked changes in T-wave shapes (38, 39). For example, the effect antipsychotic drug sertindole was more pronounced in T-wave morphology than the prolongation of QTc interval (30).

According to the International Conference on Harmonization E14 guideline defines a negative TQT study is the one having the upper one sided 95% CI for the maximum time-matched mean effect should be less than 10

ms (7). This is highly ambiguous since a fixed threshold might not be homogeneous for all drugs beyond which the TdP risk is of similar degree. To clarify this ambiguity, TQT has been investigating the possibility of including the relationship between drug concentration and QTc effect. This again is not free from faults. TdP potential sometimes does not depend on the plasma concentration, rather the rate of infusion; e.g. Almokalant and NS-7. At the similar plasma concentration level, both has been found highly torsadogenic at rapid infusion rate, but not in a slower infusion (42, 43). In addition to this, both moxifloxacin and sotalol have linear concentration-QT relationship, yet sotalol is torsadogenic at the clinically achievable concentration; whereas moxifloxacin is not proarrhythmic at the therapeutic dose (44-46). On the other hand, the TdP risk has exponential relationship with the QTc prolongation in LQTS2 patients (8) which might complicate the predictability of proarrhythmic risk from the linear concentration-QTc relationship of drugs.

In these circumstances, it is imperative to investigate T-wave morphology profile side by side of QTc in Thorough QT (TQT) study of drugs. Studies show that covariate analyses of QT and T-wave morphology increases the confidence of evaluating repolarization abnormalities (17, 28, 31). Therefore, TQT study with complementary T-wave morphology will assist in identifying the true cardio toxic effect which will strengthen the drug screening at relatively higher precision.

Objective of the Thesis

The primary objective of the thesis is to investigate and relate the concomitant changes in MAP and T-wave morphology under the effect of I_{Kr} inhibiting drug having the TdP triggering propensity. Furthermore, the most prominent T-wave morphological patterns just before the triggering of TdP arrhythmia has also been investigated from the preclinical trial data with dogs administered with supra-therapeutic dose of I_{Kr} inhibiting drug.

The work has further aimed to discover how the LQT2-like T-wave morphology descriptor parameter (MCS) can be used to indicate drug induced repolarization abnormalities in the patients with reduced repolarization reserve and patients with persistent Atrial Fibrillation. Although QTc interval is considered the gold standard for assessment of abnormal repolarization, how the MCS can represent more pronounced manifestation of drug induced repolarization abnormalities at different abnormal cardiac conditions (e.g. reduced repolarization reserve and during AF) have been investigated.

Finally, the widely used T-wave based parameter T_{peak}-T_{end} (TpTe) has been investigated if it can be used as a potential surrogate marker of drug induced repolarization abnormality beside the QT interval.

Structure of the Thesis

This thesis consists of six papers. Paperwise summery is given below:

The first paper is about the preclinical trial of sertindole at therapeutic dose with dog. It was seen that sertindole caused morphological changes was at the similar time point both in T-wave and phase-3 of the MAP.

The second paper reports how the MAP and T-wave morphology changes just before the triggering of TdP at the supra-therapeutic dose of sertindole. In this study a T-wave parameter (Relative T-wave area or RTA) has been introduced which reflects the Triangulation of the MAP. It was seen that both the RTA and triangulation of the MAP attains their maximum value just before the TdP onset.

The third paper implements the previously developed T-wave morphology parameter (RTA) on clinical data of schizophrenic patients treated with sertindole. RTA showed significantly higher effect size than that of QTc interval. We assume that, the RTA actually reflects the degree of triangulation in MAP morphology.

The fourth paper investigates the individual susceptibility of TdP by analysing LQT2-like T-wave morphology descriptor parameter (MCS) and QTc interval. It was seen that, MCS can identify the patients with reduced repolarization reserve at baseline where QTc requires the patients to be stressed with I_{Kr} inhibiting drugs to identify their susceptibility to TdP. Furthermore, covariate analysis of MCS and QTc shows significantly higher sensitivity and specificity over QTc based identification of vulnerable and not-vulnerable patients.

The fifth paper investigates the T-wave morphology in the group of patients with persistent AF. The MCS reflects the repolarization effect of the drug AZD 7009 for the treatment of AF in which QTc is exposed to higher measurement error. In addition to this, how the repolarization measurements are underestimated during AF episodes have been analysed.

The sixth paper deals with TpTe, a shorter portion of QT interval. Study from five different I_{Kr} inhibiting drugs and a group of placebo have been analysed. It was seen that, TpTe is actually a smaller part of QT interval which yields the similar proportional ratio at baseline and after the drug. Therefore, TpTe might not be a robust parameter to indicate drug induced repolarization.

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Contributions

This thesis is based on following papers:

1. Bhuiyan TA, Graff C, Thomsen MB, Struijk J. Triangulation of the monophasic action potential causes flattening of the electrocardiographic T-wave. Computing in Cardiology. 2012;39:757-760.

2. Bhuiyan TA, Graff C, Thomsen MB, Struijk J. Flattening of the Electrocardiographic T-wave is a Sign of Proarrhythmic Risk and a Reflection of Action Potential Triangulation. Computing in Cardiology. 2013; 40:353-356.

3. Bhuiyan TA, Graff C, Kanters JK, Nielsen J, Struijk J. Repolarization effects of sertindole manifest as T-wave flatness on the ECG. Computing in Cardiology. 2014;41:381-384.

4. Bhuiyan TA, Graff C, Kanters JK, Nielsen J, Stefan K, Toft E, Struijk JJ. T-wave morphology is a better predictor of patients with a history of drug induced TdP.- Article submitted at the 'Heart Rhythm'

5. The Tpeak-Tend interval as a marker of repolarization abnormality: A comparison with the QT interval for five different drugs.-Article accepted at the 'Clinical Drug Investigation'

6. Bhuiyan TA, Graff C, Kanters JK, Toft E, Edvardsson N,Struijk JJ. T-wave morphology and QT interval in atrial fibrillation.-Article submitted at the 'Drug Safety'.

Conclusion

The studies presented in this thesis primarily focus on measurement of the drug induced morphological changes related to cardiac electrophysiology, beyond the conventional QT prolongation. The altered morphology pattern of the MAP, which is indicative of the onset of TdP arrhythmia under the effect of I_{Kr} blocking drugs, has been linked to the morphology of the simultaneous surface ECG. This and similar approaches may pave the way towards new and improved cardiac safety measures for drug screening. Furthermore, it was shown that the altered T-wave morphology is not only the hallmark of drug induced repolarization abnormalities, but it also provides significant baseline information of the subjects with reduced repolarization reserve. The study thus offers new possibilities to clinical investigation of cardiotoxicity, in a clearer quantifiable way than the ambiguity of the safety margin stratified by the QT interval. Furthermore, the study also confirms that the composite score of T-wave morphology measurement (MCS) is a useful approach to drug safety screening.

This study is the first attempt to investigate the morphology measure of the MAP and ECG from simultaneous recordings in intact animals. Separate studies on the MAP and ECG have been previously reported to be surrogate marker of torsadogenic effect. The simultaneous analyses show that the changes of MAP and T-wave morphology as a whole is dose dependent and interestingly the maximum changes occur at the similar time point. This is the first indication why the T-wave is a segment with significant information of cardiotoxicity. The triangulation of the MAP has been subsequently found to be linked with the T-wave flatness and can therefore be estimated from the surface ECG and may be used to quantify and stratify the torsadogenic potential of drugs. The studies with pre-clinical data from dogs and the clinical trial with sertindole have shown obvious ground how the T-wave morphology can provide an indication of the TdP onset and thereby be a surrogate marker of drug safety profiles.

T-wave morphology is not only limited to be indicative of repolarization abnormality, it also reflects the person's propensity to develop TdP. Subjects having a history of drug induced TdP arrhythmia are more prone to drug induced abnormal repolarization than those who did not have such history. Such an aftereffect of TdP history can be explained by a reduced repolarization reserve. Repolarization reserve is a concept in which (partial) failure of one repolarizing mechanism can be compensated by other repolarizing ion channels. The T-wave morphology can significantly identify those subjects from their baseline ECG data. The distinctive morphological patterns e.g. asymmetry, flatness and notching on the

T-wave which are hallmark of abnormal repolarization, also reflects the vulnerability to the potential TdP threat. On the other hand, much less information of those abnormalities is present in the QT interval. ECGs with altered T-wave morphology but unchanged QT interval might question the acceptability of drug testing based on only the QT interval as we confirmed from the pre-clinical study that the TdP threat manifest on T-wave morphology.

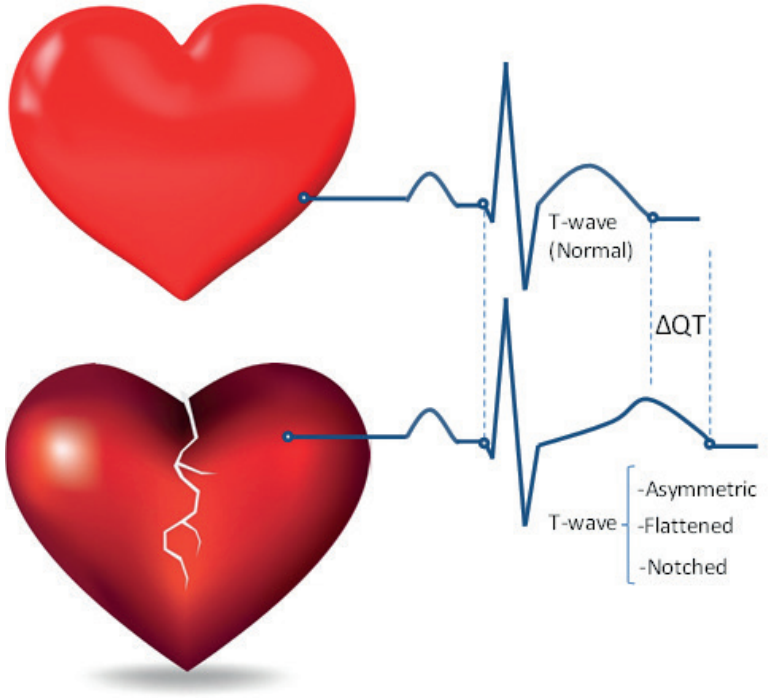
Repolarization measurement is a challenge during the episode of Atrial Fibrillation. The situation is more complicated when identifying the baseline information of the patients with atrial fibrillation. The heart rate corrected QT interval is often subjected to measurement error. The baseline information of patients is of significant importance since the AF patients are treated with class III anti-arrhythmic agents, which have effect on ventricular abnormality and thereby inducing TdP arrhythmia. Analysis shows that repolarization measurements are underestimated at the baseline of AF patients, which might pose a risk on the normal therapeutic dose of patients. QT correction formulae at the faster heart rate during AF are not straightforward; hence the T-wave morphology information may be valuable to aid the decision on the dose level for individual patients.

Finally, the T-wave based marker, TpTe is an ambiguous repolarization marker. In several I_{Kr} inhibiting drugs, it is prominent that TpTe is a smaller portion of the QT interval which reflects the partial effect of drug induced total QT prolongation. The prolongation of the TpTe interval has significant linear correlation with the QT prolongation. However, the presence of flatness and notching of T-wave complicates the estimation of T-peak and hence significant measurement variability can interpret the drug effect in thoroughly different way. Therefore TpTe as a repolarization descriptor is subjected to higher measurement variability and less reliable parameter in a statistical sense and should be used with caution while evaluating drug induced repolarization.

Individual T-wave based markers (asymmetry, flatness and notch) have been shown to be reflected by the drugs investigated in this thesis. Such markers manifest the severe abnormal repolarization which leads to TdP arrhythmia. The abnormal repolarization morphology during the torsadogenic drug effect was similar to the baseline T-wave of the patients with a reduced repolarization reserve. These observations clearly mark the necessity of covariate analysis of the T-wave.

These findings advocate the further research of using the T-wave morphology as the paramount base of drug category by their proarrhythmic potential and assessment of drug induced repolarization abnormality. Threshold of QT interval given by TQT study regulation is highly disputed and varies

significantly. Covariate analysis of T-wave and QT interval can enhance the estimation of safety margin and reduce the false positive cardio-toxic potential of newly developed medication aimed at other purposes.



SUMMARY

The purpose of this thesis is to investigate the abnormal repolarization both in the cellular and the surface ECG along with their relationship. It has been identified that the certain morphological changes of the monophasic action potential are predictor of TdP arrhythmia. Therefore the proportional changes of the surface ECG which corresponds to the arrhythmia-triggering MAP morphology is warranted to increase the confidence of determining cardiotoxicity of drugs.

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