Loratidine a potential candidate for QT prolongation.

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Under the guidance of

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Certification

This is to certify that Mr. Arindam Sarkar has carried out all research studies under my supervision at the Department of Pharmaceutical Technology at Jadavpur University, Kolkata, India, for the thesis titled " **Loratidine a potential candidate for QT prolongation"**. The ideas put into effect were original and are not a copy of any other thesis published/submitted elsewhere.

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Declaration of originality and compliance of academic ethics

The author, hereby, declares that this thesis contains original research work, as a part of his Master of Pharmacy program. The work was done entirely by the author himself and the elements in this thesis are not a copy of any other thesis published elsewhere.

All works were performed under the supervision of Prof. Sanmoy Karmakar in the Department of Pharmaceutical Technology at Jadavpur University.

All information in this document has been obtained and presented in accordance with academic and ethical conduct.

The author also declares that he has fully referenced and acknowledged all materials and results that are not original to his work, in the thesis.

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Introduction

Irregular rhythm of heartbeat is known as dysrhythmia or arrhythmia. Normal human heart rate is 50 to 100 beats per minute. Arrhythmias and abnormal heart rates don't necessarily occur together. Arrhythmias can occur with a normal heart rate, or with heart rates that are slow (called bradycardia-- less than 50 beats per minute). Arrhythmias can also occur with rapid heart rates (called tachycardia -- faster than 100 beats per minute).

There are four main types of arrhythmia: extra beats, supraventricular tachycardias, ventricular arrhythmias and bradycardias. Extra beats include premature atrial contractions, premature ventricular contractions and premature junctional contractions. Supraventricular tachycardias include atrial fibrillation, atrial flutter and paroxysmal supraventricular tachycardia. Ventricular arrhythmias include ventricular fibrillation and ventricular tachycardia. Arrhythmia occurs due to problems in the electrical conduction system of the heart. Arrhythmias is generally believed to occur over a wide age group range, although elderly are more prone to this cardiac event.

Torsades de pointes (Tdp) is a French word which literally translates to “twisting of points”; it is a polymorphic ventricular tachycardia that can result in sudden cardiac death. It was first described by Dessertenne in 1963[1]. Torsades de pointes can be transient and cause a reversible syncope, or it can deteriorate into ventricular fibrillation which may result in sudden cardiac death. The French cardiologist Dessertenne named it such due to the unique pattern of ECG in this kind of polymorphic VT, and noted its association with a markedly prolonged QT interval. The long QT interval responsible for torsades de pointes can be congenital or drug-induced. QT-interval prolongation predisposes to arrhythmia by prolonging repolarization, which induces early after-depolarizations and spatial dispersion of refractoriness.

Congenital long QT syndromes are inherited as autosomal dominant disorders with incomplete penetrance and in the past, were referred to as Romano-Ward syndrome. In rare patients with 2 abnormal copies of the genetic abnormality (particularly LQT1), the disorder is associated with congenital deafness and in the past was referred to as the Jervell and Lange-Nielsen syndrome.

Patients with long QT syndrome are prone to recurrent syncope secondary to torsades de pointes and to sudden death secondary to torsade de pointes degenerating into ventricular fibrillation.

Drug-induced long QT syndrome, more commonly, torsades de pointes VT results from antiarrhythmic drugs, usually classified as Class Ia, Ic, or III. Other drugs that can induce torsades de pointes VT include tricyclic antidepressants, phenothiazines and certain antivirals and antifungals.

The normal electrocardiogram mainly consists of a P wave, a QRS complex, a T wave and a relatively less prominent U wave. The P wave represents depolarization of the atria. Atrial depolarization spreads from the SA node towards the AV node and from the right atrium to the left atrium. The QRS complex represents the rapid depolarization of the right and left ventricles. The ventricles have a large muscle mass compared to the atria, so the QRS complex usually has a much larger amplitude than the P-wave. The T wave represents the repolarization of the ventricles. The U wave is hypothesized to be caused by the repolarization of the interventricular septum. Normally it has low amplitude, and even more often is completely absent. Apart from this, the PR interval is measured from the beginning of the P wave to the beginning of the QRS complex. This interval reflects the electrical impulse takes to travel from the sinus node through the AV node. The ST segment connects the QRS complex and the T wave; it represents the period when the ventricles are depolarized. The QT interval is measured from the beginning of the QRS complex to the end of the T wave. QT interval is solely dependent on heart rate, therefore to minimize this difference and for justified comparison corrected QT or QTc needs to be calculated. This QT or QTc interval is normally accepted as a surrogate marker for ventricular tachycardia which may or may not culminate to Torsades de Pointes.

Since all class III antiarrhtyhmics act by prolonging the APD, there is always a real risk involving TdP while using them. However, it has often been noted that all antiarrhythmic drugs which prolong QT interval do not precipitate TdP. This shows that although important, QT prolongation does not guarantee TdP. However, in recent times, due to the discovery of various non-arrhythmic drugs' action on QT interval, it has been of paramount importance to the scientific community to find out successful predictive models for drug induced TdP.

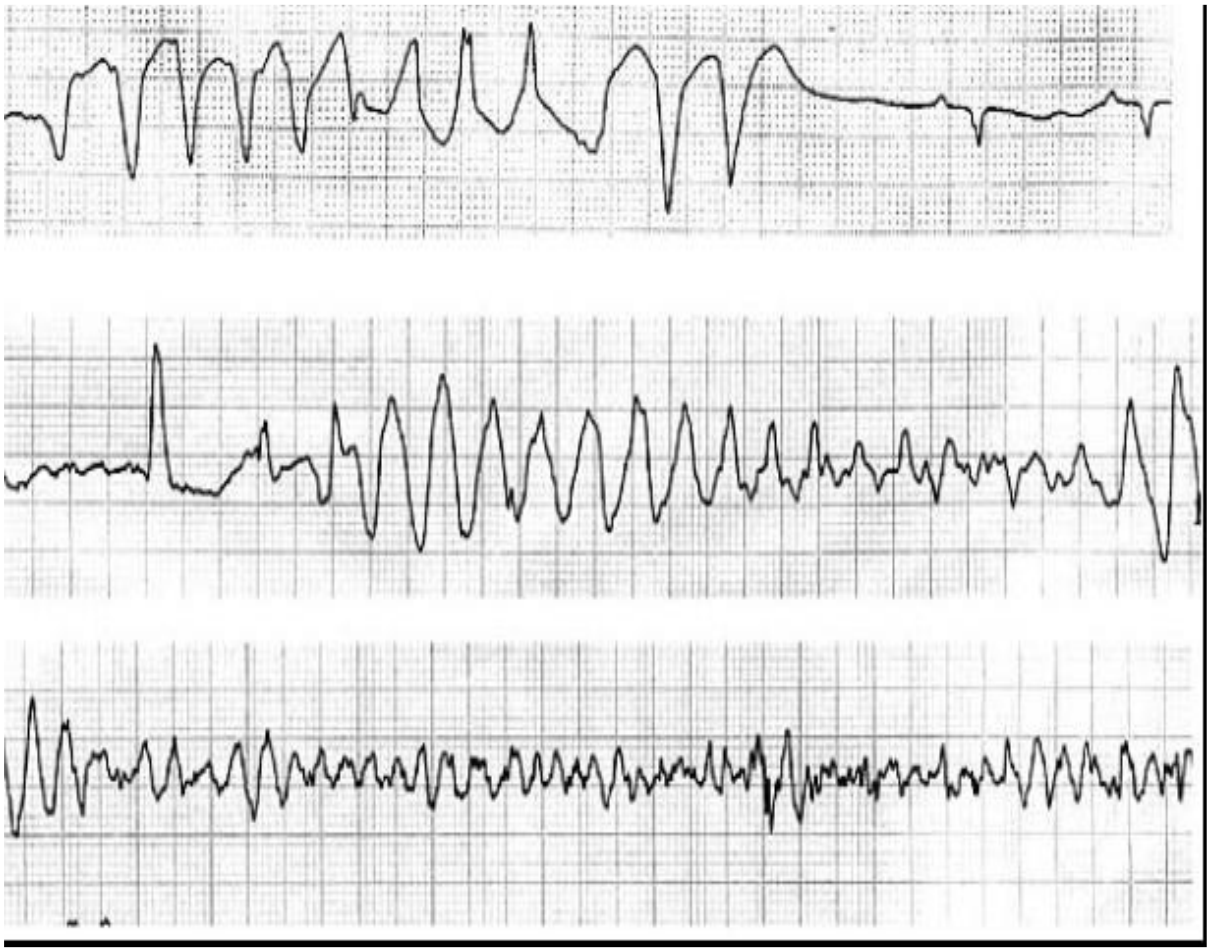


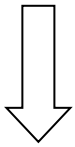
Fig :(A) Self limiting TdP. (B) and (C) TdP leading to ventricular fibrillation[2]

Objective of work

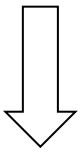
The aim and objective of this work was to investigate the potential of loratidine to induce QT prolongation in hypokalemic rats.

Plan of work

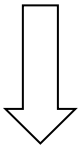
After procuring the required number of rats, the animals were acclimatised and then involved n the study.



The rats were divided in four groups (n=3), and there ECG were recorded on ‘O’ day which served as self control.



Two groups were treated with clarithromycin (70mg/kg), furosemide (10mg/kg) and loratidine (1.0mg/kg) and the remaining two groups were treated with clarithromycin (70mg/kg), furosemide (10mg/kg) and loratidine (2.0mg/kg). This study was continued for 7 days.



ECGs were recorded on day 0, 4th and 8th.

Literature review

1. Drug induced QT prolongation

The first known effect of drug induced Torsade de pointes (Tdp) was observed with quinidine syncope and was reported 100 years back [3]. Thereafter, 40 years later several such reports were published. Cardiologists were initially focused on quinidine because it was the drug of choice for the management of atrial fibrillation [4], and this prompted the realization that these dramatic episodes of syncope had 2 key features, ie prolongation of the QT interval and occurrence of Tdp ventricular tachycardia. Only in the early 1980s, it was hypothesized by Schwartz and Moss that this could be a "***forme fruste***" of the congenital Long QT syndrome[5][6]. They were of the opinion that the patients with a borderline QT prolongation could experience larger QT interval, in the event of exposure with drugs having similar pharmacological properties like that of the quinidine. In the later part of this review a genetic aspect believed to be responsible for drug induced QT prolongation has been mentioned.

As most of the initial cases reported in the 1970s and 80s were observed in patients treated for cardiac arrhythmia, it was generally assumed to be a relatively rare form of proarrhythmic event, generally associated with Class III antiarrhythmic drugs such as disopyramide and procainamide. However in the 1990s, the medical communitys` attitude towards the disease changed as it was revealed that the non sedative antihistaminic like Terfenadine can cause QT prolongation and TdP.[7]. Following this, it was revealed that drugs such as antihistaminic astemizole, gastrointestinal drug cisapride, the antibiotic erythromycin,[8] the opiates levomethadyl and methadone,[9] and many other drugs including antifungals and anticancer agents could prolong QT interval and potentiate TdP. It became clear that the risk of TdP and QT prolongation could happen with any type of drugs and immediately post marketing surveillance and stricter rules were imposed to screen drugs which could have this property. Since 1989, 14 clinically important drugs have been withdrawn from the market due to Tdp including astemizole, cisapride, grepafloxacin, terfenadine and thioridazine, and an unknown number has been stopped due to posing a concern that they might induce TdP.[10]

One of the many difficulties in measurement of QT, in ECG lies in the fact that varying heart rates decisively influences the QT interval. Accordingly, much work was done for a probable correction for acceptable normalization of QT interval.

To correctly measure QT intervals, corrected QT or QTc is generally used to nullify the variability seen in an individual's ECG. QTc can be measured in various ways, among these are,

Bazett formula: QTc= QT/(RR')1/2

Fridericia formula: QTc= QT/(RR'/3)

Framingham formula: QTc= QT + 0.154(1-RR').

Even after all this, it is often debated that QTc prolongation does not successfully predict ventricular arrhythmia and should not be taken as the sole biomarker of TdP. It has previously been argued that transmural dispersion of repolarization reserve and AP triangulation maybe more accurate for proarrhythmia prediction along with T wave alternance and QT alternance study. However, globally the study of QT prolongation till now remains the main biomarker for drug induced TdP, and a significant increase in QT interval (for men QTc>440mS and for women QTc>460mS) is thought to be a risk factor for arrhythmia where as it is generally seen that QTc>500mS precipitates arrhythmia. Whenever a drug increases QTc>60-70 mS and the increase is seen to occur rapidly, the drug is thought to be proarrhythmic.

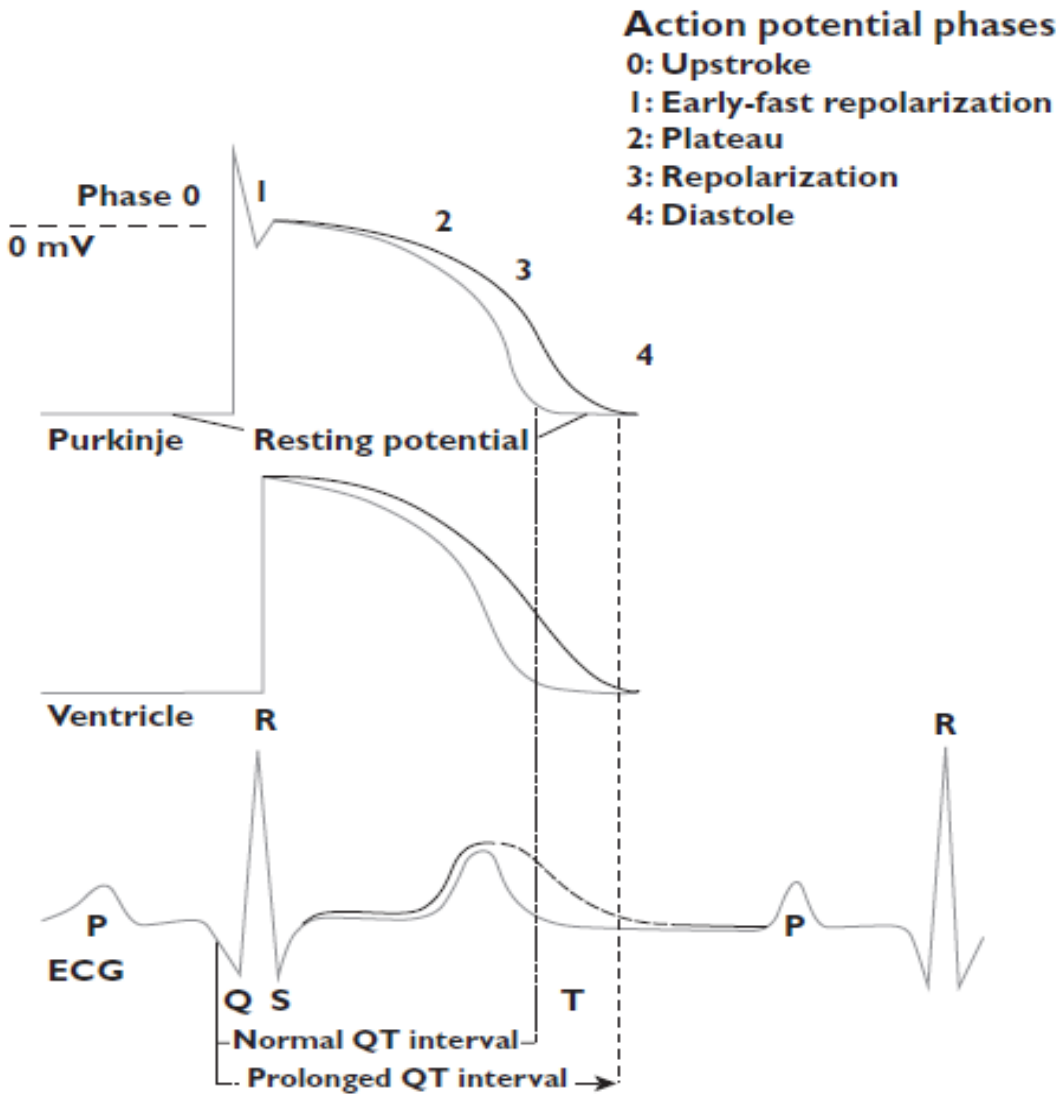


Fig 2: an ECG rhythm strip of a heartbeat in which the QT interval is normal in contrast with an abnormal heartbeat in which the QT interval is prolonged. This is correlated with the ventricular and Purkinje myocyte action potential with associated prolongation of phases 2 and 3. The hashed line represents the prolonged QT interval.

2. Existing regulatory mechanism and list of drugs

In 1999, because of increased concern for the potential scope of drug-induced TdP, the Georgetown University Centre for Education and Research on Therapeutics (GUCERT) was awarded a federal grant to evaluate the available evidence to assess drugs for their relative risk to cause TdP and make their findings freely available to the medical community and the public. The Centre subsequently moved to the University of Arizona and, in 2012, became a freestanding non-profit organization incorporated as Arizona Centre for Education and Research (AZCERT), also known as Arizona CERT. Under a contract with the FDA's Safe Use Initiative, AZCERT now maintains the Web-based lists of drugs that have a risk of QT prolongation and/or TdP. The lists were initially known as the QT drugs lists[11] and are now also available on the CredibleMeds Website[12] and are provided free to over 55,000 registered visitors from 193 countries. AZCERT has recently developed an application program interface that enables health information technology systems to have online open-source access to the lists. To prevent real or perceived conflicts of interest and to assure independence in the decision making process for TdP causality, AZCERT has been entirely supported by peer-reviewed federal awards and charitable public contributions. The methods that AZCERT uses to analyze evidence and assess TdP causality are described on the CredibleMeds Website[13] and in peer-reviewed publications.[14] Because the available evidence frequently has limitations and gaps and is often of variable quality, AZCERT uses a systematic, in-depth analysis of all available data to assess causality (see later summary). Since the lists began in 1999, over 130 new drugs have been added, and many has been moved among categories or removed. Beginning in 2012, many drugs marketed outside the United States, especially in Europe and Canada, have been reviewed, and some have been included on the lists and designated as non-United States. The analysis includes a thorough review of published studies (PubMed search using standardized search terms) and all adverse events reported to the FDA's Adverse Event Reporting System (AERS) and uses the empirical Bayesian data-mining statistical software developed for the FDA (Empirica Signal Software, Oracle Health Sciences, Redwood Shores, California) [15]. CredibleMeds places drugs in 4 categories of TdP risk. Drugs with "known risk of TdP" are those found to have convincing evidence that they can cause TdP, even when administered as recommended on the drug's FDA label. Drugs that prolong the QT interval during routine clinical use, but do not at this time have convincing evidence of TdP causality, are

classified as having a "possible risk of TdP." Beginning in 2006, the "conditional risk" category was added and includes drugs for which there is a risk of TdP but only under certain specific conditions, such as overdose, hypokalemia, hypomagnesemia, bradycardia, or when there is an interaction with another drug(s). This category also includes drugs that are associated with TdP because they have the ability to create the conditions that enable another drug to cause TdP (e.g., loop diuretic agents or metabolic inhibitors of a QT-prolonging drug). The website also posts a fourth list of drugs that should be avoided by patients with congenital LQTS, if clinically feasible. This list is generated by combining all of the drugs in the 3 risk categories listed previously and adding the adrenergic drugs that are considered to place some LQTS patients at high risk of sudden death. The process for evaluating drugs and decisions regarding their inclusion on lists are overseen by an international advisory board of clinical and pharmacological experts. The board's advice is also sought whenever conflicting evidence must be resolved or when any board member has a concern. Medications in all 4 lists are continuously monitored for new evidence and, in recent years, the lists have been revised every 4 to 8 weeks. Currently, 48 medications are on the list of drugs known to cause TdP (including 10 removed from the U.S. market but which may still be available in some countries). Another 72 are on the list for possible risk of TdP, and 32 are on the list for conditional TdP risk.

CredibleMeds has become an invaluable tool for the clinical management of patients affected by cLQTS.[17] Any experienced centre dealing with these patients refers them to the public portal of CredibleMeds and provides patients and their families with an updated list of drugs to avoid or to take only under specific circumstances. As most general practitioners and many cardiologists are unaware of the QT-prolonging potential of many cardiac and non cardiac drugs, this practice has probably saved many lives and has become an essential part of the proper management of patients with channelopathies.

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, which includes drug regulatory authorities and pharmaceutical companies in Europe, Japan and the United States, has published guidance for monitoring the effect on QT through preclinical and clinical phases of drug development[18]. Potential new drugs are routinely screened in preclinical development for interaction with the hERG-encoded

potassium channel with in vitro and in vivo studies. In the early clinical phase of drug development, a "thorough QT/QTc test" is undertaken to determine a drug's effect on QT and the dose response relationship of any effect detected.[19] Commonly, this involves testing the drug at therapeutic and supratherapeutic doses, with comparisons made against placebo as well as active controls with known QT-prolonging properties. A positive result is recorded when the upper limit of the 95% one-sided confidence interval for the largest time-matched placebo-corrected mean effect of the drug on the QTc exceeds 10ms. Generally, a positive QT/QTc test will almost always lead to a requirement by regulatory authorities for expanded ECG monitoring and safety evaluation during later stages of development.

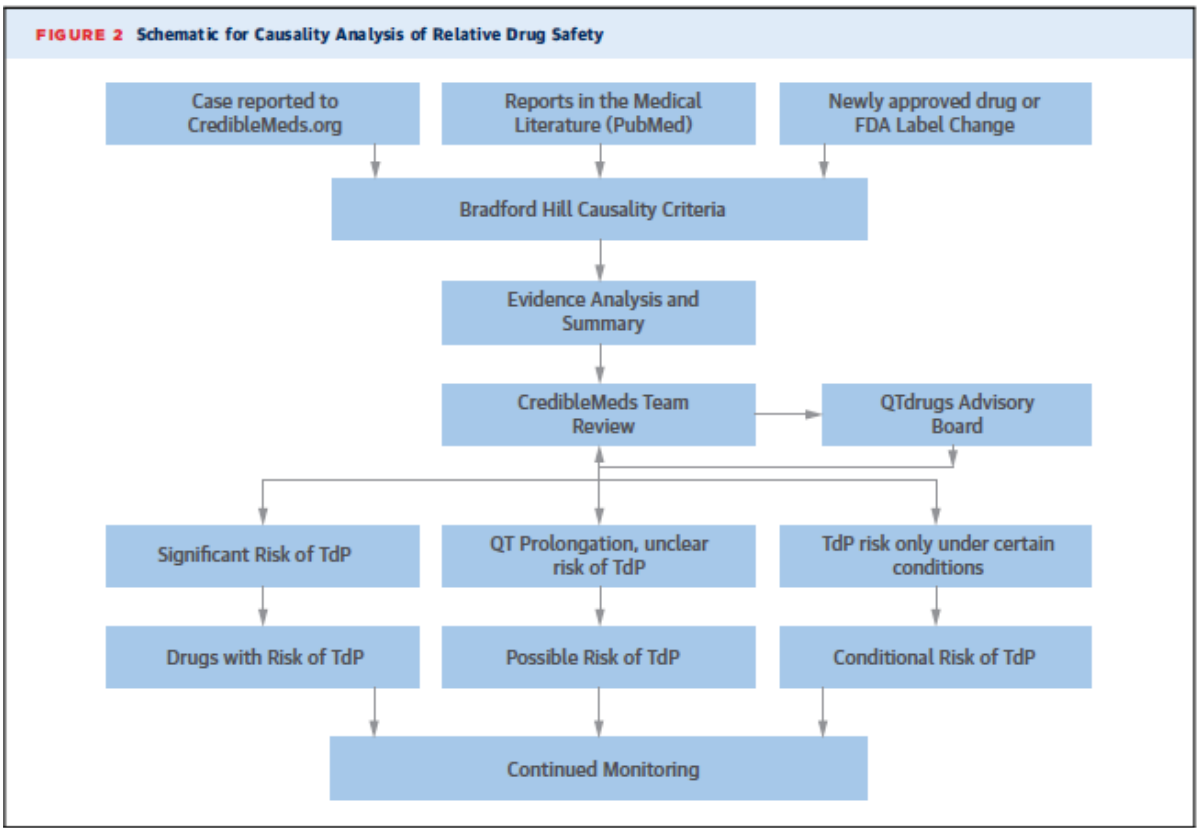


Fig 6: The flowchart shows the procedure of evaluation of drugs for TdP Risk.[20]

Drug group	Drugs associated with drug-induced arrhythmias
Anti-arrhythmics class I	Quinidine, procainamide, disopyramide, dihydroquinidine, bretyllium
Anti-arrhythmics class III	Sotalol, dofetilide, azimilide, Ibutilide, almokalant
Anti-anginals and vasodilators	Prenylamine, terodiline, lidoflazine, bepridil
Antihypertensives	Nicardipine, isradipine
Antihistamines	Terfenadine, astemizole
Serotonin agonists and antagonists	Cisapride, ketanserin
Antimicrobials	
Macrolides	Erythromycin, spiramycin, azithromycin
Antivirals	Ganciclovir, foscarnet
Fluoroquinolones	Moxifloxacin, ciprofloxacin
Antifungals	Ketoconazole, fluconazole, voriconazole
Antimalarials	Halofantrine, quinine sulphate, chloroquine
Psychiatric medications	
Tricyclic antidepressants	Amitriptyline, desipramine, imipramine, doxepin,
Antipsychotics	Thioridazine, chlorpromazine, haloperidol, droperidol
Serotonin re-uptake inhibitors	Citalopram, fluoxetine, sertraline, venlafaxine
Anti-emetic and gut motility	Metoclopramide, domperidone
Anticancer	Arsenic trioxide, vandetanibe, eribulin
Others	Probucol, methadone, pentamidine, sirolimus, lithium

Fig 7: List of drugs with potential for QT prolongation.[21]

3. The theory of repolarisation reserve

A body of cellular and molecular studies over the past 15 years has demonstrated that the fundamental molecular lesion in the drug-induced long QT syndrome (LQTS) is block of the repolarizing potassium current IKr, the "rapid" component of the repolarizing potassium current that was initially termed IK[22][23] Further, decreased IKr due to KCNH2 mutations causes type 2 congenital LQTS, one of the commonest forms of this disease[24]. Both the congenital and drug-associated form of LQTS present with QT interval prolongation and torsades de pointes, and a striking clinical feature in both is the highly variable nature of the phenotype. Every patient who are exposed to IKr-blockers doesn`t develop QT prolongation and it is also not that every patient with a loss-of-function due to mutation in KCNH2 displays QT interval prolongation. It was this clinical disconnect and the increasing recognition that normal repolarization represents a complex interaction among multiple components, that led to the formulation of the idea of "repolarization reserve." in the late 1990s [25] Recognizing that repolarization is accomplished not just by IK and ICa but also by IKr, IKs, ICa-L, ICa-T, INa-L, INCX, and so on. The concept suggests that a reduction in IKr might generate a huge effect in cells, or in patients, in whom other efficient repolarization mechanisms were absent.[26][27][28] By contrast, the same reduction in IKr might produce little change in repolarization time in settings in which other mechanisms could readily accomplish normal repolarization. The idea seems appealing, since a PubMed search identifies 209 references to "repolarization reserve" and a Google Scholar search identifies "about 5,480" hits. The term may have acquired some currency because the idea makes intuitive sense to basic and clinical electrophysiologists. However, just because it seems to sound good does not make it so, and experimental validation is a next step. One obvious possible contributor to variable repolarization reserve is variability in function of the slow component of repolarizing potassium current, IKs, generated in vivo by coexpression of the poreforming subunit encoded by KCNQ1 and the function-modifying subunit KCNE1. Indeed, initial computer simulations indicated that while reducing IKs produces minimal action potential prolongation, the extent to which IKr block prolongs action potentials is strikingly exaggerated when IKs is blocked. These simulations were then followed by experiments showing that variable IKs function could indeed play a role in modulating response to IKr block[29][30]. In addition, modeling state transitions of the KCNQ1 channel underlying IKs revealed a critical role in maintaining normal

repolarization (maximizing reserve) only when KCNQ1 was co-expressed with KCNE1; the co-expression allows the channels to rest in "pre-open" states and thus contribute maximally to the maintenance of repolarization reserve[31]

Recent computational and GWAS experiments have shown that there are many other genes and factors controlling the repolarization reserve. From computational studies it has been seen that factors such as gating, shift of the activation or inactivation potential and various other genes previously thought to be unrelated has a correlation with diLQTS. The Genome Wide Analysis Studies has shown that various new genes have a role to play in the reduction of repolarization reserve. The concomitant administration of drugs and involvement of various factors together often reduce the repolarization reserve while maintaining the baseline QT. This is important because it makes the patient vulnerable to further drugs which on small doses may prolong QT and potentiate arrhythmia.

4. Debates on considering rats as an experimental model for arrthymia study

Previously there has been dispute over the use of rat and mouse models for investigation of diLQTS. This dispute arises mainly due to two reasons. The first one arises due to the high resting heart rate of rat and mouse heart. The second and more important reason is due to variations in repolarization curve [32]. In the human heart, the Phase 0 follows a plateau attenuated by Phase 1, 2 and 3. In mouse and rats, the phase 3 is non-existent and there is no plateau phase. This gave rise to the debate whether these models were at all important in testing diLQTS. However, later reviews showed us that although there were significant differences in the nature of currents and their effects in human and rat heart [33]. the use of transgenic mice and rats provided important data that these can indeed be used as a proper model in testing diLQTS [34]. However, it was seen that there were many channels in the rat heart which were indeed present in the human heart as well, thus their interactions could properly be studied in the rat heart.

6. Genetics of drug induces QT prolongation

Since the congenital LQTS carries the same effects as diLQTS, the genetics has beenextensively studied.

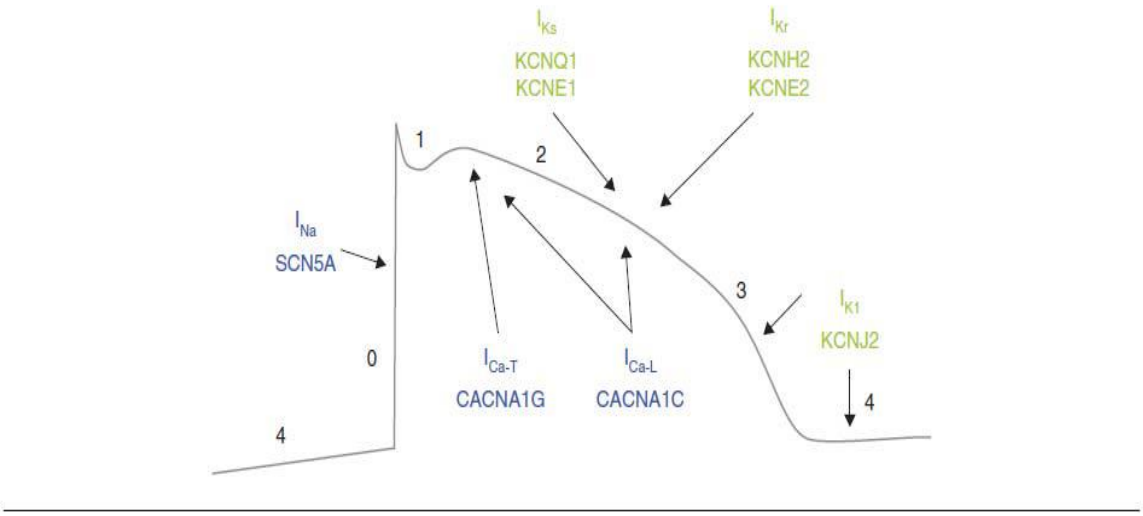


Fig 8: Role of various genes and their channels in action potential duration [35].

More than 700 mutations in at least 13 genes have been found to be associated with the cause of cLQTS. Six of these genes encode pore-forming ion channels (KCNQ1, KCNH2, SCN5A, KCNJ2, CACNA1C, and KCNJ5) and seven of these genes encode for ion channel subunits or regulatory proteins (KCNE1, KCNE2, ANKB, CAV3, SCN4B, AKAP9, and SNTA1). KCNQ1 and KCNE1 co-assemble to form IKs while KCNH2 and KCNE2 co-assemble to form IKr.
Sesti *et al*. screened the KCNE2 gene in 98 cases of drug induced arrhythmia and 200 control subjects [36]. They identified three sporadic mutations (M54T, I57T and A116V) and one more common rare variant (T8A).T8A was found in only 16 of 1010 (1.6%) control individuals and 1 of 230 (0.4%) index patients with LQTS. Functional and in silico analyses demonstrated that although the mutations produced reductions in current density, none of the three sporadic mutations altered the susceptibility to drug inhibition. On the other hand, only a 15% decrease in current density was observed with the T8A variant, and patients with this genotype have a normal QT interval at baseline [37]. Interestingly, on drug exposure this variant increased the inhibitory effects of antibiotic treatment and demonstrated that common sequence variations can be clinically silent before drug exposure yet increase the risk of DILQTS. A non-

synonymous variant, S1102Y in the SCN5A gene, was associated with an increased risk of sudden cardiac death (SCD) in African-American adults and children although it is rarer or even absent in other ethnic groups. Splawski et al. screened cases of cardiac arrhythmia (predominantly due to medication) and S1102Y heterozygosity was associated with an eight-fold higher risk of arrhythmia than in non-carriers of thevariant[38]. Functional and in silico studies of Y1102 and computational analysis of simulated action potentials generated by S1102/Y1102 channels demonstrated prolonged repolarization and early after depolarizations (EADs) associated with the Y1102 variant. At the same time, an early candidate gene study was performed in 92 subjects who had experienced, drug induced arrhythmia and 157 healthy subjects in three genes firmly associated with cLQTS. The study identified mutations in five of the 92 subjects [39]; three of them were in the SCN5A gene (G1844A, C1852T, T3748C), one in the KCNQ1 gene (C1747T) [40] and one in the KCNH2 gene (C2350T). Four polymorphisms were also identified but there was no difference in the frequency between the cases and the controls [41].

Genes encoding pore forming ion Channels	KCNQ1- IKs α subunit KCNH2- IKr α subunit SCN5A- INa α subunit KCNJ2- IK1 α subunit CACNA1C- ICaL α subunit KCNJ5- IKAch α subunit
Genes encoding ion channel subunits or regulatory proteins	KCNE1 KCNE2 ANKB CAV3 SCN4B AKAP9 SNTA1

Table 1: Genes important in cLQTS.

A candidate gene study by Paulussen et al. screened the five main cLQTS associated genes in 32 drug-induced arrhythmia patients with confirmed TdP and identified three missense variants:T8A in KCNE2, D85N in KCNE1 and P347S in KCNH2[42].T8A and D85N have been implicated with drug-induced arrhythmias in earlier studies as well as in patients suffering from QT prolongation. Three additional non-synonymous variations were found in both the controls and the cases with similar allele frequencies in the two populations in the KCNE1, KCNH2 and SCN5A genes. These variants have also been previously reported in earlier studies in patients suffering from cLQTS.[43][44][45] Kaab *et al*. confirmed the association of the KCNE1-D85N variant and drug-induced arrhythmia in 176 cases and 207 drug-exposed controls (individuals without QT prolongation when exposed to culpable medications) and 837 controls from the general population.[46] The study included 1424 single nucleotide polymorphisms (SNPs) in 18 candidate genes (which included ion channels and other high priority candidate genes) for which the subjects were genotyped. The most statistically significant associated SNP was rs7295250 in the CACNA1C gene (odds ratio 1.88, $P = 7.62 \times 10{-}4$). The strongest effect size within the study was detected for D85N (odds ratio 8.88, $P = 1.95 \times 10{-}5$). The follow-up validation study failed to replicate the initial association although a trend for the 058N variant was observed (P = 0.58).

A more recent study by Ramirez *et al*. utilized a next generation sequencing panel of 79 genes associated with arrhythmia syndromes including 13 genes which have been previously associated with cLQTS, nine additional genes shown to be involved in congenital short QT syndrome (cSQTS) and Brugada syndrome and nine other genes related to familial arrhythmia syndromes [47]. In 11/31 DILQTS (36%) subjects a novel missense mutation in genes known to be associated with congenital arrhythmia was found. In 6/26 (23%) DILQTS Caucasian subjects a conserved deleterious variation or an already identified congenital arrhythmia mutation was found. These variants were found in less than 2% of the individuals sequenced as part of the 1000 Genomes project. The conclusion from this study was that the rare variants in arrhythmia related genes, not just cLQTS genes, are responsible for a significant proportion of drug-induced arrhythmia cases. These conclusions may be limited, however, by the different platforms and depth of sequencing in the case and control groups. Overall, however, around 10% of cases may be associated with rare variation in the cLQTS genes [48]. Common variation in genes associated with QT

prolongation has also been investigated as a potential cause of drug-induced arrhythmias. The NOS1AP (CAPON) gene has been repeatedly associated with QT prolongation in healthy individuals [49] and it has also been associated with severity in patients suffering from cLQTS.[50] NOS1AP is a regulator of neuronal nitric oxide synthase (nNOS), which in turn regulates the intracellular levels of calcium and myocyte contraction in the heart.[51] NOS1AP is thought to alter cardiac repolarization by interaction with nNOS by blocking L-type calcium channels.[52] This may explain the association of NOS1AP gene variants with prolongation of the QT interval and TdP. Jamshidi *et al*. demonstrated that a common variant (rs10919035, odds ratio 5.5, P = 3.0× 104) in the NOS1AP gene which has been previously associated with the QT interval prolongation plays an important role in the risk of amiodarone-induced drug-induced arrhythmia.[53] The study included 86 cases,192 controls and 68 drug-exposed individuals with no QT prolongation. The frequency of the common variant in cases was 27.8% and in controls 7.1%. This supports the theory of repolarization reserve and its role in DITDP.

7. Recent reports in context to diLQTS

Recent advances in scientific studies have been able to give us newer insights into theissue of drug induced Torsades de Pointes. A latest study with systems pharmacology approach has given us new ideas in the role of SNPs in genes such as AKAP9, SNTA1, NOS1AP and KCNA5 which were not previously thought to be associated with TdP. It has also showed in the experiment that only 2 of the 81 drugs with target in the neighborhood had KCNH2 as their only target in the neighborhood. When the drug-KCNH2 interactions were removed from the analysis, the enrichment results were not significantly affected [54].

Another recent review tells us that instead of the density, the ratio of IKs/IKr influences the system response to perturbations.[55] It also showed that IKs is selectively sensitive to β adrenergic stimulations and high [Ca^{2+}]. These two also may work in concert to stabilize APD against AP prolongation stimuli by both increasing the GKs/GKr ratio and enhancing IKs during prolonged repolarization and shorter diastolic intervals. It also showed that NO enhancement of IKs is critical for regulating AP duration.[56]

Another study, which attempted to quantify repolarization reserve to understand interpatient variability, discovered many factors such as maximal inward

rectifier conductance, L-type Ca2+ current conductance etc to have significant effects on APD prolongation. [57]

All these studies show us that as the day progress it is increasingly becoming clearer that the importance of IKr in diLQTS has been overestimated and many other channels and genes are involved in creating this effect. Thus future studies should be done in finding more robust in vivo and in silico models which would successfully predict the ability of drugs having the potential of QT prolongation singly or in association with other factors.

1	G_{K1}	Maximal inward rectifier conductance
2	G^{Kr}	Maximal rapid delayed rectifier conductance
3	V_{K1}	Inward rectifier K+ current voltage dependant rectification
4	G_{Ca}	Maximal L type Ca2+ current conductance
5	G_{Ks}	Maximal slow delayed rectifier conductance
6	V_d	L type Ca2+ current activation
7	K_{NaK}	Maximal Na+-K+ current conduction
8	V_f	L-type Ca2+ voltage dependant current inactivation
9	V_{Kr}	Rapid delayed rectifier conductance
10	K_{NCX}	Maximal Na+/Ca2+ exchange current

TABLE: Factors influencing diTdP in a system pharmacology experiment.

8. Effect of late Sodium current on Action Potential Prolongation, some recent studies

The late sodium current, initially seen as a tiny sustained tail of sodium current, was out of the focus of research for a long time, but immediately gained increasing interest since it was linked to cardiac diseases. Up-regulation of the plateau sodium current has been implicated in multiple inherited or acquired arrhythmia syndromes or structural heart diseases. At the same time, inhibition of the currents was demonstrated to prevent or reduce arrhythmic activity in multiple pathologic models. Exponential growth in the number of research

papers and comprehensive reviews [58-64] published in the last few years indicates the great expectation on INa, L as a new, potential therapeutic target. At the present, the greatest limiting factor for the progress of this field is the lack of specific INa,L inhibitors. Development of highly specific INa,L blockers will facilitate research and provide archetype for a new class of antiarrhythmic drugs. [65]

Recent studies also suggest that CaMKII and PI3K play an important role in regulation of the late Sodium current through NaV1.5 channel [66]. It was seen that hypokalemia enhanced the CaMKII signaling in heart and thus increased late Sodium current and Action Potential [67]. Many drugs previously known to prolong the QT interval by IKr inhibition was actually found to enhance late Na+ current instead. Drugs such as dofetilide, d-sotalol, thioridazine and erythromycin were in this list.[68-70] It was also seen that chronic use of certain drugs markedly increased the action potential whereas their acute exposure failed to increase the action potential in any way. This posed a second question, do we need to add the factor of time in screening of drug induced QT prolongation studies along with other factors? [71]

MATERIALS AND METHOD

1. Chemicals and Drugs

Klacid IV 50 mg/vial (Abbott), Lasix injection 10mg/ml(sanofi) and Lormeg syrup 5mg/5ml(Alembic) were procured from near by pharmaceuticals stores.

2. Animal husbandry and maintenance

Healthy adult male Wistar rats weighing 150-200 gm were procured from M/S Chakraborty Enterprise, 3/1D Girish Vidyaratna Lane, Narkeldanga, Kolkata-700011, and used for the study. The animals were grouped and housed in cages with not more than 4 animals per cage in a controlled environment (12 h light and dark cycle, temperature of 25 ± 2ºC and 50 ± 20% relative humidity). During the period of study, the animals had free access to standard dry pellet diet (Nutrilab Rodent; Provimi) and water ad libitum. The study was conducted in accordance with the Institutional Ethical Committee (constituted under the Guidelines Committee for the Purpose of Control and Supervision of Experiments on Animals)(Nag Chaudhuri etal.,2005).

3. Animal dosing and ECG recording

IP injections and oral dosing were given following standard protocols. Before recording the ECG, the rats were anaesthetized using Ketamine 50 mg/kg and Xylazine 10 mg/kg.

4. . Statistical analysis

All data were given as Mean ± SEM in the charts.

RESULTS

1) Effect of Loratidine (1mg/kg) on clarithromycin and furo semide induced hypokalemic rat heart

Three groups of male rats (n=3) were taken and each group were administered furosemide (50mg/kg), clarithromycin (70mg/kg) and loratidine (1mg/kg) for a period of seven days and ECG were recorded on the 0, 4^{th} and 8^{th} day. The ECG recordings revealed that there was a prolongation of QT interval on day 4 but this prolongation gets reduced to a certain extend on the 8^{th} day.

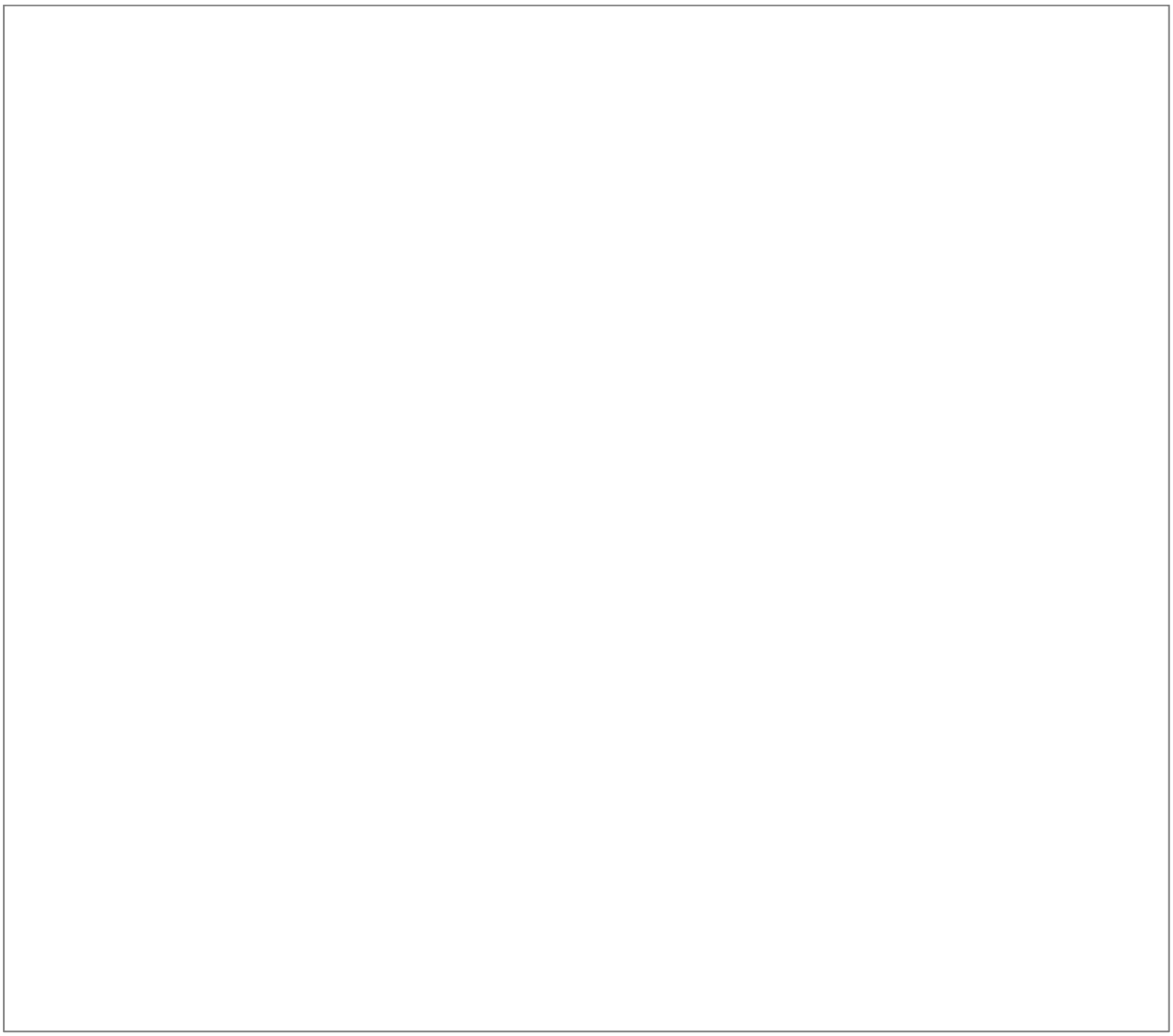


Table- The Mean ± SEM data for QTc recorded on 0, 4^{th} and 8^{th} day.

2) Effect of loratidine(2mg/kg) on clarithromycin and furosemide induced hypokalemic rat heart

Three groups of male rats (n=3) were taken and each group were administered furosemide (50mg/kg), clarithromycin (70mg/kg) and loratidine (2mg/kg) for a period of seven days and ECG were recored on the 0, 4^{th} and 8^{th} day. The ECG recordings revealed that there was a prolongation of QT interval on day 4 but this prolongation gets reduced to a certain extend on the 8^{th} day.

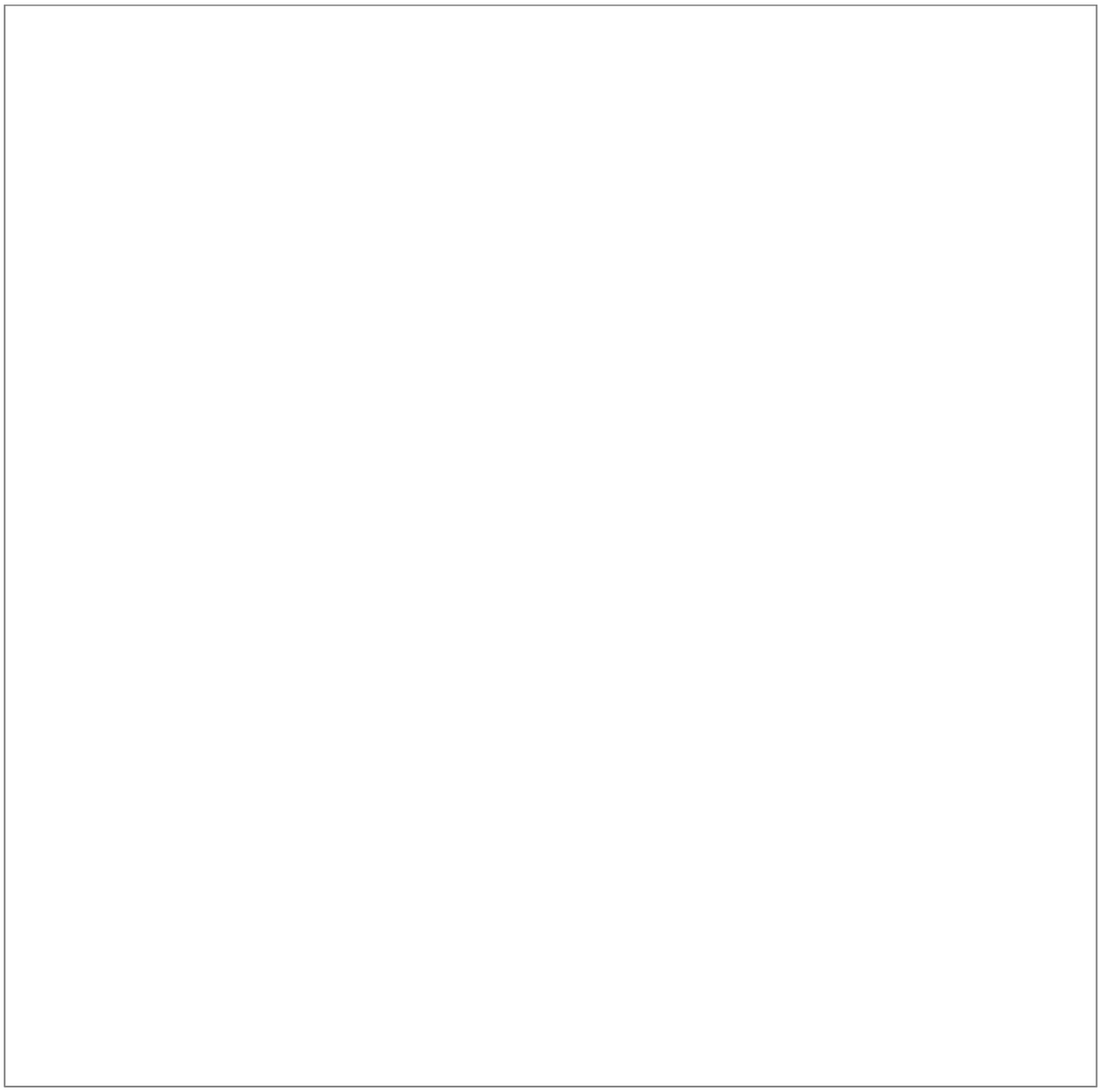


Table- The Mean ± SEM data for QTc recorded on 0, 4^{th} and 8^{th} day.

Discussion

Loratidine, a second generation anti-histamine widely used in the management of histamine induced hyperacidity. This drug is believed to be safe for use. However, in our set of experiment where the accommodation of heart to resist QT prolongation has been exhausted using co-administered of clarithromycin along side furosemide, Loratidine caused QT prolongation. The justification behind administration of clarithromycin and furosemide is that it will help to deplete the repolarise reserve thereby the intrinsic accommodation of heart against drug induced QT prolongation can be exhausted.

ECG reading revealed that there was a marked QT prolongation on 4^{th} day in comparison with the ECG taken on 0 day. But the QT interval decreases on 8^{th} day as compared to that of 4^{th} day. This particular finding was difficult to justify with present of loratidine since clarithromycin is an inhibitor of CYP3A4, while loratidine is partly metabolised by the same CYP isoenzyme. However, comparing the QTc to RR interval on both 4^{th} and 8^{th} day reading, which is considered as a clinical emergency indicating arrthymia.

Further studies that can be done

1) Tissue distribution of Loratidine with particular interest in cardiac tissuse.
2) The reason behind decrease in QT interval on 8^{th} day in comparison with the 4^{th} day can be investigated.
3) Study can be conducted to reveals the ion channels that are involved in electrical conduction of the heart which are probably leading to long QT intervals.

Conclusion

Torsades de Pointes is one of the most fatal type of cardiac arrhythmias in the world. Present advancement of medical sciences has done a lot of work to minimize drug induced QT prolongation related deaths. The role of late sodium current, and new pathways which are being discovered to prolong the action potential must be taken into consideration in the coming days while screening drugs for QT prolongation. Our work indicates the fact that common drugs which are widely used such as Loratidine might precipitate arrhythmia and prolong QT intervals when used in combination with other QT prolonging drugs or when used in patients with genetic predisposition of LQT syndromes.

Thus a more stringent screening method keeping the factor of repolarisation reserve in mind which will implement more safety measures in using the drugs. In the coming days, with the advancement in science we might understand the interaction of all these various channels which effect on the prolongation of action potential and finally a full proof screening method might come to screen the drugs.

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