Screening for drug-induced (acquired) long QT syndrome: is it time to apply new methods?

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Lande et al.\(^1\) have performed a nice investigation, throwing new light onto the diagnostic criteria of the long QT syndrome by exploring a large French family with the Romano–Ward syndrome followed-up for 25 years. In this family, as expected\(^3\), the electrocardiographic QT interval normalized when the males grew-up. In adulthood, however, the corrected QT
interval, as defined either by Bazett or Fridericia, was a poor discriminator of whether the individuals were affected, as were the popular diagnostic criteria proposed by Schwartz et al.[3] in 1993. By contrast, dynamic analysis of the QT interval had excellent negative and positive predictive values, particularly when the reversed QT/RR relationship was considered. Linkage analysis was used to identify the mutations in KvLQT1, and functional patch–clamp studies (at 35 °C) were carried out in parallel to demonstrate a reduction in the slow component of the delayed rectifier current (I_k).

Dynamic analysis of the QT interval showed, in particular, that circadian variation in the QT interval was similar in gene carriers manifesting the long QT as in those displaying a normal QT (higher QT/RR slope at night), but that the circadian variation was opposite to that observed in a group of controls (higher QT/RR slope at during the day). Based on these data, it can be hypothesized that of gene adult carriers with a normal phenotype, there are more men than women. Therefore, dynamic QT screening could be even more useful in men than women.

The authors recognize the inherent limitation of the one-pedigree approach. Whether these relationships hold in other families or mutations in other genes responsible for the long QT syndrome[4] deserves further study. Once more data have been accumulated, dynamic analysis of the QT interval might be a valuable method with which to detect silent gene carriers. Also, this method might be appealing in the search for far-reaching new avenues, in the realms of research and practical application in the debated arena of pre-clinical (and possibly clinical) safety testing of pharmacology.

To extrapolate from the results of the study by Lande et al.[1] it is tempting to speculate that drug-related torsade de points might more easily be prevented by adopting dynamic QT screening before drug prescription, aimed at searching for adult carriers of genetic abnormalities (also silent in nature) and normal phenotype.

**Drug-induced (acquired) long QT syndrome, a genetic disease?**

The long QT syndrome is a clinically heterogeneous group of disorders of cardiac repolarization, which may result from the use of drugs, or from a pathological condition with a genetic or non-genetic basis. Five loci in the human genome have been linked to the long QT syndrome in different families and specific genes have been identified at four loci[4]. In the genetic form there are long QT syndrome types 1–3 and 5 or the Romano–Ward syndrome, autosomal dominant without deafness, and types 1 and 2 of the Jervell–Lange–Nielsen syndrome, autosomal recessive with deafness. No disease gene has been identified for long QT syndrome type 4, and the long QT syndrome type 6 locus has been associated with (but not yet formally linked to) drug-induced long QT syndrome.

The essential electrophysiological mechanism underlying the long QT syndrome is a reduction in the intensity of the net outward current responsible for the repolarization process, either deriving from delayed inactivation of the inward Na+ current (I_{Na}) or decreased currents carried by one or more K+ channel. The resulting delay in the repolarization process may allow for the development of early and late after-depolarizations (particularly at the level of the Purkinje conducting system) which act as triggers for episodes of torsade de points. Calcium loading due to the first short cycle (s) of the ‘short–long–short’ series has been heavily implicated to favour the emergence of early after-depolarizations and triggered activity[4].

Basically, three major ion channel encoding genes (KvLQT1 and HERG for I_k, respectively slow and rapid, and SCN5A for I_{Na}) have been found to be sites of one or more mutations, which produce unfavourable changes in the structure of the encoded channel protein. Hence, the Na+ channel with a mutated α-subunit exhibits a markedly enhanced residual tail current in comparison to the equivalent current carried by the wild channel. This is responsible for the prolongation in action potential duration due to augmented late I_{Na}. Several mutations in the HERG gene that encodes the α-subunit of the K+ channel carrying the rapid component of the delayed rectifier current (I_k) have also been described. Electrophysiological studies performed on HERG, indicate, however, dramatic interspecies differences in this channel. For instance, the class III antiarrhythmic dofetilide is 100 times more potent in blocking HERG than its bovine equivalent BEAG channel.

Thus, very subtle changes in the protein sequence constituting a channel can dramatically affect ionic channel pharmacology. Mutations have also been identified in the α-subunit, which co-assembles with the β-subunit to form the K+ channel carrying I_k. In patients with the congenital long QT syndrome, as a result of these mutations, the fine balance between inward and outward currents, which plays a crucial role in determining a normal duration of the action potential duration, is pathologically altered. The phenotypic manifestation is a prolonged action potential duration (and QT) with a particular susceptibility to triggers such as sympathetic (arousal) and para-
sympathetic (resting) activation, hypokalaemia, and to drugs prolonging repolarization, whereas arrhythmic events are frequently of the torsade de pointes type and sudden death may ensue\(^3\).

Drug blocking or activating the normal flux of ions through their channels may modify certain aspects of the action potential duration and so affect cardiac function. Thus, blockers of Na\(^+\) channels reduce the rate of rise of the action potential (V\(_{\text{max}}\)) and can produce disturbances in cardiac conduction, which may be life threatening. Drugs increasing the current through Na\(^+\) channels prolong action potential duration, increase V\(_{\text{max}}\), prolong the QT interval and, thus, may trigger torsade de pointes and other arrhythmias. Blockers of Ca\(^2+\) channels decrease action potential duration, slow atrioventricular conduction and produce cardiac depression, whereas Ca\(^2+\) channel activators prolong action potential duration and may cause arrhythmias. Finally, K\(^+\) channel blockers prolong action potential duration and QT and can provoke arrhythmias whereas K\(^+\) channel activators shorten action potential duration and can also trigger arrhythmias. Antiarrhythmic effects of K\(^+\) channel openers in rhythm abnormalities related to delayed repolarization have, however, been reported\(^6\). More recently, Shimizu and Antzelevitch have elegantly pointed out that spontaneous and stimulation-induced torsade de pointes may be prevented with nicorandil (2 to 20 \(\mu\)mol.1\(^-1\)) when the congenital or acquired long QT syndrome is secondary to reduced I\(_{\text{Kr}}\) or I\(_{\text{Ks}}\), but less so when it is due to augmented late I\(_{\text{Na}}\)\(^7\). These latter results illustrate the complex interplay among genetics, electrophysiology and pharmacology.

There is, however, a missing epidemiological piece of evidence to complement such a complex and yet fascinating scenario. Indeed, the prevalence of silent gene carriers is unknown in the general population, and a classic epidemiological investigation might simply be non-definitive. Nevertheless, it is now imperative that new drugs on the market need to demonstrate that they do not increase the incidence of arrhythmias related to prolonged repolarization. This became a general problem, both scientific and political, when initial well-founded concerns related to some directly effective (e.g. class III agents) cardiovascular agents were extended to non-cardiovascular medicinal products\(^8\). Safety was the common thread, when it was suggested that their effects on the QT interval be investigated.

Research in terms of clinical trials is also hazardous. If we take terfenadine as a prototype agent, in which interest in terms of possible serious side-effects\(^9\), in comparison with over-the-counter antihistamines, has been renewed, it is easy to conclude that a truly prospective clinical trial would be impossible to carry out. It would require a sample size of over 5 million subjects in order to detect a small relative risk of 1:2, assuming a type I error of 0:05, power of 90% and an observed control group with life-threatening ventricular arrhythmia of 2/10 000\(^10\). It is noteworthy that the number of sudden cardiac deaths associated circumstantially with terfenadine usage and reported spontaneously to WHO was approximately two cases (a total of 69 persons) per 100 millions of daily doses sold during 1992–1996, which was a period of careful scrutiny for the cardio-toxic potential of terfenadine. It should be noted that these data might even over-estimate the cardio-toxicity of terfenadine since the spontaneous rate of cardiac events in a matched population not taking the drug was not concurrently measured, as might be expected from the intrinsic nature of spontaneous reporting systems\(^9\). Similarly, it has been estimated that the prevalence of the congenital long QT syndrome, confidently lower than 1/100 000 inhabitants, only justifies overabundance of interest due to a still high lethality, our relative ability to provide good treatment preventing sudden demise and the capacity this syndrome had to admit us into the abovementioned connections among genomic, transmembrane currents and therapeutics\(^11\). The clinical trial results of the congenital long QT syndrome may not be transferred to the general population.

The CPMP QT issue

In December 1997, the European Agency for Evaluation of Medicinal Products of the Committee for Proprietary Medicinal Products (CPMP) issued a statement (Note CPMP/986/96) entitled Points to consider: The assessment of the potential for QT interval prolongation by non-cardiovascular medicinal products. Urged by this document, while not a legal requirement, in June 1999, the European Society of Cardiology organized a Policy Conference gathering cardiologists, epidemiologists and representatives from the pharmaceutical industry and drug administrators to address the question of how to assess the safety profile of non-cardiovascular drugs with regard to their potential to prolong cardiac repolarization. On the other side of the ocean, an apparently very strict FDA guideline on clinical safety requirements for drugs prolonging QT is in preparation.

The CPMP QT document proposed a series of pre-clinical studies, recently reviewed in detail\(^8\), aimed at unveiling the QT prolongation potential of non-cardiovascular medicines and at providing reassurance concerning their safe clinical usage. The first step
is to study in vivo (in dogs) a range of escalating doses, which are complemented by in vitro electrophysiological investigations, performed using a suitable cardiac preparation and physiologically relevant conditions, while also inspecting for a reverse rate-dependency phenomenon if the compound under study is found to prolong action potential duration. If the results indicate that the novel agent does not prolong the QT interval in an unacceptable manner (say, no more than 10%), then the drug candidate can be cleared for safety assessment studies in healthy volunteers provided all other normal safety requirements are met. Under these requirements the basic assumption is made that the effects of compounds on cardiac ion channels present in the heart of the animal species selected can be directly translated to the human heart. However, the shape and duration of the cardiac action potential are features specific to each animal species\[12\]. They reflect subtle differences in type, structure, cellular distribution and the relative contribution to the generation of the cardiac action potential of the transmembrane current through the various channels. K\(^+\) channels represent the class of channels with the greatest species-dependent heterogeneity. Differences in the make-up, identity, and pharmacology of cardiac ion channels suggest that the results obtained from tissue derived from experimental animals may not adequately predict drug effects in the human myocardium\[13\]. Extrapolation of such data to human tissue requires great caution and may not always be valid.

**New methods**

Crumb and Cavero\[8\] have proposed a minimal cardiac safety package for-first-use-in-man before a candidate drug is allowed to enter phase I investigation in volunteers. Studies will be made of electrophysiological effects on well characterized native cardiac channels present in human atrium myocytes or in cloned channels expressed stably in mammalian cells, since currents passing through these channels determine the shape of the cardiac action potential. Increasing concentrations (covering 2–3 log units of the candidate drug in six experimentally viable preparations using physiological ionic solutions first, 0.1–2 Hz and at a 37 °C temperature (except for \(I_{Na}\)), together with appropriate reference compounds simultaneously, and second using certain experimental conditions which simulate some of those known to predispose to arrhythmia, such as acidosis, hypokalaemia, low resting potentials should be used, with the highest being at least 10- to 30-fold greater than the forecasted plasma concentrations necessary to obtain therapeutic activity. Crumb and Cavero proposed that this mechanistic study be performed before the canine in vivo investigation (as suggested by CPMP) since it is not expensive and need not be very time-consuming. If the candidate drug lacks substantial effects on channels responsible for QT prolongation, a classical in vivo pharmacodynamic study should be carried out in well trained dogs instrumented with telemetry systems, known to respond positively to standard drugs prolonging QT. If the in vivo studies show no evidence of feared adverse cardiovascular effects, the compound can be considered safe from a cardiac safety point of view and, thus, it may be cleared for clinical assessment.

Isolated human atrial and ventricular tissues, as obtained pre-operatively during open heart surgery may also be important in the exploration of action potential duration changes rate-dependently\[12,13\]. It is of special interest that in both tissue types at 31–33 °C, over a large range of stimulation rates (Figs 1 and 2), almost identical parameters were observed (Table 1), which enabled the conclusion that Bazett’s formula is unsuitable to fit the action potential duration/cycle length relationship. Furthermore, the parameters compared nicely with those obtained in a series of 588 middle-aged men with a normal ECG (based on Minnesota code) from one residential cohort of the Italian section of the Seven Countries Study\[14\], implying that for both human action potential duration (atrial and ventricular) and the QT interval it is possible to find an optimal mathematical relationship with cycle length. Of this relationship advantage might be taken to derive important pharmacodynamic information.

On the other hand, patients with known cardiac risks including, when ethically permissible, individuals with genetically mutated cardiac ion channels, may be selected\[8\]. The basis of this idea was that some fatal consequences of terfenadine or cisapride administration have been linked to patients with an undiagnosed cardiac risk such as a congenital long QT syndrome. Recently, it was reported that a patient reacted with a very marked QT prolongation (700 ms from baseline value of 440–480 ms) to cisapride\[15\]. This patient was a posteriori demonstrated to suffer from the congenital long QT syndrome. Recent studies have identified a large number of individuals who are silent carriers of non-clinically manifest abnormalities of cardiac repolarization\[2\]. These can be revealed by stimuli with proarhythmic potential (e.g. drugs blocking repolarization K\(^+\) channels, hypokalaemia, bradycardia, etc.).
It is clearly much more complex to transpose at the clinical level the results obtained in pre-clinical pharmacological studies. Results obtained in normal volunteers or in selected patients may not prevent later discoveries on side effects of approved drugs. We badly need screening methods, at an acceptable level of expense and reliability, to be used in both sexes and independent of actual cardiac pathology, to

Figure 1 Regression curves in 23 independent human right atrial strips between action potential duration and cycle length (138 data-points) according to four formulae. Note that suffixes of K parameters are named after the initial of the formula in which they are used. □=human right atrium at 31 °C (n=23).

Figure 2 Regression curves in three independent human left ventricular strips between action potential duration and cycle length (17 data-points) according to four formulae. Note that suffixes of K parameters are named after the initial of the formula in which they are used. ●=human left ventricle at 33 °C (n=3).
reveal the probability of undesirable QT prolongation of diverse drugs, just before prescription. This might help prevent life threatening consequences with agents predisposing to QT prolongation. Whether dynamic QT screening using the methods described by Lande et al.[1] have some potential to help, along the lines alluded to in the CMCP QT issue, when applied to both pre-clinical pharmacology and safety in prescribing selected medicines, is a new challenge awaiting keen explorers.

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References

