Gene specific therapy for arrhythmogenic disorders

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Introduction

The long QT syndrome (LQTS) is a familial disease characterized by an abnormally prolonged QT interval and by stress-mediated life-threatening ventricular arrhythmias. LQTS usually manifests itself in children and teenagers in the absence of structural cardiac abnormalities[1].

LQTS is not only an important disease for the high mortality rate when misdiagnosed, but it represents a model to understand the complex link between genetically transmitted alterations in cardiac electrophysiology, the autonomic nervous system, and sudden cardiac death. These considerations explain the interest of clinical and experimental research in LQTS, which has progressively grown from the early observations[2] to the recent development provided by the molecular understanding of the disease.

Clinical presentation and diagnosis

A familial pattern of inheritance is not apparent in approximately 20-25% of cases, which therefore have to be classified as sporadic: these individuals probably have a *de novo* mutation and will become founders of kindred with LQTS[3]. Alternatively, they may represent the only individuals with the disease phenotype among a family where other non-penetrant gene carriers are present, because of the incomplete penetrance of some mutations[4]. The clinical history is typically represented by episodes of loss of consciousness under emotional or physical stress.

Diagnostic criteria

In quite a few cases the symptoms of LQTS are so characteristic that diagnosis presents no problems for the physician aware of the disease. Borderline cases do exist and may require evaluation of the several features described above besides clinical history and surface electrocardiogram. A first diagnostic algorithm has been proposed by Schwartz et al.[5] in 1993. Unfortunately, despite the diagnostic criteria help in unifying requirements for diagnosis, they still leave open the dilemma of borderline cases: the gold standard for diagnosis of LQTS is therefore the molecular identification of the mutation in the DNA.

Toward molecular diagnosis of long QT syndrome

In the last 6 years the extensive study of LQTS families from a molecular biology standpoint has made substantial progress in the understanding of the pathophysiologic mechanisms underlying the LQTS. In 1995 the genes for chromosome 3, 7 and 11 were identified[6-10] as, *SCN5A*, the cardiac sodium channel, *HERG*, the gene encoding the rapid component of the delayed rectifier (I_K) I_{Kr}, and as *KvLQT1* encoding for the membrane ionic channel conducting I_{Ks}, the slow component of the I_K. More recently two additional genes have been identified on chromosome 21: *KCNE1* and *KCNE2*[11,12]. Overall only 1-2% of patients carry mutations in the latter genes that encode for the auxiliary protein modulating function of *KvLQT1* and *HERG* gene products.

Electrophysiologic characteristics of the mutations identified in long QT syndrome genes. *SCN5A*. The three initially identified[8,13] mutations provided the rationale for the understanding of LQT3 that still holds

true. These mutations consisted of a 9 base pair deletion (ΔKPQ) and two point mutations (R1644H and N1325S). All these three mutations affect a region important for the inactivation of the Na^+ current. Subsequently, Bennett et al.[14] characterized the ΔKPQ mutation by expressing the altered cardiac sodium channel in *Xenopus oocytes*. Mutant channels showed a sustained inward current during membrane depolarization. Dumaine et al.[15] further characterized the electrophysiologic consequences of the ΔKPQ and the two point mutations showing that all three mutations increased sodium inward current but the defects were of a different severity.

HERG. Sanguinetti et al.[16] expressed the protein codified by *HERG* in *Xenopus oocytes* and demonstrated that *HERG* encodes the rapid component of the delayed rectifier. The electrophysiologic properties of expressed *HERG* were nearly identical to the I_{Kr} current in cardiac myocytes. The same authors[17] demonstrated that *HERG* mutations produce a loss of channel function resulting in a reduced repolarizing K^+ current that may account for the clinical phenotype.

KvLQT1. Wang et al.[10] have identified the gene responsible for chromosome 11-linked LQTS (LQT1), specifically *KvLQT1*. Ten different missense mutations and intragenic deletions in *KvLQT1* were found by the authors in 16 LQTS families. *KvLQT1* proteins co-assemble with the *KCNE1* gene product to form the functional I_{Ks} conducting channel[11]. Several expression studies of *KvLQT1*-minK mutations have been performed and they showed a reduction of the I_{Ks} current. Since I_{Ks} is the most important current that allows shortening of repolarization at faster rates (i.e. during adrenergic activation) it seems logical to speculate that LQT1 individuals are more vulnerable during conditions of high adrenergic activity. Recent data by Schwartz (personal communication) have confirmed that most of the arrhythmic events in LQT1 patients occur during physical and emotional stress.

Therapy

Independently of the primary abnormality involved in its pathogenesis, the trigger for arrhythmias in most LQTS patients is a sudden sympathetic discharge[1]. Antiadrenergic interventions therefore represent the most logical therapeutic approach. Other therapeutic approaches (i.e. pacemakers, implantable cardioverter defibrillators, and non-antiadrenergic pharmacological therapies) have been also proposed. The most appealing challenge for the near future however lies in the attempt to design pharmacological interventions that may counteract the consequences of the individual mutations. Data are still preliminary, however a few strategies have been highlighted as discussed below.

Experimental basis for gene specific therapy. Based on the evidence that LQT3 is caused by alterations in the inactivation of cardiac sodium channels[14,15] and that LQT2 is caused by a reduction in the delayed rectifier potassium current[17], we attempted to develop the first cellular model for LQTS[18]. Our goal was to provide a means for assessing the effect of different interventions in two forms of the disease: LQT2 and LQT3.

We exposed guinea pig ventricular myocytes[18] to anthopleurin, a toxin that interferes with the inactivation of I_{Na}, and to dofetilide, a selective blocker of I_{Kr} in order to obtain a prolongation of cellular repolarization. Both anthopleurin and dofetilide induced a prolongation of action potential duration confirming that either a reduced I_{Kr} or a persistent inward sodium current can cause the classic phenotypic alteration of LQTS, i.e. prolongation of the QT interval. Using this experimental preparation mimicking LQT3 and LQT2, respectively, we tried to differentiate the phenotypic manifestations of these two forms of the disease by characterizing the response to the sodium channel blocker mexiletine and to rapid pacing.

Exposure to the sodium channel blocker mexiletine significantly reduced the action potential duration in cells treated with anthopleurin while it did not modify the prolongation induced by dofetilide. This demonstrated that it was possible to normalize the action potential duration by blocking the persistent inward sodium current in the LQT2 mimicking model. This finding fits in very well with the demonstration by Dumaine et al.[15] that mexiletine could reverse the changes induced by the three LQT3 mutations on *SCN5A*.

When fast pacing was performed anthopleurin treated cells showed a prompt shortening of action potential duration toward control values. Overall, these data demonstrated that pharmacological interventions aimed at mimicking phenotypic manifestations of LQT3 and LQT2 at a cellular level, result in differential responses to sodium channel blockade, and to rapid pacing. From a clinical standpoint these results might indicate that LQT3 patients could favorably respond to sodium channel blockade and to pacing that would prevent bradycardia-related QT prolongation.

Gene specific therapy for long QT syndrome patients. Based on this experimental evidence, we tested the hypothesis that mexiletine and physiologically-induced increases in heart rate would shorten the QT interval more in LQT3 than in LQT2 patients[19].

Fifteen LQTS patients who were referred to our center entered the study and were characterized genetically. Eight patients belonged to three LQTS families genetically linked to chromosome 3 (LQT3), while 7 patients belonged to two families linked to chromosome 7 (LQT2). Mexiletine significantly shortened the QT interval among LQT3 patients but not among LQT2 patients. When we examined the response to increases in heart rate, we found that LQT3

Table I. Proposed algorithm for gene specific therapy in long QT syndrome.

Genetic variant	Gene specific treatment	Rationale
LQT1	Antiadrenergic	The defective current I_{Ks} is important during adrenergic activation, antiadrenergic interventions reduce the effects of its dysfunction
LQT2	Potassium supplement Potassium sparing diuretics	The I_{Kr} current defective in these patients is augmented by increased extracellular potassium
LQT3	Sodium channel blockers	The genetic defect causes an excess of inward sodium current

patients shortened their QT interval in response to heart rate changes more than LQT2 patients and more than the healthy controls.

Conclusions

Even if data on gene specific therapy in LQTS should still be considered at a preliminary phase of development, they support important clinical considerations. LQT3 patients may not be at a particularly high risk during physical stress because during progressive sinus tachycardia, their QT intervals would markedly shorten. They might benefit less than other LQTS patients from antiadrenergic therapy while they might benefit from chronic therapy with mexiletine and pacing. On the other hand, LQT1 patients are more likely to be at risk for cardiac arrest under stressful conditions, because the arrhythmogenic effect of catecholamines would be enhanced by the lack of appropriate QT shortening when heart rate increases. LQT1 patients are likely to be protected by antiadrenergic therapy. In LQT2 patients an increase in the extracellular concentration of potassium may shorten the QT interval[20]: it is therefore possible that this subgroup of individuals may benefit from combined therapy with potassium sparing diuretics, oral potassium supplements and beta-blockers. Overall the proposed algorithm for gene specific therapy in LQTS is outlined in table I.

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