Factors of Influence on Cardiac Repolarization:
- Physiological Conditions and Factors to be Considered in Planning Clinical QT Studies -

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AGENDA

- Cardiac cell Action Potential cycle (CCC)
- Physiological links to CCC
  - Congenital “diseases” (channelopathies)
  - ANS influence: respiration, circadian cycle and other related factors
  - Metabolic factors, electrolyte balance
  - Sex & endocrinology factors
  - Cardiac memory and QT/RR hysteresis
- Measurement methods
- Which variables and co-factors to consider for data capture?

Take home messages look like this
Preface

1988: Bramah Singh published his book “Control of Cardiac Arrhythmias by Lengthening Repolarization” (Futura Publishing Company Mount Kisco, New York)

At that time it was the paramount of belief that the QT-prolongation would help to cure for any kind of arrhythmias.

THE SHOCKS

1991 CAST-Study: excess of mortality by class Ib/Ic antiarrhythmic compounds (encainide, flecainide, mexitilone)

1996 SWORD-Study: excess of mortality by class III antiarrhythmic compound oral d-Sotalol

1990s: Growing number of non-cardiac drugs with QT-related TdP
The Monophasic Action Potential of the Cells

Source: Rossi&Matturri, 1990
MAPs (Monophasic Action Potentials) are the Origin of the ECG Waveforms
Multiple Cardiac Ion Currents Interact in the Cardiac Cycle (and are also Linked to Congenital Diseases)

Cardiac cell AP cycle

 Linked to Human Disease

Animal Cardiac Effects

Current
- Sodium $I_{Na}$
- Calcium (L-type)
- Calcium (T-type)
- Na/Ca exchanger
- $I_{TO1}$ (4-AP-sensitive)
- $I_{TO2}$ (Ca$^{2+}$-activated)

Probable Clone
- SCN5A
- Cav1.2
- Cav3.1
- Na/Ca exchanger
- Kv4.2/4.3+KChIP2
- KCNQ1+KCNE1(MinK)
- HERG+KCNE2(MiRP1)
- Kv1.5(KCNA5)
- CFTR//TWIK
- Kir2.1(KCNJ2)
- Kir6.2+SUR2a/Kir3
- HCN2+HCN4

after Gerlach, 2003
Genetic Factors Affecting Repolarization

- HERG blocking drug
- High drug concentration
- Drug-drug interaction
  - 2 concurrent HERG blockers
  - Drug that inhibits metabolism of main blocker
  - SNPs that increase drug binding

SNPs that affect expression or trafficking of channels

SNPs that reduce potassium current

SNPs that increase sodium current

SNPs that affect interaction with regulatory pathways

SNP=Single Nucleotid Polymorphism
Recently detected: the Short QT Syndrome (SQTS)
Same Roots of LQTS & SQTS mechanisms

** Recently detected: the Short QT Syndrome (SQTS)**
Same Roots of LQTS & SQTS mechanisms

**Congenital diseases**

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**substitution of aspartic acid (negative charged)**
for asparagine (neutral charge)
-Splavski et al.
( Genomics 1998; 51:86-97)

**leads to a loss of function of** \( I_{kr} \)

LQTS2

**HERG (KCNH2)**

- Encodes \( I_{kr} \)

- Leads to a loss of function of \( I_{kr} \)

- N588K

**substitution of lysine (positively charged)**
for asparagine
-Brugada R et al.
( Circulation. 2004;109:30-35)

**loss of the normal rectification of the current at plateau voltages, thus**
**resulting in a huge increase of** \( I_{kr} \) **during the action potential plateau, leading to marked abbreviation of the action potential**

\( SQTS \)

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Other SQTS mechanism recently detected:

- **V307L KCNQ1 mutant affecting** \( I_{KS} \)
  revealed a-20-mV shift of the half-activation potential and an acceleration of the activation kinetics (Bellocq, Circulation 2004),

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Short QT syndrome (SQTS)

SQTS if QT<300 msec or QTcF < 320 msec

Inherited syndrome (3 forms known) linked to SCD and early deaths
Considerations for Clinical (Phase-1) Studies:

- Should we include or exclude persons with congenital inherited ion channel diseases?
- How would we assess whether our volunteers or patients are LQTS/SQTS carriers/non-carriers?
  - Most are not known to be pre genotyping diagnosis
  - ECG QT/QTc often asymptomatic
  - Family anamnese can give hints
    - Chance to unmask carriers by pharmacological or other challenge:
      - By iv low dose Ajmaline or Flecainide
      - By physical vagal challenge (neck suction)?
      - Or by naringine (never done before!)
  - Characterization by genomic testing? (very expensive & far from complete!)

- How to deal with ethical challenge?
CV+CNS-Effects Drive the Heart

Emotional influence
Vegetative reaction
Baroreceptor feedback

Respiration
Heartrate
Card.Output
Blood pressure

Vasomotoric

Sympathikus
Vagus

Neokortex
Respiratory Sinus Arrhythmia Affects RR and QT

Due to cyclic variation of RR and QT 3-5 beats are insufficient to represent true average!
Circadian Heart Rate and Repolarization Changes

Fig. 3.1 Circadian pattern of RR (open circles) and uncorrected QT intervals (closed circles).

Fig. 3.2 Circadian pattern of QTc interval obtained by the Bazett (closed squares), Fridericia (closed triangles), Framingham (open squares), Hodges (open circles), and individually optimized (closed circles, bold) heart rate corrections. (Redrawn from Smetana et al. 2003, with permission.)
Sympathovagal Condition Influence on QT

Day-night cycle („circadian cycle“)

- SCN (suprachiasmatic nucleus) governs wake-sleep cycle
- Gene expression cycle between nucleus and cytosole in SCN=pacemaker
- Day: sympathetic drive
- Night: vagal drive
- Influence on
  - RR, BP
  - QT
  - body core temperature
  - Muscle tension

QT differences caused by

- Physical activity,
- Sympathovagal regulation,
  but maybe also by body core temperature difference
Stress-level induced QT changes

- HR and plasma cortisol-level change repolarization condition
- Influence of BNP: few known
- Stress induced QTc-prolongation in deep grief situations known
- Sleep apnea
Sex & QTc

Females during fertile life phase:

• F have ~ 30 msec longer QTc than M
  – Higher risk of SCD reported for F
  – F at risk to develop TdP by drug QTc prolongation is clearly higher than M!
  – But: in general lower risk overall to suffer from CV diseases during fertile life period, CV risk increases during menopause

• QTc Prolongation F relative to M
  – begins at ~12 yr,
  – Tendency to decrease after 55 yr

• Female sexual cycle

Reason for sex differences:

• Estrogene remolds the heart cell ion channel characteristics
Sex & endocrinology factors

Circadian QTc Profiles by Sex

Smetana, 2002;
From: Malik/Camm 2004: Dynamic Electrocardiography
Testosterone Reduces QT Duration

Guinea pig myocytes patch clamp

Concentration dependent reduction of AP-interval

APD shortening by testosterone was mainly due to enhancement of slowly activating delayed rectifier K currents ($I_{Ks}$) and suppression of L-type Ca2 currents ($I_{Ca,L}$), because testosterone failed to shorten APD in the presence of an $I_{Ks}$ inhibitor, chromanol 293B, and an $I_{Ca,L}$ inhibitor, nisoldipine.

A nitric oxide (NO) scavenger and an inhibitor of NO synthase 3 (NOS3) reversed the effects of testosterone on APD, which suggests that NO released from NOS3 is responsible for the electrophysiological effects of testosterone.

EC$_{50}$: 2.1-8.7 nmol/l

Bai CX et al., Circulation. 2005;112:1701-1710.
Baseline QTc Intervals (Bazett correction) in Women During the 3 Phases of the Menstrual Cycle and in Men

Table 2. Serum Sex Hormone Levels

<table>
<thead>
<tr>
<th></th>
<th>Menses</th>
<th>Ovulation</th>
<th>Luteal</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol, pg/mL</td>
<td>37.1 (13)</td>
<td>122 (42.0)</td>
<td>123 (49.0)</td>
<td>30.8 (13.0)</td>
</tr>
<tr>
<td>Progesterone, ng/mL</td>
<td>0.6 (0.2)</td>
<td>3.8 (4.0)</td>
<td>10.2 (4.0)</td>
<td>. . .</td>
</tr>
<tr>
<td>Testosterone, pg/dL</td>
<td>27.2 (11)</td>
<td>37.4 (10.0)</td>
<td>28.8 (49.0)</td>
<td>485 (126)</td>
</tr>
</tbody>
</table>

*Data are presented as mean (SD). Ellipses indicate not applicable.
Mean Change in QTc Interval Area Under the Curve During the First Hour After Ibutilide Infusion

<table>
<thead>
<tr>
<th>Ibutilide AUC [pg x min/mL]</th>
<th>1444</th>
<th>1658</th>
<th>1531</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>repeated measures ANOVA, $P = .23$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Plasma Ibutilide concentrations after a 10-minute infusion of 0.003 mg/kg were not significantly different.

Females may react to drugs dependent on fertility cycle.


Sex & endocrinology factors
Drug Induced TdP Arrhythmia by d,l-Sotalol Depends on Sex, Dose and Renal Function

Prevalence of TdP by sex in each of four patient subgroups defined by combinations of d,l-sotalol dose and creatinine clearance (CrCl) (dichotomized at respective cut points of 4.7 mg·kg⁻¹·d⁻¹ and 50 mL/min)
Sex & Hormones & Repolarization – Arrhythmia Link

Rabbit ventricular myocytes:
effects of dofetilide on APD and EAD incidence at CL=1000 ms.
Top, Representative AP; C indicates control; Dof, 10^{-6} mol/L dofetilide. Middle, Relationship of \( \Delta APD_{90} \) to increasing dofetilide concentrations.

Bottom, Incidence of EADs induced by dofetilide. N=12, 10, 13, and 16 for female (●), male (○), OVX (▼), and ORCH (▽), respectively.

*\( P<0.05 \) vs OVX and control male; 1\( P<0.05 \) vs respective predrug control.

Castrates are “remodeled” in repolarization
M castrates (ORCH) react like F, and
F castrates (OVX) react like M!
Considerations for Clinical (Phase-1) Studies:

• Massive differences in drug reaction can be triggered by sex & endocrine situation.

• Guidelines urge to consider more females to be involved in safety testing of drugs. Practical and resource considerations may lead to conclusion not to involve female study participants too early, but definitely it will happen.

• Is the mode of action of drug candidate expected to be influenced by sex/endocrine differences?  
  Tissue/animal experiments are needed.

• When is the optimal stage of drug development to involve F?  
  And: to which degree?

• Mode of drug action can depend on sex & F cycle  
  Should we assess sexual hormone status for QT studies?  
  - sexual cycle data in F ?  
  - Testosterone levels in M?
Electrolyte Balance has to be Maintained

- **Na**: important for depolarization
- **Ca, K, Mg**: important for repolarization
  - Avoid extrema like hypokalemia and hyperkalemia

**Diarrhea and Emesis:**

- **Substitute electrolytes**
- **Possibly take extra blood samples if electrolyte status unclear**
  - Electrolytes are potential covariables to subsequent PK/PD and QT/RR modeling
  - May uncover physiological links to other biomarkers and clinical signs
Diabetes-II & FFA

Diabetes-II is triggered by obesity

Elevated plasma fatty acid (FFA) concentrations stimulate the cardiac autonomic nervous system in healthy subjects

- **FFA correlate positively with QTc in healthy volunteers!**
- **Diabetes patients typically show prolonged QTc intervals**

Corbi et al., J Clin Endocrinol Metab 87:2080–2083
Food intake causes ~10 msec QTc prolongation

Time course of heart rate (top),

Q-T interval (middle),

and QTc (bottom) during resting conditions and during 100 min of euglycemic hyperinsulinemia in 35 nondiabetic subjects.

Fasting Plasma Insuline Concentration Correlates Positively to QTc and Negatively to Serum Potassium

Top: direct relationship between resting QTc [body surface area (BSA) adjustment] and fasting plasma insulin concentrations.

Bottom: reciprocal relationship between BSA-adjusted QTc and serum potassium concentrations. Data of the basal (squares) and insulin period (filled) are shown; the regression line for the basal data (interrupted line, $r = 0.16, P < 0.04$) and that for the insulin period (full line, $r = 0.47, P = 0.003$) have similar slopes.

Insulin influences ANS and serum K+

The physiological system connecting plasma insulin concentrations with serum potassium levels and the activity of the autonomic nervous system (ANS).

(+) And (-) indicate stimulation and inhibition, respectively.

Enzyme Inhibitors May Prolong QT/QTc

• Naringenine (from grapefruit and any other citrus plant) inhibits CYP liver enzymes and prolongs QT:
  - 1 liter grapefruit juice contains >1000µmol/l naringenine glycosides
  - In a study on 10 healthy volunteers (Heidelberg Medical School) intake of 1 liter grapefruit juice caused a 12.5 (SD 4.2) msec QTcB prolongation 5 hours after ingestion. (Zitron et al. Circulation 2005;111:835-838)

According to ICH-E14 a positive control arm is required for definitive QT studies:
**Why not use grapefruit juice instead of Moxifloxacin as a positive control?**
Considerations for Clinical (Phase-1) Studies:

- **Food intake can prolong QT/QTc**
  - Change of body electrolyte situation
  - Uptake of FFA
  - Uptake of glycosis -> insulin stimulation
  - Change of ANS status

- **No grapefruit and other agrumes (and juice) consumption should be allowed**

- **High glycemic index food probably more impact than low glycemic profile**

- **Maintain electrolyte status in side effect situations**

- **Should we allow extra food / sweets intake during QT studies?**
QT Adaptation ("Cardiac Memory") non-steady state

QT interval needs time to adapt to rapid heart rate changes. This is true for both acceleration and deceleration of the cardiac rhythm.

Caveat: there is a short-term and independently long-term cardiac memory! Beat-to-beat changes: no strong memory effect!

QT Adaptation
Example of non-steady state repolarization in Holter ECG

QT= 397 msec
ECG tracing at 02:46:27
RR=1027 msec

QT= 423 msec
ECG tracing at 02:51:15
RR=1027 msec

By courtesy of Jean-Philippe Couderc
QT Adaptation and Proarrhythmic Risk

QT-RR-Hysteresis


RB=reflex bradycardia

RT=reflex tachycardia
Basic Shape of Normal ECG: Cardiac Cell Cycle Interval Parameters

Measurement considerations

- **PQ** represents the atrial depolarization duration.
- **QRS** represents the ventricular depolarization duration (contraction is very fast ~<100 ms!).
- **ST** represents the isoelectric line.
- **QT** represents the complete heart work cycle duration (depolarization + repolarization).
- **JT** represents the ventricular repolarization duration (usually >250 ms!).

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Rich vs Sparse QT Data: ECGs 10´ Parallel Recording

Holter-ECG

Triplets of Resting ECGs

Measurement considerations
Considerations on Measurement Methods

• Resting ECG should always be taken in a stable resting but wake conditions.

Persons for taking ECG:
  – Don’t move
  – Don’t speak
  – Don’t sleep – keep eyes open
  – Keep calm & without emotions

• Resting ECG measurement should take all possible beats for RR&QT assessment to ensure coverage of respiration cycle

• Resting ECG is definitely too short to allow assessment of QT-RR hysteresis

• Even ECG triplets (1-2 minute distance) are too short to allow appropriate assessment

• Best would be Holter-ECG, but this technology is still far from ideal
Considerations on Variables

- \( QT = QJ + JT_{end} \) (depolarization + repolarization interval)
- \( QJ \) (QS) and \( JT_{end} \) should always be considered as separate variables, aside of QT and QTc
- As many meaningful co-variables possible should be captured to cover influences from spurious factors, which could be included to statistical modeling
Summary of QT-influential factors I

The cardiac cycle time QT is often quoted to be primarily influenced by drugs – this is a myth!

There seems to be an optimum for QT duration, since too short as well as too long QT times make individuals vulnerable to the same reasons of cardiac death:

• Fatal arrhythmias like ventricular tachycardia and TdP
• Indicators are syncopes and
• Family history of deaths
• AF and PAF seem to be linked to QT problems
• Genotypes of LQTS and SQTS may share same gene loci, just specific mutations result in different phenotypes
Summary of QT-influential factors II

**Electrolyte imbalances** play the key role for depolarization as well as repolarization, and thus for QT (vomiting and diarrhea !!!)

**Sex and age, but also circadian changes** (including food digestion and composition) are important factors for QT duration

**Metabolic diseases matter for QT duration**, and may also be linked to imbalance of electrolytes

**Body core temperature** can be an important influential factor:
- Hyperthermia shortens QT
- Hypothermia lengthens QT (this may also be an indirect drug effect)

**Hypoxia** is a clear risk factor for QT prolongation
   (smoking, artherosclerosis, lack of physical training, CHD, CHF)

**Direct or indirect drug effects** add to the other factors
Thanks for listening to that much stuff

Anton