Join Us For The 17th Annual Ghirardelli Chocolate Festival!
Ghirardelli Square, San Francisco, CA
September 8-9, 2012, 12pm-5pm

Ghirardelli Chocolate Company is excited to invite you to the annual, two-day chocolate celebration that has transformed into a true San Francisco staple. Local vendors sample and showcase delicious treats that will delight any palate!

Non-traumatic sudden death

- ~300,000 non-traumatic sudden deaths in the US each year.¹
- In a series of consecutive sudden deaths:²
  - 75% cardiac
  - 4% PE
  - 7% respiratory
  - 1% acute stroke/bleed
  - 5% AAA
  - 8% other
- Sudden cardiac death:
  - Coronary disease in 90-95%²-⁴
  - Acute coronary thrombus in 42-74%²-⁵
  - Healed MI in 48-66%²,⁴,⁶-⁷

¹ multiple sources, none great
². Soo. Resuscitation 2001;31:257-64

Sudden death after MI

Post-MI ventricular arrhythmias

- Findings in humans
  - Pathology
  - Electrophysiology
    - Acute
    - Chronic
- Dog model of arrhythmias in the early healing phase post-MI
- Pig model of arrhythmias in “healed” infarcts

Human: post-MI pathology

- Necrosis: onset 6 hrs, peak d3-10, disappears by 6 weeks.
  - Nuclear breakdown, myocyte necrosis, inflammatory infiltrate, phagocytosis of cell breakdown products.
- Fibrosis: onset d3-10, peak d8-21, stable after 6 weeks.
  - Appearance of fibroblasts, production of collagen/ECM, new blood vessel proliferation
- Remodeling: onset d28, continuous
  - Collagen cross-linking, scar shrinkage


Human: border zone histology

Brackets enclose endocardial fibrosis (A), spared subendocardium (B), barrier infarct (C), patchy fibrosis (D). The chronic VT group had predominantly large patchy myocardial infarcts with irregular ribbons of spared subendo tissue.


Human: acute post-MI EP

- First 30 days post-MI:
  - Little knowledge of cellular EP
  - Inducibility on EP study:
    - SMVT: 46% of unselected sample, 20% of reperfused < 6hrs post-symptoms, 44% of non-reperfused, 23-28% of EF < 40%.\(^1\)\(^4\)
    - PMVT: 2-18% of EF < 40%.\(^2\)\(^4\)\(^5\)
    - VF: 4-7% of EF < 40%


Human: chronic post-MI EP

- Cellular EP (not specific to VT circuits):\(^1\)
  
<table>
<thead>
<tr>
<th>remote</th>
<th>infract BZ</th>
</tr>
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<tbody>
<tr>
<td>RMP</td>
<td>-80</td>
</tr>
<tr>
<td>APA</td>
<td>107</td>
</tr>
<tr>
<td>V-dot max</td>
<td>388</td>
</tr>
<tr>
<td>APD90</td>
<td>370</td>
</tr>
</tbody>
</table>

  - HETEROGENOUS

- Tissue conduction (not specific to VT circuits):
  - Connexin lateralization\(^2\)
  - Slow conduction more prominent lateral to fiber orientation (0.79 m/s longitudinal, 0.09 m/s transverse)\(^3\)
  - Fractionation/split potentials/late potentials do not correlate with VT site\(^4\)\(^5\)
- EP study: MUSTT 32% SMVT, 12% PMVT/VF\(^6\)

Human: gaps in knowledge

- **Early phase:**
  - Little information on cellular EP changes through the healing process
  - Suggestions from clinical trials of interactions between EP and heart failure risk markers but no solid information
- **Chronic phase:**
  - Limited information on cellular EP.
  - Available information is not specific to the VT circuit.

Dog model of acute post-MI arrhythmias

- 2-stage occlusion of mid-LAD: initial partial occlusion followed 30' later by full occlusion.\(^1\)
  - Spontaneous NSVT and AIVR 8 hrs – 5 days post-MI.
  - Inducible VT in 74-92% 1 week post-MI, decreasing to 41-67% at 2 weeks, 36-55% at 4 weeks.\(^2-3\)
- VT circuit:
  - Reentrant with spatial and temporal excitable gap\(^4\)
  - Located in surviving rim of subepicardial tissue\(^5\)
  - Multiple VT morphologies per animal (27% have multiple distinct circuits, all have multiple exit sites from a single circuit)\(^6\)

Subacute dog model: pathology

- **E** = epicardium
- **I** = infarct zone: necrotic at 5d, fatty replacement at 16m
- Not shown: endocardial fibrosis, 2-4 cell layers of surviving subendocardium

Subacute dog model: cellular EP

- **Action potential characteristics:**\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>RMP (mV)</th>
<th>V(_{\text{max}}) (V/s)</th>
<th>APA (mV)</th>
<th>APD50 (ms)</th>
<th>APD90 (ms)</th>
</tr>
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<tbody>
<tr>
<td>normal</td>
<td>-91</td>
<td>102</td>
<td>103</td>
<td>175</td>
<td>210</td>
</tr>
<tr>
<td>5 d</td>
<td>-77</td>
<td>73</td>
<td>86</td>
<td>120</td>
<td>170</td>
</tr>
<tr>
<td>2 wk</td>
<td>-88</td>
<td>104</td>
<td>101</td>
<td>192</td>
<td>115</td>
</tr>
<tr>
<td>&gt; 2m</td>
<td>-86</td>
<td>101</td>
<td>98</td>
<td>160</td>
<td>205</td>
</tr>
</tbody>
</table>

- **Tissue conduction properties:**
  - Decreased space constant in borderzone.\(^2\)
  - Decreased conduction velocity.\(^3\)
  - Central common pathway: decreased longitudinal and transverse CV
  - Outer pathway: decreased transverse CV

References:
**Subacute dog model: cellular EP**

- **Ion channels and connexins:**
  - **General borderzone cells:**
    - 3-5 days post-MI: decreased and lateralized Cx43; decreased $I_{Na}$, $I_{Ca,L}$, $I_{Ks}$, $I_{Kr}$; $I_K$ normalized and $I_{Cal}$ still reduced on day 60.
  - Mapped VT circuit:
    - Central common pathway: decreased $I_{Na}$, $I_{Ca,L}$, no change in gap junctional conductance.
    - Outer pathway: decreased $I_{Na}$, $I_{Ca,L}$, reduced side=side gap junctional conductance, no change in end=end.

**Subacute dog model: novel therapies**

- **Rotagaptide**
  - Alters Cx43 phosphorylation to preserve gap junctional conductance during ischemia
  - 3-hour Rotagaptide infusion increases connexin protein levels but has no effect on CV or VT inducibility in the 5d post-MI dog
- **Sodium channel gene therapy**
  - SNC4a (SkM1) active at less polarized membrane potential
  - Injection of AdSkM1 improves CV, decreases electrogram fractionation, reduces VT inducibility.
- **Connexin32 gene therapy**
  - Cx32 remains open at lower pH
  - Injection of AdCx32 improves CV, decreases electrogram fractionation, changes VT from polymorphic to monomorphic morphology.

**Subacute Dog model**

- **Model strengths and weaknesses:**
  - Extensive investigation of cellular EP through healing.
  - More limited investigation of VT circuit-specific changes.
  - Abundant, prominent collaterals
  - Epicardial VT circuit (prominent $I_N$, decreased Cx43).
  - Atypical scar healing with loss of VT inducibility and loss of some cellular EP changes.

- **Summary of findings:**
  - Spontaneous and inducible arrhythmias
  - Favorable conditions for reentry:
    - Reduced cellular excitability and connectivity.
    - Decreased refractory period.

**Chronic Pig model of post-MI arrhythmias**

- **EP study/barb2right/sacrifice**
- **Optical mapping and patch clamp analysis**
- **Pigs weighing 25-35kg**
- Weekly non-invasive EPS and Echo
  - Intrastimuli pacing (up to S4, BCL 250, 300, 350ms)
  - Burst pacing (CL 240-200ms)
- **gene transfer post-MI week 4**
  - Simultaneous infusion of AD into LAD/GCV
  - Invasive EP study
- **Week 4-12 termination study**
  - EP study → sacrifice
  - Optical mapping and patch clamp analysis
Chronic Pig model: infarct pathology

Pathology

(A) endocardial fibrosis
(B) spared subendocardium
(C) barrier infarct
(D) patchy fibrosis

Chronic Pig model: tissue/cellular EP

<table>
<thead>
<tr>
<th></th>
<th>RMP (mV)</th>
<th>APA (mV)</th>
<th>APD90 (ms)</th>
<th>CV (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>-73 ± 3</td>
<td>135 ± 3</td>
<td>275 ± 61</td>
<td>58 ± 22</td>
</tr>
<tr>
<td>infarct-anterior septal BZ</td>
<td>-67 ± 11</td>
<td>119 ± 11</td>
<td>340 ± 136</td>
<td>31 ± 11</td>
</tr>
<tr>
<td>infarct-lateral BZ</td>
<td>-67 ± 2</td>
<td>115 ± 10</td>
<td>311 ± 120</td>
<td>38 ± 16</td>
</tr>
</tbody>
</table>

Cx43, cadherin-merge
25% of Cx43 localizes to intercalated disk

100 µm

Chronic Pig model: EP study

- Spontaneous NSVT 1-2 days post-MI
- Sudden death in 1-2% over first 2 weeks

3D activation/entrainment mapping

Pig model: VT circuit

VT * Entrainment Pacing


Chronic Pig model: gene therapies

- Hypothesis: disrupting reentry will prevent sustained VT
  - KCNH2-G628S: dominant negative $I_{Kr}$ mutation
  - Connexin43: principle ventricular gap junction protein

Chronic Pig model: KCNH2-G628S

- Reduction in VT inducibility
- $p < 0.01$, $p < 0.05$


Chronic Pig model: connexin43

- VT inducibility decrease

Greener J Am Coll Cardiol 2012; in press.

Chronic Pig model

- Model strengths and weaknesses:
  - Pathology very similar to human. Cellular EP changes, VT circuits mirror some human data
  - Unique Purkinje fiber anatomy. VF induced more frequently than in other species.

- Summary of findings:
  - Low level spontaneous arrhythmias, Very reliable VT inducibility
  - Persistent EP changes that favor reentry:
    - Impaired RMP, APA
    - Heterogeneous repolarization
    - Increased $I_{Ca,L}$, decreased $I_{Kr}$, variable $I_{Ks}$, decreased/lateralized Cx43
  - VT can be eliminated by interventions that prevent reentry
Take-home lessons

- Limited information in humans demonstrates reentrant VT mechanism with altered cellular connectivity, excitability and repolarization.
- Animal models supplement human data with caveat that models are not perfect reflection of human situation
  - Alterations in connectivity from connexin remodeling and fibrosis
  - Alterations in excitability and repolarization from sodium, calcium and potassium current alterations.
- Interventions to disrupt reentry prevent sustained VT

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