Exploring Gene and Cell Therapies for Cardiac Arrhythmias

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The Heart as a Pump: The Circulatory System

Red is oxygenated blood
Blue is deoxygenated blood

- Right atrium
- Right ventricle
- Left atrium
- Left ventricle
- Superior vena cava
- Inferior vena cava
- Pulmonary circulation
- Systemic circulation
- Aorta
- Arteries
- Arterioles and capillaries
- Veins, venules, and venous sinuses
Cation Distribution in Excitable Cells

- **Na⁺**: 142 mEq/L (+61 mV)
- **K⁺**: 4 mEq/L (-94 mV)

Right side:

- **K⁺**: 140 mEq/L (-86 mV)
Ventricular Muscle Action Potential

- $E_m$: mV
- $I_{Na}$
- $I_{CaL}$
- $I_K$
- $I_{K1}$

1. Upstroke
2. Plateau
3. $I_f$

Resting: (200 msec)
Nerve (0.3 msec)
Heart Cells Are Electrically Connected Through Pores Called Gap Junctions

From en.Wikipedia.org/cardiac muscle

From en.Wikipedia.org/gap junction
The Heart as an Electrical Organ: The EKG

adapted from Netter, CIBA Collection, 1969
Considerations for Cardiac Disease

• Loss of muscle cells and pumping force
  – Heart attack
  – Heart failure

• Disruption of electrical signal through heart
  – As a result of muscle cell death (i.e. heart attack)
  – Independent of muscle cell death (conduction disease)

• Pharmacological Therapies
  – To improve cardiac pumping force (can’t replace dead cells)
  – To prevent rhythm disorders (may be pro-arrhythmic)

• Device Therapies
  – Ventricle assist device
  – Implantable defibrillators to stop dangerously fast heart rate
  – Electronic pacemakers to treat slow heart rate (palliative)
Gene and Cell Therapy for Cardiac Disease

• Gene therapy
  – Restore/replicate normal function by introduction of selective genes into existing heart cells within a defined region

• Cell therapy
  – Implant stem cells that have been grown and/or engineered to function as a replacement for heart cells within a defined region
Rationale for Gene/Cell

• “Heart doesn’t regenerate”, but…..
  – Can be some regeneration, but low rate (~1%/yr)
  – May be possible to stimulate regeneration
  – This remains a possible alternative to cell therapy
  – Beyond the scope of this talk

• Drugs: have limitations and side effects
  – Particularly true in the case of arrhythmia therapy

• Device: have complications and limitations
  – But are constantly improving
The Heart as a Pump:
Clinical Trials of Stem Cell Therapy

• Demonstrated procedures are safe
  – Didn’t occlude vessels or lead to rhythm disturbances
• Initial studies showed modest but measureable improvement of function (ejection fraction)
• Is result significant and sustained?
• Is there improved outcome?
• What is the mechanism?
  – Cell survival/integration?
  – Paracrine effect?
  – Will normal conduction properties be established?
Review of Repair Trials

- First clinical trial in 2002
- Most use bone marrow derived cells from the same patient
- Cell have been prepared in various ways

Literature Review and Analysis: Fisher et al, 2015

Key results: In this updated systematic review we analysed data from a total of 41 trials with over 2700 patients. Evaluation of the currently available evidence indicates that this treatment may not lead to improvement when compared to standard treatment, as measured by the frequency of deaths, heart attacks and/or heart failure requiring re-hospitalisation following treatment, as well as tests of heart function, in the short and long term.

Quality of evidence for primary outcomes: The evidence in this review is of moderate quality due to the small number of events.
The Heart as an Electrical Organ: Proof of Concept of Gene and Cell Therapy

REENTRY ARRHYTHMIAS

• What are reentry arrhythmias?
  – Involves region of slow conduction
  – Includes uni-directional block
  – Anti-arrhythmic drugs further impair conduction, causing bi-directional block to disrupt circuit
  – These drugs may also impair conduction in healthy regions

• How might gene therapy work in this case?
  – Add new ion channels to replace malfunctioning ones
  – Use alternative forms of channels to compensate for altered substrate (e.g. a depolarized setting)
A Simplified Explanation of Reentry Arrhythmia

A region of depressed conduction leads to uni-directional block and reentry

Drugs further depress conduction, leading to bi-directional block
## Major Gene Therapy Vehicles Used in Cardiovascular Applications

### A

- **Adenovirus**: 80 nm in diameter
- **AAV**: 80 nm in diameter
- **Lentivirus**: 80 nm in diameter

### B

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<thead>
<tr>
<th></th>
<th>Adenovirus</th>
<th>AAV</th>
<th>Lentivirus</th>
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<tbody>
<tr>
<td>Viral Genome</td>
<td>dsDNA</td>
<td>ss or ds DNA</td>
<td>RNA</td>
</tr>
<tr>
<td>Cloning capacity</td>
<td>7.9kb</td>
<td>&lt;5.0 kb</td>
<td>8.0 kb</td>
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<tr>
<td>Vector genome</td>
<td>episomal</td>
<td>~90% episomal &amp;</td>
<td>Integrated</td>
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<td></td>
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<td>~10 intergrated</td>
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<td>Major area of</td>
<td>Short term gene expression &amp; proof-of-principle studies</td>
<td>Long term gene expression of small genes</td>
<td>Long term gene expression of small to large genes &amp; ex vivo modification of stem cells</td>
</tr>
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<td>application</td>
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*Boink et al, JCPT 19:426, 2014*
Na Channel Schematic and Gating

Wakeling et al, INTECH Open Science, 2012
SkM1 (Nav1.4), Like Nav1.1, is Less Inactivated at Diastolic Potentials than Cardiac Isoform

Should result in greater current in diastole and hyperpolarization of action potential threshold

Na Channel Expression in Depolarized Cells

Protas et al, Cardiovasc Res 81:528, 2009
Effect of Gene Therapy on Conduction Properties Post Myocardial Infarction

QRS Duration During Normal and Premature Stimulation

Stimulation from para-septal site

Stimulation from EBZ site

Boink et al, Circ Arrhythm Electroph 5:831, 2012
Effect of Gene Therapy on Conduction Properties Post Myocardial Infarction

**Incidence and Phenotype of Induced VT**

*Boink et al, Circ Arrhythm Electroph 5:831, 2012*
Effect of Gene Therapy on Conduction Properties Post Myocardial Infarction

*Incidence and Phenotype of Induced VT*

The Heart as an Electrical Organ: 
*Proof of Concept of Gene and Cell Therapy*

**BRADYARRHYTHMIAS**

- Why replace electronic pacemakers?
- How does the native cardiac pacemaker work?
  - Normal initiation/propagation from SAN
  - Role of HCN channels
- Does HCN expression create an adequate biological pacemaker?
- How does the complexity of the native pacemaker suggest additional gene/cell therapeutic approaches?
  - Ca handling and AC1
  - Na channels
Maintaining the Heartbeat Electronically

**Disadvantages of Electronic Pacemakers**

- Poor rate responsiveness
- Implant site limitation
- Battery life; lead fracture
- Interference by various devices

*Image from ctvstexas.com website*
Electronic Pacemakers can Interact with other Devices

The Zapper: an alternative medicine treatment used to “eliminate cancer, other chronic illnesses, self-diagnosed parasites and germs”.

New England Journal of Medicine

Volume 350(16) 15 April 2004 pp 1688-1690

Hazards of an Alternative Medicine Device in a Patient with a Pacemaker
[Correspondence]

Furrer, Marcel; Naegeli, Barbara; Bertel, Osmund

The New York Times

Health

In a High-Tech World, Pacemaker Risks Rise

By ANAHAD O’CONNOR
Published: April 20, 2004
The Heart as an Electrical Organ: The EKG

adapted from Netter, CIBA Collection, 1969
Ion Channels and the Sinus Node Action Potential


Robinson & DiFrancesco, In: Foundations of Cardiac Arrhythmias, Marcel Dekker, 2000
Na Channel Schematic and Gating

Wakeling et al, INTECH Open Science, 2012
Voltage Clamp
Theory and Practice

\[ V = IR \quad \text{ohm’s law} \]
\[ I = gV \quad (g = 1/R) \]
\[ I = g(V, t)(V - V_{\text{rev}}) \]

\[ V \]

Yobas, J Micromech & Microeng 23(8), 2013
The Sinus Node Cell

Provided by DiFrancesco
Pacemaking and the HCN Gene

DiFrancesco et al, J Physiol 377:61, 1986

Rationale of HCN Based Therapeutic Pacemaker

- Depolarizing current flows upon hyperpolarization, at the end of the action potential
- Current flow terminates quickly on depolarization to minimize changes in action potential duration, which can be arrhythmogenic
- Autonomic responsiveness is intrinsic to the channel due to presence of cyclic nucleotide binding domain
HCN2 Overexpression Enhances Pacemaking Activity In Newborn Rat Ventricle Cell Culture

Native $I_f$

Overexpressed $I_{HCN2}$

Time (msec)

Pacemaker Current (pA/pF)

Qu et al, Circ Res 89:e8, 2001
Stem Cells That Could Be Used in Cardiac Cell Therapy

Embryonic stem cells driven down cardiac lineage
- Political issues in terms of availability
- Potentially neoplastic
- Require immunosuppression therapy
- Progress needed to isolate specific subtypes of cardiac-like cells

Induced pluripotent stem cells driven down cardiac lineage
- Can be produced from hair or skin of patient (no immune issue)
- Such a “designer” approach is likely to be expensive
- May be less effective when obtained from older patients

Adult mesenchymal stem cells
- Not excitable on their own, but channel genes can be expressed
- Can be modified for different purposes by expressing different proteins
- Readily available from bone marrow
- Evidence suggests they are immune privileged (no immunosuppression)
Types of Therapeutic Pacemaker

Gene-Based Therapy - **Virus**

Cell-Based Therapy – **ESC-CM**

Cell-Based Therapy - **MSC**

Tandem electronic biological pacemaking
Gene-Based Tandem Pacemaking

Biological pacing                   Electronic pacing                   Biological pacing

adapted from Bucchi et al, Circulation 114:992, 2006
Myocyte – hMSC Coupling

Valiunas et al, J Physiol 587:5211, 2009
hMSC-HCN2 Implant Functionality

adapted from Plotnikov et al, Circulation 116:706, 2007
$I_f$ traces in SAN cells

Adult

Newborn

250 pA

100 ms

Baruscotti et al, J Physiol 492:21, 1996
Effect of TTX on newborn SAN cell

Nav1.1 in situ hybridization

Baruscotti et al, J Physiol 492:21, 1996
SkM1 (Nav1.4), Like Nav1.1, is Less Inactivated at Diastolic Potentials than Cardiac Isoform

*Should result in greater current in diastole and hyperpolarization of action potential threshold*

HCN2+SkM1 Pacemaker In Vivo

Adapted from Boink et al J Am Coll Cardiol 61:1192, 2013
HCN2/SkM1- Based Therapeutic Pacemaker

- Desirable basal beating rate ~ 80 bpm
- Robust rate acceleration to ~ 130 bpm
- Complete elimination of dependence on electronic back-up
- Function critically depends on:
  1. A more negative AP threshold
  2. Injection into the LBB
Summary

• Proof of concept has been achieved for both gene and cell based HCN therapeutic pacemakers

• HCN2 delivers acceptable but not ideal physiological rates with autonomic responsiveness

• HCN mutations impact outcome but ideal in vivo treatment not yet found

• HCN combined with independent contributors derived from studies of SAN (AC1, SkM1) enhance outcome in vivo, particularly with SkM1

• Other issues remain (safety, persistence, delivery)
Remaining Issues

- Gene based
  - Safety and persistence of viral vector
- Cell based
  - ESC: neoplasia, differentiation, immune response
  - MSC: survival, migration, differentiation
- Either
  - Appropriate promoter and channel gene(s)
  - Catheter delivery system and dosage control
- Need for AV synchronization
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