AMLODIPINE THERAPY FOR IRON-OVERLOAD CARDIOMYOPATHY: THE ENDURING VALUE OF TRANSLATIONAL RESEARCH

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Amlodipine Therapy for Iron-overload Cardiomyopathy: 
*The Enduring Value of Translational Research*

Heart Failure Update, Toronto
Evolving Concepts in Heart Failure

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1. Describe the pathophysiology of iron-overload cardiomyopathy

2. Discuss the suitability of amlodipine as a therapy for this condition

3. Review existing screening and diagnostic methods for iron-overload
Iron deficiency affects almost 15% of the global population and ~ one billion people suffer from iron-deficiency anemia.

Evolution of Conservation.....Lack of an Iron Excretory Pathway

Relative Increase of Iron-Overload relative to Iron-Deficiency
World-wide distribution of iron-overload

Iron-overload

Genetic causes: Mutations in Hfe, HJV, Fpn etc.
(increased gut absorption of iron)

Non-Genetic causes: Hemoglobinopathies; MDS
(use of blood transfusions)
Causes of Mortality and Morbidity

Primary Hemochromatosis  
(less aggressive; ~50-60 yrs)  
Secondary Iron-overload  
(more aggressive; ~20-30 yrs)

1. Liver Disease  
2. Cardiac Disease  
3. Endocrinopathies  
4. Infections

Reducing Incidence of Liver Disease (Cirrhosis and Hepatocellular Carcinoma) because of decreasing rates of Hepatitis C

Screening for Hemochromatosis:

1. Serum ferritin > 200 μg/L in premenopausal women; > 300 μg/L in men and postmenopausal women
2. Fasting transferrin saturation > 45% to 50% (values greater than 60% in men and 50% in women are highly specific).
3. Genetic Testing often needed
Late Cardiac Complications of Chronic, Severe, Refractory Anemia with Hemochromatosis

By Mary Allen Engle, M.D., Marion Erlanson, M.D., and Carl H. Smith, M.D.

Circulation 1964

n=41 patients
Secondary Iron-Overload and Human Survival


Heart disease is the most important prognostic factor in determining survival
Mode of Entry of Fe into Cells

**Transferrin-Dependent** – via transferrin receptor; negative feedback; evolved later

Appearance of NTBI (Non-transferrin Bound Iron) in plasma with iron-overload conditions estimated to be 1-10 uM in patients with iron-overload

NTBI is reactive and under positive feedback

*NTBI quantitatively more important in Iron-Overload Condition*

**Transferrin-Independent (NTBI)** – via L-type Ca²⁺ Channels

Cardiomyopathy/Endocrinopathies (EC/ES coupling)
Modulation of Iron Uptake in Heart by L-Type Ca\(^{2+}\) Channel Modifiers

Possible Implications in Iron Overload

Robert G. Tsushima, Alan D. Wickenden, Ron A. Bouchard, Gavin Y. Oudit,
Peter P. Liu, Peter H. Backx

Abstract—Heart failure is the leading cause of mortality in patients with transfusional iron (Fe) overload in which myocardial iron uptake ensues via a transferrin-independent process. We examined the ability of L-type Ca\(^{2+}\) channel modifiers to alter Fe\(^{3+}\) uptake by isolated rat hearts and ventricular myocytes. Perfusion of rat hearts with 100 mmol/L \(^{59}\)Fe\(^{3+}\) and 5 mmol/L ascorbate resulted in specific \(^{59}\)Fe\(^{3+}\) uptake of 20.4±1.9 ng of Fe per gram dry wt. Abolishing myocardial electrical excitability with 20 mmol/L KCl reduced specific \(^{59}\)Fe\(^{3+}\) uptake by 60±7% (P<0.01), which suggested that a component of myocardial Fe\(^{3+}\) uptake depends on membrane voltage. Accordingly, \(^{59}\)Fe\(^{3+}\) uptake was inhibited by 10 μmol/L nifedipine (45±12%, P<0.02) and 100 μmol/L Cd\(^{2+}\) (86±3%; P<0.001) while being augmented by 100 μmol/L Bay K 8644 (61±15%, P<0.01) or 100 μmol/L isoproterenol (40±12%, P<0.05). By contrast, uptake of 100 μmol/L ferric iron (\(^{59}\)Fe\(^{3+}\)) was significantly lower (1.4±0.3 ng Fe per gram dry wt; P<0.001) compared with divalent iron. These data suggest that a component of Fe\(^{3+}\) uptake into heart occurs via the L-type Ca\(^{2+}\) channel in myocytes. To investigate this further, the effects of Fe\(^{3+}\) on cardiac myocyte L-type Ca\(^{2+}\) currents were measured. In the absence of Ca\(^{2+}\), nonactivating nifedipine-sensitive Fe\(^{3+}\) currents were recorded with 15 mmol/L [Fe\(^{2+}\)]\(_{o}\). Low concentrations of Fe\(^{3+}\) enhanced Ca\(^{2+}\) current amplitude and slowed inactivation rates, which was consistent with Fe\(^{3+}\) entry into the cell, whereas higher Fe\(^{3+}\) levels caused dose-dependent decreases in peak current. Fe\(^{3+}\) had no effect on current amplitude or decay. Combined, our data suggest that myocardial Fe\(^{3+}\) uptake occurs via L-type Ca\(^{2+}\) channels and that blockade of these channels might be useful in the treatment of patients with excessive serum iron levels. (Circ Res. 1999;84:1302-1309.)

Key Words: iron overload ■ channels ■ heart failure ■ permeability ■ Ca\(^{2+}\)
Excitation-Contraction Coupling in Cardiomyocyte

Cardiac L-type Ca$^{2+}$ channel (α-subunit)

Analytical Electron Microscopy

STEM Image

Digital Map

Energy Spectrum
L-type Calcium Channel Dependent Fe Uptake

Myocardial Fe level (µmol/g dry wt)

- Plac + Vehicle
- Fe + Vehicle
- Fe + Verapamil
- Fe + Amlodipine

- SUBACUTE
- CHRONIC

- * Significant difference
- ** Highly significant difference
Prussian Blue Staining (Iron)

- Placebo+Vehicle (0.007±0.009%)
- Iron+Vehicle (9.52±1.15%)
- Iron+Verapamil (4.98±0.76%)
- Iron+Amlodipine (5.12±0.56%)
Enhanced Iron Uptake with LTCC Overexpression

Intracellular Prussian Blue (%)

- **Plac+Vehicle**
- **Iron+Vehicle**

**CONTROL**

**TRANSGENIC**
L-type Ca$^{2+}$ channels provide a major pathway for iron entry into cardiomyocytes in iron-overload cardiomyopathy

Gavin Y Oudit$^{1,4}$, Hui Sun$^{1}$, Maria G Trivic$^{1}$, Sheryl E Koch$^{2}$, Fayez Dawood$^{1}$, Cameron Ackerley$^{2}$, Mehrdad Yazdanpanah$^{1}$, Greg J Wilson$^{2}$, Arnold Schwartz$^{3}$, Peter P Liu$^{1,4}$ & Peter H Backx$^{1,4}$

Ca$^{2+}$ channel blockers reverse iron overload by a new mechanism via divalent metal transporter-1

Susanne Ludwiczek$^{2}$, Igor Theurl$^{1}$, Martina U Muckenhalter$^{2}$, Martin Jakab$^{3,4}$, Sabine M Mair$^{1}$, Milan Theurl$^{1}$, Judit Kiss$^{2}$, Markus Paulmichl$^{3,5}$, Matthias W Hentze$^{6}$, Markus Ritter$^{3,4}$ & Guenter Weiss$^{1}$
Patients were randomized to receive amlodipine (5 mg/d) for 12 mths in a 1:2 allocation (n=10 control; n=5 amlod)

**Amlodipine Reduces Cardiac Iron Overload in Patients with Thalassemia Major: A Pilot Trial**

Juliano Lara Fernandes, MD, PhD, Erika Fontana Sampaio, MD, Kleber Fertrin, MD, PhD, Otavio Rizzi Coelho, MD, PhD, Sandra Loggetto, MD, Antonio Piga, MD, Monica Veríssimo, MD, Sara T. Saad, MD, PhD

*Internal Medicine Department, University of Campinas (Unicamp), Brazil; Centro de Hematologia São Paulo, Brazil; University of Turin, Italy; Centro Infantil Boldrini, Brazil.*
In a multicenter, double-blind, randomized, placebo-controlled trial, 62 patients were allocated to receive oral amlodipine 5 mg/day or placebo in addition to their current chelation regimen.

Precision-based Therapy safe in children
Effect of Amlodipine on the Survival of Patients With Severe Chronic Heart Failure Due to a Nonischemic Cardiomyopathy

Results of the PRAISE-2 Study (Prospective Randomized Amlodipine Survival Evaluation 2)

Milton Packer, MD, Peter Carson, MD, Uri Elkayam, MD, Marvin A. Konstam, MD, Gordon Moe, MD, Christopher O’Connor, MD, Jean-Lucien Rouleau, MD, Douglas Schocken, MD, Susan A. Anderson, MS, David L. DeMets, PhD, for the PRAISE-2 Study Group

Dallas, Texas

Conclusions

These results of the current trial, viewed together with the results from the earlier study, indicate that amlodipine does not exert favorable effects on the clinical course of patients with heart failure, regardless of the presence or absence of underlying coronary artery disease. These findings indicate the need for great caution when striking benefits are observed in subgroups of patients or in trials not primarily designed to assess such effects.

(J Am Coll Cardiol HF 2013;1:308-14) © 2013 by the American College of Cardiology Foundation
Resveratrol

- A natural polyphenol with potent signaling and antioxidant effects: SIRT1 activation deacetylates class III histones.
- Chemical Name: 3,5,4’-trihydroxy stilbene.
Active component analysis

- SERCA2a adenoviral gene therapy in EARLY Iron-overload

AAV9-SERCA2a
HELP: Human Explanted Heart Program

Explanted Heart (Adult (n=154)/Pediatric (n=31))/Healthy Donor (n=28) Heart

ABACUS Cardiovascular Science Integrated Research Core Laboratory

- Atrial/Ventricular Tissue Retrieval and Storage
- Isolation/Culture of Cardiomyocytes and Fibroblasts
- Pericardium/Valve Isolation
- Coronary Isolation and Study
- VFibrillation Optical Mapping
- Histology

- “Healthy” Donor Hearts for Research (HOPE Program)
- LVAD Apical Core Samples (n=90)
Iron-Overload Cardiomyopathy (Human)

non-failing control (NFC)  iron-overload cardiomyopathy (IOC)

Ca^{2+} cycling and metabolism

control  IOC

SERCa2a  
NCX1  
p-AMPK  t-AMPK  
p-AKT^{Thr308}  t-AKT
CONCLUSIONS

1. Iron-overload CM is prevalent and a major determinant of morbidity and mortality at a world-wide level

2. Aged HJVKO murine model is a valid pre-clinical model of iron-overload: no anemia!

3. Current Therapies
   a. Iron-chelation       b. Phlebotomy

4. Experimental Therapies
   a. Amlodipine/CCB (in clinical trials) (prevention of iron entry into the heart)
   b. Resveratrol/SIRT1 activators (protects tissue from iron-induced injury)
      > 89 ongoing clinical trials
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