# Bolus Injection of Acetylcholine Terminates Atrial Fibrillation in Rats

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*Background.* The usefulness of the currently existing approaches to treat atrial fibrillation (AF) is limited because of their relatively low effectiveness and/or potential for adverse effects. We tested the hypothesis that uniform, transient activation of muscarinic $K^+$ channels throughout the atria could destabilize and terminate the arrhythmia thereby turning the heart into the sinus rhythm.

*Aim.* To explore the effectiveness of rapidly hydrolysable cholinergic agonists for AF termination.

*Methods.* Sustained AF episodes were elicited in anesthetized Wistar rats by programmed electrical stimulation via transesophageal catheter. Rats were randomly and blindly assigned with a model drug, acetylcholine (ACh, n=17), or saline injection (n=15) either via the tail vein or into the right ventricular cavity, three minutes after the AF initiation.

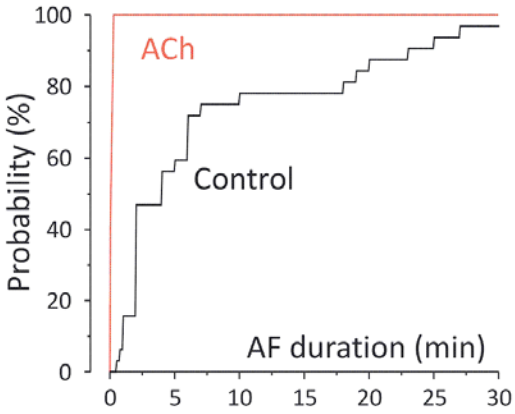


**Figure 1.** Probability density plot of AF episode duration in control (black) and following intravenous ACh administration (red).

*Results.* In all rats tested, AF was successfully converted into sinus rhythms by intravenous ACh injection, while injections of the same quantities of saline had no effect whatsoever. AF episodes were terminated almost immediately (within 8.4 ± 1.9 seconds, Fig. 1, red) following ACh administration, while the episodes in untreated AF were significantly longer (average 516 ± 132 seconds, $p < 0.0001$). The termination of AF episode was always accompanied with transient bradycardia; the sinus rhythm gradually accelerated and reached its pre-AF values within 10-20 seconds following the injection. Similar results, but with shorter recovery of sinus rhythm, were obtained with intracardiac ACh delivery (n=7).

*Conclusions.* These experiments provide first evidence that bolus administration of rapidly hydrolysable muscarinic agonist could be an effective way to pharmacologically terminate atrial fibrillation and restore sinus rhythm.

# ZnT-1, a Novel Modulator of Cardiac L-type Calcium Channels; Insights into the Molecular Mechanism

Shiri Kolt [1], Ofer Beharier [1], Liat Buzaglo [1], Lior Shaltiel [1], Daniel Gitler [1], Levi Gaber [4], Yoram Etzion [2], Amos Katz [2,3], Arie Moran [1]

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**BACKGROUND**: L-type calcium channels (LTCC), the main route of calcium entry into cardiomyocytes, are involved in various aspects of cardiac function. Modulations of LTCC activity are observed in various cardiac pathologies such as ischemia\reperfusion, cardiac hypertrophy and atrial fibrillation. We recently demonstrated that ZnT-1, a membrane protein that inhibits the LTCC without altering its expression, exists in the heart and is increased in the rat atria following acute rapid pacing as well as in the atria of AF patients. In this study we explored the molecular mechanism of ZnT-1 activity, especially in regard to possible interactions with the regulatory β-subunit of the LTCC. **METHODS AND RESULTS**: ZnT-1 induced inhibition of the LTCC was tested in HEK 293 cells and *Xenopus* oocytes. In the absent of the β-subunit ZnT-1 did not inhibit the LTCC current in *Xenopus* oocytes. Direct interaction between ZnT-1 and the LTCC β- subunit was demonstrated by co-immunoprecipitation of ZnT-1-myc and β-subunit using anti- β-subunit and anti- myc antibodies. Furthermore, Fluorescent Resonance Energy Transfer (FRET) was demonstrated in cells co-expressing $\beta_{2a}$:CFP and ZnT-1:YFP indicating molecular-range proximity between these proteins *in-situ*. In addition, changes in the cellular distribution of the ZnT-1 in cells co-expressing the β-subunit with ZnT-1:YFP were demonstrated by Total Internal Reflection Fluorescence measurements. **CONCLUTION**: The interaction between the LTCC β-subunit and ZnT-1 is an essential component in the mechanism underlying the ZnT-1 induced inhibition of the LTCC. This mechanism can serve as an important drug target for modulation of LTCC function in the diseased myocardium.

# CRP Accelerates Thrombosis by Suppressing COX-2 expression and Activity

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**Background**: C-reactive protein (CRP) is a mediator of increased thrombogenicity and thus an increased risk of vascular disease. The prostanoids, thromboxane (TXA2) and prostacyclin (PGI2) play key, yet opposing, roles in vascular homeostasis; hence, alterations or imbalances of these two prostanoids levels, are implicated as mediators of various CV diseases. The present study examined the effects of CRP on the cyclooxygenase (COX) mediated pathways in transgenic mice that express human CRP (CRPtg).

**Methods**: CRPtg and littermate C57/BL mice were subjected to femoral artery wire injury. The expression of key genes in the prostanoid pathway was measured by real time PCR and Western Blot in injured arteries and in lung tissue, at baseline, 6hr, and 24hr after injury (n=5-7/group).

**Results:** COX-2, prostacyclin synthase and prostacyclin receptor after vascular injury were significantly reduced in CRPtg while thromboxane synthase and thromboxane receptor were significantly augmented. Immunohistochemcial staining confirmed the reduced expression of COX-2 and the elevated thromboxane receptor expression in the injured arteries of CRPtg. Urinary prostacyclin metabolites were significantly reduced in CRPtg as compared with wildtypes. Aspirin therapy (30 mg/kg/day) reversed the prothrombotic effect of CRP as measured by reduced carotid thrombosis following photochemical injury and prostanoid pathway gene expression after femoral wire injury.

**Conclusions:** In mice transgenic for human CRP, arachidonic-acid cyclooxygenase pathways are modulated towards suppressed prostacyclin expression and increased thromboxane activity. These effects may promote thrombosis in response to injury and may provide rationalization for the increased incidence of vascular events that is associated with high CRP levels.

# In Vivo Engraftment of Tissue-Engineered Human Vascularized Cardiac Muscle

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Myocardial cell-replacement and tissue-engineering strategies are hampered by the lack of sources for human cardiomyocytes and by significant donor cell loss following transplantation. As a possible solution to these obstacles, we assessed the ability of 3D tissue-engineered human, vascularized, cardiac-muscle to engraft in the *in-vivo* rat heart and to promote functional vascularization.

Human embryonic stem cell-derived cardiomyocytes, alone(C), or in combination with human vascular precursor cells and embryonic fibroblasts (CHM), were seeded on degradable biopolymer-scaffolds. Synchronously contracting cardiac tissue-constructs were formed *in-vitro* that contained a dense vessel-network (CHM group). Grafting of the engineered tissue in the rat heart resulted in the formation of long-term stable grafts, showing cardiomyocyte structural maturation. Electromechanical integration between donor and host tissues was suggested by Cx43 immunostaining and electrical recordings. The formation of human and rat-derived vasculature within the scaffold was confirmed by immunostaining for SMA and human-specific-CD31. Intraventricular injection of fluorescent microspheres and lectin resulted in their incorporation by blood vessels within the scaffolds, confirming their functional perfusion capabilities. Finally, the number of vessel lumens per $mm^2$ was significantly greater in the CHM-containing scaffolds ($57\pm7$, $p<0.05$) when compared to those containing cardiomyocytes alone ($37\pm5$).

**Conclusions**: (1) Tissue-engineered human cardiac muscle, containing a dense vascular network, can be established *ex-vivo* and grafted *in-vivo* to form stable, integrated, cell-grafts. (2) The transplanted tissue-constructs showed significant vascularization, consisting of both pre-existing human- and newly-formed rat vessels. (3) The pre-existing human vessels increased scaffold vascularity and also became functional by integrating with host rat vascular network.

# Isl1 Gene Therapy – Triggering Endothelial Cells' Angiogenic Properties in a Direct and Paracrine Manner

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The LIM-homeobox transcription factor islet-1 (isl1) plays a key role in the specification of myocardial, pacemaker, endothelial, and smooth muscle cells, which are derived from the secondary heart field during heart embryogenesis. Moreover, Isl1+ precursors have the potential of self renewal and differentiation into endothelial, cardiomyocyte and smooth muscle lineages.

We investigated whether retroviral gene delivery of isl1 to endothelial cells (ECs), could promote angiogenic properties of transduced and wild type (WT) ECs.

Murine ECs were transduced to express isl1. transduced Cells' Proliferative capacity was assessed by thymidine incorporation assay and propidium iodide staining. Adhesion to fibronectin, and to monocytes was also examined. Cell based-ELISA was established to evaluate VCAM-1 and ICAM-1 expression. Angiogenesis–related cytokine secretion of transduced cells was detected using cytokine arrays. Paracrine effect on WT ECs migration and vasculogenic activity was evaluated using a Boyden chamber and tube formation on Matrigel, respectively. Eventually, the contribution of Isl1 to ECs-induced vessel formation was studied by a Matrigel plug *in vivo* assay in mice.

Isl1 expression resulted in enhanced proliferation, adhesion to fibronectin and monocytes. In addition, increased IL-1β and VEGF secretion was evident, which translated to a promoting paracrine effect on WT ECs migration and tube formation. Finally, Isl1 expressing ECs induced enhanced *in vivo* vascularization in mice, evident by immunohistochemistry.

These data suggest that isl1 cell based gene therapy approach may have a considerable therapeutic potential in promoting angiogenesis by triggering EC intrinsic proangiogenic functional properties, as well as by endowing paracrine amplification on angiogenesis.

# Percutaneous Anterior Leaflet Augmentation - a Novel Approach to Mitral Valve Repair in Ischemic Mitral Regurgitation

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**BACKGROUND:** Ischemic mitral regurgitation (IMR) is a common complex and poorly understood clinical entity, associated with poor long-term survival. Numerous surgical techniques have been developed for IMR, but none has resulted in clearly improved patient outcome. Additionally co-morbidities often associated with ageing and age itself is independent risk factors for adverse outcome after surgery. Percutaneous techniques to treat MR can reduce surgical risk and can be categorized to a) mitral annulus reshaping techniques, and b) leaflet edge-to-edge repair. We report a novel percutaneous technique and initial preclinical experience of mitral valve repair with anterior leaflet augmentation.

**METHODS AND RESULTS:** the novel percutaneous approach is based on the understanding that anterior leaflet augmentation allows relief of leaflet tethering and excellent leaflet coaptation. The procedure was tested ex vivo in three pig hearts. Mitral valve incompetence was achieved by posterior leaflet chordal shortening. The central portion of the anterior leaflet was augmented using balloon inflation and implantation of a 0.9 cm balloon-deliverable disc in the area created by balloon inflation. A flow system was used to test the presence of mitral regurgitation. In all three experiments, morphological augmentation was achieved and no signs of mitral valve incompetence (leak) were present. The concept soon will be tested in-vivo in a sheep model of Ischemic Mitral Regurgitation.

**CONCLUSIONS:** Novel percutaneous anterior leaflet augmentation for ischemic MR was feasible and resolved mitral regurgitation in ex vivo model. This novel method may be a viable option for patients with ischemic MR.