16:00 - 17:30 S21 - Basic Science Hall F

Chairs: **C. Danenberg**
M. Flugelman

16:00 **Prevention of Fatal Arrhythmia in a Catecholaminergic Polymorphic Ventricular Tachycardia Mouse Model Carrying Calsequestrin2 Mutation**

R. Alcalai [1,2], H. Wakimoto [2], M. Arad [3], L. Song [2], J. Seidman [2], C. Seidman [2]
[1] Jerusalem, [2] Boston, [3] Ramat Gan

16:15 **ZnT-1, a Novel Regulator of T- type Calcium Channels Mediating a Crosstalk Between T-type and L-type Calcium Channels.**

M. Mor [1], O. Beharier [1], Y. Etzion [2], S. Levi [1], A. Katz [2,3], A. Moran [1]
[2] Beer Sheva, [3] Ashkelon

16:30 **The Role of Sympathetic Activity in the Pause-Dependant Onset of Atrial Arrhythmia**

M. Swissa [1], S. Zhou [2], S. Lin [3], P. Chen [3], L.S. Chen [4]
[1] Rehovot , Jerusalem, [2] Los Angeles, [4] Indianapolis

16:45 **Expression of CCR3 Receptor in Patients with Acute Coronary Syndromes**

T. Greener, M. Sofia, E. Michal, G. Keren, J. George
Tel-aviv

17:00 **Improved Cardiac Electrical Stability in Exercised Myocardial Infarct Rats with Left Ventricular Hypertrophy**

H. Dor-Haim [1], C. Lotan [1], M. Horowitz [2], M. Swissa [3]
[2] Jerusalem, [3] Rehovot

17:15 **Phosphate is a Major Mediator of Aortic Valve Calcification**

S. Abedat, M. Shuvy, R. Beeri, C. Lotan
Jerusalem

Prevention of Fatal Arrhythmia in a Catecholaminergic Polymorphic Ventricular Tachycardia Mouse Model Carrying Calsequestrin2 Mutation

Ronny Alcalai [1,2], Hiroko Wakimoto [2], Michael Arad [3], Lei Song [2], Jon Seidman [2], Christine Seidman [2]

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Background Catecholamine-induced polymorphic ventricular tachycardia (CPVT) is a familial syndrome caused by mutations in the ryanodine receptor-2(RyR2) or the calsequestrin-2(CASQ2) genes and characterized by sudden death induced by exercise or emotional stress. Treatment of CPVT patients is limited given that beta-adrenergic blockers were found to be only partially effective and no other agents were broadly tested. Recent studies have shown that CPVT is mediated by increased Ca^{2+} leak through the RyR2 channel. Our aim was to determine whether agents that may inhibit intracellular Ca^{2+} leak can effectively prevent CPVT. **Methods** The efficacy of Ca^{2+} channel blockers, beta-adrenergic blockers and Mg^{2+} were tested using a CPVT mouse model carrying mutation in CASQ2 gene. We assessed the in-vivo prevalence of stress induced arrhythmia at baseline and after short and long-term drug treatment and the drug effect on contractility and Ca^{2+} transient of isolated cardiomyocytes. **Results** All study drugs reduced the frequency of stress induced ventricular arrhythmia in mutant mice. Nevertheless, only Verapamil completely prevented arrhythmia in 80% of the mice. Cardiomyocytes studies indicated that both Mg^{2+} and Verapamil inhibited sarcomere contraction, shortened the Ca^{2+} reuptake period and prolonged the caffeine induced Ca^{2+} transients of mutant cardiomyocytes. Diastolic Ca^{2+} overload and Ca^{2+} oscillations that typically present in stressed mutant myocytes were partially prevented by Mg^{2+} and more effectively by Verapamil. **Conclusion** Verapamil is the most effective agent in preventing ventricular arrhythmia in CPVT mouse model and in modifying the intracellular abnormal calcium handling of mutant cardiomyocytes, probably by inhibiting the intracellular Ca^{2+} leak. Calcium antagonists might have therapeutic value in CPVT and other RyR2 mediated arrhythmias and should be tested in human studies.

ZnT-1, a Novel Regulator of T- type Calcium Channels Mediating a Crosstalk Between T-type and L-type Calcium Channels.

Merav Mor [1], Ofer Beharier [1], Yoram Etzion [2], Shiri Levi [1], Amos Katz [2,3], Arie Moran [1]

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BACKGROUND: ZnT-1 is a transmembrane protein that was studied mainly in the context of zinc metabolism. New findings mark ZnT-1 as an inhibitor of L-type Calcium Channels (LTCC) and as a potent activator of Raf-1 in the Ras-ERK signaling pathway. Recently, we demonstrated that ZnT-1 inhibits the LTCC by direct binding to the LTCC β-subunit. In addition, we found that ZnT-1 expression in the heart is modulated by electrical pacing and ischemia\reperfusion. In the present study we explore the regulatory effects of ZnT-1 on the activity of T-type Calcium Channels (TTCC), which are known to co express with the LTCC in various cells including cardiomyocytes. **METHODS AND RESULTS:** Voltage clamp recordings in *Xenopus* oocytes revealed that ZnT-1 enhances the TTCC current (167.95±9.27 % of control, n=30, $p<0.005$). Biotinylation experiments indicated that ZnT-1 increases the surface expression of the TTCC (457.94±85.8 % of control, n=3, $p<0.005$). Overexpression of inactive Raf-1 abolish the augmentation of the TTCC current by ZnT-1 (103±4.1 % of control, n=25, $p=0.37$). In addition, we found that the ZnT-1 augmentation of the TTCC is inhibited by the expression of LTCC β-subunit. Finely, co-expression of LTCC, TTCC and ZnT-1 led to preferential inhibition of the LTCC with no effect on the TTCC. **CONCLUSION**: ZnT-1 inversely regulates the activity of TTCC and LTCC. ZnT-1 induced augmentation of TTCC activity involves activation ERK-MAPK and increased TTCC surface expression. The interaction of the LTCC β-subunit with ZnT-1 leads to a crosstalk between LTCC and TTCC. These findings suggest a key role for ZnT-1 as a regulator of cardiomyocytes excitability and calcium homeostasis.

The Role of Sympathetic Activity in the Pause-Dependant Onset of Atrial Arrhythmia

Moshe Swissa [1], Shengmei Zhou [2], Shien-Fong Lin [3], Peng-Sheng Chen [3], Lan S Chen [4]

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Background: Recent studies showed that late phase 3 early afterdepolarization (EAD) is a novel mechanism of atrial arrhythmogenesis. To induce late phase 3 EAD in vitro, long pauses and additional sympathetic stimulation are required. We sought to determine if the same sequence of events precede paroxysmal atrial tachycardias (PATs) in ambulatory dogs.

Methods: We created myocardial infarction, complete atrioventricular block and infused nerve growth factor into the left stellate ganglion (LSG) in 8 dogs. In addition, we performed simultaneous continuous (24/7) long-term recording of left stellate ganglion (LSG) nerve activity and electrocardiogram using a radiotransmitter manufactured by Data Sciences International. One pair of electrodes was used to record stellate ganglion nerve activity from the lower portion of LSG. Another pair of widely spaced bipolar electrodes was implanted in subcutaneous tissue for surface ECG recording. PAT was defined as an abrupt increase of atrial rate to more than 3 times of baseline, or greater than 200 bpm.

Results: The simultaneous recording lasted 55±40 days. PATs were recorded in all 8 dogs (mean of 7.8±5.7 episodes/dog/day). A characteristic pattern of onset of PAT was observed in 64% of the episodes. This pattern includes profound sinus bradycardia or sinus arrest for > 3 s followed by an abrupt increase of sympathetic discharge (figure1). The average rate of atrial arrhythmia was 196.2±21.7 bpm. The average of minimal atrial rate before the atrial arrhythmia initiation was 36.8±17.5 bpm.

Conclusions: Spontaneous onset of PAT in this canine model is characterized by a long pause followed by bursts of sympathetic discharges. These patterns are consistent with the induction of arrhythmia by late phase 3 EAD.

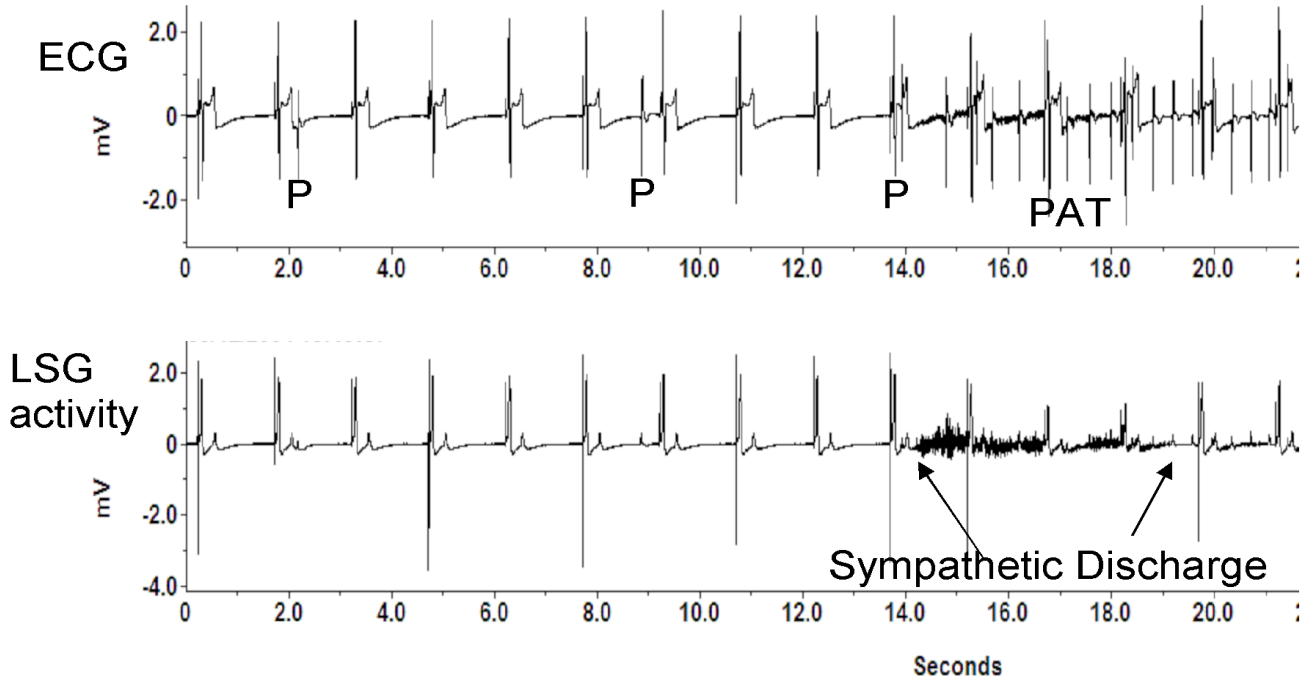

Figure 1: Profound sinus bradycardia followed by bursts of sympathetic discharge leading to PAT of 190 bpm.

Expression of CCR3 Receptor in Patients with Acute Coronary Syndromes

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OBJECTIVE: Chemokines and their receptors play an important role in atherosclerosis. CCL11 (Eotaxin) is a potent eosinophil chemoattractant that is present in atheromatous plaques. The major receptor for CCL11 is CCR3, which is found on leukocytes and on some nonleukocytic cells. In this study, we aimed to evaluate the expression of the receptor CCR3 in ACS patients compared to patients without ischemic heart disease (IHD) and the association of CCR3 levels with the extent of angiographically coronary artery disease (CAD).**METHODS AND RESULTS**: We examined 50 patients that presented with ACS and that had coronary angiography during their admission. CCR3 expression in peripheral blood mononuclear cell were significantly reduced in patients with ACS compared to patients without IHD [0.6(0.4-0.7) versus 1.2(0.6-0.15), respectively; $P<0.001$]. We did not observe a correlation between levels of CCR3 receptor and the extent of CAD. **CONCLUSIONS:** This is a first report showing that CCR3 levels are reduced during cardiac ischemia. This may point to the involvement of eotaxin and its receptor in the pathophysiology of the plaque rupture and its potential role as marker of coronary ischemia.

Improved Cardiac Electrical Stability in Exercised Myocardial Infarct Rats with Left Ventricular Hypertrophy

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Background: Aerobic training reduces the occurrence of sudden cardiac death, in patients with CHD. We hypothesized that prolonged training will alter heart substrate for arrhythmia, thus increase electrophysiological stability in myocardial infarct (MI) heart model.
Methods: Adult rats (n=30) were studied for 8 weeks. The first group (n=6) underwent LAD ligation to form MI and then trained on a treadmill for 8 weeks (TMI). A second intact group (n=8) was trained, as well, for 8 weeks (ITT). A third group (n=8) underwent LAD ligation was investigated under sedentary conditions (SMI) and a forth sham operated group (n=16) was served as sedentary control (SCN). Eventually, EPS study was performed on the isolated Langendorff perfusion system.
Results: TMI Isolated trained hearts showed 3-fold improvement in their tolerance to the induction of ventricular fibrillation (VF) in comparison to SMI. ITT, TMI and SMI hearts were significantly hypertrophied compared to SCN (15%, 18% and 20% increase respectively $P<0.01$). Effective refractory period (ERP) was significantly longer in SMI (39% increase $p<0.001$), however was normal in TMI. Aerobic training has also improved in vivo stress test measurements and ECG parameters (QTC and HRV) of TMI in comparison to SMI.
Conclusion: This study demonstrates the electrophysiological protective effect of aerobic training on MI heart model. Training has normalized the MI adverse effect on cardiac electrophysiology, despite ventricular hyperthrophy and structural remodeling of the heart. The improvement may be related to enhanced cardiac conductance and improved refractoriness induced by aerobic training.

Phosphate is a Major Mediator of Aortic Valve Calcification

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Background: Calcific aortic valve stenosis is a common disease in the elderly. The molecular and cellular mechanisms leading to this disorder remain unclear because of the absence of appropriate experimental models. In patients undergoing dialysis, aortic valve calcification (AVC) is frequently found and progress rapidly. We have previously demonstrated osteoblast transformation in a rat model of uremia.

In the present study, we explored the molecular mechanism of the differentiation of valve interstitial cells (ICs) into osteoblasts.

Methods: Primary cultures of rat aortic valve ICs were treated with phosphate (7mM) and {phosphate (7mM) +Foscarnet (0.5mM)}. Osteopontin (a major osteoblast protein) and RUNX-2 (transcription factor regulating osteoblast differentiation and function) expression was analyzed by real time PCR and western blot after 14 days. Mineralization of the cells was analyzed by Von Kossa staining.

Results: Phosphate augmented RUNX-2 and osteopontin expression after 14 days. Intense mineralization was noted (by Von Kossa staining). Foscarnet (Phosphate receptor inhibitror) reduced Osteopontin and Runx-2 expression after 14 days and there were no findings of any mineralized nodules.

Conclusions: Our findings suggest that phosphate is a major determinant of the differentiation of valve interstitial cells into osteoblasts. Additional studies are required in order to investigate the cellular and molecular mechanism involving AVC *in vivo*.