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Clopidogrel Inhibitory Effect with Omeprazole, Pantoprazole, or Famotidine- A Crossover Study

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Background: Concerns have been raised about the potential for Proton-pump inhibitors (PPIs) to blunt the efficacy of clopidogrel. Since clopidogrel's effect is influenced by the genetic activity of the CYP450 system, there is a need to examine the effect of PPI on clopidogrel resistance after cancelling genetic differences. We assessed the effects of 2 different PPI and one H2 blocker on platelet reactivity in patients treated with aspirin and clopidogrel in a crossover trial where each patient was treated with all 3 anti-acid regimens.
Methods: Patients treated with aspirin and clopidogrel were assigned to receive 3 consecutive treatment periods of one month each, in which they were treated with one of the 3 study medications. The medications tested were: Omeprazole (20mg*2/d), Famotidine (40mg*2/d) and Pantoprazole (20mg*2/d). At the end of each phase, platelet function was evaluated using the Verify Now system.
Results: Regardless of the cutoff used to define resistance, patients on Omeprazole were significantly more resistant to clopidogrel compared to famotidine or pantoprazole, respectively (48%, 33% and 31% when using the 208 PRU cutoff, p=0.04) and (37%, 17% and 23% when using the 230 PRU cutoff, p=0.003). These changes represent up to 100% increase in the number of resistant patients to clopidogrel when comparing Omeprazole to other anti acid regimens.
Conclusions: We show, for the first time, that after cancelling genetic bias that might have existed in other trials, Omeprazole is significantly associated with more clopidogrel resistance compared to other anti-acid regimens.

The Immediate Antiplatelet Effect of Prasugrel Versus Clopidogrel in STEMI Patients

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Background: Prior small retrospective studies have suggested clinical benefits of clopidogrel pre-treatment in STEMI patients undergoing PPCI. However, the anti-platelet effect of both clopidogrel and prasugrel during the narrow door-to-balloon period has not been evaluated. Our aim was to evaluate the immediate anti-platelet effect of thienopyridine pre-treatment in ST-segment elevation myocardial infarction (STEMI) patients undergoing primary percutaneous coronary intervention (PPCI).
Methods: Sixty STEMI patients undergoing PPCI were prospectively evaluated. Patients were treated with 600mg clopidogrel (N=45) or 60 mg prasugrel (N=15) loading upon admission. ADP-induced platelet aggregation (PA) was determined by light transmission aggregometry prior to thienopyridine loading, at PPCI and after 72 hours. TIMI flow prior to PPCI along with TIMI myocardial perfusion (TMP), and TIMI frame count (TFC) immediately post PPCI were determined.
Results: The two study groups were similar regarding baseline characteristics including door-to-balloon time which was 48±23 vs. 46±16 minutes (p=0.7) for the clopidogrel and the prasugrel groups respectively. Pre-loading ADP-induced PA was comparable (79±10 VS. 79±8, P=0.8), but was reduced by clopidogrel to 73±15% vs. 63±16% with prasugrel (p<0.01) at PPCI. ADP-induced PA was further reduced by clopidogrel to 47±18% vs. 27±14% with prasugrel (p<0.001) after 72 hours. Patients treated with prasugrel had better TMP (2.75±0.9 vs. 1.63±1.4, p=0.013) and better TFR count (9.8±4.1 vs. 14±7, p=0.027).
Conclusions: In STEMI patients undergoing PPCI, prasugrel compared to clopidogrel pre-treatment was associated with a more rapid and potent reduction in ADP-induced PA both at PPCI and after 72 hours. Patients treated with prasugrel had better TMP than those treated with clopidogrel suggesting better myocardial re-perfusion at PPCI.

MiRNA 106b~25 is Essential for Functional Recovery in Limb Ischemia Model by Regulating Angiogenesis

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MicroRNAs are small non-coding RNAs that regulate a wide range of physiological and pathophysiological processes by post transcriptional gene silencing. microRNA-106b~25 cluster is highly expressed in endothelial cells and adult stem cells, but it's function is not fully understood.
In the current study we investigated the effect of miR-106b~25 deletion on blood flow, using a mouse model of hind limb ischemia. In addition, we isolated bone-marrow derived stem cells from miR-106b~25 knockout mice and examined their ability to expand in-vitro, their angiogenic capacity in Matrigel tube formation assay, as well as their apoptotic response following exposure to H2O2.
We observed an up-regulation of the 106b~25 cluster following limb ischemia as evident by Real-time PCR. Interestingly, 106b~25 knockout mice had reduced blood flow compared to the wild type controls, measured by Laser-Doppler imaging. Furthermore, partial salvage of the mutant phenotype was achieved by gene delivery of miR-106b~25 to ischemic muscles. In addition, bone-marrow derived stem cells isolated from 106b~25 KO mice had reduced proliferation rate and the cells were 3-fold more sensitive to H2O2-induced apoptosis. Finally, KO bone-marrow cell culture exhibited a reduced number of Sca-1 positive cells as compared to WT cells.
In summary, we have demonstrated that the 106b~25 microRNA cluster is essential for functional recovery of ischemic muscles in mice. A few possible mechanisms were identified including augmentation of post-ischemic vascularization, reduction in apoptosis levels and regulation of bone-marrow derived Sca-1 positive cells.
miR-106b~25 gene therapy may thus stand as a novel target for future gene based therapies for regeneration of ischemic tissues.

Coupling of Transgene-Free Heart Failure-Induced Pluripotent Stem Cells with Host Cardiomyocytes

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Myocardial cell replacement therapies are hampered by the paucity of sources for human cardiomyocytes and by the expected immune rejection of allogeneic cell-grafts. Induced pluripotent stem cells (iPSCs) derived from somatic cells of patients represent a powerful tool for biomedical research and may provide a solution to these challenges.
In the current study, we aimed to derive transgene-free human iPSCs (hiPSC) from patients with advanced heart failure (HF), to induce their cardiomyocyte differentiation, and to evaluate their ability to integrate with pre-existing cardiac tissue both in-vitro and in-vivo.
Dermal fibroblasts from HF patient were reprogrammed into hiPSCs with a unique excisable single polycistronic lentiviral vector that contained four reprogramming factors (Oct4, Sox2, Klf4 and c-Myc). Subsequently, the vector was excised by the transient introduction of Cre-recombinase and eGFP, followed by a FACS sorting. The generated factor-free HF-hiPSCs were then coaxed to differentiate into cardiomyocytes. Gene expression and immunostainings confirmed their pluripotency and cardiomyocyte phenotype. Next, functional integration and synchronized electrical activities were demonstrated between HF-hiPSC derived cardiomyocytes and neonatal rat cardiomyocytes in co-culture studies. Finally, in-vivo transplantation studies in the rat heart revealed the ability of the HF-hiPSCs derived cardiomyocytes to engraft, survive, and structurally integrate with host cardiomyocytes.
In conclusion, our study demonstrates efficient reprogramming of human cells while avoiding the permanent presence of reprogramming transgenes. This represents a critical step toward the use of HF-hiPSCs for clinical purposes. Moreover, the capability of HF-hiPSC derived cardiomyocytes to couple with host cardiomyocytes both in-vitro and in-vivo is crucial for future use of these cells not only for the treatment of heart failure but also for conduction system repair (biological pacemaker approach).

Evidence for Heart Regeneration after Injury in Neonatal Mice

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Background: The human heart muscle has long been considered a terminally-differentiated organ which is unable to regenerate. Recent evidence has challenged this notion, suggesting that the heart regenerates cardiomyocytes following injury, but that this capacity is limited to a short period of time after birth. We aimed to determine whether and how neonatal mouse heart regenerates after myocardial injury.
Methods and Results: One-day-old ICR mice were anaesthetized, the chest was opened to expose the heart, and the apex was resected using iridectomy scissors. Mice were sacrificed 3 days after the operation, and the hearts were harvested, processed, immunostained and compared with normal neonatal and infarcted adult hearts. Histological and immunohistochemical examination of injured neonatal hearts revealed inflammation, granulation tissue formation, and early regeneration at the injured sites. Dedifferentiation of cardiomyocytes, represented by sarcomeric disassembly and marginalization, was evident around the injured areas. In addition, we noticed proliferation of double nuclei cardiomyocytes. The proliferating cardiomyocytes infiltrated the granulation tissue and formed a new myocardium. Interestingly, macrophage collections were found at the border zone of the regenerating myocardium.
Conclusions: Our preliminary findings suggest cardiomyocyte dedifferentiation, proliferation and regeneration in injured heart of neonatal mouse. Furthermore, the increased number of local macrophages, combined with their growth-related effects, suggests a possible active role in the regenerative process. This model could be used to study the mechanism of myocardial regeneration or repair, and to develop new regenerative therapies.

Atrial Appendages Harbor a Vast and Diverse Population of Cardiac Progenitor Cells

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Background: The regenerative capability of the adult heart has gained evidence, but the mechanism remains unknown. Cardiac progenitor cells (CPCs) are a heterogenic population and are thought to be concentrated in certain areas (e.g. ventricular apex). We hypothesized that the atrial appendages, being from an earlier stage of embryological development than the rest of the heart, contain a large amount of CPCs.
Methods: Immunostaining was performed on the frozen left atrial appendage (LAA) sections. LAAs from adult mice were harvested and digestion of explants was performed with three different concentrations of enzymes. Differentiation was induced through Dexamethasone or 5-Azacytidine. Cells were analyzed with FACS and immunostaining.
Results: The LAA contained a high number of c-Kit+ cells, from which >60% were Nkx2.5+. In culture, tissue from both appendages had an equal growth potential, but the ventricular apex showed only minimal cell growth (40x lower than the appendages, *figure*). Two distinct progenitor cell populations grew depending on the strength of enzymatic digestion: Type A, which was c-Kit+ and CD45- and Type B, which was c-Kit+ and CD45+. Nkx2.5 and GATA-4 were positive in both cell populations. Sca-1 expression was abundant in Type A cell populations (~90%), in contrast to Type B populations. Both CPC types were found from the apex-derived cells as well. Differentiation was induced to the Type A population using Dexamethasone, with organized expression of sarcomeric proteins and atrial natriuretic factor. Type B population was induced to differentiation with 5 –Azacytidine. Possibility of a mast cell contamination was ruled out by RT-PCR.
Conclusion: The atrial appendages contained multiple types and a higher concentration of CPCs than the left ventricle. Different enzymatic digestion method caused major differences in the resulting CPC population. The progenitor cells differentiated into cardiomyocytes with different mechanisms depending on their cell type.