Introduction: β-adrenergic system is altered in heart failure (HF) due to non-ischemic dilated cardiomyopathy (DCM). There are few data concerning the relative contribution of β1- and β3-adrenoceptor subtypes (β1- and β3-AR) during DCM development. We evaluated the expression and the role of each β-AR subtype in this pathology.

Methods: DCM rat model is performed by doxorubicin injections (cumulative dose: 15 mg.kg⁻¹) and validated by in vivo measurements with echocardiography-doppler. The variations of β1- and β3-AR transcript expression in left ventricle (LV) are evaluated by real-time RT-PCR. The ex vivo cardiac responses induced by selective β1-AR or non-selective β-AR stimulations are evaluated on isolated perfused heart.

Results: DCM rats present LV dilation, systolic and diastolic dysfunction (see table). Compared to controls, β1-AR transcripts and β3-AR transcripts are increased in DCM LV (+36%, n=8, p<0.05 and +358 %, n=8, p<0.05). Ex vivo parameters are summarized in the table.

Table: Basal parameters and maximum values obtained by non-selective β1-AR stimulation (isoproterenol) or selective β1-AR stimulation (SR58611A). Results are expressed by mean ± SEM:* p<0.001 vs Control, † p<0.001 vs Basal.

<table>
<thead>
<tr>
<th>Control rats (n=9-31)</th>
<th>DCM rats (n=9 in E2, 3 in E2-β3-AR KO mice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV end-diastolic diameter (mm)</td>
<td>8.82±0.33 vs 7.61±0.17 *</td>
</tr>
<tr>
<td>LV ejection fraction (%)</td>
<td>83±1.9 vs 71.2±2.8 *</td>
</tr>
<tr>
<td>LV Isovolumic relaxation time (ms)</td>
<td>21.41±1.33 vs 32.39±1.08 *</td>
</tr>
<tr>
<td>Basal ex vivo</td>
<td></td>
</tr>
<tr>
<td>DP/dt max (mmHg.s⁻¹)</td>
<td>2035±365 vs 2669±504*</td>
</tr>
<tr>
<td>DP/dt min (mmHg.s⁻¹)</td>
<td>1258±226 vs 1847±349*</td>
</tr>
<tr>
<td>Isoproterenol ex vivo (1 μM)</td>
<td></td>
</tr>
<tr>
<td>DP/dt max (mmHg.s⁻¹)</td>
<td>5263±1754 vs 4373±1383*</td>
</tr>
<tr>
<td>DP/dt min (mmHg.s⁻¹)</td>
<td>3815±1271 vs 3227±1020*</td>
</tr>
<tr>
<td>SRS58611A ex vivo (1 μM)</td>
<td></td>
</tr>
<tr>
<td>DP/dt max (mmHg.s⁻¹)</td>
<td>1722±497 vs 1930±611*</td>
</tr>
<tr>
<td>DP/dt min (mmHg.s⁻¹)</td>
<td>1027±297 vs 1138±360*</td>
</tr>
</tbody>
</table>

Conclusion: DCM induces a β1-AR gene over-expression, associated to an increase of β1-AR-induced negative inotropic and lusitropic effects. These results could partly explain the alteration of isoproterenol response in our model, suggesting that β1-AR could be a new therapeutic target in DCM.

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Endothelial Estrogen Receptor α mediates the atheroprotective action of 17β-Estradiol in LDLr deficient mice

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Background: Although estrogen administration to hysterectomized menopausal women did not prevent the occurrence of myocardial infarction in a randomized controlled trial (WHI 2004), epidemiological studies suggest and experimental results clearly demonstrate a major atheroprotective action of estrogens. The goal of the present study was to identify the cellular target(s) accounting for the estradiol (E2) beneficial action on fatty streak development.

Methods and Results: We first confirmed the key role of estrogen receptor α (ERα) in atheroprotective effect of E2 as this action was completely abolished in mice deficient both in Low Density Lipoprotein receptor (LDLr) and in ERα. Comparison of LDLr−/− mice transplanted with either ERα+/+ or ERα−/− bone marrow showed that functional ERα in the hematopoietic lineage is not required for E2 atheroprotection. We then showed that ERα floxed mice (ERαfloxb/flox) bred with the Tie2-Cre mice on the LDLr−/− background had a complete inactivation of ERα both in bone marrow and in endothelial cells. Remarkably, in this mouse model, the E2 atheroprotective action was completely abolished.

Conclusions: Altogether, this is the first in vivo demonstration that endothelial ERα represents a key target of the atheroprotective effect of E2, whereas the hematopoietic ERα is dispensable for the protective action. Selective estrogen receptor modulators that mimic this endothelial action of E2 should now be considered in hormonal treatment as well as in atheroprotection.

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Estrogen Receptor α expression in both endothelium and hematopoietic cells is required for the atheroprotective effect of estradiol on reendothelialization

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Objectives: Although reducing rates of restenosis, drug-eluting stents also impair endothelial healing, resulting in increased risk of thrombosis. Alternately, inhibition of neointimal hyperplasia is favoured by acceleration of reendothelialization. We previously showed that E2 accelerates reendothelialization through Estrogen Receptor α (ERα) and we now aimed at defining the cellular targets of this action.

Methods and Results: The respective roles of endothelial and hematopoietic cellular targets of E2 were investigated in a mouse carotid injury model, using "en face" confocal microscopy, to follow endothelial repair. Grafting ERα−/− mice with ERα+/+ bone marrow (BM) did not restore the atheroprotective effect of E2 on reendothelialization, demonstrating the necessary role of extrahematopoietic ERs. Using a cell-specific inactivation of ERα (Cre-lox recombination system), we showed that endothelial ERα plays a pivotal role in the E2 action. Finally, in wild type mice grafted with ERα−/−, the regenerative effect of E2 was abolished, demonstrating that ERα-expressing hematopoietic cells are also concomitantly required.

Conclusions: We demonstrate that endothelial ERα plays a pivotal role in E2-mediated reendothelialization. However, endothelial targeting alone is not sufficient and the concomitant stimulation of BM ERα is absolutely required. This cooperation should be now taken into account in strategies aimed at optimizing in-stent reendothelialization.

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Zac1, jointly down-regulated by preconditioning and postconditioning in a mouse model of myocardial ischemia/reperfusion: a transcriptomic approach

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Ischemic preconditioning and postconditioning are two effective therapeutic strategies for reducing infarct size in animal models and humans. The aim of our study was to compare the early regulated genes of preconditioning and postconditioning using a transcriptomic approach.

Methods: C57Bl6, Zac1−/− KO (n=7) and WT littermates (n=4) mice underwent an IR (40 min. ischemia/60 min.reperfusion) protocol. C57Bl6 mice were randomly assigned to different groups: IR (n=22); postconditioning (PostC, n=21); a protocol of 3 cycles of 1-minute reperfusion and 1-minute reoxygenation was applied at the onset of reperfusion; preconditioning (PreC, n=15; same algorithm but applied before ischemia. At the end of surgery, left ventricles were assigned to RNA extraction or infarct size assessment. Homemade mouse oligo microarrays were used for gene expression profiling (Montpellier GenomiX Facilities). Determination of area at risk (AR) and infarct size was assessed by TTC staining and planimetry.

Results: Our study revealed that despite a similar cardioprotection offered by PreC and PostC on infarct size, PostC regulates a larger number of genes compared to PreC (242 versus 40). Only 8 genes were jointly regulated by PreC and PostC and considered as putative cardioprotective key regulators. Among these candidates, Zac1 was down regulated at the transcriptional levels upon PreC and PostC. Moreover, infarct size/AR was 29%-decreased in Zac1−/− KO mice subjected to a surgical protocol of myocardial IR.

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Conclusion: Using pangenomic microarrays, we identified and compared the gene profiles of preconditioning and postconditioning versus IR in the mouse left ventricle in vivo. Among the genes jointly downregulated by preconditioning and postconditioning, Zac1 was identified as a putative cardio-protective key regulator. Indeed downregulation at the transcriptional and protein levels of Zac1 leads to cardioprotection against IR.

Combination of Sonic Hedgehog and a CXCR4 Antagonist improves functional recovery via an MMP-9-dependent Pathway after Myocardial Infarction

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Background: We have shown that the Sonic Hedgehog (Shh) embryonic signalling pathway can be reactivated in myocardial infarction (MI) in adults inducing expression of angiogenic factors. We hypothesized that combining Shh gene therapy and endothelial BM-derived pro-angiogenic cell mobilization by a CXCR4 antagonist, AMD3100 (AMD), could exert synergistic effects and would be superior to either single strategy for the treatment of MI.

Methods/results: In vivo, MI was induced in WT and GFP-bone marrow (BM) transplanted mice randomly assigned in 4 treatment groups: control; AMD (single dose, 5mg/kg s.c.); Shh (intramyocardial; 100μg Shh plasmid DNA at time of MI surgery); AMD+Shh group. Left ventricular ejection fraction (LVEF) was evaluated by echo up to 4 weeks post MI. AMD+Shh group exhibited the best LV function. Furthermore, combination of AMD with sub-therapeutic dose of Shh resulted in a significant improvement of cardiac function recovery compared to monotherapy, highlighting its synergistic effect (P<0.05). Elastic staining and immunohistological analyses demonstrated reduced infarct size and increased capillary density in the AMD+Shh group (both P<0.05). Combination therapy was also associated with significant increase in number of GFP-BS lectin BM-derived cells incorporated into the ischemic area (P<0.05). We then explored the certain potential mechanisms of the favourable effects of combination therapy. MMP-9 mRNA expression was increased in ischemic myocardium in the AMD+Shh (10-fold versus control). The positive effect on EF of combined treatment was attenuated in MMP-9 KO mice, suggesting that MMP-9 might be a key modulator of the combination therapy.

Conclusion: Pharmacological enhancement of Shh gene therapy via BM-cell mobilization by a CXCR4 antagonist is mediated via an MMP-9-dependent pathway. The combination may offer advantages in safety and feasibility by allowing lower dose gene transfer while improving outcome post-MI.

Methods and Results: Cardiac patch was based on biodegradable polysaccharide porous scaffold. After ligation of the left anterior coronary artery, the fate of 1x106 GFP+ MSC administered either using cellularized scaffold implantation or direct injection was examined at 1 and 2 months. The number of residual GFP+ cells in the sample studied was estimated on the basis of the fluorescence emitted by a defined number of GFP+ cells used for calibration. Cellularized scaffold allowed a more efficient delivery and the difference with direct injection was significant at 2 months, with respectively 2100±1300×103 and 215±85×103 residual GFP+ cells (p<0.03). Cardiac patch delivery of viable and non-viable BM mononuclear cells was achieved. Cellularized scaffold was similar whatever the administration conditions but a slight increase in the local production of vascular endothelial growth factor was observed at 2 months after patch implantation in comparison to direct injection (p<0.05). In animals having received MSC implanted on scaffold, clusters of GFP+ cells, mainly phenotypically consistent with immature MSC cells, were detected in the peri-infarct area. The increased survival using scaffold was not translated in an improved myocardial remodeling and functions with no significant difference in the LVEDD, LVESD, and FS between the 2 groups as in comparison with animals implanted with non cellularized scaffold.

Conclusions: These findings demonstrate that the implantation of cellularized grafts is safe and effective for delivering mesenchymal stem cells into damaged myocardium, and results in a better cellular engraftment compared to direct injection.

A novel polysaccharide-based porous scaffold for cell delivery into the infarcted heart

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Background: Cellular cardiomyoplasty has been proposed as an attractive strategy to repair myocardial damage. One of the crucial point is the optimal delivery strategy. In the present study, we examined the use of an implantable porous scaffold for promoting bone marrow-derived mesenchymal stem cells (MSCs) survival and functions in a rat model of acute myocardial infarction.

<table>
<thead>
<tr>
<th>Scar (mm²)</th>
<th>LVEDD (mm)</th>
<th>LVEDS (mm)</th>
<th>EF%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>6.5 (0.1)</td>
<td>3.8 (0.2)</td>
<td>76 (2.6)</td>
</tr>
<tr>
<td>AMI(1)</td>
<td>79 (4.0)</td>
<td>7.7 (0.2)*</td>
<td>5.7 (0.2)*</td>
</tr>
<tr>
<td>DM-AMI</td>
<td>83 (4.9)</td>
<td>8.5 (0.2)**</td>
<td>7.0 (0.3)**</td>
</tr>
</tbody>
</table>

*P<0.05 vs SHAM, **P<0.05 vs SHAM and AMI(1)

In AMI(1), TRβ1 and TRβ3 protein expression were not changed significantly as compared to SHAM while in DM-AMI, both TRα1 and TRβ1 were decreased 1.7 and 1.9 fold respectively as compared to SHAM, p<0.05. T3 and T4 levels were not different between groups. HYPO-AMI hearts, with scar areas comparable to AMI(2) hearts [97±4.7 vs 105±10 (10.3), p<0.05], showed a similar unfavorable functional response: EF% was found to be markedly reduced [24 (0.9) in HYPO-AMI vs 36.2 (1.0) in AMI(2), p<0.05], while LVEDS was 8.3 (0.2) for HYPO-AMI and 7.5 (0.1) for AMI(2), p<0.05. LVEDD equally increased in the 2 groups. Post ischemic cardiac remodeling is accelerated both in hypertrophic and diabetic hearts. Tissue hypothyroidism which occurs in DM after myocardial infarction, may at least in part, account for this response.

Post-ischemic cardiac remodeling is accelerated in diabetic rats: similarities to clinical and tissue hypothyroidism

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We investigated whether postischemic cardiac remodeling (REM) is accelerated in diabetic rats with possible involvement of thyroid hormone (TH) signaling in this response. Changes in TH signaling occur during REM after acute myocardial infarction (MI) and contribute to cardiac dysfunction.

Diabetes was induced in Wistar rats by streptozotcin injection (35mg/Kg i.p.). After 30 days diabetic rats (DM-AMI, n=9) were subjected to MI while control rats were either sham-operated (SHAM, n=10) or subjected to MI (AMI(1), n=10). After 2 weeks, TRα1 and TRβ1 expression and TH levels were measured. Hypothyroid rats by propyl-thioauracil administration (0.05%) in water for 3 weeks were subjected to MI (HYPO-AMI, n=6) while untreated MI rats served as controls (AMI(2), n=6). LV dimensions (LVEDD and LVEDS) and ejection fraction (EF%) were used to assess contractility and REM 2 weeks after MI using echocardiography. Cardiac function was markedly decreased in DM-AMI.

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