

Supplemental Data

Early and Short-term Triiodothyronine Supplementation Prevents Adverse Postischemic Cardiac Remodeling: Role of Transforming Growth Factor-β and Antifibrotic miRNA Signaling

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SUPPLEMENTARY MATERIALS AND METHODS

Arrhythmia Severity Scoring Rank

The severity score of ventricular arrhythmias was used as index of ischemia damage as previously described (1). The following scoring system was adopted: 0-50 ventricular ectopic beats (VEBs) score 0; 50-500 VEBs score 1; more than 500 VEBs, or one episode of spontaneously reversible ventricular tachycardia (VT) or ventricular fibrillation (VF) score 2; 2-30 episodes of spontaneously reversible VT and/or VF score 3; more than 30 episodes of spontaneously reversible VT and/or VF score 4; irreversible VF was given a score of 5. A score level falling in the rank 3 was adopted as inclusion criteria to ensure comparable I among rats.

1. Kusmic C, et al. (2014) Up-regulation of heme oxygenase-1 after infarct initiation reduces mortality, infarct size and left ventricular remodeling: experimental evidence and proof of concept. *J. Transl. Med.* 12:89.

SUPPLEMENTARY RESULTS

Post Ischemic TH Serum Levels Affect Heart Functional Parameters and TGFβ1 Signaling

To further explore the role of post-ischemic TH levels on TGFβ1 signaling, cardiac functional recovery and disease evolution, a subset of analyses were also performed in I/R-Eu rats and compared

to I/R rats developing the low T3 syndrome (I/R-L group).

As summarized in Table 1, at 3 d post ischemia the I/R-Eu rats exhibited preserved TH levels. Also, in contrast to I/R-L rats, no significant alterations of profibrotic factors and antifibrotic miRNA expression were observed in the I/R-Eu group (Table 1).

At 3 d endpoint, the I/R-Eu rats presented altered end systolic diameter (ESd) and fractional shortening (FS%), similarly

to the I/R-L group, but a better recovery of LV anterior regional contractility (sAWT%) (Fig.1 panels A-C). At 14 d endpoint, chamber geometry as well as global and regional contractility were better preserved in the I/R-Eu rats (Fig.1 panels A-C). These data confirm that in the presence of similar myocardial damage, decreased TH level in the early phase of the post ischemic wound healing affects the tissue repair molecular response and plays a key role in disease progression.

Supplementary Table S1. Serum free thyroid hormone before surgery and 3 d endpoint.

TH levels (pg/ml)	Sham	I/R-Eu	I/R-L
FT3 basal	3.0 ± 0.1	3.3 ± 0.3	2.9 ± 0.2
FT3 final	2.9 ± 0.07	3.5 ± 0.4	2.3 ± 0.05 ^{ab}
FT4 basal	11.1 ± 0.6	13.2 ± 0.6	10.9 ± 0.5
FT4 final	13.2 ± 0.5	15.0 ± 0.6	11.8 ± 0.5

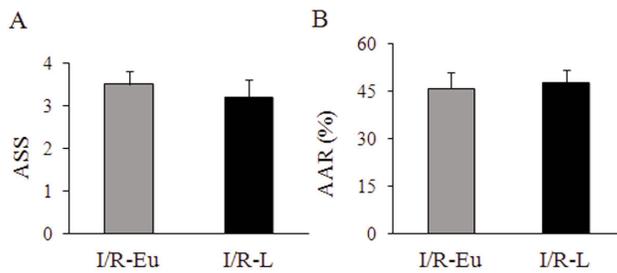
Free T3 (FT3) and free T4 (FT4) were measured before and 3 d post-I/R. Values are mean±SEM; n≥5 in each group; ^ap ≤ 0.01 vs sham basal level and respective basal level; ^bp =0.01 vs I/R-Eu at 3 d endpoint.

Supplementary Table S2. Genes and miRNAs expression in the AAR at 3 d endpoint.

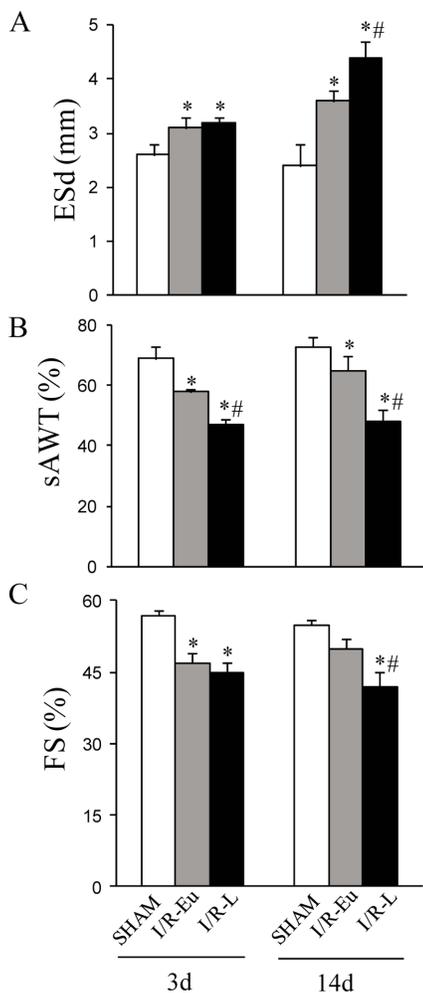
	Sham	I/R-Eu	I/R-L
Genes			
TGFβ1	0.87 ± 0.08	0.90 ± 0.3	3.6 ± 0.3 ^{ab}
CTGF	0.62 ± 0.1	0.80 ± 0.2	2.4 ± 0.1 ^{ab}
MMP2	1.2 ± 0.08	1.0 ± 0.09	5.3 ± 0.4 ^{ab}
miRNAs			
133a	1.0 ± 0.1	0.83 ± 0.2	0.35 ± 0.06 ^{ab}
30c	1.1 ± 0.7	0.82 ± 0.05	0.43 ± 0.08 ^{ab}
29a	0.98 ± 0.04	0.95 ± 0.12	0.55 ± 0.07 ^{ab}

Values are mean±SEM; n≥5 in each group; ^ap ≤ 0.0009 vs sham; ^bp ≤ 0.016 vs I/R-Eu.

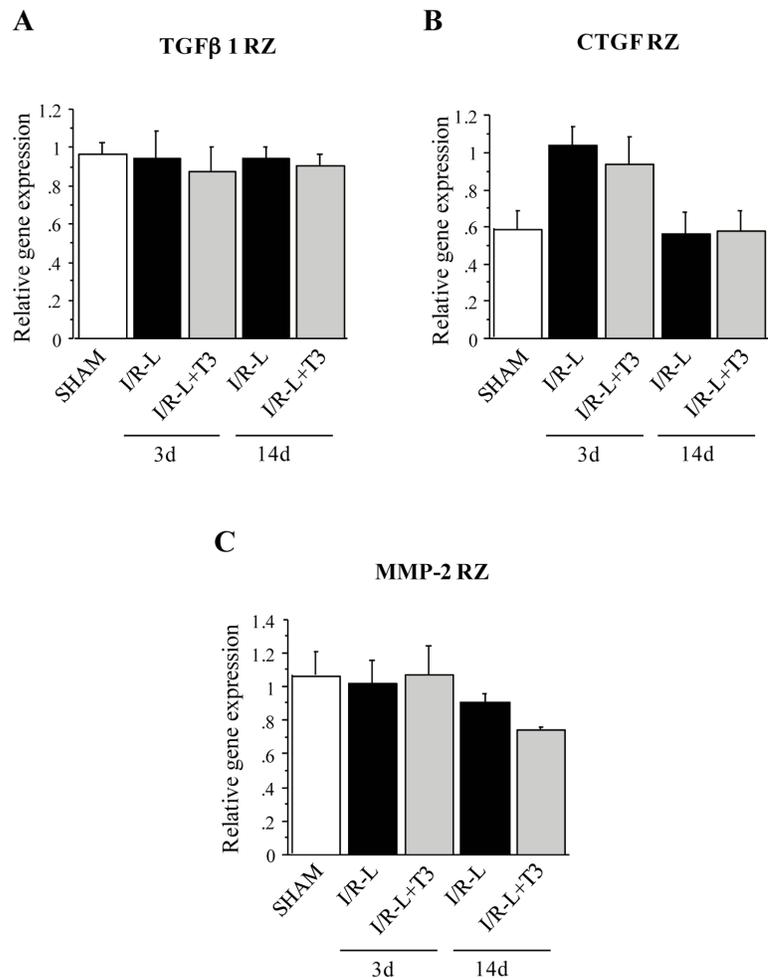
TGFβ1 ANTAGONISM BY T3 IN POST-I/R CARDIOPROTECTION



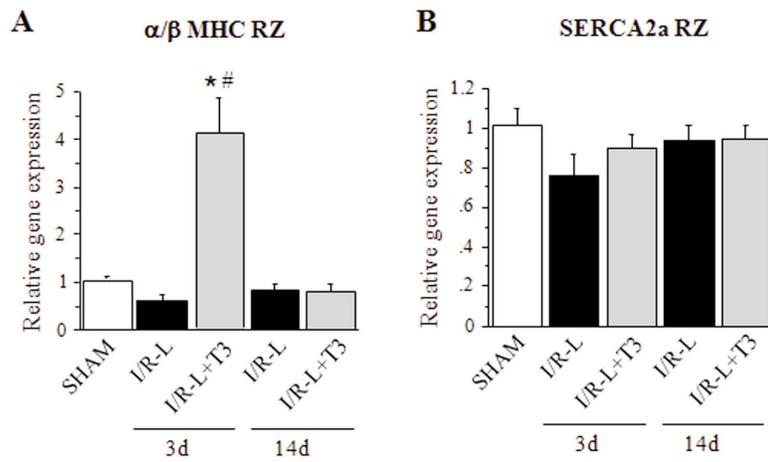
Supplementary Figure S1. Comparable infarction in the two I/R groups was indicated by similar post LAD arrhythmic severity score (ASS, n=18 in each group), (A); and similar LV area at risk (AAR, n=3) measured at 3 d post I/R, (B).



Supplementary Figure S2. Post ischemic T3 levels affect *in vivo* heart functional parameters at 3 d and 14 d endpoints. (A), left ventricular end-systolic diameter (ESd); (B), percent of systolic anterior wall thickening (sAWT%); (C), percent fractional shortening (FS%). Values are mean ± SEM; n≥6 in each group *p<0.05 vs Sham; #p≤0.01 vs I/R-Eu.



Supplementary Figure S3. Effect of T3 treatment on TGFβ1, CTGF and MMP-2 expression in the remote zone. Comparable expression level of TGFβ1 (A), CTGF (B) and MMP-2 (C) among groups were observed at both 3 d and 14 d post I/R. n≥5 in each group.



Supplementary Figure S4. Effect of T3 treatment on alpha/beta MHC ratio and SERCA2a expression in the remote zone. (A), the T3 treatment induced a significant increase of the myosin ratio at 3 d that returned to control levels at 14 d; values comparable to sham were observed in the I/R-L rats at both 3 d and 14 d. (B), comparable expression of SERCA2a was observed in all groups at both 3 d and 14 d. $n \geq 5$ in each group, * $p < 0.0001$ vs sham, # $p \leq 0.003$ vs I/R-L 3 d and 14 d and I/R-L+T3 14 d.