VCAM-1 mediates the protective effect of TNF-α preconditioning against ischemia/reperfusion injury in cultured cardiomyocytes

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Introduction

VCAM-1 is a transmembrane protein expressed in mammals whose expression is induced by TNF-α and a wide variety of pro-inflammatory stimuli in several tissues. Although the role of cardiac VCAM-1 is not fully understood, several studies have shown that its expression is increased in different cardiovascular diseases. Our previous results showed that VCAM-1 is associated with cardiomyocyte survival in a simulated ischemia model

Aim: to evaluate the protective role of VCAM-1 on TNF-α preconditioning against simulated ischemia/reperfusion (sI/R) injury in cardiomyocytes.

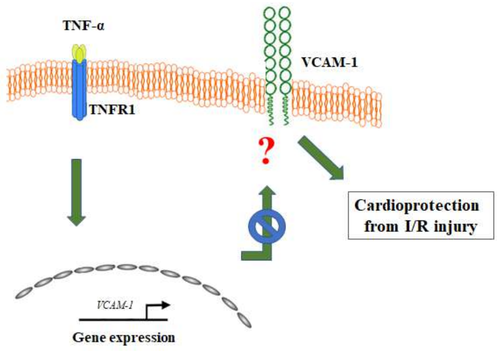

Figure 1. Aim of the study

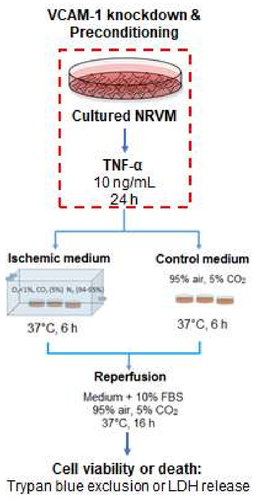

Figure 2. Experimental design

Methods

Cultured neonatal rat ventricular cardiomyocytes were treated with 10 ng/mL TNF-α. Protein and mRNA levels of VCAM-1 were measured by Western blot and RT-qPCR. VCAM-1 knockdown cardiomyocytes were pre-treated with 10 ng/mL TNF-α for 24 h and incubated under ischemic conditions for 6 h. Then, the ischemic medium was replaced by DMEM/M199 containing 10% FBS and cardiomyocytes were exposed to normoxia for 18 h. Cell death was assessed by LDH release at the end of reperfusion. Additionally, pro-survival gene expression was evaluated in VCAM-1 knockdown cardiomyocytes. Data presented as mean ± SEM. One-way or two-way ANOVA with post hoc test was performed as appropriate, p value < 0.05 was considered statistically significant.

Results

Effect of TNF-α on cell viability and VCAM-1 expression in cardiomyocytes

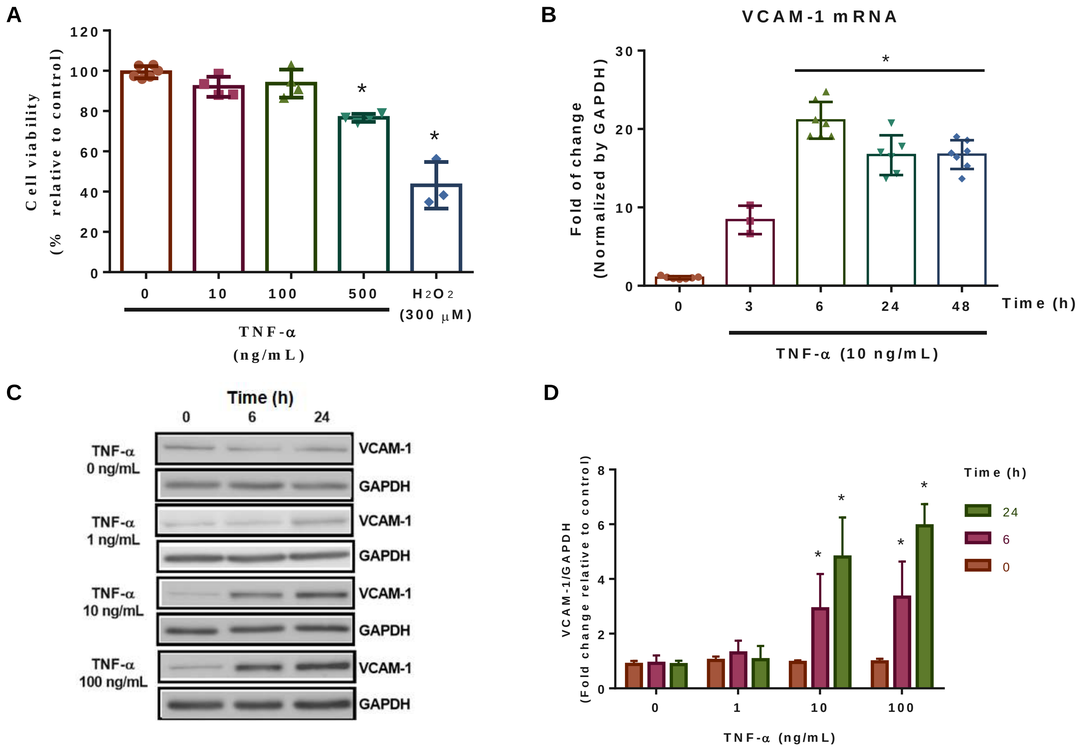

Figure 3. Effect of TNF-α on cell viability and VCAM-1 expression in cardiomyocytes. (A) Cell viability assay by trypan blue exclusion. (B) Relative mRNA levels of VCAM-1 normalized with GAPDH expression. (C) Western blot for VCAM-1, GAPDH was used as a loading control. (D) Densitometric quantification of VCAM-1 levels. *p<0,05 vs control (n≥3).

Cardioprotective effect of TNF-α on I/R injury

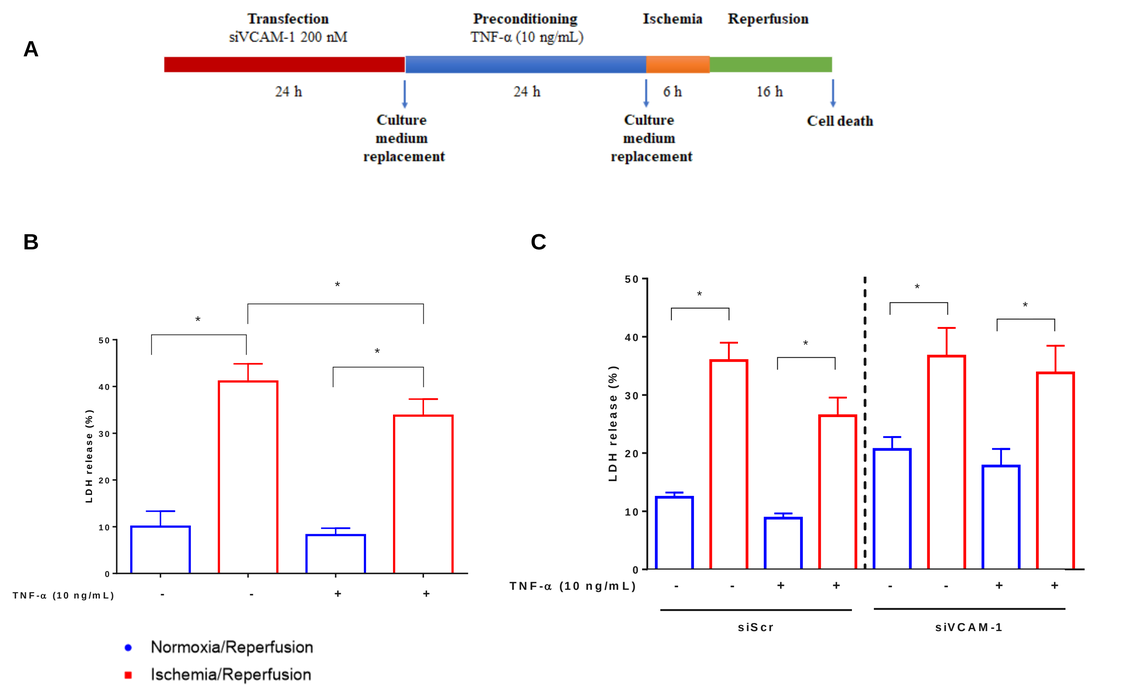

Figure 4. Cardioprotective effect of TNF-α on I/R injury. (A) Schematic representation of the experiments. (B) LDH release after TNF-α preconditioning and sI/R. (C) LDH release after VCAM-1 knockdown, TNF-α preconditioning and sI/R. *p<0,05 (n≥5).

Effect of VCAM-1 knockdown in prosurvival gene expression in cardiomyocytes

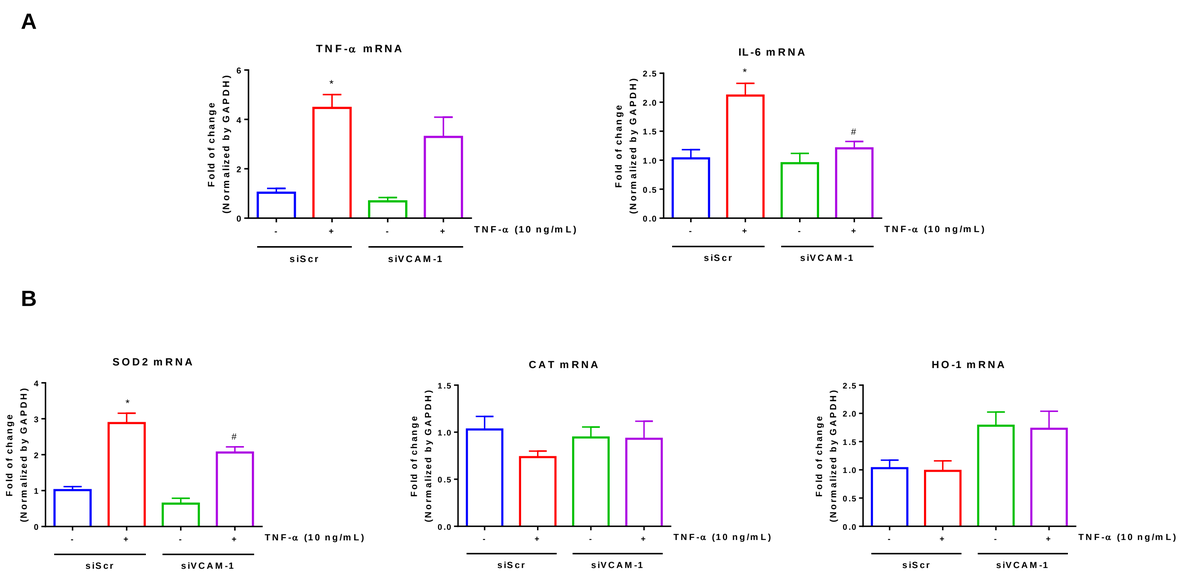

Figure 5. Effect of VCAM-1 knockdown in prosurvival gene expression in cardiomyocytes. Relative mRNA levels of (A) Pro-inflammatory genes (TNF-α and IL-6) and (B) Antioxidant genes (SOD2, CAT and HO-1), Both normalized with GAPDH expression. *p<0,05 vs control, # p<0,05 vs siScr (n=4).

Conclusions

- ✓ VCAM-1 knockdown exacerbates I/R injury in cardiomyocytes and could be mediating the protective effect of TNF-α preconditioning against sI/R damage.
- ✓ Nevertheless, further research is needed to establish the protective mechanism of VCAM-1 in cardiac I/R injury.

Acknowledgments

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