The next step in the clinical use of microRNAs: the elimination of heparin interference

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INTRODUCTION

Small noncoding RNAs (miRNA) have been described as important regulators of several physiological and pathophysiological processes in cardiology. Acute coronary syndrome (ACS) leads to alterations in circulating vascular and myocardial miRNA expression profiles. miR-126-3p is one of the abundant cardiac specific miRNA with significant positive association to acute coronary syndrome [1]. Levels of miR-126-3p in circulation are responsive to antiplatelet therapy; therefore, results of mir-126-3p measurement could be reduced in samples from these patients [2]. Heparin is a potentially confounding factor that may influence miRNA measurements in patients with acute cardiovascular disease because antiplatelet therapy (administrations of intravenous heparin) increases its endogenous levels. Interaction between heparin and antithrombin III *in vivo* cause improvement of the anticoagulant effect. It is a well-known fact that commonly used qPCR methods for miRNA detection do not recommend heparinised samples for testing due to its interference with enzymatic reaction. However, the heparin effect on endogenous mirRNAs cannot be fully explained by interference with DNA polymerases or magnesium ions. The reason could be based on heparin's ability to disrupt already formed enzyme-template complexes with sequence-dependent displacement or miRNAs compartmentalization [3]. Several different methods to minimize the effect of heparin on qPCR reactions were described. It seems to be beneficial to use heparinase treatment as an additional step in the sample preparation procedure because it is difficult to adjust the heparin interference threshold of cardiology patients unlike the physiological range of healthy individuals (0.10 – 0.24 IU/mL) [2,4]. The type of collection tube also contributes to external influence of miRNA measurement. Similar to heparin, other commonly used anticoagulants have been shown to interfere with RT-qPCR miRNAs measurement, with EDTA plasma yielding the best miRNAs profiles compared to others sample types [5].

In our pilot study we demonstrate the effect of endogenous and exogenous heparin interference to miR-126-3p quantification in two methods – RT-qPCR and enzyme immunoassay for miRNA quantification (miREIA). The difference between methods is that miREIA does not require reverse transcription which is a critical step for heparin interference.

MATERIALS AND METHODS

Sample collection

Blood samples of ACS patients (n=10) were collected into tubes with lithium heparin and K3EDTA. Heparin plasma and K3EDTA plasma were kept frozen at minus 80 °C until analysis.

RNA Isolation

Extraction was performed from 100 ul of plasma samples with added carrier RNA from bacteriophage MS2 (Roche) using the miRNeasy Mini kit (Qiagen, Germany) according to the manufacturer's protocol. Total RNA was eluted in 35 µL of nuclease-free H2O.

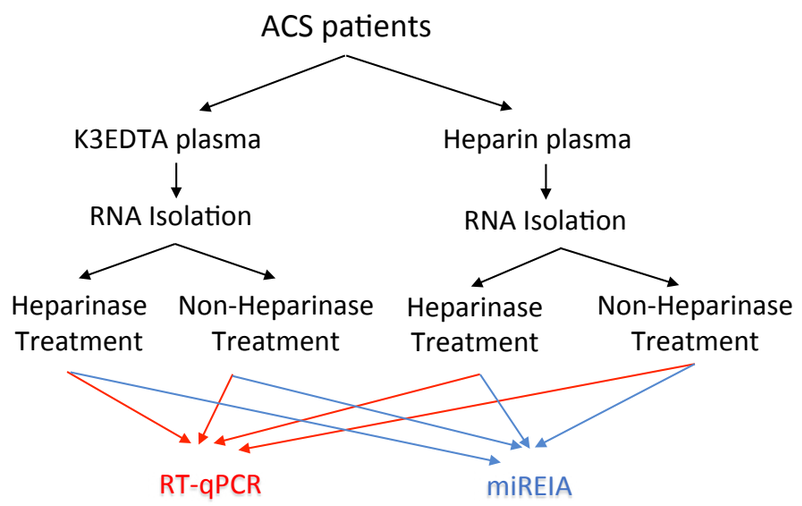
Heparinase Treatment

Each of heparin plasma and K3EDTA plasma isolates were divided into two parts. Deheparinization procedure was used for the first part of the samples: Extracted RNA was treated with heparinase, RNase inhibitor and heparinase buffer for 3 hours at 25 °C according to the procedure described by Schulte et al. (2019). For comparison, the second part of plasma isolates was treated only with heparinase buffer with RNase inhibitor.

miRNA Quantification

miR-126-3p was determined in each individual's treated or non-treated sample by RT-qPCR MiRXES ID3EAL™ miRNA qPCR assay (MIRXES, Taiwan) and immuno-based method hsa-126-3p miREIA (BioVendor, Czech Republic).

Procedure summary

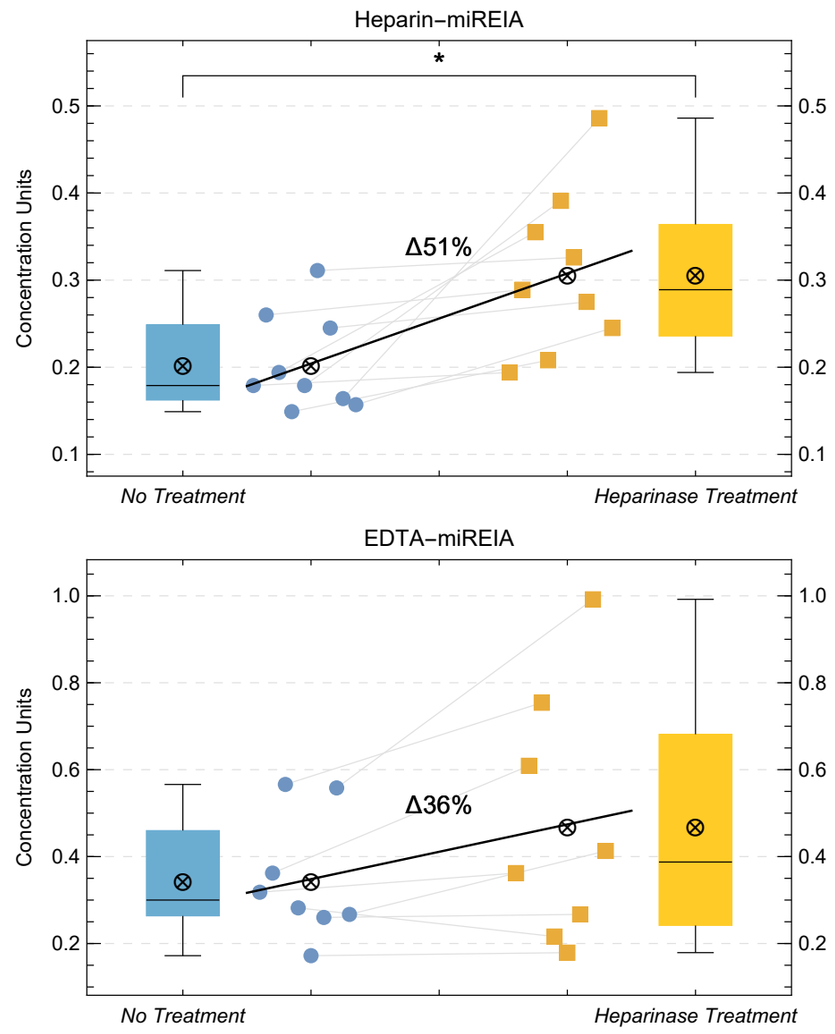


CONCLUSION

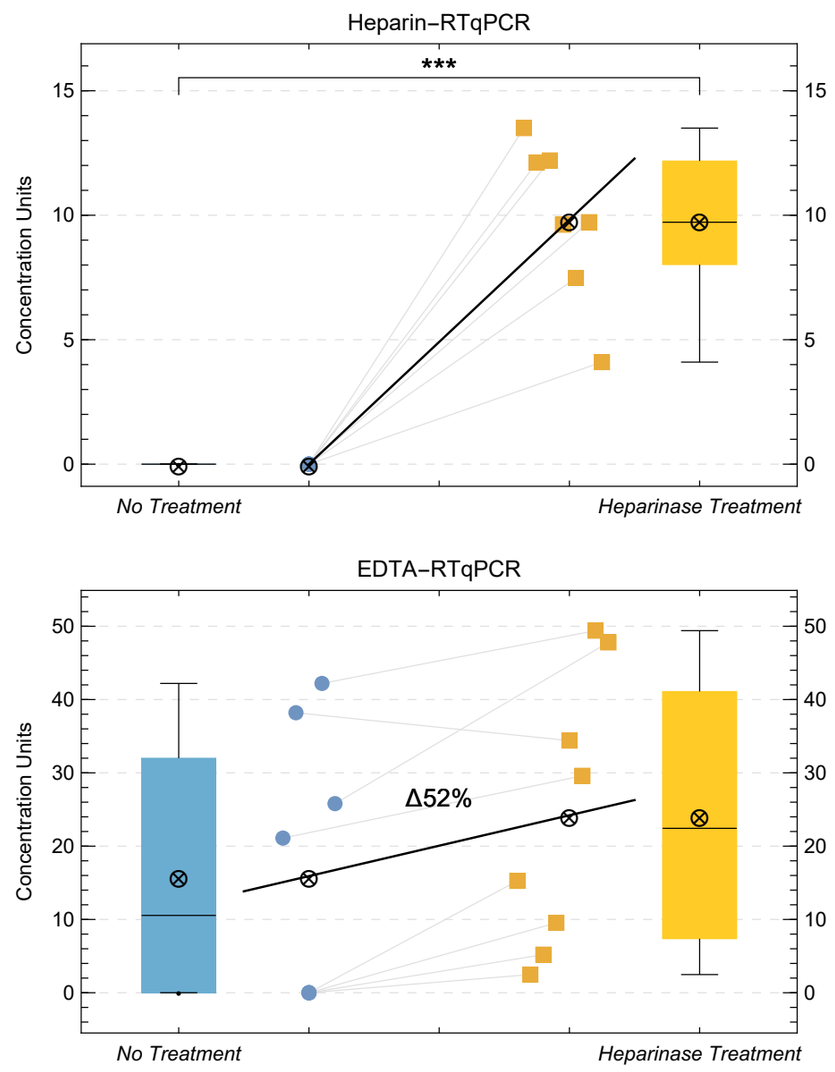
- **The results show that not only endogenous but also exogenous heparin plays an important role in mir-126-3p determination in ACS patients.**
- **Heparinase treatment increased the final miRNA concentration by 50%, improving the measurability of the samples.**
- **Heparinase treatment of samples has a significant effect on miRNA analysis in both methods – RT-qPCR and miREIA.**

RESULTS

A) miREIA



B) RT-qPCR



*Figures show box-and-whiskers plot combined with scatter plot with highlighted effects of the treatment on the estimated concentration. Statistical comparison was performed using two sample t-test or Mann-Whitney test when appropriate. Statistical significance was denoted as *, p≤0.05; **, p≤0.01; ***, p≤0.001. Symbol ⊗ represents the arithmetic mean.*

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