Heparin Selectively Affects the Quantification of MicroRNAs in Human Blood Samples

To the Editor:

MicroRNAs (miRNAs) are non-coding RNA molecules that either inhibit translational processing or mediate degradation of target mRNAs in various physiological and pathophysiological processes. miRNAs have been detected in several body fluids, such as blood plasma, and have emerged as potentially suitable biomarkers for various diseases, including cancer and myocardial infarction. Heparin is commonly used as an anticoagulant in cardiovascular diagnostics and interventions. Previous studies have demonstrated that heparin can inhibit RNA quantification in vitro by interfering with the DNA polymerase used in the quantitative PCR (qPCR) reaction. It is unclear, however, whether the biologically active concentrations of heparin achieved after treatment of patients influence the quantification of miRNAs in blood samples. We therefore assessed the effect of systemic application of heparin on the measurement of endogenous circulating miRNAs in plasma samples.

Arterial blood samples from 11 patients were obtained from the femoral artery before the cardiac catheterization procedure and at 10 and 60 min after heparin was administered (bolus, 5000 IU heparin; maintenance dose, 2500–5000 IU). We isolated RNA from EDTA-treated plasma with a TRIzol-based miRNA-isolation protocol (miRNeasy; Qiagen). We used a hydrolysis probe–based quantitative PCR method to measure several vascular-related miRNAs (miR-17, miR-34a, miR-92a, miR-126, miR-145, and miR-378). We also measured miR-1, miR-133a, miR-208, and miR-499, which have been suggested as biomarkers of acute myocardial infarction. The concentrations of miR-34a, miR-133a, miR-208, miR-378, and miR-499 were significantly and profoundly reduced from baseline by 10 min after heparin administration (P < 0.05) and remained reduced for 60 min, although only miR-34a, miR-133a, and miR-208 achieved statistically significant reductions at 60 min (P < 0.05) (Fig. 1). Furthermore, the relative concentration of circulating miR-34a was inversely correlated with the activated clotting time (r = −0.68; P = 0.01). Normalizing miRNA production in plasma is challenging because of the lack of a validated control miRNA that is not regulated. Therefore, we supplemented plasma samples with Caenorhabditis elegans miR-39 (cel-miR-39), as described previously. Surprisingly, the recombinant cel-miR-39, which we spiked into plasma samples after adding TRIzol to normalize RNA extractions, had decreased profoundly and significantly at 10 min (P = 0.001) and remained decreased by >30% after 60 min (P = 0.11). Next, we determined whether the detection of all recombinant miRNAs spiked into plasma samples is affected by heparin in the plasma. miR-499 detection was again suppressed significantly when it was added to samples obtained at 10 min [mean (SD), 65% (18%); P = 0.001] or at 60 min (P = 0.006) after heparin administration. Furthermore, miR-499 and cel-miR-39 concentrations were inversely correlated with the activated clotting time [r = −0.68 (P = 0.02), and r = −0.68 (P = 0.01), respectively]. We observed no significant interference with PCR-based detection, however, when recombinant miR-126 and miR-92a were spiked into plasma samples from patients who received heparin (all P values >0.05), suggesting that the presence of biologically active heparin influences the detection of only specific miRNAs measured by the PCR in vitro.

To determine whether heparin might interfere directly and selectively with the detection of specific miRNAs via qPCR assays, we measured recombinant miRNAs by qPCR in the presence of increasing concentrations of heparin. Heparin dose-dependently inhibi...
ited the detection of all recombinant miRNAs tested, with complete inhibition occurring at $>2 \times 10^{-5}$ IU/μL. Additionally, we determined whether heparin addition affects the detection of cel-miR-39 or the endogenous miRNAs in the samples. We spiked heparin into plasma samples from 3 healthy volunteers. Again, ex vivo addition of heparin induced dose-dependent inhibition of detection for all miRNAs, with complete inhibition observed at concentrations $>1 \times 10^{-2}$ IU/μL plasma. Given that heparin is known to interact with cations such as Mg$^{2+}$, we investigated whether increased MgCl$_2$ in the PCR can prevent the reduction in detection we observed. Although miRNA concentrations were increased in the PCR reactions with 10.5 mmol/L MgCl$_2$, plasma samples from patients treated with heparin still showed significant inhibition of miR-499 [mean at 10 min, 55% (13%); $P < 0.05$].

In summary, our data demonstrate that PCR-based measurements of circulating miRNAs need to be interpreted with caution for patients receiving heparin. Heparin strongly interferes with the detection of recombinant cel-miR-39 spiked into samples for normalization purposes. This result thus raises concerns regarding the use of this approach for normalizing data for circulating plasma miRNAs in patients receiving heparin. The development of sensitive miRNA-detection assays that are based on other PCR-free technologies (5) or the use of heparinase may circumvent this current limitation (3).

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**References**


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