



Therapeutic implication of MicroRNA-320a antagonist in attenuating blood clots formed during venous thrombosis

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Abstract

Venous thrombosis (VT) is a complex multi-factorial disease and a major health concern worldwide. Its clinical implications include deep vein thrombosis (DVT) and pulmonary embolism (PE). VT pathogenesis involves intricate interplay of various coagulants and anti-coagulants. Growing evidences from epidemiological studies have shown that many non-coding micro-RNAs play significant regulatory role in VT pathogenesis by modulating expressions of large number of gene involved in blood coagulation. Present study aimed to investigate the effect of human micro RNA (hsa-miR)-320a antagonist on thrombus formation in VT. Surgery was performed on Sprague–Dawley (SD) rats, wherein the inferior vena cava (IVC) was ligated to introduce DVT. Animals were divided into four groups (n = 5 in each group); Sham controls (Sham), IVC ligated-DVT (DVT), IVC ligated-DVT + transfection reagent (DVT-NC) and IVC ligated-DVT + miR320a antagonist (DVT-miR-320a antagonist). IVC was dissected after 6 h and 24 h of surgery to estimate thrombus weight and coagulatory parameters such as levels of D-dimer, clotting time and bleeding time. Also, ELISA based biochemical assays were formed to assess toxicity of miRNA antagonist in animals. Our experimental analysis demonstrated that there was a marked reduction in size of thrombus in hsa-miR-320a antagonist treated animals, both at 6 h and 24 h. There was a marked reduction in D-dimer levels in hsa-miR-320a antagonist treated animals. Also, blood clotting time was delayed and bleeding time was increased significantly in hsa-miR-320a antagonist treated rats compared to the non-treated and Sham rats. There was no sign of toxicity in treated group compared to control animals. Hsa-miR-320a antagonist could be promising therapeutic target for management of VT.

Keywords Venous thrombosis · MiRNA · Coagulation · IVC ligation · DVT

Abbreviations

BT	Bleeding time
CT	Clotting time
DVT	Deep vein thrombosis
IVC	Inferior vena cava
PE	Pulmonary embolism
VT	Venous thrombosis

Highlights

1. MicroRNAs regulate a large number of genes and thus play a major role in pathogenesis of complex diseases like venous thrombosis.
2. Ligation of inferior vena cava (IVC) introduces thrombus formation (DVT) in rat model.
3. Intravenous introduction of hsa-miR-320a antagonist into IVC ligated rats decrease the size and weight of thrombus significantly.
4. Hsa-miR-320a antagonist (1mg/kg body wt.) did not cause any lethality in rats and no visible signs of toxicity.
5. Hsa-miR-320a antagonist could be a promising therapeutic target for treatment of venous thrombosis.

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Introduction

Venous thrombosis (VT) is the third most common cardiovascular disease with annual incidence rate of 1–2 individuals per 1000 and having chances of long term post thrombotic complications and recurrence [1–3]. It mainly comprises of two clinical manifestation including deep vein thrombosis (DVT) and pulmonary embolism (PE), the later one being potentially fatal. Sufficient epidemiological data has revealed that VT is a complex disease governed by several acquired and inherited risk factors [4, 5]. Apart from several predisposing factors such as cancer [6], cardiovascular diseases [7], trauma [8] and surgery [9], hypoxic conditions at high altitude (HA) have been regarded as independent risk factor to venous thrombosis, increasing risk of VT occurrence upto 30 times on exposure to HA [10–12]. Hypobaric hypoxic conditions prevailing at HA trigger various haematological changes including transient hypercoagulability and increased hematocrit volume and hemoglobin due to production of erythropoietin [12–14].

The disturbance in blood flow acts as triggering factor for deep vein thrombosis [15], however the sequence of molecular and cellular events resulting in formation of thrombus remains unclear. In humans, an event of VT is not essentially linked to endothelial injury [16, 17]. In animals, ligation of inferior vena cava, restricts blood flow through the vessels, without causing endothelial injury. Thus, DVT model of animals involving IVC stenosis-induced DVT, mimics human DVT in most appropriate manner [18, 19].

Small non-coding RNAs called MicroRNAs (miRNAs) modulate gene expression at post transcriptional level either by degradation of mRNA or by translational repression of targets [20]. MiRNA binds to the 3'-untranslated region of target to form silencing complexes, thus inducing degradation [21, 22]. Each miRNA can potentially target a large number of genes and hence can modulate multiple biological pathways at the same time [23], Selbach et al. (2008). In the last one decade, number of studies has demonstrated that circulating miRNAs could serve as potential biomarkers in various diseases such as cancer, Myocardial infarction, liver diseases etc. [24–26]. Also, the critical role of specific miRNAs in vascular diseases such as atherosclerosis, vascular inflammation, coronary artery disease etc. has been highlighted [27–29]. Various bioinformatic approaches have been applied earlier to identify potential miRNAs involved in blood coagulation and VTE pathophysiology [30–33], however precise role of miRNAs in etiology of VTE is not elucidated.

Present study aims to identify potential miRNA target that can modulate genes involved in blood coagulation and could be used as therapeutic target for VTE treatment.

Materials and Methods

Bioinformatic approach to identify potential miRNA and its target genes it regulates

A thorough literature survey was done to identify the miRNAs associated with venous thrombosis, DVT and PE. Manual search was conducted on PubMed, Google Scholar and Science Direct using common key search terms. The search results were carefully analyzed to identify most suitable miRNA candidate that has been experimentally proven to play a significant role in VTE susceptibility.

Study groups

Experiments were conducted in accordance with the guidelines of Animal ethical norms laid by Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India. All experimental protocols were, including euthanasia were approved by Institutional animal ethical committee (IAEC) of Defence Institute of Physiology and Allied Sciences (DIPAS) (approval ID: IAEC/DIPAS/2021-22-Ext/06). For the present study, male Sprague–Dawley (SD) rats kept in standard laboratory conditions, weighing up to 250–300 g were selected. The study protocols were in accordance to the ARRIVE guidelines (<https://arriveguidelines.org>).

Animals used for the present study were divided into four groups randomly. Firstly, those who were operated for mid-line laprotomy and bowel manipulation without IVC ligation (Sham controls). Second set of animals were those who were operated to introduce thrombus formation by IVC ligation (DVT group). The third experimental group of animals were those in which only transfection reagent was injected (without miRNA), one hour prior to IVC ligation (DVT-NC). Lastly, fourth group of animals were injected with miR-320a antagonist after complexation with transfection reagent, invivolectamin 3.0 (Invitrogen) (DVT-miR-320a antagonist). Each experimental group comprised of five rats (n = 5). Two set of experiments were run and animals were sacrificed after 6 h and 24 h for performing thrombotic and biochemical assays (Table 1).

MiR-320a antagonist for in vivo delivery

MiRNA inhibitor (antagonist) of hsa-miR-320 (in vivo grade) was synthesized from Invitrogen, Thermo Fischer Scientific. It was suspended in transfection reagent, invivolectamine 3.0 (Invitrogen, Thermo Fischer Scientific) according to manufacturer's instructions. Briefly, to prepare 1 ml of 0.1 mg/ml complex, 100 µl of 1.2 mg/ml miRNA solution was prepared by mixing miRNA duplex

Table 1 Experimental groups and treatment of animals

Experimental groups	Time points (Hours)	No. of treated animals at each time point
SHAM-controls (Sham)	6 and 24	5
IVC ligated (DVT)	6 and 24	5
IVC ligated + transfection reagent (DVT-NC)	6 and 24	5
IVC ligated + miR-320a antagonist treatment (DVT-miR320a antagonist)	6 and 24	5

solution (2.4 mg/ml) and complexation buffer in a ratio of 1:1. Added this miRNA solution to 100 μ l of Invivolectamine 3.0, at room temperature and vortex. Incubated Invivolectamine 3.0-miRNA duplex mixture for 30 min at 50 °C. Centrifuged the tube briefly. Diluted the complex 6 folds by adding 1 ml of PBS (pH 7.4). Dose of miRNA antagonist was standardized to 1 mg/kg of animals. This dose was equivalent to 200 μ l of 0.1 mg/ml solution prepared, i.e., approx. 1 μ l/g dose for 250 g rats. Each animal was administered 200 μ l of solution (invivolectamine + DVT-miR-320a antagonist/ or distill water in case of DVT-NC group) by tail vein one hour before IVC ligation (as described by [34, 35]).

IVC ligation of rats to induce thrombus formation

Flow restriction animal model was used to for studying stasis induced thrombosis. Briefly, the animal was first allowed to acclimatize to the room environment to relieve stress. A small dose of Ketamine (100 mg/kg) and Xylazine (20 mg/kg) was injected into the animal according to its body weight to anesthetize the rats. Incision was made into a straight central body followed by opening of layer beneath and medial line of symmetry. Once the abdomen is open, the intestine and other organs were taken out and PBS was added from above to prevent its drying. To induce the thrombus in the animals, midline laprotomy was performed, blood flow was restricted by proximal ligation of the inferior vena cava (IVC) below the renal veins and lateral tributaries ligation as described by [36]. A prolene suture (non-reactive, 3.0) was used for IVC ligation below the renal veins on left and visible lateral branches [37]. The organs were then carefully placed back into the peritoneal cavity and the inner and outer layers of skin were stitched. Applied povidine-iodine ointment onto the suture to avoid post surgical infections and the animal was bandaged with adhesive tapes. Intra muscular Diclophenac (pain reliever) and Gentamycin injection (antibiotic) was given to the animals after surgery. The animals were properly marked and kept in a separate cage with food and water. Animals were kept under close watch for their physical activity till it regained consciousness.

Animals were euthanized after 6 h and 24 h of surgery and the IVC was carefully dissected. Size and weight of thrombus was measured for each rat.

Coagulatory parameters

To calculate clotting time, a small sample of blood was collected in a micro-centrifuge tube (pre-incubated at 37 °C) by retro-orbital puncture. The tube was kept in water bath set at 37 °C and was checked every 20–30 s by tilting. The time taken for blood to clot was carefully noted.

To calculate bleeding time, animals were anesthetized and tail was transacted from the tip about ~2.0 mm using a surgical blade. The transacted tail was immediately dipped in 10 ml of isotonic saline solution (0.9% NaCl) in a falcon tube. The time from transaction till the bleeding completely stopped was noted.

Biochemical assays

Biochemical markers for heart, liver and kidneys were determined using Enzyme linked immunosorbent assay (ELISA) in serum samples. These included Gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), albumin deficiency marker (NAGase) and aspartate aminotransferases AST) for liver injury; Creatinine for kidney injury; *N*-terminal pro-brain natriuretic peptide (NT-proBNP), NT-pro-atrial natriuretic peptide (NT-pro-ANP), Cardiac muscle troponin T (cTnT) and Cardiac troponin I (cTnI) for heart failure.

Thrombotic markers

Levels of circulating Prothrombin fragment 1 + 2 and levels of D-dimer, a fibrin degradation product, were measured in rat plasma using commercially available ELISA kit (Elabscience, USA).

Target gene prediction of selected miRNA

In-silico tools were used to predict miRNA gene targets. In order to gain insights into the mechanism by which hsa-miR-320 mediates genes involved in VTE, a list of target genes of hsa-miR-320 was prepared using online databases. MiRNA target prediction was done using miRWalk (<http://mirwalk.umm.uni-heidelberg.de/>), which consolidates gene targets from three different databases, Targetscan, miRDB and miRTarbase. The gene list thus obtained was subjected to pathway analysis and gene enrichment analysis using online databases.

Results

Identification of target miRNA and its role in blood coagulation

Several studies have shown significant association of a number of miRNAs with venous thrombosis. These include miR-15b-5p, miR-197-3p, miR-27b-3p, and miR-30c-5p [38, 39], hsa-miR-126-3p, hsa-miR-885-5p, hsa-miR-194-5p, and hsa-miR-192-5p [40, 41], miR-191-5p, miR-320a, miR-21-5p, miR-23a-3p, and miR-451a [42] etc. Interestingly, many a number of studies commonly reported differential

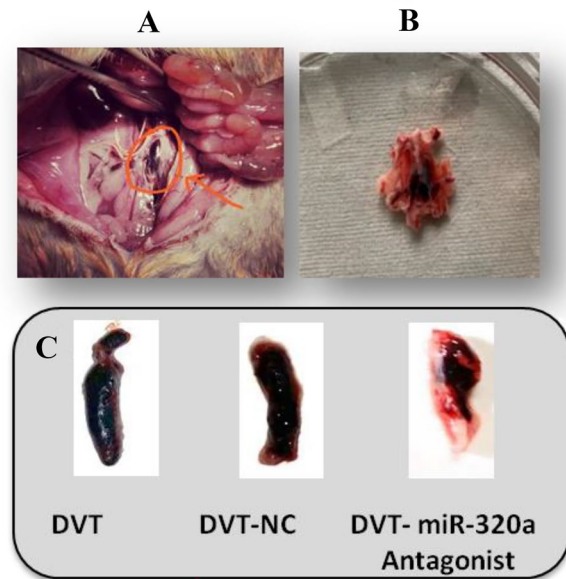


Fig. 1 **A** In vivo thrombus formation in rat after in IVC ligation; **B** Thrombus extracted from rat; **C** Thrombus formed in different animal groups; DVT, DVT-NC and DVT-miR-320a Antagonist

expression of hsa-miR-320 in VTE [42–44], (Xiao et al. 2011, Zhou et al. 2016). Significant up-regulation of hsa-miR-320a in VTE patients have been demonstrated in our previous studies [33], Srivastava et al. [32]. Thus after careful evaluation of existing data, we selected hsa-miR-320a for further study. Mature sequence of hsa-miR-320a (GCC UUCUCUCCCCGGUUCUCC) is derived from its stem loop sequence CUCCCCUCCGCCUUCUCUCCCCGG UUCUCCCCGGAGUCGGGAAAAGCUGGGUUGAGA GGGCGAAAAGGAUG, located on chromosome 8 from position 22,244,966 to 22,245,037,

on Build GRCh38. To silence the activity of hsa-miR-320a, the present study uses its antagonist (complementary to its mature miRNA sequence) in order to suppress thrombus growth.

Thrombus weight and size measurement

In general, thrombus weight is proportional to thrombus length, i.e., weight of a thrombus decreases with thrombus length. In the present study, the thrombus weight was measured using laboratory weighing balance while the thrombus length was measured longitudinally using standard scaling method. *Ex-vivo* measurement of thrombus dimensions is a limitation of the study. Figure 1 shows the marked difference in thrombus size in different experimental groups. Animals treated with hsa-miR-320a antagonist showed marked reduction in weight and size of the thrombus. While no thrombus was formed in SHAM controls, thrombus was formed in other three groups of animals and the hsa-miR-320a

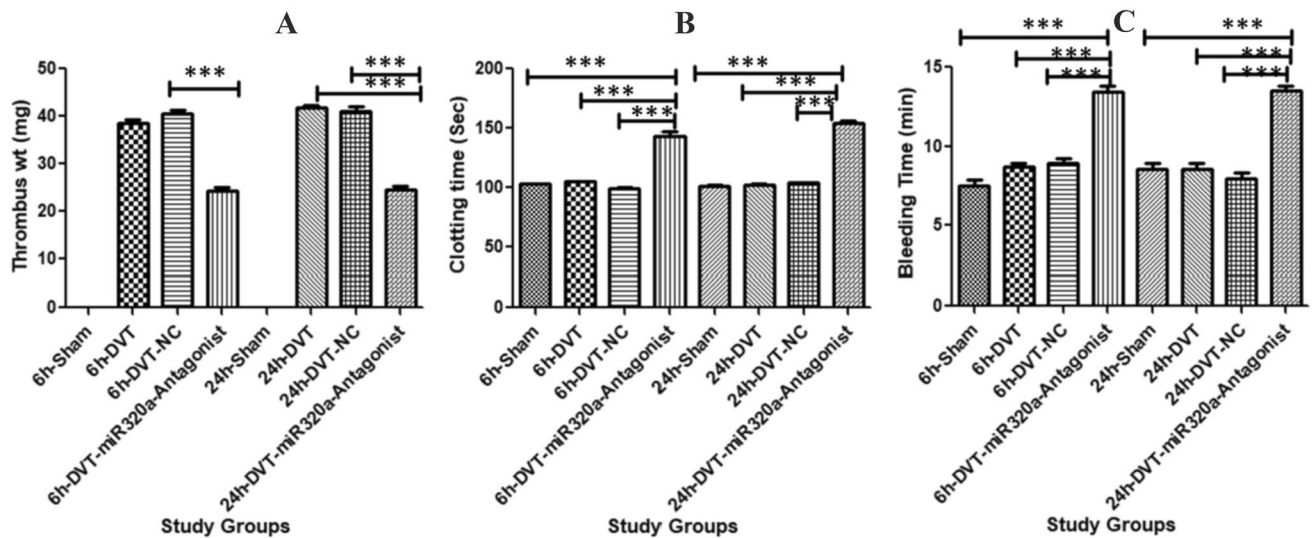


Fig. 2 Coagulation parameters: Statistical comparison of **a** thrombus weight, **b** Clotting time and **c** Bleeding time amongst four animal study groups after 6 h and 24 h of hsa-miR-320a antagonist introduction

antagonist treated animals had smallest thrombus in terms of size and average weight (~24 mg), significantly lower than the DVT (~38 mg) and DVT-NC (~41 mg) group, at both time points 6 h and 24 h. On the other hand, thrombus was of comparable size in experimental groups DVT and DVT-NC groups (Fig. 2).

Effect of systemic delivery of hsa-miR-320a antagonist

Introduction of miR-320a antagonist showed increased clotting and bleeding time in animals. Clotting time was significantly delayed in animals treated with hsa-miR-320a antagonist, both in case of 6 h and 24 h in comparison sham controls, DVT and DVT-NC groups. Another significant observation of the study was marked increase in bleeding time in hsa-miR-320a antagonist treated animals compared to other three study groups.

Toxicity of hsa-miR-320a antagonist

To study the suitability of hsa-miR-320a antagonist in in vivo delivery for VTE therapeutic potential, various toxicity assays were performed. Hsa-miR-320a antagonist did not cause lethality or any visible signs of injury in the animals. Markers indicative of liver injury such as AST, ALP, GGT and LDH did not show any significant difference in plasma levels between the different animal study groups. NAGase levels significantly changed between DVT-miR-320a antagonist treated rats and other three animal groups, both after 6 h and 24 h of introduction (Fig. 3A and B).

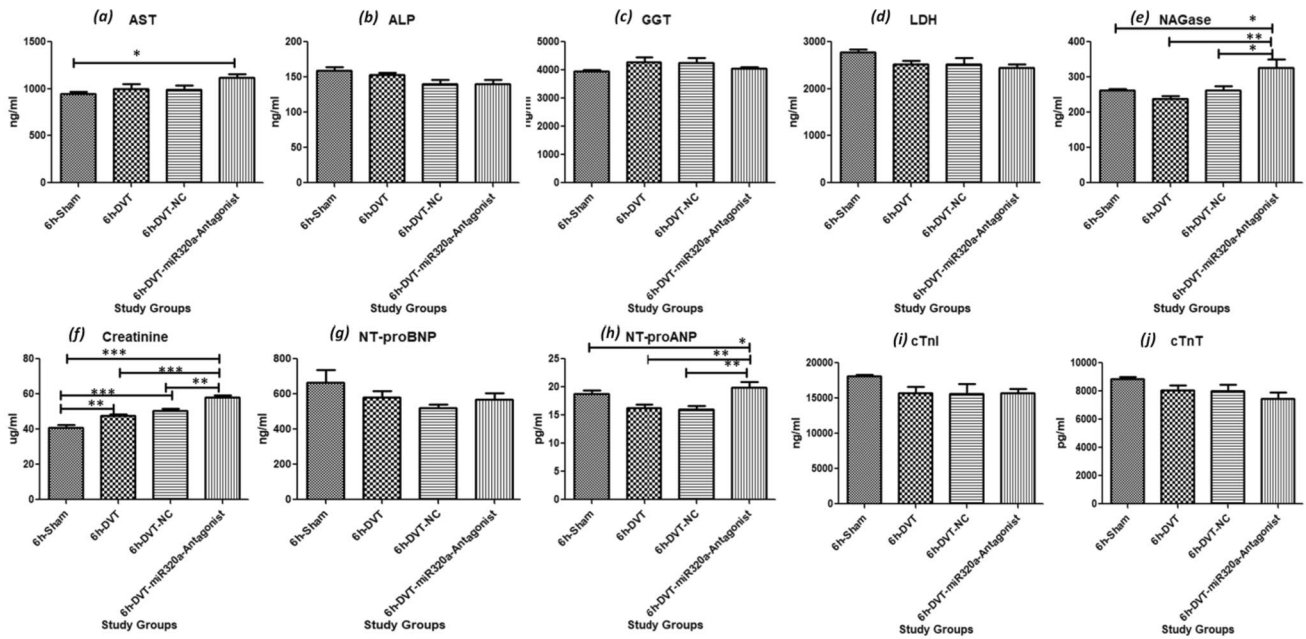
Levels of creatinine also changed significantly amongst the study groups, both at 6 h and at 24 h. There was a consistent increase in creatinine levels from sham to IVC ligated DVT rats and DVT-NC rats. Furthermore, there was a significant increase in creatinine levels in DVT-miR-320a antagonist treated rats in comparison to all other three study groups. Also, a Natriuretic peptide, NT-proANP, a marker of cardiac injury, was also significantly increased in DVT-miR-320a antagonist treated animals compared to the sham, DVT and DVT-NC rats, both at 6 h and 24 h. The significant increase in these biochemical markers could be due to physiological re-adjustments post introduction of hsa-miR-320a antagonist, however, the changes were non-lethal.

Measurement of thrombotic markers

Both D-dimer and prothrombin fragment 1 + 2 are well established markers of coagulation activation. Levels of these markers were estimated in plasma samples of rats in each study group. The average D-dimer level in sham controls was 336 ng/ml in 6 h and 356 ng/ml in 24 h. The levels of D-dimer increased beyond normal reference range in IVC ligated rats. In DVT rats, the average D-dimer levels were 788 ng/ml in 6 h and 797 ng/ml in 24 h. Similarly, for DVT-NC group D-dimer levels were 794 ng/ml and 812 ng/ml in 6 h and 24 h respectively. Interestingly, in DVT-miR-320a antagonist treated animal group, the levels of D-dimer were as low as that of sham group, i.e., 342 ng/ml in 6 h and 358 ng/ml in 24 h (Fig. 4A).

Another thrombotic biomarker, prothrombin fragment 1 + 2 levels showed exactly similar pattern of expression as

A



B

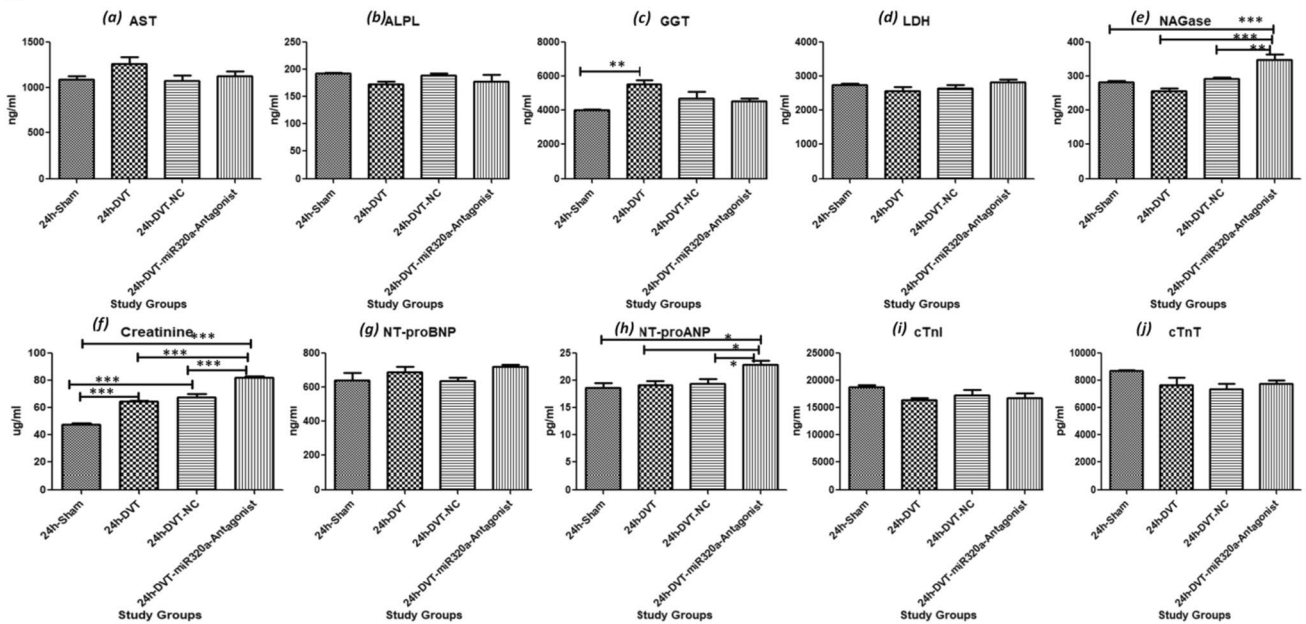


Fig. 3 A and B Biochemical assays performed on four study groups. Markers for liver injury (a) Aspartate aminotransferases (AST), (b) alkaline phosphatase (ALP), (c) Gamma-glutamyltransferase (GGT), (d) Lactate dehydrogenase (LDH) and (e) albumin deficiency marker, N-acetyl-beta-d-glucosaminidase (NAGase); Kidney Injury, (f) Creatinine; and Heart injury, (g) N-terminal pro-brain natriuretic peptide

(NT-proBNP), (h) NT-pro-atrial natriuretic peptide (NT-pro-ANP), (i) Cardiac troponin I (cTnI) and (j) Cardiac muscle troponin T (cTnT) were analyzed using ELISA. These 10 markers were evaluated in experimental sets, 6 h and 24 h. Significant differences were observed in plasma levels of NAGase, Creatinine, and NT-proANP

that of D-dimer. For sham controls, levels of prothrombin fragment 1 + 2 were 544 pg/ml and 564 pg/ml after 6 h and 24 h respectively. The average levels of this marker increased in DVT rats as well as DVT-NC rats ranging

from 830 to 864 pg/ml at both time points. However in DVT-miR-320a antagonist treated rats, there was a marked decline in prothrombin fragment 1 + 2 levels. The average

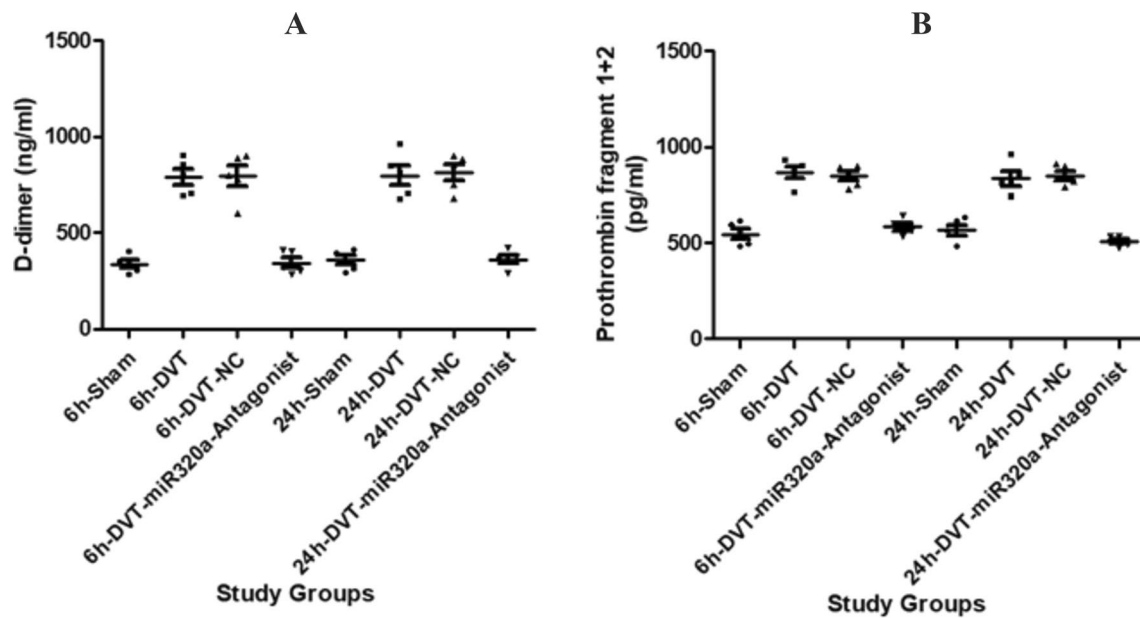


Fig. 4 Estimation of **A** D-dimer and **B** prothrombin fragment 1+2 levels in Rat plasma amongst four study groups, both after 6 h and 24 h. Both D-dimer and prothrombin fragment 1+2 were higher than normal reference range in DVT and DVT-NC group of animals

at both time points. The levels of these thrombotic indicators were within normal reference range for DVT-miR320a antagonist treated rats, close to that of sham controls, after 6 h and 24 h

estimated levels were 582 pg/ml and 504 pg/ml after 6 h and 24 h respectively (Fig. 4B).

Target validation of miR-320

Target prediction by miRWalk enlisted 63,948 gene targets, with prediction score from 1 to 0.8. After removing the duplicates from this list, 16,456 unique targets were found, which were then subjected to pathway analysis using online tool, Panther [45]. The pathway analysis showed that hsa-miR-320 modulates several genes associated with blood coagulation pathway and fibrinolytic pathway. Several anti-coagulants as well as clot dissolution genes including endothelial protein C receptor (PROCR), vitamin K-dependent protein C (PROC), vitamin K-dependent protein S (PROS), antithrombin III (SERPINC1), thrombomodulin (THBD), Urokinase plasminogen activator surface receptor (PLAUR) and tissue-type plasminogen activator (PLAT) are regulated by hsa-miR-320. Besides these, pathway analysis also demonstrated that hsa-miR-320 targets coagulation factors such as factor XI (F11), factor XII (F12), factor III (Tissue factor), factor VII (F7), factor X (F10), factor II (prothrombin) etc.

Discussion

MicroRNAs are master regulators that play a pivotal role in several cellular and biochemical pathways and hence their dysregulation has been associated with several human diseases [46, 47]. MiRNAs serve as attractive targets for developing novel therapeutic approaches which primarily involves restoration of normal miRNA function, either by using miRNA mimics or by single-stranded antisense oligonucleotides, known as anti-miRs [48, 49]. Recently, several studies have been conducted to unravel the therapeutic potential of miRNA mimics and antagonists for treatment of venous thrombosis [34, 35], Sun et al. 2019, [50] as well as several other complex diseases such as renal fibrosis [51], epilepsy [52], several types of cancers (Fu et al. 2021) and even COVID-19 [53].

Predisposition to venous thromboembolism, a complex pathological condition is significantly linked to genetic factors. It induces development of thrombosis or embolism in both arteries and veins at multiple sites [54]. Previous studies have shown association of several miRNAs with VTE such as hsa-miR-126-3p, hsa-miR-885-5p, hsa-miR-194-5p, and hsa-miR-192-5p [40, 41]. Over past one decade, there have been several animal model studies which explore modulation of miRNAs for thrombus resolution in VTE. Intravenous injection of viral vector expressing miR-150, miR-126, let-7e-5p, miR-21 and miR-483-3p [55–59] and mimics of miR-145 and miR-150 [34, 35, 38, 39] in rat model of DVT

by IVC ligation reportedly result in venous thrombus resolution in experimental DVT rats. Besides these, intravenous injection of viral vector expressing miR-103a-3p [60, 61] and mimics of miR-338-5p [62] in mouse model of DVT by IVC stenosis model have been demonstrated to reduce venous thrombus formation.

Selection of miRNA to be used in *in-vivo* studies conducted so far, have been done on the basis of either bioinformatic analysis or based on findings of previous studies on miRNA expression profile in DVT. Our research group has previously reported a significant increase in miR-320a levels in plasma of VTE patients in comparison to healthy controls [31–33]. In similar studies, significant over expression of miR-320a and miRNA-320b have reported in patients of DVT [43, 44]. Thus, miR-320 was selected to perform further *in vivo* experiments on rat model of DVT.

MiRNA-320 family comprising of miR-320a, miR-320b, miR-320, miR-320c and miR-320e is closely associated with several human diseases [63]. Previous reports have shown that miR-320 is a tumor suppressor and is significantly down-regulated in many cancers such as cervical cancer, colon cancer, gastric cancer and glioma [64–67]. These studies demonstrated that miR-320 could be used as diagnostic and prognostic biomarker in diverse range of cancers. In context of venous thrombosis, contradictory reports are available on expression of miR-320. There are evidences of hsa-miR-320 being up-regulated [32, 33, 43, 44] and down-regulated during VTE [68]. Experimental evidences in these reports establish that dysregulation of miR-320 levels are directly associated with VTE susceptibility.

MiRNA mimics and anti-miRs (antagonists) are used for miRNA modulation and thus reverse pathological miRNA expressions for variety of diseases [69]. However, integrity and stability of miRNAs in circulation is a major challenge for miRNA delivery. In the present study, invivofectamine (from Thermo Fischer Scientific) was used as transfection reagent to stabilize the miRNA-320a antagonist into living system. It is animal origin lipid based nanoparticle which facilitates *in vivo* delivery of miRNAs efficiently. We believe that, after intravenous introduction of miRNA-320a antagonist into rats through tail vein, it binds to mature circulatory miRNA-320 and thus inhibit their interaction with target gene.

The present study revealed therapeutic potential of hsa-miR-320a antagonist in treating venous thrombo-embolism. The findings of this study is a blend of network based bioinformatic tools that were used to identify potential miRNA target together with *in vivo* animal model, in which the efficacy of target miRNA as therapeutic target was validated. Our study explicitly demonstrated that intravenous administration of hsa-miR-320a antagonist reduces the size of thrombus formed due to coagulation of blood. Also, target prediction and pathway analysis of genes potentially

modulated by miR-320, showed that a large number of genes were involved in blood coagulation and fibrinolysis pathway. These included several anti-coagulants and clot dissolution genes which might be down-regulated by over-expression of miR-320 in DVT rats. Interestingly, over-expression of miR-320 has also been associated with increased platelet activity as a result of interaction with WIPF1 gene encoding for WAS/WASL-interacting protein [70]. Thus is expression may increase susceptibility towards VTE. Introduction of hsa-miR-320a antagonist in DVT rats thus inhibit the activity of mature hsa-miR-320, thereby leading to reduction in blood clot (thrombus weight). Notably, pathway analysis revealed that besides targeting genes involved in blood coagulation and fibrinolysis, miR-320 also targets genes related to hypoxic response and endothelial dysfunction. Thus further studies may also be conducted to explore its therapeutic potential in hypoxia induced VTE.

Our results demonstrate that intravenous dose of hsa-miR-320a antagonist did not show any significant signs of toxicity. Significant alterations in injury markers NAGase, Creatinine and NT-proANP, amongst the miRNA antagonist treated animal group compared to others did not cause any lethality in these animals. The marked reduction in the size of the thrombus signifies that intravenous administration of hsa-miR-320a antagonist could be an effective strategy to manage the burden of venous thrombosis. The observations of the study are preliminary in nature and further detailed studies are required with different miRNA doses and duration to validate the current findings.

Conclusion

Several miRNAs have been characterized and validated to regulate physiological processes in development of venous thrombosis. It is important to identify specific miRNA that could mitigate the size of the growing thrombus and thus provide future therapeutic target for pulmonary embolism. In this regard, introduction of hsa-miR-320a antagonist could a novel therapeutic approach for treatment of venous thrombosis.

Limitations of the study

Due to technical reasons, we could perform experiments on limited number of animals.

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Author contributions SS: experiment design, analysis and manuscript writing; IG and NG: experimental work and analysis; RV: review and editing of manuscript.

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Data availability The datasets used and analyzed during the current study available from the corresponding author on reasonable request.

Declarations

Conflict of interest The manuscript has been read and approved for submission by all authors. No potential conflict of interest.

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