Stroke

BRIEF REPORT

Daytime DNase-I Administration Protects Mice From Ischemic Stroke Without Inducing Bleeding or tPA-Induced Hemorrhagic Transformation, Even With Aspirin Pretreatment

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BACKGROUND: Acute ischemic stroke treatment typically involves tissue-type plasminogen activator (tPA) or tenecteplase, but about 50% of patients do not achieve successful reperfusion. The causes of tPA resistance, influenced by thrombus composition and timing, are not fully clear. Neutrophil extracellular traps (NETs), associated with poor outcomes and reperfusion resistance, contribute to thrombosis. DNase-I, which degrades neutrophil extracellular traps, could improve thrombolytic efficacy. However, more studies are needed to understand the impact of DNase-I in tPA-sensitive stroke models, the safety of coadministering DNase-I and tPA regarding hemorrhagic transformation (HT), optimal timing for use, and effects on aspirin-treated animals.

METHODS: We used in situ thromboembolic stroke, a tPA-sensitive model, where late tPA administration causes HT. Middle cerebral artery occlusion was induced at different zeitgeber times (ZT) to study the optimal timing for administration. DNase-I, tPA, and aspirin were administered at various times to evaluate their effects.

RESULTS: DNase-I reduced infarct volume and improved functional outcomes 24 hours post-middle cerebral artery occlusion by decreasing plasma and cortical neutrophil extracellular trap levels. DNase-I caused no bleeding or impact on HT induced by late tPA. Its protective effect was only seen when given during the daytime (rodent inactive phase; ZT4–7), not overnight (active phase; ZT13–16). Chronic aspirin pretreatment increased tPA-induced HT but did not change the protective effects of DNase-I, with or without tPA.

CONCLUSIONS: Our study demonstrates that daytime (inactive phase) DNase-I administration is a safe and effective treatment for experimental stroke. This is particularly important given the 2 ongoing clinical trials for stroke patients.

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Key Words: aspirin ■ extracellular traps ■ ischemic stroke ■ thrombosis ■ tissue-type plasminogen activator

Stroke is a leading global cause of death and disability. Reperfusion therapies like tissue-type plasminogen activator (tPA) or tenecteplase are standard treatments for acute ischemic stroke but have limitations. These therapies are suitable for selected patients, with around 50% of tPA-treated patients not achieving

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Nonstandard Abbreviations and Acronyms

HT	hemorrhagic transformation
MCAO	middle cerebral artery occlusion
NET	neutrophil extracellular trap
tPA	tissue-type plasminogen activator

successful reperfusion (tPA resistance), and tPA significantly raises the risk of hemorrhagic transformation (HT). tPA resistance is not fully understood, but thrombus composition and drug administration timing are key factors.[1] Red blood cell-rich thrombi with low von Willebrand Factor, platelets, and DNA respond better to tPA lysis, while platelet-rich thrombi, lacking red blood cells, are resistant to tPA but sensitive to DNase-1.[2]

Neutrophil activation and the release of neutrophil extracellular traps (NETs) in thrombosis are linked to poor stroke outcomes and reperfusion resistance.[3,4] DNase-I, which degrades NETs, may improve thrombolytic treatment efficacy.[3,4] Unresolved questions include its effectiveness in tPA-sensitive models, the safety of coadministration with tPA concerning HT, optimal timing, and effects on animals taking aspirin, a common antiplatelet. To investigate, a tPA-sensitive stroke model was used, demonstrating recanalization and protective effects with early thrombolysis, while late tPA administration produced HT.[5]

METHODS

A detailed description is given in the Supplemental Material. Data supporting the study's findings are available from the corresponding authors upon request. All experimental protocols were performed in accordance with the guidelines of the Animal Welfare Committee of Universidad Complutense de Madrid, Centro Nacional de Investigaciones Cardiovasculares and Consejería de Medio Ambiente y Ordenación del Territorio de la Comunidad de Madrid (Real Decreto [RD] 53/2013; PROEX 016/18) following European directives 86/609/Comunidad Económica Europea and 2010/63/European Union and reported in accordance with ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments).[6] The article adheres to the Transparency and Openness Promotion Guidelines. Middle cerebral artery occlusion (MCAO) was performed in mice using the in situ thromboembolic model.[5] DNase-I was administered 15 minutes before tPA and 12 hours later. HT was induced by late tPA administration (3 hours post-MCAO).[5] Aspirin therapy was administered 50, 26, and 2 hours before MCAO. MCAO procedures were performed at different zeitgeber times ([ZT]; ZT4–7 and 13–16). Neurological deficits were assessed using the cylinder test, infarct volume was determined by magnetic resonance imaging, and hemorrhages were analyzed by diaminobenzidine staining 24 hours after MCAO. Plasma elastase levels and cortical NETs were measured at 4 and 24 hours, respectively, post-MCAO. Statistical analyses were conducted using PRISM 9.5.0 software, with significance set at $P<0.05$.

RESULTS

Daytime DNase-I Administration Is Protective After Stroke by Reducing NET Formation

DNase-I administration reduced infarct volume and improved functional outcomes 24 hours post-MCAO (Figure 1A; $P<0.05$). Combining DNase-I with tPA did not affect infarct volume or neurological outcomes compared with vehicle or tPA groups (Figure 1A; $P>0.05$).

DNase-I treatment also decreased plasma elastase and cortical NET levels at 4 and 24 hours post-MCAO (Figure 1B through 1C; $P<0.05$). Delayed tPA administration increased cortical NETs, but DNase-I addition reduced these levels (Figure 1B). A positive correlation was found between cortical NET levels and plasma elastase concentrations ($r=0.7450$, $P<0.0001$).

The protective effect of DNase-I was noted only during the daytime (Figure 1D; inactive phase; ZT4–7; $P<0.05$), not overnight (active phase; ZT13–16; $P>0.05$). Larger infarct volumes and poorer functional outcomes were observed during the inactive phase (Figure 1D; $P<0.05$), as we demonstrated.[7] Cortical NETs and plasma elastase levels are higher in the inactive phase (Figure 1E; $P<0.05$).

Chronic aspirin pretreatment did not affect the protective effects of DNase-I, with or without tPA (Figure 1F).

Administration of DNase-I Does Not Cause Bleeding or Increase tPA-Induced HT

DNase-I did not cause bleeding (Figure 2A and 2B). Late administration of tPA resulted in HT as shown[5]; importantly, adding DNase-I did not exacerbate this bleeding (Figure 2A and 2B; $P>0.05$).

Combining DNase-I with tPA significantly improved successful arterial reperfusion rates compared with tPA alone (Figure 2C; $P<0.05$). Furthermore, the addition of DNase-I, enhancing reperfusion, significantly decreased tPA-induced bleeding volume in those who reperfused (Figure 2D; $P<0.05$).

Aspirin pretreatment increased hemorrhage volume induced by tPA (Figure 2A and 2E; $P<0.05$). Notably, in aspirin-pretreated animals, adding DNase-I to tPA reduced thrombolytic therapy-induced HT (Figure 2A and 2E; $P<0.05$).

DISCUSSION

Reperfusion therapies are the only approved treatment for stroke, but up to 50% of patients show resistance to tPA. This study explores the role of DNase-I, showing it can protect in a tPA-sensitive thrombotic stroke model by reducing NET levels. Importantly, DNase-I does not induce bleeding or exacerbate HT associated with late tPA administration.

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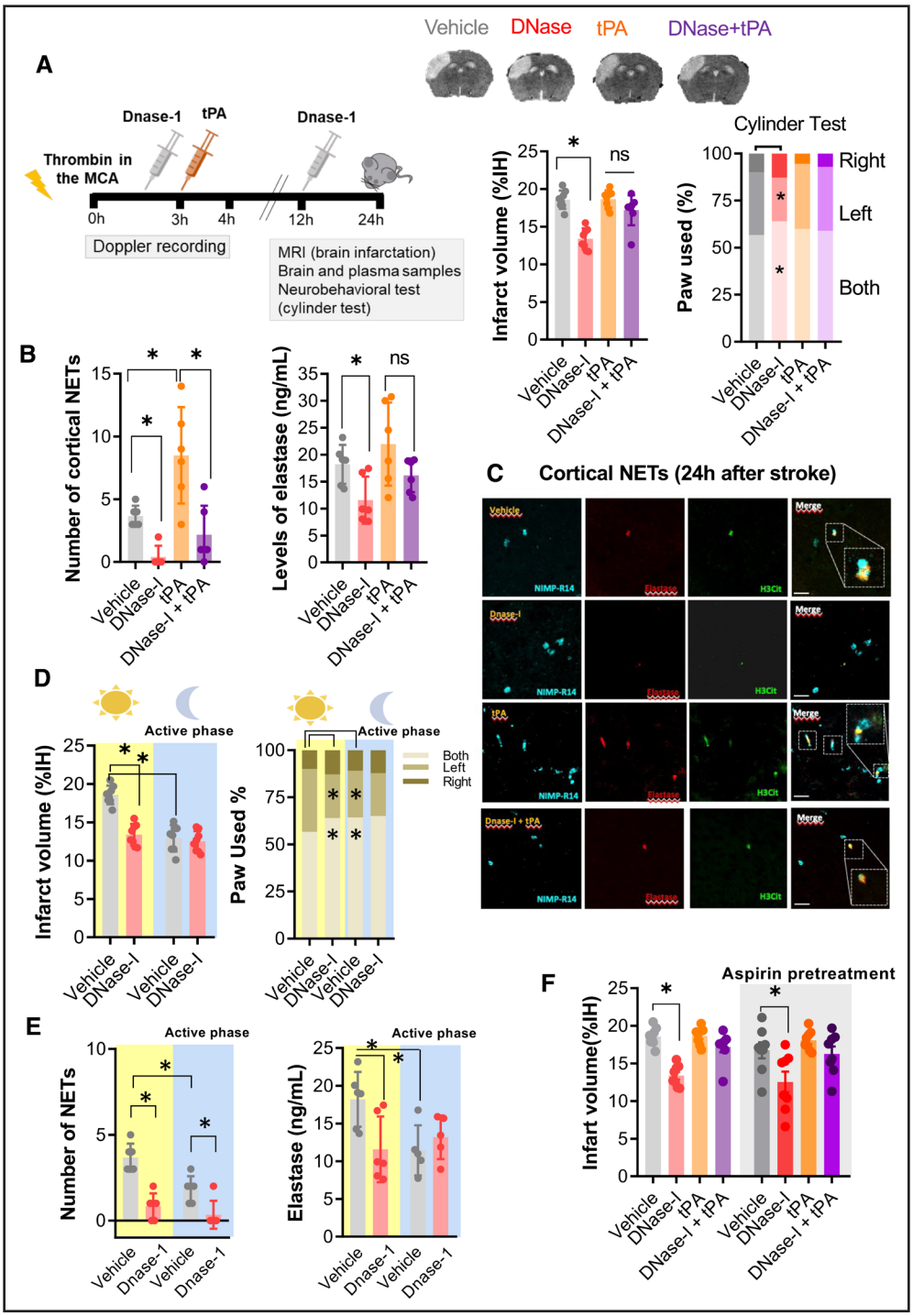

Figure 1. Daytime DNase-I administration is protective after stroke by reducing neutrophil extracellular traps (NETs).
A, Experimental design. Effects of treatments on infarct volume and neurobehavioral tests 24 hours post-middle cerebral artery occlusion (MCAO). **B** and **E**, Cortical NETs and plasma elastase measured 24 and 4 hours, respectively, post-MCAO. **C**, Representative images of cortical NETs. **D**, Circadian effects on infarct volume and neurobehavioral tests 24 hours post-MCAO. **F**, DNase/tissue-type plasminogen activator (tPA) effects, with/without aspirin pretreatment. Data are mean±SEM (n=6–8).

Additionally, its protective effects are phase-specific, occurring only during the daytime (inactive phase in rodents) and are not affected by aspirin pretreatment.

The success of thrombolysis depends largely on thrombus composition.[2] Previous studies showed that DNase-I effectively dissolves platelet-rich thrombi,[3,4] but its efficacy in tPA-sensitive thrombi is unclear and requires further study. Thus, we used a tPA-sensitive model that presents HT with delayed tPA treatment treatment.[5] The clot model offers insights beyond those

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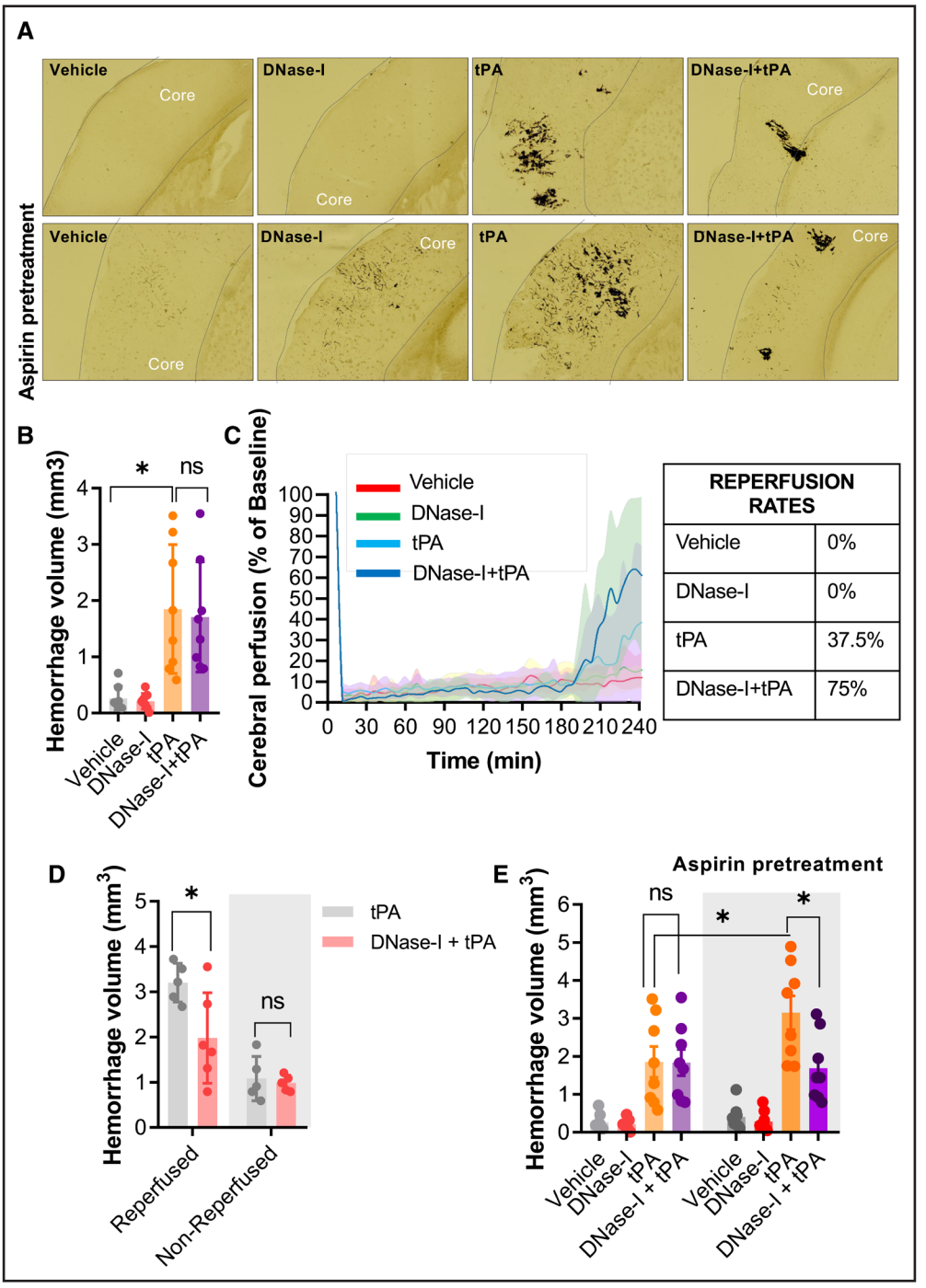

Figure 2. DNase-I administration does not cause bleeding or increase tissue-type plasminogen activator (tPA)-induced hemorrhagic transformation.
A, Staining of brain sections from all groups with/without aspirin pretreatment. **B**, Effects of treatments on hemorrhage volume 24 hours post-magnetic resonance imaging (MCAO). **C**, Cerebral perfusion and reperfusion rates measured during 4 hours post-MCAO. **D**, Effects of treatments on hemorrhage volume in reperfused/nonreperfused animals. **E**, Effects of treatments on hemorrhage volume with/without aspirin pretreatment. Data are mean±SEM (n=6–8).

provided by suture models, which focus on the effects of NETs in circulation or at the parenchymal level.[8–10] Specifically, the clot model sheds light on the role of NETs within thrombi, an area linked to tPA resistance in multiple studies, an aspect that suture models do not address. The findings indicate that DNase-I reduces NET levels and offers protection in this model, highlighting its broader therapeutic potential.

DNase-I's protective effects may arise from several mechanisms, including fibrin degradation by breaking down DNA within the thrombi.[11] It promotes NET degradation, which could provide direct benefits, as NETs

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are linked to stroke severity and mortality.[12] DNase-I might also influence infarct recanalization. While it does not alter blood flow reperfusion rates in the first 4 hours postocclusion, its potential for facilitating recanalization in subsequent hours requires further studies. Coadministration with tPA significantly improves reperfusion rates. DNase-I may also prevent microthrombosis and no-reflow phenomena in the microvasculature, as NETs are predominantly found within the brain vasculature after ischemia, suggesting their role in secondary thrombosis.[8,13,14] Studies in myocardial ischemia indicate that DNase-I combined with tPA enhances myocardial and coronary microcirculatory functions by reducing NETs.[15,16] However, the loss of DNase-I's protective effect when coadministered with tPA may be due to its inability to lower plasma NET levels and reverse microthrombosis.

DNase-I does not increase the risk of bleeding or HT, a major concern in stroke treatment. Any type of HT, including asymptomatic ones, occurs in 3% to 40% of stroke patients receiving tPA[17] and is linked to increased morbidity and mortality. NETs can damage the vascular endothelium and the blood-brain barrier, increasing vascular permeability and contributing to HT. tPA directly stimulates neutrophils to release NETs, and its removal with DNase-I reduces blood-brain barrier disruption and tPA-induced hemorrhage,[18] as seen in those animals that underwent reperfusion. These results support DNase-I's protective effects in platelet-rich thrombus models with elevated NETs.[4]

Interestingly, DNase-I's protective efficacy is diurnal, with benefits observed only during the daytime (inactive phase in rodents). This aligns with circadian influences on treatment outcomes.[19] Similar patterns occur with other treatments, such as antihypertensive and aspirin, where nighttime administration (inactive phase in humans) leads to better outcomes. The study also found that cortical NETs and plasma elastase levels, which are reduced by DNase-I, are higher during the inactive phase, indicating a link to stroke severity.

The study assessed the interaction between DNase-I and aspirin, a common treatment in stroke. Chronic aspirin did not alter DNase-I's protective effects, suggesting they can be safely used together. While aspirin increased bleeding risks with late tPA administration, DNase-I still reduced thrombolytic-induced bleeding, highlighting its safety and efficacy.

Despite promising findings, our study has limitations. It used young male animals without common comorbidities seen in stroke patients. More research is needed to explore the effects of early DNase-I and tPA coadministration, comorbidities, age and sex differences, and cerebroprotective mechanisms.

In conclusion, our results demonstrate that administration of DNase-I during the daytime (inactive phase in rodents) is an effective and safe treatment for experimental stroke. Our findings are significant, especially with 2 ongoing clinical trials investigating DNase-I to improve reperfusion rates (EXTEND-IA DNase; NCT05203224) and reduce stroke-related systemic inflammation (ReSCInD; NCT05880524).

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Supplemental Materials and Methods
ARRIVE Checklist

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