ORIGINAL ARTICLE

Effect of Recombinant Tissue-Type Plasminogen Activator on Peripheral Blood Mononuclear Cells of Patients With Alteplase-Associated Angioedema

Palestra F[1,2*], Bova M[3*], Servillo G[4], Chetta M[5], Ciardi R[1], Candelaresi P[4], Tarsitano M[5], Suffritti C[6], Gualtierotti R[6,7], Miniello S[8], Ferrara AL[1,2], Varricchi G[1,2,9], Cavallaro R[3], Andreone V[4**], Loffredo S[1,2,9,10**]

[1]Department of Translational Medical Sciences, University of Naples Federico II, Naples, Italy
[2]WAO Center of Excellence, Naples, Italy
[3]Division of Internal Medicine 2, Department of Medicine and Medical Specialties, AORN A. Cardarelli Hospital, Naples, Italy
[4]UOC of Neurology and Stroke Unit, AORN Cardarelli, Naples, Italy
[5]UOC of Medical and Laboratory Genetics, AORN Cardarelli, Naples, Italy
[6]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy
[7]University of Milan, Department of Pathophysiology and Transplantation, Milan, Italy
[8]UOC of Neurology, Sant'Anna e San Sebastiano Hospital, Caserta, Italy
[9]Center for Basic and Clinical Immunology Research (CISI), University of Naples Federico II, Naples, Italy
[10]Italian Network for Hereditary and Acquired Angioedema (ITACA), Italy
*Co–first authors.
**Co-corresponding and senior authors.

J Investig Allergol Clin Immunol 2027; Vol. 37(1)
doi: 10.18176/jiaci.1146

Abstract

Background: Recombinant tissue-type plasminogen activator (R-tPA) is a thrombolytic agent used to treat acute ischemic stroke (IS). A rare but serious adverse effect of R-tPA is angioedema, which is characterized by plasma extravasation and increased release of vasoactive factors such as bradykinin, vascular endothelial growth factor A (VEGF-A), CXCL8, angiopoietin-1 (ANGPT-1), and ANGPT-2.
Objective: To investigate whether R-tPA modulates immune cell activity differently in IS patients with and without angioedema, focusing on the release of vasoactive mediators from human peripheral blood mononuclear cells (PBMCs).
Methods: PBMCs were isolated from 7 healthy controls (HCs), 7 IS patients without angioedema, and 7 IS patients who developed angioedema during R-tPA treatment (ISAE). The production and/or release of CXCL8, VEGF-A, ANGPT-1, and ANGPT-2 following ex vivo stimulation with R-tPA was measured. Plasma levels of these mediators were also assessed in ISAE patients during both angioedema attacks and remission.
Results: R-tPA inhibited the spontaneous release of VEGF-A, ANGPT-1, and ANGPT-2 from the PBMCs of HCs and IS patients. In contrast, a significant increase in the release of these mediators after stimulation with R-tPA was observed in PBMCs from ISAE patients. Plasma concentrations of all 4 mediators were higher during angioedema attacks than in remission, with a statistically significant elevation recorded for ANGPT-2.
Conclusions: These preliminary data suggest that R-tPA–related angioedema may result from abnormal immune cell activation, leading to increased release of vasoactive mediators. This immune dysregulation may contribute to the pathophysiology of angioedema in susceptible IS patients treated with R-tPA.

Key words: Angioedema. Angiopoietins. Bradykinin. Ischemic stroke. Vascular endothelial growth factor.

Resumen

Antecedentes: El activador tisular del plasminógeno recombinante (R-tPA) es un agente trombolítico utilizado para tratar el ictus isquémico agudo (IIA). Un efecto adverso poco común pero grave del R-tPA es el angioedema (AE), caracterizado por extravasación plasmática y un aumento en la liberación de factores vasoactivos como bradicinina, factor de crecimiento endotelial vascular (VEGF-A), CXCL8, angiopoyetina-1 (ANGPT-1) y ANGPT-2.
Objetivo: Investigar si el R-tPA modula de manera diferente la actividad de las células inmunitarias en pacientes con IIA con y sin AE, centrándose en la liberación de mediadores vasoactivos por parte de células mononucleares de sangre periférica humana (CMSPs).
Métodos: Se aislaron CMSPs de 7 controles sanos (CS), 7 pacientes con IIA sin AE y 7 pacientes con IIA que desarrollaron AE durante el tratamiento con R-tPA (IIAAE). Se midió la producción y/o liberación de CXCL8, VEGF-A, ANGPT-1 y ANGPT-2 tras la estimulación ex vivo con R-tPA. También se evaluaron los niveles plasmáticos de estos mediadores en pacientes IIAAE tanto durante los episodios de AE como en remisión.

Resultados: El R-tPA inhibió la liberación espontánea de VEGF-A, ANGPT-1 y ANGPT-2 por parte de las PBMCs de los CS y pacientes con IIA. En contraste, las CMSPs de los pacientes IIAAE mostraron un aumento significativo en la liberación de estos mediadores tras la estimulación con R-tPA. Las concentraciones plasmáticas de los cuatro mediadores fueron más altas durante los episodios de AE que en remisión, siendo el aumento de ANGPT-2 estadísticamente significativo.
Conclusiones: Estos datos preliminares sugieren que el AE relacionado con R-tPA podría deberse a una activación anormal de las células inmunitarias, lo que conduce a una mayor liberación de mediadores vasoactivos. Esta desregulación inmunitaria podría contribuir a la fisiopatología del AE en pacientes con IIA susceptibles tratados con R-tPA.

Palabras clave: Angioedema. Angiopoyetinas. Bradicinina. Ictus isquémico. Factor de crecimiento endotelial vascular.

Summary box

- **What do we know about this topic?**
  Angioedema is a rare but serious complication of R-tPA administered to treat IS that can obstruct the airway. The underlying mechanism is thought to involve bradykinin, although details remain unclear.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  Our research shows that R-tPA differentially modulates immune cell vasoactive mediator release, increasing it in stroke patients with angioedema but inhibiting it in other stroke patients. This suggests a novel mechanism by which R-tPA can induce angioedema in IS patients, laying the groundwork for targeted therapies to improve management.

Introduction

Recombinant tissue-type plasminogen activator (R-tPA), also known as alteplase, is a thrombolytic drug that is widely used for the treatment of acute ischemic stroke (IS) [1]. R-tPA converts plasminogen to the proteolytic enzyme plasmin, resulting in fibrinolysis [2,3]. Activation of plasminogen and fibrinolysis may activate the kallikrein-kinin system, leading to generation of bradykinin [4,5].

While bleeding remains the primary risk associated with R-tPA therapy, angioedema is a relevant adverse effect, occurring in 0.2% to 7.9% of patients [6-9]. In the most recent classification, this rare adverse effect is referred to as drug-induced angioedema [10]. Angioedema usually manifests as transient swelling of the tongue, lips, and soft tissue of the oropharynx during or shortly after administration [6,11-13]. It is often contralateral to the ischemic hemisphere and usually resolves within 24 hours [14]. In severe cases, it can cause airway obstruction and breathing impairment (13% of patients) [14,15]. Available data suggest that angiotensin converting enzyme inhibitor (ACEI) use, female sex, hypertension, diabetes, and dyslipidemia may be risk factors for R-tPA-induced angioedema (thrombolysis-associated angioedema [TAA]) [16].

Angioedema is characterized by a rapid and temporary increase in the permeability of submucosal or subcutaneous capillaries and postcapillary venules with localized plasma extravasation due to enhanced release of bradykinin and other vasoactive factors [3,17,18]. We previously demonstrated that plasma levels of vascular endothelial growth factors (VEGFs), angiopoietin-1 (ANGPT-1), and ANGPT-2 are altered in patients with hereditary angioedema (HAE) due to C1-inhibitor deficiency (HAE-C1-INH) because of genetic variants in the *F12* gene (coding for coagulation factor XII; HAE-FXII) and other unknown factors (HAE-UNK) [18,19]. Moreover, bradykinin is a vasoactive peptide that is released mainly from high-molecular-weight kininogen (HK) by plasma kallikrein and is more quickly and intensely released during incubation of angioedema patient plasma samples with R-tPA [20]. However, data on the cellular response of IS patients who developed angioedema (ISAE) following R-tPA treatment are lacking. Consequently, we measured cleaved HK (cHK) in the plasma of ISAE patients as indirect evidence of bradykinin release upon contact system activation.

Immune cells, such as monocytes, are a source of vasoactive mediators, including bradykinin, histamine, complement components, VEGFs, and ANGPTs, whose concentrations or activities are altered in angioedema [21]. To better understand the pathophysiology of TAA, we compared the effect of R-tPA on PBMCs from healthy controls (HCs), IS patients, and ISAE patients by measuring the production and/or release of vasoactive mediators (CXCL8, VEGF-A, ANGPT-2, ANGPT-1).

Material and Methods

This was a single-center, case-control study involving 3 groups: (*1*) HCs (n=7); (*2*) IS patients treated with R-tPA who did not develop angioedema (n=7); and (*3*) TAA (ISAE, n=7). Patients were recruited from the Stroke Unit at AORN Cardarelli Hospital (Naples, Italy) between December 2021 and July 2022. HCs were selected from blood donors at the Transfusion Center of AORN Cardarelli Hospital. Specifically,

HCs were matched 1:1 to patients by sex and age (±3 years). All donors underwent the standard blood donation screening process and had no personal or family history of angioedema or other immunoallergic diseases. All participants provided their written informed consent in accordance with the Declaration of Helsinki, and the study was approved by the institutional Ethics Committee (protocol number 0001516).

Blood Sampling and Processing

Blood samples were collected during routine clinical procedures. In ISAE patients, samples were obtained both during the angioedema episode (at the end of the R-tPA infusion) and at least 1 day after resolution of symptoms (remission phase). In IS patients, samples were taken at least 1 day after onset of stroke and R-tPA treatment. For HCs, samples were collected during routine health screenings after obtaining consent. Plasma was isolated by centrifugation at 2000*g* for 20 minutes at room temperature and stored at –80°C until analysis.

Isolation and Culture of PBMCs

PBMCs were isolated from whole blood using Histopaque-1077 density gradient centrifugation following dextran sedimentation to remove erythrocytes. Cells were resuspended in RPMI 1640 medium supplemented with 5% fetal bovine serum, L-glutamine, nonessential amino acids, and antibiotics. PBMCs (2×10^6 cells per well) were incubated for 2 hours at 37°C with increasing concentrations of R-tPA (0.01-100 µg/mL) or with 0.1 µg/mL for kinetic experiments. Supernatants were collected, centrifuged, and stored at –80°C for ELISA analysis.

Measurement of Vasoactive Mediators

VEGF-A, CXCL8, ANGPT-1, and ANGPT-2 were quantified in both plasma and PBMC supernatants using commercially available ELISA kits (R&D Systems) and following the manufacturer's instructions. The results were normalized to total protein content in cell lysates and expressed as ng/mg of protein.

Complement Testing

C1-INH and C4 antigen levels and function, along with C3 and C4 levels, were measured using radial immunodiffusion (NOR-Partigen, Siemens Healthcare Diagnostics) and chromogenic assays as described. Normal values for functional C1-INH were defined as greater than 70% and assessed using a commercially available kit (Technoclone GmbH).

Kallikrein-kinin System Activation

cHK was evaluated as an indirect marker of bradykinin generation. cHK levels were quantified by SDS-PAGE followed by immunoblotting and expressed as a percentage of total HK [22].

Gene Expression Analysis

Total RNA was extracted from PBMCs using TRIzol reagent. Reverse transcription was performed, and expression levels of CXCL8, VEGFA, ANGPT-1, and ANGPT-2 were quantified by real-time PCR using SYBR Green. Gene expression was normalized to GAPDH using the $2^{-\Delta Ct}$ method.

Genetic Analysis

To exclude HAE, genomic DNA was extracted from ISAE patients and sequenced using the Clinical Exome Solution v2 platform. Variants were filtered using a virtual gene panel approach according to the criteria of the American College of Medical Genetics and Genomics and with common variant databases.

Statistical Analysis

All data are presented as mean (SEM). Comparisons between 2 groups were performed using the *t* test. Multiple group comparisons were performed using 1-way ANOVA, followed by the Dunnett or Tukey post hoc test, as appropriate. A P value ≤.05 was considered statistically significant.

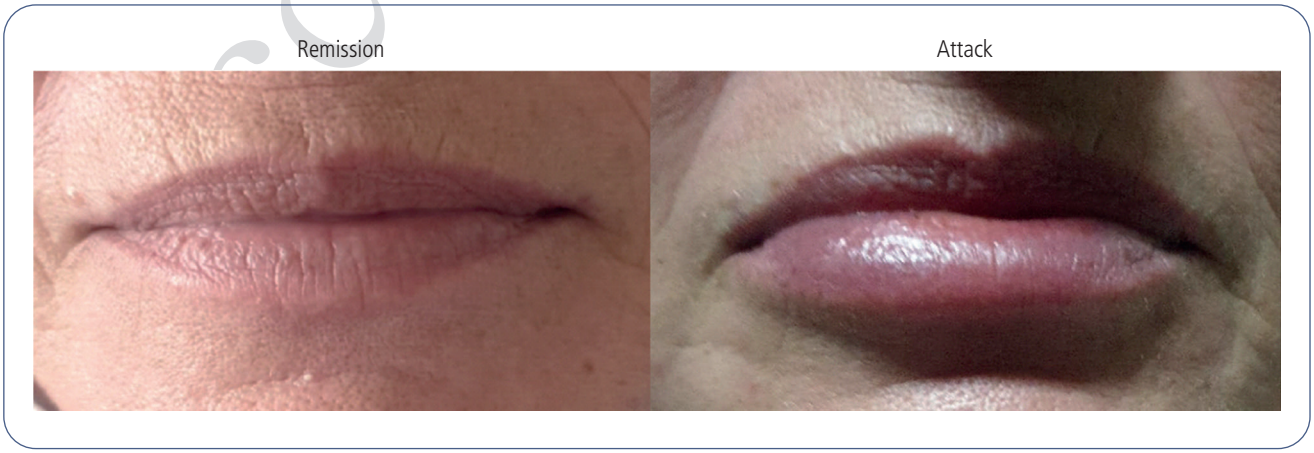

Figure 1. Photograph of patient 6 in remission (left panel) and during an angioedema attack induced by recombinant tissue-type plasminogen activator (right panel).

Results

Clinical Characteristics of ISAE Patients

From December 2021 through July 2022, 198 IS patients received R-tPA at AORN Cardarelli Hospital. Of these, 7 (3.54%) developed angioedema during or shortly after receiving R-tPA and were classified as ISAE. Angioedema typically affected the tongue, lips, or eyelids and was detected between 30 and 180 minutes following the start of thrombolysis. An example of lip involvement in patient 6 during the angioedema episode compared with remission is shown in Figure 1. In 5 out of 7 patients (71.4%), angioedema was treated with corticosteroids and/or antihistamines and resolved within 2 to 96 hours. All patients were over 18 years of age, and none had a prior history of hereditary or acquired angioedema. Detailed demographic and clinical data are provided in Table 1.

Plasma C1-INH antigen and function, as well as C4 levels, were within normal ranges in all ISAE patients. Genetic screening excluded variants in key angioedema-associated genes including *F12, ANGPT-1, PLG, KNG1*, and *MYOF*. These findings support the acquired nature of angioedema in this cohort.

R-tPA Suppresses Vasoactive Mediator Release in PBMCs from Healthy Controls

PBMCs from healthy donors were found to spontaneously release vasoactive mediators (CXCL8, VEGF-A, ANGPT-1, ANGPT-2) during a 2-hour culture (Table 2). Stimulation with increasing concentrations of R-tPA (0.01–100 µg/mL) led to dose-dependent inhibition of mediator release (Figure 2A-D). The inhibition was most pronounced for VEGF-A, ANGPT-1, and ANGPT-2, with near-complete suppression at higher concentrations.

Table 2. Spontaneous Release of Vasoactive Mediators From Peripheral Blood Mononuclear Cells of Healthy Controls.

Mediators	Proteins, ng/mg
CXCL8	36.65 (19.94)
VEGF-A	1.95 (0.73)
ANGPT-2	0.73 (0.65)
ANGPT-1	0.44 (0.47)

Peripheral blood mononuclear cells (2×10^6 cells/well) were incubated (2 hours, 37°C) in complete medium. At the end of incubation, the concentrations of CXCL8, VEGF-A, ANGPT-2, and ANGPT-1 were evaluated using ELISA. The results obtained were normalized for the total protein content in each well, which was determined in the cell lysates (0.1% Triton X-100) using the Bradford assay. Therefore, all mediator values were expressed as ng/mg of total proteins. Data are expressed as the mean (SEM) of 7 independent experiments.

In kinetic experiments using 0.1 µg/mL R-tPA, suppression of mediator release began within 5 minutes and persisted through 6 hours (Figure 3A-D). Table 3 shows the results of the Tukey multiple comparison test between individual timepoints, indicating that for ANGPT-2, the inhibition observed at 2 hours was significantly greater than at 5 minutes (P=.002) and 15 minutes (P=.003), with the most pronounced difference seen in the 2-hour vs 6-hour comparison (P=.0005). Likewise, for ANGPT-1, inhibition at 2 hours was significantly stronger than at 5 minutes (P=.0466) and at 6 hours (P=.0432). For VEGF-A, statistically significant differences were only detected between 2 hours and 6 hours (P=.0139) and between 1 hour and 6 hours (P=.0175). No significant differences between any pair of timepoints were observed for CXCL-8 (all P>.05), suggesting that its inhibition is established by 15-30 minutes and remains stable through 2 hours.

Table 1. Demographic and Clinical Features of ISAE Patients.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Sex	Female	Male	Female	Male	Male	Female	Female
Age, y	58	72	68	81	61	63	77
NIHSS	17	5	24	20	22	8	19
Ischemic stroke distribution	Left hemisphere	Right precentral gyrus	Right hemisphere	Right hemisphere	Left hemisphere	Left hemisphere	Left hemisphere
Site affected	Tongue and face	Tongue	Left tongue	Right eyelid	Left eyelid	Right lower lip	Right lower lip
R-tPA dose, mg	81	68	67	72	72	54	63
Time to detection of AE, min	60	30	120	180	180	180	120
AE treatment	Antihistamine, cortisone	Cortisone	Antihistamine, cortisone	No	No	Antihistamine, cortisone	Antihistamine, cortisone
Time to resolution of AE, h	72	2	48	Na	12	12	72
C1 f(x) (NV>60%)	110	69	73	105	100	70	78
C1 Ag (NV, 0.21-0.39 g/L)	0.47	0.39	0.37	0.29	0.27	0.30	0.40
C3 (NV, 0.9-1.8 g/L)	1.23	1.61	1.34	1.04	1.26	1.70	1.40
C4 (NV, 0.1-0.4 g/L)	0.50	0.43	0.29	0.25	0.45	0.26	0.51

Abbreviations: AE, angioedema; ISAE, ischemic stroke with angioedema; NIHSS, National Institutes of Health Stroke Scale; NV, normal value.

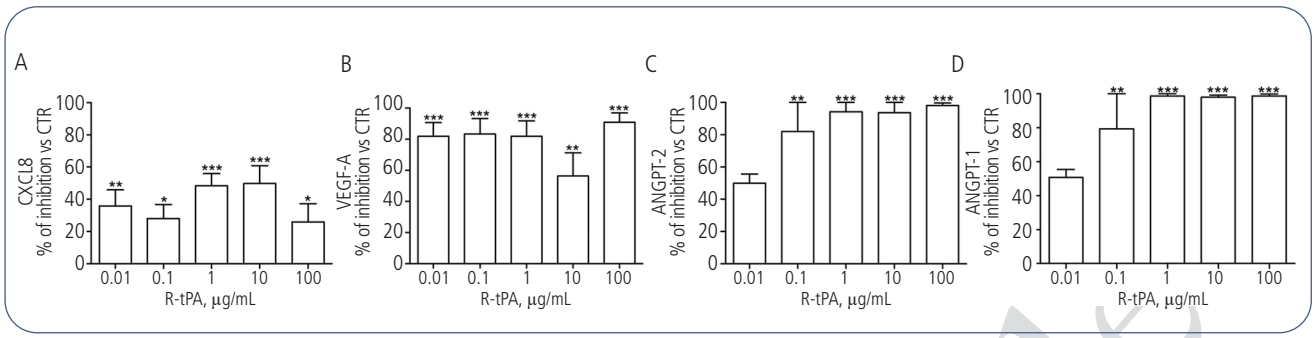

Figure 2. Inhibition percentage of spontaneous vasoactive factor release induced by R-tPA in PBMCs. PBMCs (2×10^6 cells/well) from healthy controls were stimulated (2 hours, 37°C) with RPMI alone (CTR) or increasing concentrations of R-tPA. CXCL8 (A), VEGF-A (B), ANGPT-2 (C), and ANGPT-1 (D) proteins in supernatants were evaluated using ELISA. The data are expressed as the mean (SEM) of 7 independent experiments. **P*<.05, ***P*<.01, ****P*<.001, *****P*<.0001 vs control. R-tPA indicates recombinant tissue-type plasminogen activator; PBMC, peripheral blood mononuclear cell; VEGF, vascular endothelial growth factor; ANGPT, angiopoietin.

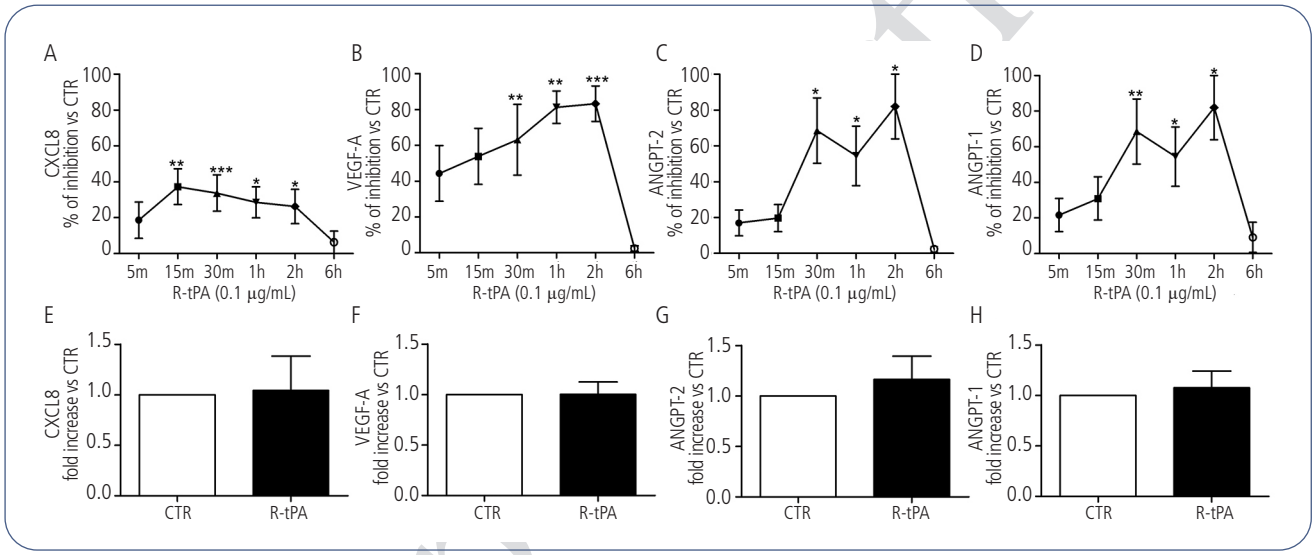

Figure 3. Kinetics of inhibition of spontaneous vasoactive factor release induced by R-tPA and effects of R-tPA on mRNA expression for CXCL8, VEGF-A, ANGPT-2, and ANGPT-1 in PBMCs. PBMCs (2×10^6 cells/well) were stimulated (from 5 minutes to 6 hours, 37°C) with medium alone (CTR) or with R-tPA (0.1 µg/mL). CXCL8 (A), VEGF-A (B), ANGPT-2 (C), and ANGPT-1 (D) proteins in supernatants were evaluated using ELISA. PBMCs (2×10^6 cells well) were incubated (6 hours, 37°C) in the absence of R-tPA (CTR) or in the presence of R-tPA (0.1 µg/mL). At the end of incubation, CXCL8 (E), VEGF-A (F), ANGPT-2 (G), and ANGPT-1 (H) mRNAs were determined using quantitative RT-PCR. The data are expressed as the mean (SEM) of 7 independent experiments. **P*<.05, ***P*<.01, ****P*<.001 vs respective control. R-tPA indicates recombinant tissue-type plasminogen activator; VEGF, vascular endothelial growth factor; ANGPT, angiopoietin; PBMC, peripheral blood mononuclear cell.

No significant changes in gene expression were observed for CXCL8, VEGFA, ANGPT-1, or ANGPT-2 mRNA after stimulation with R-tPA (Figure 3E-H), suggesting post-transcriptional regulation or inhibition of secretion.

Differential Effects of R-tPA on PBMCs from IS and ISAE Patients

PBMCs from IS patients (n=7) responded similarly to those from HCs: R-tPA treatment suppressed release of VEGF-A, ANGPT-1, and ANGPT-2 (Figure 4B-D), while CXCL8 levels remained unchanged (Figure 4A).

In contrast, PBMCs from ISAE patients (n=7) responded to R-tPA, with a significant increase in the release of VEGF-A, ANGPT-1, and ANGPT-2 (Figure 4F-H). CXCL8 levels were unaffected (Figure 4E). Fold-change analysis confirmed that R-tPA selectively enhanced vasoactive mediator secretion in ISAE-derived PBMCs, in contrast to the suppressive effect observed in IS and HC samples (Figure 4I).

Plasma Levels of Vasoactive Mediators in ISAE Patients During Angioedema and Remission

At baseline (≥1 day after the stroke and outside angioedema episodes), plasma levels of CXCL8, VEGF-A, ANGPT-1, and ANGPT-2 did not differ significantly between IS and ISAE patients (Figure 5A-D).

During angioedema attacks, plasma levels of all 4 mediators tended to increase compared to remission (Figure 5E-H). Of these, only ANGPT-2 reached statistical significance (*P*<.05),

possibly owing to sample size limitations. These data suggest transient upregulation of vascular permeability factors during angioedema episodes.

Increased cHK Levels During Angioedema in ISAE Patients

To indirectly assess generation of bradykinin, plasma levels of cHK (% of total HK) were measured in ISAE patients during angioedema attacks and during remission. As shown in Figure 6, cHK levels were modestly elevated during angioedema episodes, indicating enhanced activation of the kallikrein–kinin system.

Discussion

This study demonstrates that R-tPA exerts distinct effects on PBMCs depending on the donor source, namely, HCs, IS patients, and ISAE. Specifically, R-tPA suppressed the spontaneous release of vasoactive mediators from the PBMCs of HCs and IS patients. In contrast, the PBMCs from ISAE

Table 3. Pairwise Statistical Comparisons Between Timepoints After Stimulation With R-tPA.

Cytokine	Timepoints compared	*P* value	Significance	Cytokine	Timepoints compared	*P* value	Significance
CXCL8	5 min vs 15 min	.6495	NS	ANGPT-2	5 min vs 15 min	.9999	NS
	5 min vs 30 min	.8909	NS		5 min vs 30 min	.0205	*
	5 min vs 1 h	.9807	NS		5 min vs 1h	.1637	NS
	5 min vs 2 h	.9942	NS		5 min vs 2 h	.002	**
	5 min vs 6 h	.9714	NS		5 min vs 6 h	.8758	NS
	15 min vs 30 min	.9998	NS		15 min vs 30 min	.032	*
	15 min vs 1 h	.9886	NS		15 min vs 1 h	.2282	NS
	15 min vs 2 h	.9684	NS		15 min vs 2 h	.0032	**
	15 min vs 6 h	.4063	NS		15 min vs 6 h	.7764	NS
	30 min vs 1 h	.9994	NS		30 min vs 1 h	.9691	NS
	30 min vs 2 h	.9968	NS		30 min vs 2 h	.9748	NS
	30 min vs 6 h	.6312	NS		30 min vs 6 h	.0043	**
	1 h vs 2 h	>.9999	NS		1 h vs 2 h	.6528	NS
	1 h vs 6 h	.8025	NS		1 h vs 6 h	.038	*
	2 h vs 6 h	.8643	NS		2 h vs 6 h	.0005	***
VEGF-A	5 min vs 15 min	.9945	NS	ANGPT-1	5 min vs 15 min	.9885	NS
	5 min vs 30 min	.9415	NS		5 min vs 30 min	.1929	NS
	5 min vs 1 h	.4846	NS		5 min vs 1 h	.5618	NS
	5 min vs 2 h	.426	NS		5 min vs 2 h	.0466	*
	5 min vs 6 h	.3419	NS		5 min vs 6 h	.987	NS
	15 min vs 30 min	.9975	NS		15 min vs 30 min	.4121	NS
	15 min vs 1 h	.7677	NS		15 min vs 1 h	.8341	NS
	15 min vs 2 h	.7118	NS		15 min vs 2 h	.1279	NS
	15 min vs 6 h	.1507	NS		15 min vs 6 h	.8736	NS
	30 min vs 1 h	.9682	NS		30 min vs 1 h	.9902	NS
	30 min vs 2 h	.9507	NS		30 min vs 2 h	.9921	NS
	30 min vs 6 h	.1134	NS		30 min vs 6 h	.1469	NS
	1 h vs 2 h	>.9999	NS		1 h vs 2 h	.8453	NS
	1 h vs 6 h	.0175	*		1 h vs 6 h	.4041	NS
	2 h vs 6 h	.0139	*		2 h vs 6 h	.0432	*

Abbreviation: NS, nonsignificant

Peripheral blood mononuclear cells were stimulated with R-tPA (0.1 µg/mL) from 5 minutes to 6 hours, and mediator release (CXCL8, VEGF-A, ANGPT-2, ANGPT-1) was assessed using ELISA as shown in Figure 2A-D. Multiple comparisons between timepoints were performed using 1-way repeated measures ANOVA followed by the Tukey multiple comparisons test. **P*<.05, ***P*<.01, ****P*<.001 vs indicated timepoint.

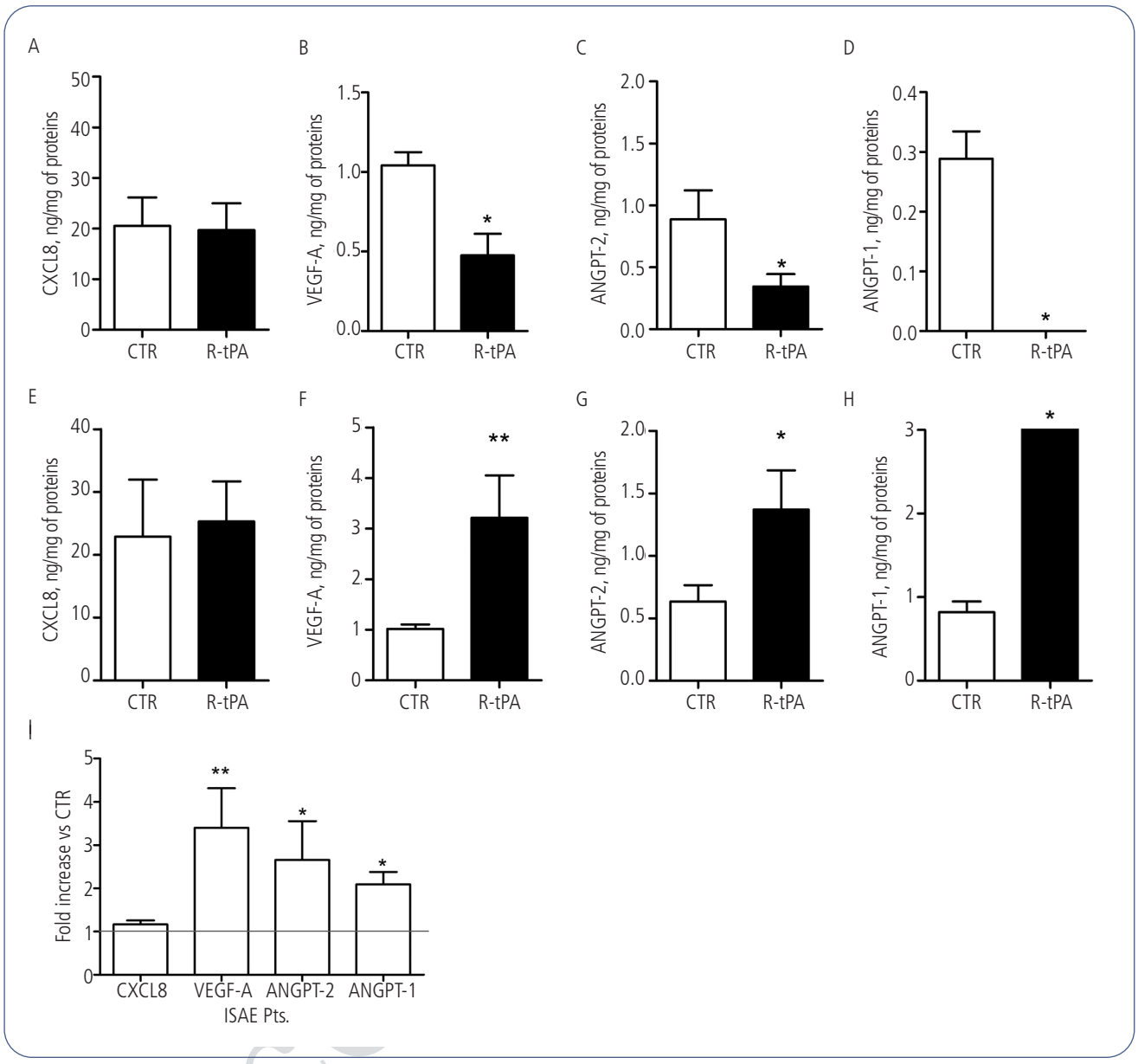

Figure 4. Effect of R-tPA on vasoactive factor release in PBMCs from IS and ISAE patients. PBMCs (2×10^6 cells/well) of 7 IS and 7 ISAE patients were stimulated (2 hours, 37°C) with medium alone (CTR) or R-tPA (0.1 µg/mL). CXCL8 (A), VEGF-A (B), ANGPT-2 (C), and ANGPT-1 (D) in supernatants were evaluated using ELISA. The results obtained were normalized for the total protein content in each well, determined in the cell lysates (0.1% Triton X-100) using the Bradford assay. Therefore, all mediator values were expressed as ng/mg of total proteins. (I) Effect of R-tPA on vasoactive mediator release of the PBMCs of ISAE patients measured as fold increase vs control. **P*<.05, ***P*<.01 vs control. R-tPA indicates recombinant tissue-type plasminogen activator; PBMC, peripheral blood mononuclear cell; IS, ischemic stroke; ISAE, ischemic stroke with angioedema; VEGF, vascular endothelial growth factor; ANGPT, angiopoietin.

patients exhibited increased release of VEGF-A, ANGPT-1, and ANGPT-2 following stimulation with R-tPA. These findings are further supported by elevated plasma levels of vasoactive mediators during angioedema attacks, especially ANGPT-2.

The 2024 global consensus (91 experts from 35 countries) updated the nomenclature and classification of angioedema, including drug-induced angioedema, which encompasses adverse reactions from agents such as R-tPA [10]. While the mechanisms of drug-induced angioedema remain incompletely understood, our results provide new insight into immune-mediated pathways that may contribute to its development.

Previous studies have reported serious allergic reactions, including angioedema, in the context of thrombolytic therapy with R-tPA (alteplase) for conditions such as IS, myocardial infarction, and deep vein thrombosis [11,13,23]. Although considered rare, TAA is a potentially life-threatening complication, with reported incidence ranging from 0.2% to 7.9% [6-9]. At our center, AORN Cardarelli Hospital, 13 of 1158 patients with IS treated with R-tPA between August 2017 and July 2022 developed TAA (1.12%). Interestingly, since

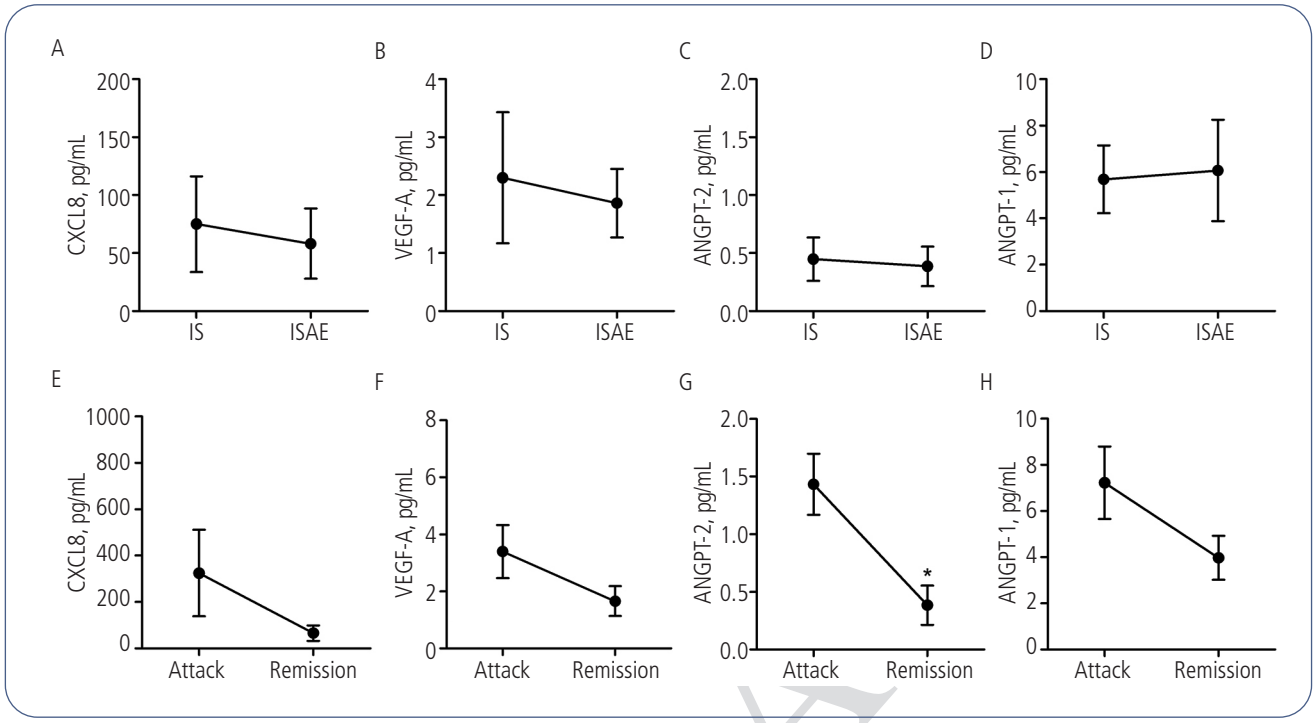

Figure 5. Plasma concentrations of CXCL8, VEGF-A, and ANGPTs in patients with IS and ISAE.
Concentrations of CXCL8 (A), VEGF-A (B), ANGPT-2 (C), ANGPT-1 (D) in 7 IS and 7 ISAE patients at least 1 day after the ischemic stroke and R-tPA treatment. Levels of CXCL8 (E), VEGF-A (F), ANGPT-2 (G), and ANGPT-1 (H) in ISAE patients during the angioedema attack (at the end of R-tPA treatment) and in remission. A *P* value ≤.05 was considered statistically significant. **P*<.05 vs attack. VEGF indicates vascular endothelial growth factor; ANGPT, angiopoietin; IS, ischemic stroke; ISAE, ischemic stroke with angioedema.

December 2021, the frequency of TAA increased to 3.54%, likely reflecting increased awareness and multidisciplinary collaboration between immunologists and neurologists, as previously reported [1,11-14]. In our cohort, angioedema typically developed within 2 hours of the R-tPA infusion and presented on the face, affecting the eyelids, lips, and tongue. We also report one of the few cases of a patient with eyelid angioedema following administration of R-tPA [6]. Moreover, it cannot be excluded that some patients developed nonvisible TAA (eg, bowel) [24] and, consequently, may have been missed.

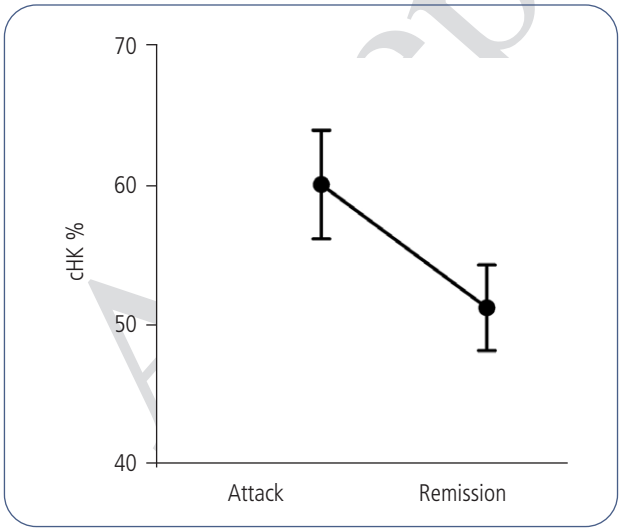

Figure 6. Plasma cHK in ISAE patients in remission and during angioedema attack. cHK measured by means of immunoblotting in plasma collected from ISAE patients during the angioedema attack (at the end of R-tPA treatment) and in remission. cHK indicates cleaved high-molecular-weight kininogen; ISAE, ischemic stroke with angioedema.

Contrary to published data, TAA in the study population was not lateralized relative to the stroke hemisphere, nor did it compromise the airways. Five of the 7 patients were treated with corticosteroids and antihistamines, and symptoms resolved across a variable timeframe (2 hours to 4 days). Bradykinin-mediated angioedema, such as that induced by ACEIs, is typically unresponsive to treatment with antihistamines, corticosteroids, and epinephrine [25]. Our findings align with published cases reporting improvement following administration of icatibant, (a synthetic bradykinin ß2-receptor antagonist) [26,27], fresh frozen plasma, or C1-INH concentrate [9,28].

The current guidelines for the management of patients with acute IS from the American Heart Association/American Stroke Association recommend airway protection when necessary and suggest using treatments targeting mast cell– and bradykinin-driven pathways. However, no specific protocols for management of TAA have been established. More data are needed to offer specialists clear rules for treatment of TAA [29].

In patients with hereditary HAE, plasma levels of vasoactive mediators such as bradykinin, ANGPTs, and VEGFs are often altered during both symptomatic and asymptomatic phases [17-19]. In contrast, our ISAE patients exhibited no difference in plasma mediator levels during remission compared

with IS controls, suggesting the absence of a predisposing "vascular preconditioning" state. Instead, plasma concentrations of ANGPT-2 increased significantly during angioedema attacks, with a similar trend observed for VEGF-A, ANGPT-1, and CXCL8. Although the limited sample size may have underpowered these comparisons, the results suggest transient mediator surges in response to immune activation.

Given the rapid onset and short half-life of bradykinin, direct measurement remains challenging [30]. To circumvent this, we assessed cHK as a surrogate marker of bradykinin release. As shown previously [31], elevated cHK during angioedema episodes supports involvement of the kallikrein–kinin system in the pathogenesis of TAA. Our findings echo preclinical and clinical studies showing that R-tPA generates large quantities of bradykinin through proteolytic cleavage of HK [4,32-34]. Importantly, only 33% of the ISAE patients in our study were receiving ACEIs at the time of their stroke, despite 85.7% being hypertensive, indicating that ACEI use is not the sole risk factor for TAA [16].

Emerging evidence implicates the role of the innate and adaptive immune responses in the pathophysiology of angioedema, with leukocytes contributing directly or indirectly, serving as both the source of vasoactive mediators and target of several stimuli [21]. Our study demonstrates that R-tPA rapidly inhibits the spontaneous release of CXCL8, VEGF-A, ANGPT-1, and ANGPT-2 from the PBMCs of HC and IS patients, with effects observable within 5 minutes of incubation and disappearing after 6 hours. mRNA levels of these vasoactive mediators were unaffected, suggesting that R-tPA may block degranulation or intracellular trafficking rather than transcription.

Interestingly, this inhibitory effect was reversed in ISAE-derived PBMCs, where stimulation with R-tPA enhanced the release of VEGF-A, ANGPT-1, and ANGPT-2. The induction time (1 hour) of vasoactive mediators by R-tPA reflected the clinical onset of TAA. While the cellular source remains unclear, further studies will be needed to clarify it.

Finally, genetic testing using a clinical exome (CES_V2) ruled out hereditary forms of angioedema in all ISAE. No pathogenic variations were detected in genes linked to HAE with normal C1-INH. This strengthens the hypothesis that TAA results from an acquired, stimulus-specific dysregulation of immune function as opposed to an underlying genetic defect.

In conclusion, our data suggest that TAA in IS patients could be due to rapid and selective activation of immune cells in susceptible patients, leading to increased release of vasoactive mediators. These mediators may then amplify vascular endothelial hyperpermeability through surface receptor engagement, culminating in the tissue edema characteristic of TAA.

Acknowledgments

We thank nurses Barbara Amoroso and Maria Maione for their contribution. We acknowledge Content Ed Net for editorial support. This service was offered freely for the project "Rare Disease Writing: tips and tricks". The project is unconditionally sponsored by BioCryst srl.

The study was partially supported by the Italian Ministry of Health – Bando Ricerca Corrente, Italian Ministry of Health ['Piano Nazionale Complementare Ecosistema Innovativo della Salute - Hub Life Science-Diagnostica Avanzata (HLS-DA)' - PNC-E3-2022-23683266 - 'INNOVA'], Italian Ministry of Education and Research - MUR ('Dipartimenti di Eccellenza' Programme 2023–27 - Dept. of Pathophysiology and Transplantation, Università degli Studi di Milano).

Funding

This work was supported in part by grants IIR-ITA-002138 from Shire (S.L.).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Data Availability

All data are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

ORCID

Francesco Palestra https://orcid.org/0000-0001-6145-7475
Maria Bova https://orcid.org/0000-0002-7655-0696
Giovanna Servillo https://orcid.org/0009-0008-5302-4889
Massimiliano Chetta https://orcid.org/0000-0001-6300-0604
Renato Ciardi https://orcid.org/0000-0003-3815-426X
Paolo Candelaresi https://orcid.org/0000-0003-2477-840x
Marina Tarsitano https://orcid.org/0009-0003-3217-1074
Chiara Suffritti https://orcid.org/0000-0002-8872-8842
Roberta Gualtierotti https://orcid.org/0000-0001-6465-7624
Stefania Miniello https://orcid.org/0000-0003-2875-4800
Anne Lise Ferrara https://orcid.org/0000-0003-1839-8873
Gilda Varricchi https://orcid.org/0000-0002-9285-4657
Raimondo Cavallaro https://orcid.org/0009-0008-7223-9615
Vincenzo Andreone https://orcid.org/0000-0002-9590-9414
Stefania Loffredo https://orcid.org/0000-0002-5871-1898

References

1. Dhillon S. Alteplase: a review of its use in the management of acute ischaemic stroke. CNS Drugs. 2012;26(10):899-926.
2. Gurman P, Miranda OR, Nathan A, Washington C, Rosen Y, Elman NM. Recombinant tissue plasminogen activators (rtPA): a review. Clin Pharmacol Ther. 2015;97(3):274-85.
3. Shoemaker LR, Schurman SJ, Donaldson VH, Davis AE, 3rd. Hereditary angioneurotic oedema: characterization of plasma kinin and vascular permeability-enhancing activities. Clin Exp Immunol. 1994;95(1):22-8.
4. Gauberti M, Potzeha F, Vivien D, Martinez de Lizarrondo S. Impact of Bradykinin Generation During Thrombolysis in Ischemic Stroke. Front Med (Lausanne). 2018;(5):195.
5. Del Rosso M, Fibbi G, Pucci M, Margheri F, Serrati S. The plasminogen activation system in inflammation. Front Biosci. 2008;13:4667-86.
6. Minami C, Araki R, Hamamoto T, Yamada H. [Orolingual Angioedema after Recombinant Tissue Plasminogen Activator Treatment in Acute Cardiogenic Cerebral Embolism Patient Using Olmesartan: A Case Report]. Yakugaku Zasshi. 2022;142(1):85-9.

7. Rathbun KM. Angioedema after thrombolysis with tissue plasminogen activator: an airway emergency. Oxf Med Case Reports. 2019;2019(1):omy112.
8. Wang YX, Li YQ, Chen Y, Zhang CH, Dong Z, Wang Z, et al. Analysis of related factors of orolingual angioedema after rt-PA intravenous thrombolytic therapy. Eur Rev Med Pharmacol Sci. 2018;22(5):1478-84.
9. Mazzoli CA, MID Angelo, Simonetti L, Cirillo L, Zini A, Gentile M, et al. Angioedema after rt-PA infusion led to airway emergency: a case report of rescue treatment with fresh frozen plasma. Braz J Anesthesiol. 2023;73(2):223-6.
10. Reshef A, Buttgereit T, Betschel SD, Caballero T, Farkas H, Grumach AS, et al. Definition, acronyms, nomenclature, and classification of angioedema (DANCE): AAAAI, ACAAI, ACARE, and APAAACI DANCE consensus. J Allergy Clin Immunol. 2024;154(2):398-411 e1.
11. Bozkurt S, Arslan ED, Kose A, Ayrik C, Yilmaz A, Dundar GA. Lingual angioedema after alteplase treatment in a patient with acute ischemic stroke. World J Emerg Med. 2015;6(1):74-6.
12. Engelter ST, Fluri F, Buitrago-Tellez C, Marsch S, Steck AJ, Ruegg S, et al. Life-threatening orolingual angioedema during thrombolysis in acute ischemic stroke. J Neurol. 2005;252(10):1167-70.
13. Frohlich K, Macha K, Gerner ST, Bobinger T, Schmidt M, Dorfler A, et al. Angioedema in Stroke Patients With Thrombolysis. Stroke. 2019;50(7):1682-7.
14. Hurford R, Rezvani S, Kreimei M, Herbert A, Vail A, Parry-Jones AR, et al. Incidence, predictors and clinical characteristics of orolingual angio-oedema complicating thrombolysis with tissue plasminogen activator for ischaemic stroke. J Neurol Neurosurg Psychiatry. 2015;86(5):520-3.
15. Ottomeyer C, Sick C, Hennerici MG, Szabo K. [Orolingual angioedema under systemic thrombolysis with rt-PA: an underestimated side effect]. Nervenarzt. 2009;80(4):459-63.
16. Mas-Serrano M, Garcia-Pastor A, Iglesias-Mohedano AM, Diaz-Otero F, Vazquez-Alen P, Fernandez-Bullido Y, et al. Related factors with orolingual angioedema after intravenous alteplase in acute ischemic stroke: results from a single-center cohort and meta-analysis. Neurol Sci. 2022;43(1):441-52.
17. Ferrara AL, Bova M, Petraroli A, Veszeli N, Galdiero MR, Braile M, et al. Hereditary angioedema attack: what happens to vasoactive mediators? Int Immunopharmacol. 2020;78:106079.
18. Loffredo S, Bova M, Suffritti C, Borriello F, Zanichelli A, Petraroli A, et al. Elevated plasma levels of vascular permeability factors in C1 inhibitor-deficient hereditary angioedema. Allergy. 2016;71(7):989-96.
19. Bova M, Suffritti C, Bafunno V, Loffredo S, Cordisco G, Del Giacco S, et al. Impaired control of the contact system in hereditary angioedema with normal C1-inhibitor. Allergy. 2020;75(6):1394-403.
20. Marceau F, Bachelard H, Charest-Morin X, Hebert J, Rivard GE. In Vitro Modeling of Bradykinin-Mediated Angioedema States. Pharmaceuticals (Basel). 2020;13(9):201.
21. Ferrara AL, Cristinziano L, Petraroli A, Bova M, Gigliotti MC, Marcella S, et al. Roles of Immune Cells in Hereditary Angioedema. Clin Rev Allergy Immunol. 2021;60(3):369-82.
22. Suffritti C, Zanichelli A, Maggioni L, Bonanni E, Cugno M, Cicardi M. High-molecular-weight kininogen cleavage correlates with disease states in the bradykinin-mediated angioedema due to hereditary C1-inhibitor deficiency. Clin Exp Allergy. 2014;44(12):1503-14.
23. Hill MD, Barber PA, Takahashi J, Demchuk AM, Feasby TE, Buchan AM. Anaphylactoid reactions and angioedema during alteplase treatment of acute ischemic stroke. CMAJ. 2000;162(9):1281-4.
24. Yakhkind A, Lang AE, Montalvo M, Beland MD, Cutting S. Gastrointestinal Angioedema as a Side Effect of Alteplase for Acute Stroke. J Vasc Interv Radiol. 2020;31(11):1921-4.
25. Mormile I, Suffritti C, Bova M. Exploring the management of recurrent angioedema caused by different mechanisms. Curr Opin Allergy Clin Immunol. 2025;25(1):47-57.
26. Cheong E, Dodd L, Smith W, Kleinig T. Icatibant as a Potential Treatment of Life-Threatening Alteplase-Induced Angioedema. J Stroke Cerebrovasc Dis. 2018;27(2):e36-e37.
27. Mas-Serrano M, Garcia-Pastor A, Tornero-Molina P, Vazquez-Alen P, Palacios-Mendoza MA, Gil-Nunez AC. [Treatment of alteplase-induced orolingual angioedema by means of icatibant]. Rev Neurol. 2019;69(6):261-2.
28. Pahs L, Droege C, Kneale H, Pancioli A. A Novel Approach to the Treatment of Orolingual Angioedema After Tissue Plasminogen Activator Administration. Ann Emerg Med. 2016;68(3):345-8.
29. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the Early Management of Patients With Acute Ischemic Stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. Stroke. 2019;50(12):e344-e418.
30. Nussberger J, Cugno M, Amstutz C, Cicardi M, Pellacani A, Agostoni A. Plasma bradykinin in angio-oedema. Lancet. 1998;351(9117):1693-7.
31. Marcos-Contreras OA, Martinez de Lizarrondo S, Bardou I, Orset C, Pruvost M, Anfray A, et al. Hyperfibrinolysis increases blood-brain barrier permeability by a plasmin- and bradykinin-dependent mechanism. Blood. 2016;128(20):2423-34.
32. Kaplan AP. Bradykinin-mediated diseases. Chem Immunol Allergy. 2014;100:140-7.
33. Caballero T, Baeza ML, Cabanas R, Campos A, Cimbollek S, Gomez-Traseira C, et al. Consensus statement on the diagnosis, management, and treatment of angioedema mediated by bradykinin. Part II. Treatment, follow-up, and special situations. J Investig Allergol Clin Immunol. 2011;21(6):422-41.
34. Molinaro G, Gervais N, Adam A. Biochemical basis of angioedema associated with recombinant tissue plasminogen activator treatment: an in vitro experimental approach. Stroke. 2002;33(6):1712-6.

Manuscript received March 13, 2025; accepted for publication October 16, 2025.

Stefania Loffredo

E-mail: stefania.loffredo2@unina.it;
stefanialoffredo@hotmail.com

Vincenzo Andreone

E-mail: vincenzo.andreone@aocardarelli.it