Article

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Enhancing Ischemic Stroke Diagnosis Using Transferrin-Conjugated Iodine Contrast Agent Liposomes: A Comparative Computed Tomography Angiography Study

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Background: Rapid and precise imaging is critical for timely therapeutic interventions that minimize brain damage in ischemic stroke. Conventional iodine-based contrast agents (ICA) enhance vascular visibility but fail to delineate cellular and molecular alterations in ischemic tissues. To bridge this gap, we developed Transferrin-Conjugated Iodine Contrast Agent Liposomes (TICA), a novel targeted agent designed to bind transferrin receptors upregulated in ischemic brain regions.

Methods: TICA was synthesized to target transferrin receptors overexpressed during ischemic injury. Its efficacy was evaluated using a comprehensive set of imaging and histological techniques in a rat Middle Cerebral Artery Occlusion (MCAO) model. Serial Computed Tomography (CT) imaging was conducted to evaluate the clarity and extent of ischemic regions. Triphenyltetrazolium chloride (TTC) staining was performed to verify the extent of ischemic damage, while immunohistochemical analysis quantified transferrin receptor expression in brain tissue. Additionally, Terminal deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) staining was employed to quantify cellular apoptosis in ischemic regions. Results obtained with TICA were compared against those from standard ICA to demonstrate the enhanced capability of TICA in identifying and characterizing ischemic damage.

Results: Initial CT imaging demonstrated that TICA provided clearer and more comprehensive visualization of ischemic regions than ICA. Longitudinal imaging further showed that TICA consistently delineated ischemic areas with greater spatial precision, findings corroborated by histopathological analysis. Collectively, these findings indicate that TICA improves both the initial identification and temporal monitoring of ischemic injury.

Conclusion: TICA represents a significant advancement in ischemic stroke imaging by offering a targeted, pathophysiology-aligned visualization of ischemic tissue. Its application improves diagnostic accuracy and may enhance treatment planning, potentially improving clinical outcomes in stroke management.

Keywords: ischemic stroke; computed tomography angiography; transferrin-conjugated liposomes iodine contrast agents; middle cerebral artery occlusion

Introduction

Ischemic stroke, which occurs when blood flow to a region of the brain is obstructed, remains a major global health concern and a leading cause of mortality and long-term disability [1–3]. According to recent data, approximately 7.8 million new cases of ischemic stroke occur worldwide each year, accounting for 62.4% of all stroke cases [4]. This condition not only contributes to significant healthcare expenditures but also imposes immense social and economic burdens on patients, families, and healthcare systems [1,5]. The rapid onset of ischemic stroke necessitates equally swift medical interventions to restore cerebral perfusion and minimize irreversible brain damage [6–8]. The multifaceted pathophysiology of stroke, characterized by a cascade of biological processes such as neuronal death, inflammation, and vascular disruption, underscores the urgent need for precise and rapid diagnostic approaches [9–11].

Although Nuclear Magnetic Resonance (NMR) imaging offers detailed visualization of brain structures due to its superior image continuity and spatial resolution, its elevated operational costs and prolonged acquisition times make it less viable for rapid diagnostic applications required in acute stroke management [12,13]. In contrast, Computed Tomography (CT) Angiography (CTA) has emerged as a pivotal diagnostic tool in emergency stroke care due to its rapid imaging capability [14–16]. CTA allows the prompt visualization of cerebral vasculature, providing essential information within therapeutic time windows necessary for

effective stroke management [17–19]. However, it is less sensitive than diffusion-weighted imaging (DWI) for detecting ischemic lesions [20] and less accurate than digital subtraction angiography (DSA) in the diagnosis of both immediate and delayed penetrating cerebrovascular injury [21]. Despite these limitations, the speed of CTA remains a decisive advantage, as it can deliver vital diagnostic insights much faster than NMR, facilitating immediate clinical decisions that are paramount for minimizing stroke-induced brain damage [14].

Traditional iodine-based contrast agents (ICA) employed in CTA, such as Iomeprol, enhance vascular visualization but often fail to capture cellular or molecular changes specific to ischemic regions [22–25]. While these agents effectively delineate blood flow and vascular architecture, they provide limited information regarding the underlying pathophysiological processes within ischemic tissue [26,27].

To overcome these constraints, this study introduces an innovative approach: Transferrin-Conjugated Iodine Contrast Agent Liposomes (TICA) [28]. TICA leverages the specificity of transferrin, a protein that is upregulated in response to ischemia-reperfusion injury associated with iron-mediated cell death [28,29]. Following ischemic stroke, hallmark features of ferroptosis, lipid peroxidation and iron accumulation are observed, accompanied by altered expression of ferroptosis-related genes (*GPX4*, *ACSL4*, and *SLC7A11*). These findings suggest that ferroptosis plays a key role in ischemic stroke pathology and may provide a novel avenue for therapeutic intervention in ischemic stroke [30]. By incorporating iodine contrast within liposomes targeted to transferrin receptors, TICA aims to provide more detailed and pathologically relevant imaging. This targeted delivery is hypothesized to enhance the visualization of ischemic regions, yielding clearer insights into the extent and localization of cerebral injury.

By focusing on the design and application of TICA for CTA in ischemic stroke, this study aims to bridge the gap between conventional imaging techniques and the demand for precise, pathologically relevant diagnostic tools. The study not only aims to improve the precision and clarity of ischemic lesion imaging but also strives to align imaging performance with the underlying biological mechanisms of ischemic injury, supporting more informed and effective therapeutic decision-making.

Materials and Methods

Animals

Thirty-three 7-week-old male Sprague-Dawley (SD) rats, weighing around 250–300 g, were procured from Hangzhou Medical College (Hangzhou, China). The animals were housed under standard laboratory conditions with a 12-hour light/dark cycle at 22 °C and 55% relative humidity. Rats had *ad libitum* access to water and standard rodent chow. All animal experiments were performed in accordance with institutional guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of Zhejiang Baiyue Biotechnology Co., Ltd. (Protocol No. ZJBYLA-IACUC-20240308). The study design, experimental procedures, and reporting of results adhered to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines to ensure high-quality, transparent, and reproducible research.

Middle Cerebral Artery Occlusion (MCAO)

Rats were anesthetized by intubation and mechanically ventilated with 3.0 vol% sevoflurane (28523-86-6, Sigma-Aldrich, St. Louis, MI, USA) in a gas mixture of 30% oxygen and 70% nitrogen [31]. The right middle cerebral artery was occluded using a 0.38 mm diameter silicone-coated monofilament (MSRC40B200PK50, Conduct Science, Skokie, IL, USA), following sequential ligation and transection of the upper thyroid artery, lower thyroid artery, pterigopalatine artery, and external carotid artery [32]. After 60 minutes of ischemia, the filament was withdrawn to allow reperfusion. Inhaled and exhaled concentrations of sevoflurane, oxygen, and carbon dioxide were continuously monitored using a Capnomac Ultima device (Datex-Ohmeda, Madison, WI, USA).

Physiological Parameter Monitoring

Body temperature was continuously monitored via rectally inserted thermoprobe and maintained at 37.5 °C using a servo-controlled heating pad (Harvard Apparatus, Hollis, MA, USA). Plasma glucose levels were measured with an Accu-Check Sensor (Roche-Diagnostic, Basel, Switzerland). Hemoglobin levels were monitored using a B-Haemoglobin Analyzer (HemoCue AB, Shanghai, China), and arterial blood gases were analyzed with an ABL5 analyzer (Radiometer Medical, Copenhagen, Denmark). These physiological parameters were recorded before and after surgery to ensure systemic stability and to minimize variability in response to cerebral ischemia (Table 1).

Contrast Agent Preparation

Two contrast agents were employed in this study. The first, an iodine-based contrast agent (ICA; lomeprol, 612.4 mg/mL, V08AB10, Milan, Italy), served as a standard control comparison. It is highly water-soluble and contains 300 mg/mL organically bound iodine, used as the imaging tracer [28]. The second, Transferrin-conjugated Iodine Contrast Agent Liposomes (TICA; transferrin:phospholipid molar ratio 1:60; phospholipid concentration 0.2 mM), was formulated for targeted delivery to ischemic brain regions overexpressing transferrin receptors.

Liposomes were prepared using a thin-film hydration technique with a lipid mixture containing cholesterol (57-

Table 1. Physiological monitoring data of experimental animals before and after Middle Cerebral Artery Occlusion (MCAO) modeling.

Parameter	Time point	Sham group	MCAO group	MCAO + TICA group	*p*-value	*F*-value
Body temperature (°C)	before modeling	37.52 ± 0.30	37.50 ± 0.24	37.45 ± 0.19	0.779	0.254
	after modeling	37.55 ± 0.19	37.52 ± 0.30	37.47 ± 0.34	0.877	0.132
Blood glucose (mmol/L)	before modeling	4.20 ± 0.37	4.10 ± 0.35	4.30 ± 0.40	0.667	0.417
	after modeling	4.13 ± 0.36	4.18 ± 0.40	4.15 ± 0.29	0.969	0.032
Hemoglobin (g/L)	before modeling	141.50 ± 13.20	140.80 ± 13.97	141.20 ± 13.60	0.996	0.004
	after modeling	142.00 ± 13.61	141.50 ± 13.90	139.43 ± 10.46	0.934	0.068
pO_2 (mmHg)	before modeling	95.17 ± 5.98	94.83 ± 5.04	97.00 ± 6.20	0.785	0.246
	after modeling	94.00 ± 5.14	96.00 ± 5.76	95.17 ± 5.31	0.815	0.207
pCO_2 (mmHg)	before modeling	38.62 ± 2.43	40.22 ± 2.79	39.40 ± 2.50	0.573	0.579
	after modeling	39.50 ± 2.50	40.62 ± 2.70	38.92 ± 2.17	0.495	0.736

TICA, Transferrin-Conjugated Iodine Contrast Agent Liposomes; pO_2, partial pressure of oxygen; pCO_2, partial pressure of carbon dioxide.

88-5, Sangon Biotech, Shanghai, China), phospholipids (11145, Sigma-Aldrich, St. Louis, MI, USA), and polyethylene glycol (PEG)-modified lipids (61909-81-7, Sangon Biotech, Shanghai, China) dissolved in chloroform (67-66-3, Sigma-Aldrich, St. Louis, MI, USA) [28]. After solvent evaporation, the thin lipid film was hydrated with an aqueous iodine contrast solution and extruded through polycarbonate membranes (WHA70604713, Sigma-Aldrich, St. Louis, MI, USA) to yield uniform liposomes [28]. Transferrin was subsequently conjugated to the surface of these liposomes to enable receptor-mediated targeting of ischemic brain tissue.

CT Imaging

Initial imaging was conducted immediately post-surgery for all groups. Before scanning, rats were anesthetized with ketamine (80 mg/kg; 1867-66-9, Sigma-Aldrich, St. Louis, MI, USA) and diazepam (10 mg/kg; 439-14-5, Sigma-Aldrich, St. Louis, MI, USA) to minimize movement and stress. Animals received either ICA or TICA via convection-enhanced delivery using an infusion syringe pump (NE-300, New Era Pump Systems, Inc., Farmingdale, NY, USA) over 30 minutes at 0.33 mL/min, with a total infusion volume of 20 µL. CT scans were obtained using the Bruker SkyScan 1276 (Bruker Corporation, Billerica, MA, USA), optimized for small-animal imaging to provide optimal contrast resolution. Standardized imaging protocols included pre-contrast and post-contrast acquisitions. Image analysis was conducted using Analyze 14.0 software (AnalyzeDirect, Inc., Overland Park, KS, USA), focusing on quantifying areas of contrast enhancement and differentiating ischemic from non-ischemic tissues. Regions of interest (ROIs) were manually defined around ischemic areas, and contrast intensity was quantitatively assessed.

Grouping

First, rats were randomly assigned to three groups (n = 3 per group): Sham (sham-operated rats), MCAO (rats subjected to MCAO), and MCAO + TICA (rats subjected to MCAO and treated with TICA).

Subsequently, rats were randomly divided into four experimental groups (n = 3 per group): Sham + TICA (sham-operated rats receiving TICA liposomes), MCAO + TICA (MCAO rats treated with TICA), Sham + ICA (sham-operated rats receiving standard ICA), and MCAO + ICA (MCAO rats treated with ICA).

Finally, serial imaging was performed at 1, 3, 5, and 7 days post-MCAO (1 d, 3 d, 5 d, 7 d groups; n = 3 per group) using both TICA and ICA to assess temporal changes in ischemic lesion development.

Triphenyltetrazolium Chloride (TTC) Staining

TTC staining was performed to evaluate ischemic damage in two independent experiments involving different rat groups and time points. In the first experiment, TTC staining was used immediately post-surgery to evaluate infarct size in the Sham (animals subjected to sham surgery without MCAO or TICA treatment), MCAO (animals subjected to MCAO without any contrast agent), and MCAO + TICA (animals subjected to MCAO and treated with TICA) groups. In the second longitudinal experiment, TTC staining was used to assess the temporal progression of ischemic damage in the 1 d, 3 d, 5 d, and 7 d groups, with the relative ischemic area calculated based on the estimated 40% brain volume initially affected on day 1.

For the staining procedure, rats were anesthetized with 3% isoflurane (26675-46-7, Sigma-Aldrich, St. Louis, MI, USA), euthanized by cervical dislocation, and their brains were sectioned coronally at 2 mm thickness. The slices were incubated in 2% TTC solution (17779, Sigma-Aldrich, St. Louis, MI, USA) at 37 °C for 30 minutes and subsequently fixed in 10% buffered formalin (E672001-

0001, Sangon Biotech, Shanghai, China). The stained sections were photographed, and images analyzed to quantify unstained (infarcted) versus stained (viable) brain regions.

Infarct rate (%) = (Infarct volume/total volume) × 100%.

Immunohistochemistry (IHC)

Immunohistochemistry was performed to examine the expression of transferrin in rat brain tissues under different experimental conditions and time points. Two independent experiments were conducted. In the first, immediately post-surgery, transferrin expression was analyzed in Sham, MCAO, and MCAO + TICA groups to determine the treatment-related changes in transferrin regulation between ischemic and non-ischemic tissues. In the second study, transferrin expression was analyzed in the 1 d, 3 d, 5 d, and 7 d groups.

Brain tissues were fixed overnight in 10% buffered formalin at room temperature, dehydrated through a graded ethanol series, cleared in xylene (A530011-0500, Sangon Biotech, Shanghai, China), and embedded in paraffin (A601801-0500, Sangon Biotech, Shanghai, China). Sections (5 µm) were cut, deparaffinized, and rehydrated. For immunostaining, sections were blocked with 1% bovine serum albumin (BSA) (10711454001, Sigma-Aldrich, St. Louis, MI, USA) for 1 hour at room temperature, followed by overnight incubation at 4 °C with an anti-transferrin primary antibody (sc-365871, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Sections were then incubated with an Horseradish Peroxidase (HRP)-conjugated anti-mouse IgG secondary antibody (sc-2005, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 hour at room temperature. After washing, the chromogenic reaction was developed using diaminobenzidine (DAB) (91-95-2, Sigma-Aldrich, St. Louis, MI, USA), counterstained with hematoxylin (H9627, Sigma-Aldrich, St. Louis, MI, USA), and visualized under a BX53 microscope (Olympus Life Science, Tokyo, Japan) at 400× magnification with a 100 µm scale bar.

Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) Staining

TUNEL staining was utilized to detect apoptotic cells within brain tissue sections from the 1 d, 3 d, 5 d, and 7 d groups. Following fixation in 10% buffered formalin and paraffin embedding, 5 µm sections were prepared, deparaffinized in xylene, and rehydrated through graded alcohols. Sections were then permeabilized with 20 µg/mL proteinase K (P8107S, New England Biolabs, Ipswich, MA, USA) for 15 minutes. Staining was conducted using a commercial TUNEL detection kit (C1091, Beyotime, Shanghai, China) following the manufacturer's instructions. After TUNEL staining, signal development was achieved with DAB, followed by counterstaining with hematoxylin. Stained sections were examined under a BX53 microscope at 400× magnification, with a scale bar of 100 µm.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA). Quantitative variables were expressed as mean ± standard deviation (SD). Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons. Pearson correlation analysis was applied to evaluate linear relationships. A p-value < 0.05 was considered statistically significant.

Results

Initial Imaging and Histological Assessments

Initial CT imaging demonstrated no signs of ischemia in the Sham + TICA and Sham + ICA groups (Fig. 1A), confirming the absence of cerebral infarction in these control animals. Conversely, the MCAO + TICA group exhibited a more clearly defined and extensive ischemic region compared with the MCAO + ICA group (Fig. 1A), indicating that TICA enhances the clarity and precision of ischemic area visualization.

Consistent with these imaging results, TTC staining performed to evaluate ischemic injury revealed extensive ischemia in both the MCAO and MCAO + TICA groups (Fig. 1B), validating the imaging findings with histopathological evidence. A thin slice taken from the largest ischemic area in the MCAO group was subjected to immunohistochemistry, which revealed a significant increase in transferrin expression, indicated by brown staining, within the ischemic regions of both the MCAO and MCAO + TICA groups, corresponding spatially to the areas of ischemic damage (Fig. 1C).

Longitudinal Imaging and Pathological Progression

Serial CT scans obtained at 1, 3, 5, and 7 days post-MCAO showed a gradual reduction of the ischemic area in both the TICA-treated and ICA-treated groups (Fig. 2A,B). However, a statistically significant reduction was observed only at day 7 for TICA-treated animals and at days 5 and 7 for ICA-treated animals compared with day 1 (Fig. 2A,B, $p < 0.05$). This trend was supported by longitudinal TTC staining of the MCAO group, which showed that the ischemic areas at 1, 3, 5, and 7 days closely paralleled the CT scan findings obtained using TICA (Fig. 2C). A statistically significant reduction in ischemic area was also observed on day 7 compared to day 1 (Fig. 2C, $p < 0.05$).

Molecular Responses and Cellular Apoptosis

In the MCAO group, transferrin expression was closely monitored at 1-, 3-, 5-, and 7-days post-surgery. Immunohistochemical analysis indicated that transferrin (indicated by brown staining) persisted throughout the seven-day period post-surgery, with staining intensity corresponding closely to the ischemic regions (Fig. 3A).

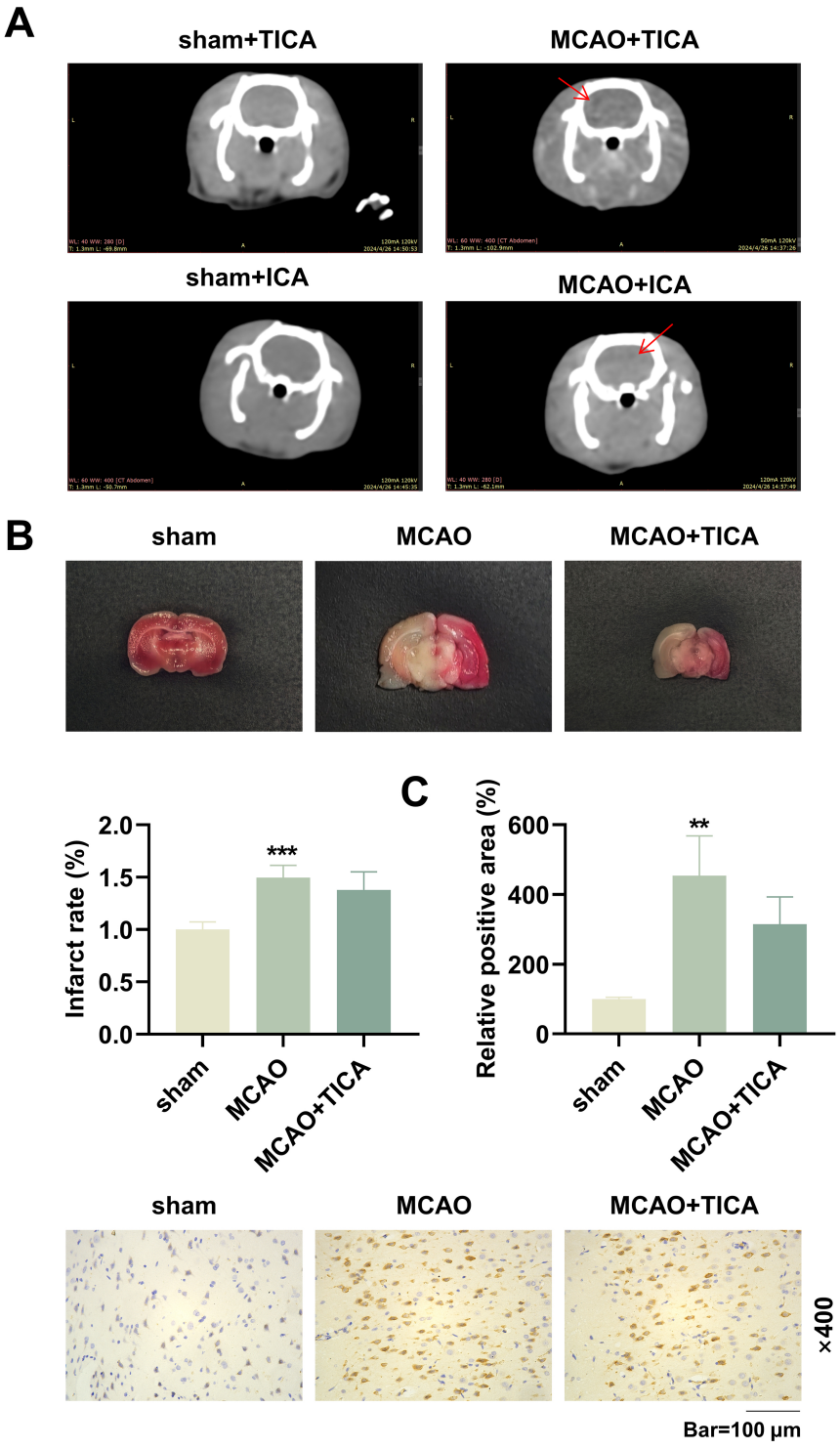

Fig. 1. Diagnostic imaging and histological assessment of middle cerebral artery occlusion (MCAO). (A) Computed tomography (CT) images of Sham + transferrin-conjugated iodine contrast agent liposomes (TICA), MCAO + TICA, Sham + iodine contrast agents (ICA), and MCAO + ICA groups showing areas assessed for ischemic damage. Images capture different cerebral regions under investigation. Red arrows indicate ischemic areas appearing as low-density shadows. (B) Triphenyltetrazolium chloride (TTC) staining of brain sections from Sham, MCAO, and MCAO + TICA groups. Red staining indicates viable tissue, while pale or white areas indicate infarcted (ischemic) tissue. (C) Immunohistochemical (IHC) staining for transferrin in brain tissues from Sham, MCAO, and MCAO + TICA groups. Brown staining indicates positive transferrin expression. Images captured at 400× magnification with a scale bar of 100 μm. Data are presented as mean ± SD (n = 3 rats/group). $^{**}p < 0.01$, $^{***}p < 0.001$ vs. Sham.

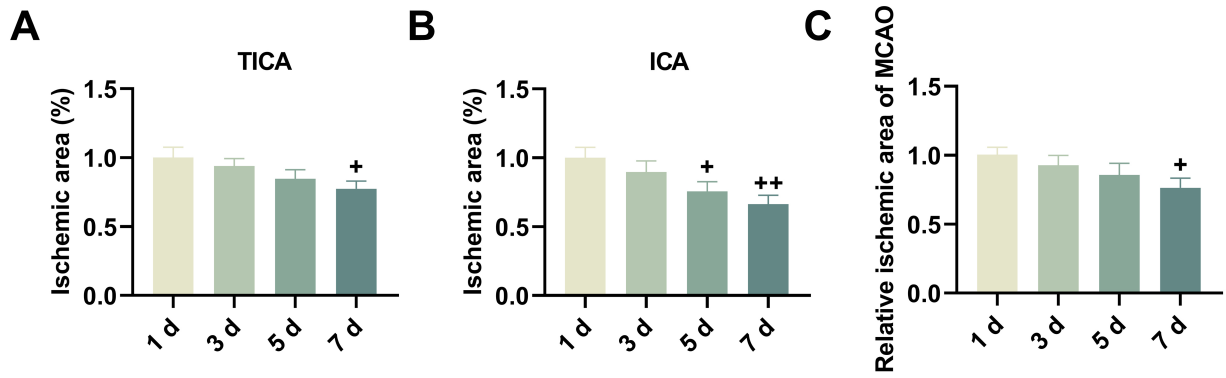

Fig. 2. Quantitative analysis of ischemic damage progression over time in different treatment groups. (A) Percentage of ischemic area in brains treated with TICA at 1, 3, 5, and 7 days post-MCAO, quantified by CT imaging. (B) Percentage of ischemic area in brains treated with ICA at 1, 3, 5, and 7 days determined by CT imaging. (C) Relative ischemic area in the MCAO group over time (1, 3, 5, and 7 days) determined by TTC staining. Data are presented as mean ± standard deviation (SD) (n = 3 rats/group). $^{+}p < 0.05$, $^{++}p < 0.01$ vs. 1 d.

Concurrently, TUNEL staining conducted at the same time points (1, 3, 5, and 7 days post-MCAO) revealed that the extent of cellular apoptosis (indicated by brown nuclear staining) within the affected regions of the brain was directly proportional to transferrin expression levels (Fig. 3B,C), showing a strong positive correlation ($r = 0.9213$, $p < 0.001$).

Discussion

The primary finding of this study, that TICA provides enhanced clarity and accuracy in imaging ischemic areas, significantly advances current understanding of targeted diagnostic approaches in stroke management. Unlike traditional ICA used in CTA, which primarily delineates vascular structures, TICA is designed to visualize molecular and cellular changes within ischemic regions. This enhanced imaging capacity for detailed visualization not only supports the hypothesis that targeted liposomal delivery systems can improve diagnostic precision but also underscores a potential paradigm shift in how contrast agents are conventionally perceived and utilized in clinical neuroimaging.

Compared with previous literature, the performance of TICA represents a significant breakthrough in stroke diagnostics. Traditional contrast agents cannot meet the diverse needs of disease diagnosis and treatment [33]. These agents typically enhance vascular structures in a generalized manner, lacking the capacity to reveal specific cellular or molecular changes that occur during acute ischemic events, and it is necessary to develop new types of contrast agents.

Transferrin molecules targeted transferrin receptor that is overexpressed in the blood-brain barrier [34]. This molecular specificity enables the contrast agent to not only visualize vascular abnormalities associated with strokes but also to highlight the underlying pathophysiological changes within the ischemic tissue. Such detailed visualization is essential for determining the extent of cerebral injury and for guiding timely and effective therapeutic interventions, particularly given the rapid progression of ischemic damage and the limited time frame within which effective treatments can be administered. This approach allows clinicians and researchers to visualize the direct molecular effects of therapeutic interventions, potentially informing the development of more effective treatments. When contrasted with conventional non-targeted contrast agents, the targeting capabilities of TICA become even more compelling, providing anatomical and molecular information within the same diagnostic framework.

In addition, the use of TICA may alter the standard procedures of stroke management protocols [35]. The current dependence on broad-spectrum imaging techniques often results in diagnostic ambiguity, leading to undertreatment or over-treatment, as these modalities may inadequately differentiate between affected and unaffected brain tissues [14]. By providing more refined and spatially accurate assessments, TICA introduces the potential for personalized and precision-guided therapeutic approaches that could substantially improve patient outcomes.

The findings of this study support the initial hypothesis that transferrin-targeted liposomes can more accurately reflect the dynamic progression of ischemic damage compared with conventional contrast agents. Furthermore, the observed gradual decrease in ischemic areas over time in serial TICA imaging underscores its potential utility not only in the early detection of ischemia but also in longitudinal monitoring of therapeutic efficacy and recovery processes.

However, several translational challenges must be acknowledged before considering clinical application. Significant interspecies differences exist between rodent and human neurovascular systems, especially regarding transferrin receptor expression patterns, distribution density, and

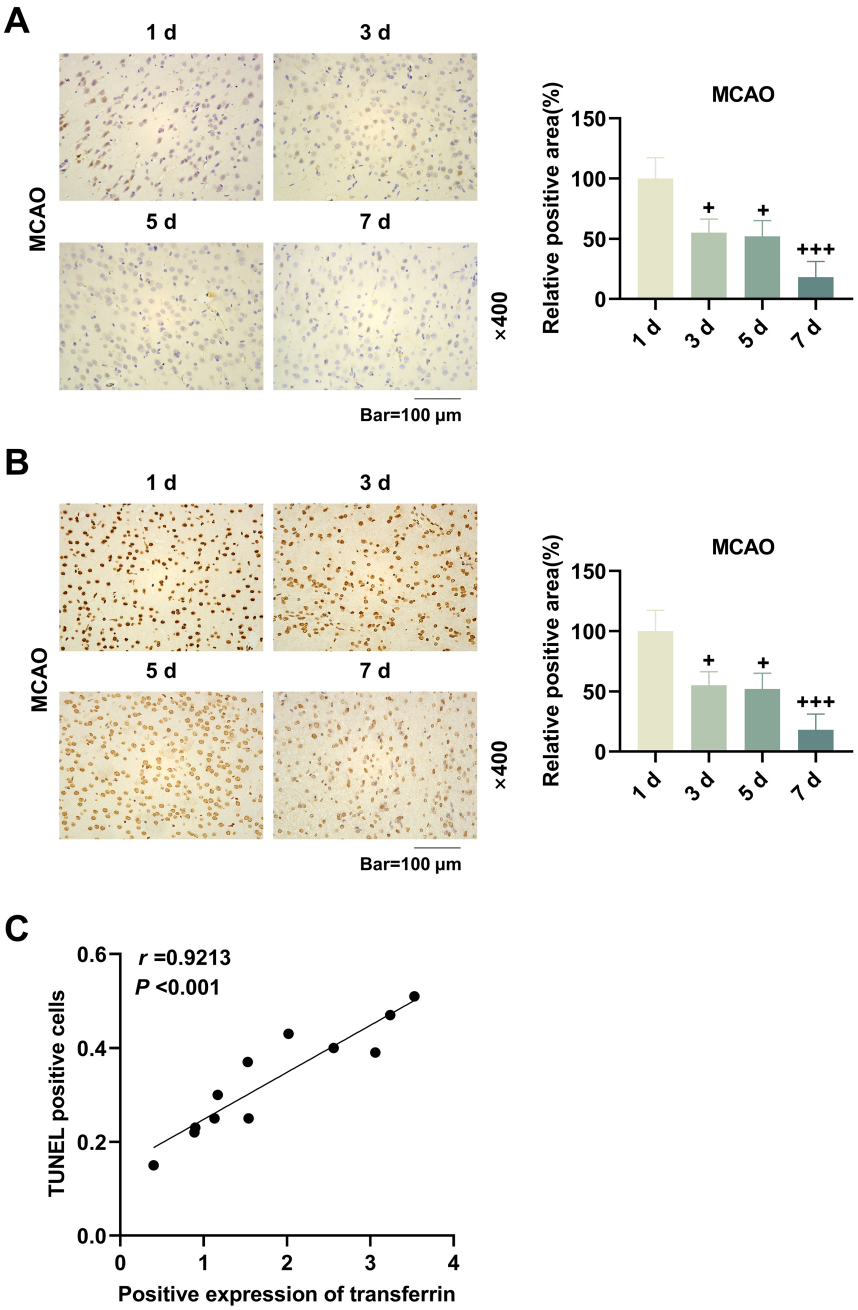

Fig. 3. Longitudinal analysis of transferrin expression and cellular apoptosis in the MCAO group post-stroke. (A) Immunohistochemical staining for transferrin in brain tissues at 1, 3, 5, and 7 days post-MCAO. Brown staining denotes positive transferrin expression. Images were obtained at 400× magnification with a scale bar of 100 μm. (B) Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining for cellular apoptotic cells within the infarct region at 1, 3, 5, and 7 days post-MCAO. Brown staining indicates apoptotic cells. Images were captured at 400× magnification with a scale bar of 100 μm. (C) Pearson correlation analysis between transferrin expression and apoptotic cell distribution based on IHC and TUNEL staining data ($r = 0.9213$, $p < 0.001$. Data are presented as mean ± SD (n = 3 rats/group). $^{+}p < 0.05$, $^{+++}p < 0.001$ vs. 1 d.

responsiveness to ischemic injury. Furthermore, the extrapolation of dosing regimens across species requires careful evaluation of pharmacokinetic and pharmacodynamic parameters that may vary substantially between rodents and humans. The optimization of convention-enhanced delivery parameters, including infusion volume, rate, and spatial distribution, represents an additional critical translational hurdle that requires systematic investigation in preclinical and clinical studies.

While TICA demonstrates promising advancements, several mechanistic questions remain unresolved. The precise molecular pathways through which transferrin targeting enhances ischemic tissue specificity are yet to be fully elucidated. Future research should aim to clarify these mechanisms and explore the potential application of TICA in other forms of vascular injury or neurodegenerative injury. Moreover, adapting similar targeted imaging strategies for alternative biomarkers could expand the utility of this approach across a broader spectrum of pathologies.

This study is not without limitations. While the low dose and localized delivery via convention-enhanced delivery (CED) likely minimize systemic exposure, the definitive safety profile of TICA warrants rigorous evaluation in future investigations. Dedicated toxicological studies should include comprehensive hematological, biochemical, and histopathological analyses of major organs under acute and chronic exposure conditions to establish their safety margin. Furthermore, comparative assessment of TICA and ICA imaging outcomes at multiple time points post-modeling should be undertaken in future work to delineate temporal dynamics more precisely. Additionally, direct physicochemical characterization data, such as conjugation efficiency and precise iodine loading, for the specific TICA batches used in this study, were not obtained due to experimental constraints. Future studies will prioritize these essential characterizations to provide direct and reproducible evidence. Moving forward, expanding the scope of this research to include multicenter collaborations and comparative studies across diverse stroke subtypes may provide more comprehensive data and support the widespread clinical adoption of TICA.

Conclusion

In conclusion, while the present findings underscore the enhanced diagnostic capabilities of transferrin-targeted liposomes in ischemic stroke imaging, they also call for cautious optimism. The promising associations and mechanisms identified in this study highlight the potential of TICA to advance molecular imaging toward greater precision and pathophysiological relevance. However, further investigation and validation through mechanistic, safety, and large-scale clinical studies are imperative to truly realize its potential in improving the diagnosis, monitoring, and management of ischemic stroke.

Availability of Data and Materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Author Contributions

HLY and LQY designed the research study; LQY and DL performed the research; JY and WZZ collected and analyzed the data. DL has been involved in drafting the manuscript and all authors have been involved in revising it critically for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

All experimentation involving animals was conducted in accordance with institutional guidelines for the care and use of laboratory animals and received approval from the Institutional Animal Care and Use Committee (IACUC) of Zhejiang Baiyue Biotechnology Co., Ltd. (ZJBYLA-IACUC-20240308).

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Conflict of Interest

The authors declare no conflict of interest.

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