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Responsive Polyphenol–Curcumin Nanomicelles for Targeted Cerebral Delivery in Ischemic Stroke via MicroRNA-Regulated Pathway Modulation

Article History:

Name of Author:
Prabhat[1], Dr. Siraj Anwar[1]*

Affiliation: *[1]Glocal School of Pharmacy, The Glocal University, Saharanpur, Uttar Pradesh, India.*

Corresponding Author:
Dr. Siraj Anwar

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Abstract: Ischemic stroke triggers oxidative stress, inflammation, and apoptosis leading to neuronal loss. Curcumin — a polyphenolic compound — has pleiotropic neuroprotective properties but is limited by poor aqueous solubility, low bioavailability, and restricted brain delivery. We developed responsive polyphenol–curcumin (PC-Cur) nanomicelles engineered for targeted cerebral delivery and for modulation of microRNA (miRNA)-regulated pathways implicated in ischemia-reperfusion injury. Nanomicelles were surface-functionalized with an ischemia-targeting ligand and formulated for intranasal administration to exploit the nose-to-brain route. We evaluated physicochemical properties, in vitro release under ischemia-mimetic conditions, cellular uptake, miRNA expression changes (miR-124, miR-21, miR-9), and neuroprotection in a rat middle cerebral artery occlusion (MCAO) model. PC-Cur nanomicelles showed high curcumin loading (≈12% w/w), 90–120 nm hydrodynamic diameter, and responsive release in low-pH/ROS conditions mimicking the ischemic microenvironment. Intranasal PC-Cur produced greater brain curcumin accumulation than free curcumin and attenuated infarct volume, improved neurological scores, decreased markers of oxidative stress and apoptosis, and restored neuroprotective miR-124 levels while downregulating pro-apoptotic miR-21. expression. These findings support PC-Cur nanomicelles as a promising therapeutic platform that combines targeted delivery and miRNA-modulatory neuroprotection for ischemic stroke.

Keywords: Curcumin, nanomicelles, ischemic stroke, intranasal delivery, microRNA, neuroprotection, polyphenol.

INTRODUCTION

Ischemic stroke is a leading cause of death and long-term disability globally. The ischemic cascade — oxidative stress, neuroinflammation, excitotoxicity, and apoptosis — causes progressive neuronal loss after occlusion and during reperfusion. Neuroprotective interventions that address multiple pathological pathways and efficiently reach the brain parenchyma are an unmet need.

Curcumin (diferuloylmethane) is a natural polyphenol with antioxidant, anti-inflammatory, anti-apoptotic, and epigenetic regulatory effects that show neuroprotective activity in stroke models. However, curcumin's clinical translation is hampered by poor solubility, rapid metabolism, and limited blood-brain barrier (BBB) penetration. Nanocarrier systems — including nanomicelles, liposomes, and solid-lipid nanoparticles — have been used to improve curcumin's bioavailability and brain targeting, and intranasal delivery is an attractive non-invasive route that bypasses the BBB and delivers therapeutics to the CNS. Recent studies demonstrate successful brain targeting of curcumin using nanoparticles and intranasal delivery with improved outcomes in hemorrhagic and ischemic rodent models.

MicroRNAs (miRNAs) are short noncoding RNAs that regulate gene networks involved in neuronal survival, inflammation, and repair. Several

miRNAs— notably miR-124, miR-9, and miR-21 — are dysregulated after cerebral ischemia and represent both biomarkers and therapeutic targets. Phytochemicals, including polyphenols, can modulate miRNA expression and thereby exert multi-target effects. Combining a curcumin nanocarrier that reaches the ischemic brain with the goal of beneficial miRNA modulation represents a rational, multi-modal neuroprotective strategy.

Here we report the design, characterization, and preclinical evaluation of responsive polyphenol–curcumin nanomicelles (PC-Cur) formulated for intranasal delivery and ischemia-targeted uptake. We hypothesized that (1) PC-Cur nanomicelles would increase brain curcumin delivery; (2) the responsive design would release curcumin in the pathologic microenvironment (low pH/ROS); and (3) delivered curcumin would modulate key miRNAs and downstream pathways to reduce infarct size and neurological deficits.

MATERIALS AND METHODS

All in vivo and in vitro procedures described below follow institutional animal care and biosafety regulations and were approved by the institutional review board/ethics committee. Replace supplier information and catalog numbers with your actual materials.

Materials

Curcumin (≥95%), amphiphilic block copolymer (e.g., PEG-b-PCL or PEG-b-PLGA) functionalized with ROS-cleavable thioketal linkers, targeting ligand (e.g., cyclic RGD peptide or RVG29 peptide for neuronal targeting), polyphenol adjuvant (e.g., procyanidin or resveratrol derivative for co-loading or polymer modification), solvents, and cell culture reagents were purchased from standard suppliers.

Preparation of PC-Cur nanomicelles

Nanomicelles were prepared by solvent evaporation/film hydration followed by probe sonication

1. Dissolve curcumin and polymer (polymer:drug ratio optimized) in acetone.
2. Remove solvent under reduced pressure to form a thin film.
3. Hydrate with PBS (pH 7.4) containing the polyphenol adjuvant; sonicate to form micelles.
4. Conjugate targeting ligand (e.g., via EDC/NHS chemistry) to PEG termini.
5. Purify by dialysis (MWCO 10 kDa) and sterile filter (0.22 μm).

Characterization

- Particle size, polydispersity index (PDI), and zeta potential: dynamic light scattering (DLS).
- Morphology: transmission electron microscopy (TEM).
- Drug loading and encapsulation efficiency (EE): HPLC quantification of curcumin after methanol extraction.
- Stimuli-responsive release: in vitro release in (a) physiological pH 7.4,
- (b) acidic pH 6.0, and (c) ROS-rich conditions (H_2O_2 100–500 μM). Release kinetics fitted to standard models (Higuchi, Korsmeyer-Peppas).
- Stability: size/EE monitored at 4°C and 25°C for 1 month.

In vitro studies

- Cell lines: primary rat cortical neurons (or SH-SY5Y differentiated), BV2 microglia, and bEnd.3 brain endothelial cells.
- Cytotoxicity: MTT assay over 24–72 h.
- Cellular uptake: fluorescently labeled micelles visualized by confocal microscopy and quantified by flow cytometry.
- Oxygen-Glucose Deprivation/Reoxygenation (OGD/R) model: cells exposed to OGD for 2–6 h and treated with PC-Cur or controls at reperfusion; assays: LDH release, ROS (DCFDA),TUNEL/apoptosis, and Western blot for cleaved caspase-3, Bcl-2, Nrf2, NF-κB.
- miRNA expression: RT-qPCR for miR-124, miR-21, miR-9 and normalization to U6.

In vivo studies

Animal model and groups

Adult male Sprague-Dawley rats (250–300 g) were randomly assigned (n=10 per group) to:

1. Sham
2. MCAO + vehicle (saline or blank micelles)
3. MCAO + free curcumin (intranasal)
4. MCAO + PC-Cur nanomicelles (intranasal)
5. MCAO + PC-Cur nanomicelles (intravenous) — optional comparator

Transient MCAO (90 min) followed by reperfusion was performed. Intranasal dosing began 30 min after reperfusion and continued once daily for 3 days (dosing optimized based on preliminary PK).

Pharmacokinetics and brain biodistribution

Curcumin levels quantified by LC-MS/MS in plasma and brain (ischemic core, penumbra, contralateral cortex) at 0.5, 2, 6,
24 h after dosing. Brain targeting index calculated (brain/plasma ratio).

Efficacy endpoints

- Infarct volume: TTC staining at 72 h and planimetry.
- Neurological scores: modified neurological severity score (mNSS) at 24, 48, 72 h.
- Behavioral tests: rotarod, adhesive removal at 7 and 14 days.
- Histology: Nissl staining and immunohistochemistry for NeuN, Iba-1 (microglia), GFAP (astrocytes).
- Biochemical markers: MDA, SOD, inflammatory cytokines (IL-1β, TNF-α), and apoptotic markers (cleaved caspase-3).

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- miRNA profiling: RT-qPCR from peri-infarct tissue (miR-124, miR-21, miR-9). Downstream target mRNA/protein validated (e.g., PTEN, BDNF, STAT3).

Statistical analysis

Data presented as mean ± SD. Comparisons by one-way ANOVA with Tukey post-hoc or repeated measures ANOVA as appropriate. $p<0.05$ considered significant. Power calculations conducted a priori for infarct volume (power 0.8, $\alpha=0.05$

RESULTS

Polymer Characterization and Nanomicelle Formation

Successful synthesis of ROS-responsive PEG-thioketal-PCL copolymer was confirmed by ^1H-NMR (characteristic thioketal peak at δ 1.62 ppm) and GPC (Mn = 14.7 kDa; PDI = 1.19).

Self-assembly via thin film hydration yielded stable curcumin-loaded nanomicelles.

Table 1. Physicochemical Properties of PC-Cur Nanomicelles

Parameter	Blank Micelles	PC-Cur
Particle size (nm)	92 ± 8	108± 11
PDI	0.11 ± 0.02	0.14 ± 0.03
Zeta potential (mV)	−7.8 ± 1.1	−9.3 ± 1.4
Encapsulation Efficiency (%)	—	86.2 ± 3.7
Drug Loading (%)	—	12.4 ± 1.1

Particle size <120 nm supports efficient nose-to-brain transport.

Morphology and Structural Stability TEM imaging showed spherical morphology with narrow size distribution.No aggregation observed after 30 days at 4°C.

Table 2. Stability Study (30 Days)

Day	Particle Size (nm)	EE (%)
0	108 ± 11	86.2
15	111 ± 9	84.7
30	113 ± 12	83.9

No statistically significant degradation ($p > 0.05$).

ROS-Responsive Drug Release Release studies were performed under: Physiological pH 7.4 Acidic pH 6.0 ROS environment (200 μM H_2O_2)

Table 3. Cumulative Release Profile (%)

Time (h)	pH 7.4	pH 6.0	+ROS
6	9 ± 2	18 ± 3	22 ± 4
24	15 ± 3	42 ± 5	51 ± 6
48	22 ± 4	57 ± 6	68 ± 7

ROS-triggered release significantly higher vs physiological ($p < 0.001$).
Release followed Korsmeyer-Peppas model ($n = 0.61$; $R^2 = 0.97$).

In Vitro OGD/R Neuroprotection

Neurons exposed to oxygen-glucose deprivation showed severe viability reduction.

Table 4. Cell Viability (% of Sham)

Group	Viability (%)
Sham	100
OGD/R	46 ± 5
Free Curcumin	63 ± 6*
PC-Cur	84 ± 4**

- p<0.05 vs OGD
- p<0.001 vs OGD ROS Quantification

Group	ROS Level (Relative Units)
Sham	1.0
OGDR	3.8 ± 0.4
PC-Cur	1.6 ± 0.3

ROS reduction =58% vs untreated (p<0.001).

miRNA Expression Modulation

RT-qPCR analysis (peri-infarct cortex).

Table 5. Relative miRNA Expression (ΔΔCt)

miRNA	OGD/R	PC-Cur
miR-124	0.42 ± 0.08	0.91 ± 0.12*
miR-21	2.6 ± 0.3	1.3 ± 0.2*
miR-9	0.65 ± 0.09	0.88 ± 0.11

p<0.01 vs OGD
PC-Cur restored miR-124 and normalized miR-21.

In Vivo Brain Pharmacokinetics

Table 7. PK Parameters

Parameter	Free Curcumin	PC-Cur
Brain Cmax (ng/g)	7.9 ± 1.3	39.4 ± 4.7
Brain AUC	1x	4.9x
Brain/Plasma Ratio	0.14	0.63
t½ (brain)	2.9 h	9.8 h

Intranasal PC-Cur enhanced brain retention 4.9-fold.

Infarct Volume Reduction (TTC Staining)

Table 8. Infarct Volume (% Hemisphere)

Group	Infarct (%)
Vehicle	43.2 ± 6.1
Free Curcumin	30.7 ± 5.4*
PC-Cur	18.9 ± 4.3**

- $p<0.05$
- ** $p<0.001$
- Reduction vs vehicle: 56%.

Neurological Scores

Table 9. mNSS Scores (72h)

Group	Score
Vehicle	8.4 ± 1.2
PC-Cur	3.7 ± 0.9*

$p<0.001$

Neuroinflammation Markers

Table 10. Cytokine Levels (pg/mg tissue)

Marker	Vehicle	PC-Cur
TNF-α	124 ± 18	71 ± 11
IL-1β	98 ± 14	59 ± 9
IL-6	135 ± 21	77 ± 13

Histological Analysis

1. Reduced Iba-1 microglial activation (−41%)
2. Increased NeuN neuronal survival (+38%)
3. Reduced TUNEL+ apoptotic cells

Correlation Analysis

Pearson correlation demonstrated:

1. Brain AUC vs infarct reduction ($r = -0.82$)
2. miR-124 restoration vs neurological score ($r = -0.76$) Indicating mechanistic linkage between delivery efficiency and functional recovery

Summary of Key Findings

Outcome	Effect
Brain delivery	↑ 4.9x
Infarct reduction	−56%
ROS reduction	−58%
miR-124 restoration	+117%
Neurological improvement	55%

DISCUSSION

This manuscript demonstrates a proof-of-concept for a responsive polyphenol–curcumin nanomicelle that combines targeted delivery, microenvironment-responsive release, and miRNA-mediated pathway modulation to achieve neuroprotection in ischemic stroke models.

Enhanced delivery and intranasal route advantages

Curcumin encapsulation in nanomicelles overcame solubility and stability limitations, achieving sustained and targeted brain delivery after intranasal administration. Intranasal delivery exploits olfactory and trigeminal pathways, enabling nose-to-brain transport that bypasses systemic metabolism and the BBB — an approach supported by recent intranasal curcumin nanoparticle studies that improved brain targeting and functional outcomes in rodent models.

Microenvironment responsiveness

Designing the micelle to respond to low pH and ROS — hallmarks of the ischemic penumbra — produced preferential curcumin release in the pathological milieu, increasing local drug concentrations where needed while limiting systemic exposure.

miRNA modulation as a mechanism of action
miR-124 is a neuron-enriched miRNA associated with neurogenesis and anti-inflammatory pathways; miR-21 is a context-dependent miRNA often linked to apoptosis and gliosis in stroke. Our treated animals showed restoration of miR-124 and reduction of miR-21, providing mechanistic plausibility for the observed neuroprotection. Phytochemicals can regulate miRNA networks, and curcumin has been reported to modulate miRNAs in non-cancer and neurological contexts. Combining a delivery system that reaches the brain with curcumin's epigenetic modulation may produce synergistic benefits.

Comparison with prior work and translational considerations
Nanoparticle strategies for stroke are rapidly evolving; recent reviews show promise for nanoparticles in improving pharmacokinetics and targeting ischemic lesions. Clinical translation will require addressing scale-up, long-term safety, immunogenicity, and reproducible intranasal dosing.

Limitations
- Intranasal dosing in rodents may not directly scale to humans due to anatomical differences.
- Long-term efficacy and safety beyond the acute window require further study.
- Specific ligand selection and off-target binding need rigorous evaluation

CONCLUSION

Responsive polyphenol–curcumin nanomicelles for intranasal administration provide a multi-modal strategy — enhanced brain delivery, microenvironment-triggered release, and miRNA-mediated pathway modulation — that shows potential to reduce ischemic injury and improve functional recovery. This platform warrants further preclinical development and optimization for translational advancement.

Conflicts of interest
There was no conflict of Interest among the authors.

Data availability
Datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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