

Intravenous Delivery of Angiopep-Functionalized Polypropylenimine Dendriplex Enhances Gene Expression in the Brain

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Background: The application of gene therapy for treating neurological disorders, including brain cancer, Parkinson's, and Alzheimer's disease, is significantly limited by the current shortage of gene vectors that can effectively cross the blood-brain barrier (BBB) following intravenous administration. Recent studies demonstrated that angiopep-2 can enhance the delivery of therapeutic agents across the BBB through receptor-mediated endocytosis. This study therefore explores the potential of angiopep-2-conjugated generation-3 diaminobutyric polypropylenimine (DAB) dendrimer (DAB-Ang) as nanocarrier for brain-targeted gene delivery.

Methods: Angiopep-2 was conjugated to DAB dendrimer and evaluated in terms of DNA condensation ability, particle size, surface charge, and structural morphology. The cellular uptake was studied in vitro using bEnd.3 brain endothelial cells, and the in vivo efficacy of DAB-Ang dendriplexes for brain gene expression was evaluated in BALB/c mice following intravenous administration.

Results: DAB-Ang dendrimer successfully condensed up to 90% of DNA, forming stable spherical dendriplexes with sizes under 240 nm and positive zeta potentials. In vitro, DAB-Ang dendriplex achieved a 9-fold higher cellular uptake in brain endothelial cells in comparison to the unmodified complex, predominantly through clathrin-mediated endocytosis and macropinocytosis. In vivo studies showed significantly increased gene expression in the brain following DAB-Ang dendriplex treatment, achieving 1.8-fold and 3.2-fold higher expression in comparison to DAB dendriplex and naked DNA, respectively, with minimal off-target effects.

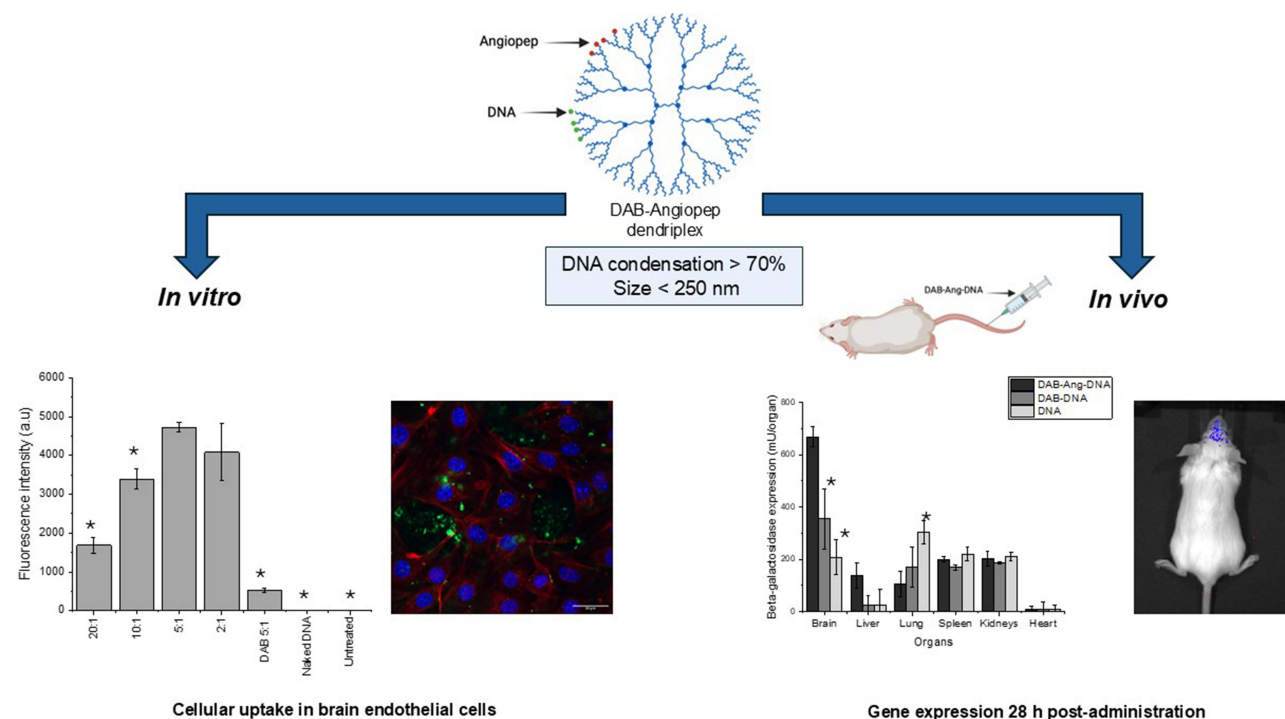
Conclusion: Angiopep-2-conjugated DAB dendrimer demonstrated high specificity and efficacy in facilitating gene delivery to the brain, offering a promising platform for therapeutic applications in neurological disorders.

Keywords: dendrimer, angiopep, brain delivery, gene expression, blood-brain barrier

Introduction

Disorders of the central nervous system (CNS), such as brain tumors, neurodegenerative conditions, and infections, often have a poor prognosis due to the challenges in achieving efficient delivery of therapeutic agents to the brain.¹ A primary obstacle in drug delivery to the central nervous system (CNS) is the blood-brain barrier (BBB), a physiological barrier composed of specialized endothelial cells and other cell types with unique macrovascular properties.² This barrier tightly regulates the transport of ions and molecules across the blood-brain interface, playing a crucial role in protecting the CNS from toxins and pathogens while maintaining CNS homeostasis, which is essential for neural function.³ However, this stringent regulation also significantly impedes the delivery of therapeutic agents to the central nervous system.

Graphical Abstract



To address the challenges posed by the BBB, extensive research has focused on modifying the physicochemical properties of therapeutic compounds, exploring alternative administration routes such as direct injections into the brain parenchyma or intranasal delivery, and developing nanocarriers capable of crossing the blood–brain barrier and transporting therapies to the brain.^{4,5} Given the genetic underpinnings of many CNS disorders, gene therapy has shown great potential as a therapeutic strategy. However, the lack of effective and safe delivery approaches that enable gene transfer across the BBB to brain tissue limits the promise of gene therapy for managing CNS-related conditions.^{6–8} It is therefore crucial to develop safe and efficient gene delivery nanocarriers that can cross the BBB and effectively transport therapeutic genes to the brain.

Dendrimers, polymeric molecules comprising a central core structure with uniform, regularly branching units with functional surface groups, have attracted interest as effective vectors for delivering therapeutic nucleic acids due to their unique branched architecture, low polydispersity index, ease of production, and the high level of control over their surface functionality.² Amongst dendrimers, generation-3 diaminobutyric polypropylenimine (DAB) dendrimer has been reported to be safer and more effective in gene transfection compared to higher-generation dendrimers.^{9–17} Additionally, generation-3 DAB dendrimer conjugated to the brain targeting ligands transferrin and lactoferrin has shown enhanced gene expression in the brain compared to other major organs following intravenous injection in mice.^{18–20}

Among various targeting moieties, angiopep-2, a 19-amino-acid oligopeptide which binds to the low-density lipoprotein receptor-related protein-1 (LRP1) overexpressed on the BBB,²¹ has been shown to significantly improve nanocarrier penetration across the BBB.^{22,23} Angiopep-2 has been conjugated to various nanoscale systems to promote effective brain delivery of therapeutic nucleic acids. For example, Gao et al demonstrated that angiopep-conjugated, poly L-lysine-bearing polyethylenimine (PEI-PLL) nanoparticles were able to successfully cross the BBB and deliver suicide genes to the striatum and cortex.²⁴ Another study showed that an angiopep-bearing polyamidoamine (PAMAM)

dendriplex was more effective than its unmodified counterpart in crossing the BBB in vitro as well as in vivo.²⁵ Similarly, Huang et al showed that an angiopep- and polyethylene glycol (PEG)-conjugated PAMAM dendrimer improved transport of DNA into brain and glioma tissues compared to unmodified PAMAM dendrimer and naked DNA.²⁶ Angiopep-2 has also been conjugated to nucleic acid-loaded cationic liposomes, enhancing the DNA delivery and gene expression within the brain and glioblastoma compared to unmodified liposomes.²⁷ Angiopep-conjugated PAMAM dendrimer has also been explored for delivery of therapeutics to the brain. For example, Sahoo et al demonstrated that an angiopep-functionalized PAMAM dendrimer significantly improved temozolomide uptake in brain tissue versus the free drug solution following systemic administration in a rat model.²⁸ However, to our knowledge, angiopep-modified DAB dendrimers have not yet been investigated for brain delivery.

This study therefore aims to investigate whether angiopep-conjugated DAB dendrimer can enhance gene delivery to brain tissue. Specifically, it involves the synthesis and characterization of DAB dendrimer functionalized with angiopep-2, and the assessment of their efficacy in transporting plasmid DNA to the brain under in vitro and in vivo conditions.

Materials and Methods

Cell Lines and Reagents

Generation-3 diaminobutyric polypropylenimine (DAB) dendrimer was purchased from SyMO-Chem (Eindhoven, Netherlands). Angiopep-2 was obtained from Biomatik (Wilmington, DE, USA).

SnakeSkin[®] dialysis tubing with a molecular weight cut-off of 3.5 kDa, N-(γ -maleimidobutyryloxy) succinimide ester (GMBS), paraformaldehyde, sucrose, normal goat serum, gelatine, and Dulbecco's Modified Eagle's Medium (DMEM) were obtained from ThermoFisher Scientific (Waltham, MA, USA). Plasmids encoding β -galactosidase (pCMVSPORT β -galactosidase) and the Quanti-iT[®] PicoGreen[®] dsDNA reagent were sourced from Invitrogen (Paisley, UK).

Plasmids encoding TNF α (pORF9-mTNF α) and tdTomato (pCMV-tdTomato) were sourced from InvivoGen (San Diego, CA, USA) and Clontech (Mountain View, CA, USA), respectively. The luciferase expression plasmid (pEF1 α -Luc) was generated by inserting the elongation factor 1 alpha (EF1 α) promoter, derived from the pEF1/Myc-His vector (Invitrogen, Paisley, UK), into the promoterless pGL3-Basic vector (Promega, Southampton, UK). All plasmids were amplified in *E. coli* and purified using the Endotoxin-free Giga Prep[®] Kit (Qiagen, Hilden, Germany).

D-luciferin (firefly) was purchased from Caliper Life Sciences (Hopkinton, MA, USA). Passive lysis buffer and Vectashield[®] medium with 4',6-diamidino-2-phenylindole (DAPI) for nuclear staining were from Promega (Southampton, UK) and Vector Laboratories (Peterborough, UK), respectively. The Label IT[®] Fluorescein Nucleic Acid Labeling Kit was sourced from Cambridge Bioscience (Cambridge, UK). Alexa Fluor[®] 647 probe, Alexa Fluor[®] 594 (goat anti-rabbit), Fluoromount-G[®], DAPI, and 7-hydroxy-9H-(1,3-dichloro-9,9-dimethyl-acridin-2-one (DDAO) were from Invitrogen (Paisley, UK). Lidocaine was purchased from Aspen Pharmacare (Durban, South Africa), and pentobarbital was obtained from Vetoquinol (Towcester, United Kingdom). The anti-DsRed (rabbit) was sourced from Clontech (Mountain View, CA, USA). The bEnd.3 murine brain endothelial cell line was obtained from LGC Standards (Teddington, UK). Any additional reagents not specified above were sourced from Sigma Aldrich (Poole, UK).

Synthesis of Angiopep-Conjugated DAB Dendrimer

Angiopep-conjugated DAB (DAB-Ang) dendrimer was prepared through a two-step synthetic method adapted from Hermanson (2013)²⁹ (Figure 1). First, DAB (20 mg) was dissolved in 2 mL of buffer containing 50 mM sodium phosphate and 0.15 M sodium chloride at pH 7.4. N-(γ -maleimidobutyryloxy) succinimide ester (GMBS) (0.67 mL, 10 mg/mL in dimethylsulfoxide (DMSO)) was then added to the DAB solution and stirred to ensure mixing for one hour at 25°C. Separately, angiopep-2 (20 mg) was dissolved in a 20 mL solution of Milli-Q grade ultrapure water (resistivity: 15.0 M Ω -cm) and acetonitrile in a 3:1 ratio. The DAB-bearing GMBS solution was then added to the angiopep-2 solution as a whole, and the pH was adjusted to 6.5 with 0.1M HCl. The resulting mixture was stirred for 16 hours at 25 °C (Figure 1). The final compound was purified using SnakeSkin dialysis membrane with a 3500 Da molecular weight cut-off, dialyzed against 1.8 L of ultrapure water at 25°C, with the water being replaced twice throughout dialysis. The

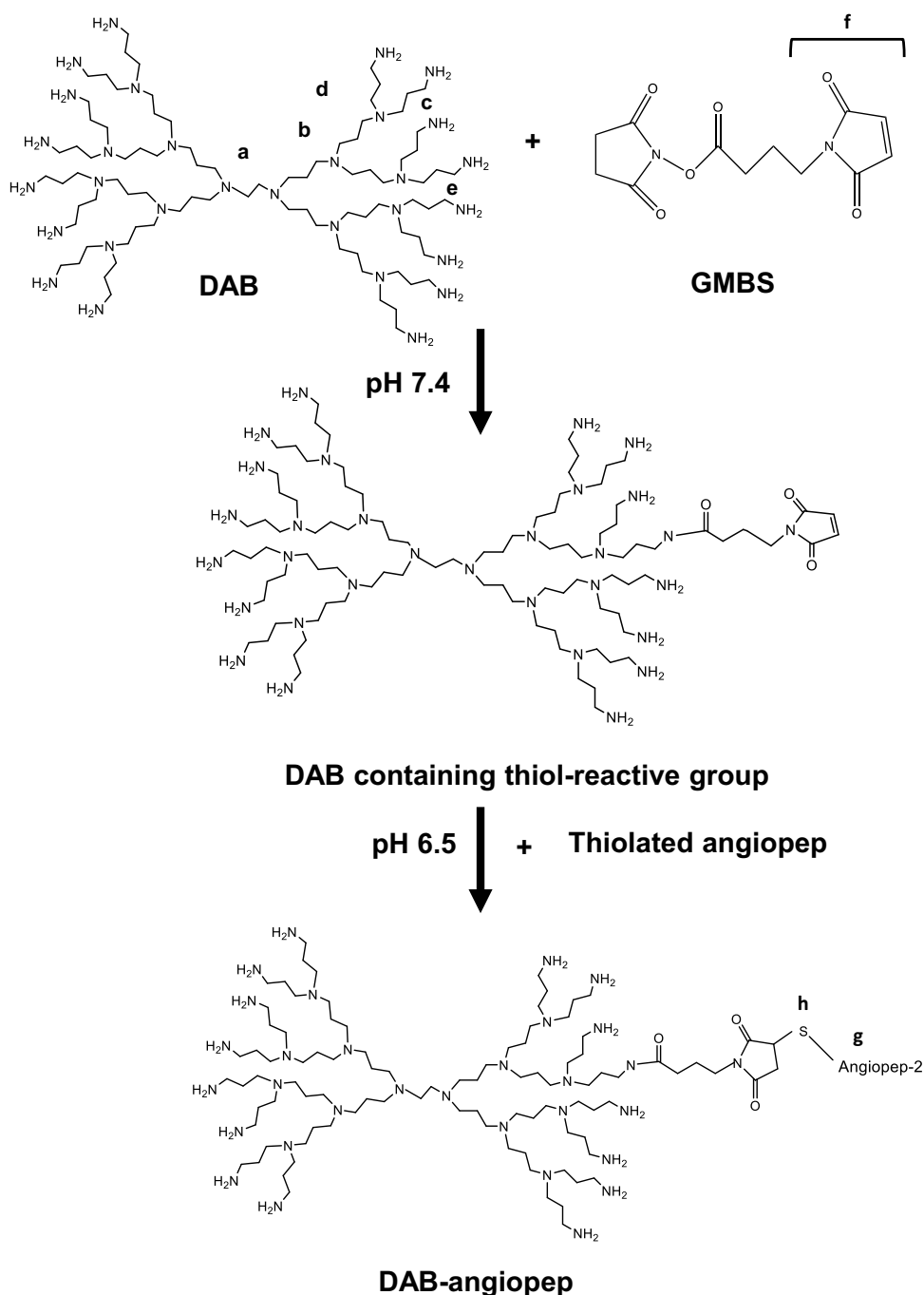


Figure 1 Synthesis of angiopep-bearing DAB dendrimer.

collected solution was subsequently frozen and lyophilized with a Christ Epsilon 2–4 LSC[®] freeze dryer (Osterode am Harz, Germany) for 48 h. The synthesis of angiopep-bearing DAB was confirmed via ¹H NMR spectroscopy performed on a Jeol Oxford NMR AS 600[®] spectrometer (Peabody, MA, USA).

The molecular weight of DAB-angiopep dendrimer was determined by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry using a Shimadzu Axima Performance[®] mass spectrometer (Shimadzu, Kyoto, Japan), where a Class 3B laser operating at 337 nm was used to generate the ions before being accelerated to 20 kV. 2,5-Dihydroxybenzoic acid (DHB) was used as the matrix, which was prepared at a concentration of 10 mg/mL in methanol. The sample was prepared by thoroughly mixing 3 μ L DAB-angiopep solution in ultrapure water with the 3 μ L

matrix solution in 1/1 (v/v) ratio. One μL of this sample mixture was then placed onto the MALDI sample plate and left for air-drying before measurement using linear mode with 3-point calibration. Mass spectrum of DAB-angiopep mainly exhibited singly charged molecular ions $[M]^+$ with their fragmented products.

DNA Condensation

The efficiency of DAB-Ang in condensing plasmid DNA was assessed both qualitatively using a gel retardation assay and quantitatively using a PicoGreen assay.

For the qualitative assessment, DAB-Ang dendriplex was prepared using dendrimer: DNA weight ratios between 20:1 and 0.5:1, with a fixed DNA concentration of 10 $\mu\text{g/mL}$. Each sample (15 μL), combined with 2 μL of loading buffer, was applied to a 0.8% (w/v) agarose gel prepared in 1 $\times$ Tris-Borate- EDTA (TBE) (89 mM Tris base, 89 mM boric acid, 2 mM Na_2EDTA , pH 8.3) and containing ethidium bromide (0.4 $\mu\text{g/mL}$). The gel was run using 1 $\times$ TBE as the running buffer and HyperLadder[®] 1kb as the DNA size marker, at 50 V for 1 hour. It was subsequently imaged under UV illumination to observe DNA condensation.

To quantitatively assess the DNA condensing capability of the DAB-Ang dendrimer, 500 μL of DNA solution (10 $\mu\text{g/mL}$ in Tris-EDTA (TE) buffer) was mixed with 500 μL of dendrimer solutions prepared at various concentrations in the same buffer. This resulted in dendriplexes formed at dendrimer: DNA weight ratios of 20:1, 10:1, 5:1, 2:1, 1:1, and 0.5:1. Throughout the experiment, the DNA concentration in all complexes was consistently maintained at 10 $\mu\text{g/mL}$. Subsequently, 1 mL of PicoGreen reagent, diluted 200-fold in the same buffer, was added to the dendriplexes. The fluorescence intensity of PicoGreen was recorded at multiple time points (0, 1, 2, 4, 6, and 24 hours) using a Varian Cary Eclipse[®] spectrofluorometer (Palo Alto, CA, USA), with excitation and emission wavelengths set at 480 nm and 520 nm, respectively.

Morphology of Dendriplexes

Transmission electron microscopy (TEM) was used to examine the morphology of angiopep-functionalized DAB dendriplexes. Both DAB-angiopep and unmodified DAB dendriplexes were formulated at a dendrimer: DNA weight ratio of 5:1, using a dendrimer concentration of 400 $\mu\text{g/mL}$ in 5% glucose solution. These preparations were then diluted 100-fold with ultrapure water. A 5 μL aliquot of the final diluted sample was applied to a carbon-coated copper grid (400 mesh size) and left to air dry overnight. Transmission electron microscopy was conducted using a Jeol JEM-1400 Flash[®] microscope (Jeol, Peabody, MA, USA) operated at an accelerating voltage of 80 kV. Images were acquired with a Jeol Flash[®] CCD camera using TEM Center[®] software version 1.7.26.3016 (Jeol, Peabody, MA, USA).

Size and Zeta Potential of Dendriplexes

Photon correlation spectroscopy was used to determine the size and zeta potential of the dendriplexes. Dendrimer solutions (500 μL in 5% glucose solution) were prepared at concentrations to yield dendriplexes of dendrimer: DNA weight ratios of 20:1, 10:1, 5:1, 2:1, 1:1, and 0.5:1, with each ratio prepared in quadruplicate. DNA solution (500 μL at 100 $\mu\text{g/mL}$ in 5% glucose solution) was added to the dendrimer samples by pipetting and thoroughly mixing. Measurements of particle size and zeta potential were carried out at 37°C using a Malvern Zetasizer Nano-ZS[®] (Malvern Instruments, Malvern, UK).

Cellular Uptake

Confocal microscopy was used to qualitatively evaluate the cellular uptake of fluorescein-labelled DNA complexed with DAB-Ang. Cells were seeded onto coverslips placed in 6-well plates at a density of 100,000 cells per well and incubated for 24 hours. Subsequently, they were treated with DAB-Ang dendrimer complexed with fluorescein-labelled DNA encoding β -galactosidase at dendrimer: DNA weight ratios of 20:1, 10:1, 5:1, and 2:1. As a positive control, cells were exposed to DAB-DNA complex at a dendrimer: DNA ratio of 5:1, while naked DNA-treated and untreated cells served as negative controls. The DNA concentration was maintained at 2.5 μg per well throughout the experiment. To prepare the slides for imaging, each well was rinsed three times with 3 mL of phosphate-buffered saline (PBS) followed by fixation with 3 mL of 3.7% w/v formaldehyde solution for 15 min at 25°C. The wells were then washed again three times

with 3 mL of PBS and incubated with 3 mL of 0.1% Triton X-100 solution for 15 minutes to permeabilize the cells. To minimize non-specific binding, the cells were blocked using 3 mL of 1% w/v bovine serum albumin (BSA) in PBS for 30 minutes at 25 °C. Alexa Fluor 647 dye (1 unit) was diluted with 200 µL of PBS, added to the wells, and incubated for 45 min at 25°C. The cells were subsequently mounted on glass slides using Vectashield mounting medium containing DAPI and incubated in the dark at 25 °C for 2 hours prior to imaging. Confocal microscopy was performed using a Leica TCS SP5[®] microscope (Wetzlar, Germany). DAPI-stained nuclei were excited with a 405 nm laser (emission bandwidth: 415–491 nm), fluorescein-labelled DNA was excited at 514 nm (emission bandwidth: 550–620 nm), and Alexa Fluor 647, which stained the cell membranes, was excited at 633 nm (emission bandwidth: 650–685 nm).

Cellular uptake was also quantitatively assessed using flow cytometry. Plasmid DNA was fluorescently labelled with fluorescein using the Label IT Nucleic Acid Labelling Kit, in accordance with the manufacturer's protocol.

Cells were seeded in 6-well plates at a density of 100,000 cells per well and incubated for 24 hours. Treatments were applied under the same conditions used for the confocal microscopy experiments. After 24 hours of incubation, the cells were washed three times with 3 mL of PBS. Single-cell suspensions were prepared by adding 250 µL of trypsin per well, incubating at 37 °C for 5 minutes, and then neutralizing with 500 µL of FACS buffer (PBS buffer supplemented with 0.5% BSA and 2 mM EDTA). Fluorescence intensity was then measured using an Attune NxT[®] flow cytometer (Thermo Fisher Scientific, Waltham, MA, USA), analyzing 10,000 gated events per sample.

Mechanism of Cellular Uptake

The mechanism of cellular uptake of DAB-Ang dendriplex was investigated using inhibitors targeting major endocytic pathways. Cells were seeded in 6-well plates at a density of 100,000 cells/well and incubated at 37°C for 24 hours. They were then pre-treated for 20 minutes at 37 °C with various inhibitors: free angiopep (80 µmol/L), colchicine (10 µmol/L), phenylarsine oxide (10 or 20 µmol/L), filipin (5 or 10 µg/mL), and poly-L-lysine (40 µg/mL). Following this pre-treatment, DAB-Ang dendrimer complexed with fluorescein-labelled DNA at a dendrimer: DNA weight ratio of 5:1 was added to the cells and incubated for 24 hours. The DNA concentration was consistently maintained at 2.5 µg/well throughout the experiment. After incubation, the samples were processed for confocal microscopy and flow cytometry as described above.

Biodistribution of Gene Expression

Female BALB/c mice were housed in groups of five under controlled conditions (19–23 °C) with a 12-hour light/dark cycle. Standard laboratory mouse diet and water were available *ad libitum*. All experimental procedures were conducted in accordance with the UK Home Office regulations (PPL0688944, PPL7467535) and received approval from the institutional ethics committee (Animal Welfare and Ethical Review Body (AWERB), University of Strathclyde).

To determine the optimal treatment duration for achieving maximal gene expression in the brain, female BALB/c mice received a single intravenous injection of DAB-Ang dendrimer complexed with a luciferase expression plasmid at a dendrimer: DNA ratio of 5:1 (50 µg of DNA). At designated intervals post-injection, the mice were administered the luciferase substrate D-luciferin (150 mg/kg) intraperitoneally and anesthetized using isoflurane. Bioluminescence imaging was performed using the IVIS Spectrum[®] system (PerkinElmer, Waltham, MA, USA) to assess biodistribution. Emitted light was recorded for 2 minutes using Living Image[®] software, with pseudo-color overlays depicting the spatial distribution of photon emissions within the animals. All images were captured using identical illumination settings to ensure consistency.

A similar procedure was followed to evaluate gene expression levels among the DAB-Ang dendriplex, unmodified DAB dendriplex, and naked plasmid DNA. Imaging was performed at the previously determined optimal time point for peak gene expression.

Biodistribution was assessed using a β -galactosidase reporter gene expression assay. Groups of mice (n=5) received a single intravenous injection of either DAB-Ang dendriplex encoding β -galactosidase, unmodified DAB dendriplex with the same plasmid, or naked plasmid DNA. At the previously identified peak expression point, animals were euthanized, and major organs, including brain, heart, lungs, spleen, liver, and kidneys, were harvested and immediately frozen in liquid nitrogen. Tissues were weighed prior to further processing for analysis. To homogenize the organs, 1 mL of freshly

prepared homogenization buffer (2 mL for the liver) (consisting of 20 $\mu\text{L/mL}$ protease inhibitor cocktail, 40 $\mu\text{L/mL}$ of 50 mM phenylmethylsulfonyl fluoride and 200 $\mu\text{L/mL}$ passive lysis buffer in ultrapure water) was added to the organs. The tissues were homogenized using a Bead ruptor 4[®] homogenizer (OMNI international, GA, USA). A 100 μL aliquot of the homogenized tissue was then mixed with 300 μL of reaction buffer, consisting of 25 $\mu\text{g/mL}$ DDAO galactoside, 33 $\mu\text{L/mL}$ of 50 mM phenylmethylsulfonyl fluoride, 33 $\mu\text{L/mL}$ protease inhibitor cocktail, and 67 $\mu\text{g/mL}$ maltose in PBS. The mixture was incubated at 37 °C for 90 minutes (reduced to 45 minutes for liver tissue) with vortexing performed at 15-minute intervals. After incubation, 200 μL of the mixture was heated to 95 °C for 2 minutes, then mixed with 800 μL of isopropanol to extract the reaction product. The samples were vortexed and shaken at 5 °C for 20 minutes, followed by centrifugation to separate the reaction product. Fluorescence in the resulting supernatant was measured using a Varian Cary Eclipse spectrofluorometer (Palo Alto, CA, USA), with excitation and emission wavelengths set at 630 nm and 650 nm, respectively.

Distribution of Gene Expression Within the Brain

To evaluate the distribution of gene expression within the brain, four mice received a single intravenous injection of DAB-Ang complexed to plasmid DNA encoding either tdTomato ($n = 2$) or a control gene, β -galactosidase ($n = 2$), at a dendrimer: DNA weight ratio of 5:1 (with 50 μg of DNA). Additional groups were administered naked plasmid DNA encoding either tdTomato or β -galactosidase. The DNA dose was standardized at 50 μg across all treatment groups. Twenty-eight hours after injection, mice were anesthetized via intraperitoneal administration of 0.1 mL pentobarbital (Dolethal[®]). Transcardial perfusion was then performed using 0.1 M PBS, followed by 4% paraformaldehyde in 0.1 M PBS. The brains were removed and immersed in the same fixative solution overnight at 4 °C, and then transferred to a 30% sucrose solution for storage at the same temperature.

For histological staining and imaging, the brains were sectioned coronally at a thickness of 80 μm using a Leica SM2010R[®] microtome (Wetzlar, Germany) and placed in wells containing 0.1 M PBS. The sections were incubated in a blocking solution consisting of 10% normal goat serum (NGS) in 0.3% Triton-X in PBS (PBST) for 1 hour at room temperature. Subsequently, the sections were incubated overnight at 4 °C with the primary antibody (rabbit anti-DsRed, 1:1000) diluted in 3% NGS/PBST. After three washes with 350 μL PBS, the brain sections were incubated with a secondary antibody (Alexa Fluor 594 goat anti-rabbit, 1:1000) diluted in 3% NGS/PBST for 2 hours at room temperature in the dark. Following another set of three PBS washes (350 μL each), the sections were stained with DAPI (1:1000 in PBS) for 10 minutes at room temperature, then washed again. The sections were then mounted on glass slides using a 0.5% gelatin solution and allowed to dry overnight in the dark. On the following day, the sections were cover-slipped with Fluoromount-G, an antifade mounting medium. Imaging was carried out with a Nikon Eclipse E600[®] epifluorescence upright microscope (Tokyo, Japan), and image analysis was performed using ImageJ software.

Statistical Analysis

Results were presented as mean $\pm$ standard error of the mean (SEM). Statistical significance was evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. For paired comparisons, unpaired t -tests were applied (Minitab[®] software, State College, PE). A p -value below 0.05 was considered statistically significant.

Results

Synthesis of Angiopep-Conjugated DAB Dendrimer

The successful conjugation of angiopep-2 to the DAB dendrimer via an amide bond (formed between the amino groups of the GMBS crosslinker and the thiol groups of the thiolated angiopep) was confirmed by ¹H-NMR spectroscopy (Figure 2). The NMR spectra were analyzed for DAB, the GMBS cross linker, angiopep-2, and the final DAB-Ang compound. Successful conjugation was confirmed as follows: ¹H-NMR (D_2O): δ DAB ($\text{N-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}$) at 1.43 ppm (a), δ DAB ($\text{N-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}$) at the peak at 1.59 ppm (b); δ DAB ($\text{N-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}$) at 2.48 ppm (c); δ DAB ($\text{N-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}$) at 2.62 ppm (d); δ DAB ($\text{N-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2$) at 2.98 ppm (e). For the GMBS cross-

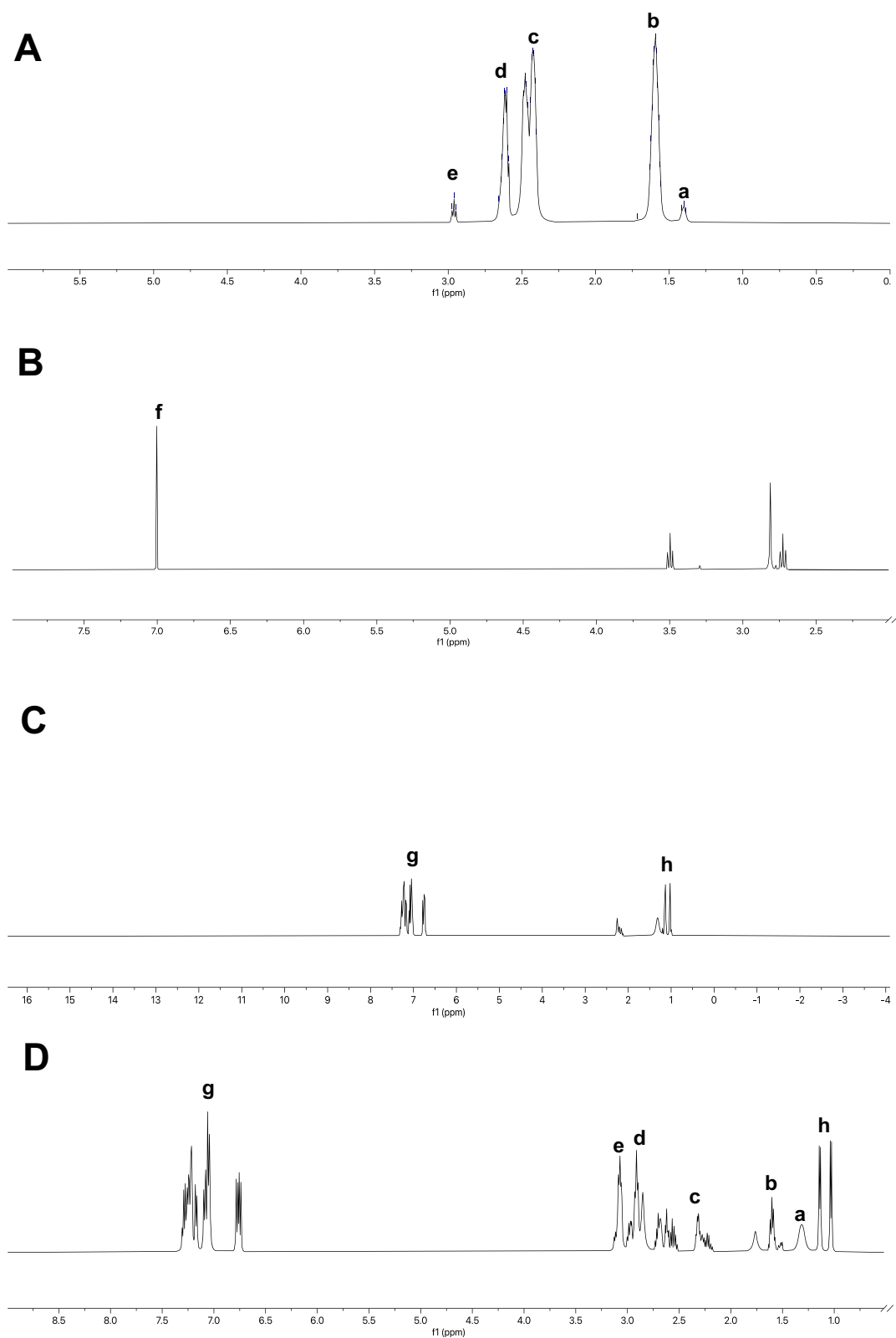


Figure 2 ^1H -NMR spectra of DAB (A), GMBS (B), angioprep-2 (C), and DAB-Ang (D).

linker, characteristic peaks were observed at 2.72, 2.81, and 3.5 ppm, corresponding to protons on the carbon backbone, while the proton signal from the maleimide group appeared at 7 ppm (f). The characteristics peaks of thiolated angioprep were observed in the region between 6.7 and 7.2 ppm (g), corresponding to the benzene rings of angioprep. The hydrogen of the sulfur in the thiol group was observed at 1.17 ppm (h), and the protons of the carbons adjacent to the sulfur appeared at 2.15 ppm. Further evidence of successful conjugation was provided by the disappearance of the sharp peak at 7 ppm in the GMBS spectrum, which corresponds to the maleimide proton. This loss indicates the formation of a thioether bond between the maleimide group of GMBS and the thiol group of thiolated angioprep (Figure 2).

The molecular weight of DAB-angioprep dendrimer was assigned by analyzing MALDI-TOF mass spectrum (Figure 3), which mainly exhibited singly charged molecular ions $[M]^+$ with its fragmented products. It was 4779.25 Da, indicating that the ratio of the conjugated angioprep per dendrimer was nearly 1 (Figure 3). Conjugation of the peptide on one amine group of dendrimer helped to retain more primary amine groups on the surface of the dendrimer, leaving them free for complexation of nucleic acids.

DNA Condensation

The DNA condensation capacity of the DAB-Ang dendrimer was evaluated using a gel retardation assay (Figure 4). At dendrimer: DNA weight ratios of 20:1, 10:1, 5:1, and 2:1, no DNA migration was observed on the gel, indicating that the DAB-Ang dendrimer effectively condensed the DNA at these ratios, resulting in minimal free DNA available to migrate through the gel. In contrast, at dendrimer: DNA weight ratios of 1:1 and 0.5:1, a migration pattern similar to that of naked DNA was observed. At the 0.5:1 ratio, the presence of a diffuse smear rather than distinct bands suggests partial complexation, suggesting that the DNA was not efficiently complexed to the polymer and was able to migrate through the gel (Figure 4A).

These findings were further confirmed and quantified using the PicoGreen assay (Figure 3B). The DAB-Ang dendrimer successfully condensed $77.7 \pm 0.43\%$, $74.1 \pm 0.2\%$, $70.9 \pm 0.1\%$, and $66.4 \pm 0.3\%$ of DNA at dendrimer: DNA weight ratios of 20:1, 10:1, 5:1, and 2:1, respectively, immediately after complexation ($T=0$). Over time, DNA condensation gradually increased, reaching $90.4 \pm 0.5\%$, $89.5 \pm 2.0\%$, $90.9 \pm 0.8\%$, and $85.4 \pm 3.6\%$, respectively, and the dendriplexes remained stable over 24 hours. In contrast, lower DNA condensation levels of $25.6 \pm 1.2\%$ and $30.4 \pm 0.1\%$ of DNA were detected at the lower dendrimer: DNA weight ratios of 1:1 and 0.5:1 (Figure 4B).

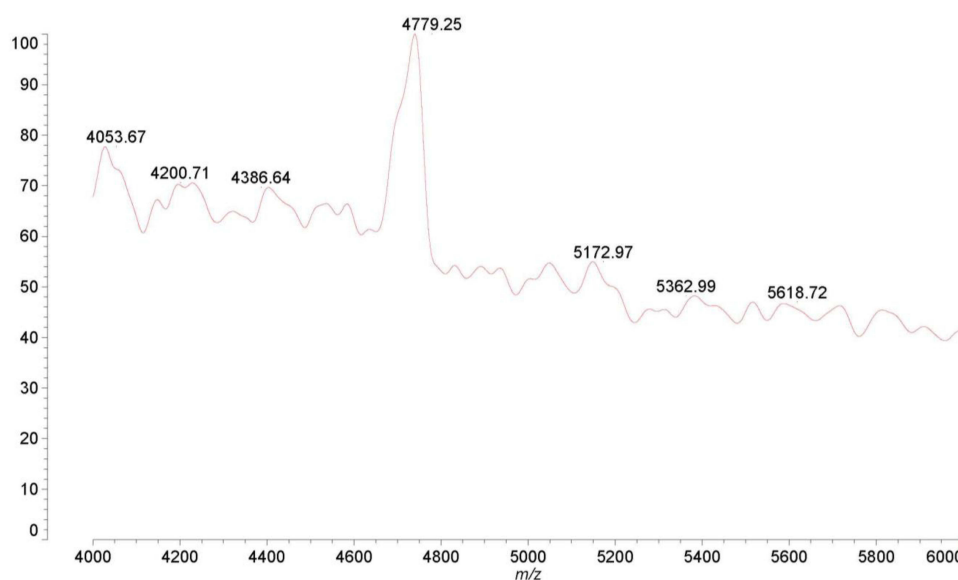


Figure 3 MALDI-TOF MS spectrum of DAB-angioprep.

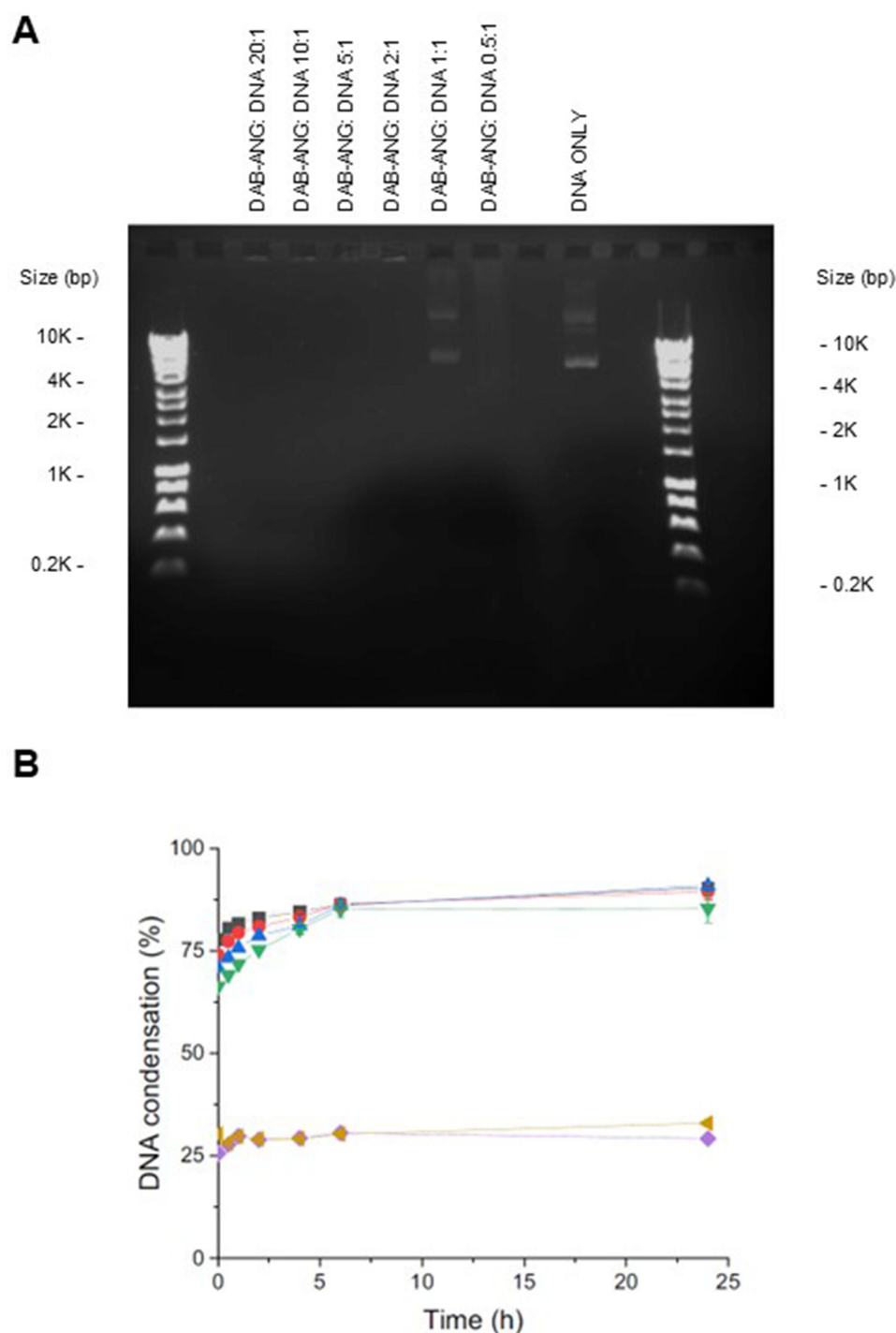


Figure 4 Evaluation of DNA condensation efficacy of DAB-Ang at various dendrimer: DNA weight ratios using gel retardation assay (**A**) and PicoGreen assay (**B**) (dendrimer: DNA weight ratios 20:1 (black), 10:1 (red), 5:1 (blue), 2:1 (green), 1:1 (yellow), and 0.5:1 (violet)) (n=4).

Morphology of Dendriplexes

TEM analysis of the DAB-Ang dendriplex showed spherical nanoparticles with an average diameter below 200 nm, similar to the size observed for the unmodified DAB dendriplex (Figure 5).

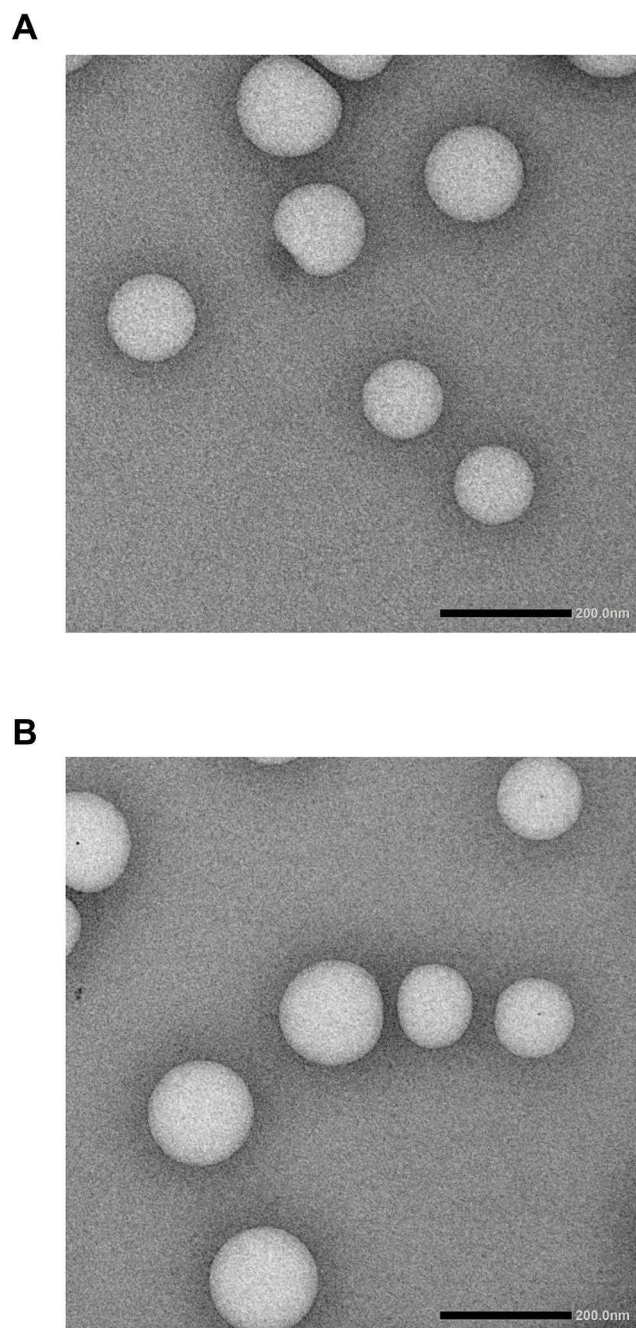


Figure 5 TEM images of DAB-Ang dendriplex (A) and DAB dendriplex (B).

Size and Zeta Potential of Dendriplexes

Across all tested weight ratios, the DAB-Ang dendriplexes displayed average sizes below 300 nm (Figure 6). The smallest particles, measuring 65 ± 4 nm, were observed at a dendrimer: DNA ratio of 5:1, whereas the largest, 241 ± 34 nm, were observed at a 20:1 ratio. A low polydispersity index (less than 0.3) was obtained at all the tested ratios, indicating a uniform particle size distribution.

The zeta potential analysis showed a consistent positive charge of 35.7 ± 1.5 mV, 29.51 ± 3.1 mV, and 30.03 ± 1.4 mV for dendriplexes at dendrimer: DNA ratios of 20:1, 10:1, and 5:1, respectively. However, at lower dendrimer: DNA weight ratios of 2:1, 1:1, and 0.5:1, a notable decrease in charge was observed, resulting in negative zeta potentials of -16.7 ± 5.0 mV, -27.7 ± 3.0 mV, and -28.6 ± 1.4 mV, respectively. These values were close to the zeta potential of naked

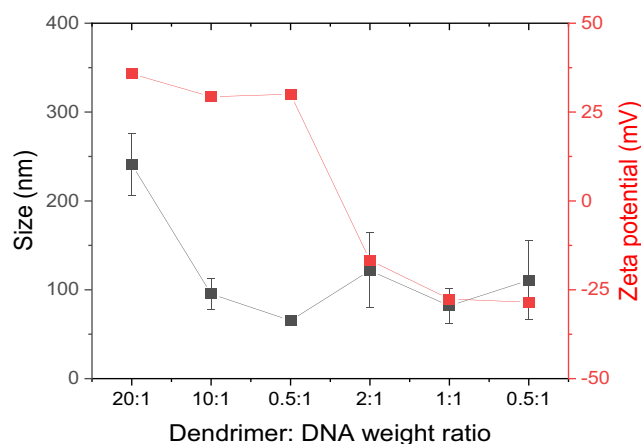


Figure 6 Size and zeta potential of DAB-Ang dendriplex at various dendrimer: DNA weight ratios (n=4).

DNA (-36.5 ± 3.0 mV). At higher dendrimer: DNA weight ratios, most of the DNA was complexed to the polymer, leaving minimal free DNA in the solution and resulting in a positive zeta potential. In contrast, lower dendrimer: DNA weight ratios resulted in a negative zeta potential, attributed to the presence of unbound, negatively charged DNA in the solution, indicating reduced condensation efficacy at these ratios (Figure 6).

Cellular Uptake

The cellular uptake of DAB-Ang dendriplexes carrying fluorescein-labelled DNA was initially assessed by confocal microscopy in bEnd.3 murine brain capillary endothelial cells (Figure 7). Cells treated with DAB-Ang dendriplexes at dendrimer-to-DNA weight ratios of 20:1, 10:1, and 5:1 exhibited markedly greater fluorescence intensity than those treated with unmodified DAB dendriplex or naked DNA.

Flow cytometry analysis supported these findings, showing that DAB-Ang dendriplexes achieved higher uptake than the positive control (DAB dendriplex) across all ratios tested (Figure 8). The peak uptake occurred at a dendrimer: DNA ratio of 5:1, with a fluorescence intensity of 4729 ± 113 a.u., which was approximately 10-fold higher than that of the positive control (529 ± 54 a.u.).

Mechanism of Cellular Uptake

To investigate and confirm the cellular uptake mechanism of dendriplexes in bEnd.3 cells, the cells were pre-treated with various inhibitors targeting specific cellular uptake pathways.

Confocal microscopy revealed that the green fluorescence of the fluorescein-labelled DNA was visible for all tested inhibitors, with variable intensities indicating the degree of inhibition (Figure 9). Cells pre-treated with angiopep-2 peptide, poly-L-lysine, or filipin exhibited higher fluorescence intensity, whereas those pre-treated with chlorpromazine or colchicine showed lower fluorescence levels (Figure 9).

Flow cytometry confirmed these findings (Figure 10). Colchicine, which inhibits macropinocytosis, and chlorpromazine, a clathrin-mediated endocytosis inhibitor, resulted in the highest reduction in uptake in bEnd.3 cells, with decreases of 87% and 81%, respectively. Filipin, an inhibitor of caveolae-mediated processes in clathrin-independent endocytosis, along with angiopep-2 peptide (a substrate for LRP1 receptor) and poly L-lysine, an inhibitor of cationic molecule uptake, induced a lower degree of inhibition, approximately 55.5%.

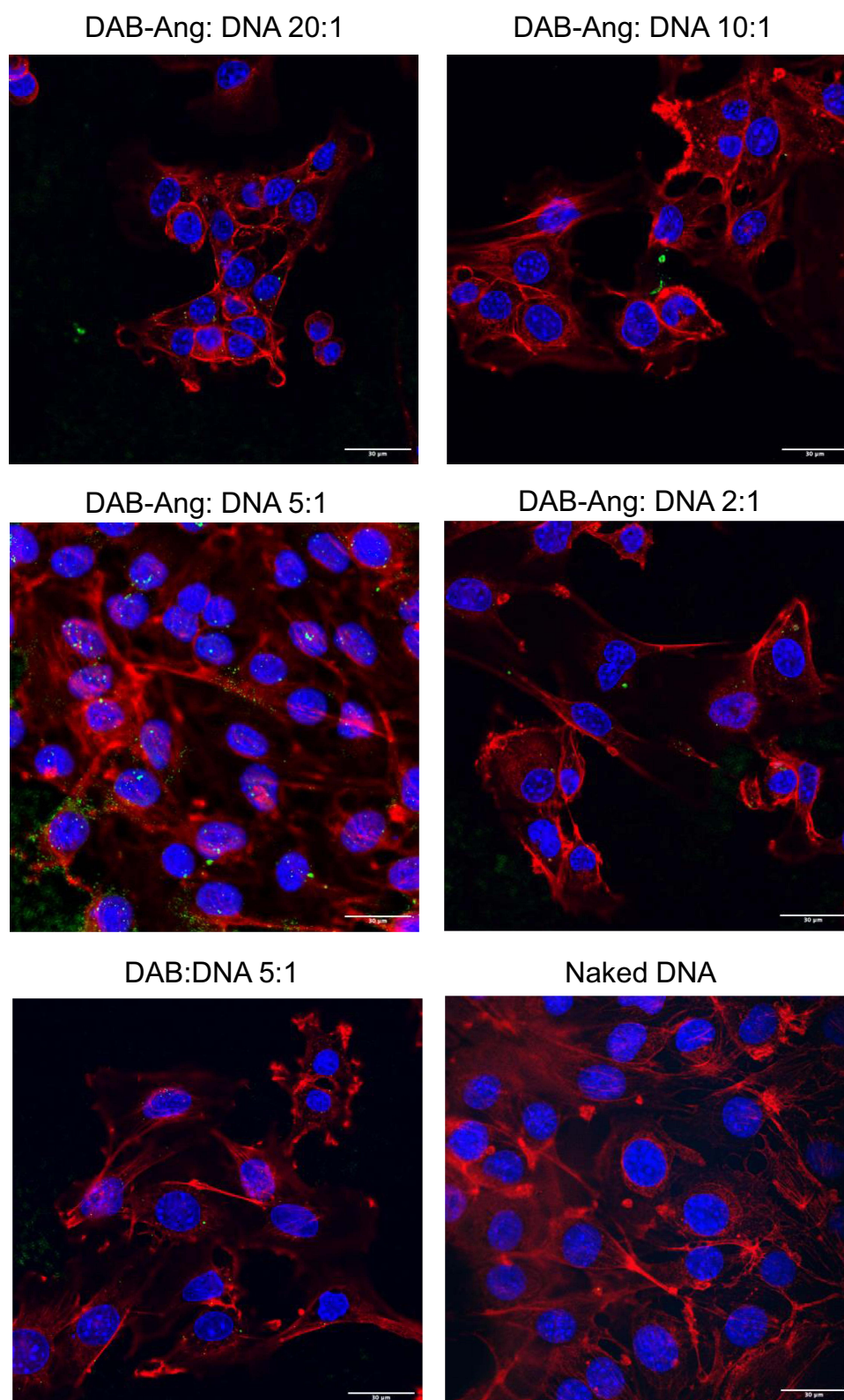


Figure 7 Confocal microscopy imaging of the cellular uptake of fluorescein-labelled DNA (2.5 µg/well), either complexed to DAB-Ang dendrimer, DAB dendrimer, or in solution after 24 h incubation with Bend.3 cells. (Blue: nuclei stained with DAPI (excitation: 405 nm laser line, bandwidth: 415–491 nm), green: fluorescein-labelled DNA (excitation: 514 nm laser line, bandwidth: 550–620 nm), red: cell wall (excitation: 633 nm laser line (bandwidth: 650–685 nm)) (Bar: 30 µm).

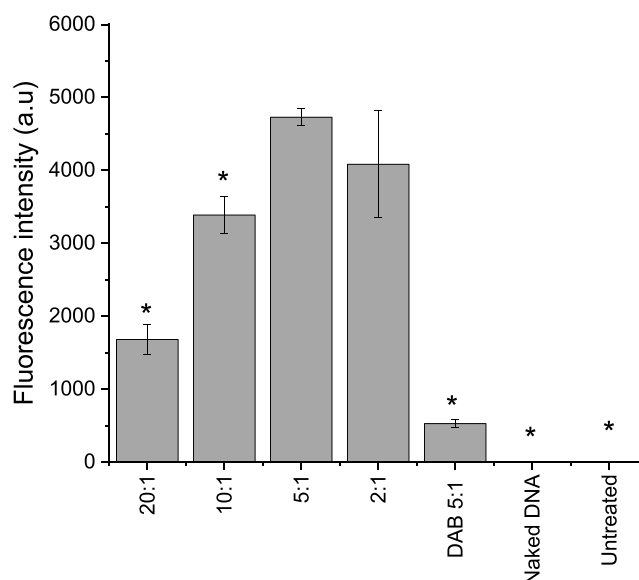


Figure 8 Fluorescence emission intensity as a quantitative measurement of the cellular uptake of fluorescein-labelled DNA (2.5 μ g/well) either complexed to DAB-Ang dendrimer, DAB dendrimer, or in solution after 24 h incubation with Bend.3 cells. Results are expressed as the mean $\pm$ SEM of $n=3$, *: $P \leq 0.05$ versus the highest level of gene expression.

Biodistribution of Gene Expression

The distribution of gene expression following intravenous administration of DAB-Ang dendriplex encoding luciferase was initially evaluated using bioluminescence imaging at various time points. Expression was predominantly observed in the brain of the mice, with the peak level detected 28 h post-injection (Figure 11).

Subsequently, gene expression levels from DAB-Ang dendriplex were compared with those from unmodified DAB dendriplex and DNA solution at 26 h and 28 h post-administration (Figure 12). The results indicated that DAB-Ang dendriplex treatment resulted in the highest gene expression in the brain. Moreover, no detectable luciferase expression was observed in organs other than brain, nor was any expression detected in the brain before 24 h or after 32 h. This may be due to the detection limits of the imaging method, which likely enabled visualization only of tissues with the strongest luminescent signals.

These findings were further supported by quantitative analysis of gene expression in major organs using a β -galactosidase reporter gene expression assay (Figure 13). Intravenous delivery of the DAB-Ang dendriplex led to predominant gene expression in the brain (667 ± 38 mU β -galactosidase per organ), followed by the kidneys (202 ± 29 mU) and the spleen (200 ± 11 mU). Lower expression levels were detected in the liver (139 ± 48 mU), lungs (107 ± 47 mU), and heart (9 ± 11 mU β -galactosidase per organ). Although nanocarriers administered systemically are often captured by the mononuclear phagocyte system in the liver and spleen, our results show minimal β -galactosidase expression in these organs. This suggests that, while some degree of accumulation may occur, it does not lead to productive gene expression, likely due to limited transfection efficiency in non-target cells or intracellular barriers to nuclear delivery.

The conjugation of angiopep to the DAB dendrimer significantly enhanced gene expression in the brain, showing a 1.8-fold increase compared to the DAB dendriplex (354 ± 113 mU β -galactosidase per organ) and a 3.2-fold increase relative to the DNA solution (208 ± 65 mU). No significant differences in β -galactosidase expression were observed in other organs following treatment with the DAB-Ang dendriplex compared to the DAB dendriplex. However, the DAB-Ang dendriplex led to a significant reduction in gene expression in the lungs compared to that observed with the DNA solution, which resulted in 304 ± 44 mU β -galactosidase per organ.

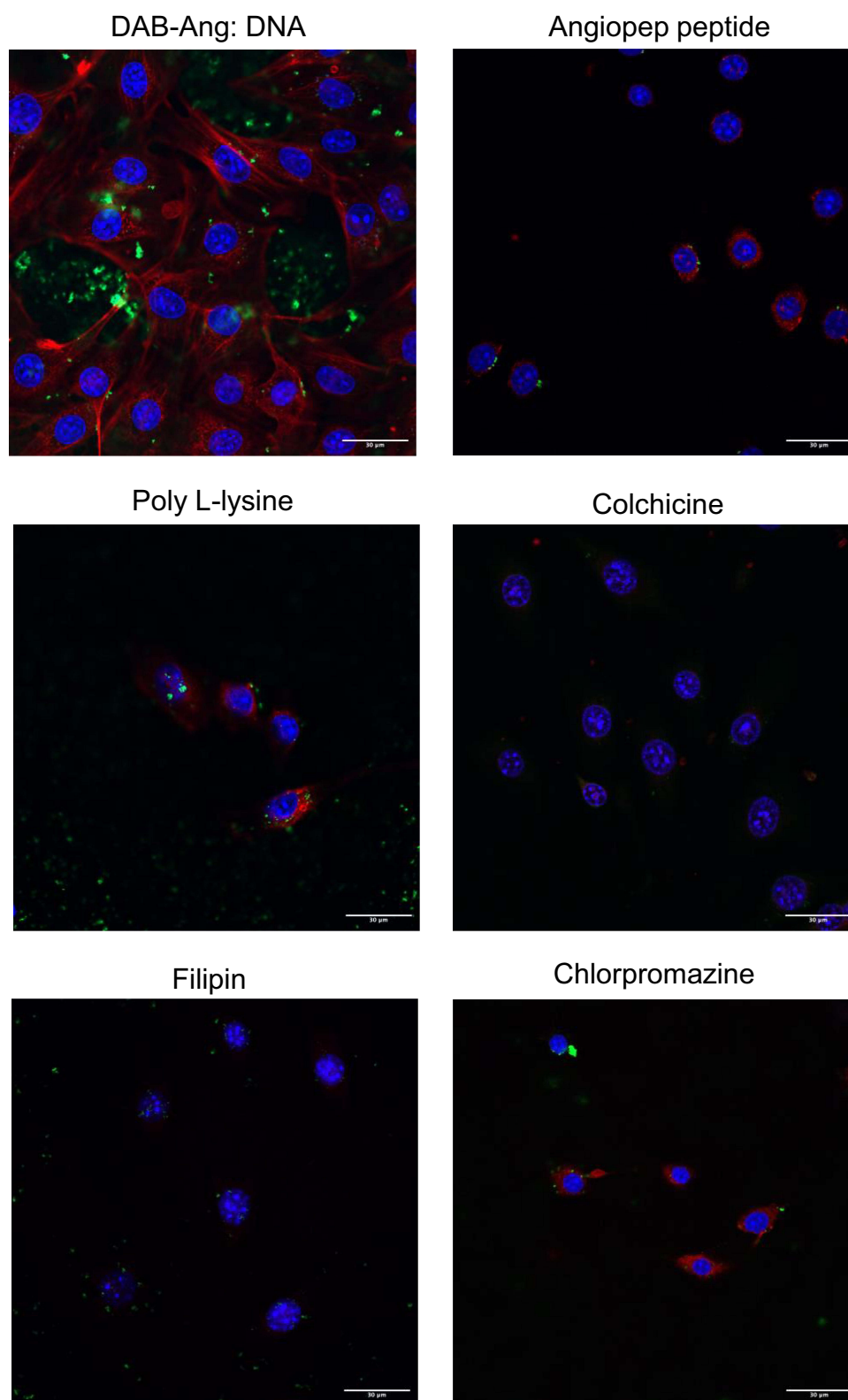


Figure 9 Confocal microscopy imaging for the assessment of the mechanism of cellular uptake of fluorescein-labelled DNA (2.5 µg/mL) complexed with DAB-Ang in Bend.3 cells after pre-treatment with various cellular uptake inhibitors. (Blue: nuclei stained with DAPI, green: fluorescein-labelled DNA, red: cell wall stained with Alexa Fluor 647 probe) (Bar: 30 µm).

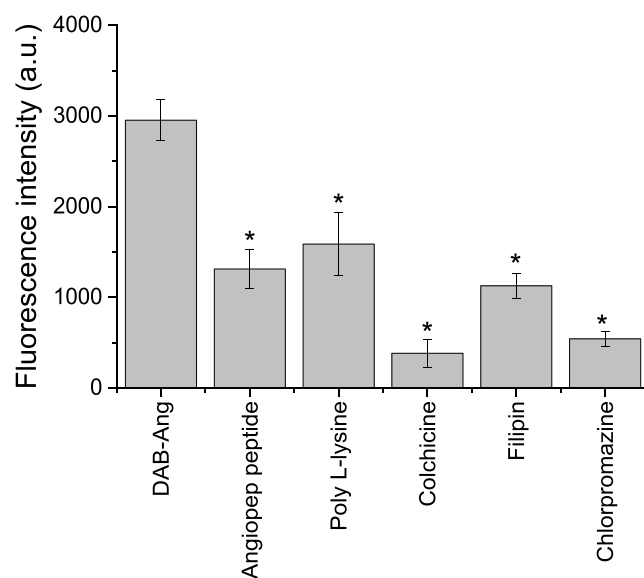


Figure 10 Fluorescence emission intensity as a quantitative measurement of the cellular uptake of fluorescein-labelled DNA (2.5 $\mu\text{g}/\text{well}$) complexed with DAB-Ang dendrimer in Bend.3 cells after pre-treatment with various cellular uptake inhibitors ($n = 3$), *: $p < 0.05$ compared with the positive control (no inhibitor).

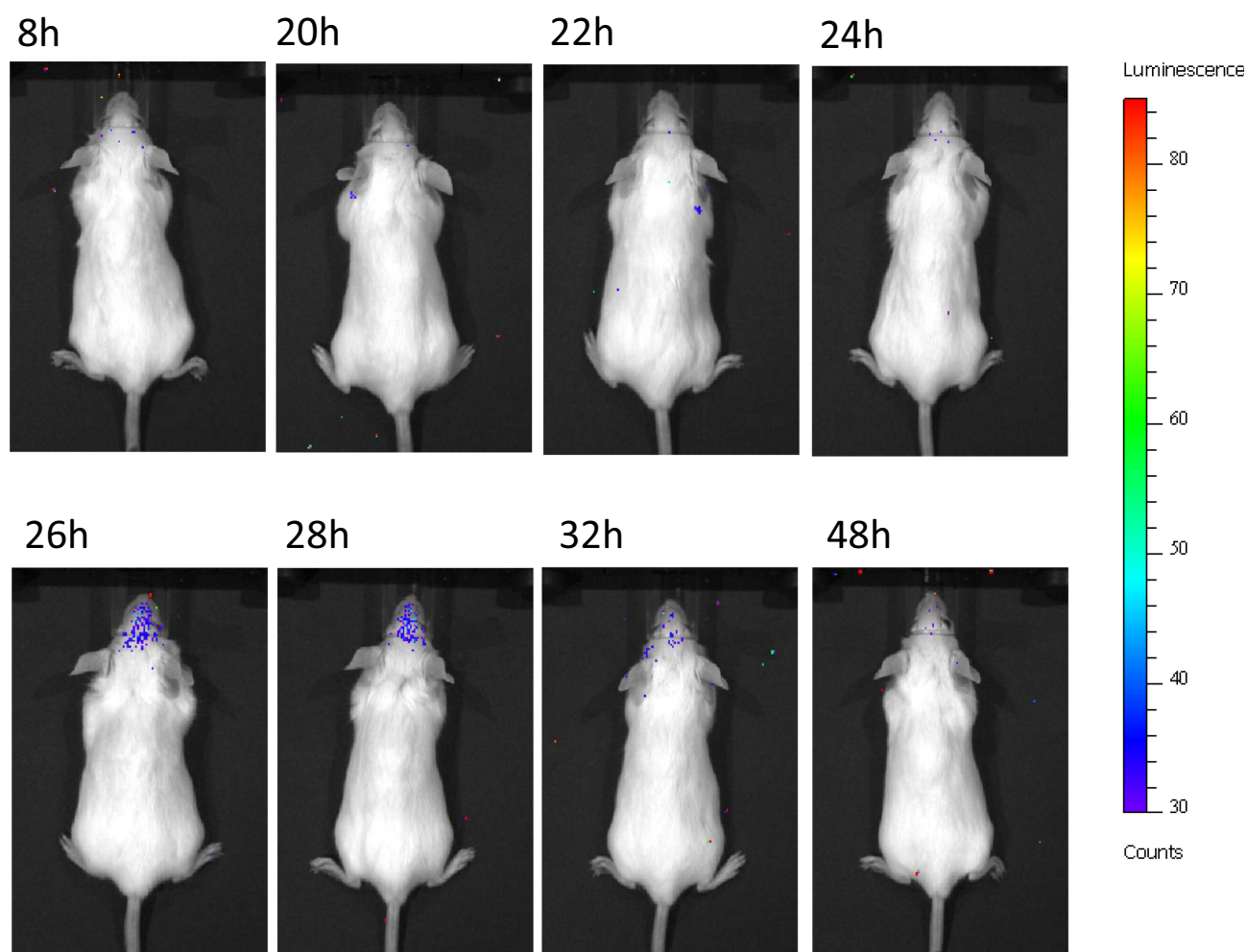


Figure 11 Bioluminescence imaging of gene expression following intravenous administration of DAB-Ang dendriplex (50 μg DNA administered). The mice were imaged using the IVIS Spectrum at various time points post-injection. The scale indicates surface radiance (photons/s/cm²/steradian).

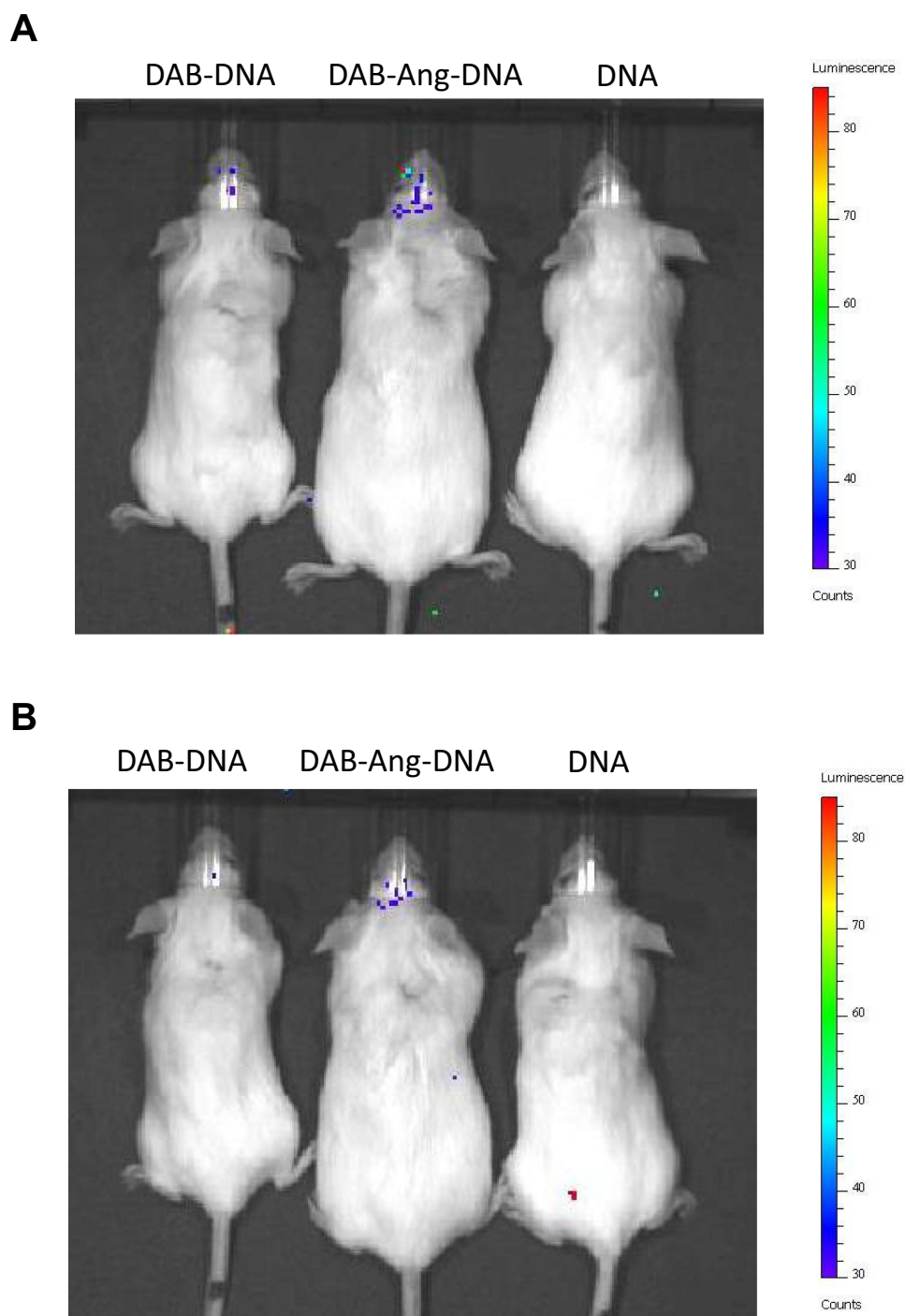


Figure 12 Bioluminescence imaging of gene expression after intravenous administration of DAB-Ang dendriplex, DAB dendriplex and DNA solution (50 μ g DNA administered). The mice were imaged using the IVIS Spectrum 26 h (**A**) and 28 h (**B**) post-injection. The scale indicates surface radiance (photons/s/cm²/steradian).

Distribution of Gene Expression Within the Brain

In a separate experiment, the distribution of gene expression within the brain was histologically and qualitatively evaluated using DAB-Ang dendriplex encoding either tdTomato or the control gene β -galactosidase (Figure 14). The red fluorescence of tdTomato was observed across multiple regions, including the striatum, habenula, neocortex, and pons. The fluorescence appeared diffusely localized within cell membranes. Compared to the control, fluorescence signals were consistently stronger across regions, indicating that these signals were not due to autofluorescence.

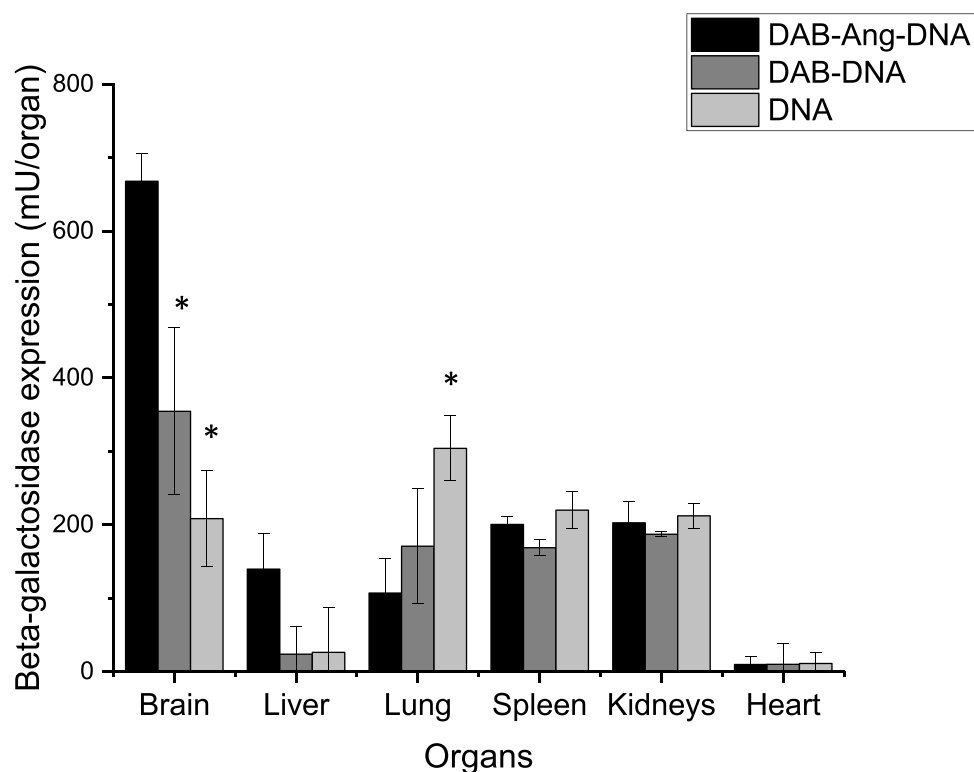


Figure 13 Biodistribution of gene expression after a single intravenous administration of DAB-Ang dendriplex, DAB dendriplex, and DNA solution (50 μ g DNA administered). Results were expressed as milliunits β -galactosidase per organ (n=5). *: $P < 0.05$ compared with angiopep-bearing DAB dendriplex for each organ.

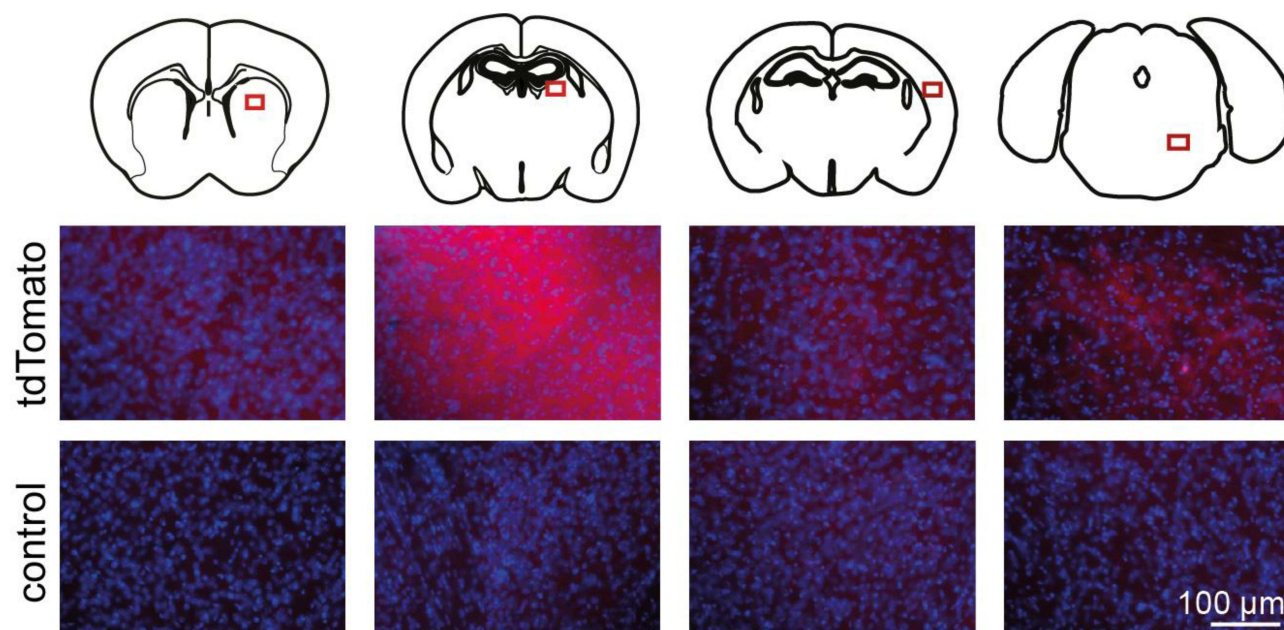


Figure 14 Epifluorescence microscopy imaging of gene expression distribution within the anterior, median, and posterior brain regions following a single intravenous injection of DAB-Ang dendriplex encoding tdTomato or a control gene (β -galactosidase) (50 μ g DNA administered) (Blue: nuclei stained with DAPI (excitation: 365 nm, emission bandwidth: 435–485 nm); red: tdTomato expression (excitation bandwidth: 530–635 nm, emission bandwidth: 605–655 nm)).

Discussion

Gene therapy has emerged as a promising therapeutic modality, driven by advancements in genetic and epigenetic profiling of central nervous system disorders.³⁰ However, its clinical application faces significant hurdles, primarily due to the inherent instability of nucleic acids and their vulnerability to enzymatic degradation in the bloodstream.⁸ The use of gene therapy to treat CNS disorders is further challenged by the presence of the BBB, which restricts the transport of therapeutic agents to the brain. This highlights the importance of developing safe and efficacious gene delivery systems capable of crossing the BBB.³¹

Among non-viral gene delivery systems, DAB dendrimers are particularly promising due to their small size, high gene expression efficacy, and the ability to modify their surfaces with ligands targeting receptors highly expressed on the BBB endothelial cells.^{9,19,20}

The DAB dendrimer used in this study promotes endosomal escape via the “proton sponge” effect, a mechanism involving buffering of endosomal acidification by the dendrimer’s amine groups. This leads to osmotic swelling and endosomal membrane disruption, resulting in cytosolic release of the nanocarrier–DNA complex.^{32,33} Angiopep-2 peptide has been identified as a potent brain-targeting ligand owing to its capacity to cross the BBB through the interaction with LRP-1 receptors, enabling targeted and effective delivery. Various dendrimers modified with angiopep have shown significant promise in drug and gene delivery to the brain, enhancing delivery efficiency.^{23,34} While DAB dendrimers have demonstrated superior results in brain gene delivery compared to other nanocarriers,^{9,19,20} the potential of angiopep-modified DAB dendrimer for targeted delivery remains unexplored. Therefore, this study explored the potential of angiopep-conjugated DAB dendrimer as a gene delivery system for targeting the brain.

Firstly, the conjugation of angiopep-2 to DAB dendrimer was assessed using ¹H-NMR. The presence of the protons corresponding to the benzene of the angiopep amino acids in the ¹H-NMR spectrum confirmed the successful grafting of angiopep to the dendrimer. For effective gene delivery, DAB-Ang dendrimer must efficiently condense nucleic acids. The positively charged terminal NH₂ groups of DAB dendrimer facilitate this through electrostatic interactions with negatively charged DNA phosphate groups.³⁵ Surface modification can however partially shield these positive charges, necessitating evaluation of condensation efficacy post-modification. DAB-Ang dendrimer maintained a high condensation efficacy of over 70% across various dendrimer: DNA weight ratios (20:1, 10:1, 5:1, and 2:1), with the condensation remaining stable for 24 hours.

The lack of complete DNA condensation at lower dendrimer: DNA weight ratios of 1:1 and 0.5:1 was most likely due to inadequate electrostatic interactions. At these ratios, the amount of dendrimer present was insufficient to fully neutralize and bind the DNA strands. This resulted in partially or uncomplexed DNA remaining in the solution, which was shown from the free DNA bands in the gel and the negative surface charges measured by zeta potential analysis. These observations confirm that higher dendrimer: DNA ratios are required for efficient DNA complexation and stable dendriplex formation.

Key properties of delivery systems, such as size, surface charge, and morphology, are known to play a critical role in nanoparticle interactions with cells and their accumulation in tissues, especially within the brain. Studies indicated that nanoparticles with sizes ranging from 10 to 200 nm are most effective for crossing the BBB and ensuring adequate brain accumulation.³⁶ The size of DAB-Ang dendriplexes ranged from 65 ± 5 nm at a dendrimer: DNA weight ratio of 5:1 to 241 ± 34 nm at a dendrimer: DNA weight ratio of 20:1. A small polydispersity index (PDI) ranging from 0.22 ± 0.04 to 0.35 ± 0.05 was observed at these ratios, reflecting a narrow size distribution and high uniformity in particle size. At those ratios, dendriplexes displayed a positive surface charge, thought to improve their ability to cross the BBB. While neutral nanoparticles have shown a longer circulating time compared to charged nanoparticles, cationic nanoparticles have shown an enhanced ability to cross the BBB via adsorptive-mediated transcytosis. This is due to the presence of proteoglycans on the BBB endothelium, resulting in a higher density of negative charges on the BBB endothelium compared to blood components and human umbilical vein endothelial cells.³⁶

Particle shape has also been shown to significantly impact the capacity of a delivery system to reach the brain by affecting cellular uptake and biodistribution. Spherical nanoparticles are advantageous for targeted delivery due to their uniform shape and size and consistent distribution and targeting, facilitating BBB penetration. Furthermore, their ability

to evade the mononuclear phagocytic system (MPS) increases circulation time and bioavailability.³⁶ TEM imaging of DAB-Ang complexed to DNA confirmed the formation of spherical dendriplex with a size smaller than 200 nm.

In vitro biological studies demonstrated that DAB-Ang dendriplex achieved a 9-fold higher cellular uptake compared to unmodified DAB dendriplex. These findings are in accordance with those reported by Gao et al, who observed a 3.2-fold increase in cellular uptake for poly (l-lysine)-grafted polyethylenimine (PEI-PLL) nanoparticles conjugated to angiopep-2, compared to non-modified nanoparticles in an in vitro BBB model.²⁴ However, DAB-Ang achieved a 500-fold higher DNA uptake than naked DNA, significantly higher than the 20.1-fold increase reported for PEI-PLL.²⁴

When compared to DAB dendrimers modified with other targeting ligands, such as lactoferrin and transferrin, DAB-Ang showed superior enhancement in DNA uptake. DAB-Tf and DAB-Lf dendriplexes exhibited a 1.4-fold and 2.1-fold increase in DNA uptake, respectively, compared to unmodified DAB dendriplex.^{19,20} In contrast, DAB-Ang achieved a 9-fold increase, underscoring its superior performance as a gene delivery vector. The mechanism of cellular uptake for delivery systems is affected by various factors, including their size, surface shape, charge, modifications, and the type of target cell. While the four endocytosis pathways contributed to the uptake of DAB-Ang dendriplex, its uptake was predominantly mediated by clathrin-mediated pinocytosis and macropinocytosis, the primary pathways in brain endothelial cells.³⁷ These findings are consistent with observations for angiopep-modified PAMAM-dendrimers, where the highest inhibition of uptake occurred in cells pre-treated with colchicine and phenylarsine oxide, inhibitors of macropinocytosis and clathrin-mediated pinocytosis.²⁵

While in vitro studies are of high importance for evaluating variables and optimizing treatment conditions, in vivo studies are crucial for assessing the efficacy of gene delivery vector in crossing the BBB. They consider systemic factors such as immune responses, metabolism, and tissue-specific interactions, which are essential for understanding the full impact and efficacy of gene delivery systems.

In vivo gene expression studies revealed that gene expression in the brain was highest 28 h after intravenous administration of DAB-Ang dendriplex, with significantly higher levels than those detected in other organs, and higher than observed with unmodified DAB dendriplex or naked DNA.

Biodistribution analysis confirmed that the brain gene expression of DNA complexed with DAB-Ang was 1.9-fold higher than with DAB dendriplex and 3.2-fold higher than with naked DNA. Furthermore, gene expression in the brain was at least 3.3-fold higher than in any other organs following administration of DAB-Ang dendriplex. Notably, lung gene expression from DAB-Ang dendriplex was significantly reduced compared to that achieved with naked DNA. These findings demonstrate that DAB-Ang dendriplex exhibits high specificity and efficacy for brain-targeted gene delivery, as well as high gene expression efficacy. Comparable results have been reported for DAB dendrimers modified with other ligands, such as lactoferrin. For instance, DAB-Lf dendriplex increased gene expression of DNA by 6.4-fold compared to unmodified DAB dendriplex. However, the level of gene expression in the brain achieved with DAB-Ang dendriplex was significantly higher than that with DAB-Lf dendriplex, at 667.8 ± 38.6 mU β -galactosidase per organ compared to 116.1 ± 0.1 mU β -galactosidase per organ, respectively.²⁰ Despite this, DAB-Lf dendriplex reduced gene expression in the lungs and kidneys compared to unmodified DAB, whereas no significant difference in off-target expression was observed between DAB-Ang and unmodified DAB in these organs. Similarly, a study by Ke et al demonstrated that angiopep-conjugated PAMAM dendrimers significantly enhanced gene expression in the brain compared to unmodified DAB dendrimer and naked DNA. However, unlike DAB-Ang dendriplex, PAMAM-Ang dendriplex showed high accumulation in the spleen.²⁵ The negligible gene expression observed in liver and spleen, despite the known tendency for nanoparticle accumulation in these organs, may be attributed to the low efficacy of gene expression in phagocytic or non-dividing cells. Moreover, the DAB-Ang delivery system was designed to facilitate brain targeting via LRP1-mediated transcytosis, potentially reducing uptake or functional delivery in peripheral tissues. These findings highlight the specificity and functional selectivity of the delivery system.

This study used a single-dose intravenous administration of the DAB-Ang dendriplex to evaluate brain-targeted gene delivery via LRP1-mediated transcytosis. It is acknowledged that high-dose or repeated administration of LRP1-targeting ligands may influence receptor expression, internalization, or recycling, potentially leading to desensitization or altered signaling. Future studies will aim to evaluate the effects of repeated dosing on LRP1 trafficking and downstream pathways.

Alternative nanocarriers, including nanoparticles and liposomes, have also been widely investigated for gene delivery to the brain. Gao et al reported that angiopep-conjugated poly (L-lysine)-grafted polyethylenimine nanoparticles demonstrated superior gene expression in the brain compared to their unconjugated counterparts. However, this enhancement was accompanied by increased off-target DNA accumulation in the spleen, kidneys, and liver, likely due to non-specific interactions between the cationic nanoparticles and anionic regions on cytoplasmic membranes.²⁴ Similar results were obtained with angiopep-modified liposomes, where fluorescence imaging showed enhanced Cy5-siRNA uptake in the brain compared to unmodified liposomes, but with high fluorescence also observed in other organs.²⁷

These findings highlight the unique potential of DAB-Ang dendriplex as a highly specific and effective vector for brain-targeted gene delivery, achieving superior brain gene expression compared to other non-viral delivery systems. Additionally, gene expression within the brain was localized to critical brain regions, including the striatum, sensory cortex, and pons. This underscores the promising potential of DAB-Ang dendriplex for targeting and treating disorders that affect these specific brain regions. Future studies will involve co-labelling with established markers such as NeuN, GFAP, and Iba1 to investigate the cellular tropism of the DAB-Ang system *in vivo*.

While this study focused on proof-of-concept gene delivery using reporter constructs, future work will explore the delivery of therapeutic genes such as brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), or anti-inflammatory cytokines, to evaluate the functional and disease-modifying potential of the DAB-Ang nanocarrier in models of neurological disease. These studies will be essential to demonstrate the translational relevance of this platform for non-invasive gene therapy in the CNS.

Conclusion

The conjugation of angiopep-2 to DAB dendriplex significantly increased DNA uptake in bEnd.3 brain endothelial cells *in vitro* compared to the unmodified dendriplex. *In vivo*, intravenous administration of the angiopep-functionalized DAB dendriplex resulted in markedly higher and more specific gene expression in the brain compared to the unmodified dendriplex and naked DNA, with minimal off-target effects in other major organs. These findings establish the angiopep-2-modified DAB dendrimer as a highly promising nanocarrier for effective and targeted gene delivery to the brain, offering potential for therapeutic applications in central nervous system disorders.

Abbreviations

ANOVA, one-way analysis of variance; BBB, blood-brain barrier; BSA, bovine serum albumin; CNS, central nervous system; DAB, generation-3 diaminobutyric polypropylenimine dendrimer; DAB-Ang, generation-3 diaminobutyric polypropylenimine dendrimer conjugated to angiopep-2; DAPI, 4',6-diamidino-2-phenylindole; DDAO, 7-hydroxy-9H-(1,3-dichloro-9,9-dimethyl-acridin-2-one; DMEM, Dulbecco's Modified Eagle's Medium; DMSO, dimethylsulfoxide; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; EF1 α , elongation factor 1 alpha; GMBS, N-(γ -maleimidobutyryloxy) succinimide ester; LRP1, low-density lipoprotein receptor-related protein-1; MPS, mononuclear phagocytic system; MWCO, molecular weight cut-off; NGS, normal goat serum; PAMAM, polyamidoamine; PBS, phosphate-buffered saline; PBST: phosphate-buffered saline containing Triton-X; PEG, polyethylene glycol; PEI-PLL, polyethylenimine conjugated to poly L-lysine; PDI, polydispersity index; SEM, standard error of the mean; TBE: Tris-borate- EDTA; TE, Tris-EDTA; TEM, transmission electron microscopy.

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Disclosure

The authors report no conflicts of interest in this work.

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