Biological Effect Tetra-Branched Anti-TNF-Peptide and Coating Ratio-Dependent Penetration of the Peptide-Conjugated Cerium\(^{3/4+}\) Cation-Stabilized Gamma-Maghemite Nanoparticles into Rat Inner Ear after Transtympanic Injection Visualized By MRI

Zou J\(^1,3\), Peng B\(^2\), Ostrovsky S\(^4\), Li B\(^3\), Li C\(^2\), Kettunen M\(^1\), Lellouche J\(^P\), and Pyykkö I\(^3\)

\(^1\)Department of Otolaryngology-Head and Neck Surgery, Center for Otolaryngology-Head & Neck Surgery of Chinese PLA, Shanghai Hospital, Second Military Medical University, Shanghai, China
\(^2\)Hebei Bio-high Technology Deve CO., LTD, Shijiazhuang, Hebei Province, China
\(^3\)Hearing and Balance Research Unit, Field of Oto-laryngology, School of Medicine, University of Tampere, Tampere, Finland
\(^4\)Laboratory of Nanoscale Materials and Systems, Department of Chemistry, Bar-Ilan University, Ramat-Gan, Israel
\(^5\)A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland

Both authors contributed equally

*Corresponding author: Zou J, Department of Otolaryngology-Head and Neck Surgery, Center for Otolaryngology-Head & Neck Surgery of Chinese PLA, Shanghai Hospital, Second Military Medical University, Shanghai, China, Hearing and Balance Research Unit, Field of Oto-laryngology, School of Medicine, University of Tampere, Tampere, Finland, Tel: +86 21 311 62021, E-mail: Jing.Zou@staff.uta.fi


Received Date: August 23, 2017 Accepted Date: October 25, 2017 Published Date: October 28, 2017

Abstract

**Objective:** To evaluate the biological efficacy of TBATP and their distribution in the inner ear after transtympanic injection.

**Methods:** TBATP was synthesized through standard Fmoc solid phase synthesis. A model of TNF-α-induced apoptosis was established in human umbilical vein endothelial cells (HUVECs). The peptides were covalently attached onto CAN-\(γ\)-Fe\(_2\)O\(_3\) NPs. Nanoparticle suspension was injected to rat middle ear cavity. Distribution of CAN-\(γ\)-Fe\(_2\)O\(_3\) NPs in the inner ear was detected using a 7.0 T MRI machine in combination with Prussian blue staining.

**Results:** TBATP almost fully suppressed the inhibitory effect on HUVECs induced by TNF-α while the linear one reduced less than half of the effect. CAN-\(γ\)-Fe\(_2\)O\(_3\) NPs conjugated to TBATP at a peptide weight ratio of 10% but not 50% efficiently entered the inner ear at 3 h through 2 w post-middle ear administrations and most pronounced at 2 w.

**Conclusion:** The tetra-branched anti-TNF-α peptide (TBATP) is capable of suppressing the inhibitory effect on HUVECs induced by TNF-α, and is visualized by MRI after conjugating to super-paramagnetic Ce\(^{3+}\) cation-doped maghemite nanoparticles arising from ceric ammonium nitrate-mediated doping oxidation of starting magnetite nanoparticles (CAN-\(γ\)-Fe\(_2\)O\(_3\) NPs).

**Keywords:** Peptide; Functional maghemite nanoparticles; Inner ear; Magnetic resonance imaging; Biological barrier

**List of Abbreviations:** CAN-\(γ\)-Fe\(_2\)O\(_3\) NPs: Super-paramagnetic maghemite (\(γ\)-Fe\(_2\)O\(_3\)) nanoparticles using ceric ammonium nitrate (CAN)-mediated oxidation of starting magnetite nanoparticles (CAN-\(γ\)-Fe\(_2\)O\(_3\) NPs).

Introduction

The cascades of tumor necrosis factor-α (TNF-α) have been implicated in the pathological progress of noise induced hearing loss, sudden sensorineural hearing loss, age related hearing loss, and neonatal hyperbilirubinaemia induce neural toxicity [1-6]. Application of TNF-α blocker (antibody) has been reported to improve the auditory function in noise induced hearing loss,
autoimmune sensorineural hearing loss [7-9]. However, antibodies against TNF-α have too large molecular weight to access the therapeutic target. Peptides are smaller in size, more stable during storage, and easier to manipulate than antibodies. To simulate antibody in structure, novel tetra-branched anti-TNF-α peptide selected using phage library with capability of inhibiting human TNF-α binding to its receptors were developed [10]. It is clinically relevant to develop a similar therapeutics for treating the inner ear diseases.

The intratympanic delivery of therapeutics is a currently used approach in clinical practice with advantages of avoiding systemic adverse effects and reducing the administration dosage to the individual. In vivo MRI studies demonstrated that intratympanic delivery induced more gadolinium chelate passage into the perimodiolar lymph and lateral wall of mouse cochlea than did the intravenous injection attributed to potential porous structures of medial wall of the scala tympani and spiral ligament extracellular space [11]. An efficient passage of agents into the modiolus and lateral wall would raise the possibility that drug delivery to these areas of the cochlea might be favored by intratympanic route. In vivo tracking distribution of therapeutics in the targeting organ is a reliable method to follow the dynamics of the delivered agents. We recently disclosed the preparation of highly hydrophilic anti-aggregative super-paramagnetic maghemite (γ-Fe₃O₄) nanoparticles (NPs) using ceric ammonium nitrate (CAN)-mediated oxidation of starting magnetite (Fe₃O₄) NPs (CAN-γ-Fe₃O₄ NPs), which were highly stable aqueous suspensions/ferrofluids due to a unique ultrasound-mediated doping process of the Fe₃O₄ NP surface using lanthanide Ce³⁺ cations [12]. We have also demonstrated that the novel CAN-γ-Fe₃O₄ NPs is a strong T₁, MRI contrast agent and penetrates both round and oval windows, which has potential application in the molecular imaging of the inner ear [13].

The present study aimed to develop feasible agents for the inner ear therapy as well as diagnosis, and acquire information about their passing through the round and oval windows after conjugation with MRI-traceable nanoparticles. Tetra-branched anti-TNF-α peptides were developed according to a previous report with modifications [10]. Biological effect of the peptides in inhibiting TNF-α induced cellular impairment was analyzed in cell cultures. The specific peptides were conjugated to CAN-γ-Fe₃O₄ NPs at various ratios and the resulting dynamic distributions in the inner ear after middle ear administration have been evaluated in rats using in vivo MRI.

Materials and Methods

Synthesization and Characterization of Tetra-Branched Anti-TNF-α Peptide

The sequences of effective peptide were HIIHDDLRRYYGW, and that of scramble peptide were HDYLHRILGYDW. The selected peptides were synthesized in a tetrameric form (Figure 1) through standard Fmoc solid phase synthesis, building the tetramer on a three-lysine core and polyethylene glycol tail [10]. The synthesis was performed on a Sophas P1 S Solid Phase Peptide Synthesizer (Zinsser Analytic GmbH, Frankfurt, Germany) using [Fmoc-Lys(Fmoc)]₂-Lys-βAla-2Cl Resin (0.15 mmol/g) (Shanghai Top-Peptide Biotechnology Co. Ltd., Shanghai, China), Fmoc-amino acids (0.5 mol/L), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (0.25 mol/L), N,N-Diisopropylethylamine (0.3 mol/L) (GL Biochem Co. Ltd., Shanghai, China) through rinse, deprotection, and ligase circles.

For characterization, the peptide-resin complexes were pyrolyzed at room temperature for 3 h in the following solutions, trilfluoroacetic acid: 3-hydroxytoluene: thioanisole: H₂O: ethane-1,2-dithiol=82.5: 5: 5: 2.5. After removing the resin through filtration, the peptides were sedimentated using ice ether, centrifugated at 2362.5 g (4 °C) for 7 times, and then lyophilized. The samples (1 mg/mL, 50 μl) were run on an Agilent HPLC 1100 equipped with a column of Agilent 300SB-C18 4.6*250mm. The mobile phase A was composed of 0.1% trifluoroacetic acid in H₂O, and mobile phase B was composed of 0.1% trifluoroacetic acid in acetonitrile. The elution gradient was created by mixing A and B at various ratio at different time points. B: A=1/4, 2/3, 3/2, 9/1, and 1/4 at 0, 15, 25, 30, and 35 min. 5μm (Agilent Technologies, California, USA) at an elution speed of 1.0 mL/min and temperature of 40 °C.

Impact of Tetra-Branched Anti-TNF-α Peptide on Cellular Toxicity Induced by TNF-α

Firstly, a cellular model of apoptosis was established in human umbilical vein endothelial cells (HUVECs) using TNF-α (Supplementary material 1). After optimization with concentrations of TNF-α for establishing the cell model of apoptosis in HUVECs, the impact of tetra-branched anti-TNF-α peptide on cellular toxicity induced by TNF-α was then analyzed. HUVECs were cultivated as above mentioned. When the cells propagated for 24 h and formed a monolayer confluent, 100 μL of the following...
solutions were added to each well with 6 repeats. A. Negative control without any treatment; B. TNF-α (40 ng/mL)+tetra-branched anti-TNF-α peptide (0 µg/ mL); C. TNF-α (40 ng/mL)+tetra-branched anti-TNF-α peptide (25 µg/ mL); D. TNF-α (40 ng/ mL)+tetra-branched anti-TNF-α peptide (50 µg/ mL); E. TNF-α (40 ng/mL)+tetra-branched anti-TNF-α peptide (100 µg/ mL); F. TNF-α (40 ng/mL)+tetra-branched anti-TNF-α peptide (200 µg/ mL); G. TNF-α (40 ng/mL)+tetra-branched anti-TNF-α peptide (400 µg/ mL). The baseline reference and MTT assay were performed as above mentioned.

After optimization for concentrations for inhibiting apoptosis in HUVECs, intervention effect of tetra-branched anti-TNF-α peptide was compared with that of tetra-branched scrambled peptides, linear specific anti-TNF-α peptide, and linear scrambled peptides. HUVECs were cultivated as above mentioned. When the cells propagated for 24 h and formed a monolayer confluent, 100 µL of the following solutions were added to each well with 6 repeats. A. Negative control without any treatment; B. TNF-α (40 ng/mL); C. TNF-α (40 ng/mL)+tetra-branched anti-TNF-α peptide (200 µg/ mL); D. TNF-α (40 ng/mL)+tetra-branched scrambled peptides (200 µg/ mL); E. TNF-α (40 ng/mL)+linear anti-TNF-α peptide (800 µg/ mL); F. TNF-α (40 ng/mL)+linear scrambled peptides (800 µg/ mL). The baseline reference MTT assays were performed as described in Supplementary material 1.

**Preparation and Characterization of Tetra-Branched anti-TNF-α-Peptide-Conjugated CAN-γ-Fe₂O₃ NPs**

All the specific chemicals and reagents (analytical grade and/or highest purity level) used in this study, i.e., FeCl₃·6H₂O, FeCl₃·4H₂O, NH₄OH (ACS reagent, 28-30%), ceric ammonium nitrate (CAN, CeIV(NH₃)₆(NO₃)₃) have been purchased from Sigma-Aldrich (Israel) and have been used without any further purification. Starting neat magnetite (Fe₃O₄) nanoparticles (Fe₃O₄ NPs, basic Massart hydrolytic method) were prepared as nanocarrier (Supplementary material 2).

**Experimental Procedure for Nanocarrier Fabrication with Tetra-Branched Anti-TNF-α-Peptide Covalent Attachment to CAN-γ-Fe₂O₃ NPs**

Corresponding CAN-γ-Fe₂O₃ NPs aqueous suspension (1 mL, Fe=1.96 mg/mL –ICP-AES measurement) was diluted to 25.0 mL using milliQ-purified H₂O. Then, 0.196 mg and 0.98 mg of tetra-branched anti-TNF-α peptide (peptide/Fe Wt ratio: 10% and 50% respectively) were added to the NP suspension as an aqueous solution and the medium was shaken overnight at 15 °C (orbital shaker). At completion of peptide contacting/NP surface decoration, resulting tetra-branched anti-TNF-α peptide₎₀% - CAN-γ-Fe₂O₃ NPs and tetra-branched anti-TNF-α peptide₃₀% - CAN-γ-Fe₂O₃ were washed 3 times (3 x 10 mL ddH₂O) using an Amicon® Ultra-15 centrifugal filter device (100K) operated at 4,000 rpm (5 min) to afford cleaned tetra-branched anti-TNF-α peptide₅₀% - CAN-γ-Fe₂O₃ and tetra-branched anti-TNF-α peptide₃₀% - CAN-γ-Fe₂O₃ NPs. Covalent contact of tetra-branched anti-TNF-α peptide (10% and 50% wt ratio) with CAN-γ-Fe₂O₃ NPs procedure was performed as discussed above.

**Middle Ear Administration of Tetra-Branched Anti-TNF-α-Peptide-Conjugated CAN-γ-Fe₂O₃ NPs and MRI of Rat Inner Ear**

Four male Sprague Dawley rats (8 ears), weighing between 300 and 350 grams, were maintained in the animal laboratory of A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland. The animals were randomly assigned into 2 groups (NP10, NP-50) (Table 1). The left ear was utilized for the delivery, and the right side was used as a negative control (NC group in Table 1). All the animal experiments have been approved by the Ethical Committee of University of Tampere (permission: ESVI/3033/04.10.03/2011). Animal care and experimental procedures were conducted in accordance with European legislation. Throughout the experiments, the animals were anesthetized with a 5% isoflurane–oxygen mixture for induction and a 2% isoflurane–oxygen mixture for maintenance via a facemask. Intramuscular injection of enrofloxacin (Baytril”vet, Orion, Turku, Finland) at a dose of 10 mg/kg was applied before the intratympanic administration of NPs to prevent potential infection. The animal’s eyes were protected with Viscoatears” (Novartis Healthcare A/S, Copenhagen, Denmark). The peptide-conjugated CAN-γ-Fe2O3 NPs were administered to the middle ear cavity with an injection tube by touching the surface of the tympanic medial wall according to a previously reported procedure [13,14]. A total volume of 10 µL of undiluted CAN-γ-Fe2O3 NP suspension was injected onto the medial wall of the middle ear cavity. The animals remained in the lateral position with the injected ear upward for 15 min before MRI study. After the last MRI measurement, the animals were decapitated for Prussian blue staining for iron in the tissue (Supplementary material 3) (Table 1).

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Number of ears</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NP-10</td>
</tr>
<tr>
<td>MRI</td>
<td>2</td>
</tr>
<tr>
<td>Prussian blue Staining</td>
<td>2</td>
</tr>
</tbody>
</table>

* MRI studies were performed at the time points of 3 h, 1 d, and 2 w post-middle ear administrations. NC: negative control; NP-10: CAN-γ-Fe₂O₃ NPs were conjugated to tetra-branched anti-TNF-α peptide at 10% peptide ratio; NP-50: CAN-γ-Fe₂O₃ NPs were conjugated to tetra-branched anti-TNF-α peptide at 50% peptide ratio. *The right ear of each animal was used as NC in all studies.

**Table 1:** Assignments of rat ears in MRI measurements and histology post-middle ear administration of tetra-branched anti-TNF-α peptide-conjugated CAN-γ-Fe₂O₃ NPs.
Statistics

In cell culture study, inhibition ratios on cell viability by different treatments were compared using one-way analysis of variance. Values of \( p < 0.05 \) were accepted as statistically significant.

Results

Characterization of Tetra-Branched Anti-TNF-α Peptide and the Inhibiting Effect on Apoptosis of HUVECs Induced By TNF-α

Purity of both tetra-branched anti-TNF-α and scramble peptides was approved by high performance liquid chromatography that demonstrated the unique specific peak of the peptides (Figure 2). The other sharp peaks might be associated with incomplete cracking of the protecting group for the side chain according to experience. A cell model of apoptosis was successfully established in HUVECs using TNF-α, which showed inhibition on viability of the cells after 24 h incubation in a concentration-dependent response. TNF-α protein at 40 ng/mL induced an average apoptosis of 30.1% of the cells and was selected as the optimized concentration for the following experiments (Figure 3A). Tetra-branched anti-TNF-α peptide suppressed the inhibitory effect on HUVECs induced by TNF-α in a dosage-dependent reaction, and 200 μg/mL of the peptides demonstrated the maximum effect of 69.2% that was used for comparison with other peptides in the following study (Figure 3B). The linear anti-TNF-α peptide also suppressed the inhibitory effect on HUVECs induced by TNF-α but significant lower than did the tetra-branched anti-TNF-α peptide (Figure 3C).

Characterization of Tetra-Branched Anti-TNF-α-Peptide-Conjugated CAN-γ-Fe₂O₃ NPs

The neat CAN-γ-Fe₂O₃ NPs has an average DLS size of 45.8 nm, and every 10% peptides conjugated to the NPs increased around 5 nm to the DLS diameter of the NPs. The neat CAN-γ-Fe₂O₃ NPs has an average ζ potential of +48 mV, and every 10% peptides conjugated to the NPs reduced approximately 5 mV of ζ potential (Table 2, Figure 4).
<table>
<thead>
<tr>
<th>NPs</th>
<th>DLS (nm)</th>
<th>ζ potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAN-γ-Fe₂O₃</td>
<td>45.8</td>
<td>+48.0</td>
</tr>
<tr>
<td>0310A10% -CAN-γ-Fe₂O₃</td>
<td>49.68</td>
<td>+43.8</td>
</tr>
<tr>
<td>0310A50% -CAN-γ-Fe₂O₃</td>
<td>70.63</td>
<td>+24.8</td>
</tr>
</tbody>
</table>

CAN-γ-Fe₂O₃: neat NPs without peptide conjugation; 0310A₉₀% -CAN-γ-Fe₂O₃: covalent contact of tetra-branched anti-TNF-a peptide at 10%wt ratio with CAN-γ-Fe₂O₃ NPs; 0310A₅₀% -CAN-γ-Fe₂O₃: covalent contact of tetra-branched anti-TNF-a peptide at 50%wt ratio with CAN-γ-Fe₂O₃ NPs.

Table 2: DLS and ζ potential results for neat CAN-γ-Fe₂O₃ NPs (Ce³⁺)-doped maghemite (γ-Fe₂O₃) and peptide-conjugated NPs

Dynamic Distribution of Tetra-Branched Anti-TNF-Peptide-Conjugated CAN-γ-Fe₂O₃ NPs in Rat Inner Ear Shown by MRI

Significant morphological changes were detected in rat inner ear at 3 h through 2 w post-middle ear administrations of tetra-branched anti-TNF-a-peptide-conjugated CAN-γ-Fe₂O₃ NPs at 10% peptide ratio, which demonstrated that area of the ipsilateral cochlea basal turn, vestibule including the ampulla of semicircular canals shrunk in comparison to the contralateral ear (Figure 5). The changes were most pronounced at 2 w after administration, with typical wiping out of the perilymphatic spaces in the basal turn and hook region of the cochlea, and vestibule (Figure 6). This phenomenon was verified by injection of superparamagnetic iron oxide nanoparticles hierarchically coated with oleic acid and Pluronic® F127 copolymers into the scala tympani of rats.

Figure 5: Efficient rat inner ear entry of tetra-branched anti-TNF-a-peptide-conjugated CAN-γ-Fe₂O₃ NPs at 10% peptide ratio shown by MRI. The bright area of inner ear fluids in the basal turn of cochlea (1st), vestibule (Vest), and ampulla of semicircular canal on the delivery side (L) were smaller than that on the contralateral side (R) 3 h after administration, which progressed one day later (1 d) and remained stable in 2 w. A: Cochlear MRI at 3 h after administration; B: Vestibular MRI at 3 h post-administration; C: Cochlear MRI on 1 d after administration; D: Vestibular MRI on 1 d post-administration; E: Cochlear MRI on 2 w after administration; F: Vestibular MRI on 2 w post-administration. 2nd: second turn of cochlea; L: Left; R: Right. Scale bar=1.0 mm

Figure 4: DLS and ζ potential results for neat CAN-γ-Fe₂O₃ NPs (Ce³⁺)-doped maghemite (γ-Fe₂O₃) and peptide-conjugated NPs. 0310A₁₀% -CAN-γ-Fe₂O₃: covalent contact of tetra-branched anti-TNF-a peptide at 10%wt ratio with CAN-γ-Fe₂O₃ NPs; 0310A₅₀% -CAN-γ-Fe₂O₃: covalent contact of tetra-branched anti-TNF-a peptide at 50% wt ratio with CAN-γ-Fe₂O₃ NPs.
signal in the endolymphatic spaces of the cochlear hook region and vestibule including ampulla became visibly significantly brighter than that in the contralateral side (Figure 6) [15]. Similar morphology was visualized in both inner ears of rats receiving middle ear administration of tetra-branched anti-TNF-α-peptide-conjugated CAN-γ-Fe\(_2\)O\(_3\) NPs at 50% peptide ratio. However, no cell population or structure specific distribution of CAN-γ-Fe\(_2\)O\(_3\) NPs conjugated to tetra-branched anti-TNF-α peptide was observed (Figure 7).

**Figure 6:** Signal changes in rat inner ear on 1 d post-middle ear administration of tetra-branched anti-TNF-α-peptide-conjugated CAN-γ-Fe\(_2\)O\(_3\) NPs at 10% peptide ratio shown by MRI. The narrow strips in the cochlear basal turn and hook region of the delivery ear (L) suggest the scala media (2) while the perilymph signal in the scala vestibuli (1) and scala tympani (3) were wiped out due to distribution of tetra-branched anti-TNF-α-peptide-conjugated CAN-γ-Fe\(_2\)O\(_3\) NPs (A). The remnant endolymph signal in vestibule (Vest) (B), ampulla (Am) of the semicircular canal, and hook region of cochlea (H) (C) became bright. LSCC: Lateral semicircular canal; L: Left; R: Right. Scale bar=1.0 mm

**Figure 7:** Insignificant rat inner ear entry of tetra-branched anti-TNF-α-peptide-conjugated CAN-γ-Fe\(_2\)O\(_3\) NPs at 50% peptide ratio shown by MRI. The cochlea and vestibule (Vest) showed symmetric images on both the delivery (L) and contralateral (R) sides at the time points of 3 h (A, B), 1 d (C, D) and 2 w (E, F). Am: ampulla of the semicircular canal; 1st: basal turn of cochlea; 2nd: second turn of cochlea; L: Left; R: Right. Scale bar=1.0 mm

Inner ear distributions of tetra-branched anti-TNF-α-peptide-conjugated CAN-γ-Fe\(_2\)O\(_3\) NPs were histologically detected using Prussian blue staining (Figure 8 and 9). In accordance with the MRI results, CAN-γ-Fe\(_2\)O\(_3\) NPs conjugated to tetra-branched anti-TNF-α peptide at 50% peptide ratio did not sufficiently enter the inner ear (Figure 9).
The present study demonstrated that the tetra-branched anti-TNF-α peptide significantly inhibited apoptosis in HUVECs induced by TNF-α, with an inhibitory effect that was significantly greater than did the linear anti-TNF-α peptide. Both in vivo MRI and histology displayed that CAN-γ-Fe$_2$O$_3$ NPs conjugated to tetra-branched anti-TNF-α peptide at peptide ratio of 10% but not 50% efficiently entered the inner ear and distributed mainly in the perilymphatic spaces after administration in rat middle ear. It indicated that the previously reported novel CAN-γ-Fe$_2$O$_3$ NPs had the capacity to carry the novel tetra-branched anti-TNF-α peptide from middle ear into the inner ear [13]. It is possible that the tetra-branched anti-TNF-α peptides are able to pass the middle-inner ear barriers and enter the inner ear by themselves, however, conjugating the peptides onto CAN-γ-Fe$_2$O$_3$ NPs endowed the tetra-branched anti-TNF-α peptide a characteristic of visibility by MRI that will be potentially used as a theranostics agent.

**Discussion**

In the in vitro study, the tetra-branched anti-TNF-α peptide almost fully suppressed the inhibitory effect on HUVECs induced by TNF-α while the linear anti-TNF-α peptide reduced less than half of the TNF-α effect (Figure 5). The branched anti-TNF-α peptide was proven to inhibit TNF-α binding to human melanoma cells expressing TNF-α receptors. However, protecting effect of the tetra-branched anti-TNF-α peptide on cells against TNF-α receptor signaling activation has not been reported before [10]. TNF-α-specific peptide synthesized in linear and tetrameric form showed practically the same binding kinetics to human TNF-α [10]. The tetra-branched anti-TNF-α peptide suppressed the TNF-α-induced apoptosis in HUVECs more efficiently than did the linear one might be interpreted as following. The TNF-α-specific peptide and TNF-α receptor may bind to human TNF-α at different sites, and the TNF-α-specific peptide-complexed human TNF-α retains the affinity to TNF-α receptor; the tetra-branched anti-TNF-α peptide has larger spatial volume than the linear one due to the 3-dimensional conformation, and has higher possibility to block the
TNF-α-receptor binding site on the human TNF-α. The present work provided data showing that the novel tetra-branched anti-TNF-α peptide has potential to be applied in the clinic treatment of diseases associated with TNF-α receptor signaling activation.

In vivo tracking of the tetra-branched anti-TNF-α peptide will provide information on dynamics of the novel peptides in the inner ear, which is a critical issue with respect to quality control of therapeutic study. The novel CAN-γ-Fe₃O₄ NPs are a strong T₂-contrast agent attributed by superparamagnetic effect and provides an ideal tool to track the kinetics of therapeutics in the inner ear especially the perilymph and endolymph that are the major buffering systems of the inner ear [13]. The present experiment showed that tetra-branched anti-TNF-α peptide accumulated in the inner ear maximally 1 day after middle ear administration and remained in place for at least 2 weeks. The kinetics of CAN-γ-Fe₃O₄ NPs conjugated to tetra-branched anti-TNF-α peptide at a peptide ratio of 10% demonstrated to be significantly slower than that of neat CAN-γ-Fe₃O₄ NPs [13]. This further indicated that the observed dynamic distribution of CAN-γ-Fe₃O₄ NPs in rat inner ear represented the kinetics of tetra-branched anti-TNF-α peptide rather than the behavior of neat CAN-γ-Fe₃O₄ NPs.

Conclusion

In conclusion, the present study demonstrated that the tetra-branched anti-TNF-α peptide reduced apoptosis in HUVECs induced by TNF-α more efficiently than did the linear one. CAN-γ-Fe₃O₄ NPs conjugated to tetra-branched anti-TNF-α peptide at a peptide ratio of 10% but not 50% efficiently entered the inner ear and distributed in the perilymphatic spaces after administration in rat middle ear. It suggested that the previously reported novel CAN-γ-Fe₃O₄ NPs have the capacity to carry the novel tetra-branched anti-TNF-α peptide from middle ear into the inner ear. Coordinately conjugating the peptides onto CAN-γ-Fe₃O₄ NPs endowed the tetra-branched anti-TNF-α peptide a characteristic of visibility by MRI that will be potentially used as a theranostics agent. The present study therefore significantly broadened the application of both the novel peptide and CAN-γ-Fe₃O₄ NPs.

Acknowledgements

The authors thank Yong Xiong for his participating in high performance liquid chromatography of the peptides. This study was supported by China-EU collaborative project sponsored by Ministry of Science and Technology of China (contract grant number: 0102013DFFE33160).

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

Submit your next manuscript to Annex Publishers and benefit from:

- Easy online submission process
- Rapid peer review process
- Online article availability soon after acceptance for publication
- Open access: articles available free online
- More accessibility of the articles to the readers/researchers within the field
- Better discount on subsequent article submission

Submit your manuscript at