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Antinociceptive properties of nasal delivery of Neurotoxin-loaded nanoparticles coated with polysorbate-80

Yeping Ruan[a,*], Li Yao[a], Bingbing Zhang[a], Shuijuan Zhang[a], Jianyou Guo[b]

[a] College of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou 310053, PR China
[b] Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, Beijing 100101, PR China

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ABSTRACT

Neurotoxin-1 (NT) is an analgesic peptide which is endowed an exceptional specificity of action that blocks transmission of the nerve impulse. The aim of this study was to evaluate the potential application of nanoparticles technology as drug carrier system for the nasal delivery of NT. Mice were administered intranasally (i.n.) with NT (NT-P-NP), free NT solution (F-NT) and intravenously (i.v.) with NT (IV-NT) respectively. The NT levels in animal brain and antinociceptive activity of NT were analyzed. The result on brain transport showed that nanoparticles could exert enhanced delivery of NT into the brain significantly after i.n. administration. The results of antinociceptive activity showed that NT-P-NP increased immobility in the open-field test, both phases of formalin test were significantly inhibited by the NT-P-NP and NT-P-NP significantly inhibited the reaction time to thermal stimuli at 60 and 90 min. Both NT-P-NP and IV-NT were able to inhibit constrictions in acetic acid-induced writhing reaction. These data suggest that NT-loaded nanoparticles coated with polysorbate-80 could generate a significant improvement of drug levels in the brain. Intranasal administration of Neurotoxin-1 entrapped in nanoparticles coated with polysorbate-80 is an attractive alternative to intravenous administration.

1. Introduction

Neurotoxin-1 (NT), an analgesic peptide which was separated from the venom of Naja naja atra [10,18], is endowed an exceptional specificity of action that block transmission of the nerve impulse by binding to the α-subunit of the nicotinic acetylcholine receptor at the postsynaptic level of the neuromuscular junction [11,15,19]. However, daily injections of NT are still the most accepted route of administration. In some cases, in order to have a better control, multiple daily injections are necessary. To eliminate the pain [13] and fear [12] associated with injections and increase patient compliance, researchers have actively worked to identify administration routes or alternatives that are painless and non-invasive.

The use of a delivery system for nasal administration is a promising alternative to both daily injections and long term continuous subcutaneous infusion of NT. Convenience and compliance in patients will improve by avoiding daily injections. The mucosal layers of the nasal cavity provide access to the bloodstream and are often marked by relative large surface area for absorption.

It has been reported that nanoparticles overcoated with polysorbate-80 (P-80-NP) were capable of transporting the loaded drugs across the blood–brain barrier (BBB) after administration, which functioned as a tool to deliver drugs to the brain. The aim of this study was to evaluate the potential application of P-80-NP as drug carrier system for the nasal delivery of NT and demonstrate whether the NT-loaded P-80-NP (NT-P-NP) also presents antinociceptive properties.

2. Materials and methods

2.1. Chemicals

NT-1 (molecular mass 6952.19 Da; purity of 99.3%), consisted of 62 amino acid residues, was separated from the venom of Naja kaouthia (Yunnan, China). It was provided by Kunming Institute of Zoology, the Chinese Academy of Sciences. Polysorbate-80 was products from Huadong Medicine Group Co. Polylactide homopolymers (PLA) (m.w. of 11 kDa) was purchased from Sigma. Other reagents were analytical grade.

2.2. Animals

Kunming strain of Swiss mice weighing 20–22 g were purchased from the Experimental Animal Center, Zhejiang University. The

* Corresponding author. Tel.: +86 571 86613521; fax: +86 571 86613500.
E-mail address: Yepingruan@yeah.net (Y. Ruan).

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mice were maintained at room temperature under alternating natural light/dark photoperiod, and had access to standard laboratory food and fresh water *ad libitum*. This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals. Care was taken to minimize discomfort, distress, and pain to the animals.

2.3. Preparation of nanoparticles

The procedures used for preparing the nanoparticles were performed according to the method introduced by Qiaoyuan Cheng [14]. Briefly, a 50 μL of solution (pH 2.5 adjusted with 0.1 N HCl) containing 56 μg NT was emulsified in 1 mL of PLA in ethyl acetate (50 mg/mL) by sonication on an ice-bath for 30 s (40 W). Then, 2 mL of aqueous sodium cholate solution (1%, w/v) was added and the resulting (w/o)/w emulsion was sonicated for 20 s (40 W). The double emulsion was diluted in 100 mL aqueous sodium cholate solution (0.3%, w/v) and the solvent was rapidly eliminated by evaporation under vacuum. Finally, the nanoparticles were isolated by centrifugation (22,000 × *g*, 30 min), washed three times with water and lyophilized. Polysorbate-80 was added at a ratio of 1:1 (w/w) to reconstitute nanoparticles and incubated for 30 min.

2.4. Experimental design

The mice were randomly divided into four groups ($n = 10$, each group); groups 1 and 2 mice were administered intranasally with NT-P-NP (45 mg lyophilized nanoparticles were dissolved in 0.15 mL PBS with addition of 1:1 (w/w) polysorbate-80, 17 mg NT/kg) and free NT solution (F-NT, 17 mg NT/kg), respectively. Group 3 was treated (17 mg NT/kg) intravenously with NT-P-NP (IV-NT). Group 4 was administered intranasally with distilled water used as controls. After each experiment, the animals were anesthesitized with ethyl ether and sacrificed by decapitation [9]. The brain was quickly dissected and stored at −20 °C. The amount of drug in brain was measured by high-pressure liquid chromatography analysis.

2.5. Determination of Neurotoxin-1

The procedures used for detecting the NT were performed according to the method introduced by Yan [21,22]. Briefly, about 200 mg of brain sample was homogenized and homogenate sample was then mixed with 10 mL of ethanol–water (3:7, v/v), shaken briefly and sonicated for 1 h. The extracts were centrifuged at 15,000 × *g* for 10 min and the supernatant filtered through a 0.45 μm membrane. The solution was derivatized immediately. Samples were derivatized in 0.5 M $NaHCO_3$ within 2 min at ambient temperature by adding 50 mM para-nitro-benzyloxycarbonyl chloride (PNZ-Cl) in acetonitrile. The PNZ-derivatives were quantified by high-pressure liquid chromatography/ultraviolet absorption (LC-10ATvp liquid chromatograph; SHIMADZU) at a wavelength of 260 nm, and a Luna-C18 column (250 mm × 4.6 mm I.D.), 5 μm particle diameter (Phenomenex, USA) was used. SCL-10Avp system control SPD-10Avp UV–vis detector, FRC-10A fraction collector, CTO-2AS thermostat and 7725 injection valve was used in this study. A N3000 workstation (Zhejiang University, China) was used to collect and analyze data. The detection limit was evaluated by injecting successively lower concentrations until a signal-to-noise of 3:1 was achieved. Limits were 6.0 ng/mL.

2.6. Open field test

The open field area was made of acrylic (transparent walls and black floor, 60 cm × 60 cm × 50 cm). The open field was used to evaluate the exploration activity of the animal. After 30 min of administration each mouse was placed in the center of the arena and the movement was measured for 10 min to assess total distance traveled and the time spent in the center zone (area > 12 cm from walls). Total distance traveled was indicative of general activity level, whereas the percent time spent in the center was used as an index of the anxiety state of mice [17].

2.7. Formalin test

Formalin test has been used as a model for tonic pain and localized inflammatory pain. 20 μL of a 1%-formalin solution was injected into the right hind paw of mice, and the licking time was recorded after the first 5 min (1st phase, corresponding to a direct chemical stimulation of nociceptors) and after 20 min (2nd phase, involving inflammation), for 5 min each time [4,6].

2.8. Hot-plate test

In this test, mice were pre-selected according to their reactions to a thermal stimulus (jumping or licking of hind limbs when placed on a hot plate at 55 °C). Latency times were recorded immediately before and 30, 60 and 90 min after drug administration, up to a maximum time of 40 s to avoid paw lesions [20].

2.9. Writhing test

Mice were treated with NT-P-NP, FNT and IV-NT 60 min before receiving a 0.6% acetic acid injection (10 mL/kg, *ip*). The number of contractions or writhing, determined by abdominal muscle contractions and hind paw extension was recorded for 20 min, starting 10 min after the administration of acetic acid [8].

2.10. Data analysis

All data were analyzed by a one-way analysis of variance, and the differences between means were established by Duncan's multiple-range test. The data represents means and standard deviations. The differences between NT-P-NP and IV-NT groups were also analyzed by Paired-samples *t*-test. The significant level of 5% ($p < 0.05$) was used as the minimum acceptable probability for the difference between the means.

3. Results

Fig. 1 shows the NT (peptide) concentrations in mice brain after different administration in mice. When the mice were given NT solution intranasally, the concentration of NT (peptide) in the brain was 6.26 ± 0.23 ng/mL. When the mice were treated intravenously

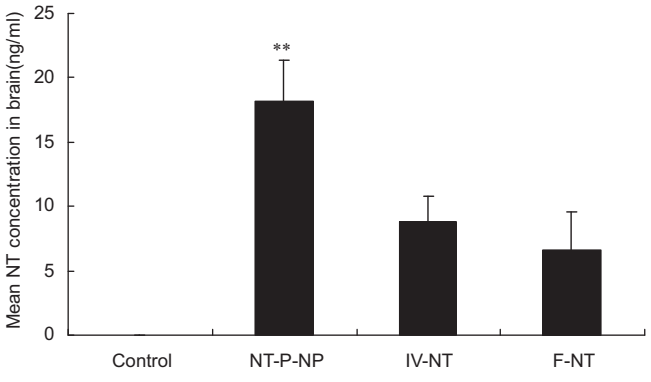

Fig. 1. Mean NT concentration in brain (ng/ml). Values are shown as means ± SD. **$p < 0.01$ vs. IV-NT and F-NT group.