Evaluation of pulmonary toxicity of zinc-doped magnetite nanoparticle in mice after intragastric administration

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Abstract: Zn^{2+} doped magnetite nanoparticles ($Zn_aFe_bO_4$ NPs) have higher magnetic susceptibility than conventional magnetite nanoparticles (Fe_3O_4 NPs). Dimercaptosuccinic acid (DMSA)-coated $Zn_{0.4}Fe_{2.6}O_4$ nanoparticles ($Zn_{0.4}Fe_{2.6}O_4$ NPs) were synthesized. The pulmonary toxicity of DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs in mice was evaluated after oral administration for one month. No abnormal activities among the mice were observed in $Zn_{0.4}Fe_{2.6}O_4$ NPs-treated group during the whole experiment process. The accumulation of Fe in the lungs was observed after oral administration of DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs in mice. The accumulation of Fe in the lungs resulted in an increase of coefficient of lung, but did not cause obvious pulmonary injury, except for a very slight inflammatory response in the tissue. The results show that low toxic DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs may be used as drug delivery or imaging contrast agents by oral route.

Key words: magnetite nanoparticle; Zn; toxicity; histopathology; intragastric administration

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$Zn_{0.4}Fe_{2.6}O_4$ 纳米颗粒对小鼠肺毒性效应

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摘要: 锌掺杂的磁性纳米颗粒($Zn_aFe_bO_4$ NPs)比常规的磁性纳米颗粒(Fe_3O_4 NPs)具有更高的磁化率. 合成了二巯基丁二酸(DMSA)包裹的 $Zn_{0.4}Fe_{2.6}O_4$ 纳米颗粒(NPs),并研究了 $Zn_{0.4}Fe_{2.6}O_4$ NPs 灌胃给药对小鼠肺的毒性效应. 在整个灌胃给药一个月的实验过程中,对照组和实验组的小鼠均无异常现象发生. 灌胃给药 $Zn_{0.4}Fe_{2.6}O_4$ NPs 以后,Fe 在小鼠的肺内聚积. 肺内 Fe 的聚积导致肺的脏器系数增加. $Zn_{0.4}Fe_{2.6}O_4$ NPs 灌胃给药除了引起肺组织非常轻微的炎症反应以外,没有对肺产生明显的损伤. 实验结果表明, DMSA 包裹的

$Zn_{0.4}Fe_{2.6}O_4$ NPs 毒性低,可以作为口服的药物载体或成像的造影剂.

关键词:磁性纳米颗粒;Zn;毒性;组织切片;灌胃

0 Introduction

Nanomaterials have been widely used in various fields due to their unique physicochemical properties including large surface area, small size and high reactivity. Among a wide variety of nanomaterials, magnetite nanoparticles (MNP) have been widely proposed for biomedical applications, including drug delivery, magnetic resonance imaging and hyperthermia[1], mainly due to their unique magnetic properties. With the wide range of applications of MNP, there comes an increasing concern about its potential risk to human health[2-4].

Fe_3O_4 nanoparticles, as one of typical manufactured nanomaterials, are produced on an industrial scale. Therefore, they are related to environmental and occupational exposure. Previous studies demonstrated that Fe_3O_4 nanoparticles (9 nm) were mainly distributed in the lung, liver and spleen of mice by intravenous injection with a single dose of 163.60 mg/kg[5]. Because the lung is one of the main target organs of MNP, an increasing number of reports describing MNP toxicity on lungs have been published. Baratli et al.[6] showed that Fe_3O_4 nanoparticles (9 nm) did not show any toxicity on mitochondrial after exposure of normal lungs of rats to 100, 200, 300 and 500 $\mu g/mL$. Cho et al.[7] reported that Cy5.5-conjugated thermally cross-linked superparamagnetic iron oxide nanoparticles (TCL-SPION) (36 nm) (5.4 mg/kg) may mildly induce pulmonary inflammation in mice by intratracheal instillation, but they have no toxicity under the concentration of 1.8 mg/kg. Totsuka et al.[8] demonstrated that inflammatory responses involved in the genotoxicity were induced in the lungs of mice after intratracheal instillation of nanosized-magnetite with single dose of 0.05 or 0.2 mg per animal. These studies were undertaken to determine MNP-induced pulmonary toxicity by intratracheal inhalation/instillation or intravenous injection. However, the MNP-induced pulmonary toxicity by oral administration is still unclear. MNP can be used as oral targeted drug delivery and MRI contrast agents[9-10]. Thus it is necessary to determine the pulmonary toxicity of MNP by oral route.

Lee et al.[11] reported that Zn doped Fe_3O_4 nanoparticles show higher magnetization values than that of Fe_3O_4 nanoparticles and are more appropriate than Fe_3O_4 nanoparticles in biomedical application. In this study, dimercaptosuccinic acid (DMSA)-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs with high saturation magnetization values were synthesized easily and economically. A preliminary study was undertaken concerning the pulmonary uptake and toxicity of DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ after intragastric administration in mice for one month. It might be useful for determining the risks involved in the biomedicine regarding lung use of this type of MNP.

1 Experimental

1.1 Synthesis and characterization of DMSA-MNP

The synthesis of DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ nanoparticles (11 nm) was achieved as described by Liang et al.[12] with slight modifications. $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$ and $ZnSO_4$ were dissolved in 20 mL water to give a 1.73×10^{-3} mol Fe^{2+} and 2.67×10^{-4} mol Zn^{2+} precursor. Then, 10 mL oleic acid, 1 g NaOH and 10 mL ethanol were mixed by stirring at room temperature to get an even solution. Thereafter, the Fe^{2+} precursor was added to the mixed solution. After stirring for a few minutes, the precipitate turned brown. The mixed reactants were transferred into a 50 mL autoclave, sealed, and heated at 230 ℃ for 15 h. The system was then allowed to cool to room temperature. The products deposited at the bottom

of the vessel, and cyclohexane was added to the solution to collect the nanoparticles. The organic surfactants on the nanoparticle surface were double-exchanged with DMSA to make the nanoparticles completely dispersed in the aqueous medium. The DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ suspensions were stored at 4 ℃ until toxicity testing. The size, shape and distribution of as-synthesized nanoparticles were investigated by transmission electron microscopy (TEM) (JEM-2010, JEOL Ltd, Japan). The chemical interaction between hydrophobic $Zn_{0.4}Fe_{2.6}O_4$ and DMSA were investigated by Fourier transform infrared spectroscopy (FTIR, Thermo, Nicolet 8700, USA). Iron oxide powder samples were dried at 60 ℃ under vacuum for 24 h and then were prepared by pressing it into a KBr pellet. The element contents of the nanoparticles were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (OPTIMA 7300DV, PerkinElmer, USA). Magnetic properties were determined using a quantum design superconducting quantum interference device (SQUID) magnetometer (SQUID-VSM, Quantum Design, USA) at 300 K.

1.2 Treatment of animals

Imprinting control region mice (ICR) (30 g ± 2 g, 6～8 weeks old) were obtained from Shanghai Slac Laboratory Animal Co., Ltd (China). Mice were maintained under controlled conventional conditions (12 h light/dark cycle; temperature 22 ℃ ± 2 ℃; relative humidity 50% ± 5%). Animals were provided with sterilized food and distilled water ad libitum. All procedures using laboratory animals were reviewed and approved by the Animal Ethical Committee of University of Science and Technology of China.

After one week of acclimation, mice were randomly assigned into two groups (10 male mice in each group): control group (0.9% saline) and experimental group (50 mg $Zn_{0.4}Fe_{2.6}O_4$ NPs per kg body weight per day). $Zn_{0.4}Fe_{2.6}O_4$ NPs were suspended in physiological saline and stirred for 5 min and then sonicated for 30 min at 90 W (KQ2200DE ultrasonicator, Kunshan Ultrasonic Instrument Co. Ltd., China) before the injection into mice. Chemicals were given to mice via intragastric administration at a dose of 10 mL per kg body weight per day over a period of 30 days. Clinical observations were recorded daily during the study period. After an overnight fast, the mice were sacrificed after being anesthetized for appraising the biochemical factors. After weighing the body and lungs, the coefficient of lung to body weight was calculated as the ratio of lung (mg, wet weight) to body weight (g).

1.3 Histopathological examination

For histopathological evaluation, the lung tissue was removed from the mice with or without nanoparticles administration and fixed in 10% (volume fraction) formalin, embedded in paraffin blocks, then sliced into 5 mm thick sections. The sections were stained with hematoxylin and eosin (H&E) and then photographs were obtained with an optical microscope (IX-81, Olympus, Japan).

1.4 Analysis of Fe and Zn biodistribution

The lung tissues (0.1～0.3 g) were chemically digested using ultrapure nitric acid overnight, and then heated at 130 ℃ to remove the remaining nitric acid, followed by additional 0.5 mL of 70% perchloric acid. Then, the solutions were heated again until only inorganic content was left, subsequently cooled to room temperature. Finally, the samples were diluted to 3 mL with 2% (volume fraction) nitric acid and filtered through a 0.22 μm cellulose membrane filter for analysis. The Fe and Zn contents in the lung were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Perkin Elmer Corporation, USA).
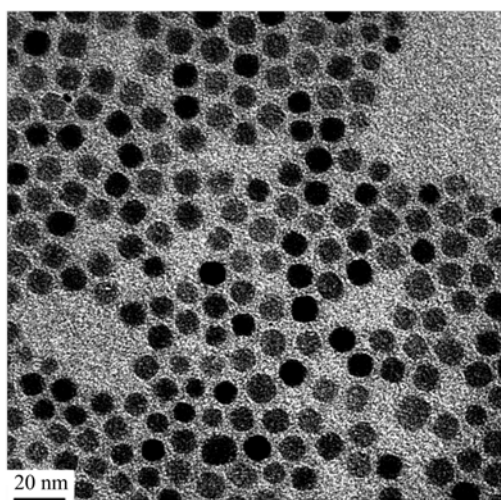
1.5 Statistics analysis

Values were expressed as means ± standard deviation (SD, $n = 10$). All the results were subjected to one-way analysis of variance (ANOVA) test using SPSS 16.0 and the Dunnett's test (SPSS Inc., Chicago, IL), where $p < 0.05$

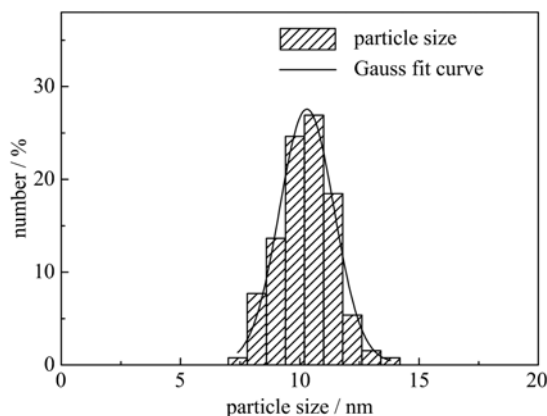was considered to indicate statistical significance for all tests.

2 Results

2.1 Synthesis and characterization of $Zn_{0.4}Fe_{2.6}O_4$ NPs

Monodisperse $Zn_{0.4}Fe_{2.6}O_4$ NPs were synthesized by using a simple and low-cost hydrothermal method based on an oleic acid/alcohol/water system. The size and morphology of as-synthesized $Zn_{0.4}Fe_{2.6}O_4$ NPs were analyzed by TEM. As illustrated in Fig. 1(a), the particles have high monodispersity and a nearly spherical shape. The average size of the $Zn_{0.4}Fe_{2.6}O_4$ NPs is 11.0 nm ± 0.5 nm. Size distribution histogram (Fig. 1(b)) was obtained via considering 411 particles and was fitted using a log-normal distribution. The log-normal size distribution indicates that $Zn_{0.4}Fe_{2.6}O_4$ NPs have a crystal habit[13].

Because DMSA coated nanoparticles display high colloidal stability in aqueous media, surfactants on the oleic acid coated nanoparticle surface were exchanged with DMSA molecules. FTIR spectroscopy in Fig. 2(a) provides information about iron oxide phase and the coating molecules. The sharp peak at 582 cm^{-1} in both a and b curve belongs to the spinel structure and the stretching mode of Fe—O in Fe_3O_4[14]. In the oleic acid coated $Zn_{0.4}Fe_{2.6}O_4$ NPs curve, the characteristic bands at 1 425 and 1 577 cm^{-1} of COO— symmetric and asymmetric stretching and 2 853 and 2 924 cm^{-1} of C—H symmetric and asymmetric stretching verify the adsorption of oleic

(a) TEM image analysis of the particle morphology and size

(b) Size distribution histogram

Fig. 1   Characterization of $Zn_{0.4}Fe_{2.6}O_4$ NPs
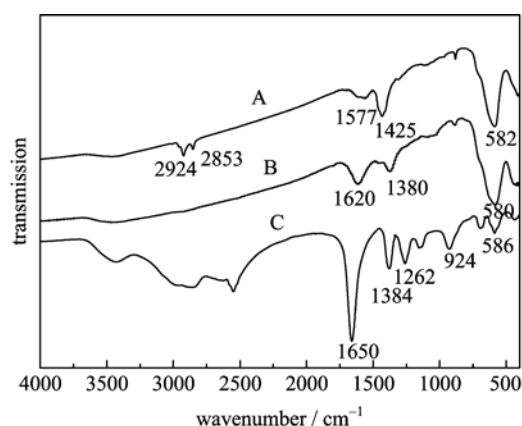
(a) Fourier transform infrared spectroscopy of $Zn_{0.4}Fe_{2.6}O_4$ NPs (A), oleic acid-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs and DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs (B) and DMSA (C)
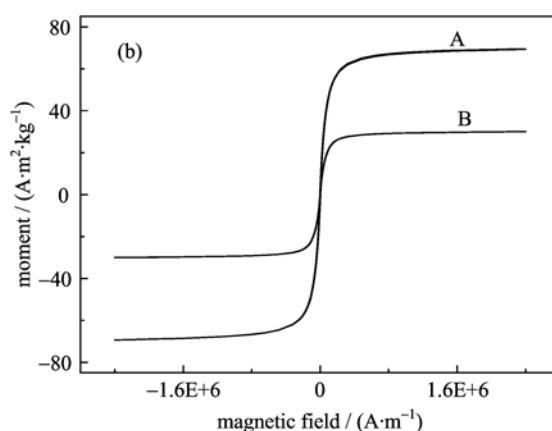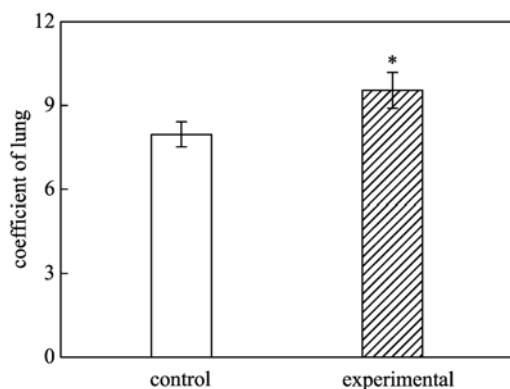

(b) Magnetization curves of $Zn_{0.4}Fe_{2.6}O_4$ NPs (A) and Fe_3O_4 NPs (B)

Fig. 2   Characterization of DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs

acid molecules on the surface of $Zn_{0.4}Fe_{2.6}O_4$ NPs. In the DMSA coated $Zn_{0.4}Fe_{2.6}O_4$ NPs curve，1 620 and 1 380 cm^{-1} peaks can be attributed to the asymmetry and symmetry stresses of the COO group of DMSA，respectively，which correspond to the 1 650 and 1 384 cm^{-1} peaks in the DMSA curve（Fig. 2（a））[15]. Based on the FTIR spectra，DMSA is believed to coat the surface of the as-prepared $Zn_{0.4}Fe_{2.6}O_4$ NPs. The obtained nanoparticles can form stable aqueous suspension for several months. ICP-AES analysis indicates that the Fe to Zn mole ratio of the sample is 2.63 : 0.38. The magnetic saturation value of $Zn_{0.4}Fe_{2.6}O_4$ NPs measured by using SQUID is 69.5 A · m^2 · kg^{-1}，which is higher than Fe_3O_4 NPs（29.5 A · m^2 · k g^{-1}）（Fig. 2（b））.
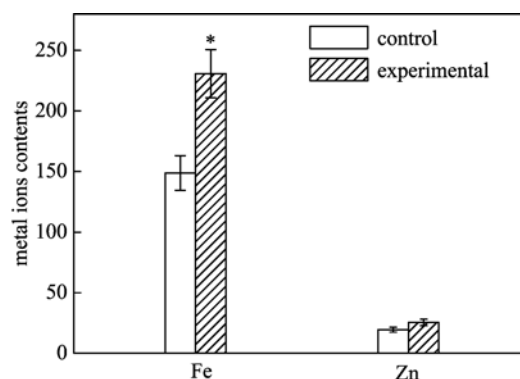
2.2 Coefficient of lungs and contents of Fe and Zn

During the 30 d study period，no animals showed any abnormal daily activity and symptoms except that several mice in $Zn_{0.4}Fe_{2.6}O_4$ NPs-treated group appeared to breathe audibly during the last week of the experiment. Compared to the control group，the coefficient of lung（Fig. 3）in $Zn_{0.4}Fe_{2.6}O_4$ NPs-treated group increased significantly（$p < 0.05$）. The contents of Zn and Fe in lungs were determined by ICP-AES. As shown in Fig. 4，the content of Fe in $Zn_{0.4}Fe_{2.6}O_4$ NPs-treated group was higher than that of the control group（$p < 0.05$），but the contents of Zn detected in the control and $Zn_{0.4}Fe_{2.6}O_4$ NPs-

Data are shown as mean±SD（$n=10$）；＊ $p < 0.05$

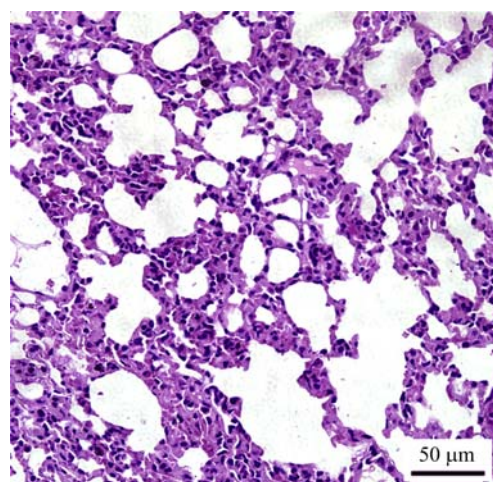**Fig. 4 The contents of Fe and Zn in the lung of the mice after oral exposure to $Zn_{0.4}Fe_{2.6}O_4$ NPs**

treated groups were nearly similar.
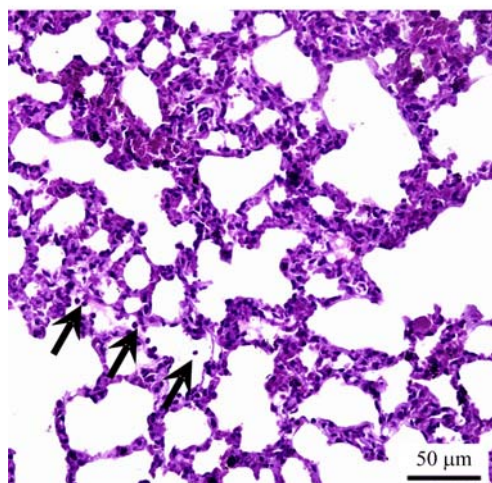
2.3 Histopathological analysis of tissues

For evaluating the toxicity of DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs，histopathological assessments were performed on the lung tissue. As shown in Fig. 5，there were clear pulmonary alveoli in the control group. Administration of DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs caused no significant inflammatory cell infiltration，no vascular congestion，alveolar edema and alveolar collapse. The tissues were comparatively the same as that in the control group except that there were several inflammatory lymphocytes scattered within a few alveoli. This result indicates no significant adverse effects of DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs on the lung after long-term oral administration.

3 Discussion

For biocompatible in biological applications and localization in a specific area，proper surface modifications of magnetic particles are needed. DMSA is nontoxic and therefore considered as a biocompatible surface coating agent[16]. Previous studies show that the DMSA coating on MNP，which serves as a barrier against direct contact between nanoparticles and tissues for inhibiting a potential toxic effect，preferentially targets DMSA-MNP to the lung after intravenous injection[17]. Our results also show that high accumulation of Fe occurred in the lungs after

Data are shown as mean±SD（$n=10$）；＊ $p < 0.05$

Fig. 3 Coefficient of the lung after oral exposure to $Zn_{0.4}Fe_{2.6}O_4$ NPs

(a) The control group shows normal pulmonary alveoli

(b) The treated group shows several inflammation cells
(lymphocytes) scattered in alveoli (black arrows)

**Fig. 5　Lung histopathology of mice after oral
exposure to $Zn_{0.4}Fe_{2.6}O_4$ NPs**

long-term oral administration of DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs. It indicates that the surface modifications of nanoparticles may affect their in vivo targeting. In addition, the lung is enriched with the reticuloendothelial system, which contains amounts of macrophages involved in the uptake of foreign particulates[18]. Therefore, DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs is a favorable candidate as a drug-carrier or imaging agent for lung targeting.

Lin et al.[19] reported that nano-Fe_3O_4 could produce mild to moderate persistent lung inflammatory responses by intratracheal instillation at a dose of 2 or 10 mg/kg once every 2 d for 35 d.

Srinivas et al.[20] showed that the rats exposed to Fe_3O_4 NPs（640 mg Fe_3O_4 NPs/m^3）showed cytotoxicity via oxidative stress. The present results indicate that DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs did not cause significant histopathological changes in the lungs of mice, except for an increase in the coefficient of the lung, which might be caused by accumulated Fe-induced slight inflammation in lungs after long-term exposure. The absence of abnormal activities of mice in the treated groups during the whole experiment process also indicates that DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs have no significant toxic effect on the mice lungs via oral administration. These results together suggest that DMSA coating on MNP can attenuate the MNP-induced toxicity in lungs. A similar study demonstrated that DMSA-coated MNP accumulated in lung tissues by intravenous injection with no significant signs of toxicity[21]. The injected dosage of DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ in this study is substantially higher than that required in ordinary biomedical applications, such as diagnostic liver MRI（20 μmol Fe/kg）[22]. Therefore, low toxic DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs may be an appropriate candidate as drug delivery or imaging contrast agents by oral route. Further experiments are necessary to clarify the mechanism underlying lung toxicity of DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs and confirm their safety for clinical application.

4　Conclusion

In the present study, the accumulation of Fe in the lung was observed after oral administration of DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs in mice for one month. The accumulation of Fe in the lung did not cause obvious pulmonary injury, except for a very slight inflammatory response in the tissue. Low toxic DMSA-coated $Zn_{0.4}Fe_{2.6}O_4$ NPs may be used in biomedical science.

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