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Evaluation of local anesthetic effects of Lidocaine-Ibuprofen ionic liquid stabilized silver nanoparticles in Male Swiss mice

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A B S T R A C T

A simple approach for the synthesis of Lidocaine-Ibuprofen ionic liquid stabilized silver nanoparticles (IL-AgNPs) was reported in this work. The shape, size and surface morphology of the Lidocaine-Ibuprofen ionic liquid stabilized AgNPs were characterized by using spectroscopic and microscopic techniques such as Ultraviolet-visible spectroscopy (UV–Visible), X-ray diffraction (XRD) analysis, Selected area electron diffraction (SAED), Transmission electron microscopy (TEM). TEM analysis showed the formation of 20–30 nm size of IL-AgNPs with very clear lattice fringes. SAED pattern confirmed the highly crystalline nature of fabricated IL stabilized AgNPs. EDS results confirmed the formation of nanosilver. The fabricated IL-AgNPs were studied for their local anesthetic effect in rats. The results of local anesthetic effect showed that the time for onset of action by IL-AgNPs is 10 min, which is significantly higher than that for EMLA. Further, tactile test results confirmed the stronger and faster local anesthetic effect of IL-AgNPs when compared to that of EMLA.

1. Introduction

In recent times, noble metal nanoparticles has increased attention of researchers all over the world because of their extraordinary electronic, optical and magnetic features. Due to their exceptional properties, Silver nanoparticles (AgNPs) possess applications in different research areas of science and technology such as pharmaceuticals [1], sensing [2], optoelectronics [3], electronics [4], catalysis [5,6], photonics [7], antimicrobial products [8,9] and therapeutics [10–12].

Moreover, the AgNPs possess anti-microbial and anti-fungal activity and hence AgNPs based products are being available in the market with low production costs. For instance, AgNPs have been used in bandages, food packing, deodorizers, coatings and in disinfectants [13,14]. The most traditional preparation methods for AgNPs involves the photo reduction [15], polyol process [16,17], microwave irradiation [18], acrylate/citrate reduction [19–22], and plants [23]. One of the major drawbacks of this method involves the use of toxic chemicals which are expensive and requires high energy.

Therefore, there is a requirement for the new synthetic methods for NPs that involves environmental friendly and safe experimental procedures with high scale of production without using toxic chemicals. In literature, various biomolecules such as amino acids [24], vitamins [25] and plant polyphenols are being used for the NPs production. Therefore, the demand for the green synthetic approaches for the NPs that are useful for biological and medical applications is growing day by day.

In this work, we reported the Lidocaine-Ibuprofen ionic liquid stabilized AgNPs. Also, the ionic liquid (IL) stabilized nanoparticles were characterized by various spectroscopic and microscopic techniques. The present work also involves the local anesthetic effect of Lidocaine-Ibuprofen ionic liquid stabilized AgNPs in male rats.

2. Experimental Section

2.1. Materials

$AgNO_3$, Lidocaine-Ibuprofen ionic liquid, NaOH and all other biochemicals and solvents were obtained from Sigma Aldrich chemicals, Shanghai, China. Male hairless rats (280–340 g weight, CD hairless rats, Shanghai) were used for in vivo studies.

2.2. Preparation of Silver Nanoparticles

AgNPs were synthesized by mixing 50 mg ionic liquid in 10 mL of milli Q water followed by the addition of 1 mL of 10 mmol/L $AgNO_3$ aqueous solution and the resulting solution was maintained a pH of 10 using 0.1 M NaOH. To the above solution, 0.5 mL of 0.1 mol/L of $NaBH_4$ was gradually added and allowed for vigorously stirring for about 30 min under room temperature conditions. The solution was

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centrifuged to obtain the precipitate which was later washed several times with milli Q water followed by drying in oven at 60 °C under vacuum conditions.

2.3. Local Anesthetic Effect of Silver Nanoparticles

Thermal and tactile tests were conducted to know the delivery of AgNPs into the skin and to know their local anesthetic effect in male hairless rats. During the thermal test, the rats were adapted to the test cubicles for about half an hour before the experiment. A Qtip was used to administer the IL stabilized AgNPs onto the surface of a hind-paw of rats. After 10 min, the superfluous AgNPs were removed and local anesthetic effect was measured with time using the hot-plate test. During the experiments, the apparatus was maintained with thermocouple and timer to sustain a constant temperature of about 55 °C with time. For each trial of experiments, both the paws were tested with a time gap of 1 min. The response time was known by considering the time essential for lifting or licking the paw from the hot surface. The experiment was strictly followed with automatic stop of the heat stimulus if the animal did not remove its paw within a time of about 20 s. To get baseline latency, each rat was served with its own negative control and the same rat was used for local anesthetic study experiments. The average time of paw withdrawal was considered as the response latency of the rat. EMLA (Eutectic mixture of local anesthetics) was used as positive control during these experiments.

During tactile test, about 1 mL of AgNPs was administered to the middle of rat tail. A Von Frey Hair test was used to know the dermal anesthesia at various times, which measures touch sensation threshold limit. To perform the above test, a larger diameters size of Von Frey hairs were administered to the tail where the local anesthetic was studied till the tactile threshold was found. To avoid the bucklings, an automatic force is produced by each hair and hence it is possible to determine the minimum force required to evoke a tail flicking. About 5 s interval was maintained to apply for each hair for 10 times applications. Finally, the tactile threshold limit was known by considering the tail flicking response in 8 of the 10 trials.

2.4. Characterization

Ultraviolet visible (UV–vis) spectroscopic data of synthesized colloidal AgNPs was obtained as a function of wavelength range of 200–900 nm using Elico SL 196 spectrophotometer instrument at a resolution of 1 nm. Philips CM200, Netherlands instrument was used to know the morphology and size of the fabricated NPs by transmission electron microscope (TEM) analysis. About 115 kV acceleration voltage was used for TEM measurements. The samples for analysis were made by depositing the AgNPs solution onto the surface of copper grid and dried before analysis.

3. Results and Discussion

When the Lidocaine-Ibuprofen ionic liquid was added to $AgNO_3$ solution, the colour of the reaction mixture is changed from pale yellow to brown after stirring of about 15 min, representing the formation of AgNPs. Fig. 1 represented the optical absorbance peak for fabricated AgNPs at 435 nm found in the visible region which is characteristic to the surface plasmon resonance of ionic liquid stabilized silver nanoparticles indicating the transformation of Ag^+ ions to Ag^0 stage. From the literature, it is known that basic pH enhance the reduction potential features of reducing agents [26] and hence the pH of the reaction solution was maintained to 10 during the synthesis of AgNPs.

The formation of AgNPs is also confirmed by XRD analysis (Fig. 2). From XRD analysis it is represented the presence of three X-ray diffraction peaks with corresponding indexing planes are shown which located at 38.1° (111), 44.1° (200) and 64.1° (220), signifying the face centred cubic nano silver. XRD results showed that the fabricated nano silver has a calculated lattice constant of about 4.086 Å. The obtained results are well matched with the Joint Committee on Powder Diffraction Standards data base with corresponding standard JCPDS file No. 04-0783. The average grain size of fabricated silver nano colloids is calculated from the following Scherr's formula and is found to be 35 nm.

Scherr's formula, $d = (0.9\lambda \times 180^{\circ})/\text{ß}\cos$

Further, the morphology and size of fabricated AgNPs are studied by using TEM analysis. Fig. 3 showed the TEM images of AgNPs stabilized by Lidocaine-Ibuprofen ionic liquid. From figure, it is found that the size of IL stabilized AgNPs is found to be 20–30 nm. Fig. 3B represented the high resolution TEM image of single AgNPs showing clear lattice fringes which indicated the highly crystalline nature of ionic liquid stabilized AgNPs. Additionally, Fig. 3C showed the selected area electron diffraction (SAED) pattern of AgNPs with sharp diffraction spots indicating the crystalline nature of AgNPs. However, similar kind of SAED pattern for AgNPs was already reported in literature [24].

Fig. 4 showed the EDS spectrum of AgNPs. The EDS spectrum represented the strong signal peak of silver. It is also showed a peak of carbon element which is due to the carbon grid that is used for sample preparation of analysis. Further, the presence of silver peak confirmed the formation of silver nanoparticles.

Further the capping of IL onto the surface of NPs was known by using FTIR analysis. Fig. S1 showed the FTIR results of fabricated AgNPs. The vibrational band found at 3426 cm^{-1} is because of O—H stretching vibrations. The other vibrational bands located at 2870 and 2954 cm^{-1} were characteristic to methylene and methyl stretching vibrations, correspondingly. The C═O stretching vibrations were recognised with presence of bands at 2338 and 1651 cm^{-1}. Additionally, the presence of band with high intensity at 1589 cm^{-1} and low intense band at 1512 cm^{-1} are corresponding to vibrations of C═C functional group. Also, the bands found at 1438 and 795 cm^{-1} are due to C—H deformation vibrations. All these results confirmed the stabilization of AgNPs with IL.

3.1. Local Anesthetic Effect

Fig. 5 showed the thermal anesthetic analysis of IL stabilized AgNPs. Before starting the thermal text, the average paw withdrawal latency is found to be 9.0 s. The negative control group, with no administration of AgNPs, represented a slightly lesser latency time, which is not significant. On the other hand, the positive control group, with administration of EMLA, it is found that no change in latency time was observed for about 40 min time. It is also found an increase in the latency time after 1 h, followed by a slow decrease with time, representing that EMLA required about 1 h time to induce noteworthy local anesthesia effect in the rat skin. It is found that the application of AgNPs, raised the latency time significantly at the first data point at 20 min. Similarly, paw withdrawal response showed a steady raise in the latency time during the course of the first 20 min. It is also found that the latency time was significantly higher after 10 min, when compared to that at 50 min. It is also comparable with latency value at 60 min after administration of EMLA. From these results, it can be concluded that the time for onset of action by AgNPs is 10 min, which is knowingly higher than that for EMLA.

Fig. 6 showed the tactile anesthetic analysis of AgNPs. Von Frey Hair test was conducted to know the local anesthesia (tactile sensation) in the tail of rat. During this experiments, nylon filaments of different diameter are stressed against the rat's skin to know the threshold of sensation. This experiment was performed with a controlled force and a flick of the rat's tail is observed to know threshold of sensation. When no anesthetic was administered, the tail flick sensation threshold is found to be constant over time at 5.3 g. On the other hand, the threshold sensation is raised over 60 min when EMLA is applied, which later decrease over following 60 min representing a reduced local anesthetic effect. Also, the threshold sensation value do not show a