International Journal of Biological Macromolecules 103 (2017) 379–384

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

In vivo evaluation of toxicity and antiviral activity of polyrhodanine nanoparticles by using the chicken embryo model

Ahmad Nazaktabar[a], Mohammad Soleimani Lashkenari[b,*], Atefeh Araghi[a,*], Mohsen Ghorbani[c], Hannaneh Golshahi[d]

[a] Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran
[b] Faculty of Engineering Thechnologies, Amol University of Special Modern Technologies, Amol, Iran
[c] Faculty of Chemical Engineering, Babol University of Technology, Babol, Iran
[d] Department of Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

ARTICLE INFO

Article history:
Received 2 February 2017
Received in revised form 9 April 2017
Accepted 15 May 2017
Available online 17 May 2017

Keywords:
Polyrodanine
Nanoparticles
Chemical polymerization
Toxicity
Antiviral activity

ABSTRACT

Evaluation of the potential cytotoxicity of polyrhodanine nanoparticles is an important factor for its biological applications. In current study, for the first time histopathological and biochemical analysis of polyrhodanine besides of its antiviral activity against Newcastle disease virus (NDV) were examined on chicken embryo model. Polyrhodanine was synthesized by the chemical oxidative polymerization method. The obtained nanoparticles were characterized by scanning electron microscopy (SEM), and Fourier transform infrared (FTIR). Different doses of polyrhodanine nanoparticles were injected into the albumen in 4-day-old embryonic eggs for groups: (0.1 ppm, 1 ppm, 10 ppm and 100 ppm), while the Control group received only normal saline. The gross examination of chicks revealed no abnormality. No pathological changes were detected in microscopical examination of the liver, kidney, spleen, heart, bursa of Fabricius and central nervous system tissues. Blood serum biochemical indices showed no significant differences between control and treatment groups. Interestingly, polyrhodanine nanoparticles showed strong antiviral activity against NDV *in ovo*. These preliminary findings suggest that polyrhodanine nanoparticles without any toxicity effect could be utilized in controlling Newcastle disease in chickens.

1. Introduction

In recent decades the unique characteristics of conducting polymers e.g. low cost, environmental and thermal stability, good conductivity, easy synthetic route, and biocompatibility, attract significant attention of many research groups [1,2]. Among the different structures of conductive polymers, polyrhodanine has been studied in extensive range of research fields. Rhodanine and its derivatives were reported to have various biological properties such as antidiabetic, anticonvulsant, antihistaminic, antimicrobial and antiviral activities [3–8]. Furthermore, they also used as heavy metal absorbent and anticorrosion agent [9–11].

Polyrhodanine with active functional groups such as amine, sulfide, carbonothioyl and carbonyl and/or amide and dithiocarbamate can act as an advantaged scaffold for drugs and antimicrobial agents which make it an attractive candidate for biomedical application [12]. The active functional groups present in rhodanine ring make it possible to synthesize compounds by various biological interests [13]. Biocompatibility of well-known conductive polymers such as polypyrrole and polyaniline has been extensively studied in several *in-vivo* and *in-vitro* systems [14,15].

Newcastle disease is one of the most important diseases of poultry industry which beside of causing high mortality, enormously impacts the economy of this industry and in this sense it is the most important disease in the poultry industry. It has been demonstrated that one of the most important ways of spreading of Newcastle disease virus (NDV) to new geographical areas and introducing to poultry farms is contaminated water and feed and appropriate hygienic practices play an important role in dramatically reduction of spreading of the virus [16,17]. Polyrhodanine was also suggested for antimicrobial applications, such as antimicrobial additives, food-packaging, and antimicrobial coatings. Therefore, evaluating biocompatibility properties of polyrhodanine can be

* Corresponding authors.
E-mail addresses: m.soleimani@ausmt.ac.ir (M.S. Lashkenari), a.araghi@ausmt.ac.ir (A. Araghi).

http://dx.doi.org/10.1016/j.ijbiomac.2017.05.069
0141-8130/

used for broadening its applications in biological sciences and industry (including use as a disinfectant additive and etc).

To the best of our knowledge, nothing has been reported on the assessing biocompatibility of pristine polyrhodanine. In this regard, the purpose of the present study firstly was to synthesis polyrhodanine nanoparticles and evaluation its effects on embryo growth and development, secondly antiviral activity of polyrhodanine nanoparticles against NDV was assessed *in ovo*.

2. Material and methods

Rhodanine (97%) was purchased from Merck, Germany. Potassium permanganate ($KMnO_4$) (99%) was purchased from Chadwell Heath Essex England. All these chemicals were used as received without any further Purification. Distilled water was used throughout the experiment.

2.1. Fabrication of polyrhodanine

Polyrhodanine nanoparticles were synthesized by the chemical oxidative polymerization method. Polymerization was done for 20 h at room temperature and under vigorous magnetic stirring due to high shear flow during the polymerization. In a typical experiment, 0.15 gr rhodanine was dissolved in 50 ml distilled water. Along with stirring, the mixture was heated slowly until rhodanine monomers, which are insoluble in water at room temperature, completely dissolve (usually occurs between 60 and 70 °C). In order to prevent aggregation and adherence of monomers, the heated solution was left to cool down to about 35–40 °C. The polymerization process was immediately started; when the oxidant solution consisting of 0.5 g $KMnO_4$ dissolved in 50 ml deionized water was added drop wise.

After polymerization, the synthesized product was obtained by centrifugal precipitation (30 min with 6000 rpm) and washed for several times with distilled water to remove the impurities (monomer, low molecular weight oligomer and unreacted oxidant). Afterward, obtained products were dried at 40°C in an oven for 48 h and stored for further experiments. The surface morphology of polyrhodanine was characterized using Scanning electron microscopy (SEM; KYKY EM3200- China). The molecular structure of the synthesized polyrhodanine was investigated using Fourier transform infrared (FTIR) spectroscopy (Bruker, karlsrohe, Germany).

2.2. Animal model

One hundred fertile eggs were obtained from a broiler breeder farm (Ross 308 strain). The eggs were incubated at 37.5 °C and 65% relative humidity. On the 3th day of incubation, eggs were candled, unfertilized eggs and dead embryos were removed from examination. In the 4th day of incubation embryonated eggs were randomly divided into five groups: 1) control group (without injection), 2) group received 0.1 ppm polyrhodanine, 3) group received 1 ppm polyrhodanine, 4) group received 10 ppm polyrhodanine, and 5) group received 100 ppm polyrhodanine. Administration was done into the egg-albumen with 0.1 ml of mentioned doses according to the Beck method [18]. To avoid contamination, all injections were carried out in a clean room and all the equipments were sterilized. The injection site was sealed with paraffin and the eggs were returned into the hatchery and kept at the temperature of 37 °C until hatch.

2.3. Blood serum biochemical parameters and tissue harvesting

After hatching blood samples were collected and centrifuged, serum of each sample was separated, and kept at −20 °C until serum biochemical analysis. Moreover kidney, spleen, liver, heart, bursa of Fabricius and brain tissues from the newly hatched chick were taken out (n = 50) and fixed in 10% buffered formalin for histological examination.

2.4. Assessment of antiviral activity

Antiviral activity evaluation of polyrhodanine was carried out *in ovo* assay measuring its potential to reduce the EID50 index of a field strain of NDV isolated from pigeon during an outbreak of Newcastle disease featured by respiratory and neurological disorders in 2009 in Tehran province, Iran.

Determining of EID50 index of NDV was performed as described by previously. Sixty 9-day old fertile eggs were obtained from a broiler breeder farm (Ross 308 strain) and were randomly allocated into 4 groups as is described below. Group 1 was consisting of 25 embryonated eggs (dividing into 5 groups of 5 eggs) which were dedicated to control of EID50. Group 2 consisting of 25 embryonated eggs and was organized similar to group 1 dedicating to challenge with polyrhodanine-treated virus. Groups 3 and 4 each consisting of 5 eggs were received 0.1 ml of 100 ppm polyrhodanine or remained un-inoculated, respectively.

To measure the effect of polyrhodanine on NDV, ten-fold serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-3}, 10^{-4} and 10^{-5}) of a suspension of virus containing of 2000 EID50 per 0.1 ml were prepared with sterilized phosphate buffer saline (PBS) in two separate rows of micro-tubes, then 500 μl of 200 ppm concentration of polyrhodanine were added to 500 μl of each diluted virus suspensions of one of two rows of micro-tubes. The contents of the other row received only the solvent used to prepare polyrhodanine (PBS). Following by pipetting well, all mixtures were incubated at 4 °C for one hour. After that, 100 μl of each mixture was inoculated to allantoic cavity of 5 embryonated eggs of each group according to description in paragraph above. Then, all eggs were returned to incubator (temperature 35° C and humidity 65%) and were daily candled to examine the viability of embryos. Five days after inoculation the allantoic fluid of all eggs were harvested and presence of the virus were tested by a standard hemagglutination (HA) test for NDV titration using 1% suspension of chicken red blood cell. The embryo infection dose 50 (EID50) were determined by the Reed and Muench method.

3. Results

3.1. Particle size and morphology of nanoparticles

In Fig. 1 morphology of the synthesized polyrhodanine is shown. As it was anticipated polyrhodanine shows a uniformly dispersed spherical particles morphology with average diameter of 50–200 nm where majority of the particles are <80 nm in diameter [19].

3.2. FTIR spectroscopy

In order to confirm the polymerization of rhodanine monomers FTIR was employed. Fig. 2 shows FTIR spectra of the polyrhodanine synthesized with oxidation polymerization and characteristic peak locations related to the corresponding chemical bonds of polyrhodanine are listed in Table 1.

The peak at 1195 cm^{-1} attribute to the C—O^- stretching vibration. A band at 1438 cm^{-1} is assigned to the C=N^+ stretching in the polymer chain. These peak at 1667 cm^{-1} correspond to the stretching vibration of C =C groups in the polymer chain. The absorption peak at 3412 cm^{-1} is for the hydroxyl group (–OH). There is a tiny dip in the spectra at 2353 cm^{-1} due to the presence of atmospheric CO_2. As highlighted by Song et al., from the FTIR analyses